NEUROBEHAVIOURAL AND HISTOMORPHOLOGICAL EFFECTS OF HYOSCYAMINE FRACTION OF *Datura stramonium* SEEDS ON THE HIPPOCAMPUS AND DENTATE GYRUS OF DEVELOPING AND ADULT WISTAR RATS

BY

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FACULTY OF BASIC MEDICAL SCIENCES,
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MARCH, 2021

DECLARATION

I declare that the work in this thesisentitled "**Neurobehavioural and Histomorphological Effects of Hyoscyamine Fraction of *Datura stramonium* Seeds on the Hippocampus and Dentate Gyrus of Developing and Adult Wistar Rats**" has been performed by me in the Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, Ahmadu Bello University Zaria. The information from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

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CERTIFICATION

This thesis entitled "**Neurobehavioural and Histomorphological Effects of Hyoscyamine Fraction of *Datura stramonium* Seeds on the Hippocampus and Dentate Gyrus of Developing and Adult Wistar Rats**" by**Idris Tela ABDU** meets the regulations governing the award of the degree of Doctor of Philosophy (PhD) in Human Anatomy of the Ahmadu Bello University Zaria and is approved for its' contribution to knowledge and literary presentation.

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ABSTRACT

Datura stramonium (*D. stramonium*) is one of the well-known traditional plants with both poisonous and therapeutic properties due to the presence of tropane alkaloids. Hyoscyamine is one of such alkaloids with suspected teratogenic tendencies,however, received little attention in the literature.The study aimed to evaluate the effects of hyoscyamine fractionof*D. stramonium*seeds on the neurobehavioural and histomorphology of developing hippocampus and dentate gyrus of Wistar rats. Two thousand grams of fresh and mature *D. stramonium*seeds wereprocured, extracted andfractioned to detect the presence and quantify the amount of hyoscyamine using thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) analyses respectively. Thirty-two (32) healthy Wistar rats comprised of adult males and females (1:1), weighed 150-250 g were procured. The oestrus cycles of the female rats were synchronized and the proestrus phases determined by vaginal cytology. Eight (8)Wistar ratscomprised of malesand females(1:1) were randomly selected and mated into four different groupsfor treatments at gestation, lactation, adolescence and adulthood respectively.Each group was further divided equally into control and treatment subgroups. A total of one hundred and twenty (120) litters were obtained after mating. Thirty (30) litters fromgroup I received200 mg/kg in *utero*of normal saline and the hyoscyamine fraction of *D. stramonium* seeds ona gestational day (GD)15-20 respectively. Sixty (60)litters obtained from the group II and III were treated withnormal saline, 400, 800 mg/kg hyoscyamine fraction on a postnatal day (PND)0-21 andPND22-42respectively.Thirty (30) litters obtained in group IV were further subdivided into fourgroups and treated withnormal saline, 200, 400 and 800 mg/kg bodyweight of the fraction on PND56-77 respectively. Litters ingroup I was physically observed for gross

congenital malformation of physical growth indices (eyes-opening, teeth eruption, bilateral ear detachment and hair growth). Functional battery tests for motor reflex using surface righting reflex (SRR), cliff avoidance (CAV) and negative geotaxis (NEG) was conducted on the PND4, 5 and 6 respectively to assess the effects of the fraction on motor reflexesinthe Wistar rat pups. Triple horizontal bar test (3HBT), Morris Water Maze (MWM) and Novel Objects Recognition (NORT) tests were used to assess motor coordination, spatial learning and memory and recognition ability inall groups when theyreachedadulthood. The animals were sacrificed at end of every phase of the treatment and in adulthood. The hippocampus was harvested for histology, stereology, Atomic Absorption Spectrophotometry of selected trace/heavy elements (Cu, Ni and Zn), and gene expressions studies. The data obtained were expressed as Mean±SEM using Minitab 17 statistical package. $P<0.05$ was considered statistically significant.

The results showed that 42 grams (2.10%) of crude ethanol extract of *D. stramonium*seeds were obtained from two thousand grams of the sample. Also,18 grams (0.01%) hyoscyamine fraction obtained from the crude extract and yielded 24.09 µg/dl hyoscyamine.No statistically significant difference ($p> 0.05$) in the physical growth indices observed, however, a statistically significant difference ($p< 0.05$) SRR, CAV and NEG were observed between the groups. There wasalso a statistically significant decrease in bodyweight, NORT, Discrimination Index (DI), Novelty Preferences, in Prenatal, Adolescence and Adulthood exposure and MWM test paradigms ($p< 0.05$). The neuronal numbersin region CA1, CA3 and dentate gyrus (DG) ofthe hippocampus, surface areas and volumes of the hippocampus in the treated groups significantly differed ($p<0.001$) from the control groups. The expressions of the *DLG3* and *NeuroD1* genes

were also significantly downregulated (p<0.001) in the treated groups when compared to the control. Significant differences in the amounts of the Cu, Ni and Zn (p<0.005) were also observed in the treated groups during lactation, adolescence, and adulthood. A variable degree of neurodegenerationand lesions such as pyknosis, cytoplasmic vacuolation and nuclear hyperchromasia were observed in the treated groups.

In conclusion, ingestion of hyoscyamine fraction of *D. stramonium*seeds at any stage of development decreased the bodyweight, impaired motor reflexes, altered the trace elements balance Cu, Ni and Zn balance, impaired recognitionability, spatial learning and memory and downregulated the expressions of *NeuroD1* and *DLG3* in the hippocampus of Wistar rats and neurodegenerations.

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LIST OF ABBREVIATIONS

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2D: Two - dimensional 67

3D: Three - dimensional 67

AAS: Atomic absorption spectrum 109

ABU: Ahmadu Bello University 84

AChE: Actylcholinestrase 53

AD: Alzheimer's Disease 8

ALS: Amyotrophic Lateral Sclerosis 8

ALT: Alanine aminotransferase 25

AST: Aspartate Aminotransferase 25

BAW: Butanol Acetic Water 77

BDNF: Brain-derived neurotrophic factor 336

BUK: Bayero University Kano v

CA: Cornu Ammonis 211

CAV: Cliff avoidance 93

cDNA: Complementary DNA 114

CE: Coefficient of Error 119

CN: Cranial nerve 44

CNS: Central nervous system 51

Co: Cobalt 335

Cr: Chromium 8

CREB: cAMP-response element-binding protein 336

CT: Cycle threshold 204

DG: Dentate gyrus 211

DGCs: Dentate gyrus cells 45

dH_2O: Distilled water 101

DI: Discrimination index 333

DLG3: Disc large gene 3 viii

DNA: Deoxyribonucleic acid 114

Ds: *D.stramonium* 124

ECETOC: European Centre for Ecotoxicologyand Toxicity of Chemicals 6

EtOH: Ethanol 101

Fob: Familiar object 186

FUI: Italian Pharmacopoeia 64

GABA: Gamma amino butyric acid 52

GC: Granular cell 253

GCL: Granular cell layer 251

GUK: Guanylate kinase 47

HC: Hippocampus 225

HD: Huntington's Disease 8

HPLC: High-Performance Liquid Chromatography vi

IHC: Immunohistochemistry 65

$K_2Cr_2O_7$: Potassium dichromate 63

LOD: Limit of detection 127

LOQ: Limit of quantification 127

mAChRs: Muscarinic acetylcholine receptors 333
MAGUK: Membrane Associated Guanylate Kinases 46
MATH: Mammalian atonal homolog 49
Mg: Magnesium 8
ML: Molecular layer 253
MWM: Morris Water Maze 12
N: North 107
NaCl: Sodium chloride 86
N-CH_3: Methylated Nitrogen 14
NeuroD1: Neurogenic differentiation ..... passim
NGE: Negative geotaxis 93
Nob: Novel object 186
NORT: Novel object recognition test vii
NTP: National toxicology programme 330
OECD: Organization for Economic Cooperation and Development 57
OP: Organophosphate 25
PBDEs: Polybrominated diphenyl ethers 56
PBS: Phosphate buffered saline 109
PC: Pyramidal cell 245
PCR: Polymerase Chain Reaction 116
PD: Parkinson's Disease 8
PFC: Prefrontal cortex 6, 347
PND: Postnatal day 3

PPI: Protein-protein interaction 47

PSD: Postsynaptic density 48

PTU: Propylthiouracil 56

RNA: Ribonucleic acid 114

SGZ: Subgranular zone 45

SO: Stratum oriens 232, 234, 236

SR: Stratum radiatum 232, 234, 236

SRR: Surface righting reflex 93

St: Standard L-hyoscyamine sulphate 124

SW: South-west 107

TA: Tropane alkaloid 333

TLC: Thin Layer Chromatography vi

USEPA: United Nation Environmental Protection Agency 57

USP: United State Pharmacopoeia 64

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Daturastramonium (*D. stramonium*) also called inHausa (*Zakami*);Yoruba*Apikan/Gegemu*); and Igbo (*Myaramuo*) (Steenkamp, 2004; Babalola, 2014)is one of the well-known traditional plants from the *Solanaceae* family with both poisonous and medicinal properties (Basu and Lal, 1947). It has been used as aherbal medicine for relieving coughing, asthma and controlling pain for a long time (Busia, 2005). *D.stramonium*originated from the tropical areas of Central and South America (Henderson, 1995) and it is now a cosmopolitan weed in temperate regions. *Datura*plant owns toxic and poisonous features (Wahlström and Nordberg, 1992). All parts of *Datura*are toxic, but the highest concentration of toxic compoundsstored in ripe seeds (Shagal *et al.,* 2012). *D. stramonium*contains a variety of alkaloids, including atropine and scopolamine that can cause anticholinergic poisoning, if taken in large dosages (Friedman and Levin, 1989; Brown and Taylor, 2006).Atropine is found to have more exciting properties, while scopolamine has more relaxing and hallucinogenic properties (Weitz, 2003). The concentration of these components may vary with species and the environment where it is grown. So, the range of toxicity is unpredictable from plant to plant. The seeds are responsible for the anticholinergic toxicity of the plant (Joshi, 2003). Atropine and scopolamine are competitive antagonists of muscarinic cholinergic receptors and central nervous system depressants (Brown and Taylor, 2006). Symptoms of *D. stramonium*toxicity include both peripheral (dry mucosa, flushed skin, mydriasis and blurred vision, thirst, swallowing difficulty, urinary retention and tachycardia) and central (agitation, combative behaviour, hallucination, delirium, seizure and coma)

(Busia, 2005). Lately, this plant has become an increasingly widespread narcotic and local anaesthetic drug in many countries (Akal *et al.,* 2014). Many young people use its seeds by smoking for hallucinogenic effects which causes serious organ and tissue poisoning (Schulman and Bolton, 1998). Diverse reactions have been reported.*Datura*poisoning occurs because of contamination of food and decoction prepared from herbal prescriptions or when these plants species are eaten accidentally (Devi, 2011).

The developing foetus underlies daily changes during the process of prenatal ontogeny, which is generally highly predictable in mice (Wahlsten, 1974). Still, the exact timing of developmental events and consequently the exact end of the prenatal phase can vary, leading to a delayed or advanced onset of the postnatal phase in a range of about one day (Williams and Scott, 1954). Post-partum variation concerning the exact timing of developmental milestones expands to several days and even months as the mice grow older. Different strains of inbred mice do further extend or shorten certain life periods or have shifted distinct developmental events (Fox, 1965; Nelson *et al.,* 1990; Moore *et al.,* 2011, Yuan *et al.,* 2012). Also, distinct environmental cues might delay or increase the speed of maturation (Wittingham and Wood, 1983).

The prenatal phase of development is a period that fertilized egg divides and a variety of cell types, specialized tissues, and organs are formed. The processes during embryogenesis include coordinated cell division, cell specialization, cell migration, and genetically programmed cell death (Prather and First, 1988). Of special interest for any behavioural development process following the neurulation which begins around

embryonic day eight or nine (Copp *et al.*, 2013) with the closure of the neural tube. The neural tube develops into the spinal cord and the brain, orchestrated by coordinated and precisely timed gene expression of thousands of genes (Liscovitch and Chechik, 2013).

In the early postnatal phase of development, environmental modifications are the most critical challenges in the successful survival of a mouse. Morphological changes such as the appearance of hairs occur around three days of age (Okon, 1970), but temperature regulation continues to be solely dependent on the environment as well as the quality of the nest, maternal care, and position within the litter (Okon, 1970; Sales and Skinner, 2009). Early neurobehavioural tasks learning has been conducted successfully with mice at postnatal day (PND 3) with full retention of learning after 24 hours which could only be detected in mice from PND 9 onwards (Alleva and D' Udine, 1987). Ultrasonic vocalisation eliciting maternal care begins after birth and increases in intensity until around PND 8 (Elwood and Keeling, 1982). The ears open on PND 3 (Theiler, 1972) and the ability to be conditioned to auditory cues are present as early as on PND 4 (Williams and Scott, 1954). Complete development of the inner ear structures takes more time and is completed at PND 13 (Theiler, 1972). The eyes around 11-13 days postnatally (Williams and Scott, 1954; Theiler, 1972). Before the PND 16, mice pups are fed by nursing only. Nursing continues until the age of about 22 days and is successively complemented by solid food. The rapid development of behavioural abilities and reflexes begins at birth. First complex motor abilities like forelimb grasping, placing of hind limbs, standing, and self-grooming develop continuously over the first six days of life (Fox, 1965). The early postnatal period ends around PND 21-25 with the completion of weaning and thus the mouse becomes independent from the mother (Le Roy *et al.*, 2001).

The maturation of nutritional and behavioural independence is a change in the life of young mice that marks one of the focal points in development (Tsuda *et al.,* 2011). Adolescence is the developmental stage that marks the transition from childhood to adulthood. During this phase, significant hormonal and behavioural changes, as well as substantial alterations in structural and neurobiological remodelling, occur together in the cortical and limbic circuits (Romeo *et al.,* 2002). This change leads to cognitive, emotional, social, and sexual maturation (Sisk and Zehr, 2005; Spear, 2000). The period of adolescence can be subdivided into three intervals, early- (pre-pubescent, PND 22-34), mid- (pubescent, PND 35-47) and late adolescence (sexually mature, PND 48-60) (Laviola *et al.,* 2003; Adriani *et al*., 2004). The adolescence phase has a unique difference substantially from adulthood. For instance, there is a general trend of increased open field activity (Hefner and Holmes, 2007; Moore*et al.,* 2011; Blaney *et al.,* 2013; Molenhuis *et al.,* 2014), increased food consumption which is accompanied by high energy expenditure (Moore *et al.,* 2011) throughout all three stages of adolescence well into adulthood. It was demonstrated that adolescent mice (PND 26-28) often exhibit a highly flexible behaviour compared to adults (PND 60-70) in a multiple-choice reversal learning test (Johnson and Wilbrecht, 2011). In line with these findings are observations of increased risk-taking (Laviola *et al.,* 2003) and sensitivity to drugs and alcohol consumption (Adriani *et al.,* 2004; Hefner and Holmes, 2007) during adolescence. Overall, changes in exploration, risk-taking behaviour, and behavioural flexibility reflect the preparation for becoming independent (Spear, 2000). Adulthood is accompanied by behavioural maturity, ability to mate and successfully give birth and raise offspring. Although is debatable that mice at PND 55 – 60 are considered at adulthood or late

adolescence stage, female mice earliest mating starts around PND 35, at PND 50 half of the female mice paired with a male were mated and 100% were mated at PND 60 (Chehab *et al.,* 1997). In male mice, the balano-preputial-separation, which allows pheromonal communication, is often used as an external indicator for completed puberty and the first sign of adulthood. However, the balano-preputial-separation is usually detectable as early as around PND 30 (Qiu *et al.,* 2013), which is long before mating attempts, can be observed and thus falls clearly into adolescence. Therefore, exposure of the rat at any of the development phases to obnoxious chemicals or neurotoxicants may lead to silent, delayed or chronic neurotoxicity that could result in abnormal birth, permanent damage or death.

'Neurulation' refers to the coordinated morphogenetic stages during chordate embryogenesis that gives rise to the neural tube, which through continued growth and differentiation ultimately develops into the brain and spinal cord(Rubenstein *et al.,* 1998). During neurulation, the neural plate folds upon itself to form the neural tube (Zohn and Sarkar, 2008). Different regions of the neural tube areformed by two different processes: primary and secondary neurulation. In primary neurulation, the flat neural plate proliferates and invaginates, creasing inward until the elevating edges come in contact and fuse to form a cylindrical, hollow neural tube. Secondary neurulation occurs in the caudal section where the solid cord of cells sinks into the embryo and cavitates to form a separate hollow tube in the tailbud. The tubes formed by primary and secondary neurulation eventually connect (Rubenstein *et al.,* 1998; Zohn and Sarkar, 2008; Zohn, 2012; Copp *et al.,* 2013). The ontogenetic development of the cerebral cortex of the brain is a process of astonishing complexity. Every cubic millimetre of cortical tissue in the

order of 10^6 neurons must be wired appropriately for their respective functions such as the analysis of sensory inputs, the storage of skills and memory, or motor control (Wolf and Geisel, 1998). The cerebral cortex develops from a morphologically uniform neuroepithelium located in the "dorsal" part of the telencephalic vesicles, or pallium (Puelles *et al.*, 2000). The pallium is further subdivided into the hippocampal formation (archicortex), neocortex, olfactory/piriform cortex (paleocortex), and claustrum/pallial amygdala (Puelles *et al.*, 2000). Each of these large domains is further subdivided. For example, within the neocortex, rostral regions regulate motor and executive functions (that is, prefrontal cortex), whereas caudal regions regulate sensory processing somatosensation, audition and vision). The prefrontal cortex (PFC) is a neocortical region that has critical roles in higher cognitive functions and has been implicated in neuropsychiatric disorders. The PFC is divided into dorsal, medial and orbital regions (Ongür and Price, 2000; Price, 2006).

Neurotoxicity has been defined as "any adverse effect on the chemistry, structure or function of the nervous system, during development or at maturity, induced by chemical or physical influences" (Costa, 1998). An adverse effect is "any treatment-related change which interferes with normal function and compromises adaptation to the environment" [European Center for Ecotoxicology and Toxicology of Chemicals (ECETOC), 1992]. Thus, adverse effects can be considered as morphological changes such as neuronopathy (a loss of neurons), axonopathy (a degeneration of the neuronal axon), myelinopathy (a loss of the glial cells surrounding the axon), or other gliopathies, would be considered adverse, even if structural and/or functional changes were mild or transitory. Also, neurochemical changes, also in the absence of structural damage, should be considered

adverse, even if they are transient and reversible, as they lead to impaired function. There is a potential difference between the developing and the mature nervous system neurotoxicity. This underscores the fact that developmental neurotoxicity is an important aspect of neurotoxicology. Many known human neurotoxicants are indeed developmental neurotoxicants (Grandjean and Landrigan, 2006), and in most cases, the developing nervous system is more sensitive to toxicants than the adult nervous system (Crofton, 2008). Neurotoxicity can also occur as a result of indirect effects, such as damage to hepatic or cardiovascular structures, or because of interference with the endocrine systems. Some chemicals may have multiple modes of action and may affect the nervous system both directly and indirectly. For example, some halogenated compounds may interact directly with brain cells, and also affect the development of the nervous system by altering thyroid hormone homeostasis (Costa and Giordano, 2007; Crofton, 2008).

Trace elements play important roles in chemical, biological, metabolic, and enzymatic reactions in the living cells of plants, animals and human beings (Hashmi *et al.*, 2007). However, the release of trace metals through human activities into the environment has increased over the years and the excess of these metals in the environment has been reported to be extremely dangerous to human health (Olowoyo *et al.*, 2010). The uptake of trace elements by plant varies and depends largely on several factors, such as soil pH and organic matter content (Logan *et al.*, 1997). Plant uptake is one of the major routes of food chain exposure to trace elements in the soil. Numerous studies have been conducted worldwide to determine heavy metal levels in medicinal plants and plant-based products (Annan *et al.*, 2010; Maharia *et al.*, 2010; Ebrahim *et al.*, 2012). The broad use of traditional medicine by rural communities due to the accessibility and affordability of

herbal medicine has also necessitated further research into the uptake and translocation pattern of trace metals by some medicinal plants grown in the urban areas (Ajasa *et al.,* 2004). While heavy metals have been detected in African traditional remedies few studies have documented the commonly available metals used in African traditional medicine or investigated the reason behind such usage (Ernst, 2002; Dargan *et al.,* 2008). Hexavalent chromium (Cr(VI), a class 1 carcinogen, is commonly used in South African traditional medicines and known locally as *ndonya*. Poisonings from traditional medicine products containing heavy metals are well documented (Ernst, 2002; Dargan *et al.,* 2008). The toxic effects of heavy metals are due to their hindrance with the regular body biochemistry in normal metabolic processes (Duruibe *et al.,* 2007). Arsenic, Cr and magnesium (Mg) are the heavy metals most frequently implicated in morbidity and death in South Africa (Steenkamp *et al.,* 2002). A report revealed that a seven-month-old infant was hospitalized after the intake of traditional medicine which resulted in a severe case of multiple metal poisoning (Steenkamp *et al.,* 2002). It is known that numerous traditional medicines give rise to severe renal pathology, the mechanism of which is uncertain but which could be associated with heavy metal toxicity (Steenkamp *et al.,* 2000).

Neuronal injury and neurodegenerationare hallmark pathologies of a variety of acute and chronic neurological conditions such as traumatic brain injury, stroke and epilepsy. Neuronal injury or cell death refers to many acute and chronic neurological conditions, including Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD), stroke, trauma, epilepsy, multiple sclerosis and amyotrophic lateral sclerosis (ALS) (West *et al.,* 1991). The stereology technique allows for the reliable quantitative description of a 3D object to be made from 2D measurements. This is achieved by

creating a *Z*-stack of 2D images to create a 3D tissue model (West *et al.,* 1991; West, 2012a). Stereology utilizes random, systematic sampling to provide unbiased and quantitative data (Mayhew, 2014). Modern design-based stereology has been an important and efficient tool in many applications of optical and confocal microscopy. There is a multitude of uses for stereology in many biomedical fields including histology, and neuroanatomy to accurately quantify the number of cells, the length of fibres; and the area and volume of biological structures or regions (Mayhew, 2014; Bronoosh *et al.,* 2015).

Cell polarity is essential to the function of many cell types, such as epithelial cells and neurons. The Discs large (*Dlg*) scaffolding protein was identified in Drosophila as a major regulator of basolateral epithelial identity. Four *Dlg* orthologs (*Dlg*1 through 4) are found in vertebrates and mutations in the human *DLG*3 gene is associated with X-linked mental retardation (Lee *et al.,* 1995). It was reported that *DLG*3 controls apical epithelial polarity and tight junction formation and contributes to neural induction in mouse development (Van Campenhout *et al.,* 2011).*NeuroD*1 is involved in neurogenesis. It can convert embryonic epidermal cells into fully differentiated neurons in Xenopus and promotes premature cell-cycle exit and differentiation of neural precursor cells (Lee *et al.,* 1995).

1.2. Statement of the Research Problem

Medicinal plants play important roles in the treatment of human diseases, but the unguarded rise in the use of herbal medicines, deliberate ingestion and accidental contaminations raises a question as to how safe are these preparations for unborn foetuses.*Daturastramonium*is one of the well-known traditional plants in the folklore with both poisonous and medicinal properties (Diker, 2007; Pandey, 2011) due to the alkaloids present. Accidental contamination or deliberate ingestion of any part of this plant especially the seeds have been the major source of toxicity which often results infatal consequences. This is due to alkaloids present, especially hyoscyamine which possesses both anticholinergic and suspected teratogenic properties (Hanson, 2008). Several studies have been conducted ontoxicological effects of *D. stramonium*seeds focused mainly on its acute toxicityin adult Wistar rats,little attention was, however, paid tothe alkaloidfractions especially hyoscyamine on the hippocampus and dentate gyrus of developing and adult Wistar rats.

1.3. Justification of the Study

Although Nigeria has laws concerning foods, drugs, and cosmetics, the laws are not effective in hinderingthe procurement ofherbs,which many have considered safe, better and sometimes more effective than most conventional or allopathic medicines.With the poor health system and exorbitant charges in our hospitals, many patientswould resort to the use of the local medicinal herbs such as *D. stramonium*to meet up with their health needs without considering the possible harmful effects that may arise from such action. *D. stramonium* is a hallucinogenic herbthat iseasily accessible. The seeds of this plant are commonly abusedespecially by the youths as a substitute to the conventionalpsychedelic and hypnotic drugs, which are more often expensive and restricted by the law,to get them "high" (Vearrier and Greenberg, 2010). This study willdetermine the possible consequences associated with the unguarded consumption of the local herbs both at prenatal and postnatal stages of development about the physical body growth and histomorphology in the hippocampus of Wistar rats. It may also give a clue on the teratogenic exposure in experimental animals as an important approach to understanding how environmental insults at particular developmental stages can lead to complex brain malformations/development and subsequent outcome of neurobehavioral disorders. This may serve as a tool for legal practitioners to re-invigorate the existing laws prohibiting drugs and related substances abuse.

1.4. Aim and Objectives

1.4.1. Aim of the study

The aim was to evaluate the effects of hyoscyamine fraction of *D. stramonium*seeds on the neurobehaviour and histomorphology of hippocampus and dentate gyrus in developing and adult Wistar rats.

1.4.2. Objectives of the study

The objectives of the study were to evaluate;

i. the phytoconstituents and quantifythe hyoscyamine fraction of the crude ethanol extract of the seeds of *D. stramonium* using High-Performance Liquid Chromatography (HPLC).

ii. the effect of hyoscyamine fraction ingestion of*Daturastramonium* seeds on physical growth and body weights in Wistar rats.

iii. the effects of the hyoscyamine fraction of *D. stramonium* seedson the motor coordination in theWistar rat pups using surface righting reflex, negative geotaxis, cliff avoidance and triple horizontal bars tests paradigms.

iv. the effect of the hyoscyamine fractionof *D. stramonium* seedson the spatial learning and memory in adult Wistar rat fed with *D. stramonium* seeds using Novel Object Recognition (NOR) and Morris Water Maze (MWM) tests paradigms.

v. the effects of hyoscyamine fraction of *Daturastramonium* seeds on the histomorphology of hippocampus in Wistarrats using Hematoxylin and Eosin stain.

vi. the effect of hyoscyamine fractionof *D. stramonium* seeds ingestion on the quantity of trace/heavy elements (Cu, Ni, Pb, and Zn) in the hippocampus of Wistar rats using Atomic Absorption Spectrophotometry (AAS500).

vii. the expression of *DLG3* and *NeuroD*1 genes in the hippocampus Wistar rats exposed to graded doses of hyoscyamine fraction of*D. stramonium* seeds using quantitative Real-Time Polymerase Chain Reaction (qRT – PCR).

viii. estimate the neuronal numbers, thicknesses, areas and volumes of Dentate gyrus (DG) of the hippocampus of Wistar rats treated with hyoscyamine fraction of*D. stramonium* seeds using unbiased Cavalieri Estimator Counting Frame, Physical Fractionator Grids of *STEPanizer[v1.0]* and *AperioImageScope [v12.4.0.5043]* stereological tools.

1.5.The Hypotheses of the Study

Alternative Hypothesis (H_a): Ingestion or exposure to hyoscyamine fraction of*D. stramonium* seeds impairs;

i. motor reflexes and coordinationin Wistar rats.

ii. spatial learning and memory functionsof the hippocampusin Wistar rats.

iii. quantity of selected trace elements (Cu, Ni, Pb and Zn) in the hippocampus in Wistar rats.

iv. expression of the neurogenic differentiation 1(*NeuroD1*) and disc large homolog 3(*Dlg3*) in the hippocampus of Wistar rats.

v. stereological indices (neuronal numbers, thicknesses, areas and volumes) of the hippocampus and dentate gyrus in Wistar rats.

vi. histologyof the hippocampus (CA1 and CA3) and dentate gyrus in Wistar rats.

CHAPTER TWO

2.1.Literature Review

2.1.1.*Daturastramonium*

Medicinalplants have always played a major role in the treatment of human traumas and diseases worldwide. The increasingdemand for medicinal plant in both developed and developing countries is due to the growing recognition of natural product. Herbal medicine is an important part of both the traditional and modern system of medicines (Kirtikarand Basu, 1994). *Daturastramonium* (*D. stramonium*) is a widespread annual plant from the Solanaceae family. It is one of the widely well-knownlocal medicinal herbs. It is a wild-growing flowering plant and was investigated as a local source fortropane alkaloids which contain a methylated nitrogen atom ($N\text{-}CH_3$) and include the anticholinergic drugs atropine, hyoscyamine and scopolamine. In ancient civilization, it was traditionally used for religious visionary purposes throughout the world and witchcraft in medievalEurope. The god Lord Shiva was known to smoke *Cannabis* and *Datura*. People still provide the small thorn apple during festivals and special days asofferings in Shivaicons at temples. The leaves extract is taken orally for the treatment of asthma and sinus infections,and stripped bark is applied externally to treat swellings,burns and ulcers. The incidence of*D. stramonium*poisoningis sporadic with a cluster of poisoning cases in the 1990sand 2000s, the United States media reported some casesoccurring mostly among adolescents and young adults dyingor becoming seriously ill from ingestion. Some medicinaluses of the plant are its anti-inflammatory property ofall parts of the plant,stimulation of the central nervoussystem, respiratory decongestion, treatment of dental andskin infections, alopecia and the treatment of toothache.It is a hallucinogenic plant that causes serious poisoning.Consumption of any part of the plant

may result in asevere anticholinergic reaction that may lead to toxicityand occasionally cause diagnostic difficulties. Cases ofpoisoning have been reported after eating the berries.Death may occur from heart failure after ingesting 125seeds because the seeds contain the highest concentration of atropine and have a rapid onset of action, thus may be potentiallyuseful as an alternative to atropine for the treatment of themuscarinic symptoms of organophosphate toxicity and someof the central anticholinergic effects. The wide distribution,the strong toxicity and the potential foroccurrence infoodstuffs are responsible for the numerous incidents inhumans(Das *et al.,* 2012). *Datura* genus distributes over tropical and warmtemperate regions of the world. About ten species of *Datura*are found, of which *Daturainnoxia* and *D. stramonium*arethe most important drug plants. *Datura* has long been knownas a medicinal plant and as a plant hallucinogen all overthe world. Pre-historic use of *Datura* in medicinal andceremonial rituals could be observed in aboriginal in Indiansub-continent(Parashuram, 2011). The therapeutic activities of most plantsare due to the presence of one or more of such componentsas alkaloids, tannins, saponins and cardiac glycosides.

The phytochemical screening revealed the presence ofsaponins, tannins, steroids, alkaloids, flavonoids, phenolsand glycosides(Shagal *et al.,* 2012).Atropine and scopolamine are competitiveantagonists of muscarinic cholinergic receptors and arecentral nervous system depressants. All parts of the plantare toxic, but the highest amount of alkaloids is containedin the ripe seeds (Oseni, *et al,* 2011; Shagal *et al.,* 2012). Many cases of accidental poisoning by *D. stramonium*have been reported when these plants wereeaten accidentally (Devi *et al.,* 2011).

2.1.2.Regional and other names of *D. stramonium*

Sanskrit: Umatta-virkshaha, English: Thorn apple, Hindi: Sadah-*Datura*, Safed *Datura*, Tamil: Umatai Arab: Jonz-masal, Gujrat: Dhatoria, Bengali: Dhattura, Malayalam: Maraummam,Marathi: Kanaka,

2.1.3.Taxonomic classification of *D. stramonium*

Kingdom: Plantae

Phylum: Magnoliophyta

Subphylum: Angiospermae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Solanales

Family: Solanaceae

Genus: *Datura*

Species: *Daturastramonium*

2.1.4. Botanical description

Plant appearance

*D. stramonium*is an annual plant (plate I). The stem isherbaceous, branched and glabrous or only lightly hairy. Bycultivation, the plant reaches a height of about one meter(Nadkarni and Nadkarni, 1996; Jaraldand Edwin, 2007).The branching stems are spreading, leafy, stout, erect, smoothand pale yellowish-green in colour, branching repeatedly ina forked manner. Leaves are hairy, big, simple dentate, ovalglabrous, apposite veins of leaves are pale black, stalked,4 – 6inches long, ovate and pale green. The upper surface isdark and greyish-green, generally smooth, the under surfacepaler, and when dried, minutely wrinkled (plate Ia). *D. stramonium*bears funnel-shaped, white

or purple colouredflowers, with 5 stamens and a superior ovary (plate Ib). Theaverage length of the flower is about 3 inches. The calyx is long,tubular and somewhat swollen below and very sharply fiveangled surmounted by five sharp teeth. Corolla is funnel-shaped. The stem stalk is pale blue or greenish-white. Seeds are black, kidney shape and flat (Gupta, 2008; Gary *et al.*, 2005). Fruits are as large as walnuts and full of thorns (hence the English name "thorn apple") (plate Ic). The plant is a strong narcotic but has a peculiar action on the human which renders it very valuable as medicines. The whole plant is poisonous and the seeds are the most active; neither drying nor boiling destroys the poisonous properties. The symptoms of acute Jimsonweed poisoning included dryness of the mouth and extreme thirst, dryness of the skin, pupil dilate ion, impaired vision, urinary retention, rapid heartbeat, confusion, restlessness, hallucinations, and lossofconsciousness(Das*et al.*, 2012).

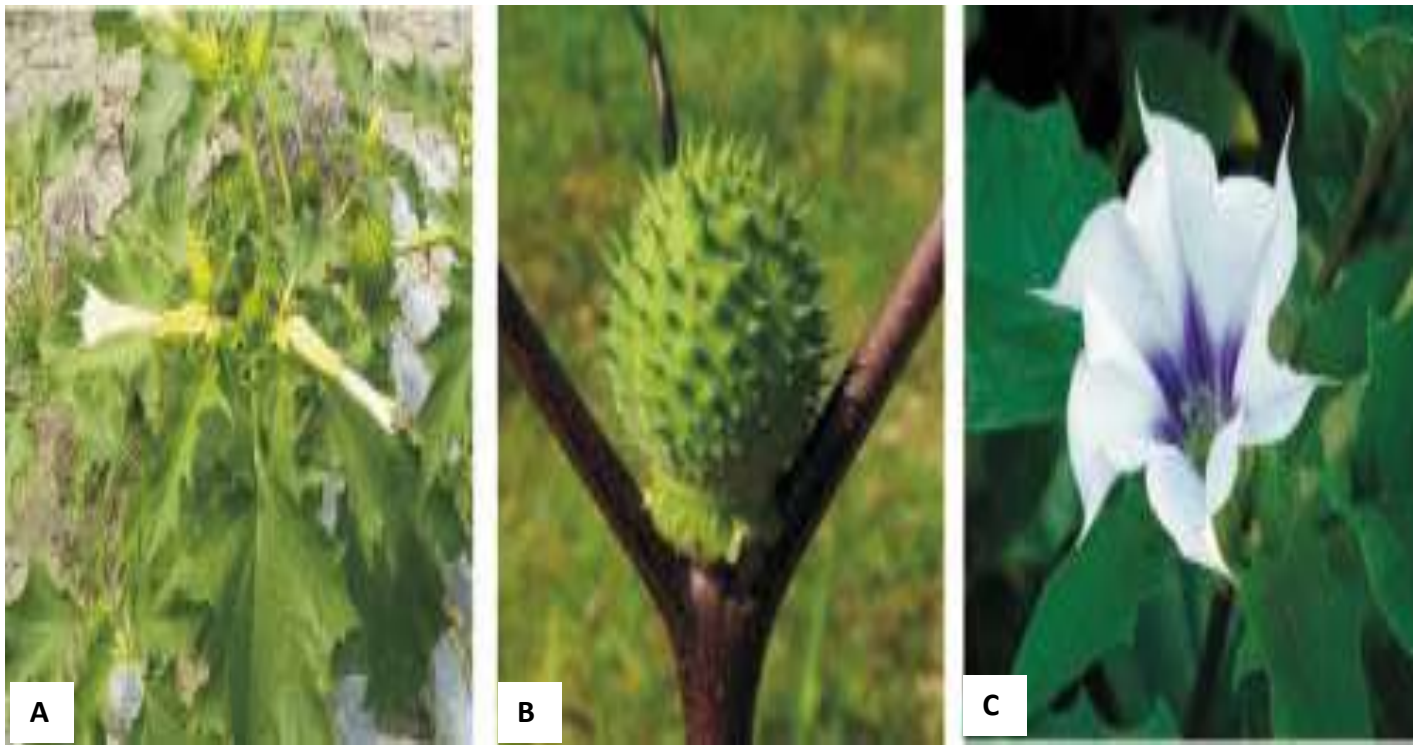


Plate I: *D. stramonium*Plant

A: Leaves, **B**: Seeds; **C**: Flower (Adapted from Soni *et al.*, 2012)

2.1.5.Distributions of *D. stramonium*

*D. stramonium*is probably originated in Caspian Sea territories and spread to Europe in the first century. At present, it grows in waste places in Europe, Asia, America and South Africa. *D. stramonium*is cultivated in Germany, France, Hungary, South America and throughout the world (Jarald and Edwin, 2007).

2.1.6. Ethnomedical uses

Plant-derived drugs come into use in modern medicine through the uses of plant material as an indigenous cure in folklore or traditional systems of medicine. The leaves of *D. stramonium*L. are used for the relief of headache and vapour of leaf infusion is used to relieve the pain of rheumatism and gout. The smoke from the burning leaf is inhaled for the relief of asthma and bronchitis. European remedy of *D. stramonium*for haemorrhoid is to steam the part over boiling water containing a leaf. The fruit juice is applied to the scalp for the treatment of falling hair and dandruff. It is also applied to smooth painful wounds and sores. Seeds and leaves of *D. stramonium*were used to sedate hysterical and psychotic patients, also to treat insomnia (Pandey *et al.,* 2011). *D. stramonium*was used as a hallucinogenicherb. It is also used to relax the smooth muscles of the bronchial tube and asthmatic bronchial spasm. It was reported that *D. stramonium*was used internally to treat madness, epilepsy and depression. Externally it forms the basis of ointment for burns and rheumatism (Guarrera, 1999). It is also used in the treatment of Parkinsonism and haemorrhoids. Its leaves, applied after roasting, are useful in relieving pain. The bitter narcotic plant relieves pain and encourages the healing process. The seeds of the plant are medicinally the most active. Externally, the plant is used as a poultice in treating fistulas, abscesses wounds and severeneuralgia. Scopolamine is also found in the plant,

which makes it a potent cholinergic-blocking hallucinogen that has been used to calm schizoid patients. Its leaves, containing hyoscyamine and atropine, can be used as an immensely powerful mind-altering drug. The seeds of *Datura* are analgesic, anthelmintic and anti-inflammatory and as such,they are used in the treatment of stomach and intestinal pain that results from worm infestation, toothache, and fever from inflammation. The juice of its fruit is applied to the scalp, to treat dandruff and falling hair. The growing plant works as an insect repellant, which protects neighbouring plants from insects(Das *et al.,* 2012). *D. stramonium*is mostly used as anthelmintics and antiparasitic in Marche, Abruzzo and Latium. Records of continued use of the plant in these sectors were collected from farmers and shepherds (mostly old people) (Ertekin *et al.,* 2005; Shagal *et al.,* 2012).

2.1.7.Phytochemicals of *D. stramonium*

The major tropane alkaloids hyoscyamine and scopolamine and several minor tropane alkaloids have been identified in *Datura* species. Typical examples of minor alkaloids in *D. stramonium*are tigloidin, aposcopolamine, apoatropin, hyoscyamine N-oxide and scopolamine N-oxide. 6-α-ditigloyloxytropane and 7-hydroxyhyoscyamine are reported for the first time in this species (Das *et al.,* 2012).The production of hyoscyamine and scopolamine in *D. stramonium*has been investigated in the different plant parts, at different stages of their life cycle. The maximum contents were found in the stems and leaves of young plants, hyoscyamine being always the predominant component. These compounds were included in many pharmacopoeias because of their anticholinergic activities (Shagal *et al.,* 2012). *D. stramonium*containsa variety of alkaloids including atropine, hyoscyamine and scopolamine (Ivancheva*et al.,* 2006).

2.1.8.The pharmacological activity of *D. stramonium*

Antiasthmatic activity

*D. stramonium*in asthma treatment and possible effects on prenatal development were studied. Exposure of the foetus to *D. stramonium*when a mother uses it for asthma willcause acontinuous release of acetylcholine, resulting in thedesensitization of nicotinic receptors; this could ultimatelyresult in permanent damage to the foetus. Itwasconcluded that this African herbal remedy should be usedwith caution during pregnancy(Pretorius and Marx, 2006).

Anticholinergic activity

The alkaloids found in *D. stramonium,* are organic esters used clinically as anticholinergic agents. Jimson weed has been reported as a drug of abuse and has been involved in the accidental poisoning of humans and animals. Symptoms of acute jimson weed poisoning included dryness of the mouth and extreme thirst, dryness of the skin, pupil dilation and impaired vision, urinary retention, rapid heartbeat, confusion, restlessness, hallucinations, and loss of consciousness. Anticholinergic syndrome results from the inhibition of central and peripheral muscarinic neurotransmission (Taha and Mahdi, 1984; Boumba *et al.,* 2007; Diker *et al.,* 2007).

Acaricidal, repellent and oviposition deterrent properties

The ethanol extracts obtained from both leaf and seed in *D. stramonium*(Solanaceae) were investigated for acaricidal, repellent and oviposition deterrent properties against adult two-spotted spider mites (T. urticae Koch) (Acari: Tetranychidae) under laboratory conditions. Leaf and seed extracts, which were applied in 167.25 and 145.75 g/L concentrations, respectively (using a Petri leaf disc-spray tower method), caused 98% and 25% mortality among spidermite adults after 48 h. These results suggest that *D.*

*stramonium*extracts could be used to manage the two-spotted spider mite (Kumral *et al.*, 2009).

Antimicrobial Activity

The methanol extracts of *D. stramonium*and *Daturainoxia* showed activity against Gram-positive bacteria in a dose-dependent manner. Little or no antimicrobial activity was found against Escherichia coli and Pseudomonasaeruginosa (Takhi *et al.*, 2011). The anti-microbial activity of combined crude ethanolic extract of *D. stramonium*,*Terminalia arjuna* and *Withania somnifera* in cup plate diffusion method for antibacterial and antifungal activity. The extracts were subjected to screening to detect potential antimicrobial activity against *Staphylococcus aureus, Bacillus subtilus, Escherichia coli, Klebsiella pneumoniae, Micrococcus luteus*and *Candida albicans* with comparing Ciprofloxacin standard drug (Sharma and Sharma, 2010).

Anticancer activity

An integrated approach is needed to manage cancer using the growing body of knowledge gained through scientific developments. Thousands of herbal and traditional compounds are being screened worldwide to validate their use as anticancerous drugs. *D. stramonium*isa therapeutic dose of 0.05-0.10 g was used to cure cancer. Likely unsafe produce vomiting, hypertension, loss of consciousness may lead to coma but may interact with anticholinergic drugs (Balachandran and Govindarajan, 2005).

Antiinflammatory activity

Coriandrum sativum (*C. sativum*), *D. stramonium*and *Azadirachta indica* (*A. indica*) are traditionally used in the treatment of inflammation. Ethanolic extracts of fruits of

C.sativum leaves of *D. stramonium.* Ethanolic extracts of fruits of C. sativum, leaves of *D. stramonium*and A. indica were subjected to preliminary screening for anti-inflammatory activity in albino rats. All ethanolic extracts exhibited significant anti-inflammatory activity comparable to the standard drug diclofenac sodium against the carrageenan-induced rat paw oedema method. Among these plant, A. indica showed maximum anti-inflammatory activity per hour (Gupta *et al.,* 2008).

Larvicidal and mosquito repellent activities

Ethanolic extracts of leaves of *D. stramonium*were evaluated for larvicidal and mosquito repellent activities against Aedes aegypti, Anopheles stephensi and *Culex quinquefasciatus*. The LD_{50} values for larvicidal activity were found to be 86.25, 16.07 and 6.25 mg/L against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* respectively. The ethanolic leaves extract of *D. stramonium*provided complete protection time (mosquito repellency) of 2.7, 71.7 and 117.7 minutes against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* at higher concentration (1%) (Swathi *et al.,* 2012).

Pesticide toxicity

Extract of *D. stramonium*was effective in countering the toxicity of the cypermethrin pesticide toxicity (Das *et al.,* 2003). Antifungal activity of a concoction brewed from *D. stramonium,Calotropis gigantea*, A. indica (neem) and cow manure (T1) followed by methanol-water (70/30 v/v) extracts of *D. stramonium,Calotropis gigantea* and *A. indica* T2 against Fusarium mangiferae. The study proved that the concoction-brewed compost T1 is effective, inexpensive, easy to prepare and constitutes a sustainable and eco-

friendlyapproach to control floral malformation in mango when it is sprayed at bud break stage and again at fruit set stage (Usha *et al.,* 2009).

Vibriocidal activity

A simple in vitro screening assay was employed for the standard strain of *Vibrio cholerae*, 12 isolates of *Vibrio cholerae*non-O1, and *Vibrio parahaemolyticus*. Aqueous and organic solvent extracts of different parts of the plants were investigated by using the disk diffusion method.Extracts from 16 medicinal plants were selected on account of the reported traditional uses for the treatment of cholera and gastrointestinal diseases, and they were assayed for vibriocidalactivities. The results indicated that *Lawsonia inermis, Saraca indica, Syzygium cumini, Terminalia belerica, Allium sativum,* and *D. stramonium*served as broad-spectrum vibriocidal agents (Sharma *et al.,* 2009).

Biopesticide with antifungal activity

Biopesticides (leaf extracts) obtained from eight plants (*Vitex negundo, Polyanthalongifolia, Vinca rosea, Withania somnifera, Lawsonia inermis, Adhotoda zylanica, D. stramonium*and*Hyptis suaveolens*) showed antifungal activities against the fungal pathogen (*Fusarium oxysporum*) of wilt of pigeon pea (*Cajanuscajan* L.). Both in vivo and in vitro higher concentration of ethanolic leaf extracts of all eight plants showed complete inhibition in linear growth and sporulation in test fungi (Khandare and Salve, 2011).

2.1.9.Toxicity studies using D. *stramonium*

Toxicity studies of ethanol extract of the leaves of *D. stramonium*in rats. Two doses of 50 and 200 mg/kg of the extract were administered to the rats for five weeks. Parameters studied were the indices of liver and kidney function and some biochemical and

haematological parameters. Feed intake, final body weight, serum AST, ALT, bilirubin, total protein, urea and the electrolyte studied were not affected by the extract administration. Serum creatinine levels were however significantly raised in the rats administered with ethanol extract at the dose of 200 mg/kg body weight. The biochemical and haematological parameters were also affected (Giadado *et al.*, 2007). The effects of acute, subacute and chronic administration of alkaloids atropine and scopolamine, the main constituents of the active principle of *D. stramonium*, with toxic properties, were studied in male Albino Wistar rats. After acute i.p. administration of dose 100 mg/kg of total alkaloids to the seeds of *D. stramonium*, there were no remarkable changes in general appearance and no deaths occurred in any experimental group. Twenty-fourhours after total alkaloids of seeds, a significant reduction in indices of the liver, spleen brain and kidney function and some biochemical and haematological parameters were observed. The red blood cells, hematocrit, haemoglobin and white blood cells were significantly higher in the treated groups than in the control group. A subacute study for four weeks showed no resulting mortality or signs of toxicity. In a chronic study, the synthetic alkaloids administered i.p. at daily doses of 4.2 mg/kg of atropine and 1.6 mg/kg of scopolamine, did not produce death. However, diarrhoea and hypoactivity were observed. The relative weight of the liver was significantly less than that of the control group (Bouzidi *et al.*, 2011).

Use as a protective agent in severe organophosphate toxicity

Treatment of patients following an organophosphate (OP) exposure can deplete a hospital's entire supply of atropine. Given the possibility of multiple severe exposures after a terrorist attack using OP nerve agents, there exists a need for either greater

atropine stores or the development of alternative antidotes. Jimsonweed (*D. stramonium*)contains atropine and other anticholinergic compounds and is common and readily available. It is used recreationally for its central anticholinergic effects and is easy to be made into an extract by boiling thecrushed seeds. The extract has a rapid onset of effects and may be useful for the treatment of OP poisoning. Pretreatment with *D. stramoniumextracts* significantly increases survival following severe dichlorvos exposure (Bania *et al.*, 2004).

2.1.10.Biochemical analysis of Datura*stramonium* extract

*Daturastramonium*is an annual plant that belongs to the *Solanaceae*family and emits an unpleasant odour because of the presence of tropane alkaloids (Kumar,2009).*Daturastramonium*is a toxic plant; also, the toxicity is given by the presence of alkaloids: hyoscyamine, atropine and scopolamine. Iranbakhsh *et al.* (2006) determined the quantities of scopolamine and atropine in different stages of growth. The largest amount of scopolamine is found in leaves in the vegetative period, and the smallest amount is found in the root in the vegetative stage. Also, the greatest quantity of atropine is found in the petiole in the vegetative period, and the smallest quantity is found in the seed (Sreenivasa *et al.,* 2012). The total concentration of alkaloids in *Daturastramonium*leaves is 0.2 – 0.5%. More than 70 alkaloids were identified in different parts of the plant, but the main alkaloids are hyoscyamine, atropine, scopolamine (Schmelzer and Gurib – Fakin, 2008; Mann, 2008). Hyoscyamine is a major component when the plant is highly developed. Besides this, the plant also contains scopolamine, atropine, tropine, belladonnine, leucine, glutamic acid, enzymes, citric acid, malic acid, etheric oil, mineral salts, etc.

Atropineis a tropane alkaloid, an isomer of hyoscyamine. It is found in different concentrations in *Hyoscyamus niger, Daturastramonium, Brugmansia suaveolens, Atropa belladonna, Duboisia myoporoides*. The molecular formula is $C_{17}H_{23}NO_3$. Atropine has two major actions. One of the actions affects the central nervous system (CNS) and provokes airway stimulation. On the other hand, this alkaloid depresses the smooth muscle and the secretory glands which are innervated by the parasympathetic nerves. The central action of atropine is sedative (in paralysis, agitation, Parkinson disease). Peripheral action of atropine refers especially to the secretion of the respiratory glands, bronchial muscles, heart, gastrointestinal tract, urinary tract (Kasture and Wandodkar, 2008). When administrated orally, the atropine is absorbed well in the organism and is eliminated rapidly, the excretion is complete in approximately 24 hours. The normal dose should be 0.4 mg/day by the oral tract or parenteral. If the dose is more than 3 mg it can provoke mental and behavioural changes.

Atropine biosynthesis begins with the amino acid L-phenylalanine. Through transamination, the– NH_2 group is transferred and it forms pyruvic acid who in turn is reduced to phenyl lactic acid. Through a series of reaction tropine and litorin is formed which undergoes a rearrangement initiated by the enzyme *p450* forming hyoscyamine aldehyde. Through a dehydrogenation reaction hyoscyamine is formed and through racemization passes into atropine (Behçet, 2014). Atropine is cleaved by the atropinesterase enzyme to the tropic acid in the roots of the plant, *Daturastramonium.*

Hyoscyamine(daturine) is an ester of tropic acid with atropine, being amino alcohol. It is a secondary metabolite also found in the leaves of *D. stramonium.* The molecular formula

is $C_{17}H_{23}NO_3$. After the plant is dried, the structural component is modified and hyoscyamine goes through the levogirous isomer called atropine. Hyoscyamine is an antagonist of muscarinic acetylcholine receptors having 98% of the anticholinergic power of atropine. The place and the hyoscyamine biosynthesis in the plant are similar to atropine (Roberts and Wink, 2013). Hyoscyamine is used in gastrointestinal disorders, spasms, ulcers, pancreatitis, cholics and cystitis. Also, it is used in heart problems as well as controlling Parkinson's disease symptoms (Schmelzer and Gurib – Fakin, 2008).

Scopolamine(hyoscine) is a toxic alkaloid met at species of the *Solanaceae* family respectively in *Hyoscyamus niger, Brugmansia suaveolens, Daturastramonium, Duboisia myoporoides, Atropa belladonna.* Scopolamine takes part of the tropane alkaloids class, alkaloids that contain nitrogen in heterocycle and are synthesized from amino. The molecular formula is $C_{17}H_{21}NO_4$. Scopolamine is a hallucinogenic substance if administered in high doses, which can even lead to coma or body death (Sweta and Lakshimi, 2015). It has medical properties, frequent are used for motion sickness, is administrated after surgery because it prevents nausea and vomiting, also it is used as a sedative. Recently it is useful as a detoxifier because it has significantly reduced the desire for the use of heroin and its effects (Liu *et al.,* 2013). It is used in intestinal cramps (Alvarez and Marconi, 2011), it has pharmacological properties on the CNS, on the gastrointestinal system, in shortness of breath, and it is also administered to people suffering from Parkinson's disease (Yang *et al.,* 2014). Scopolamine takes part in the class of anaesthetic drugs, adjuvants, antiemetics, gastrointestinal and anti-vertigo. The pathway of penetration into the body is transdermal. The scopolamine biosynthesis can be obtained in 3 steps: a) Beginning from the ornithine amino acid in the presence of the

enzyme ornithine decarboxylase is obtained putrescine which in the presence of the enzyme putrescine N-methyl transferase result in the N – methyl putrescine. Through a spontaneous reaction,tropinone is formed, which in the presence of the tropinone reductase 1 enzyme leads to the obtaining of tropine. b) Starting from shikimic acid, phenylalanine, phenyl pyruvic acid and tropic acid are obtained. c) The products from step a and b (tropine and tropic acid) lead to the obtaining of hyoscyamine which is transformed into scopolamine by hyoscyamine 6 β hydroxylase enzyme (Facchini, 2001).

The place where biosynthesis begins can be determined through locating alkaloid enzyme of synthesis (Indra, 2012). Thus, the *h6h* enzyme which converts hyoscyamine into scopolamine and *PMT* enzyme which initiates the transformation of putrescine into N-methyl putrescine are found in the pericycle of the root. The pericycle is a thin layer that is found in between the endoderm and the phloem (Dubrovsky and Rost, 2012) and scopolamine is synthesized in the pericycle being translocated through the xylem to aerial parts where it accumulates in the vacuoles (Chandra, 2012; Roberts, 2007; Hashimoto *et al.,* 1991). The mechanism of alkaloids translocation from the root to the aerial parts is controvertible (Kanegae, 1994). Scopolamine translocation towards aerial parts of plants is realized with the help of the xylem. More precisely from the pericycle, the scopolamine passes into the xylem. Scopolamine will betranslocated with sap xylem to aerial parts of the plant (Kandoth *et al.,* 2010). An important factor on which scopolamine depends is light. Studies made on root crops of *Scopolia carniolica* indicated the fact that the roots kept in darkness strongly stimulate the synthesis of scopolamine. In this article, it was realized a qualitative and quantitative chemical analysis of the ethanolic extract of *Daturastramonium*by two methods: Thin-layer chromatography (CSS) and High-

Performance Liquid Chromatography with reversed-phase (HPLC). That was determined the antimicrobial activity of the *Datura*extract both on *Gram-positive*and *Gram-negative*bacteria.

2.2. Phases of Development in Rats

The developing foetus underlies daily changes during the process of prenatal ontogeny, which is generally highly predictable in mice (Wahlsten, 1974). Still, the exact timing of developmental events and consequently the exact end of the prenatal phase can vary, leading to a delayed or advanced onset of the postnatal phase in a range of about one day (Williams and Scott, 1954). After being born the variation concerning the exact timing of developmental milestones expands to several days and even months as the mice grow older. Different strains of inbred mice do further extend or shorten certain life periods or have shifted distinct developmental events (Fox, 1965; Moore *et al.,* 2011, Yuan *et al.,* 2012; Nelson*et al.,* 1990). Also, distinct environmental cues might delay or increase the speed of maturation (Wittingham and Wood, 1983). Thus, the time of the onset and end of the following can differ up to several days depending on the criteria and strain of mice used. Be this as it may, in principle all events are harmoniously coordinated and occur in the same order in every individual (Le Roy *et al.,* 2001). Within each phase, there can be salient developmental moments (e.g. SRY gene expression leading to sexual differentiation (Berta *et al.,* 1990). There is five-stage of development in mice. These include the followings; that is, the prenatal phase, the early postnatal phase, adolescence, adulthood, and the post-reproductive phase.

2.2.1. The prenatal phase

After conception, a single cell develops to give rise to a complex, multicellular organism over 19 days. During this period the fertilized egg divides and a variety of cell types, specialized tissues, and organs are shaped. Processes during embryogenesis include coordinated cell division, cell specialization, cell migration, and genetically programmed cell death (Prather and First, 1988). Of special interest for any behavioural development process following the neurulation which begins around embryonic day 8.5 (Copp *et al.*, 2003) with the closure of the neural tube. The neural tube develops into the spinal cord and the brain, orchestrated by coordinated and precisely timed gene expression of thousands of genes (Liscovitch and Chechik, 2013). During the maturation of neuronal tissues, the brain circuits are highly plastic. In male mice at embryonic day 11.5 the SRY gene transcription peaks (Hacker *et al.*, 1995) and initiates sexual differentiation with the induction of Leydig cells secreting testosterone. Interestingly, there is an inter-uterine transfer of testosterone between foetuses. Thereby individuals are affected by the number of neighbouring males leading to developmental differences in physiology and behaviour later in life (vom Saal and Bronson, 1980). Generally, it is assumed that changes in the uterine environment are capable to exert long-lasting changes in behaviour and physiology (Cratty *et al.*, 1995; Seckl, 2004; Champagne and Curley, 2009; Bodden *et al.*, 2015). Already during prenatal development, motor-sensory and cognitive behaviours can be observed in rodents (Busnel *et al.*, 1992; Smotherman and Robinson, 1985). Testing learning abilities in utero is challenging and was conducted during the last day of uterine life in rats in a taste/odour aversion learning task (Smotherman and Robinson, 1985). In mice, possibly due to size limitations, tasks investigating early learning have

been conducted more focused on the postnatal phase, with successful conditioning as early as PND 3 (Alleva and D' Udine, 1987).

2.2.2. The early postnatal phase

After birth, environmental changes are the most dramatic challenges during the life of a mouse. Temperature and nutrition that were being constantly supplied by the mother are now a factor that cannot be determined predictably. More so, they have to struggle for a livelihood by interacting with siblings to compete for the available resources. Although mouse pups are altricial and are thus born in highly immature sense organs, they do have whiskers and tactile ability as well as olfactory and thermal cues on their first day of life (Latham and Mason, 2004). The hair begins appearing around three days of age but thermoregulation continues to be largely dependent upon ambient temperature as well as the quality of the nest, maternal care, and position within the litter (Okon, 1970; Sales and Skinner, 2009). Learning tasks have been conducted successfully with mice at PND 3 although full retention of learning after 24 hours could only be detected in mice from PND 9 onwards (Alleva and D' Udine, 1987). Ultrasonic vocalisation eliciting maternal care begins after birth and increases in intensity until around PND 8 (Elwood and Keeling, 1982). The ears open on PND 3 (Theiler, 1972) and the ability to be conditioned to auditory cues are present as early as on PND 4 (Williams and Scott, 1954). Still, the complete development of the inner ear structures takes more time and is completed at PND 13 (Theiler, 1972). The eyes open around that time, too, in the range of PND 11-13 (Williams and Scott, 1954; Theiler, 1972). Until around day 16, mice pups are fed by nursing only. Nursing continues until the age of about 22 days and is successively complemented by solid food. The digestive system of newborn mice is also dependent on

maternal care and pups produce ultrasonic wriggling calls to demand licking of the trunk by the mother for stimulating digestion, urination and defecation (Ehret and Bernecker, 1986). From PND 0 onwards a rapid development of behavioural abilities and reflexes begins. First complex motor abilities like forelimb grasping, placing of hind limbs, standing, and self-grooming develop continuously over the first six days of life (Fox, 1965). First agonistic traits, that is, biting and defensive posture can be elicited between PND 12-14. With full sensory perception available, young mice following PND 14 are most of their time engaged in exploratory and investigatory behaviour. Along with developing motor abilities, the pups begin to leave the nest and explore the surroundings. As a consequence, affiliative behaviour towards the nestmates, like huddling, decreases while exploration and jumping behaviour increases (Terranova and Laviola, 1998).

On PND 15 a behavioural phenomenon is observable which is known as the ‘hoppy’ or ‘popcorn’ stage and is manifested in vigorous jumping (Wahlsten, 1974) based on the synchronous contraction of fore and hind limb extensors (Patel and Hillard, 2001). This behaviour is shown with or without observable disturbing stimuli. In the absence of any noticeable stimuli, running and jumping can be considered solitary play behaviour (Walker and Byers, 1991). If this behaviour is shown in response to external stimuli, it was proposed to be adaptive for avoiding predators. As sensory perception is not entirely completed, young mice are unable to discriminate between harmful and harmless stimuli. Therefore, jumping behaviour serves as an alternative response to predators until optimal adult strategies that would require advanced physiological and behavioural skills are developed (Williams and Scott, 1954; Patel and Hillard, 2001; Walker and Byers, 1991; Sorsby *et al.*, 1954; Henderson, 1981). The stage peaks at around PND 20 showing an

inverted u-shaped curve over the early postnatal period (Terranova and Laviola, 1998) and according to Scott and Williams (1954) the hoppy-phase ends at PND 25.

The early postnatal period ends about PND 21-25 with the completion of weaning and thus independence from the mother (Le Roy *et al.,* 2001). The maturation of nutritional and behavioural independence is a change in the life of young mice that marks one of the focal points in development (Tsuda *et al.,* 2011).

Differences in maternal care can lead to epigenetic changes during this phase of early development which can persist through life (Champagne and Curley, 2009; Szyf *et al.,* 2007). Tsuda *et al.*(2011) demonstrate that stress caused by maternal separation during this life stage (2 weeks after birth) reduces plasma testosterone levels, decreases arginine-vasopressin and increases oxytocin immunoreactivity in the paraventricular nucleus of males. Such hormonal changes can lead to long term behavioural changes like an increase in aggressiveness of adolescent males. Thus, the age at which experimental subjects are separated from their mother can have great influences on a variety of behavioural traits later in life as has been shown in rats (Smith, 1991) as well as in mice (Terranova and Laviola, 1995).

2.2.3. The Adolescence phase

Adolescence is the developmental transition from childhood to adulthood. During this phase, a multitude of hormonal and behavioural changes as well as alterations in neurobiological structures occur together with a substantial remodelling of cortical and limbic circuits (Romeo *et al.,* 2002). This variety of changes leads to cognitive, emotional, social, and sexual maturation (Spear, 2000; Sisk and Zehr, 2005). The temporal boundaries of adolescence in rodents, the exact beginning and the end, are not

precisely defined. Several authors, however, see it roughly as the range from weaning (PND 22-25) to adulthood (PND 55-65).The period of adolescence can be subdivided into three intervals, early- (prepubescent, PND 22-34), mid- (pubescent, PND 35-47) and late adolescence (sexually mature, PND 48-60) (Laviola *et al.,* 2003; Adriani *et al*., 2004), which will be characterized in more detail in the following subsections.

Generally, the phase of adolescence is characterised by behaviour and physiology that differs substantially from adulthood, e.g. there is a general trend of increased open field activity throughout all three stages of adolescence well into adulthood (Hefner and Holmes, 2007;Moore *et al.,* 2011; Blaney *et al.,* 2013;Molenhuis *et al.,* 2014). Increased food consumption during this phase is accompanied by high energy expenditure (Moore *et al.,* 2011). Furthermore, stressors cause greater behavioural changes than in previous or following periods of life. For example, four-week-old mice exposed to social or restraint stress showed an increase in anxiety measured in an elevated plus-maze. In contrast, anxiety was unchanged in stressed versus unstressed individuals tested at eight weeks of age (Stone and Quartermain, 1997). One reason for this higher reactivity to stressful events might be maturational changes in the hypothalamic-pituitary-adrenal (HPA) axis (Lynn and Brown, 2010) which is most likely more sensitive to stress during adolescence (Andersen, 2003). Vulnerability to stress is thus linked to the state of sexual maturity (Stone and Quartermain, 1997).

The stage of early adolescence starts at weaning and is characterized by the onset of sexual maturation and an increase in growth hormones which peak at PND 28-30 (Alba and Salvatori, 2004). In female mice, the vagina is closed at birth and opens at around PND 26. In male mice, spermatogenesis starts around day 21 with elongated spermatozoa

present at around day 35 (Krishnamurthy *et al.*, 2001). Characteristic behavioural and physiological patterns like greater exploration, lower anxiety and a lowered stress reactivity (Adriani and Laviola, 2004) sum up to an increased risk-taking behaviour shortly after weaning when mice begin to explore the surrounding area (Macrì *et al.*, 2002). Aggressive behaviour is further developed beginning with competition over food at this stage (Williams and Scott, 1954).

During mid-adolescence, mice become fully fertile (Jean-Faucher *et al.*, 1978). Sexual maturity, however, precedes behavioural maturity (Fox, 1965). Pheromone production in male mice is detectable in this phase of puberty (Drickamer and Murphy, 1978) and elevated secretion of gonadal steroid hormones occurs (Sisk and Zehr, 2005). Additionally, male mice begin to display severe conspecific-related aggression, although aggressive behaviour *per se* is not observed in all individuals before late adolescence (Tsuda *et al.*, 2011).

In late adolescence mice start to disperse, that is, dispersal in males is around PND 49 and in females around PND 71 (Groo *et al.*, 2012). Males are generally more likely to disperse than females although such a sex bias was not found in all studies (Pocock *et al.*, 2005). Going along with this, agonistic behaviours are further developed coinciding with social encounters with unfamiliar conspecifics under natural conditions (Terranova *et al.*, 1998). Social interest measured as time spent near an unfamiliar mouse, however, decreases slightly compared to the phase of early adolescence (Chen *et al.*, 2009).

Experiences throughout adolescence also have profound effects on behaviour later in life. Mice exposed to chronic social stress between PND 28-77 showed increased anxiety-like

behaviour in adulthood (Schmidt *et al.*, 2007). Such stressful experiences may induce serious changes in metabolism which can lead to impairments of hippocampus-dependent cognitive function or alterations of body fat distribution in adulthood involved in metabolic diseases (Schmidt *et al.*, 2009; Sterlemann *et al.*, 2009).

Regarding anxiety measured in the open field or elevated plus maze, prepubescent mice (PND 24) exhibit more fearful behaviour than adult mice (PND 75) (Balsevich *et al.*,2014). Locomotor behaviour in the open field was found to increase from the early and mid-adolescent phase to adulthood (Hefner and Holmes, 2007; Moore *et al.*, 2011). In contrast, Macri *et al.*(2002) describe that mice of both sexes are more explorative and less anxious at PND 48 compared to adult mice at PND 61. Johnson and Wilbrecht (2011) were able to demonstrate that adolescent mice (PND 26-28) often exhibit a highly flexible behaviour compared to adults (PND 60-70) in a multiple-choice reversal learning test. In line with these findings are observations of increased risk-taking (Laviola *et al.*, 2003) and sensitivity to drugs and alcohol consumption (Adriani *et al.*, 2004; Hefner and Holmes, 2007) during adolescence. Overall, changes in exploration, risk-taking behaviour, and behavioural flexibility reflect the preparation for becoming independent (Spear, 2000).

2.2.4. The Adulthood Phase

In adulthood sexual maturity is accompanied by behavioural maturity, that is, mice are capable of mating and successfully siring and raising offspring. Although it might be debatable whether or not an individual mouse at PND 55-65 is to be considered as an early adult or as being in late adolescence, adulthood is successfully defined as the

abilityto sire offspring successfully. In female mice,the earliest mating starts around PND 35, at PND 50 half of the female mice paired with a male were mated and 100% were mated at PND 60 (Chehab *et al.,* 1997). In male mice, the balanopreputial-separation, which allows pheromonal communication, is often used as an external indicator for completed puberty and the first sign of adulthood. However, the balanopreputial-separation is usually detectable as early as around PND 30 (Qiu *et al.,* 2013), which is long before mating attempts, can be observed and thus falls clearly into adolescence. In general, pheromonal communication is correlated with the rank of male mice with subdominant adults depositing more scent marks than juveniles but less than dominant adult male mice (Hurst, 1990). Thus, scent-marking allows priming to avoid costly aggressive interactions (Hurst, 1993). Not only strain differences, but also the presence of other adult females or males can significantly bring forward or delay sexual maturation (Vandenbergh, 1971). Around PND 60 the intratesticular testosterone levels of males reach adult levels (Keene *et al.,* 2002). Thus, commonly PND 60 is defined as the onset of adulthood where male and female mice possess a fully developed body as well as the full behavioural repertoire of the species. Behavioural changes within the adult phase seem to happen more or less continuously and no apparent developmental physiological changes are present in healthy adult mice. Activity measured in a motor activity chamber (Joyal *et al.,* 2000; Thifault *et al.,* 2001), as well as anxiety (Thifault *et al.,* 2001) and exploratory tendencies (Sprott, 1975), seem to decrease slowly and in a more or less linear fashion. Unfortunately, statistical proof for these observed effects is rare due to a lack of tests on repeatability explicitly done with data of adult mice, although the raw data are often available (e.g. (Joyal *et al.,* 2000; Szenczi *et al.,* 2012). The general trends,

however, seem to be in line with statistically confirmed findings of Marquette and Schneider (1991). They found the vitality of mice, in general, to decline over 2 to 24 months of age regardless of individual differences in the experience. Notwithstanding this decline, the authors also report that activity in the open field remains stable in the same individuals within this period. The overall decrease in performance might be due to deterioration of neuronal mediators as well as a reduction of muscles and muscle flexibility. Also, divergent results found in different traits and testing setups might be due to differences in the relative importance of changes in brain and physiology (Marquette and Schneider, 1991). Good evidence for individual stability in performance comes from (Rödel *et al.*,2011), who found female outbred mice (strain RjHan: NMR1) to remain stable in their anxiety-related behaviour measured in an elevated plus-maze at PND 90 and PND 135, regardless of changes in their reproductive state. In inbred mice, behavioural stability was demonstrated in a study (Lewejohann *et al.*, 2011) by showing that male mice (strain C57BL/6N) were stable in their anxiety-related behaviour measured in an elevated plus-maze around PND 60 and PND 90. Interestingly, both studies report behavioural consistencies despite considerable inter-individual variability. Possibly some of the variability is experimentally induced by either including two groups of females with different breeding experiences (Rödel *et al.,* 2011) or different subgroups of mice that were housed in either stable or unstable social groups (Lewejohann *et al.*, 2011).

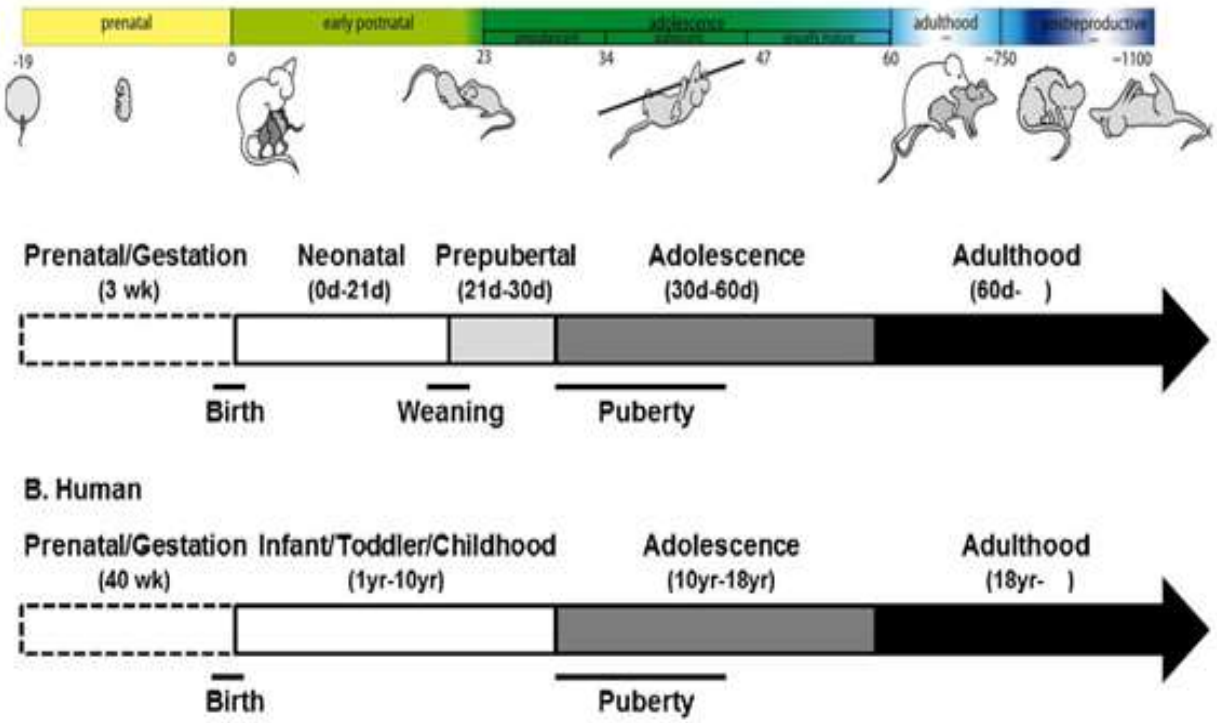
2.2.5. The Post-reproductive phase

In female mice, the last possible parturition after which no more offspring are born can be seen as the onset of the post-reproductive phase. Cessation of male mouse fertility correspondingly is indicated by the inability to sire further offspring, although

spermatozoa might still be produced at least in low numbers (Parkening, 1989). Wild house mice are known to be fertile until they reach an age of about 700 days. The fertile period was found to be significantly reduced to 570 days when wild mice were inbred for one generation (Flurkey *et al.*, 2007). The length of the post-reproductive phase is not only determined by the end of fertility but naturally also by the age at which the animals die. Substantial variation in lifespan has been found between different strains of mice. While surprisingly little is known about ageing in wild mice (Garratt *et al.*, 2011), the mean lifespan of different inbred strains is well documented, and according to Lhotellier and Cohensalmon, (1991) and Yuan *et al.*(2011), ages between 256 (AKR/J), 718 (DBA/2J), and 914 (C57BL/6J) days in females and 272 (AKR/J), 741 (NZB/B1NJ), 901 (C57BL/6J) in males are common. Ageing, in general, is also dependent upon life history experiences. For example, stress was shown to significantly reduce telomeric lengths and is therefore suspected to decrease life expectancy (Kotrschal *et al.*, 2007).

However, regardless of the environmentally induced individual differences as well as the genetically anchored between-strain differences, there is a considerable period between the loss of reproductive ability and death. This period has been estimated to be 204±32 days in 29 female and 83±29 days in 25 male F1 hybrids of C57BL6/Jlco female and CBA/Jlco male mice (Tarin *et al.*, 2014). Throughout the post-reproductive period, the central nervous system alters its structure by behavioural changes. The cerebellum and hippocampus, for example, are impaired in electrophysiological processes and comprise altered synapses or reduced cell numbers. Such changes lead to a declining functionality, consistent with behavioural changes in humans and other animals (Barnes, 1979; Swanson *et al.*, 1982; Cheal, 1986; Lhotellier and Cohensalmon, 1991; Altun *et al.*, 2007;

Kennard and Woodruff-Pak, 2011). In mice, studies that recorded exploratory behaviour in the open field measured as surface units crossed per time interval found a decrease in the behaviour when comparing adult mice with mice at an age of at least 22.5 months (Elias *et al.,* 1975; Sprott, 1975). Along the same line, a comparison of exploration behaviour in two consecutive trials in a hole- board test revealed an increase of exploration when tested in 5 months old adults. On the other hand, when the same test was conducted with aged mice (28 months), decreased exploration was found. These age-related changes in exploration behaviour have additionally been linked to changes in the hippocampal serotonergic system and are one physiological change contributing to an alteration of exploration in mice with age (Brennan *et al.,* 1981). Generally speaking, the explorative tendencies of mice seem to decrease with age, although Brennan *et al. (*1981) did not find these differences between 4 and 24-month-old male mice of the A/J strain. Besides exploration, activity decreases in ageing individuals. As reviewed in detail in Lhotellier and Cohen-Salmon (1991), ageing in mice is accompanied by a decline in motor capability comparable to motor disorders that have been found to appear along with human ageing. A decline in activity has been found across all strains of mice but might vary in its onset between strains. A decrease in exploration and locomotion is assumed to be a key factor that determines the number of age-dependent performance decreases in other contexts, e.g. in cognitive performance (Fahlstrom *et al.*, 2011).



A. Rodents

Figure 2. I. Timelines of development of physiology and behaviour in the lifetime of rodents (rats and mice, (A) andhuman (B).

Abbreviations: d, day; wk., week. Adapted from (Eiland and Romeo, 2013; Brust *et al.*, 2015).

2.3.Early Central Nervous System Development in Mammals

'Neurulation' refers to the coordinated morphogenetic stages during chordate embryogenesis that gives rise to the neural tube, which through continued growth and differentiation ultimately develops into the brain and spinal cord. During neurulation, the neural plate folds upon itself to form the neural tube. Different regions of the neural tube form by two different processes: primary and secondary neurulation. In primary neurulation, the flat neural plate proliferates and invaginates, creasing inward until the elevating edges come in contact and fuse to form a cylindrical, hollow neural tube. Secondary neurulation occurs in the caudal section where the solid cord of cells sinks into the embryo and cavitates to form a separate hollow tube in the tailbud. The tubes formed by primary and secondary neurulation eventually connect (Rubenstein *et al.,* 1998; Zohn and Sarkar, 2008; Zohn, 2012; Copp and Greene, 2013).

Briefly, the neural plate develops as a thickened region of embryonic ectoderm overlying the notochord and prechordal plate (Rubenstein *et al.*, 1998). Following neural plate formation, the neural groove develops along the midline (Theiler, 1989), and the lateral edges of the neural plate elevate to form neural folds that ultimately will meet and undergo fusion at the dorsal midline, giving rise to the closed neural tube. Closure of the neural tube is initiated in the cervical region at the hindbrain-cervical boundary and then proceeds cranially and caudally until the ends are sealed; the rostral (anterior) neuropore closes slightly before the caudal (posterior) neuropore. The cephalic end of the neural

tube subsequently dilates to form the three primary brain vesicles: prosencephalon (forebrain), mesencephalon (midbrain), and rhombencephalon (hindbrain). Shortly thereafter, the prosencephalon subdivides into the telencephalon (cerebral hemispheres) and diencephalon (consisting chiefly of the thalamus and hypothalamus). The mesencephalon (midbrain) is the most rostral brain domain in which the neural boundaries for organizing brain nuclei (that is, the alar and basal plates, the sulcus limitans) remain distinct. The rhombencephalon subdivides into the metencephalon (cerebellum and pons) and the myelencephalon (medulla oblongata). The post-cephalic region of the neural tube remains a hollow cylinder and develops into the spinal cord. The spinal cord also is organized using the alar and basal plates. Extensions from the brain (cranial nerves [CN]) and spinal cord (peripheral nerves) reach the periphery, often after passing through one or two ganglia (Theiler, 1989; Kaufman, 1992;Jacobowitz and Abbot, 1998; Paxinos *et al.,* 2007; Schambra, 2008; Graham *et al.,* 2015).

2.3.1.Neurogenesis,learning and memory in the adult Mammals

As it had been well accepted that adult neurogenesis occurs throughout life, the regulation and function of adult neurogenesis in physiological and pathological conditions has received much attention over the last decade, and it remains a topic of intense investigation and discussion in Neuroscience Research (Ming and Song, 2005; Braun and Jessberger, 2014). Decades of research have demonstrated that hippocampal neurogenesis plays an important role in the learning and memory process. Hippocampal neurogenesis can be modulated by environmental factors that are correlated with cognitive functions. For example, living in an enriched environment increases the survival of immature neurons younger than 3 weeks of age (Tashiro *et al.,* 2007).

Physical exercises, such as voluntary running, can promote SGZ cells proliferation in adult animals and rescue impaired hippocampal neurogenesis (van Praag *et al.,* 1999a; Lafenetre *et al.,* 2010). Both environmental enrichment and voluntary running can improve performance in learning and memory tasks (van Praag *et al.,* 1999b; Bruel-Jungerman *et al.,* 2005). Besides, learning depending on the hippocampus can regulate SGZ neurogenesis in a complex fashion. Different maturation states of newborn DGCs and different phases of learning in the Morris Water Maze (MWM) can positively or negatively influence hippocampal neurogenesis. For instance, learning the MWM promotes the survival of 7-day-old neuronsin SGZ but induces the apoptosis of 3-day-old newborn cells (Dupret *et al.,* 2007). An early phase of MWM training does not affect cell proliferation; conversely, the late phase increases the proliferation of neural progenitors and induces the apoptosis of new cells generated in the early phase (Dobrossy *et al.,* 2003).

2.3.2.Hippocampusneurogenesis and mood disorders

Hippocampus neurogenesis plays a central part in the regulation of emotional behaviour given the following points: (1) stress, which is capable of exacerbating depression in rodents and humans, decreases SGZ cell proliferation and induces cell death and dendritic shrinkage (Mahar *et al.,* 2014); (2) magnetic resonance imaging studies have revealed a volumetric reduction or loss of neurons in the hippocampus of depressed patients (Fotuhi *et al.,* 2012); (3) antidepressants cannot only increase the number of hippocampal early progenitors but also reverse the stress-induced decrease in neurogenesis(Boldrini *et al.,* 2012); and (4) hippocampal neurogenesis is required for the antidepressant-like activity of imipramine and fluoxetine, which are typical

antidepressants (Santarelli *et al.*, 2003). However, some studies have provided conflicting results. For example, some behavioural effects of fluoxetine are independent of SGZ neurogenesis (David *et al.*, 2009), and the direct ablation of neurogenesis does not always precipitate depression-like effects (Hanson *et al.*, 2011). Eisch and Petrik (2012) proposed that the differences are due to the diverse and dynamic factors that regulate neurogenesis.

2.4.Role of *DLG3*Expression in Memory

Cell polarity is essential to the function of many cell types, such as epithelial cells and neurons. The Discs large (*Dlg*) scaffolding protein was identified in Drosophila as a major regulator of basolateral epithelial identity. Four *Dlg* orthologs (*Dlg*1 through 4) are found in vertebrates and mutations in the human*DLG*3 gene is associated with X-linked mental retardation. Recently, hasfound that *DLG*3 controls apical epithelial polarity and tight junction formation and contributes to neural induction in mouse development (Van Campenhout *et al.,* 2011). During evolution, *DLG*3 acquired specific PPxY motifs, which bind to the WW domains of the E3 ubiquitin ligases, Nedd4 and Nedd4-2. This interaction results in monoubiquitination of *DLG*3, leading to directed microtubule-dependent protein trafficking, via the exocyst complex, in different polarized cell types. Directed trafficking of *DLG*3 plays an important role, during both mammalian development and in adulthood, in the establishment and maintenance of specialized apical cell junctions, such as tight junctions in epithelial cells and synapses in neurons.

In the vertebrates, deciphering Scrib/Lgl/*Dlg* functions in apical-basal polarity establishment is challenging due to gene duplication during evolution, as one Scribble but

two Lgls (Lgl1 and Lgl2) and four *Dlg*s (*Dlg*1 through 4) orthologs have been identified (Assémat *et al.,* 2008). The *Dlg*s belong to the MAGUK (Membrane Associated Guanylate Kinases) family of scaffolding proteins, characterized by the presence of three different protein-protein interaction (PPI) domains, including the Postsynaptic density 95/Discs large/Zonula occludens-1 (PDZ), the SRC Homology 3 Domain(SH3) and the Guanylate Kinase (GUK) domains. The *Dlg*s act as molecular scaffolds to build multi-protein complexes that establish and maintain specific membrane regions. In humans, mutations in the *DLG*3/SAP102 (synaptic associated protein 102) gene go hand-in-hand with X-linked mental retardation. This genetic disorder leads to synaptic dysfunction and learning and memory impairments (Tarpey *et al.*, 2004; Cuthbert *et al.*, 2007; Zanni *et al.*, 2010; Gardoni, 2008).Recently it was found that *DLG*3 inactivation in mice causes midgestational lethality (Van Campenhout *et al.*, 2011;Cox *et al.*, 2010), the earliest phenotype during embryonic development described so far in the mammalian *Dlg* family. At the end of gastrulation, the *DLG*3 mutant mouse embryos present with several developmental defects of variable severity, such as an absence of embryonic turning, posterior truncation and forebrain deletion. These abnormalities are a consequence of polarity defects in the embryonic late-gastrula organizer tissues; namely, the definitive endoderm and the axial mesendoderm. During development, the organizer is essential for anterior neural induction and head formation (Ang and Rossant, 1994; Kinder *et al.*, 2001). It was also found that *DLG*3 acts cell-autonomously in the epithelial cells of the late-gastrula organizer to establish apicobasal cell polarity and maintain tissue integrity. These findings suggest that human X-linked mental retardation caused by *DLG*3 mutations might be due to neural induction failure during early embryonic

development.Furthermore, some of the *Dlg*s were found to have functionally diverged during evolution from the common Drosophila ancestor. *DLG*3 is located in the cytoplasm and along the cell membrane, with a peak in distribution at the level of the apical membrane. These observations strongly contrast with the idea that the *Dlgs* function exclusively at the basolateral membrane, a hypothesis that is based on studies of *Dlg* in Drosophila. In the brain, the *Dlgs* cluster at synapses in the presynaptic active zone and postsynaptic densities (PSD) in a highly dynamic manner, both spatially and temporally (Sans *et al.*, 2000).

Important clues to understanding the mechanisms of *DLG*3-mediated cell polarity and junction establishment came from the analysis of the protein interactome.*DLG*3 is part of an apical trafficking complex in epithelial cells (Zuo *et al.,* 2009; Kinzel *et al.,* 2010), which contains the motor protein dynein (Burgess *et al.,* 2003) and the exocyst proteins Sec6 and Sec8 (Sans *et al.,* 2003). Additionally, proteins important for apical polarity establishment, such as the enzymes PP1a (Traweger *et al.,* 2008) and the tight junction protein TJAP-1, interact with *DLG3*. Interestingly, *DLG*3 is the only *Dlg* family member containing PPxY motifs that bind to the WW domain of Nedd4 and Nedd4-2 E3 ubiquitin ancestor. *DLG3* is located in the cytoplasm and along the cell membrane, with a peak in distribution at the level of the apical membrane. These observations strongly contrast with the idea that the *Dlg*s function exclusively at the basolateral membrane, a hypothesis that is based on studies of *Dlg* in Drosophila. In the brain, the *Dlg*s cluster at synapses in the presynaptic active zone and postsynaptic densities (PSD) in a highly dynamic manner, both spatially and temporally (Sans *et al.,* 2000).

2.5.Role of *NeuroD1*Expression in Cortical Differentiation

*NeuroD*1 is involved in neurogenesis. It can convert embryonic epidermal cells into fully differentiated neurons in *Xenopus* and promotes premature cell-cycle exit and differentiation of neural precursor cells (Lee *et al.,* 1995). By crossing *NeuroD*1-deficient mice with either transgenic mice expressing *NeuroD*1 in b cells or (mammalian atonal homolog) MATH deficient mice, Miyata *et al. (*1999) and Schwab *et al. (*2000) demonstrated that *NeuroD*1 deletion in the central nervous system leads to depletion of the cerebellar granule cells and loss of the dentate gyrus in the hippocampus. Furthermore, *NeuroD*1 is involved in the differentiation of peripheral neurons. Thus, *NeuroD*1-deficient mice exhibit neuronal cell loss in the inner ear (Kim *et al.,* 2001; Liu, 2000). In the retina of *NeuroD*1-deficient mice, amacrine cell differentiation is perturbed and the number of bipolar interneurons decreases (Morrow *et al.,* 1999). Furthermore, it has recently been reported that *NeuroD*1 plays an essential role in the maintenance of neural precursor cells in adult neurogenesis: inducible stem cell-specific deletion of *NeuroD*1 causes fewer newborn neurons in the hippocampus and the olfactory bulb (Gao*et al.,* 2009; Kuwabara *et al.,* 2009).

2.6.Developmental Neurotoxicity

Neurotoxicity has been defined as “any adverse effect on the chemistry, structure or function of the nervous system, during development or at maturity, induced by chemical or physical influences” (Costa, 1998). An adverse effect is “any treatment-related change which interferes with normal function and compromises adaptation to the environment”

[European Center for Ecotoxicology and Toxicology of Chemicals (ECETOC), 1992]. Thus, most morphological changes such as neuronopathy(a loss of neurons), axonopathy (a degeneration of the neuronalaxon), myelinopathy (a loss of the glial cells surrounding the axon), or other gliopathies, would be considered adverse, even if structural and/or functional changes were mild or transitory. Besides, neurochemical changes, also in the absence ofstructural damage, should also be considered adverse, even if they are transient and reversible, as they lead to impaired function. The general definition of neurotoxicity also points out a potential difference between the developing and the mature nervous system, to underscore the fact that developmental neurotoxicity is an important aspect of neurotoxicology. Many known human neurotoxicants are indeed developmental neurotoxicants (Grandjean and Landrigan, 2006), and in most, but not all cases, the developing nervous system is more sensitive to toxicants than the adult nervous system. Neurotoxicity can also occur as a result of indirect effects, such as damage to hepatic or cardiovascular structures, or because of interference with the endocrine systems. Some chemicals may have multiple modes of action and may affect the nervous system both directly and indirectly. For example, some halogenatedcompounds may interact directly with brain cells, and also affect the development of the nervous system by altering thyroid hormone homeostasis (Costa and Giordano, 2007;Crofton, 2008).

2.7. Biochemical Mode of Action of Poisonous Secondary Metabolites

Toxins and poisons are classified into four categories according to their oral toxicity determined in rat experiments: class Ia: extremely hazardous (5 mg or less per kg body weight); Ib: highly hazardous (5 to50 mg/kg body weight); class II: moderately hazardous (50 to 500 mg/kg body weight) and class III: slightly hazardous (500 mg and more per kg

body weight). It is important to recall that the dose is very important; already Paracelsus (1493 – 1541) had postulated in 1537 "*sola dosis facet venenum"* (it is the dose that makes a poison) besides inherent toxic properties (Mutschler *et al.*, 2008; Wink and van Wyk,2008).

2.7.1.Developmental Neurotoxicants

There are approximately 200 chemicals thatare neurotoxic in humans (Grandjean and Landrigan, 2006), and for many more, there is at least some evidence of neurotoxicity deriving from animal studies. Of these, several are developmental neurotoxicants. However, of over 80,000 chemicals on the market,only a handful (about 200) have undergone developmentalneurotoxicity testing according to the established guidelines(Makris *et al.,* 2009) As said, the developing brain is often more sensitive thanthe adult brain to toxic insult; thus, neurotoxicity is observedat much lower exposure levels. This is the case, for example,of lead or methylmercury. In several cases, the effects ofdevelopmental chemical exposure are different from thoseobserved in adult, upon similar exposure; this is the case, forexample, of ethanol or valproate. In some cases, developmentalexposure to neurotoxicants results in morphologicalalteration of the CNS, with accompanying changes in functions(Costa *et al.,* 2004). However, in several instances, functional changesmay be the result of subtler biochemical/molecularalterations without major structural abnormalities. Exposureto chemicals that may adversely affect the nervoussystem has been suggested to be associated with many developmental disabilities (learning disabilities, attention-deficit hyperactivity disorder, dyslexia, sensory deficits,mental retardation, and autism spectrum

disorders) whichare diagnosed in children at an alarmingly increasing rate(Grandjean and Landrigan, 2006; Van Den Hazel *et al.,* 2006; Miodovnik, 2011).

Neurotoxinscan affect important ion channelsof neuronal cells, such as Na^+, K^+, and Ca^{2+} channels, either by activating or inactivating them permanently. Both actions will stop neuronal signal transduction and thus block the activity of the central nervous system (CNS) but also neuromuscular signalling (Wink 2003a, 2000; Alberts *et al.,* 2008; Mutschler *et al.,* 2008), which eventually leads to paralysis ofboth striated and smooth muscles of heart, lungs and skeleton. A special case is the Na^+, K^+-ATPase, which is the most important ion pump in neuronal and other cells to maintain an ion gradient important for action potentials and transport mechanisms (Alberts *et al.,* 2008; Mutschler *et al.,* 2008). Cardiac glycosides, which occur in several plant families and even in toad skins (genus *Bufo*) are strong inhibitors of this pump. Because this pump is extremely important, cardiac glycosides are considered to be toxins of class Ia.Neuroreceptorsare another prime target for many alkaloids, which structurally resemble the endogenous neurotransmitters, such as acetylcholine, dopamine, noradrenaline, serotonin, adrenaline, GABA or glutamate (Wink 1993a, 2000; Alberts *et al.,* 2008; Mutschler *et al.,* 2008).

The neuroactive alkaloids can either function as agonists, that overstimulate a neuroreceptor or as antagonists, which would block a certain neuroreceptor. Agonists and antagonists can cause excitation, hallucinations, and general CNS disturbances, which would put an herbivore into deep sleep or coma; higher doses would lead to death by either cardiac or respiratory arrest (Mutschler *et al.,* 2008). An herbivore that is on a "trip" has no chance in the wild; it would either fall from trees or cliffs or would be easy

prey for all sorts of enemies. The production of neuroreceptor modulators is quite safe for the plant producing them because plants do not have neuroreceptors and thus cannot poison themselves with the defence chemicals.Some alkaloids inhibit the enzymes that break down neurotransmitters, such as cholinesterase (AChE) and monoamine oxidase (MAO)(Wink 2003a, 2000). These toxins have similar toxic properties as secondary metabolites that are neuroreceptor agonists since they would lead to a higher concentration of neurotransmitters in the synaptic cleft. Higher doses would lead to death by either cardiac or respiratory arrest (Mutschler *et al.,* 2008).

2.7.2.Silent Neurotoxicity, Indirect Neurotoxicity, and Long-Term Effects

Manifestations of the neurotoxiceffect upon developmental exposure either *in utero,* during lactation, or in early childhood, are usually seen shortly after exposure. Yet, the evidence is emerging that the deleterious effects of toxicants may not become clinically evident for some months, or even several years after exposure. This period during which the individual may manifest no evidence of toxicity is referred to as the "silent" period processes (Reuhl, 1991; Grandjean, 2008). Silent toxicity is defined as a persistent biochemical or morphological injury that remains clinically unapparent unless unmasked by experimental or natural processes (Reuhl, 1991; Grandjean, 2008). Silent toxicity may be compared to the process of carcinogenesis, in which molecular and cellular damage occurs years if not decades before any clinical manifestation of the disease (Weiss, 1991). In the case of neurotoxicity, an example of silent toxicity is represented by the Parkinsonism-dementia known as Guam's disease, in which latencies of decades have been reported before alleged still undefined exposures and clinical signs (Zhang *et al.,* 1990). Similarly, bovine spongiform encephalopathy (mad cow disease), a variant of

Creutzfeld-Jacob disease, has been shown to have a latency of decades (MacKnight, 2001). The delay between exposure and clinical expression of neurotoxic injury may be ascribed to various causes. For example, a selected population of neuronsmay be affected, but the known plasticity of the brain would compensate for such loss. However, further exposure to exogenous influences (e.g., stress, disease, additional chemical exposure), or the natural ageing process, would unmask the existing deficit. Alternatively, the organism may be initially able to compensate for a certain deficit, but progressive loss of function would eventually overcome the functional reserve and plasticity of the brain (Reuhl, 1991).

The possibility that such a latent period between exposure and clinical manifestation would occur in the context of development is even more probable. The concept that adult disease may have a fetal origin has been introduced by David Barker and is known as the "Barker hypothesis" (Godfrey and Barker, 2001). Exposure to chemicals may cause direct damage or alter developmental programming, whose resulting functional deficits become apparent later in life. A famous example is thatof diethylstilbestrol, in which *in utero* exposure leads to an increase in vaginal adenocarcinoma around the time of puberty (Swan, 2000). In the case of developmental neurotoxicity, an example is represented by the findings of experimental studies on prenatal infection. Perinatal exposure of rats to the Gram (−) bacteriotoxin lipopolysaccharide causes a 30% loss in dopaminergic neurons in the substantia nigra and persistent injury to the dopaminergic system (Ling *et al.,* 2001 and Fan *et al.,* 2011), suggesting that, in humans, prenatal infections occurring at the appropriate gestational age would result in the birth of an individual with fewer

dopaminergic neurons. This would be initially inconsequential, as clinical signs of Parkinson's disease are not apparent until about 80% of dopaminergic neuronsare lost. However, this early-life damage would predispose an individual to develop Parkinson's disease as the ageing process brings along a normal progressive loss of dopaminergic neurons(Landrigan *et al.,* 2005). Developmental exposures to certain pesticides, such as the herbicide paraquat and the fungicide maneb, which also target dopaminergic neurons, have also been implicated in the later development of Parkinson's disease (Cory-Slechta *et al.,* 2005). Similarly, developmental exposure to the now-banned organochlorine insecticide dieldrin has been shown to cause long-lasting alterations of the dopaminergic system, typical of a silent dopaminergic dysfunction (Richardson *et al.,* 2006).

On some occasions, early mild damage may worsen as the individual matures and ages. For instance, neurotoxic effects of developmental exposure to MeHg may be unmasked only during ageing (Newland and Rasmussen, 2000 and Weiss *et al.,* 2002). *In utero* exposure to methylazoxymethanol, which causes microencephaly, caused a premature decline in cognitive functions (Lee and Rabe, 1992), and the neurotoxic effects of neonatal exposure to triethyltin, a glial neurotoxicant, were exacerbated by ageing (Barone *et al.,* 1995). In other situations, this may not be the case, yet the neurotoxic effects of developmental exposure appear to be irreversible, and even if they do not worsen with age, they are certainly long-lasting, as shown, for example, for PCB-126 (Vitalone *et al.,* 2010). Perturbations of cognitive functions due to developmental Pb exposure remain for life (White *et al.,* 2007), and perinatal Pb exposure has been associated with the development of schizophrenia and Alzheimer's disease (Zawia and

Basha*et al.,* 2005; Opler *et al.,* 2008). It has been suggested that exposure to Pb during critical periods of brain development may impact the expression and regulation of amyloid precursor protein later in life, potentially altering the course of amyloidogenesis, thereby acting as a risk factor for the onset of Alzheimer's disease-like pathology (Wu *et al.,* 2008; Basha *et al.,* 2005; Basha and Reddy, 2010).

The development of the nervous system can also be influenced by chemicals that may not interact directly with neuronal and glial cells but may act instead, or beside, as endocrine disruptors. For example, as indicated in a previous section, PBDEs are thought to exert developmental neurotoxicity by two generals, and not mutually exclusive, modes of action, one related to effects on thyroid hormones, and the other involving direct effects of PBDEs on the developing brain (Costa and Giordano, 2008). Thyroid hormones are known to play a relevant role in brain development (Chan and Rovet, 2003), and hypothyroidism has been associated with a large number of neuroanatomical and behavioural effects (Haddow *et al.,* 1999; Zoeller and Crofton, 2005). PBDEs have been shown to perturb the thyroid system both during development, leading to a reduction of circulating thyroid hormone (Zhou *et al.,* 2002). Though the exact mechanism(s) underlying these effects are unclear, reduction in thyroid hormones may contribute to developmental neurotoxicity of PBDEs, though effects have been seen also in the absence of thyroid hormone changes (Gee *et al.,* 2008). Experimental compounds such as propylthiouracil(PTU), which inhibits thyroid hormone synthesis and causes hypothyroidism, are clear developmental neurotoxicants, as evidenced by behavioural and morphological alterations in the CNS (Zoeller and Crofton, 2005; Gilbert, 2011). Decreases of T4 following developmental exposure to PBDEs or PCBs range from 10 to

60%. As decrements in neurological development in children of mothers with a 25% decrease in T4 have been reported (Haddow *et al.,* 1999), the effects of these chemicals on thyroid hormones may well contribute to their developmental neurotoxicity.

In addition to chemicals affecting thyroid hormones, other endocrine disruptors may also exert adverse effects on the developing nervous system (Masuo and Ishido, 2011). Much attention has for example been devoted to bisphenol-A, a plasticizer used in several industrial and consumer products, as several studies have shown that this compound when given perinatally to experimental animals, causes various behavioural and morphological alterations (Nakamura *et al.,* 2007; Viberg, *et al.,* 2011 and Nakamura *et al.,* 2012). In contrast to these findings, however, a developmental neurotoxicity test, done according to USEPA and OECD guidelines, provided negative results (Stump *et al.,* 2010).

2.8.The Vulnerabilityof the Developing Nervous System

Several lines of evidence suggest that the developing nervous system may bemore susceptible, and/or differentially susceptible, to toxic insult than the adult nervous system. Different parts of the CNS develop at different stages; cell proliferation, migration, and differentiation contribute to the formation of definite brain structures, in which the correct number of cells in the proper location is necessary for proper function (Bayer *et al.,* 1993; Rodier, 1994). Even within a single brain region, subpopulations of neuronsmay have a different rate of development; for example, in the cerebellum, Purkinje cells develop early (embryonic days 13–15 in the rat, corresponding to gestational weeks 5–7 in humans), while granule cells are generated much later (postnatal

days 4–19 in the rat, corresponding to gestational weeks 24–36) (Bayers*et al.,* 1993). Failure in cell proliferation or cell migration because of exposure to toxic insults (e.g., irradiation or methylmercury) has profound deleterious effects on the developing brain (Costa*et al*., 2004). Though neurons maintain the ability to make new synapses throughout life, the period of brain development when synaptogenesis occurs is critical for the formation of the basic circuitry of the nervous system (Rodier, 1995). Furthermore, in the developing nervous system, neurotransmittersmay have a function other than neurotransmissions, such as modulation of cell proliferation, survival, and differentiation (Nguyen *et al.,* 2001). Thus, any toxicant that interferes with neurotransmission during development may cause permanent defects in the CNS.

Neurogenesis produces more neurons than those found in the mature nervous system, and excess neuronsare pruned by finely regulated apoptotic processes at different developmental times (Johnson and Deckwerth, 1993). Any chemical interfering with apoptotic processes may trigger degeneration of neuronsthat would not otherwise have been deleted or may promote the survival of unnecessary cells (Ikonomidou *et al.,* 2001). Also, pruning, defined as loss of synapses, occurs physiologically in the developing brain, and chemicals interfering with this process (which is longer lasting than the neuronal loss to apoptosis) would have the most significant adverse effects on brain functions (Webb *et al.,* 2001). While all these considerations relate to the development of neurons, it has become apparent that glial cells (astrocytes, oligodendrocytes, and microglia) also play a relevant role in brain development and may be the target of toxic action (Aschner and Costa, 2005). Indeed, several chemicals (e.g., alcohol, nicotine, and certain pesticides) exert profound neurotoxic effects when exposure occurs during the

brain growth spurt, characterized by extensive glial cell proliferation and maturation. In addition to all sensitive processes described, the developing brain is distinguished by the absence of a blood-brain barrier. The development of this barrier is a gradual process, beginning *in utero* and reaching completion around postnatal month six in humans (Rodier, 1995). The incomplete development of the blood-brain barrier allows several endogenous and exogenous chemicals, normally excluded from the brain, to freely enter the developing brain.

2.9.The HeavyElements in Medicinal Plants

Trace element plays an important role in chemical, biological, metabolic, and enzymatic reactions in the living cells of plants, animals and human beings (Hashmi *et al.,* 2007). However, the release of trace metals through human activities into the environment has increased over the years and the excessof these metals in the environment has been reported to be extremely dangerous to human health (Olowoyo *et al.,* 2010). The accumulation of trace metals by plants is one of the most serious environmental concerns lately. This is a result of the harmful effects of toxic metals on animals and human health.Heavy metal contamination in agricultural environments can come from the atmospheric fall-out, pesticide formulations, and contamination by chemical fertilizers and irrigation with water of poor quality (Marcovecchio *et al.,* 2007). Heavy metals rank high amongst the chief contaminants of leafy vegetables and medicinal plants (Ajasa *et al.,* 2004). The uptake of trace elements by plant varies and depends largely on several factors, such as soil pH and organic matter content (Logan *et al.,* 1997). Plant uptake is one of the major routes of food chain exposure to trace elements in the soil.

Despite this problem and limited knowledge of its dangers by the consumer, demands for better quality and less expensive agricultural products are increasing especially in urban cities (Mapanda *et al.,* 2007). Some consumers consider undamaged, dark green and big leaves as characteristics of good quality leafyvegetables. However, the external morphology of vegetables cannot guarantee safety from contamination especially when farming activities are carried out around industrial areas. Evidence of severe poisoning caused by some metal compounds and the proven carcinogenicity of some metal ions has fostered intensive research into the different uptake and translocation pattern in food crops (Vélez *et al.,* 2004).The broad use of traditional medicine by rural communities due to the accessibility and affordability of herbal medicine has also necessitated further research into the uptake and translocation pattern of trace metals by some medicinal plants grown in the urban areas (Ajasa *et al.*, 2004).

2.9.1.Heavy metals in medicinal plants and plant-based products

Numerous studies have been conducted worldwide to determine heavy metal levels in medicinal plants and plant-based products (Annan *et al.,* 2010; Ebrahim *et al.,* 2012; Maharia *et al.,* 2010). Both developed and developing countries have shown high levels of potentially toxic heavy metals in products available to the public (Denholm, 2010; Garvey *et al.,* 2001). Such products are not only from local sources but are often imported (Saper *et al.,* 2008). A study examining heavy metal content in traditional Asian herbal remedies purchased in the United States, Vietnam and China revealed that the majority of products had detectable levels of heavy metals, with nearly 74% containing amounts greater than current recommended public health guidelines (Garvey *et al.,* 2001). An effective solution to the importation of traditional medicines containing heavy

metals presents a great challenge (Shaw, 1998; Saper *et al.*, 2008). These products are expected to be imported in small quantities by numerous different routes, including via the postal service and with intercontinental travellers (Denholm, 2010).

2.9.2.Intentional uses of heavy metals for medicinal purposes

Heavy metals are a regular and deliberate component of traditional remedies worldwide; therefore, the use of the term ‘contamination’concerning the occurrence of heavy metals in traditional remedies is disingenuous (Ernst, 2002). In traditional Chinese medicine, heavy metals have been used for numerous health complaints. For example, Hg is part of certain preparations under the terminology of ‘*cinnabaris’* (mercury sulfide), ‘calomel’ (mercury chloride) or ‘hydrargyriaoxydumrubrum’ (mercury oxide) (Ernst, 2002). Allopathic medical practitioners are sceptical about the use of Hg for therapy —a perception not supported by traditional medicine practitioners (Kamath *et al.,* 2012). In Indiantraditional medicine, otherwise known as Ayurveda, bhasma (calcified powder/ash) are herbo-mineral or herbo-metallic formulations. The segment of Ayurvedathat involves bhasma is referred to as ‘Rasa Sastra’. Buddhist Philosopher Nagarjuna who was deemed the father of metallic medicine in India used metals and minerals in the form of bhasma as remedial agents from the preceding 8th century A.D (Senthil *et al.,* 2011). Metals mixed with organic compounds derived from plant extracts render them biocompatible according to ancient traditions (Senthil *et al.,* 2011; Kamath *et al.,* 2012). Recent studies have shown the scientific processes behind the detoxification of metals during the processing of such herbo-metallic formulations (Kamath *et al.,* 2012). Although heavy metal salts are an additive of African traditional medicines, no such epistemology has

been documented to explain the alleviation of potentially toxic substances. However, this is not to say that such a theory does not exist.

While heavy metals have been detected in African traditional remedies few studies have documented the commonly available metals used in African traditional medicine or investigatedthe reason behind such usage. Hexavalent chromium (Cr (VI), a class 1 carcinogen, is commonly used in South African traditional medicines and known locally as *ndonya*. A study by Sewram *et al.*(2010) revealed that 72% of traditional health practitioners (n=395) prescribed *ndonya*-containing medicines for healing purposes. A large number (50%) admitted to substituting *ndonya* with copper sulphate crystals if *ndonya* was not available. The issue of toxicity depended on the method of preparation and modeof administration whilst the pharmacological effects were said to be altered by the food eaten as well as individual tolerance. The concentration of Cr(VI) in traditional medicine preparations varied considerably from 4 μg/L–53 g/L. Such results are cause for concern and warrants toxicity awareness.

2.9.3.Poisoning from heavy metal contamination of traditional medicine products

Medicinal plants are both a rich source of essential metal ions and a potentially dangerous source of non-essential metals (Narendhirakannan *et al.,* 2005; Singh and Garg, 1997). Poisonings from traditional medicine products containing heavy metals are well documented (Dargan *et al.,* 2008; Ernst, 2002). The toxic effects of heavy metals are due to their hindrance with the regular body biochemistry in normal metabolic processes (Duruibe *et al.,* 2007). Arsenic, Cr and magnesium (Mg) are the heavy metals most frequently implicated in morbidity and death in South Africa (Steenkamp *et al.,* 2002).

An investigation of the Johannesburg forensic record over 5 years (1991–1995) identified 206 cases in which a traditional remedy was either declared to be the grounds for death or was found to be present in a case of poisoning with an unidentified substance (Stewart *et al.,* 1999). Heavy metals were responsible for 10% of these poisonings. A study by Steenkamp *et al. (*2000) on heavy metal concentrations in plants, plant-based remedies and urine from patients treated with traditional remedies concluded that out of the 12 concoctions investigated, copper (Cu) levels were extremely high in 4 of the concoctions. A large number of patients (34%) showed elevated zinc (Zn) concentrations. In one of the patients, the Zn concentration was 10 times the upper limit of the reference range. After a week of vomiting with hepatomegaly, and dehydration, the patient died of hepatic failure. A further report revealed that a seven-month-old infant was hospitalized after the intake of traditional medicine which resulted in a severe case of multiple metal poisoning (Steenkamp *et al.,* 2002). It is known that numerous traditional medicines give rise to severe renal pathology, the mechanism of which is uncertain but which could be associated with heavy metal toxicity (Steenkamp *et al.,* 2000). It has been suggested that potassium dichromate ($K_2Cr_2O_7$) toxicity should be suspected in cases of unexplained renal failure (Woods *et al.,* 1990). Woods *et al.*(1990) reported seven cases of dichromate poisoning after the use of purgative solutions obtained from traditional healers. One patient who ingested dichromate died from a massive gastrointestinal haemorrhage. Six patients took dichromate solutions as rectal enemas, two were left with impaired renal function and one patient required a permanent colostomy caused by extensive peri-anal necrosis. Similar reports have indicated that Cr(VI)-containing traditional remedies have been the cause of poisonings and morbidity in young children (Steenkamp *et al.,* 2002).

In Nigeria, a study reported high levels of heavy metal in blood from unknown sources (Ibeto and Okoye, 2010) and the introduction of African traditional medicines cannot be ruled out.

Unfortunately, customarily African traditional medicine products do not contain details such as place of production/manufacture thus there is no accountability for adverse reactions. Strict regulations concerning classification and labelling may prevent further poisonings. Moreover, certain substances should not be available to the general public for self-administration such as $K_2Cr_2O_7$. Regrettably, adverse reactions and poisoning profile of traditional medicine are not well documented and substances are often inadequately categorized. For example, potassium permanganate which could be classified as a household chemical or antiseptic is also used in South African traditional medicine however this substance may be incorrectly profiled with regards to causative agents in acute and chronic poisonings (Balme *et al.*, 2012).

2.9.4.International standards regarding heavy metals in traditional medicines

Health, safety and quality declarations are key features concerning regulatory requirements and standards globally. However, there are immense discrepancies between countries regarding regulatory requirements to pledge the safety and quality of plant-based products (Diederichs *et al.*, 2006). Several regulations have already been established worldwide for medicinal plants and related marketed herbal products such as the US Pharmacopoeia (USP), Italian Pharmacopoeia (FUI), and European Pharmacopoeia (Ph. Eur.).Moreover, there are legal frameworks at national and/or regional levels that are designed to regulate the quality of plant-based products (Sarma *et*

al., 2012). Before 1988, only 14 WHO the Member States had regulations relating to herbal medicine products but by 2003, this had increased to 53 Member States (37%). Of those without laws/regulations, 49% declared that suchregulations were in the process of being developed (WHO, 2005). Several countries, including Canada, China, Malaysia, Singapore and Thailand, have developed their national guidelines to ensure satisfactory levels of heavy metals in medicinal plants and plant-based products (Table 3). The WHO (1998) recommends maximum permissible levels in raw materials for Cd and Pb which amount to 0.3 and 10 mg kg−1, respectively. Even though certain essential elements can be toxic at high levels; the WHO limits for these metals have not yet been established.

2.10.Histopathology and Tissue Evaluation

Immunohistochemistry is an integral technique in many laboratories for diagnostic and research purposes. In the last decade, there is a tremendous improvement in IHC techniques making it an effective adjuvant to H and E diagnosis in a majority of tumour cases through the establishment of a definitive diagnosis. It has also provided insight into tumourhistopathogenesis and has contributed to a more accurate determination of patient prognosis. It is a technique for identifying cellular or tissue constituents (antigens) using antigen-antibody interactions, the site of antibody binding being identified either by direct labelling of the antibody or by use of a secondary labelling method (Jackson and Blythe, 2008).

Monoclonal antibodies are a homogenous population of antibodies for a single epitope, produced by an individual clone of plasma cells. Mice are most frequently used for their production. Polyclonal antibodies are a mixture of different molecules with varying

affinities and even specificities, to a range of antigens. These are produced by different cells and are immunochemically dissimilar (Jackson and Blythe, 2008).

Antibodies react to specific regions (epitopes or antigenic determinants) of a target molecule. Each molecule has several different epitopes as well as multiples of each epitope. Polyclonal antibodies can react with several types of epitopes within the antigen. Monoclonal antibodies react only with a single kind of epitope. While it would appear at first that polyclonal would be the better choice, monoclonal often provides greater specificity 25for the target molecule. An epitope is not necessarily unique to a particular antigen, it simply occurs in abundance on that molecule. There is a probability that the same epitope (sequence of amino acids) can be found elsewhere. Increasing the number of target epitopes, as in the use of polyclonal, only increases the chance for staining molecules other than the one against which the antibody was raised (Key, 2009).

Epitopes are recognized by their shape, among other characteristics. When that shape is distorted, it may becomeunrecognizable to the antibody. Formaldehyde and alcohol are not likely to compromise the shape of the epitope. Instead, by changing the shape of the entire molecule they hide the epitope. Successful antigen retrieval uncovers hidden epitopes (Key, 2009).

2.11.Neurostereology and Quantification of Neuronal Injury and Neurodegeneration

Quantification of absolute neuron and interneuron counts in various brain regions using an optimized neuro-stereology protocol is essential to understanding the impact of neurological insults or disease progression on neuronal survival and neurodegeneration(West *et al.,* 1991). The conventional qualitative scoring-based

protocols are superficial and less reliable for use in studies of neuroprotection evaluations. Over the years, advances in neuro-stereology methods have been developed to allow for unbiased total estimates of cell numbers in a specific brain region (Keuker *et al.*, 2001). These methods depend on the combination of the optical disector and fractionator sampling scheme. Before the introduction of the disector (Sterio, 1984), neuronal nuclei counting was based on isolated two-dimensional (2D) probes, as neuronal cells only contain one nucleus. Several such two-dimensional probe methods were described previously (Floderus, 1944; Abercrombie, 1946; Weibel, 1969; Rose and Rohrlich, 1987; West *et al.*, 1991). However, the number of nuclei in the brain is a zero-dimensional quantity, hence the various 2D probe methods led to bias. Those concerns were solved by the implementation of the optical disector, a 3D probe (Sterio, 1984; Harding *et al.*, 1993).The Stereology techniqueallowsforthe reliablequantitative description ofa 3D object tobe made from 2Dmeasurements. This is achieved by creating a *Z*-stack of2Dimagesto create a 3Dtissue model (West *et al.*, 1991; West, 2012a). Stereology utilizes random, systematic sampling to provide unbiased and quantitative data. Modern design-basedstereology has been an important and efficient tool in many applications of optical and confocal microscopy. There is a multitude of uses for stereology inmanybiomedicalfieldsincludinghistology, bone andneuroanatomytoaccuratelyquantifythenumberof cells, the length of fibres; andtheareaandvolumeofbiological structures orregions (Mayhew, 2014; Bronoosh *et al.*, 2015). Sampling isoneofthemostimportantstatistics-based concepts in stereology (Cruz-Orive andWeibel, 1981; West, 2012b). The application of sampling in stereology is found in every step of the process—from Cavalieri's estimation of volume to the optical

fractionator. The Cavalieri estimator is commonly used to estimate the volume of a specific region of the brain such as the hippocampus; however; it can be used to find the volume of any irregular shape of the tissue. The estimator isfrequently used in conjunction with the optical disector and optical fractionator to estimate densities such as cell number density, length density, or surface density. The optical disector uses a reference volume generated by two parallel sections separated by a known distance to count cells in a 3D space under the microscope (Sterio, 1984; Cruz-Orive, 2004; Kaplan *et al.,* 2012). The counts are considered unbiased as they are unaffected by variations such as size, shape, and orientation of the particles being counted (Sterio, 1984; West *et al.,* 1991; Harding *et al.,* 1993). Subsequently, as the sample size increases, the unbiased cell number estimate for a given reference volume more closely approaches the true value (Kaplan *et al.,* 2012).

CHAPTER THREE

3.0.MATERIALS AND METHOD

3.1.Identification of Plant and Seed Procurement

After obtaining ethical approval from the AhmaduBello University Committee on Animal Use and Care(**ABUCAUC/2018/042**),fresh *Daturastramonium* plantwas collected from Sharada residential area of Kano MunicipalLocal Government, Kano State, Nigeria. The mature seeds in their pods were carefully collected in a cleaned polythene bag and taken to the herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria for identification. The plant was identified and given a voucher number (**VN108**). More of the identified plant seed pods were later collected and peeled off.The seeds were separated from the chaffs, washed with tap water and air-dried under shade. The dried seeds wereweighed using a digital weighing machine (LB-1000, China Sensitivity, 0.1 gram), grounded and pulverized into fine powder by an electronicgrinder. The powdered sample was collected into a sterile cellophane bag and kept in a cool dry place until the period ofextraction.



Plate II. Collection and Identification of *D. stramonium*seeds

A: *D. stramonium* plant and seeds at Sharada residential area of Kano Municipal LGA, Kano State, Nigeria, **B**: Dried *D. stramonium* seeds in pods under shade, **C**: De-husk *D. stramonium* seeds.**D**: Dried *D. stramonium* seeds being grounded to powder.

3.2.Crude Extraction of *Daturastramonium* Seeds

The extraction was carried out using cold maceration in the Department of Pharmacognosy, Faculty of Pharmacy, Ahmadu Bello University Zaria according to Djilani *et al*. (2006) protocol. Two thousand grams of the pulverized seeds powder was obtained for the study. For every, 200g of pulverized seeds was extracted in 1, 500 ml of 70% (v/v) ethanol at room temperature for 72 hrs. The extract was filtered and the solvent was evaporated in a waterbath at 50 °C (figure 2b). The residue, dissolved in H_2O and acidified with H_2SO_4 to pH 3-4, was extracted with petroleum ether and diethyl ether to remove lipophilic, acidic, and neutral material. After basifying the aqueous solution to p^H 9-10 with NH_4OH (25%, m/m), it was extracted with chloroform. The extract washed with distilled water to neutral pH, dried with Na_2SO_4, and concentrated to dryness under reduced pressure to obtain crude alkaloids. Preliminary phytochemical analysis was carried out and the percentage yield of the crude extract was determined using the formula below;

$$\% \text{ Yield} = \frac{Weig\,ht\ of\ the\ extract}{Initial\ \ weig\,ht\ of\ the\ sample} \times 100$$

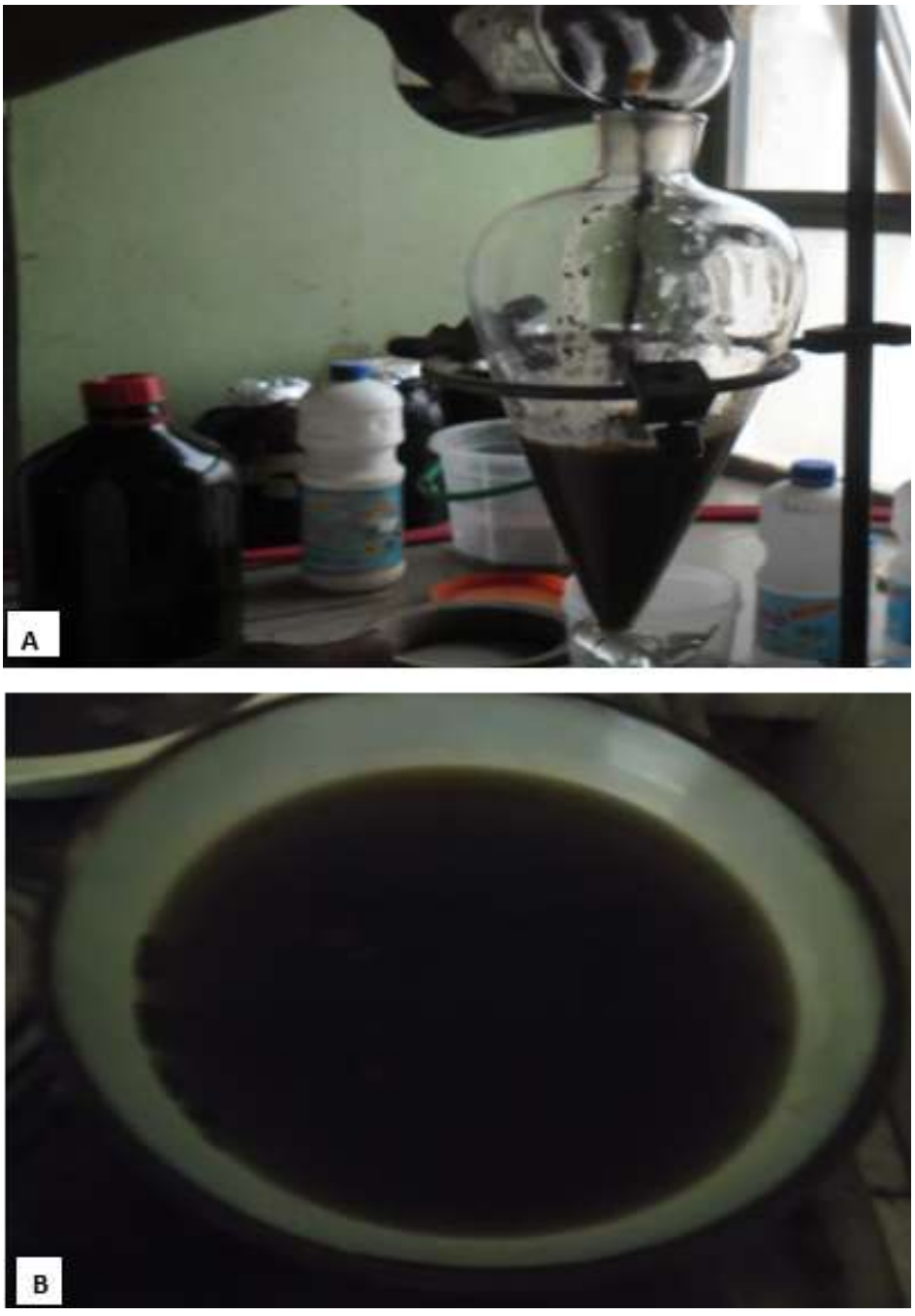


Plate III. Crude extraction of *D. stramonium*seeds

A: Ethanol extraction of crude *D. stramonium* seeds using cold maceration, **B**: Crude ethanol extract of *D. stramonium* seeds on water-bath (50 ^{0}C).

3.3 Phytochemical Analysis of the Crude Seeds Extract

3.3.1 Test for Alkaloids

Mayer's test:

To a 2 ml of the plant sample extract, 2 drops of Mayer's reagent are added along the sides of the test tube. The appearance of a white creamy precipitate indicates the presence of alkaloids (Evans, 2014).

Wagner's test:

Two drops of Wagner's reagent are added to a 2 ml of plantsample extract along the sides of the test tube. A reddish-brown precipitate confirms the test as positive (Wagner, 1993).

Dragendoff's test

To a 2 ml ofplantsample extract in the test tube, 1 ml of Dragendoff's reagent was added drop by drop,formation of a reddish-brown precipitate indicates the presence of alkaloids (Evans, 2014).

3.3.2 Test for Carbohydrates

Molisch's test:

To a 2 ml of plant sample extract, two drops of alcoholic solution of α- naphthol was added. The mixture was shaken well and few drops of concentrated sulphuric acid was added slowly along the sides of the test tube. A violet ring indicates the presence of carbohydrates (Yasuma and Ichikawa, 1953).

Fehling's test:

To a 2 ml of the plant sample extract, 0.5 ml of Fehling's reagent was added. The mixture is heated on a boiling (70 ^{0}C) water bath for 2 minutes. A characteristic brick-red precipitate indicates the presence of reducing sugar (Evans, 2014).

3.3.3. Test for Saponins

Frothing test:

50 mg of the plant sample extract was diluted with 5 ml of distilled water and made up to 20 ml. The suspension was gently shaken in a graduated cylinder for 15 minutes. About2 cm layer of foam indicates the presence of saponins (Kokate, 1999).

3.3.4. Test for Glycosides

Keller – Killiani test:

2 ml of the plant sample extract was dissolved in a mixture of 1 % ferric sulphate solution in (5%) glacial acetic acid. Two drops of concentrated sulphuric acid were added to the mixture and a blue colourdevelops due to the presence of deoxy sugar(Evans, 2014).

3.3.5. Test for Tannins

Ferric Chloride test:

50 mg of the plant sample extract wasdissolved in 5 ml of distilled water. Two drops of neutral 5%, ferric chloride solution was added. The dark green colour indicates the presence of a phenolic compound(Mace, 1963).

3.3.6. Test for Flavonoids

Alkaline reagent test:

2 ml of the aqueous solution of the plant sample extract was treated with 10% ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids(Evans, 2014).

3.3.7. Test for Steroids

Libermann-Burchard's test:

50 mg of the plant sample extract was dissolvedin 2 ml acetic anhydride. 2 drops of concentrated sulphuric acid was added slowly along the sides of the test tube. An array of colour change suggests the presence of phytosterols.

3.4. Fractionation of the Hyoscyamine from the Crude Seeds Extract

The fractionation was carried out in the Department of Pharmacognosy, Faculty of Pharmacy, Ahmadu Bello University Zaria according to Salamah and Ningsih (2017) protocol. Five grams of the viscous extract was dissolved in 10 ml of water (figure 3a). Then, the solution was poured into a separating funnel, 10 ml of chloroform was added, and shaken to solve with two phases, namely water, and chloroform. These two phases were separated and collected. This was repeated until the chloroform phase had the same colour as the chloroform solvent. The chloroform was then evaporated and recrystallized to obtain the hyoscyamine fraction (figure 3e). The alkaloid was analyzed with UV-Vis spectrophotometric method. The fractionation was carried out at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria.

$$\% \text{ Yield} = \frac{Weight\ of\ the\ fraction}{Initial\ \ weight\ of\ the\ sample} \times 100$$

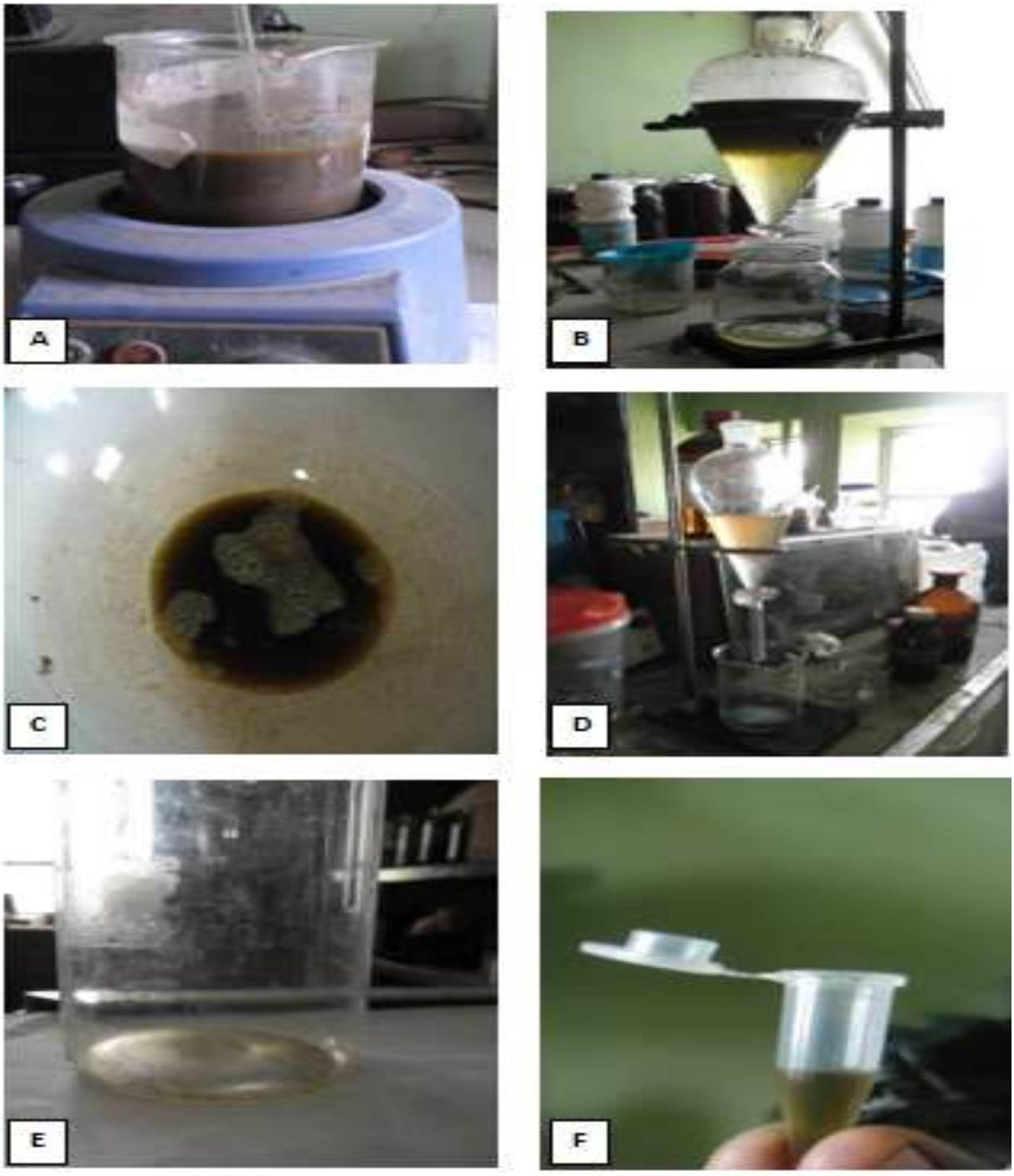


Plate IV. fractionation of Hyoscyamine fraction of *D. stramonium*

A: Crude ethanol extract of*D. stramonium* seeds dissolved in hot distilled water, **B**: Basification, partitioning and dissolution of the crude extract of *D. stramonium* seeds with ammonia, hydrochloric acid and chloroform, **C**: Evaporation the of fraction in water-bath (50 ^{0}C), **D**: Re-purification of the acid layer with ammonia, **E**: Evaporation of the chloroform layer, **F**: Hyoscyamine fraction of *D. stramonium* seeds in Eppendorf's tube.

3.5. The Thin Layer Chromatography (TLC)

With a pencil, a thin mark was made at the bottom of the pre-coated silica plate about 0.5 cm from the bottom to serve as the origin of the applied sample spots. Then, samples solutions (standard l-hyoscyamine powder and fraction aliquot) were then applied separately and parallel to each other on the spots marked at the baseline at equal distances respectively.The mobile phase Butanol – Acetic acid – Water (BAW) in ratios of 4:1:1 was poured into the TLC chamber to a level a few centimetres above the chamber bottom. A moistened filter paper in the mobile phase was placed on the inner wall of the chamber to maintain equal humidity (and also thereby avoids edge effect).Now, the plate prepared with the sample spot was placed in the TLC chamber so that the side of the plate with the sample line was facing the mobile phase. Then the chamber was closed with a lid. The plate was then immersed, such that the sample spots were well above the level of the mobile phase, but not immersed in the solvent. This was then allowed for spots to sufficiently develop to the solvent front of about 1 cm before the plate apex. After about 1 hour, the plate was removed, sprayed Dragendorff's reagent and heated in an oven for5 minutes. The platewasvisualized in a visible light chamber for the presence of the spots and calculation of Rf value (Hahn-Deinstrop, 2007).

$$\text{Retention factor (RF) value} = \frac{\text{Distance travelled by the spot}}{\text{Distance travelled by the solvent front}}$$

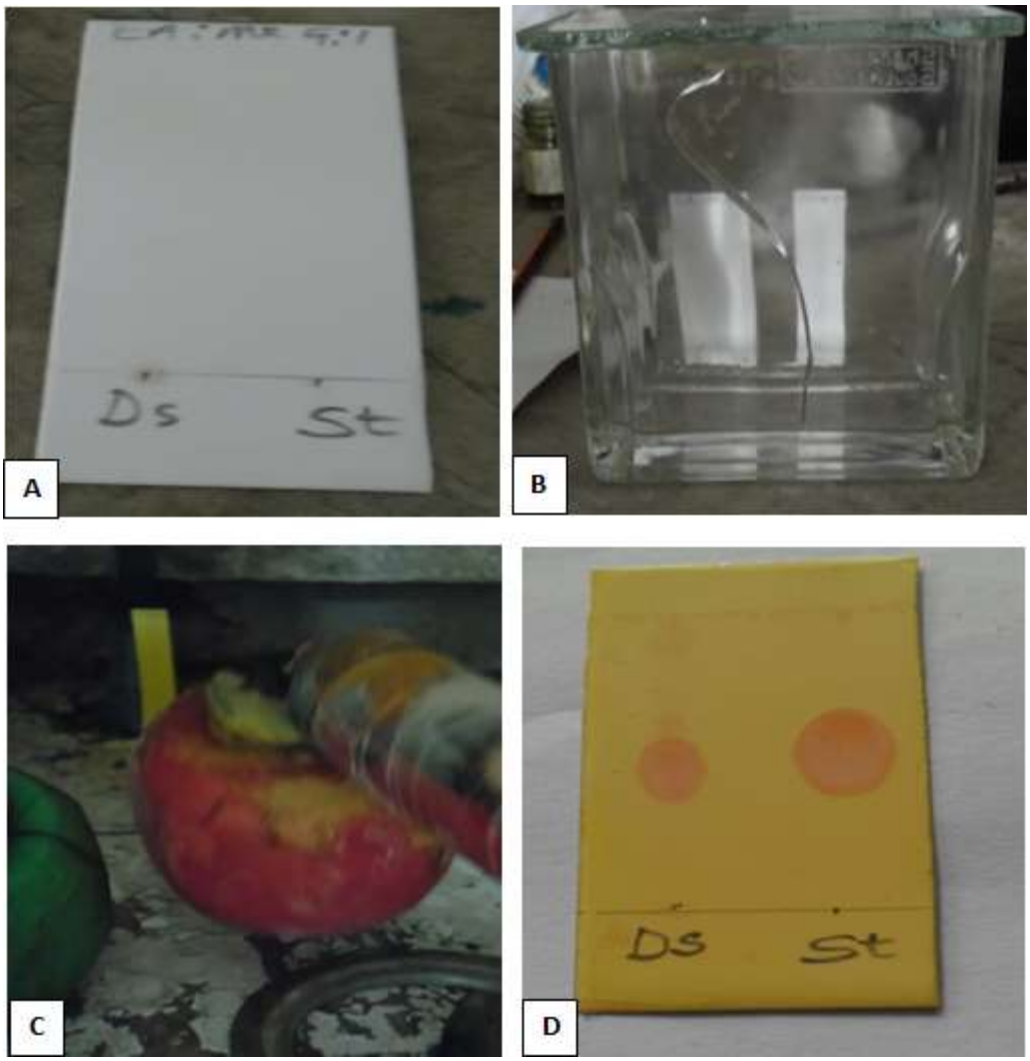


Plate V. Thin Layer Chromatography (TLC)of Hyoscyamine fraction

A: Plane chromatographic plate; **B**: A chromatographic tank containing plates of standard powder and sample, **C**: Spraying of the chromatographic plate with Dragendorf's reagent in a chamber, **D**: Sprayed chromatographic plate showing orange-coloured spots of hyoscyamine in the standard powder (St) and the *D. stramonium* sample (Ds).

3.6.HPLC Analysis and Quantification of the Hyoscyamine fraction

3.6.1.Samples preparation

A 1mg of standard *l*-hyoscyamine (Sigma, St. Louis, MO, USA) was weighed using an electronic chemical balance (Shimadzu, USA) and was dissolved in 10 ml of standard methanol to make a stock solution of 100 µg/l. A 6.4 ml of the stock solution was added to 10 ml of the methanol to make a 64 µg/l concentration. Two more concentrations of 32 and 16 µg/l of the standard solution was formed by serial dilution to allow for a total of four graded solutions for quantifying the hyoscyamine concentration amount in the fraction of the sample.

3.6.2. High-Performance Liquid Chromatography (HPLC)Conditions

A reversed-phase Techsphere 50DS C_{18}HPLC column (25 cm × 4.6 mm i.d.) particle size 5 µm, Supelco, Bellefonte, PA, USA) with oven temperature, 40°C in conjunction with a UV adsorption detector operating at 270 nm was employed. The mobile phase was a mixture of 20%acetonitrile and 45 % methanol, 35 % water (H_2O)and 0.1 mol/L phosphoric acidswhich adjusted the pH to 7.0 and were used at a flow rate of 1 ml/min. The calibration curves for hyoscyamine were constructed by plotting the peak area of samples versus their concentrations.

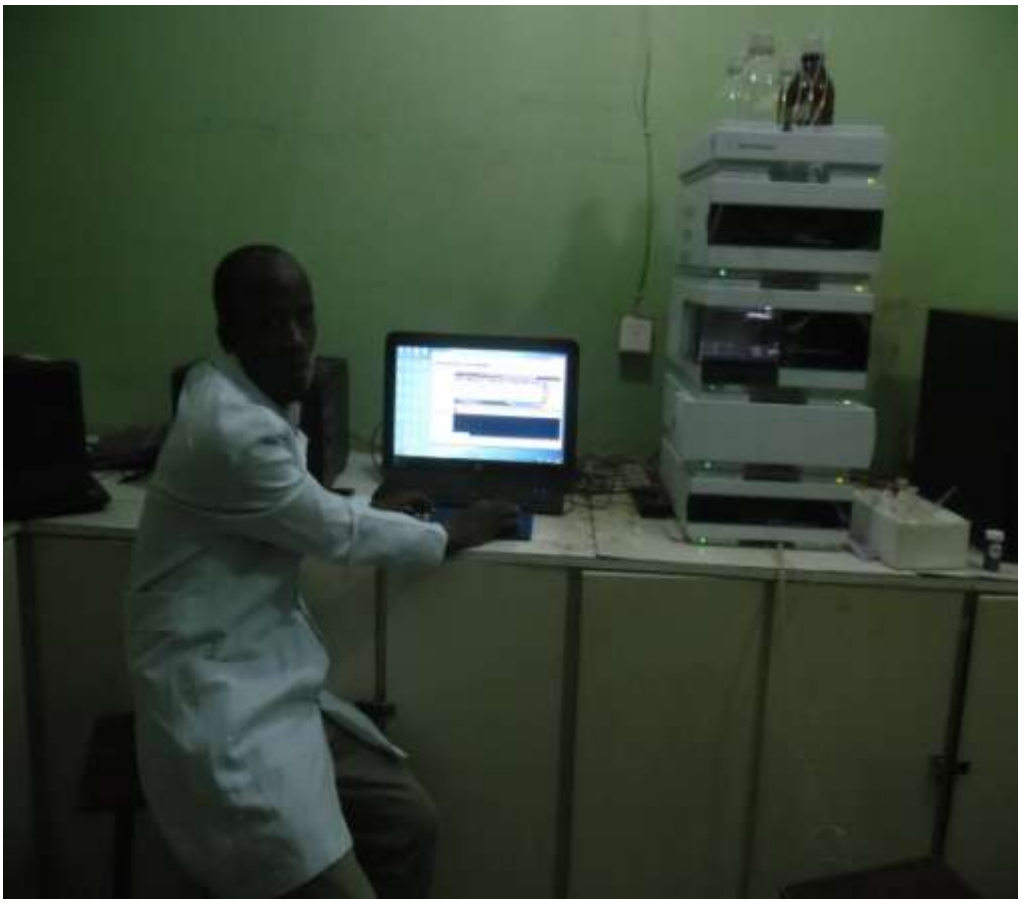


Plate VI. Quantification of the Hyoscyamine fraction by HPLC
At the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ahmadu Bello University, Zaria, 2019.

3.7. Acute Toxicity Test and LD_{50} Determination of the Fraction

The test was carried out at the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria. The hyoscyamine fraction of *Daturastramonium*seeds that contained 11.04 µg was used to calculate the LD_{50} using Lorke's (1983)method. The study was carried out in two phases using 12 rats. During the first phase, 9 rats wererandomly selected into three groups each. Their weights weretaken and received 10,100 and 1000 mg/kg for the 1^{st}, $2^{nd}$and 3^{rd} groups respectively. The animals were observed for neurotoxicity symptoms. Twenty – four hours (24 hrs) later, the second phase of the study was undertaken using three (3) different sets of animals, as no mortality was recorded in the previous phase. During this phase, doses of 1600, 2900 and 5000 mg/kg were given to the three rats (one rat per dose) and further observed for neurotoxicity symptoms.neurotoxicity symptoms were observed, but no mortality was recorded even at the highest dose, therefore considered hyoscyamine fractionsafein Wistar rats at these dosages for the study.

3.8. Dosage Calculation and Administration

A stock solution of 500 mg/kg of the hyoscyamine fraction of *D. stramonium*was prepared by dissolving 5 g of the hyoscyamine fraction in 10 ml distilled water and two drops of Tween 80. The dose for each animal in a group was determined as follows:

$$\text{Dose (ml)} = \frac{\text{Selected dose (mg/kg)} \times \text{Bodyweight (kg)}}{\text{Stock solution (mg/ml)}}$$



Plate VII. Dose preparation

A: An amount of hyoscyamine fraction of*D. stramonium*seeds being weighed for dose preparation using Mettler P162 digital Weighing balance**B**: Dissolving the hyoscyamine fraction of*D. stramonium* seeds for dose preparation.

3.9. Animals Procurement and Handling

Thirty-two (32) healthy Wistar rats of weight range between 150-250 g comprised of equal numbers of adult males and females wereprocured from the Animal House of the Anatomy Department, Faculty of Basic Medical Sciences, Bayero University, Kano. The animals were transported to the Animal House of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University (ABU) Zaria. The malesweresеparated from the female, housed and allowed to acclimatize for two weeks at ambient temperature, with alternate day and night natural cycles condition. Rat chow (*Vital feed*®) and tap water weremade available to the animal's*ad libitum*. All the procedures to be carried out were following the approved guidelines of Ahmadu Bello University (ABU) Zaria Animal's Right and Ethics Committee for Scientific Research Purposes.



Plate VIII. Animal Procurement and Handling

3.10.Animal Synchronization and Determination of Oestrus Cycle

To synchronize the animals' cycles, each of the female rats was injected with 0.01 mg/kg*Zoladex*®(3.6mg AstraZeneca) intraperitoneally, andthe oestrous cycle in the female animals was determined by microscopic examination of the vaginal smear (Byers, 2012). Twenty-fourafter synchronization,vaginal smears were collected by vaginal lavage (Michelle*et al.,* 2015) using a 1 ml plastic pipette filled with 10 µl of normal saline (NaCl 0.9%). The tip of the pipette was gently but superficially inserted into the rat vagina (Marcondes*et al.*,2002). The vaginal fluid was carefully aspirated and placed on a cleaned glass slide. A different glass slide was used for each rat. An unstained material was observed under a light microscope, without the use of the condenser lens, at 10 and 40 x objective lenses (Marcondes*et al.,* 2002). Three types of cells were recognized; round and nucleated cells (epithelial cells), irregular cells without nuclei(cornified cells), and little round cells (leukocytes). The proportion among them was used for the determination of the oestrous cycle phases (Marcondes*et al.*,2002).

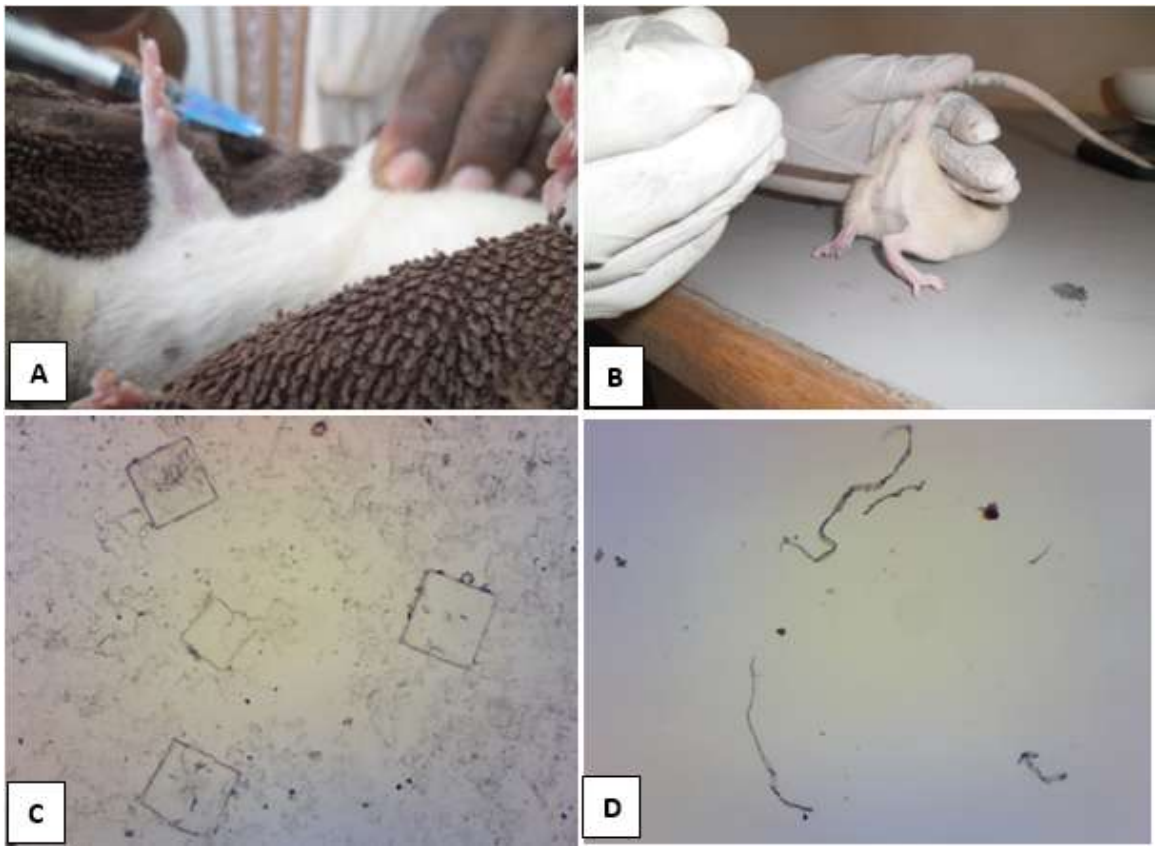


Plate IX. Synchronization and Determination of the Animals Oestrus cycles

A: Synchronization of the adult female virgin Wistar ratcycle with 0.01 mg/kg *Zoladex* (3.6 mg AstraZeneca), **B**: Collection of vaginal smears for cytology, **C**: Proestrus phase in the female virgin Wistar rats, **D**: Remnant of the sperm tails collected early morning after overnight mating with adult male Wistar rats.

3.11. Experimental Design

3.11.1.Animal grouping and mating

Thirty-two (32) healthy Wistar rats comprised adult males and females (1:1), weighed 150-250 g were used for the study. The animals were randomly selected and divided into four groups (I, II, III and IV), each comprised of eight males and females Wistar rats in equal ratio for treatment at gestation (GD15-20), lactation (PND 0-21), adolescence (PND 22-49) and adulthood (PND56-77) respectively. Each group was further divided equally into control and treatment subgroups. The animals from each group were allowed to mate freely and evidence of mating was established by the presence of sperm tails in the vaginal smears collected from each female rat twenty-four hours after the mating. Furthermore, abdominal palpation was carried out on GD12-13 (Rosssell *et al*., 2020) to avoid error due to pseudopregnancy. Once pregnancy was detected, the rats wereisolated and transferred to maternity cages for themto litter. Animals from the same group werekept closely together but in separate cages. A total of one hundred and forty (140) litters were obtained after the mating, thirty (30) litters from each group.Only male rats were used in group IV and further divided equally into four subgroups fornormal saline, low, medium and high dosetreatments of the fraction.



Plate X. Animals grouping and mating

3.11.2. Dose administration

i. Group I: received 200 mg/kg in *utero* of normal saline, while the treated group received 200 mg/kg hyoscyamine fraction of *D. stramonium* seeds on a gestational day (GD)15-20 respectively.

ii. Group II: twenty (20) litters,ten from each subgroup received 400 mg/kg normal saline and 400 mg/kg received hyoscyamine fraction of *D. stramonium* seeds through lactation on a postnatal day (PND)0-21 respectively.

iii. Group III: twenty (20) adolescent rats, ten from each subgroup received 800 mg/kg normal saline and 400 mg/kg received hyoscyamine fraction of *D. stramonium* seeds orally on a postnatal day (PND)22-49 respectively.

iv. Group IV: forty (40) adult Wistar rats, ten from each subgroup received equivalent bodyweight of normal saline,200, 400 and 800 mg/kg hyoscyamine fraction of *D. stramonium* seeds orally on a postnatal day (PND)56-77 respectively.

Table 3.1. **Duration, Dosage and Route of Administration of Hyoscyamine Fraction of *D. stramonium* seeds in Wistar rats**

		Control	Treatment Dose (mg/kg)			
Group	**Duration**	**Normal saline**	**200**	**400**	**800**	**Route**
I	GD15-20	✓	✓			Orally
II	PND0-21	✓		✓		Orally
III	PND22-42	✓			✓	Orally
IV	PND56-77	✓	✓	✓	✓	Orally

3.11. 3.Physical Observation

Growth and Physical Development Indices

Each pup was observed daily for the appearance of developmental landmarks (teeth eruption, eye-opening, bilateral ear detachment and hair growth), and general health and behaviour weekly throughout lactation, and once every two weeks thereafter until adulthood. Weights were measured daily for each rat from PND 8 to PND 75 using LB – 1000 Compact Bowl Digital Scale (China). Starting on PND 4, the appearance of pigmentation, bilateral ear detachment, hair growth,eye-opening, and teeth eruptionwere recorded(Adams, 1986).

3.12.Early Developmental Neurotoxicity Test

3.12.1.Pre-weaning tests

Pups weretested for reflex development and neuromotor ability using tests of surface rightingreflex (SRR), cliff avoidance (CAV) and negative geotaxis (NGE) (Adams, 1986; Moser, 1999). Reflex testing began in the morning and was concluded at least one hour before dosing to minimize any possible acute effects of hyoscyamine on reflexes.

Surface righting reflex

This began from PND 4. A pup wasplaced in a supine position and the time to right onto all four paws was measured using a stopwatch for 30 seconds. This test continued, with one trial per day, until the rats meet the criterion of 3-second latency or less on two consecutive days (Gallavan, 1999; Gray, 2000).

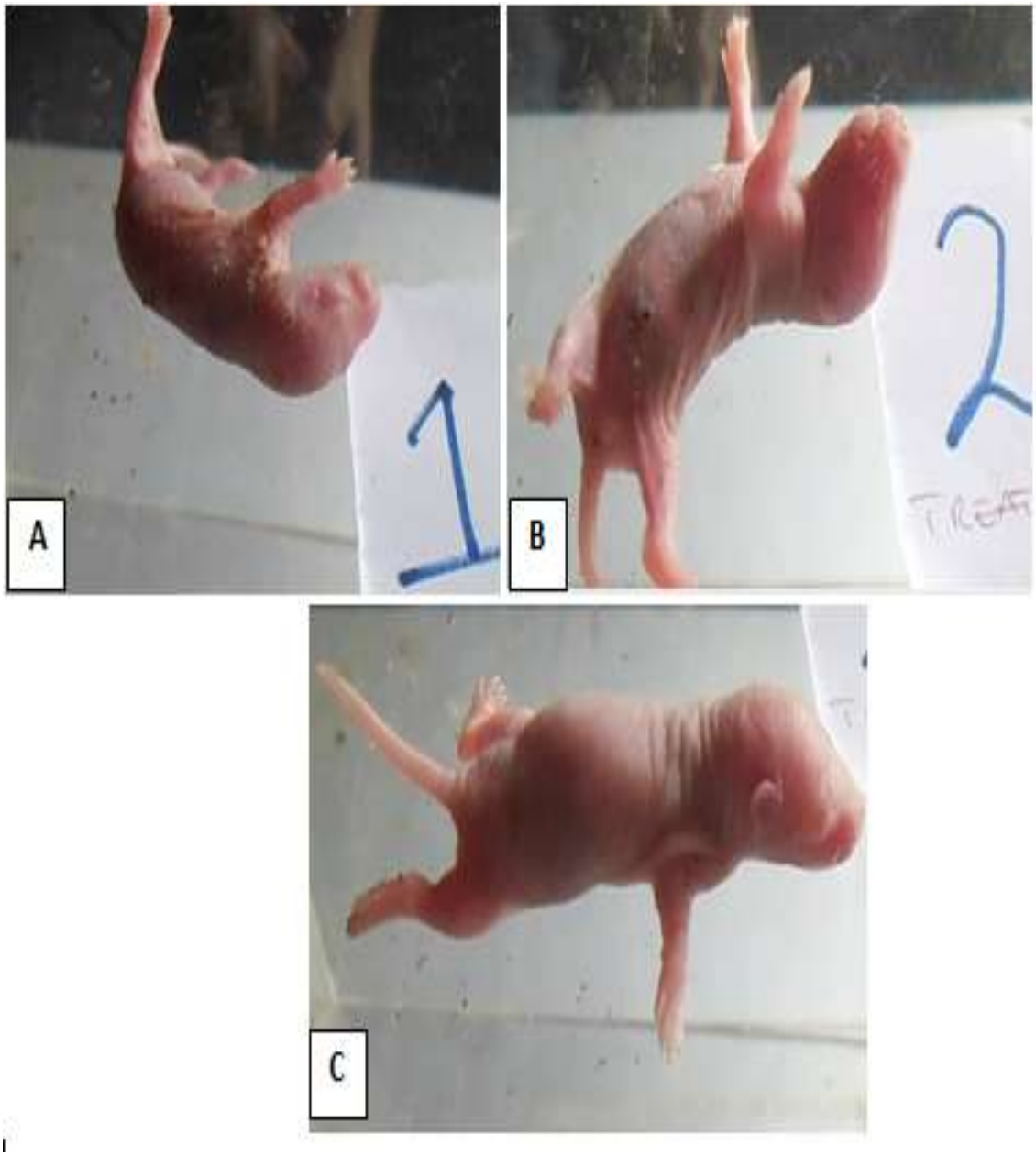


Plate XI. Surface righting reflex test

A: Wistar rat pup performing surface righting reflex neuromotor coordination test, **B**: A hyoscyamine fraction treated Wistar rat performing surface righting reflex neuromotor coordination test, **C**: Wistar rat on its four paws after performing surface righting reflex neuromotor coordination test.

Cliff avoidance

The testing began from PND 6. The pups were tested for this task using a flat plexiglass surface that was raised to a height of 23 cm above the lab bench. Each pup was placed with front paws and snout over the edge and the time to turn 180° away from the cliff face was recorded. Cliff avoidance was measured daily until a latency of 6 sec or less was met for two consecutive days (Gallavan, 1999; Gray, 2000). Each pup was tested in 2 trials each test day, with a cut-off time of 30 sec allowed for each trial.

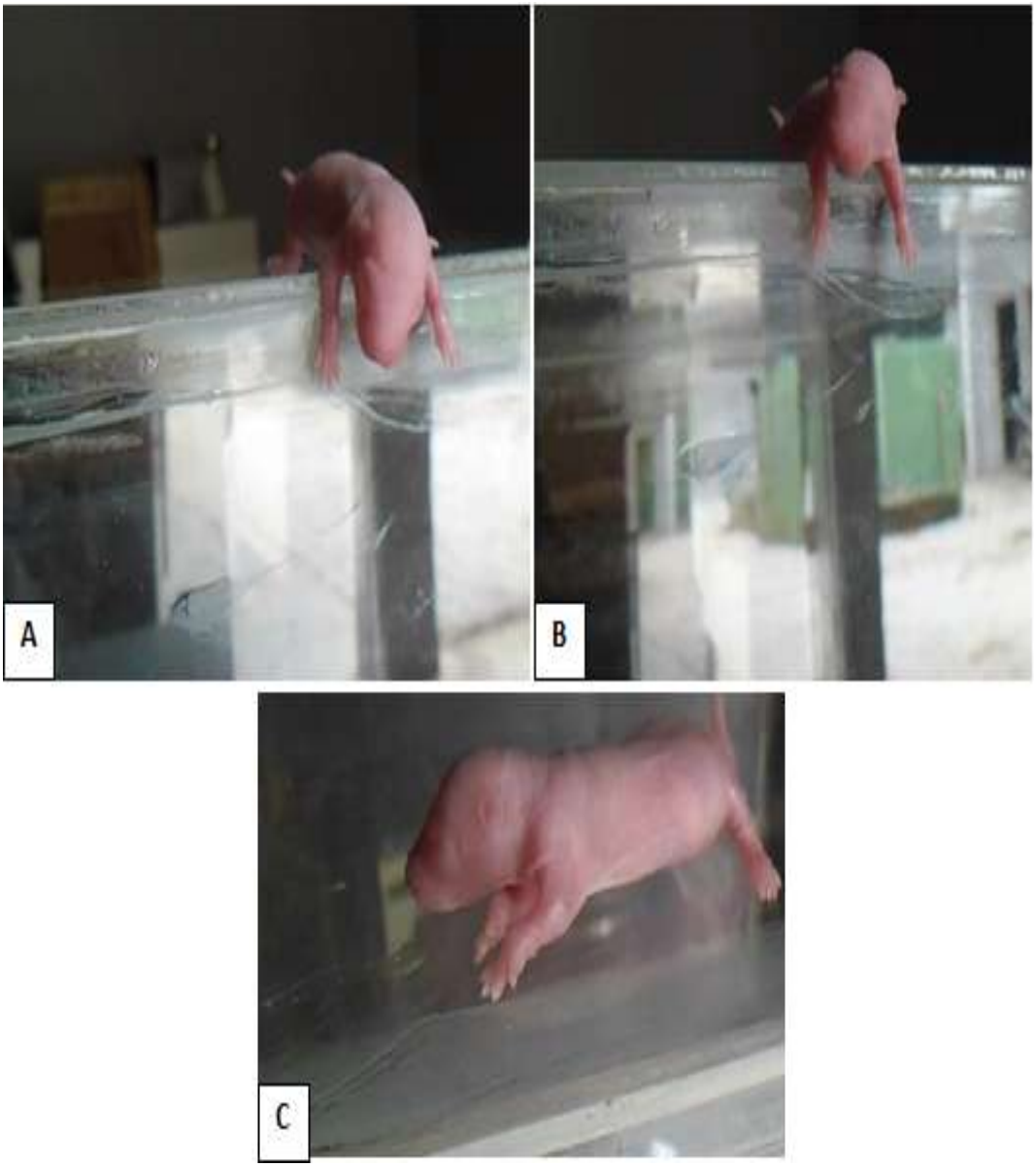


Plate XII. Cliff Avoidance test

A: A Wistar rat pup with paws and snout at the edge of a vertical cliff performing cliff avoidance reflex test of neuromotor coordination, **B**: A Wistar rat pup struggling to back away from the cliff, **C**: A Wistar rat pup backed 180^0away from the cliff during the test.

Negative geotaxis

This began from PND 7. The pups were assessed for this task by placing their heads down on a 30° - 40° rough surface inclined plane and the time taken to turn 180° to face upward was measured. Pups were tested daily until a latency of 6 sec or less is reached for two consecutive days.

For all reflex tests, if a pup did not complete the task within 30 sec, the test was terminated and the rats were returned to their cages (Moser, 1999; Motz and Alberts, 2005).

The order of reflex testing for each day consisted of cliff avoidance, followed by negative geotaxis, then surface righting reflex, with a rest period of at least 15 min between tests.

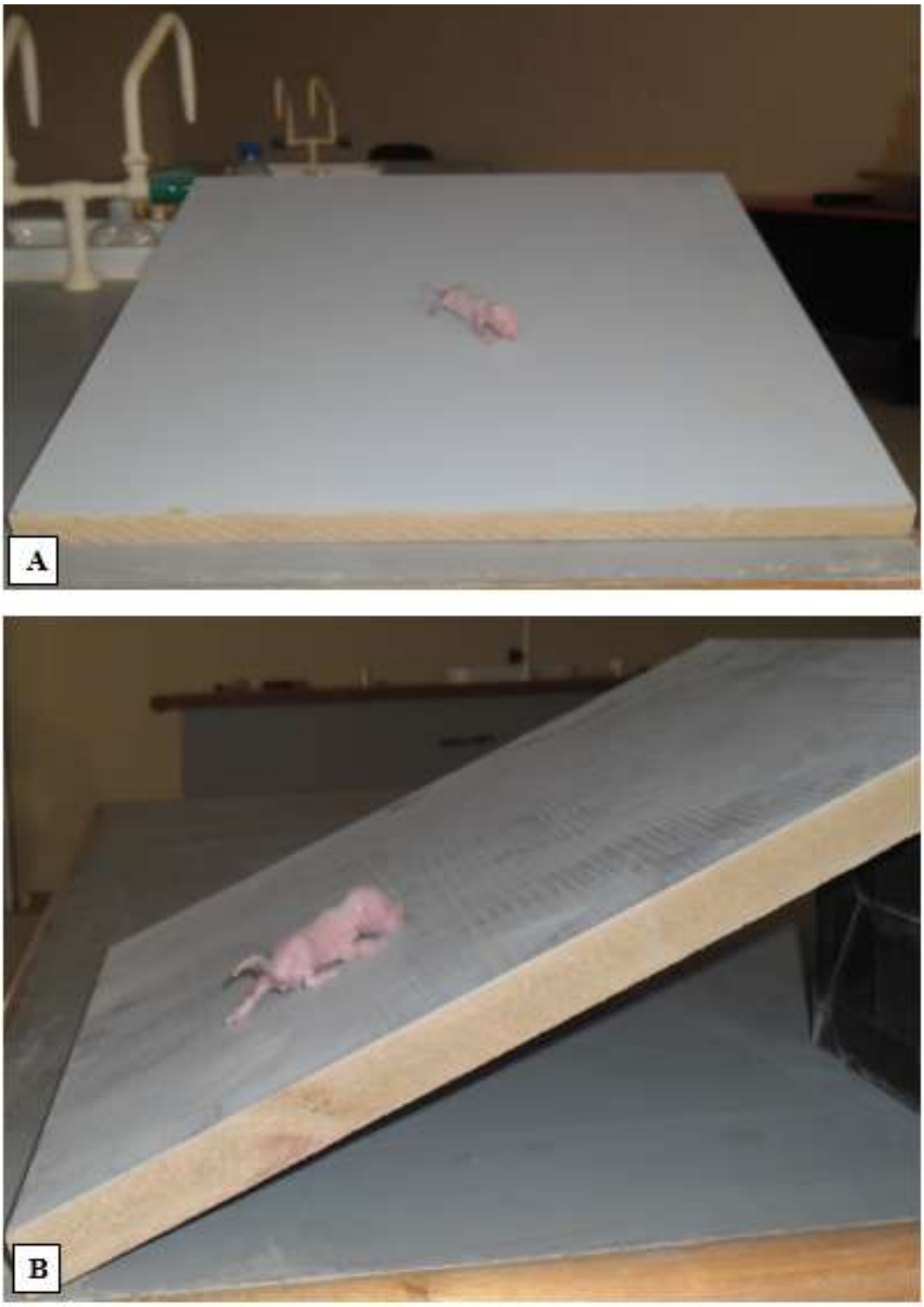


Plate XIII. Negative Geotaxis Test

A: Wistar rat pup placed head-down for negative geotaxis test of neuromotor coordination, **B**: Wistar rat pup backed 180^0away from the plane during the test.

3.12.2.Post-weaning Tests

Triple horizontal bars

This was conducted according to Deacon (2013) during the adolescent stages (PND45-46) of all the treated groups before adulthood and after treatment of the groups treated in adolescence and adulthood. The barsconsisted of three cylindrical metals of different diameter improvised using ironrods. The bars were 38 cm long-held horizontally by a wooden support column at each end 49 cm above a bench. Three bars diameters were 2, 4 and 6 mm respectively. The 2 mm bar was the standard used for the training, whereas the 4 and 6mm rods respectively were used for the test.

During the test, a ratwas held by the tail, was placed on the bench in front of the apparatus, slide quickly backwards about 20 cm (this aligns it perpendicular to the bar), rapidly raised and let to grasp the horizontal bar at the central point with its forepaws only, and release the tail, simultaneously starting the stopwatch. The maximum test time (cut-off time) is 30 sec. Any rat falls before 5 sec, the procedure was repeated three time as this could be due to poor placing until the rat gets a > 5-sec score. For scoring the horizontal bars tests, any rat:

Falling between 1-5 sec = 1, Falling between 6-10 sec = 2, Falling between 11-20 sec = 3, Falling between 21-30 sec = 4, Falling after 30 sec = 5

Placing one forepaw on bar support without falling = 5

The total time scored by each rat from the 2, 4 and 6 mm was added respectively and the final score from all groups were cumulated as a total of 2, 4 and 6 mm respectively.

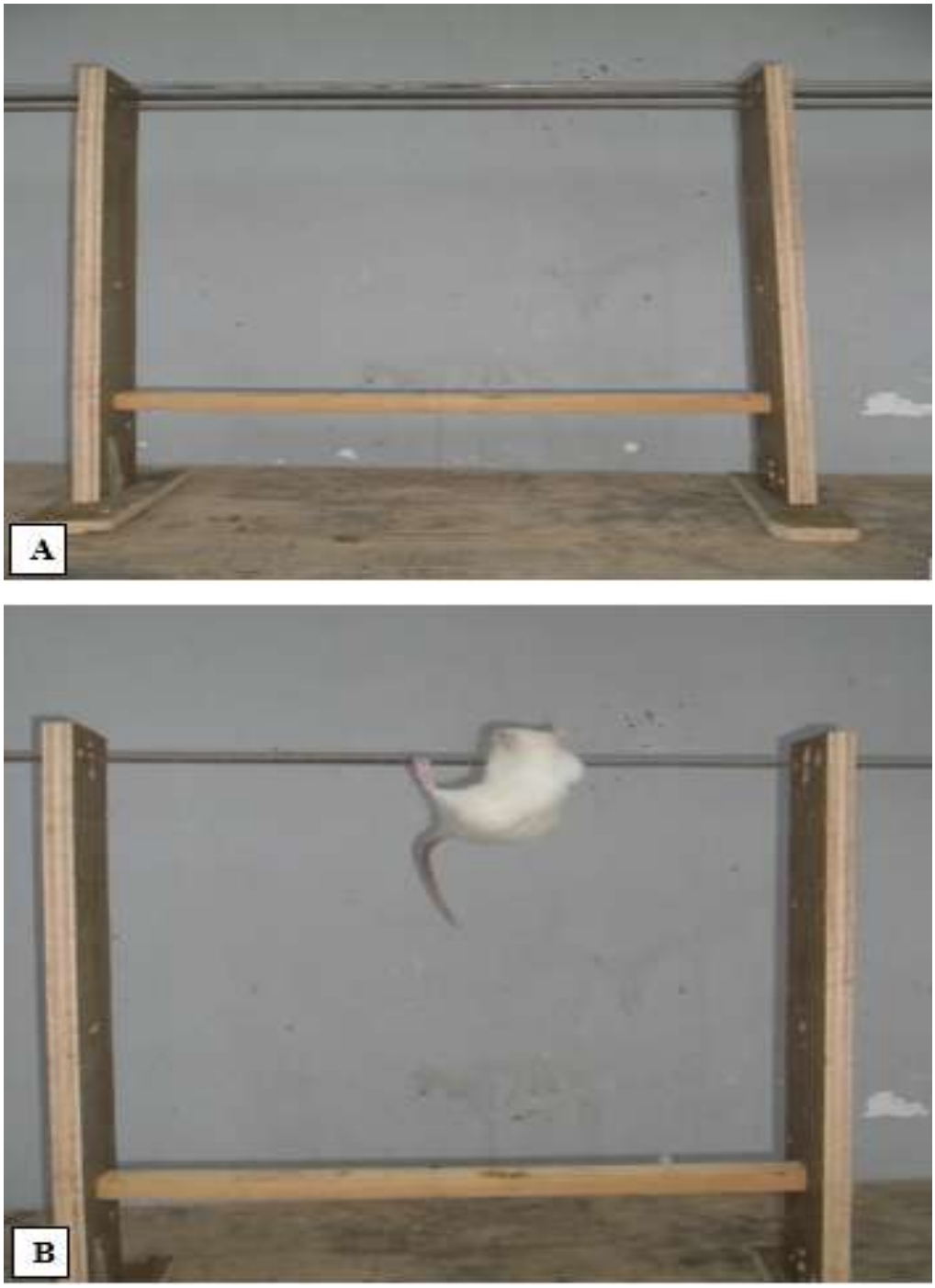


Plate XIV. Triple Horizontal Bar (THB) test

A: Triple horizontal bars for neuromotor coordination in adult Wistar rats model consisted of three metal bars; 2 mm, 4 mm and 6 mm diameters respectively. **B**: A Wistar performing the task.

3.12.3.Spatial Learning and Memory Test

Novel Object Recognition Test (NORT)

This was conducted in adulthood (PND 61 – 75). The test consisted of three phases that are, (habituation, sampling and test) which was completed in two days. For each phase of this test, the open field arena was thoroughly cleaned with an unscented bleach germicidal wipe, 70% Ethanol (EtOH) followed by distilled water (dH_2O) before initial use.

The Habituation Phase:

A day before object exposure, the rats were habituated to the open field arena in a 50x50 cm wooden box. Before the commencement of the habituation session, SONY® (Model DCR – PJ5E) digital and a video camerawas used for proper recording of a rat inthe maze.Rats were removed from the rack and gently placed on a table close to the proximity of the arena.Oneat a time was gently removed from the home cage and placedin the centre of the arena. The video recording system was turned on and the rat was allowed to freely explore the arena for 10 minutes. After every habituation session, the rat was takenback into the home cage and the arena thoroughly cleanedwith an unscented bleachgermicidal wipe, 70% EtOH followed by dH2O before another session began (Jablonski *et al.*, 2013).This was repeated for all the rats until all got habituatedto the arena.Afterwards,the video of the arena habituated by all the rats was analyzed. Any rat with stereotyped behaviour or biases in the time spent in particular regions of the arena was excluded from further experimentation**.**

The Sampling/Familiarization phase:

Before the start of this phase, two identical objects (A_1 and A_2)were placed into the 50 x 50 cm wooden boxarena and fix to the floor with cello-tape putty so that animalscould not move the objects. The objects were aligned diagonally opposite to each other along the walls with enough distance between the walls and objects so that the rats could freely explore the objects from all angles.The data acquisition system, SONY® (Model DCR – PJ5E) digital and a video camerawas used for proper recording of a rat inthe maze.Rats were removed from the rack and gently placed on a table close to the proximity of the arena.One rat at a time was gently removed from the home cage and gently placedin the centre of the arena. The video recording system was turned on and the rat was allowed to freely explorefor 5 minutes. At the end of each session, the rat wasgently removed and placed back into its home cage. The arena and objects were thoroughly cleaned with 70% EtOH and dH_2O (Jablonski *et al.*, 2013). This was repeated until all rats familiarized themselves with the objects. The recorded video of this phase was analyzed.Object explorations were counted once the mouse oriented toward the object, the snout was very close to the object, orpawning the object for at least 1 second. The object bias score was calculated as;

$$\text{Bias score (A or B)} = \frac{TimeExpoloringObjectAorB}{TotalTimeExploringObjects} \times 100$$

Any rat that exhibited an object bias score below 20% or above 80% was excluded fromfurther experimentation.

The Test Phase:

This phase was performed 2-3 h after completion of the sample phase. Before the start ofthis phase session, two un-identical objects labelled object A for the familiar and object B for the novel object respectively were placedinto the 50 x 50 cm wooden boxarena and fix to the floor so that the animals could not move theobjects. The objects were placed in the same position as in the sample phase. The video camera was set up. Rats were removed from the rack and gently placed on a table close to the proximity of the arena. A rat at a time was gently removed from the home cage and gently placed the rat in the centre of the arena. The video was turned on and the rat was allowed to freely explore the objects for 5 minutes. At the end of the session, rats were gently placed back into their home cage. The process was repeated for the rats and the scoring condition remained the same in the sample phase (Jablonski *et al.*, 2013). The novelty preference and discrimination index (DI) were calculated as follows;

$$\text{Novelty preference} = \frac{TNovel}{TFamiliar + TNovel} \times 100$$

(Ennaceur and Delacour, 1988)

$$\text{Discrimination index (DI)} = \frac{TNovel - TFamiliar}{TFamiliar + TNovel}$$

Where;

T_{Novel} = Time taking for Exploring Novel Object

$T_{Familiar}$ = Time taking for Exploring Familiar Object

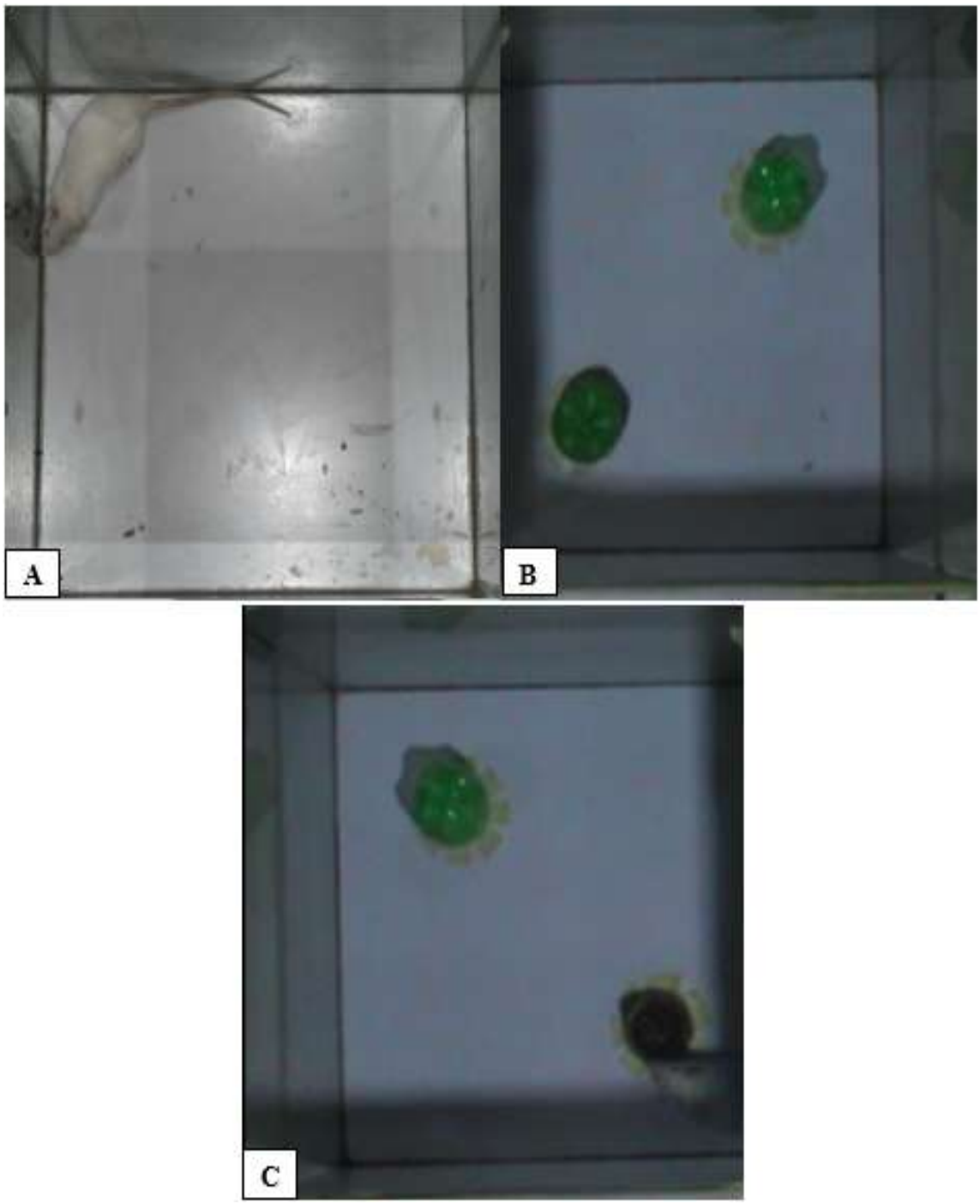


Plate XV. Novel Object Recognition Test (NORT)

A: An adult Wistar during habituation phase in Novel Object Recognition test paradigm
B: A sampling phase in Novel Object Recognition test paradigm
C: An adult Wistar rat during the test phase in the Novel Object Recognition test paradigm

***Morris Water Maze*(MWM)**

This was carried out also in adulthood (PND78-83) for six (6) consecutive days. During this test spatial learning and retention wastested using a modified Morris method (1984). The apparatus consisted of a circular pool with a diameter of 100cm and a depth of 60cmbearing a platform 20 cm long and 12 cm diameter. The inner surface of the tank was painted black and thenfilled with water at 22-25^{o}C deep enough to expose about 1 inch of the platform above the water surface. The video device was placed to capture the entire pool and the platform in it.

During the pre-training (Day 1), the rats were transferred from their housing facility to the behaviour room and kept in an area where they were unable to see the pool or spatialcues. They were allowed to adjust to the new environment for about 30 minutes before testing. To begin testing, a rat was lifted from the home cage by the base of the tail and gently placed into the clean and clear water, facing the edge of the pool. The rat was allowed to swim and find the platform within the 60 sec cut-off in the absence of anybody in the room. This was repeated for all the rats at five different locations of the platforms in the pool. As indicated in the protocol of Qing *et al*. (2008) and shown in table 3.2.

Table 3.2. Morris Water Maze protocol for Spatial Learning and Memory in Wistar rats

Trial	Day 1		Day 2	Day 3	Day 4	Day 5	Day 6
	Platform location	**Starting Direction**	**Platform location: SW Starting Direction as follows:**				**No platform**
Trial 1	SW	S	W	N	N	E	N
Trial 2	NW	N	S	W	E	S	
Trial 3	NE	S	N	E	W	W	
Trial 4	Centre	E	E	W	S	E	
Trial 5	SE	W	S	S	N	N	

Adapted from (Qing *et al.*, 2008)

In the training (Day 2 – 5), the test procedure was the same as day 1 only that the platform was completely immersed 1 inch below the water in the pool and mixed with non – toxic (methylene blue) ink to hide the platform. The platform also was maintained in one quadrant (SW) throughout the trial periods and the days. The methylene blue was added to hide the platform enough so the rats found it difficult to identify it easily.

In the test (day 6), the same protocol was observed as in the previous days, only that the platform was removed and a singletrial was carried out by releasing the animals from the farthest side of the quadrant (N).

At the end of each day, the video records of all the trials were analyzed for the escape latency and period spent in the escape latency, and time spent in each quadrant.

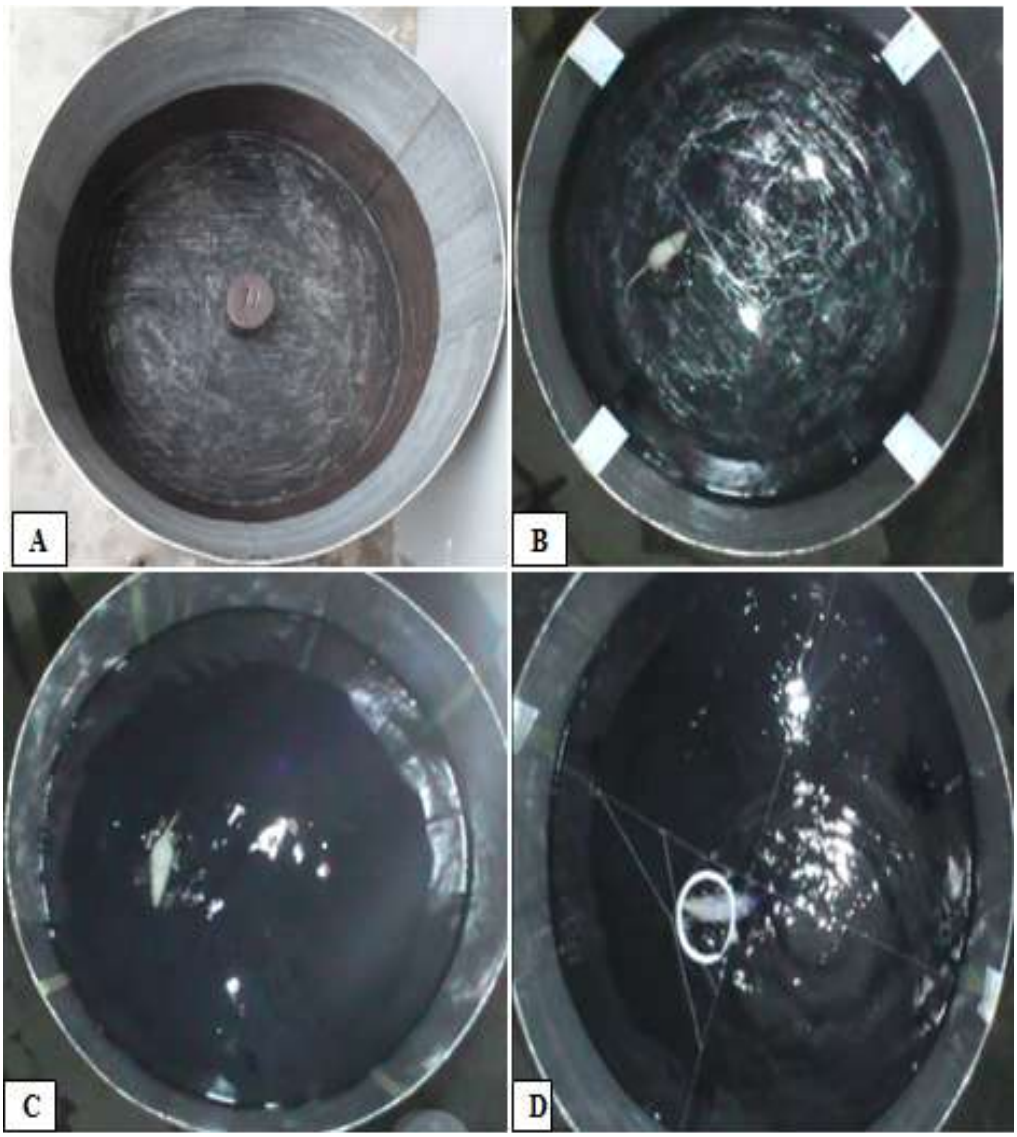


Plate XVI. Morris Water Maze test paradigm

A: Morris Water Maze (MWM) paradigm for spatial learning and memory test with a centrally placed escape platform, **B**: Wistar rat performing the MWM task in a clean transparent warm water during the 1st day training day, **C**: Wistar rat performing the MWM task in methylene-blue opaque water during the 2nd – 5th training days, **D**: A Wistar rat performing the MWM task in methylene-blue opaque water during the test phase (no platform).

3.13. Animal Sacrifice and Tissue Preparation

Five Wistar rats were sacrificed each at the end of the gestational (PND1), lactational (PND22) and adolescence (PND42) phases of the treatment only by cervical dislocation for histology. At maturity (PND85),ten Wistarrats from each group including those treated in adulthood wereeuthanized using 75% Ketamine (10 mg/ml USP) anaesthesia. Five of the brain tissues were preserved in Bouin's fluidfor histology and the remaining five were preserved in P^H 7.4 PBS for AAS) analysis respectively except the group treated in gestation. Five brain tissues from the subgroups treated in adulthood only were also preserved in the PBSfor genes expression analysis.

3.14.Atomic Absorption Spectrum Analysis (AAS)

This was carried out according to Helrich (1990) protocol. Five adult brain tissues from each group, except for the gestation preserved in p^H 7.4 PBS were homogenized in 10 ml normal saline for the AAS analysis in theDepartment of Soil Science, Faculty of Agriculture, Ahmadu Bello University, Zaria. 650 ml concentrated nitric was added to80 ml perchloric and 20 ml sulphuric acid to form an acids mixture. 1 ml each of the homogenate samples from each group was added into 100 ml beakers to digest the tissue. 30 ml of the acid mixture was added to the beaker and placed on a hot plate to digest the tissue till the brown fumes of nitric acid escaped and dense white fumes formed. The heating continued until the content reduced to about 10 ml volume. The content was then washed with distilled water in a 50 ml volumetric flask to make it up the mark. The Cu, Ni, Pb and Zn were determined using atomic absorption spectrophotometer (AAS500 Pg).

3.15.Dissection of Hippocampus

The brain was placed in a Petri dish containing ice-cold PBS and cut along the longitudinal fissure of the cerebrum using a surgical blade. The regions posterior to lambda (midbrain, hindbrain, and cerebellum) was cut off. The cerebral hemisphere was placed medial side up and, using forceps, the diencephalon was carefully removed (thalamus and hypothalamus) with the aid of a magnifying lens. This exposed the medial side of the hippocampus, thus allowing for visualization of the dentate gyrus. A sharp needle-tip was inserted into each side of the dentate gyrus and the needle was slide superficially along the septo-temporal axis of the hippocampus to isolate the dentate gyrus.The hippocampus was picked up using a needle and placed in a sample tube that contained RNALater solution (Hagihara*et al.,* 2009). This was immediately frozen in -20^{o}C for gene expression study.

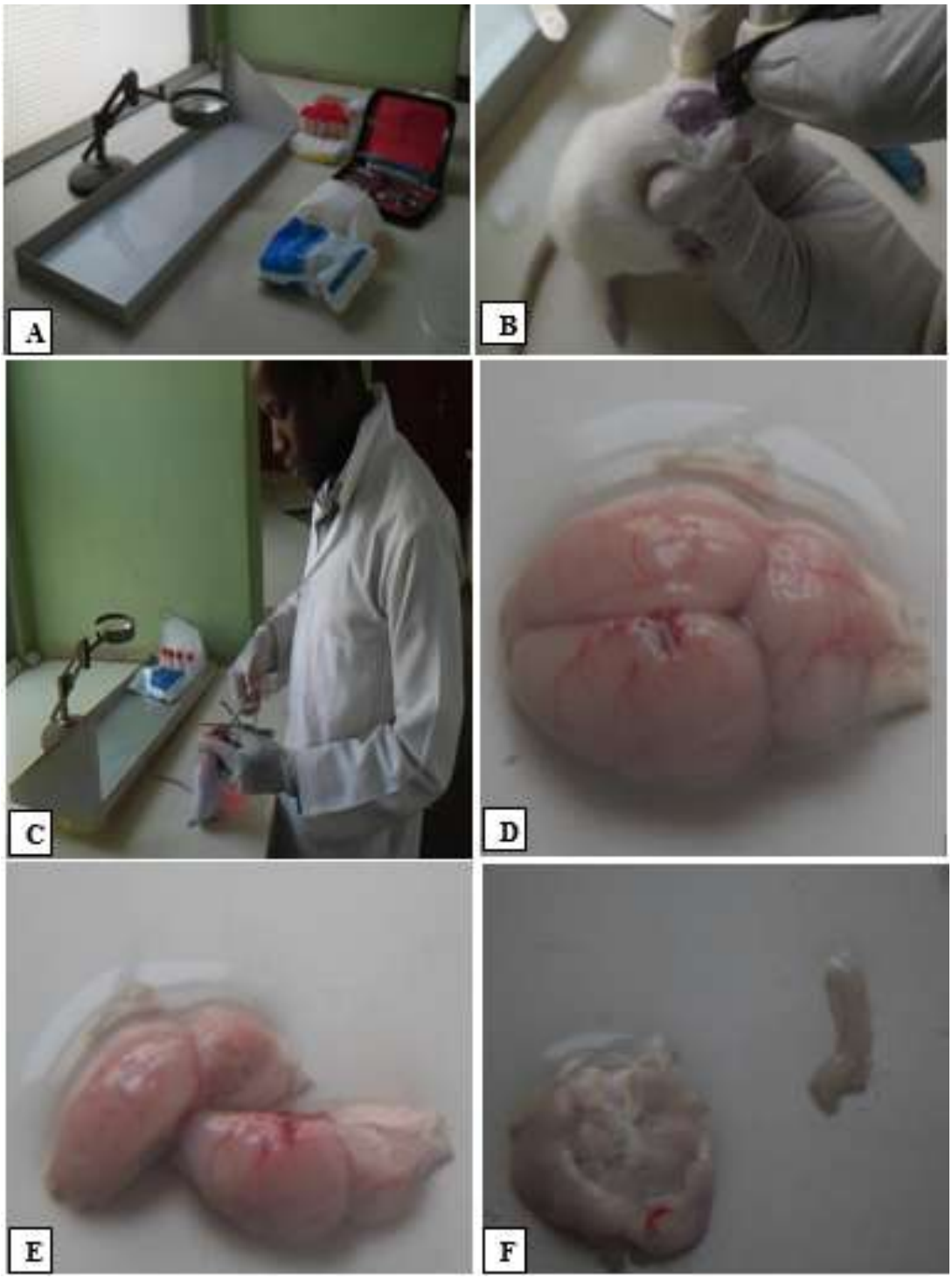


Plate XVII. Dissection of Hippocampus

A: Dissection on a table, **B**: Dissecting rat's scalp using a blade, **C**: Excision of the rat skull with scissors, **D**: Dissected rat's brain in iced phosphate-buffered saline, **E**: Sagitally dissected rat's brain in iced phosphate-buffered saline, **F**: Isolated rat's hippocampus in iced phosphate-buffered saline.

3.16.Gene Expression and Analysis

3.16.1 Primer Design

The two targeted genes discs large Membrane Associated Guanylate Kinase (MAGUK) scaffold protein 3 (*DLG*3) and Neuronal differentiation 1 (*NeuroD1*) were designed from the NCBI website. The primer sequence was placed for order from the USA, through the DNA labs limited, Kaduna State, Nigeria.

Table 3.3. Primers for quantitative Real-Time PCR Analysis

Genes	**Forward primer (+)**	**Reverse primer (-)**	**Size**	**Accession Number**
**NeuroD1*	CGTGTGCAAGGAATGAATCG	ACGTCAGTTTCACCATTCCC	20	NM_0192118.2
**DLG3*	CCAAGATCCATGACTTGCGA	GTGTTGGTTTAGTGCAGGGA	20	NM_031639.1
***β – Actin*	GGTGTGATGGTGGGTATGGG	CAGTTGGTGACAATGCCGTG	20	NM_031144.3

Note: *Target gene, **Reference gene.

3.16.2.Tissue Lysis and cDNA Synthesis

The hippocampus of the rats treated in adulthood only wasused for the gene expression study. The tissues were lyzed and theirRNAs extractedusing AccuPrep® Genomic DNAand RNAExtraction kits respectively according to the manufacturer's instruction. After extractions, the RNAswere purified using TURBO DNA – *free* (Ambion) to remove the contaminated genomic DNA. cDNA was synthesized using Invitrogen protocol (SuperScript II Reverse Transcriptase) and 1 µg of DNase – treated total RNA was used as the starting material.



Plate XVIII. Tissue lysis and cDNA synthesis

A: Dissected hippocampus being transferred into a sample bottle, **B**: Samples of hippocampus frozen at -20^{0}C for gene expression study, **C**: Hippocampus lysis for DNA extraction, **D**: DNA extraction DNA, **E**: Conversion of DNA to cDNA, **F**: Amplification of cDNA for gene expression study.

3.16.3.Quantitative Real-Time PCR of cDNA

The RT – PCR experiment was performed using a Lightcycler 2.0 rapid thermal cycler system (Roche). PCR reactions were set upper sample using 3 µL cDNA, 1µmol/L each primer, 50 µL Quantitect SYBR Green PCR Master Mix (Qiagen). The conductionsof the PCR machine were;pre - denaturation 1 cycle of 95^{o}C for 10min, denaturation 35 – 40 cycles of 94^{o}C for 15 sec, Annealing: 45^{o}C for 10sec, 1 cycle of extension, 72^{o}C for 5 minutes. The resulting amplicons were checked for specificity and size in agarose gels. The data generated were analyzed for cycle threshold and fold change using ($2^{-\Delta\Delta CT}$) *Livak's* method (2001). Beta-actinin was used as endogenous controls (that is, House Keeping Gene).



Plate XIX. Quantitative Real-Time PCR of cDNA

A and B: Addition of Mastermix reagent to cDNA for gene expression study, **C**: Lightcycler 2.0 rapid system (Roche) for gene expression study, **D**: RT-qPCR and a display monitor for gene expression study, **E**: A screen of a display monitor of showing RT-PCR undergoing cycles of reactions, **F**: Fluorogram of the cycle threshold (ct) values by the RT-qPCR machine.

3.17. Histological Studies

The tissues were processed at the Department of Pathology, Ahmadu Bello University Teaching Hospital (ABUTH), Shika, Zaria, Kaduna state, Nigeria. The brain tissues were dehydrated in different grades of alcohol and cleared in xylene using an automatic processing machine (Shandon Southern Duplex Processor). The tissues were then infiltrated with paraffin wax and blocked in the coronal plane. Serial sections of the blocks were taken at 8 μm with a (Leitz Wetzlar) microtome, mounted on glass slides and allowed to dry overnight. The staining technique employed was hematoxylin and eosin in paraffin sections Lillie (1976). Sections were then observed under a light Olympus Binocular Microscope (Ch-20i, Uttar Pradesh, India) high magnifications (x 40) and micrographs were taken with the help of Celestron® eyepiece digital camera (EC 3.0 MP, China). Coronal sections of the hippocampus were observed in the treated rats and compared to the controls.

3.18. Stereology and HistomorphometryStudies

A total of 36coronal sections from each group (6-7 sections/animal) were captured using Olympus Binocular Microscope (Ch-20i, Uttar Pradesh, India)in each subdivision of the hippocampus (CA1, CA3) and ectal limb of the dentate gyrus (DGEC,). In each animal, the first section was chosen randomly at the level approximately −3.3 mm posterior to bregma and every fifth section (40 μm caudal to the first one) was taken subsequently to avoid repeat measuring of the cells according to the systematic uniform random sampling (SURS) procedure (Gundersen and Jensen, 1987).Approximate70 neurons per region per animal

were analyzed (Isgor*et al*., 2004).The photomicrograph files were imported into Cavalieri'sunbiased estimator and physical fractionator of*STEPanizer[v1.0]* software(Tschanz*et al*., 2011) for the estimation of the volume and neuronal numbers respectively.

Counting frames with areas of 0.01 x 0.01mm^2 (10, 000 μm^2) was used for CA (CA1 and CA3 regions) and DG (DGEC) regions, respectively. The total numbers of neuronal nuclear profiles falling in the counting frames of micrographs were counted. All counting procedures were carried out using the "Forbidden Line" rule (Gundersen, 1977).Also, the thicknesses and surface areas of the hippocampusand dentate gyri were determinedusing *AperioImageScope [v12.4.0.5043]*.

Thus, neuronal numbers were calculated as;

i. N = 1/asf × 1/ssf ×$\sum_{i=1}^{n} Pi$

Where, $1/asf$= area sampling fraction, 1/ssf = section sampling fraction

ii. Volume =$\frac{1}{ssf} \times a(p)\ t^{-} \sum_{i=1}^{n} Pi$

Where, $\sum_{i=1}^{n} Pi$= sum of allpoints hitting the tissue,

t = was the distance between the sections, and

a (p) = the area of the tissuecovered by each point.

The error variance of the Cavalieri estimator is based on a mathematical model from Gundersen and Jensen (1987).

Coefficient of error (CE) = $\frac{\sqrt{VarSURS + Varnoise}}{\sum_{i=1}^{n} Pi}$

$Var_{SURS} = \alpha_i (3 \times (A - Var_{noise}) - 4 \times B+C)$

$Var_{noise} = 0.0724 \times \left(\frac{b}{\sqrt{a}}\right) \times \sqrt{n \sum_{i=1}^{n} Pi}$

$\left(\frac{b}{\sqrt{a}}\right) = 5$

$A=\sum_{i=1}^{n} PiPi$

$B = \sum_{i}^{n} Pi + 1$

$C = \sum_{i=1}^{n} Pi + 2$

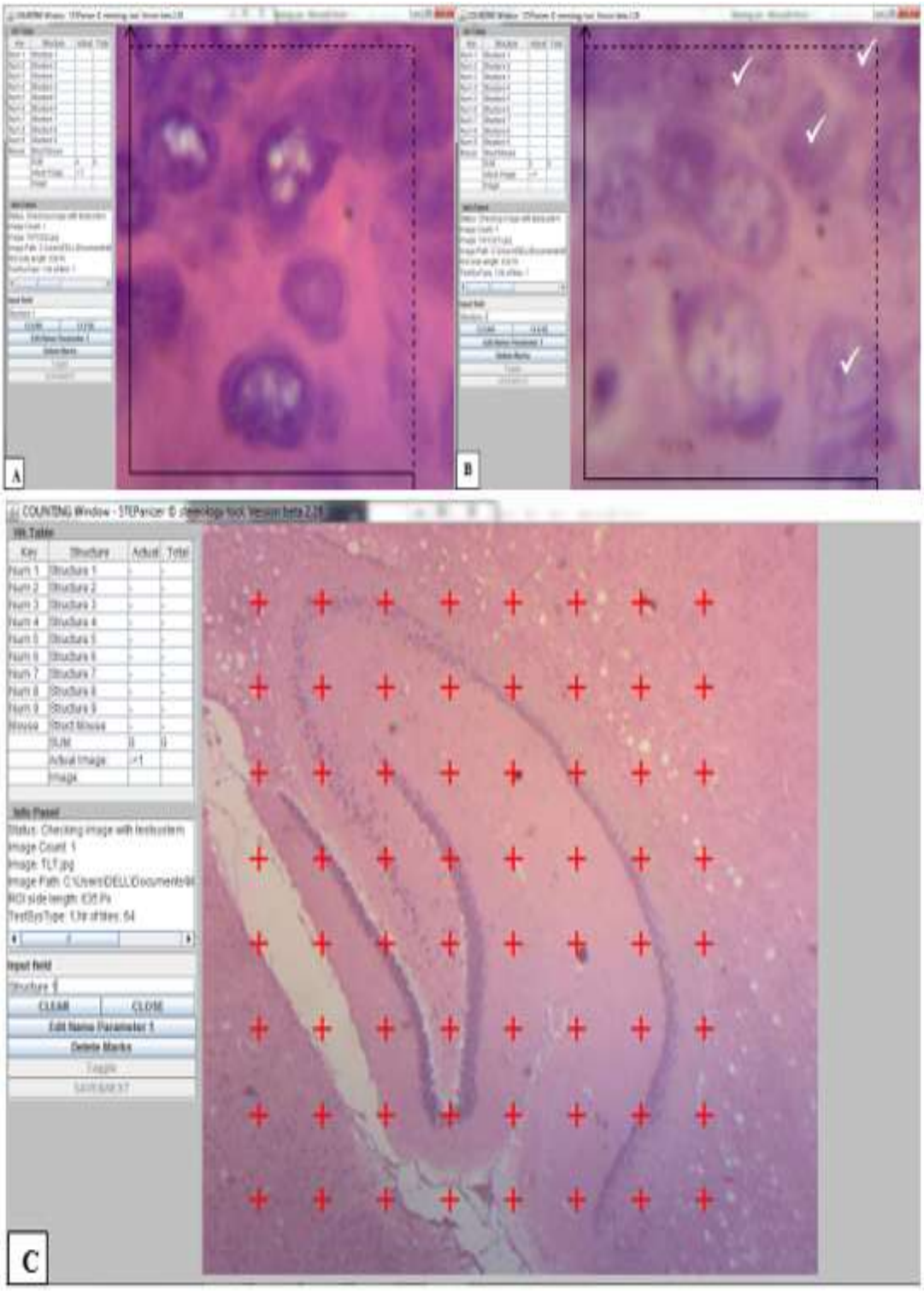


Plate XX. Estimation of Neuronal numbers and Hippocampus volume

Aand B:Cavalieri's physical fractionator for the neuronal number estimation, look-up section (left), and reference section (right), **C**: Cavalieri's unbiased counting grid for volume estimation.

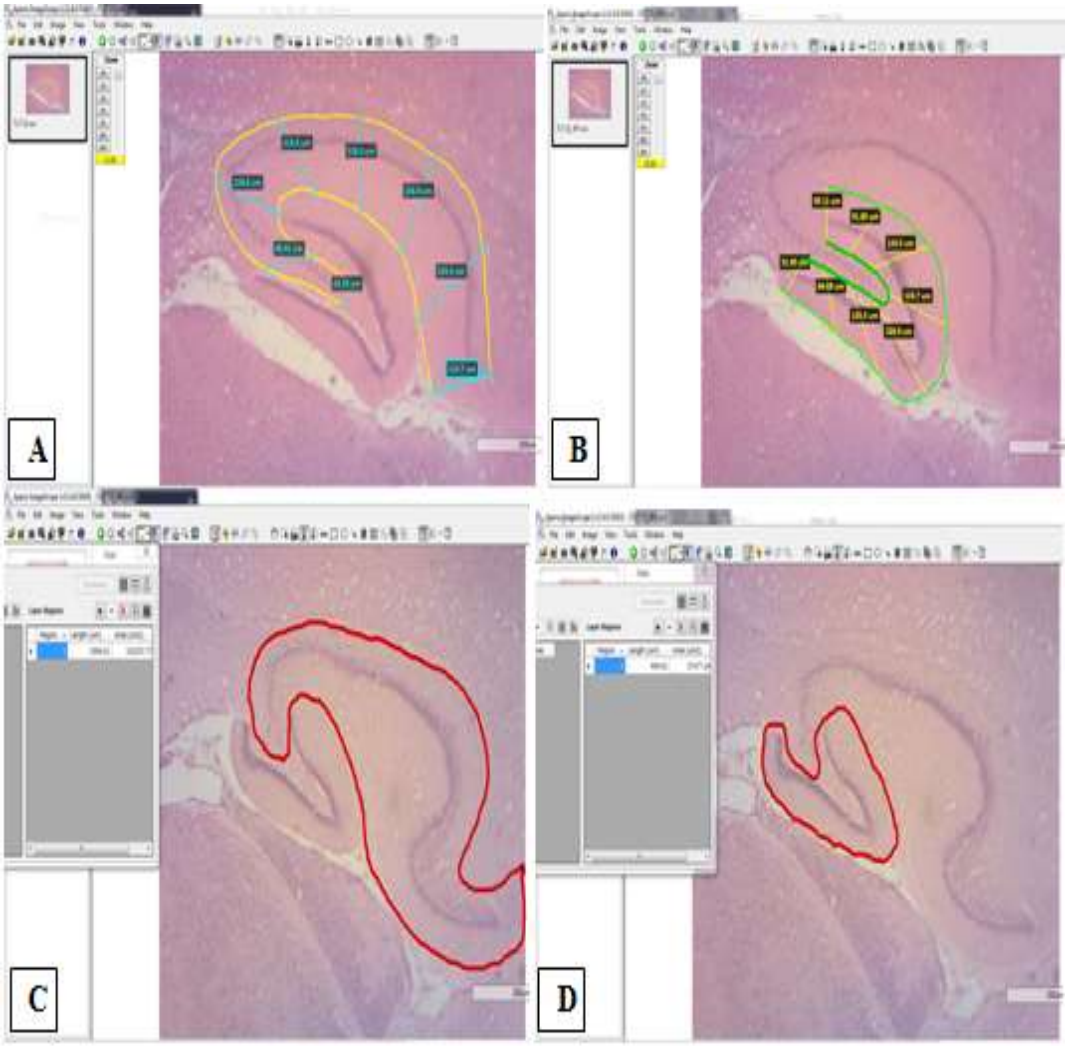


Plate XXI. Estimation of thicknesses and surface areas of Hippocampus and Dentate gyrus

A: An outline of the hippocampus for the estimation of thickness, **B**: An outline of dentate gyrus for the estimation of thickness, **C**: An outline of the hippocampus for the estimation of surface area, **D**: An outline of dentate gyrus for the estimation of surface area.

3.19Limitations

i. Only one type of histology stain (H and E) was used for the study.

ii. Both histology and stereological studies were manually done.

iii. Only one housekeeping gene was used for the gene expression study.

iv. Insufficient fund to use immunohistochemistry stains to demonstrate the expression of the genes and other histological features of developing neuronal cells at every developmental stage.

v. The paraffin wax method of slide processing was used to prepare the slide sections instead of cryosections.

3.20. Statistical Analyses

Data obtained wereexpressed as Mean±SEM. Pearson's Chi-Square test, Student's*t*-test, one – way ANOVA and Repeated Measures Analysis of Variance (Repeated ANOVA) followed by Fisher's exact post-hoc test wascarried out to find the association and compare the mean differences between the experimental groups.*Minitab* (version 17) software was used to analyze the results and $p< 0.05$ was considered statistically significant. All charts and graphs were plotted using *GraphPad prism 8*.

CHAPTER FOUR
4.0. RESULT

The two thousand (2000) gramsof the *D. stramonium*seeds yielded 42 grams (2.10%) of the crude extract. Also,18 grams (0.01%) hyoscyamine fraction obtained from the crude extract and 24.09 µg/dl hyoscyamine was quantified from the fraction.The results of phytochemical screening of the crude *D. stramonium* seeds in table 4.1 showed the presence of alkaloids, carbohydrates, saponins, cardiac glycosides, steroids, triterpenes were all detected except for flavonoidsand tannins which were not detected.

PlateXXII shows two orange spots of the hyoscyamine in the extract and standard powder were qualitativelyidentified using thin-layer chromatography. The thin layer chromatographyshows orange spot colour in both the standard powder (**St**)and a relatively small spot (**Ds**) of the extract on the right. Comparatively, this showed that the chromatogram of the samplewhich resembles that of the standard as regards position, size and colour was truly the hyoscyamine compound in the sample. And therefore, the presence of tropane hyoscyamine alkaloids was identified in the extract while the intensity of the belts suggested that the hyoscyamine was present. The retention factor (rf) value determined were 0.53 and 0.48 for the standard powder and the sample hyoscyamine fraction respectively.

Table 4.1. PhytochemicalScreening ofthe Crude *Daturastramonium* seeds

Test	Result	Confirmation
Alkaloids		
a. *Wagner's test:*	A reddish-brown precipitate was formed	+
b. *Dragendorff's test:*	A reddish-brown precipitate was formed	+
c. *Mayer's test:*	A whitish creamy precipitate was formed	+
Carbohydrates		
a. *Molisch's test:*	A violet ring was formed	+
b. *Fehling's test:*	Abrick-red precipitate was formed	+
Saponins		
a. *Frothing test:*	A thin layer of foam was formed	+
Cardiac glycosides		
a. *Keller – Killiani test:*	A blue colouration was formed	+
Tannins		
a. *Ferric Chloride test:*	A dark green colouration was not formed	-
Flavonoids		
a. *Alkaline reagent test*	A yellow fluorescence was not formed	-
Steroids and Triterpenes		
a. *Liebermann - Bucchard*	A green colouration was formed	+

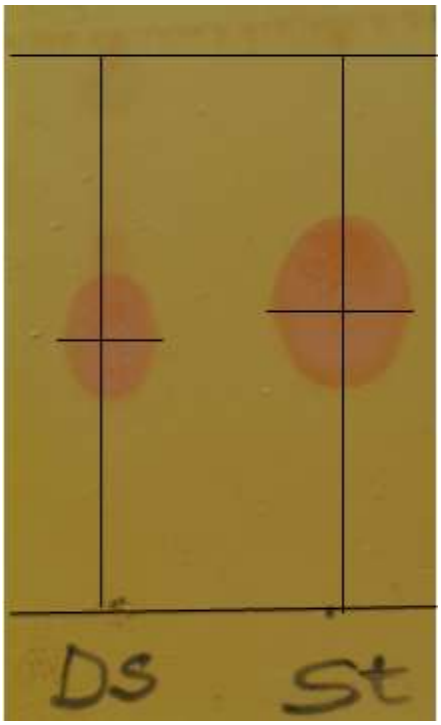


Plate XXII. Thin Layer Chromatogram for Hyoscyamine Fraction and Standard Powder

Table 4.2. shows the graded doses of standard ļ–hyoscyamine powder,peak areas and retention time produced by the HPLC for the quantification of hyoscyamine in the *D. stramonium*seeds. The peak areas increased with an increase in the concentration of the standard powders.

Figure 4.1. showed the standard curve of the peak areas constructedagainst concentration for the calibration of the hyoscyamine obtained in the sample. The equation of a straight line was established, where **x** represents the concentration [µg/ml], while **y** = the area of the peak from the sample. Using the equation, the quantity of hyoscyamine compound in the ethanol extract of *D. stramonium*seeds was calculated at the moment when the area of the sample peak was reached. The peak area of hyoscyamine fraction was taken from the chromatogram of *D. stramonium*seeds extract.

Figure 4.2 shows the overlapped HPLC chromatograms of the standards (hyoscyamine) (A), as well as the chloroform, a fraction (B) from the *D. stramonium*. The retention time for the hyoscyamine standard is 1.66 – 1.98 min. A good correlation of linearity has been achieved (n = 3; R^2 = 0.9995) and, in the range of 1–10 µg/ml for hyoscyamine and a limit of detection (LOD) and quantification (LOQ), 0.75 µg/mland 2.26 µg/ml respectively.Based on the HPLC chromatogram, the hyoscyamine came out from the sample at 1.683 minutes.

Table 4.2.The Quantity of Standard Hyoscyamine Powder Samples and Areas

Quantity (µg/ml)	Peak Areas (mUA*S)	Time/min
16	09.4576	1.664
32	10.1167	1.667
64	11.1797	1.660

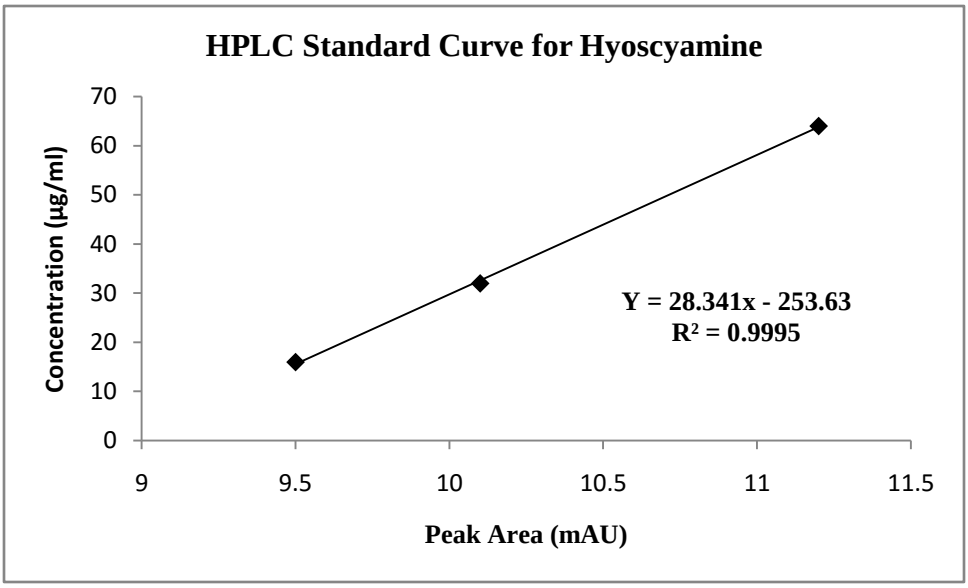


Figure 4.1. Standard Curve of l-Hyoscyamine Powder

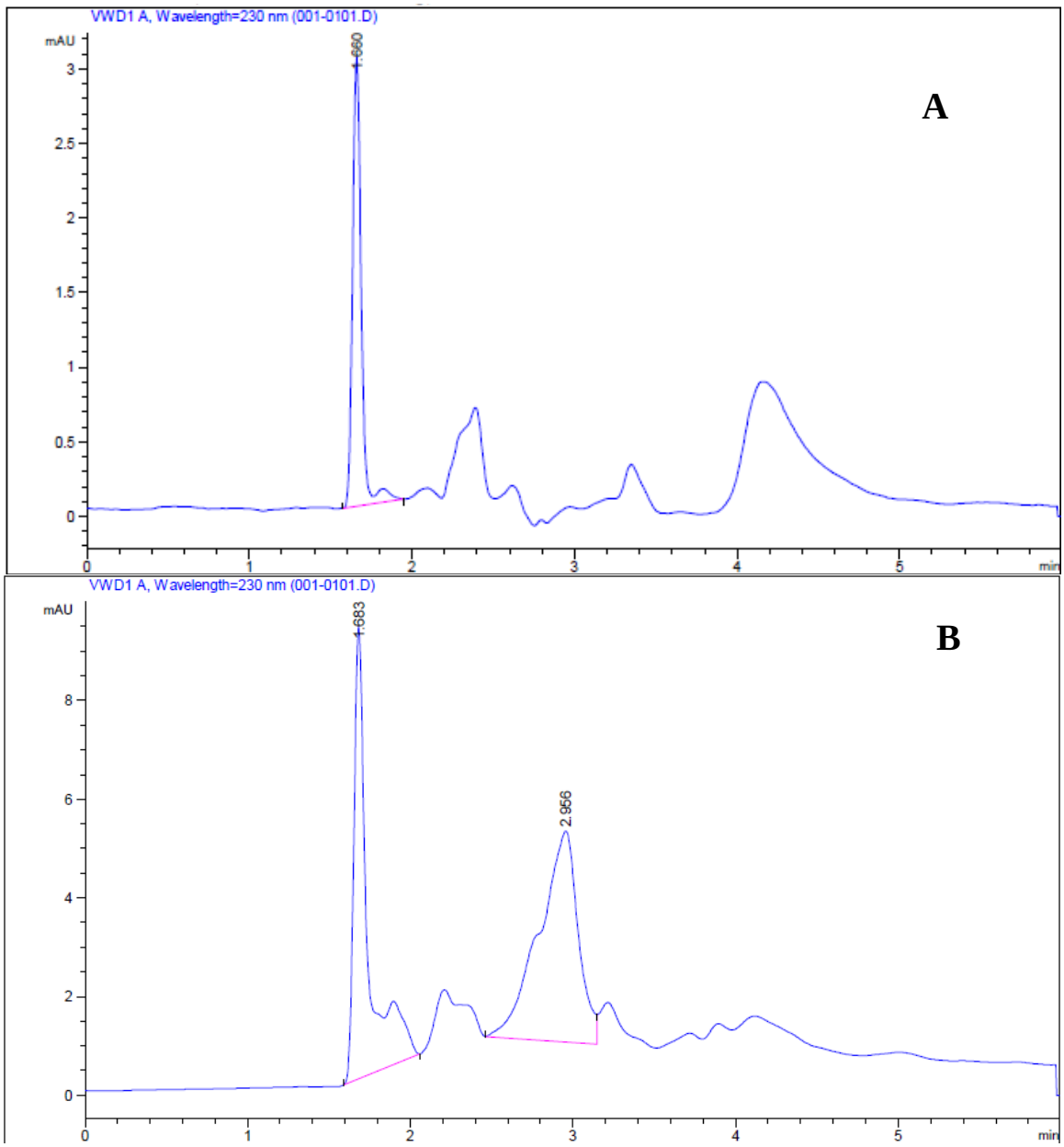


Figure 4.2.HPLC Chromatogram of Hyoscyamine

A: l-hyoscyamine of Standard Powder,**B**: Fraction of *D. stramonium*Seeds.

Table 4.3., shows the quantity of the hyoscyamine fraction of the ethanol extract of *D. stramonium*.The absorption peak for hyoscyamine was at 1.683 minutes. Comparing the result with the absorption peak of standard hyoscyamine shows that the sample was correctly identified. Using the equations from the standard curves of standards solutions and that of the peak area from the extract, the alkaloids quantity in µL of the sample was calculated. Based on this, the concentration of the hyoscyamine alkaloid was calculated in the fraction of the *D. stramonium*seeds. The concentration of the hyoscyamine in the seeds was given in table 4.3. The results of the quantitative analysis showed a significantly little content of hyoscyamine.

Table 4.3. Quantity of Hyoscyamine in the *D. stramonium* seeds Fraction

	Sample (µg)	**Hyoscyamine (µg)**
Purified fraction	8250	11.04
Raw fraction	18000	24.09

4.1. Bodyweight Measurement of Rats

There was a continuous increase in the bodyweight of both the control and prenatally treated rats used in the study (Fig. 4.3). The increased bodyweight continued progressively from the 1stto the 7th week and plateaued between the 7th and 8th weeks with mean bodyweights of 93.28±1.80 and 73.65±1.20 grams at the 7th week between the control and the treated groups respectively. There was no difference in the bodyweight between the groups first two weeks, however, a significant difference ($p < 0.001$) was observed from the 3rd week (PND21) to the end of the study (PND85).This was steeply maintained to the end of the experiment.

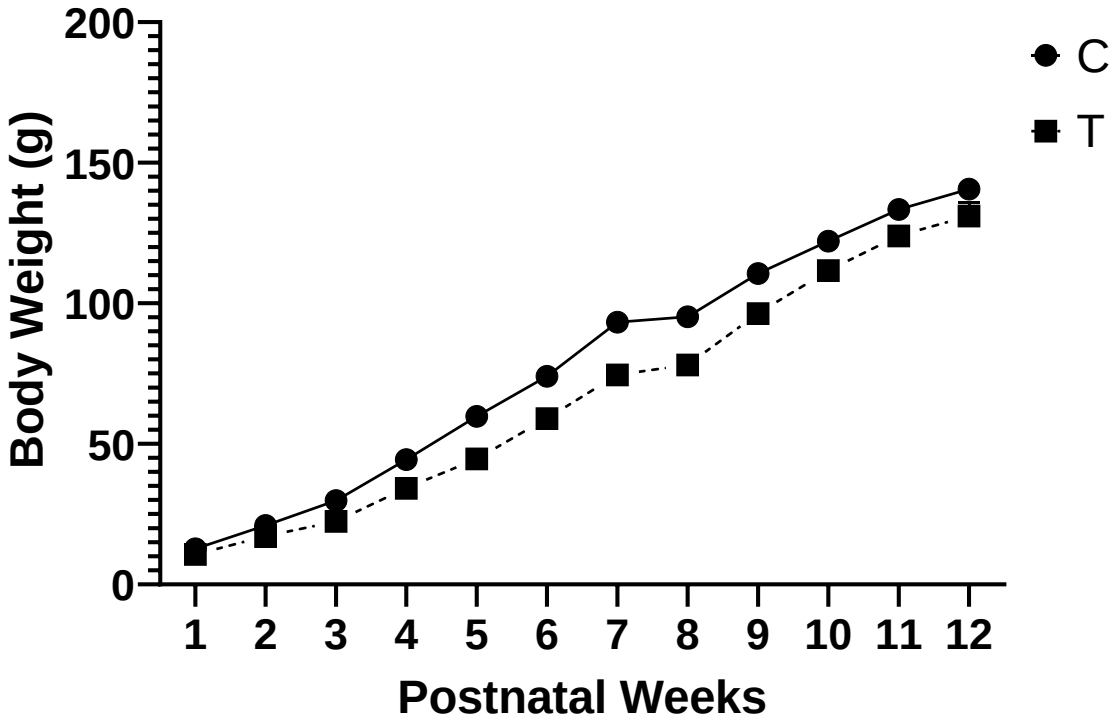


Figure 4.3. Bodyweight Patterns in Wistar rats treated with 200 mg/kg hyoscyamine fraction of *D. stramonium* seeds in gestation

4.2. Neurobehavioural Studies

Figure 4.4 shows the results of comparisons tests between motor coordination and prenatal exposure to hyoscyamine fraction of *D. stramonium* seeds in adulthood. There was no statistically significant difference (p =0.642) in the motor coordination ability between the control and treated group.

The result in figure 4.5 shows results of a comparison test of triple horizontal bar test between the control and treated groups of Wistar rats treated hyoscyamine fraction of *D. stramonium*during lactation. The control group indicated a higher level of motor coordination, however, no statistically significant difference (p =0.138) in the motor coordination ability between the groups.

Figure 4.6 shows results of comparisons in horizontal bar test in adolescent Wistar rats treated with hyoscyamine fraction of *D. stramonium* seeds. The control group indicated a higher level of motor coordination ability compare to the treated which was statistically significant (p <0.05) between them.

Figure 4.7 shows the results of the comparisons test in triple horizontal bars test in Wistar rats treated with graded doses of hyoscyamine fraction of *D. stramonium*seeds. There was no statistically significant difference between the treated groups in the neuromotor coordination ability [F (3, 20) = 2.55, p = 0.085]. However, the Fisher Pair-wise comparisonspost-hocanalysis observed significant ability (p = 0.002) in the coordination ability in the group treated with 400 mg/kgcompared to the control.

4.2.1. Triple Horizontal Bar Test

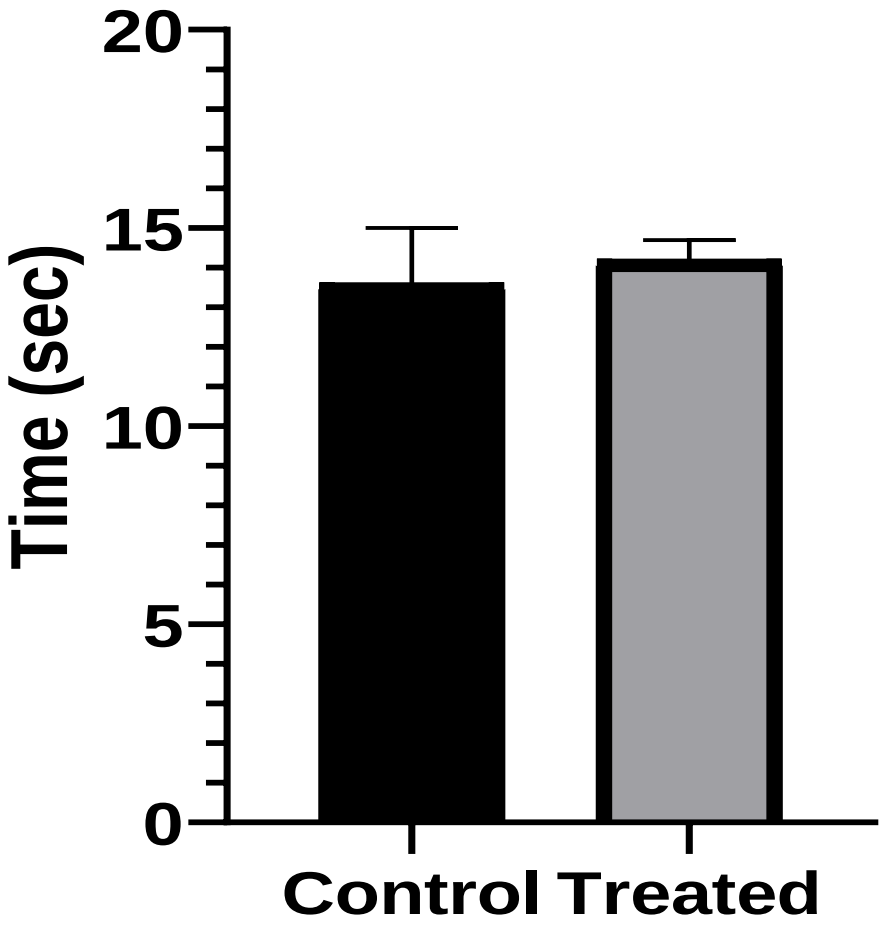


Figure 4.4. Triple Horizontal Bar test in Wistar rats treated with 200 mg/kg hyoscyamine fraction of *D. stramonium* seeds in gestation

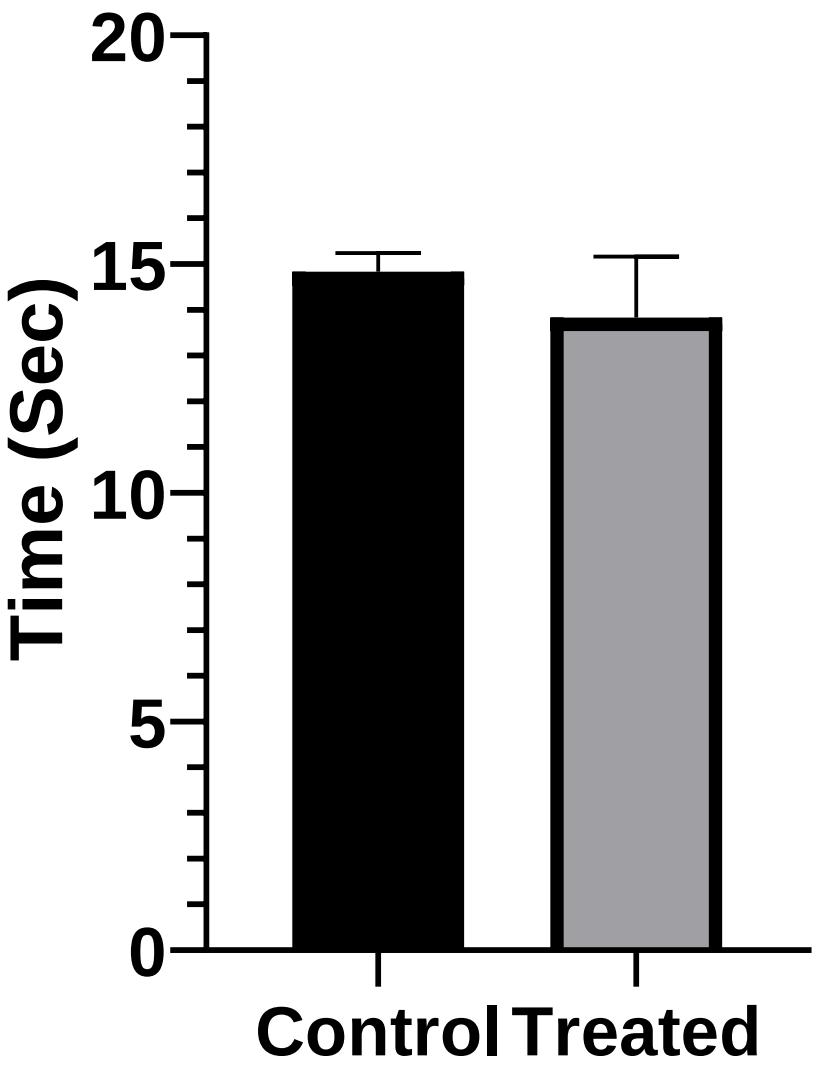


Figure 4.5. Triple Horizontal Bar test in Wistar rats treated with 400 mg/kg hyoscyamine fraction of *D. stramonium* seeds in lactation

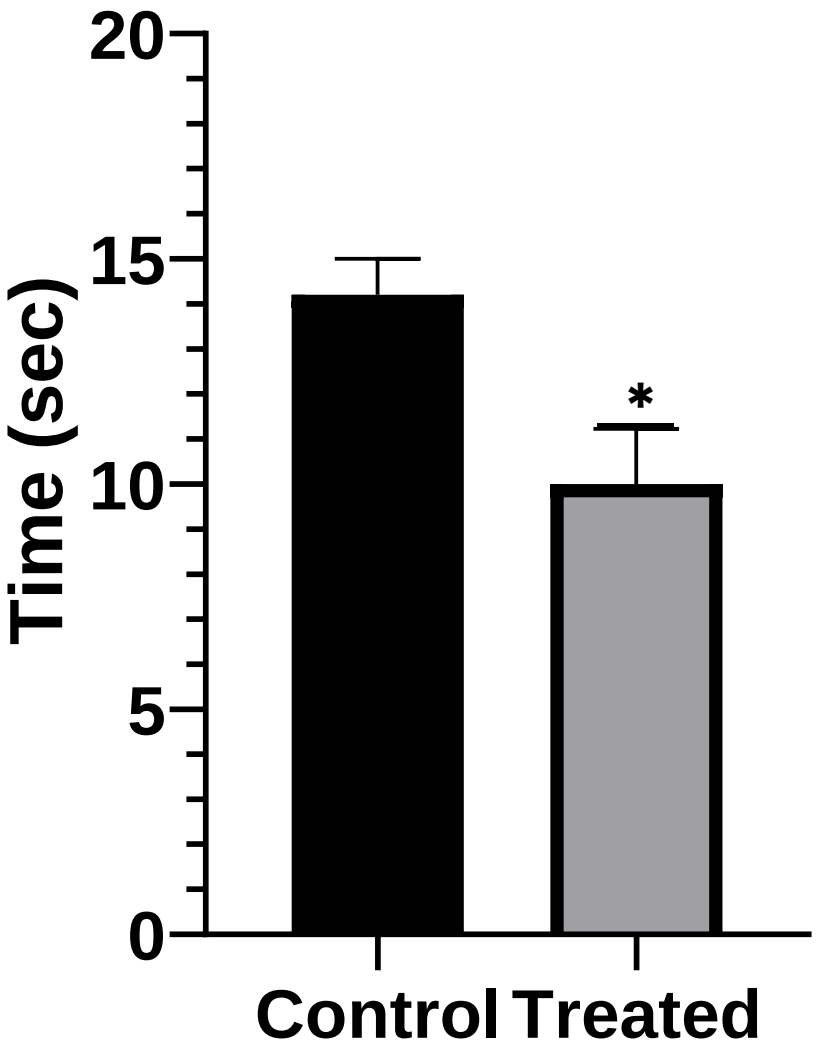


Figure 4.6. Triple Horizontal Bar test in Wistar rats treated with 800 mg/kg hyoscyamine fraction of *D. stramonium* seedsinAdolescence

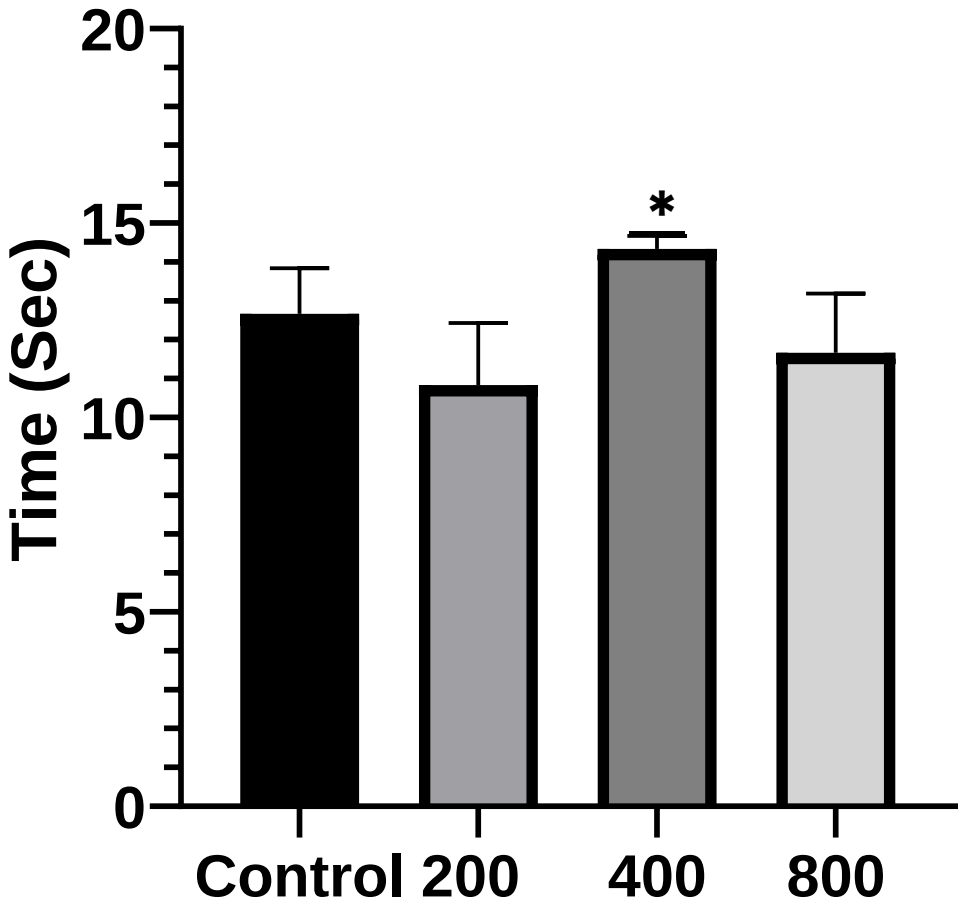


Figure 4.7. Triple Horizontal Bar test in adultWistar rats treated with 200, 400 and 800 mg/kg hyoscyamine fraction of *D. stramonium* seeds in adulthood.

4.2.2. Novel Object Recognition Test (NORT)

Figure 4.8 shows a percentage exploration time in the sampling phase of the novel object recognition test (NORT) using two identical objects (A1 and A2) between the controls and prenatally exposed, 200 mg/kg hyoscyamine fraction treated group. There was no statistically significant difference ($p > 0.05$) in the exploration time.

The result in figure 4.9 shows a percentage exploration time in the sampling phase of the novel objects recognition test (NORT) using two identical objects (A1 and A2) between the controls and lactational exposed, 400 mg/kg hyoscyamine fraction groups. There was no statistically significant difference ($p < 0.05$) in the exploration time within the two groups.

The percentage exploration time of sampling phase in novel objects recognition test (NORT) using two identical objects (A1 and A2) between the controls and adolescent, 800 mg/kg hyoscyamine treated groups was shown in figure 4.10. There was no statistically significant difference ($p > 0.05$) in the exploration time between the two groupsfor the object's explorations.

Figure 4.11 shows the percentage exploration time in the sampling phase of the novel objects recognition test (NORT) using two identical objects (A1 and A2) between the control, and adult Wistar rats treated with 200, 400 and 800 mg/kg hyoscyamine treated groups. There was no statistically significant difference ($p >0.05$) in the exploration time concerning objects exploration. Any rat that scored less than or above 20 or 80% respectively was excluded for the testphase of the experiment.

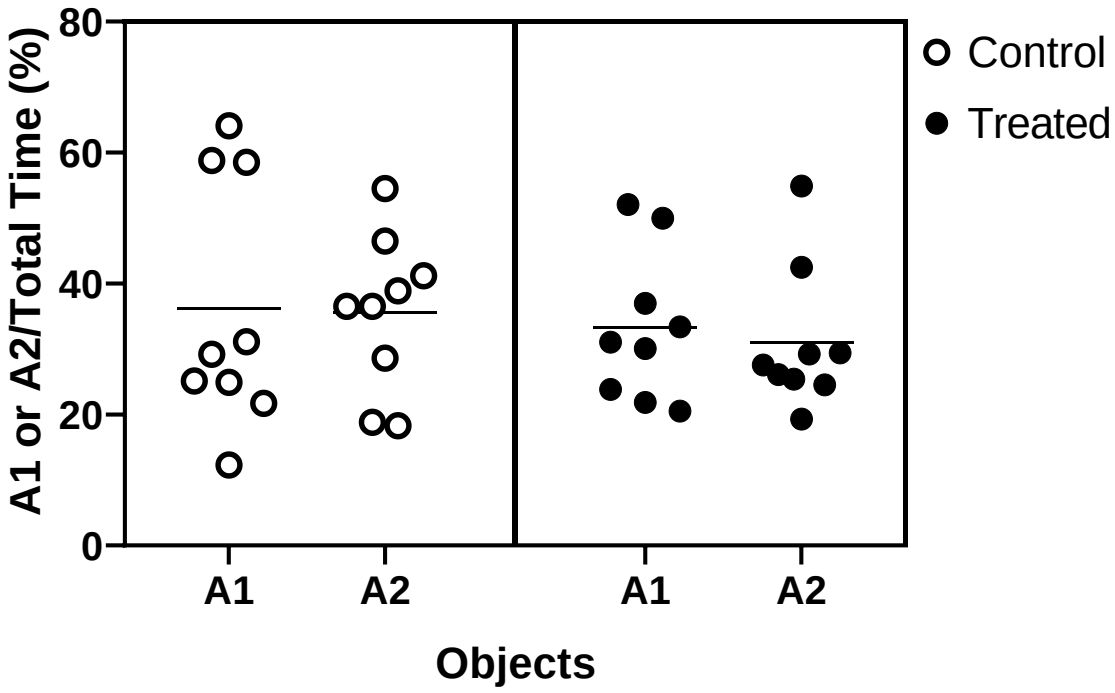


Figure 4.8. Sampling Phase of Novel Object Recognition Test in Wistar Rats treated in Gestation

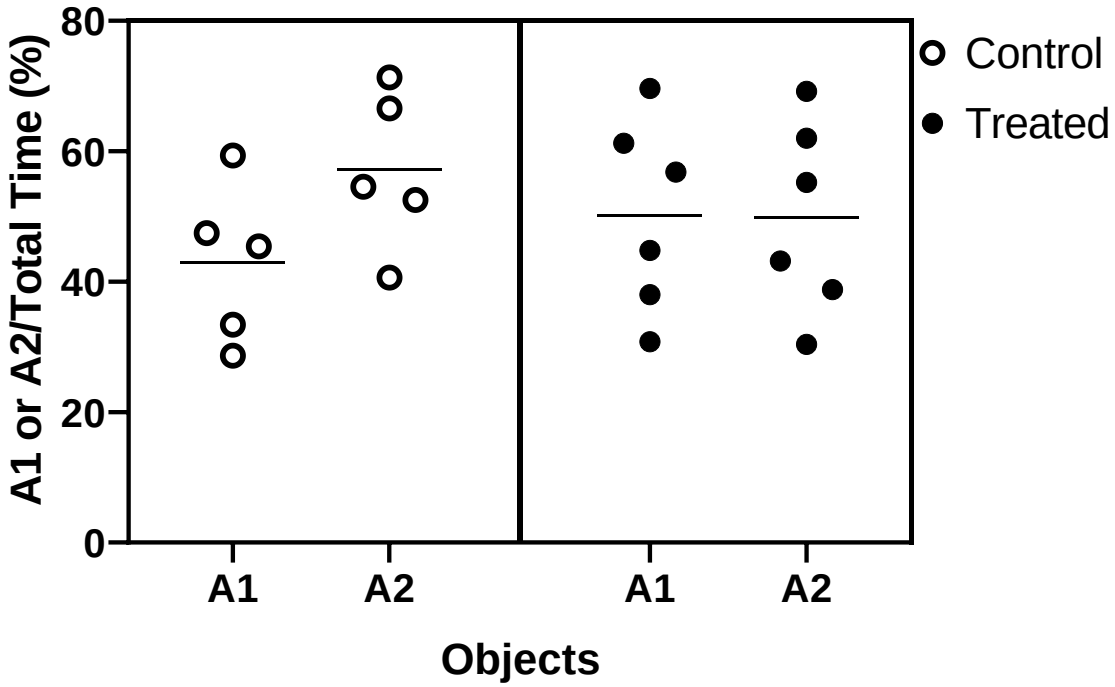


Figure 4. 9. Sampling phase of Novel Object Recognition TestinLactation

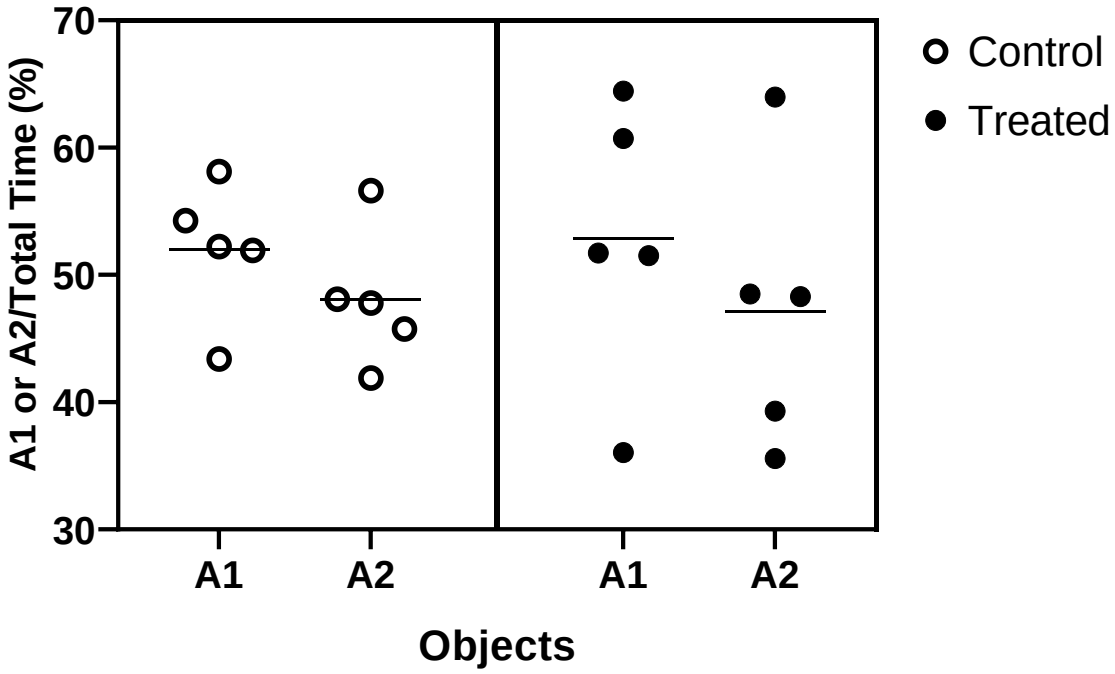


Figure 4.10. Sampling phase of Novel Object Recognitionof Wistar Rats Treated in Adolescence

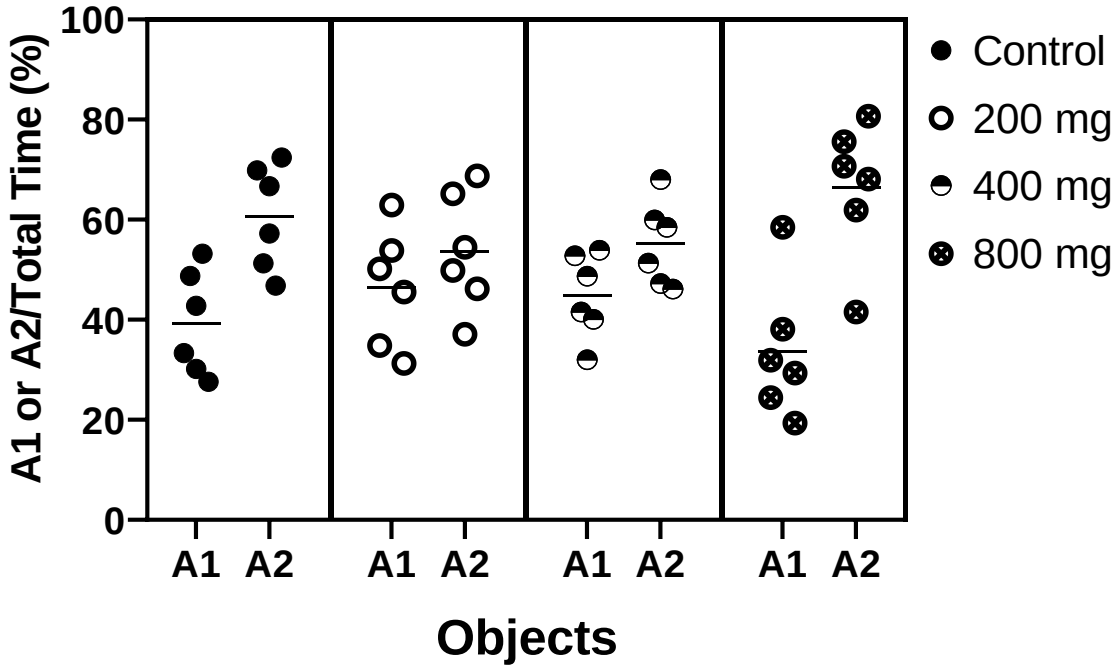


Figure 4.11. Sampling Phase of Novel Object Recognition Test in Adult Wistar RatsTreated in Adulthood

Figure 4.12 shows the resultof the testphase in a novel object recognition test experiment between the control and prenatally treated hyoscyamine fraction groups using two non-identical objects, one previously known (familiar A) and other unknown (non-familiar B). The control group showed a significant delay in exploring the non-familiar (B) object (p = 0.012) compared to the familiar object (A). On the other hand, no statistically significant difference (p = 0.325) was observed in the time took to explore the familiar (A) and non-familiar (B) objects in the treated groups.

The result shows a testphase in the novel object recognition test experiment between the control and Wistar rats treated with 400 mg/kg hyoscyamine fractionof *D. stramonium*groups using two non-identical objects; one previously known (familiar A) and previously unknown (non-familiar B) was shown in figure 4.13. The control group spent a significant (p = 0.049) amount of time exploring the non-familiar object (B) compared to the familiar (A), whereas the treated group, showed no statistically significant difference (p = 0.238) in the time taken to explore known (A) and unknown (B) objects.

In figure 4.14 was the result shows the test-phase in novel object recognition test experiment between the control and adolescent Wistar rats treated with 800 mg/kg hyoscyamine fractionof *D. stramonium*seeds groups using two non-identical objects: A which was previously known (familiar A) and unknown (non-familiar B) which was previously unknown. The control group spent a significant amount of time exploring the non-familiar (B) object (p = 0.047) than the time taken to explore the familiar (A) one.

However, the treated group showed no statistically significant difference (p = 0.845) in the exploration time between the objects.

Figure 4.15 shows results of comparisonsof the test-phase Wistar rats treated with graded doses of hyoscyamine fraction of *D. stramonium* seeds using two non-identical objects, familiar object (A) and non-familiar object (B) in adulthood. There was significant difference in the exploration time between the group [F (3, 40) = 9.80, p = 0.003]. The control group showed a statistically significant time (p = 0.031) exploring the unknown object (B) compared to the known object (A). The exploration time between the two objects for all the treated groups was, however, not significant (p > 0.05). For the low dose treated group p = 0.191, medium dose, p = 0.406 and for the high dose, p = 0.071 respectively.

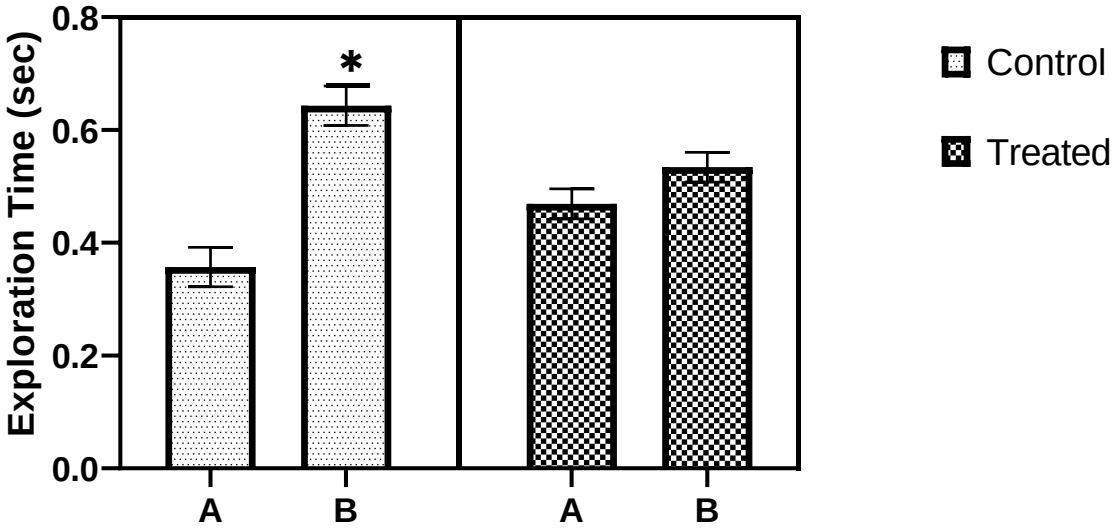


Figure 4.12. The Test Phase of Novel Object Recognition in Wistar Rats Treated 200 mg/kg hyoscyamine fraction of *D. stramonium* seeds in gestation

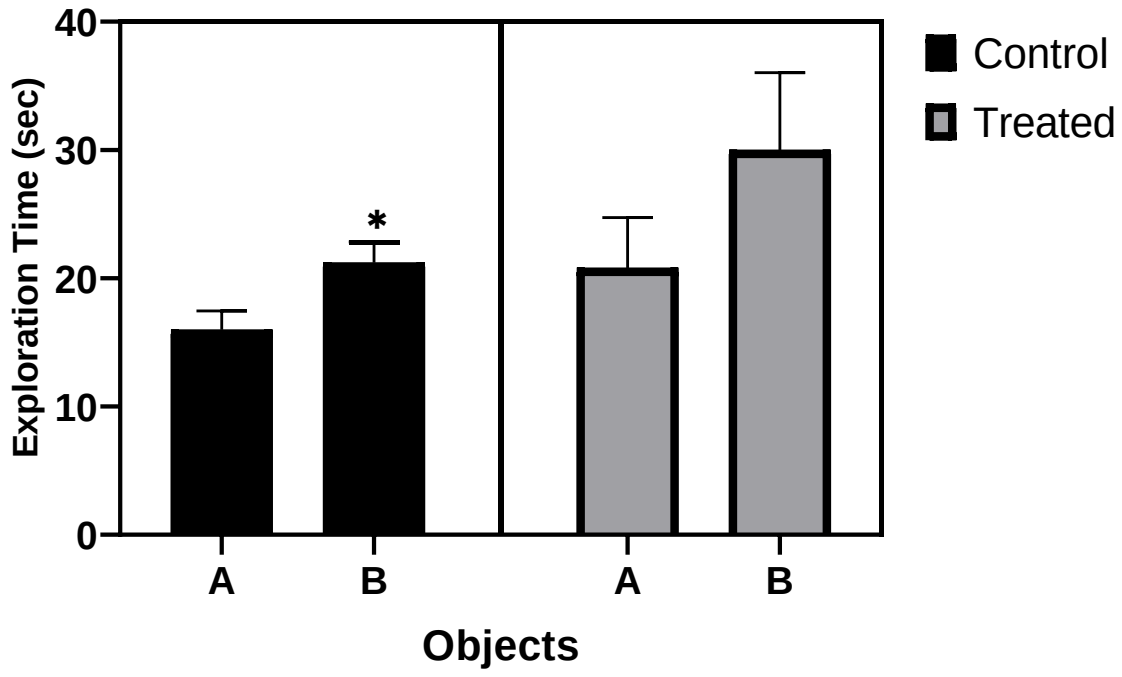


Figure 4.13. The Test Phase of Novel Object Recognition in Wistar Rats treated with 400 mg/kg hyoscyamine fraction of *D. stramonium* seedsinlactation

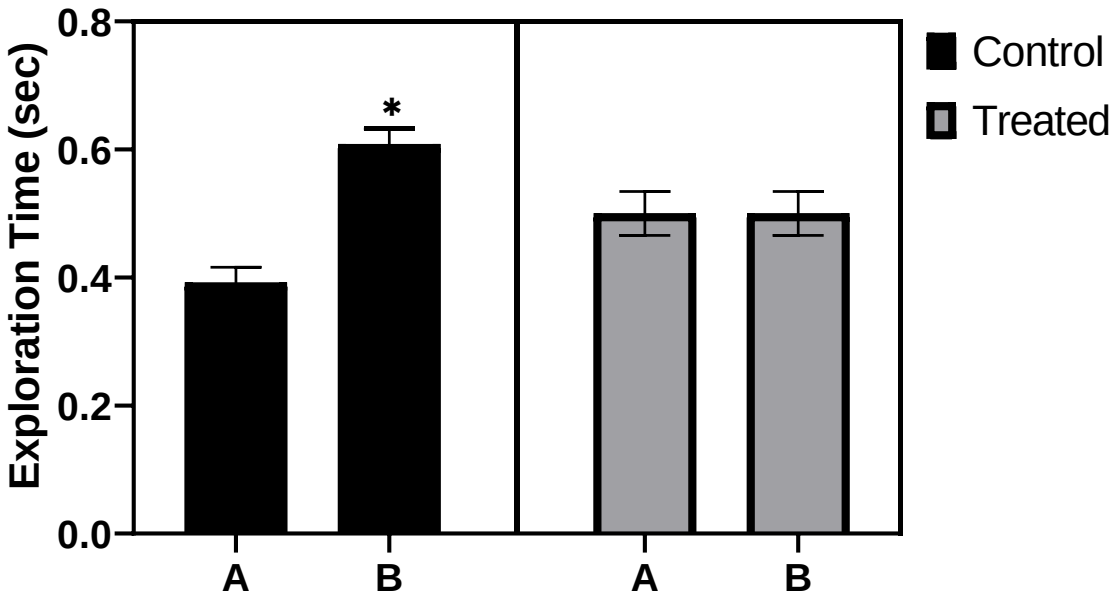


Figure 4.14. The Test Phase of Novel Object Recognitionof Wistar rats treated with 800 mg/kg hyoscyamine fraction of *D. stramonium* seedsinadolescence

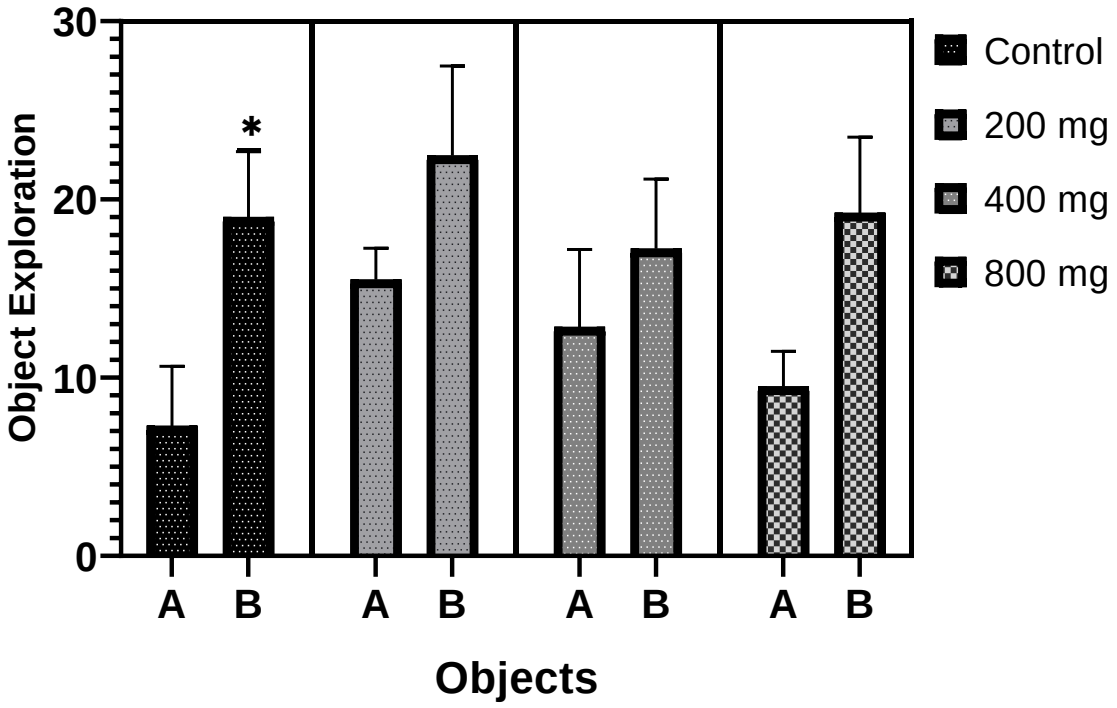


Figure 4.15. The Test Phase of Novel Object Recognitionin adult Wistar rats treated with 200, 400 and 800 mg/kg hyoscyamine fraction of *D. stramonium* seedsin adulthood

Figure 4.16. shows the results of comparisons test for discrimination index (DI) in Wistar rats prenatally exposed to hyoscyamine fraction*D. stramonium* seeds.The treated groups showed a significant decrease in discrimination ability when compared to the control (p = 0.024).

Figure 4.17 showscomparisons test for discrimination index in Wistar rats exposed to hyoscyamine fractionof *D. stramonium*seeds through lactation. No statistically significant difference (p >0.05) in the discrimination ability between the groups was observed.

Figure 4.18 presents the comparisons test resultsof novel object discrimination indexin Wistar rats treated with hyoscyamine fractionof *D. stramonium*seeds in adolescence. The control group showed a statistically significant difference (p = 0.023) in the discrimination ability when compared to treated groups.

Figure 4.19 presents the comparisons test result for novel object discrimination index in adult Wistar rats treated with hyoscyamine fraction of *D. stramonium* seeds.No significant difference [F (3, 20) = 2.01, p = 0.144] in the novel object discrimination ability between treated groups. However, the post-hoc test showed that the control group discriminate against the novel object significantly (p = 0.030) better when compared to the treated group. The inability to discriminate between the familiar and non–familiar objects of the treated groups increased in a dose-dependent manner.

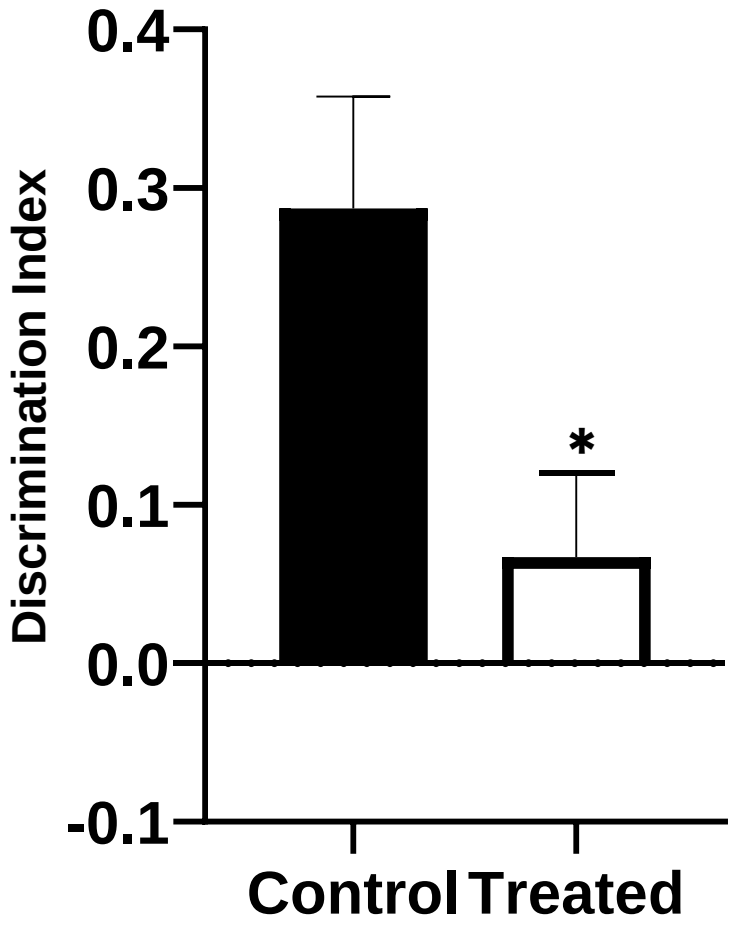


Figure 4.16. Discrimination Index of Novel Object Recognition Test in Wistar rats treated with 200 mg/kg hyoscyamine fraction of *D. stramonium* seedsin Gestation

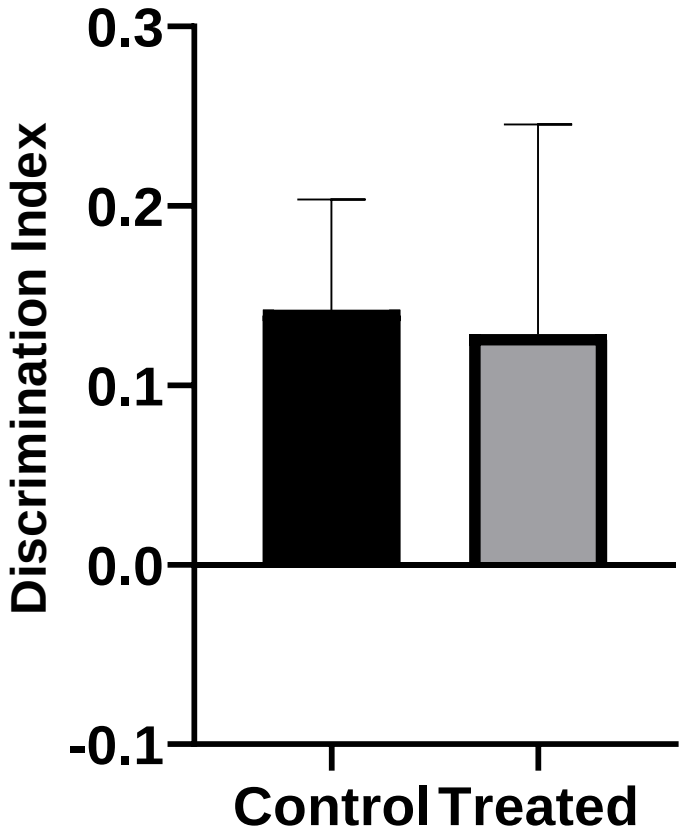


Figure 4.17. Discrimination Index of Novel Object Recognition Test inWistar ratstreated with 400 mg/kg hyoscyamine fraction of *D. stramonium* seedsinLactation.

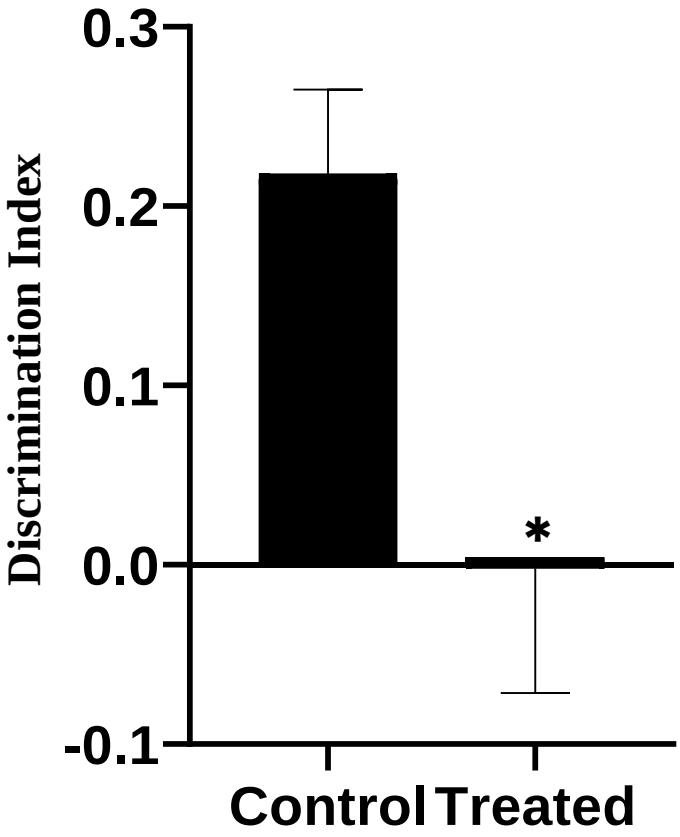


Figure 4.18. Discrimination Index of Novel Object Recognition test in Wistar rats treated with 800 mg/kg hyoscyamine fraction of *D. stramonium* seedsin adolescence

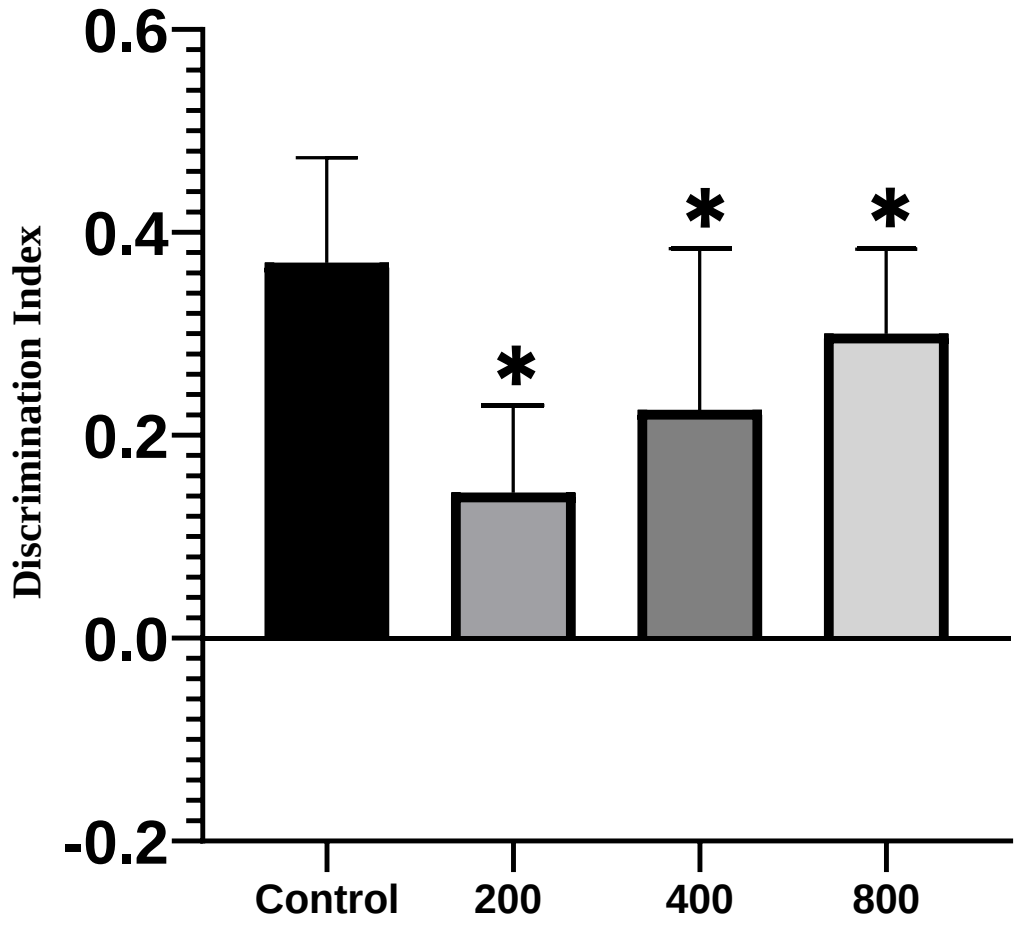


Figure 4.19. Discrimination Index of Novel Object Recognition Test in adult Wistar rats treated with 200, 400 and 800 mg/kg hyoscyamine fraction of *D. stramonium* seeds in adulthood.

Figure 4.20 shows the compared results ofthe novelty preference in Wistar rats prenatally exposed to hyoscyamine fraction of *D. stramonium*seeds and control group. A significant (p = 0.022) difference in the percentage novelty preference was observed between the groups. The control group preferred the novel object compared to the treated group.

In figure 4.21 presents the result of comparisons for the novelty preference in Wistar rats exposed to hyoscyamine of *D. stramonium*seeds through lactation. Although the percentage novelty preference was slightly higher in the treated group, no statistically significant (p = 0.411) difference in the preference for the novel object between the two groups was observed. Thus, the control and the treatment had an equal preference to the novel object.

Figure 4.22 shows comparisons of novelty preference in Wistar rats treated with hyoscyamine fraction *D. stramonium* seeds in adolescence. A higher percentage of the novelty preference which was statistically significant (p = 0.023), was observed in the control group compared to the treated group. The control groups showed more affinity to the novel object than the treated group.

Figure 4.23 presents the result of the comparisonsof percentage novelty preference in Wistar rats treated with graded doses of hyoscyamine fractionof *D. stramonium* seeds in adulthood. There was no significant difference [F (3, 20) = 2.01, p = 0.144] in the novel object preference between the treated group, however,a significant decrease in the novelty preference (p = 0.030) was observed when compared to the control group. The

novelty preference increased in a dose-dependent manner in the treated groups though not statistically significant ($p > 0.05$).

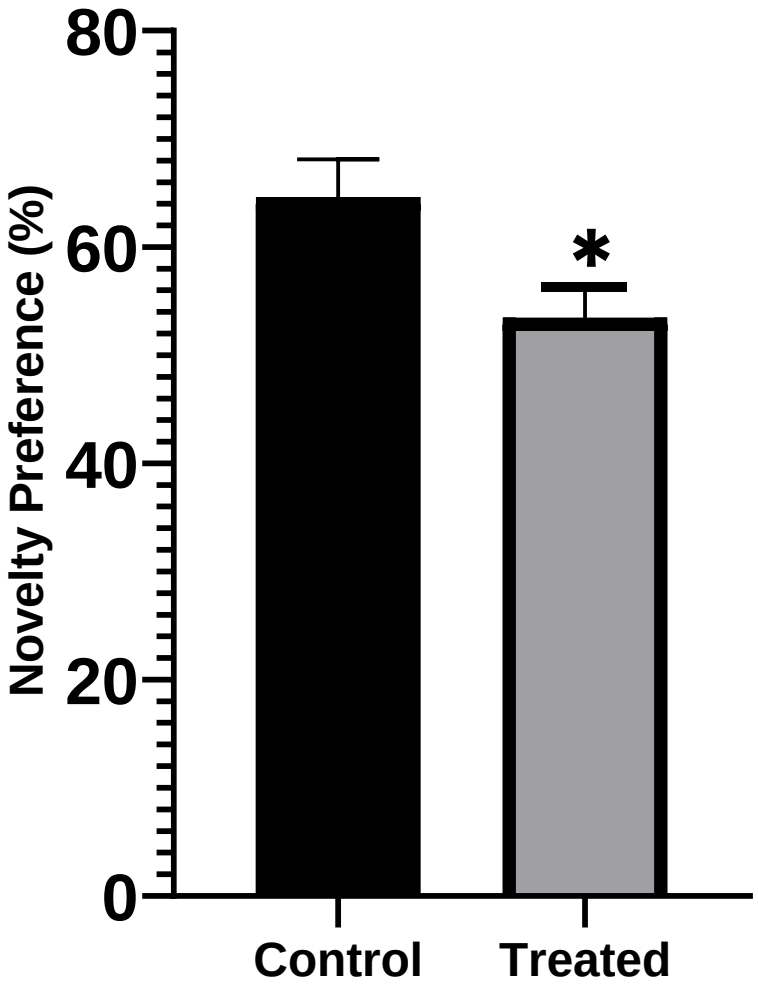


Figure 4.20. Novelty Preference in Wistar rats treated with 200 mg/kg hyoscyamine fraction of *D. stramonium* seedsin gestation.

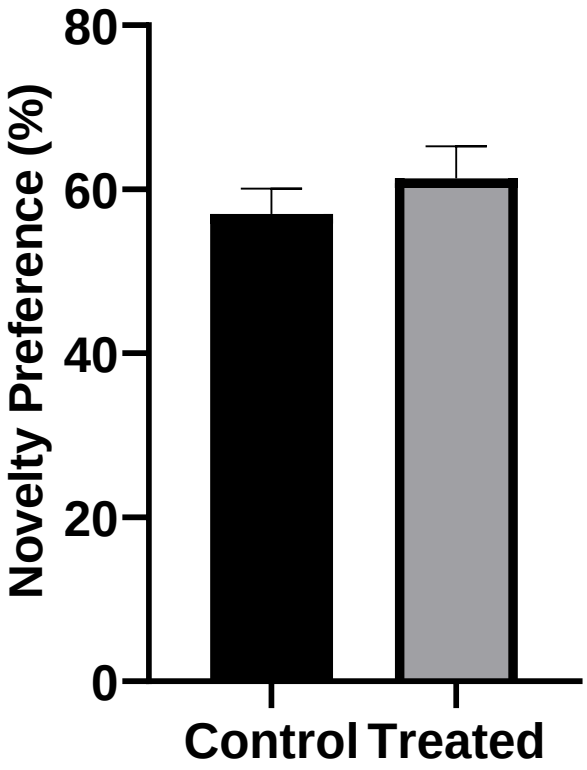


Figure 4.21. Novelty Preference in Wistar rats treated with 400 mg/kg hyoscyamine fraction of *D. stramonium* seedsinLactation

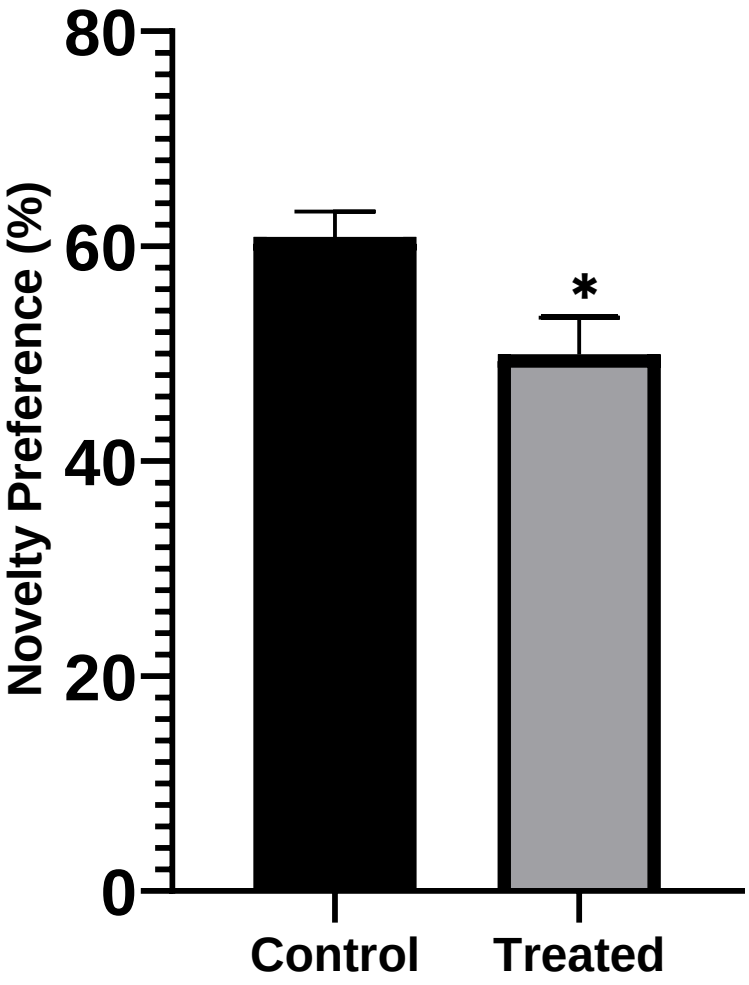


Figure 4.22. Novelty Preference Wistar rats treated with 800 mg/kg hyoscyamine fraction of *D. stramonium* seedsinadolescence

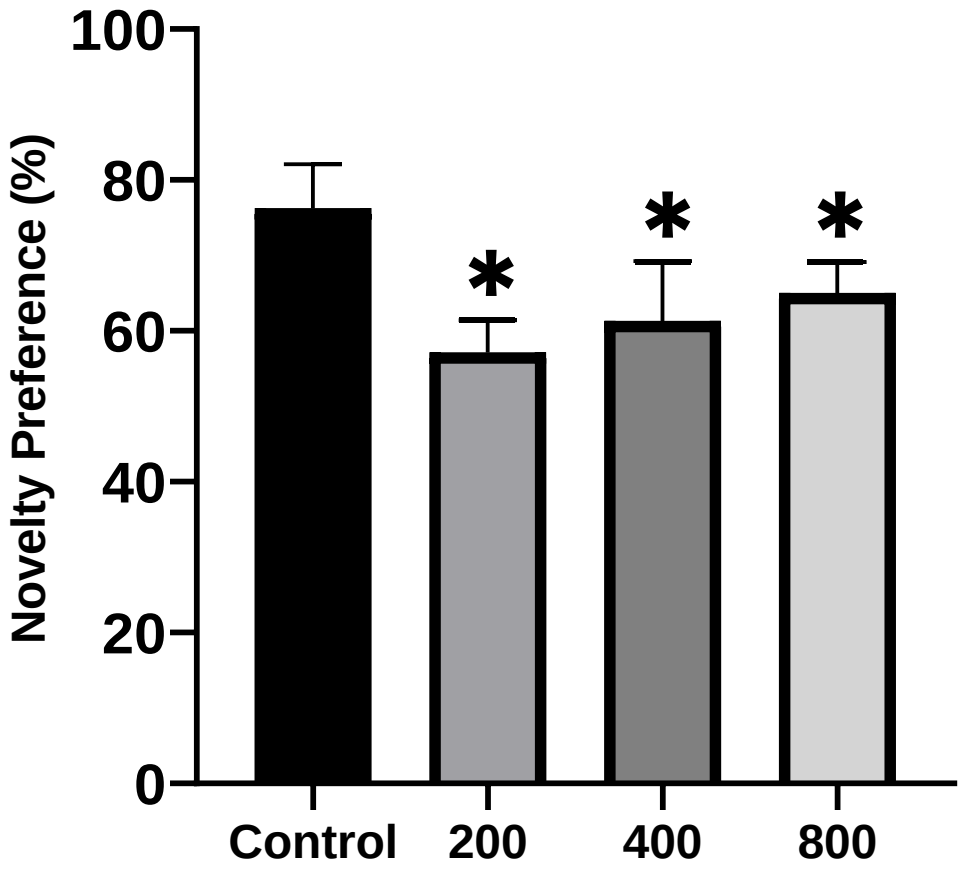


Figure 4.23. Novelty Preference in Wistar rats treated with 200, 400 and 800 mg/kg hyoscyamine fraction of *D. stramonium* seedsinadulthood

4.2.3. Morris Water Maze

Figure 4.24 shows the comparisons for spatial learning and memory test by days and prenatal exposure to hyoscyamine fractionof *D. stramonium* seeds in Wistar rats. There was statistically significant difference in the learning ability within the days [F (5, 120) = 18.59, $p< 0.001$] and between the groups and days [F (5, 120) = 18.59, $p< 0.047$]. The post-hoc test, however, shows that during the first day of the five days training, no significant difference was observed in the escape latency between the groups ($p = 0.437$). Although the escape latency was slightly shorter in the control compared to the treated group on the second day, there was still no statistically significant ($p = 0.086$) difference observed. From the third through the fifth day the escape latency also showed some levels of persistently however no statistically significant ($p > 0.05$) difference between the groups, as they consistently had virtually equal escape latencies. On the sixth day (test day), however, higher escape latency time was observed in the treated group compared to the control which was statistically significant ($p = 0.001$) between the groups.

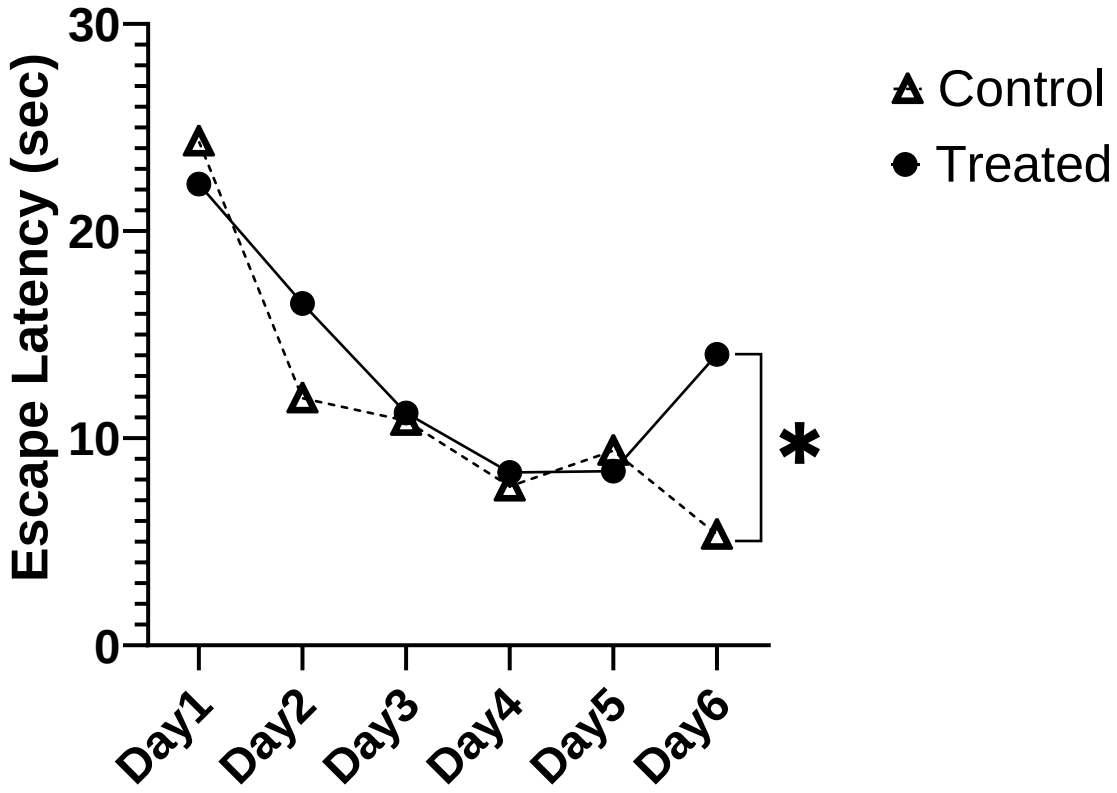


Figure 4.24. Morris Water Maze test in Wistar rats treated with 200 mg/kg hyoscyamine fraction of *D. stramonium* seeds in gestation

Figure 4.25 shows the comparisons for spatial learning and memory test by days and lactational exposure to hyoscyamine fraction of *D. stramonium* seeds in Wistar rats. No significant differences between groups [F (1, 54) = 0.02, p = 0.875] or between groups and days [F (5, 54) = 0.64, p = 0.670] were observed. The post-hoc test further revealed that although in the first and third training days the treated groups showed lesser escape latencies when compared to the controls, there was however no statistically significant (p > 0.05) differences in the spatial learning when compared to the control. Similar observations were observed in the control on the second and fifth training days of training (p > 0.05). On the fourth training day, the two groups almost had equal escape latency times which were not significant statistically (p = 0.799). On the sixth day (probe), the escape latency time was shorter in the treated compared to the control group but not significant statistically (p = 0.626).

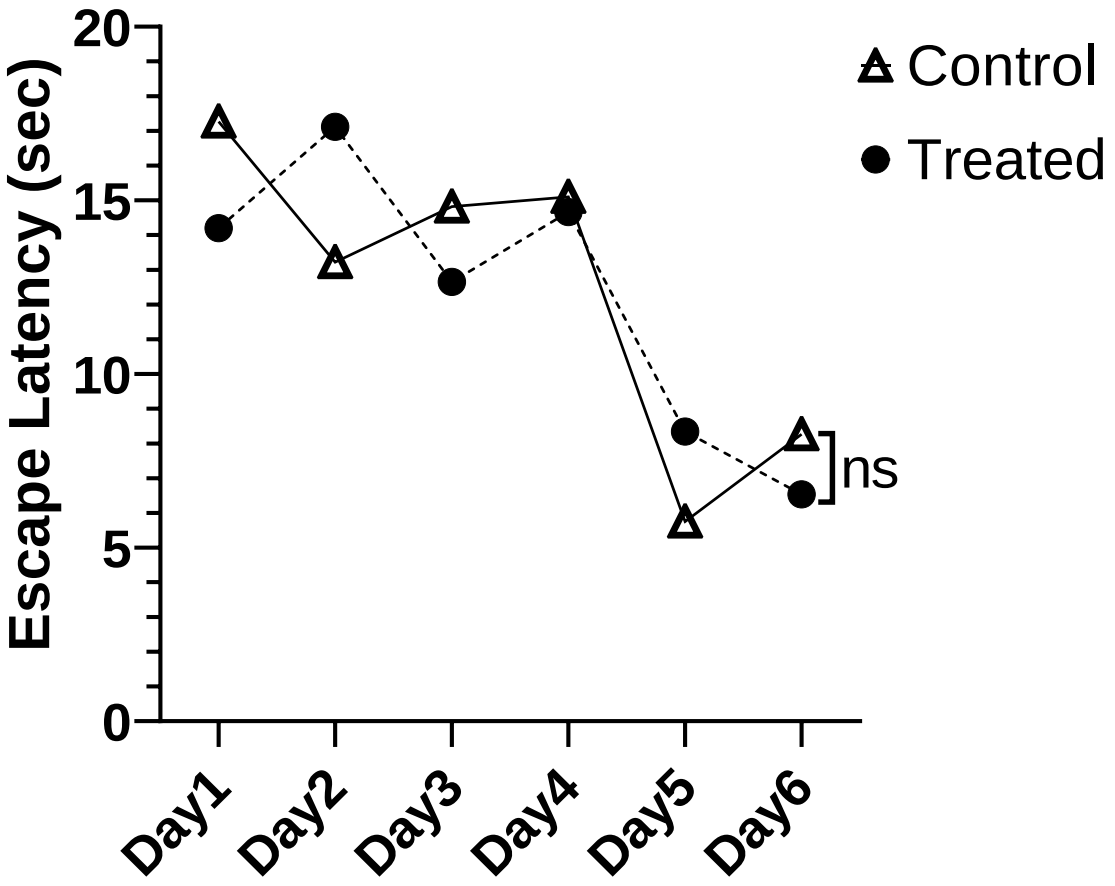


Figure 4.25. Morris Water Maze test in Wistar rats treated with 400 mg/kg hyoscyamine fraction of *D. stramonium* seeds inlactation

Figure 4.26 shows the comparisons for spatial learning and memory test by days and oral ingestion of hyoscyamine fraction of *D. stramonium* seeds in Wistar rats during adolescence. There was no statistically significant difference between the groups [F (1, 48) = 0.49, p = 0.487] or between the groups and days [F (5, 48) = 0.62, p = 0.685]. During the first and fourth days of the training, the two groups virtually had equal escape latency time with no statistically significant value (p = 0.713). In the first and third training days, the treated groups showed lesser escape latency times when compared to their respective controls on the same days and still not significant statistically ($p > 0.05$). Similar observations were made in the second and fifth days of the escape latencies ($p > 0.05$) although the control group had a shorter time. In the third and sixth day (probe) days, the treated groups spent lesser escape latencies which were not significant ($p > 0.05$) in comparison to their respective controls.

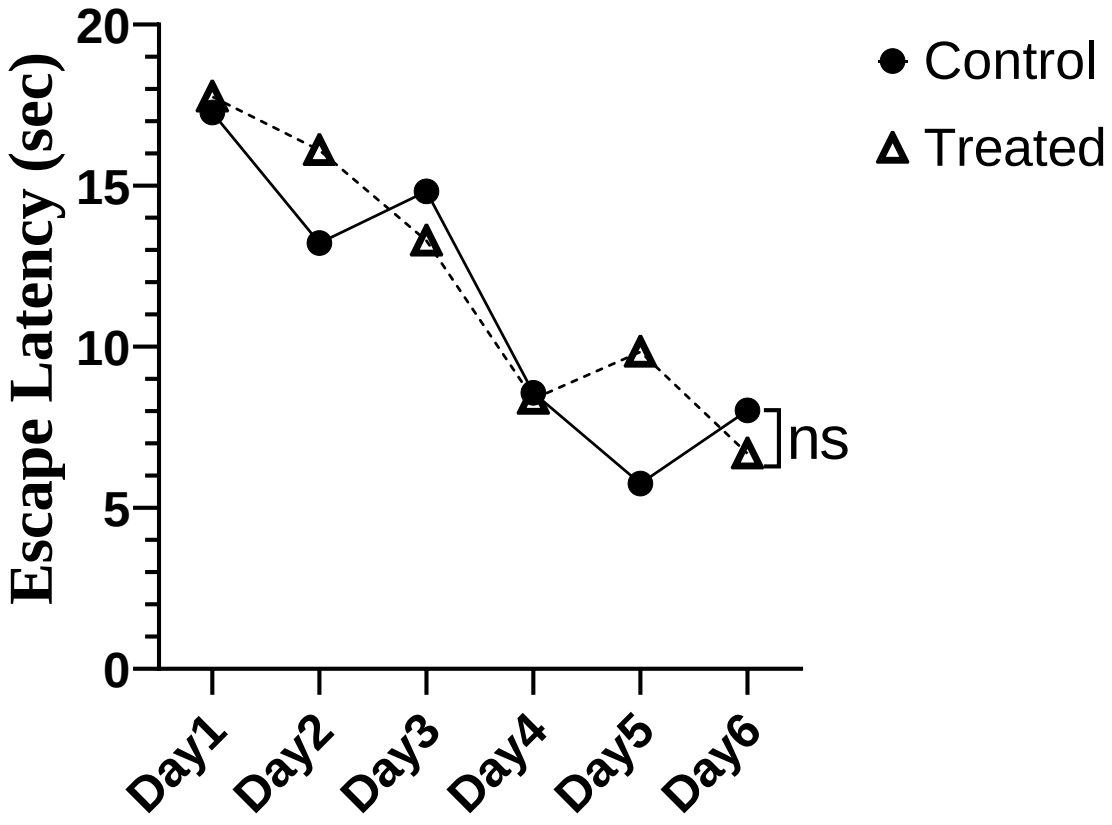


Figure 4.26. Morris Water Maze test in Wistar rats treated with 800 mg/kg hyoscyamine fraction of *D. stramonium* seedsinadolescence

Figure 4.27 shows the comparisons for spatial learning and memory test by days and oral ingestion of graded hyoscyamine fraction of *D. stramonium* seeds in adult Wistar rats. There was statistically significant difference between the groups [F (3, 120) = 3.01, p = 0.033] and between days [5 (5, 120) = 14.80, p< 0.001] for the group treated with 200 mg/kg. The post-hoc test shows that in the first three days of the five days training, control groups showed a consistent shorter duration of escape latencies which were statistically not significant (p > 0.05) when compared tothe treated groups in the same days. Conversely, the escape latencies of the fourth and fifth days among the treated groups were slightly shorter however, not significant statistically (p > 0.05) when compared to their respective controls. On the sixth day which was the test – day, the escape latency of the control was significantly lower statistically (p = 0.008) when compared to the control.

For the group treated with 400 mg/kg also, a statistically significant difference between the groups [F (3, 120) = 3.01, p = 0.033] and days [5 (5, 120) = 14.80, p< 0.001] was observed. There was a consistent flow pattern of escape latencies between the two groups with the controls taking the leads in the first five training days. Although the difference in the latency time was prominent on the fourth day it is not statistically significant (p = 0.628) between the groups. It took the treated group a longer duration in the sixth day to arrive at the platform when compared to the control, hence statistically significant (p = 0.023).

Finally, the figure also showed the comparisons of 800 mg/kg hyoscyamine fraction treated adult Wistar rats. There was statistically significant difference between the treated group [F (3, 120) = 3.01, p = 0.033] andbetween days [5 (5, 120) = 14.80, p< 0.001]. the control group took lesser escape latency time during the first and third training days compared to the control. Although the difference in the escape latency time was prominent on the third day, however, no statistically significant difference (p> 0.05) was observed between the groups. The control group showed higher escape latency on the fifth day but lower and statistically significant escape time (p> 0.041) on the sixth day.

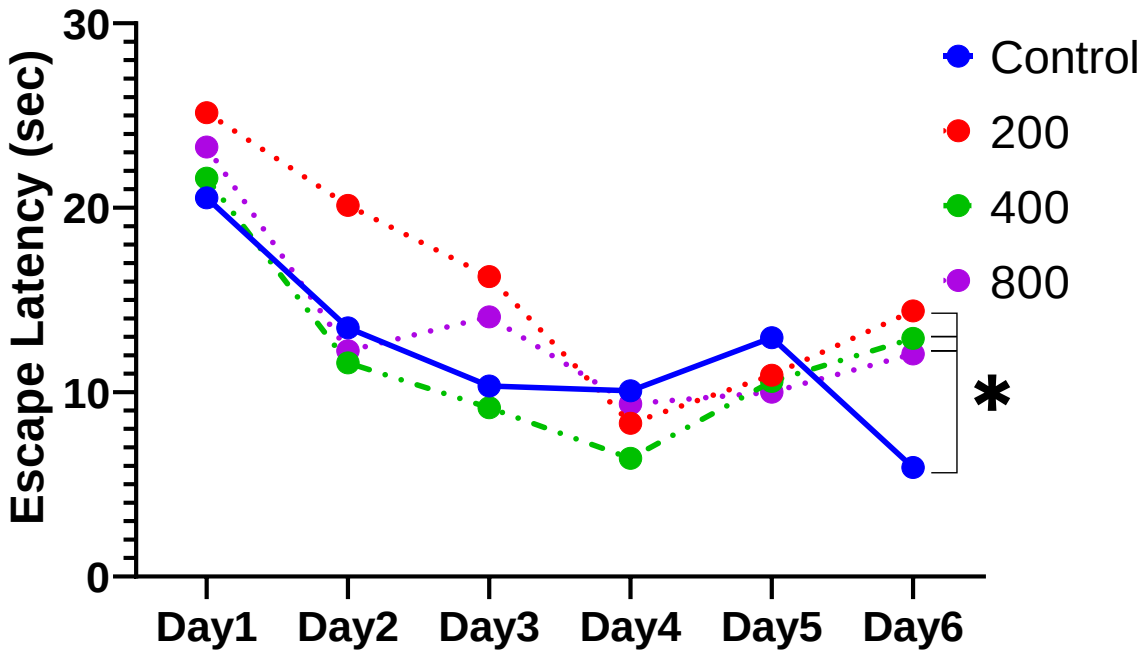


Figure 4.27. Morris Water Maze test in Wistar rats treated with 200, 400 and 800 mg/kg hyoscyamine fraction of *D. stramonium* seedsinadulthood

Figure 4.28 shows comparisons in folds change in the expressions of the neurogenic differentiation1 (*NeuroD1*) gene in adult Wistar rats treated with graded doses of hyoscyamine fraction of *D. stramonium* seeds orally. There was a significant ($p < 0.05$) down-regulation in the expression of the gene across groups. Although the regulation was in a non-monotonic dose responses fashion, a significant difference in the expression ($p < 0.05$) was equally observed between the treated groups ($p<0.001$).

Figure 4.29 shows comparisons in folds change in the expressions of the Disc large homolog 3 (*DLG3*) gene in adult Wistar ratstreated with graded doses of hyoscyamine fraction of *D. stramonium* seeds orally. There was significant ($p < 0.05$) down-regulation in the expression of the gene across groups compared to the control. Although the regulation was in a non-monotonic dose responses fashion, a significant difference in the expression ($p < 0.05$) was equally observed between the treated groups ($p<0.001$).

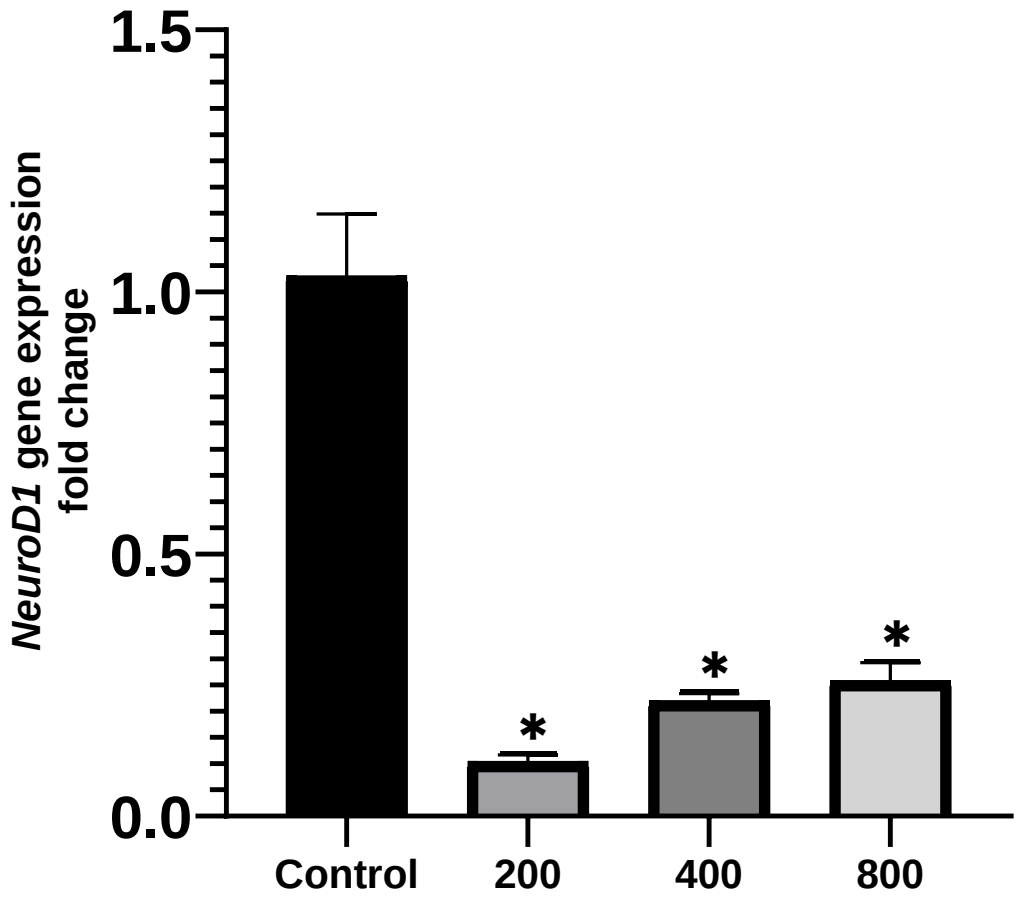


Figure 4.28. *NeuroD1*gene Fold Changes in adult Wistar rats treated with 200, 400 and 800 mg/kg hyoscyamine fraction of *D. stramonium* seeds in adulthood

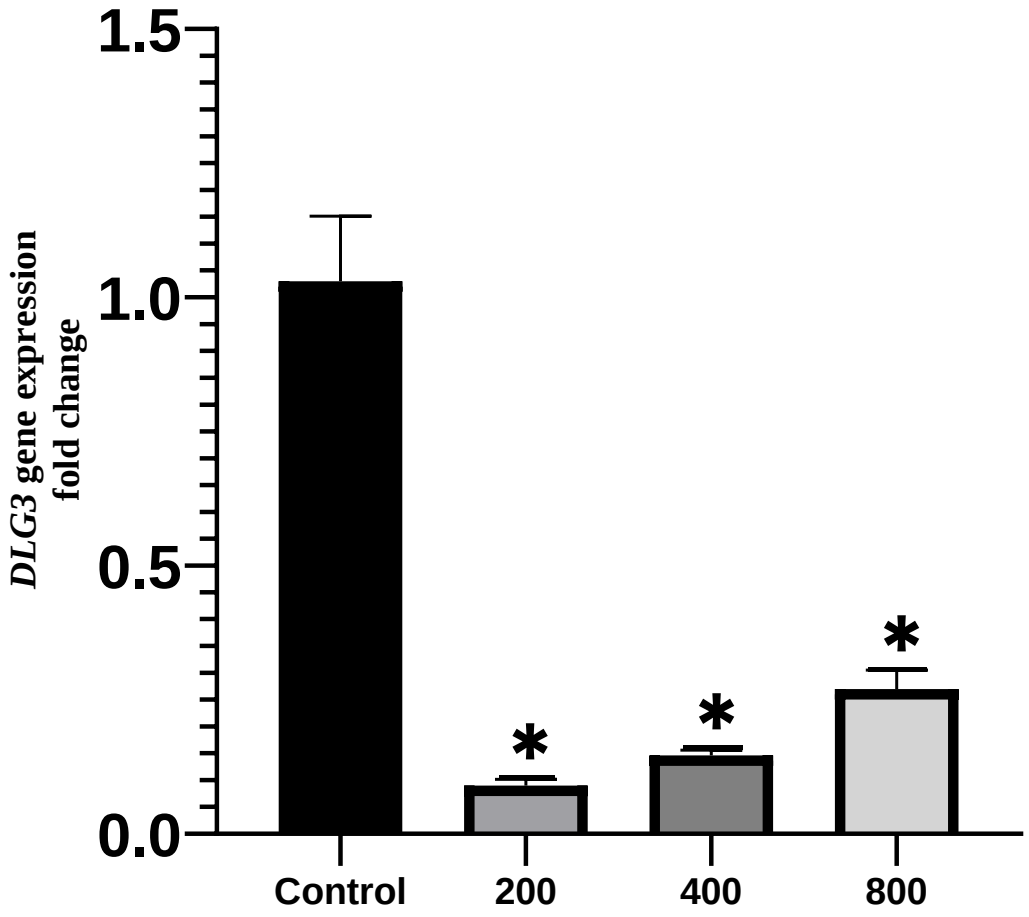


Figure 4.29. *DLG3*gene Fold Changes in adult Wistar rats treated with 200, 400 and 800 mg/kg hyoscyamine fraction of *D. stramonium* seeds in adulthood

4.4. Trace ElementsStudies

Figure 4. 30shows comparisonsbetween exposure to hyoscyamine fraction of *D. stramonium* seeds and trace elements (Cu, Ni, Pb and Zn)in Wistar rats' hippocampus after treatment inlactation and adolescence respectively. The result shows a significant decrease ($p < 0.022$) in the quantity of Pb in the treated groups and also a significant increase ($p < 0.014$) in the Ni but only in the lactation treated group. The quantities of the remaining elements were however not significant statistically ($p > 0.05$) between the treated and control groups. Comparatively, the figure also shows the relative effect of the hyoscyamine fraction with regards to the exposure time between the lactation and adolescence periods. There was no statistically significant difference in the amount of the trace elements quantified in the hippocampus tissues collected between the groups, except for Ni which showed a statistically significant decrease ($p < 0.05$).

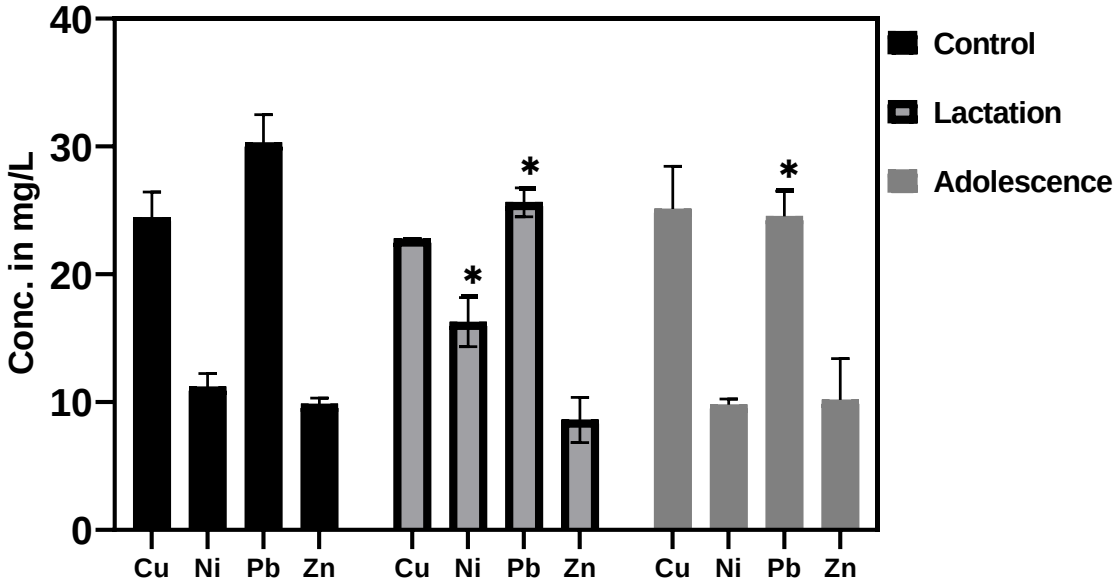


Figure 4.30. Trace Elements in the hippocampus of Wistar rats treated with 400 and 800 mg/kg hyoscyamine fraction of *D.stramonium* seeds in lactation and adolescence respectively.

Figure 4.31 shows comparisons between oral ingestion of hyoscyamine fraction of *D. stramonium* seeds and quantity of trace elements (Cu, Ni, Pb and Zn) in adult Wistar rats' hippocampus after treatment in adulthood.Generally, there was statistically significant differences between the groups and between the quantity of the trace elements exposure [$F(3, 64) = 58.36$, $p < 0.001$]. There was,however, no statistically significant difference in the amount of the selected elements in the hippocampus of the group that received the least dose except for Ni and Pb ($p < 0.05$). Equally, selected elements in the group treated with medium-dose were not statistically significant from the control group except for the Ni ($p < 0.001$). For the group that received the highest dose, however, all the selected trace elements differed statistically and significantly from the control except for the Zn ($p = 0.638$).

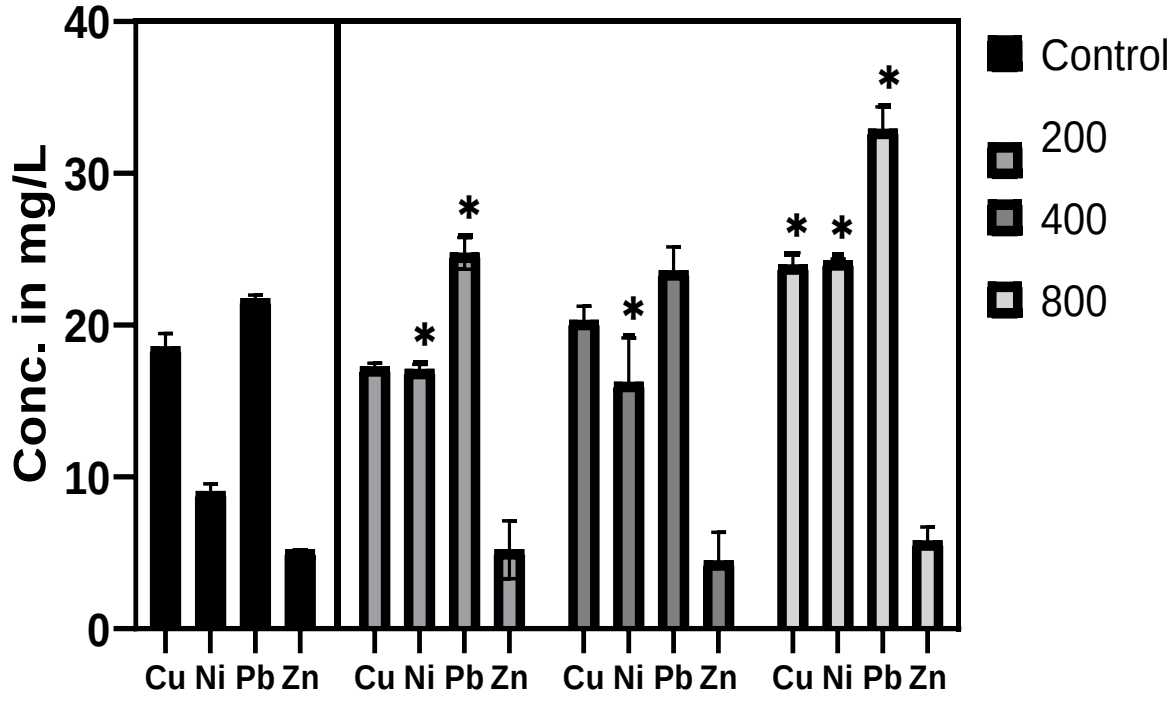


Figure 4.31. Trace Elementsin the hippocampus of Wistar rats treated with 200, 400 and 800 mg/kg hyoscyamine fraction of *D. stramonium* seeds inadulthood

Table 4.4shows associations between prenatal exposure to hyoscyamine fraction of *D. stramonium*seeds andphysical growth indices (ears-detachment, hair growth, eye-opening, and teeth eruption)in Wistar rat pups. No significant ($p > 0.05$) association between the exposure to the fraction and ears detachment, hair growth, eye-opening or teeth eruption between the control and treated pups. The bilateral ears-detachment was not observed before PND 3 of the neonatal pups, observed on the PND 4 in both groups. The hair growth was present in the treated group on the PND 8but observed in the control group at PND 7. The eyes-opening in the control group (PND 13) preceded that of the treated group by a day, (PND 14). Teeth-eruption between the groups was observed on the same day (PND 18).

Table 4.4. Associations Between Prenatal Exposure to Hyoscyamine Fraction of *D. stramonium* Seeds and Physical Body Growth Indices in Wistar Rats Pups

Features	Response	Test	Control	Treatment	*p*-value
Ear Detachment	No	(Observed)	3.00	3.00	1.000
		(Expected)	3.00	3.00	
		Contribution to χ^2	0.00	0.00	
	Yes	Yes (Observed)	18.00	18.00	
		(Expected)	18.00	18.00	
		Contribution to χ^2	0.00	0.00	
		Total	21	21	
Hair growth	No	(Observed)	7.00	8.00	0.747
		(Expected)	7.50	7.50	
		Contribution to χ^2	0.03	0.03	
	Yes	(Observed)	14.00	13.00	
		(Expected)	13.50	13.50	
		Contribution to χ^2	0.02	0.02	
		Total	21	21	
Eye-opening	No	(Observed)	13	14	0.747
		(Expected)	13.50	13.50	
		Contribution to χ^2	0.02	0.02	
	Yes	(Observed)	8.00	7	
		(Expected)	7.50	7.50	
		Contribution to χ^2	0.03	0.03	
		Total	21	21	
Teeth eruption	No	(Observed)	18.00	18.00	1.000
		(Expected)	18	18	
		Contribution to χ^2	0.00	0.00	
	Yes	(Observed)	3.00	3.00	
		(Expected)	3.00	3.00	
		Contribution to χ^2	0.00	0.00	
		Total	21	21	

In table 4.5 comparisons between prenatal exposure to hyoscyamine fraction of *D. stramonium* seeds and motor reflexes in Wistar rat pups. The result showed astatistically significant difference([F= (4, 54) = 5.11, p = 0.002) in the surface righting reflex response between days of the experiment and groups. However, nosignificant difference was observed (p > 0.05) between groups or between groups and days except in PND 5 where the treated group showed a significant delay in the surface righting reflex from the control (p = 0.002). This shows a progressive decline in the righting reflex time within groups with days.

The table also shows that the cliff avoidance in the Wistar rats' pups. There was no statistically significant difference([F= (3, 36) = 2.34, p = 0.090) in the response time for the cliff avoidance between the groups and days of the experiment except for PND 6 of the experiment, where the treated group showed a statistically significant decrease in the cliff avoidance compared to the control p = 0.043).

Also, the table showed that no statistically significant difference ([F= (1, 27) = 0.79, p =0.466)between the control and treated Wistar rat pups was observed in the negative geotaxis test. The Fisher's post-hoc multiple comparisons further indicated that the first 4 weeks did not show a statistically significantdifference between the groups except for the 5^{th} week where the difference emerged (p = 0.005).

Table 4.5.Comparison of Functional Battery Tests in Wistar Rats Pups

Test	Postnatal Day	Group	Mean ± SEM	*t* – value	*p*-value
Surface Righting Reflex	5	Control (n=5)	3.08 ± 0.58	3.20	0.002
		Treated (n=5)	7.22 ± 3.50		
	6	Control (n=5)	4.48 ± 1.20	-0.34	0.734
		Treated (n=5)	4.08 ± 1.30		
	7	Control (n=5)	2.00 ± 0.41	-0.03	0.979
		Treated (n=5)	1.96 ± 0.59		
	28 (4^{th} Week)	Control (n=5)	0.52 ± 0.06	-0.07	0.946
		Treated (n=5)	0.36 ± 0.04		
	35 (5^{th} Week)	Control (n=5)	0.46 ± 0.07	-0.02	0.983
		Treated (n=5)	0.50 ± 0.03		
Cliff Avoidance	6	Control (n=5)	03.28 ± 0.47	2.10	0.043
		Treated (n=5)	08.52 ± 4.32		
	7	Control (n=5)	01.72 ± 0.18	0.41	0.687
		Treated (n=5)	02.73 ± 0.32		
	28 (4^{th} Week)	Control (n=5)	02.08 ± 0.74	-0.19	0.849
		Treated (n=5)	01.60 ± 0.18		
	35 (5^{th} Week)	Control (n=5)	03.36 ± 0.74	0.85	0.401
		Treated (n=5)	05.48 ± 0.85		
Negative Geotaxis	7	Control (n=5)	12.94 ± 2.68	1.97	0.059
		Treated (n=5)	23.12 ± 4.30		
	28 (4^{th} Week)	Control (n=5)	10.32 ± 3.23	1.28	0.211
		Treated (n=5)	16.95 ± 4.33		
	35 (5^{th} Week)	Control (n=5)	08.64 ± 3.73	3.04	0.005
		Treated (n=5)	24.37 ± 2.71		

A comparisons test for the bodyweights between control and prenatally exposed hyoscyamine fraction Wistar rat pups were shown on table 4.6 from the early postnatal period of 1^{st} – 12^{th} week. Bodyweight data for control and treated Wistar rats were not significantly different in the first two weeks of birth. However, from the fourth week onward to adulthood, the weights of the treated groups were significantly lower than those of controls [F (1,108) = 137.73, p <0.001]. There were no observed physical abnormalities in the growing and adult animals of either group during the study. This trend was maintained throughout the study. Equally, significance difference between the weeks [F (1, 11) = 756.97, p <0.001] and groups vs. weeks [F (1, 11) = 2.70, p = 0.004] was also observed between the control and the treated groups.

Table 4.6. Comparisons of Bodyweight Measurements in Wistar rats treatedat Gestation

Week	Dose (mg/kg)	Group	Mean ± SEM	*t* – value	*p*-value
1st	Normal saline	Control (n = 5)	12.68 ± 0.30	-0.66	0.513
	200	Treated (n = 6)	10.60 ± 0.31		
2nd	Normal saline	Control (n = 5)	20.96 ± 0.58	-1.28	0.203
	200	Treated (n = 6)	16.90 ± 0.40		
3rd	Normal saline	Control (n = 5)	29.86 ± 0.61	-2.21	0.030
	200	Treated (n = 6)	22.87 ± 0.66		
4th	Normal saline	Control (n = 5)	44.40 ± 1.30	-3.41	0.001
	200	Treated (n = 6)	33.58 ± 0.69		
5th	Normal saline	Control (n = 5)	59.80 ± 1.40	-4.85	<0.001
	200	Treated (n = 6)	44.43 ± 0.77		
6th	Normal saline	Control (n = 5)	74.04 ± 1.50	-4.62	<0.001
	200	Treated (n = 6)	59.40 ± 1.00		
7th	Normal saline	Control (n = 5)	93.28 ± 1.80	-6.19	<0.001
	200	Treated (n = 6)	73.65 ± 1.20		
8th	Normal saline	Control (n = 5)	95.24 ± 1.80	-5.04	<0.001
	200	Treated (n = 6)	79.25 ± 2.20		
9th	Normal saline	Control (n = 5)	110.56 ± 2.50	-4.19	<0.001
	200	Treated (n = 6)	97.27 ± 2.40		
10th	Normal saline	Control (n = 5)	122.12 ± 3.20	-3.10	0.002
	200	Treated (n = 6)	112.30 ± 3.70		
11th	Normal saline	Control (n = 5)	133.38 ± 3.10	-2.52	0.013
	200	Treated (n = 6)	125.40 ± 3.80		
12th	Normal saline	Control (n = 5)	140.62 ± 3.50	-2.60	0.011
	200	Treated (n = 6)	132.40 ± 4.70		

Table 4.7 presents comparisons between controls andhyoscyamine fraction*D. stramonium* seeds treated Wistar Rats at gestation, lactation and adolescence stages respectivelyfor triple horizontal bars test. Exposure in gestation and lactationshow no statistically significant difference from their respective controls ($p > 0.05$) however, in the adolescence treated group a statistically significant ($p = 0.005$) decrease in the motor coordination task was observed when compared to the control. The comparisons for the group treated in the adulthood did not differ significantly between the groups [$F(3, 20) = 2.52$, $p = 0.085$], except for the group that received 400 mg/kg of the fraction which indicated a significant difference ($p = 0.02$) from the control group.

Table 4.7. Comparison of Triple Horizontal Bars Test in Wistar Rats

Stage of treatment	Dose (mg/kg)	Group	Mean ± SEM	*t* – value	*p*-value
Gestation	Normal saline	Control (n = 5)	13.60 ± 1.40	-0.50	0.642
	200	Treated (n = 6)	14.33 ± 0.42		
Lactation	Normal saline	Control (n = 6)	14.83 ± 0.17	1.76	0.138
	400	Treated (n = 6)	13.83 ± 0.54		
Adolescence	Normal saline	Control (n = 5)	14.20 ± 0.80	3.96	0.005
	800	Treated (n = 6)	10.33 ± 0.56		
Adulthood	Normal saline	Control (n = 6)	12.67 ± 1.20		
	200	Treated (n = 6)	10.83 ± 1.60	0.44	0.664
	400	Treated (n = 6)	14.33 ± 0.33	2.52	0.020
	800	Treated (n = 6)	11.67 ± 1.50	0.46	0.652

Table 4.8. provides results comparisons between familiar and novel objects recognition tests (NORT) in Wistar rats treated with hyoscyamine fraction of *D. stramonium* seedsin gestation, lactation, adolescence and adulthood stages respectively. The mean time it took to explore the novel object (Nob) were significantly ($p < 0.05$) higher than that taken for the familiar object (Fob) in all the control groups when compared to their respective treated groups. Although the meantime took to explore the novel object (Nob) in the treated groups for the gestation, lactation, and adolescence groups were slightly higher compared to the familiar object (Fob), there was, however, no statistically significant difference in the time spent to explore the two objects ($p > 0.05$). The one – way ANOVA with fisher's post-hoc pair-wise comparisons test for familiar and novel objects exploration in Wistar rats treated with 200, 400 and 800 mg/kg graded doses of hyoscyamine fraction respectively in adulthood showed a significant increase in mean exploration time for the novel object (Nob) compared to the familiar one (Fob) in the control group($p= 0.031$). Although the exploration times for the novel object (Nob) in the treated groups were still higher compared to the familiar object (Fob), there was, however, no statistically significant difference in exploration time between the two objects ($p > 0.05$) in the groups.

Table 4.8. Comparison of Familiar and Novel Objects Exploration in Wistar Rats

Stage of Treatment	Dose(mg/kg)	Group	Mean ± SEM		*t* – value	*p*-value
			Fob	Nob		
Gestation	200	Control (n = 6)	22.80 ± 4.00	38.00 ± 3.70	-2.79	0.012
		Treated (n = 6)	42.40 ± 4.00	47.70 ± 3.40	-1.01	0.325
Lactation	400	Control (n = 6)	15.97 ± 1.50	21.21 ± 1.60	-2.37	0.049
		Treated (n = 6)	22.73 ± 4.20	34.93 ± 4.20	-2.04	0.080
Adolescence	800	Control (n = 6)	13.23 ± 2.00	23.49 ± 3.60	2.50	0.047
		Treated (n = 6)	10.82 ± 3.60	12.10 ± 5.20	-0.20	0.845
Adulthood	N. saline	Control (n = 6)	7.29 ± 3.40	18.99 ± 3.70	-2.24	0.031
	200	Treated (n = 6)	15.49 ± 1.80	22.50 ± 5.00	-0.67	0.508
	400	Treated (n = 6)	12.80 ± 4.40	17.23 ± 3.90	-1.18	0.247
	800	Treated (n = 6)	09.50 ± 2.00	19.20 ± 4.30	-1.81	0.077

Fob: Familiar Object, **Nob:** Novel Object

Table 4.9 showed the results of comparisons of novelty preferences in Wistar rats treated with hyoscyamine fraction of *D. stramonium* seeds in gestation, lactation, adolescence and adulthood stages respectively. The novelty preferences for the control groups were significant ($p < 0.05$) statistically at gestation and adolescence stages when compared to the respective treated groups respectively. The novelty preference for the control in the lactation group, however, was not statistically significant ($p = 0.411$) when compared to the treated group. The results of one – way ANOVA with Fisher's post-hoc pair-wise comparisons test for novelty preference in Wistar rats treated at adulthood showed significant difference [$F(3, 20) = 2.01$, $p > 0.05$] in the novelty preference of the control group was higher when compared to the treated groups. However, the result of the post-hoc Pair-wise comparisons test for novelty preference shows a significant decrease ($p < 0.030$) in the 200 mg/kg treated group.

Table 4.9. Comparison of Novelty Preference in Wistar Rats

Stage of Treatment	Dose (mg/kg)	Group	Mean ± SEM	*t* – value	*p*-value
Gestation	200	Control (n = 5)	64.60 ± 3.50	2.54	0.022
		Treated (n = 5)	53.44 ± 2.60		
Lactation	400	Control (n = 5)	57.01 ± 3.10	-0.87	0.411
		Treated (n = 5)	61.34 ± 3.90		
Adolescence	800	Control (n = 5)	63.63 ± 3.20	2.91	0.023
		Treated (n = 5)	49.92 ± 3.40		
Adulthood	Normal saline	Control (n = 5)	76.21 ± 5.87		
	200	Treated (n = 5)	57.11 ± 4.30	-2.34	0.030
	400	Treated (n = 5)	61.28 ± 7.95	1.83	0.083
	800	Treated (n = 5)	64.97 ± 4.16	1.38	0.184

Table 4.10, shows the results of comparisons of discrimination indexes (DI) of Wistar rats treated with hyoscyamine fraction of *D. stramonium* seeds in gestation, lactation, adolescence and adulthood stages respectively. The discrimination index for the control groups was significant ($p < 0.05$) statistically at gestation and adolescence stages when compared to the respective treated groups. The discrimination index for the control in the lactation group however not significant ($p = 0.411$) compared to the treated group. The one – way ANOVA test with Fisher's post-hoc pair-wise comparisons for discrimination indexin Wistar rats treated at adulthood showed no statistically significant difference between the group [$F (3, 20) = 2.01$, $p = 0.144$]. The result of the post-hoc pairwise comparisons test for discrimination indexshows a statistically significant decrease ($p < 0.030$) in discrimination ability in the group that received the least dose.

Table 4.10. Comparisons of Discrimination Index in Wistar Rats

Stage of Treatment	Dose(mg/kg)	Group	Mean ± SEM	*t* – value	*p*-value
Gestation	200	Control (n = 6)	0.29 ± 0.07	2.50	0.024
		Treated (n = 6)	0.07 ± 0.05		
Lactation	400	Control (n = 6)	0.14 ± 0.06	-0.87	0.411
		Treated (n = 6)	0.23 ± 0.08		
Adolescence	800	Control (n = 6)	0.27 ± 0.07	2.91	0.023
		Treated (n = 6)	-0.002 ± 0.07		
Adulthood	Normal saline	Control (n = 6)	0.52 ± 0.12		
	200	Treated (n = 6)	0.14 ± 0.09	-2.34	0.030
	400	Treated (n = 6)	0.23 ± 0.16	1.83	0.083
	800	Treated (n = 6)	0.30 ± 0.08	1.38	0.184

Table 4.11, was descriptive statistics for escape latency of Morris Water Maze (MWM) in Wistar rats treated with hyoscyamine fraction during gestation. There was a progressive decline in the meantime of the escape latency in the first four training days in both control and treated groups. However, on the fifth day, latency time for the control went up to 09.40 ± 1.80 seconds whereas, that of the treated maintained at 08.41 ± 0.92 respectively. On the sixth day (probe), the latency period of the control groups was 5.35 ± 0.568 seconds and that of the treated group was 14.05 ± 2.74 seconds.

There was a progressive decline in the meantime of the escape latency from the first five days (training period) in the control group of the animals treated during lactation. Comparatively, the treated group shows an inconsistent flow of the latency time within the same days. In both cases, the mean times taken for the latency were 08.26 ± 3.15 and 06.53 ± 1.51 seconds respectively. The maximum latency period observed on the sixth day (probe day) for the control and treatment were 26.41and 16.45 and the minimum was 9.53 and 2.91 seconds respectively.

Descriptive statistics for escape latency of the Morris Water Maze (MWM) in the Wistar rats treated with hyoscyamine fraction and control in the adolescence showed a progressive decline in the meantime of the escape latency from the training period first to fifth days. During the test day (sixth day) however, the latency period was slightly higher in the control compared to the treated group. Comparatively, the treated group also shows a similar trend until the fifth day where the latency period of escape slightly increased. The maximum latency period observed on the sixth day (probe day) for the control and treatment was 16.34 and 12.34 seconds and the minimum were 02.40 and 2.75 seconds respectively.

Table 4.11. Descriptive Statistics of Escape Latency of Morris Water Maze (MWM) in Wistar Rats in Gestation, Lactation and Adolescence

Stage of Treatment	Variable	Dose (mg/kg)	Day	Mean ± SEM	Min.	Max.
Gestation	Control	N. saline	1	24.33 ± 2.04	16.05	34.41
			2	11.93 ± 1.60	6.21	20.23
			3	10.85 ± 1.52	3.42	17.43
			4	07.68 ± 0.65	4.81	11.27
			5	09.40 ± 1.80	3.52	22.90
			6	5.35 ± 0.568	2.97	08.69
	Treated	200	1	22.27 ± 2.81	4.03	36.36
			2	16.51 ± 2.40	4.80	28.53
			3	11.22 ± 1.46	5.18	23.64
			4	08.35 ± 1.29	4.28	20.26
			5	08.41 ± 0.92	4.69	15.10
			6	14.05 ± 2.74	2.57	26.13
Lactation	Control		1	17.27 ± 3.50	09.53	26.41
			2	13.22 ± 1.02	10.00	15.76
			3	14.82 ± 1.99	07.46	18.20
			4	15.10 ± 1.68	10.02	19.66
			5	05.75 ± 0.92	03.80	8.742
			6	08.26 ± 3.15	02.40	16.34
	Treated	400	1	14.20 ± 1.05	10.05	16.45
			2	17.12 ± 4.32	04.62	32.93
			3	12.65 ± 3.55	04.12	29.29
			4	14.20 ± 1.05	10.05	16.45
			5	08.34 ± 2.07	03.83	18.10
			6	06.53 ± 1.51	02.91	11.35
Adolescence	Control		1	17.27 ± 3.50	09.53	26.41
			2	13.22 ± 1.02	10.00	15.76
			3	14.82 ± 1.99	07.46	18.20
			4	08.57 ± 2.07	04.01	14.79
			5	05.75 ± 0.92	03.80	8.742
			6	08.02 ± 3.04	02.40	16.34
	Treated	800	1	18.35 ± 2.63	10.90	25.37
			2	16.10 ± 1.16	12.95	19.49
			3	13.29 ± 1.75	06.47	16.56
			4	08.38 ± 0.51	07.10	9.982
			5	09.84 ± 2.12	06.67	18.02
			6	06.68 ± 1.76	02.75	12.34

N. saline: Normal saline

Descriptive statistics for escape latency of Morris Water Maze (MWM) in Wistar rats treated with hyoscyamine fraction and control during adulthood was presented in table 4.12. The control group shows a progressive decline in the meantime of the escape latency by the days from first through the fourth days respectively. During the test day (sixth day) however, the latency period was 12.95 ± 2.40 seconds. The trend was however not similar in the treated groups. While the maximum time spent by the control was 29.74 seconds, the minimum time for the same control group was 2.13 seconds on the sixth day (probe).

Table 4.12. Descriptive Statistics for Escape latency of Morris Water Maze (MWM) in Wistar Rats at Adulthood

Stage of Treatment	Group	Day	Mean ± SEM	Min.	Max.
Adulthood	Control	1	20.53 ± 2.89	08.23	29.74
	200		25.15 ± 3.28	16.17	39.45
	400		21.60 ± 3.10	13.77	30.89
	800		23.29 ± 4.72	11.79	41.90
	Control	2	13.49 ± 2.21	07.56	21.40
	200		20.13 ± 3.91	09.93	36.92
	400		11.59 ± 2.50	04.20	22.39
	800		12.25 ± 1.64	07.34	17.54
	Control	3	10.33 ± 1.26	04.44	12.87
	200		16.26 ± 3.70	07.38	28.64
	400		09.16 ± 1.81	04.41	16.56
	800		14.08 ± 3.27	04.72	23.64
	Control	4	10.07 ± 1.39	07.39	16.14
	200		08.31 ± 0.93	04.37	10.62
	400		06.41 ± 1.02	03.97	09.63
	800		09.37 ± 1.14	05.70	14.05
	Control	5	12.95 ± 2.40	04.58	19.58
	200		10.92 ± 1.10	07.72	15.32
	400		10.58 ± 2.13	04.22	16.70
	800		10.00 ± 0.98	07.31	13.03
	Control	6	03.61 ± 0.51	02.13	04.66
	200		14.41 ± 3.55	06.37	25.56
	400		12.92 ± 3.28	03.22	22.47
	800		12.09 ± 2.60	06.69	23.84

Table 4.13 shows the results of comparisons of Morris Water Maze (MWM) test in Wistar rats treated with hyoscyamine fraction of *D. stramonium* seeds in gestation, lactation and adolescence stages respectively. There was no statistically significant difference between the treated groups [F (1, 120) = 3.02, p = 0.085]. However, statistically significant difference was observed between the days [F (5, 120) = 18.59, p <0.001] and within the groups [F (5, 120) = 2.33, p = 0.047] respectively.

Table 4.13. Comparisons of Morris Water Maze in Wistar Rats in Gestation, Lactation and Adolescence

Stage of Treatment	Dose (mg/kg)	Day	Group	Mean ± SEM	*t* – value	*p*-value
Gestation	200	1	Control (n = 5)	24.33 ± 2.04	-0.78	0.437
			Treated (n = 5)	22.27 ± 2.81		
		2	Control (n = 5)	11.93 ± 1.60	1.73	0.086
			Treated (n = 5)	16.51 ± 2.40		
		3	Control (n = 5)	10.85 ± 1.52	0.14	0.887
			Treated (n = 5)	11.22 ± 1.46		
		4	Control (n = 5)	07.68 ± 0.65	0.25	0.802
			Treated (n = 5)	08.35 ± 1.29		
		5	Control (n = 5)	09.40 ± 1.80	-0.38	0.708
			Treated (n = 5)	08.41 ± 0.92		
		6	Control (n = 5)	05.35 ± 0.57	3.29	0.001
			Treated (n = 5)	14.05 ± 2.74		
Lactation	400	1	Control (n = 5)	17.27 ± 3.50	-0.87	0.387
			Treated (n = 5)	14.20 ± 1.05		
		2	Control (n = 5)	13.22 ± 1.02	1.11	0.272
			Treated (n = 5)	17.12 ± 4.32		
		3	Control (n = 5)	14.82 ± 1.99	-0.62	0.540
			Treated (n = 5)	12.65 ± 3.55		
		4	Control (n = 5)	15.10 ± 1.68	-0.26	0.799
			Treated (n = 5)	14.20 ± 1.05		
		5	Control (n = 5)	05.75 ± 0.92	0.74	0.463
			Treated (n = 5)	08.34 ± 2.07		
		6	Control (n = 5)	08.26 ± 3.15	-0.49	0.626
			Treated (n = 5)	06.53 ± 1.51		
Adolescence	800	1	Control (n = 5)	17.27 ± 3.50	-0.37	0.713
			Treated (n = 5)	18.35 ± 2.63		
		2	Control (n = 5)	13.22 ± 1.02	-0.99	0.327
			Treated (n = 5)	16.10 ± 1.16		
		3	Control (n = 5)	14.82 ± 1.99	0.53	0.602
			Treated (n = 5)	13.29 ± 1.75		
		4	Control (n = 5)	08.57 ± 2.07	0.07	0.948
			Treated (n = 5)	08.38 ± 0.51		
		5	Control (n = 5)	05.75 ± 0.92	-1.41	0.166
			Treated (n = 5)	09.84 ± 2.12		
		6	Control (n = 5)	08.02 ± 3.04	0.46	0.648
			Treated (n = 5)	06.68 ± 1.76		

Table 4.14 shows the results of comparisons of Morris Water Maze (MWM) test in Wistar rats treated with graded doses of hyoscyamine fraction of *D. stramonium* seeds in adulthood. There was statistically significant difference between the groups [F (3, 120) = 3.01, p = 0.033] and between the days [F (5, 120) = 14.8, p <0.001]. No significant difference was observed between groups and days [F (15, 120) = 1.03, p = 0.430] respectively. The post-hoc analysis shows that the significant difference in escape latency between groups at day six was higher in the order of least, (p < 0.008), medium (p < 0.023) and high doses (p < 0.041) respectively.

Table 4.14. Comparisons of Morris Water Maze (MWM) in Wistar at Adulthood

Stage	Day	Group/Dose (mg/kg)	Mean ± SEM	*t* – value	*p*-Value
Adulthood	1	Normal saline	20.53 ± 2.89		
		200	25.15 ± 3.28	1.27	0.206
		400	21.60 ± 3.10	0.30	0.768
		800	23.29 ± 4.72	0.76	0.450
	2	Normal saline	13.49 ± 2.21		
		200	20.13 ± 3.91	1.83	0.070
		400	11.59 ± 2.50	-0.52	0.602
		800	12.25 ± 1.64	-0.34	0.735
	3	Normal saline	10.33 ± 1.26		
		200	16.26 ± 3.70	1.63	0.105
		400	09.16 ± 1.81	-0.32	0.747
		800	14.08 ± 3.27	1.03	0.303
	4	Normal saline	10.07 ± 1.39		
		200	08.31 ± 0.93	-0.49	0.628
		400	06.41 ± 1.02	-1.01	0.315
		800	09.37 ± 1.14	-0.19	0.847
	5	Normal saline	12.95 ± 2.40		
		200	10.92 ± 1.10	-0.56	0.578
		400	10.58 ± 2.13	-0.65	0.515
		800	10.00 ± 0.98	-0.81	0.418
	6	Normal saline	03.61 ± 0.51		
		200	14.41 ± 3.55	2.71	0.008
		400	12.92 ± 3.28	2.30	0.023
		800	12.09 ± 2.60	2.07	0.041

Table 4.15 showed results of comparisons between the quantities of trace elements (Cu, Zn, Pb and Ni) and ingestion of hyoscyamine fraction of *D. stramonium*in lactation and adolescence in Wistar rats hippocampusrespectively. The result shows significant difference in the quantity within the elements [F (3, 48) = 112.14, p <0.001] and also between the elements quantity and groups [F (6, 48) = 3.54, p = 0.006]. However, no significant difference in the quantity of the elements was observed between the treated groups [F (2, 48) = 1.25, p = 0.295]. The post-hoc analysis showed a significant increase in the quantities of Ni and Pbat lactational treatment ($p < 0.05$), but the amount decreases at adolescence significantly (p = 0.005). The amount of Pb also statistically significant decrease but, in a dose-dependent manner between the groups. However, a significant difference in the quantities of Cu and Zn were observed across the groups. The table also showed comparisons test for Atomic Absorption Spectrophotometry (AAS) in the quantification of selected heavy metal (Cu, Ni, Pb and Zn) in Wistar rats hippocampus treated with 200, 400 and 800 mg/kg doses of hyoscyamine fraction at adulthood. There was significant difference in the quantity of the elements between the groups [F (3, 64) = 58.36, p <0.001], within elements [F (3, 64) = 374.27, p <0.001] and also between the groups and the quantity of the trace elements [F (9, 64) = 10.28, p <0.001]. While the post – hoc analyses also show that the quantity of Ni increased significantly ($p < 0.001$) from the control, and that the quantity of Zn remained partly constant between the groups. The quantity of Pb also increased significantly ($p < 0.05$) from the control in all the treated groups except for the medium group which was treated with 400 mg/kg of the hyoscyamine fraction (p = 0.150). Although the quantity of the Cu increased in a dose-

dependent manner, a statistically significant increase ($p < 0.001$) was only observed in the group treated with the highest dose of the hyoscyamine fraction.

Table 4.15. Comparisons of the Numberof TraceElements in the Hippocampus of Wistar Rats

Stage of treatment	Elements (mg/l)	Dose(mg/kg)	Mean ± SEM		*t* – value	*p*-value
			Control (n=6)	Treated (n=6)		
Lactation	Cu	400	24.45 ± 1.99	22.76 ± 0.03	-0.85	0.399
	Ni		11.20 ± 1.03	16.25 ± 1.92	2.56	0.014
	Pb		30.30 ± 2.19	25.63 ± 1.13	-2.37	0.022
	Zn		09.85 ± 0.47	08.60 ± 1.77	-0.63	0.529
Adolescence	Cu	800	24.45 ± 1.99	25.10 ± 1.50	-0.33	0.743
	Ni		11.20 ± 1.03	09.80 ± 0.20	0.71	0.481
	Pb		30.30 ± 2.19	24.53 ± 0.88	2.93	0.005
	Zn		09.85 ± 0.47	10.18 ± 1.44	-0.16	0.870
Adulthood	Cu	200	18.55 ± 0.89	17.25 ± 0.25	1.02	0.309
	Ni		09.03 ± 0.53	17.08 ± 0.35	-6.35	<0.001
	Pb		21.73 ± 0.26	24.73 ± 1.04	-2.37	0.021
	Zn		05.18 ± 0.01	05.20 ± 1.90	-0.02	0.984
	Cu	400	18.55 ± 0.89	20.30 ± 0.43	-1.38	0.172
	Ni		09.03 ± 0.53	16.23 ± 1.31	-5.68	<0.001
	Pb		21.73 ± 0.26	23.58 ± 0.70	-1.46	0.150
	Zn		05.18 ± 0.01	04.45 ± 0.85	0.57	0.570
	Cu	800	18.55 ± 0.89	23.95 ± 0.83	-4.26	<0.001
	Ni		09.03 ± 0.53	24.23 ± 0.15	-11.98	<0.001
	Pb		21.73 ± 0.26	32.90 ± 1.48	-8.81	<0.001
	Zn		05.18 ± 0.01	05.78 ± 0.93	-0.47	0.638

Cu: Copper, Ni: Nickel, Pb: Lead, Zn: Zinc

Table 4.16 showeddescriptive statisticsof the cycle threshold (Ct) values of *β-Actin*, *NeuroD1* and *DLG3* genes of the hippocampus in Wistar rats treated with 200, 400 and 800 mg/kg doses of hyoscyamine fraction at adulthood. The Mean ± SEM values for *β-Actin* were 24.58 ± 0.01, for *NeuroD1*, 25.39 ± 0.05 and for 23.27 ± 0.00. While the least value for *β-Actin* was 19.99 ± 0.03 observed in the 200 mg/kg treated group, 23.72 ± 0.06 and 22.10 ± 0.03 values were observed least in the 400 mg/kg treated groups for *NeuroD1* and *DLG3* genes respectively.

Table 4.16. Descriptive Statistics of the Cycle Threshold (Ct) values of *β-Actin*,*NeuroD1* and *DLG3*Genes of the Hippocampus in Wistar Rats

Gene	Group	Dose (mg/kg)	Mean ± SEM	Min.	Max.
***β-Actin**	Control	Normal saline	24.58 ± 0.01	24.55	24.63
	Treated	200	19.99 ± 0.03	19.92	20.05
	Treated	400	20.41 ± 0.19	19.76	20.73
	Treated	800	21.89 ± 0.05	21.72	22.01
***NeuroD1**	Control	Normal saline	25.39 ± 0.05	25.30	25.56
	Treated	200	24.33 ± 0.11	24.05	24.60
	Treated	400	23.72 ± 0.06	23.53	23.84
	Treated	800	25.07 ± 0.02	25.02	25.12
***DLG3**	Control	Normal saline	23.27 ± 0.01	23.26	23.27
	Treated	200	22.30 ± 0.08	22.14	22.60
	Treated	400	22.10 ± 0.03	22.01	22.16
	Treated	800	22.88 ± 0.02	22.83	22.95

* House – Keeping Gene, **Gene of Interest

Table 4.17 showed comparisons of cycle threshold (Ct) values of *β-Actin*, *NeuroD1* and *DLG3*in the hippocampustreated with graded doses of hyoscyamine fraction*D. stramonium* seeds in adulthood. There was a statistically significant difference [F (3, 16) = 454.94, $p < 0.001$] between the samples in the groups. The post – hoc analysis further showed that the ct values for the *β-Actin* in the control were statistically higher ($p < 0.001$) compared to all the treated groups. Likewise, the ct value in *NeuroD1* significantly differed [F (3, 16) = 122.61, $p < 0.001$] across the groups. Also, when compared to the treated groups, the amount of the ct values in the control was significantly higher ($p < 0.001$). The ct values of the *DLG3* also increased significantly [F (3, 16) = 1625.34, $p < 0.001$] in the control compared to the treated groups. The post-hoc test result also showed that the interaction between the groups was also significant ($p < 0.001$).

Table 4.17. Comparisons of Cycle Threshold (Ct) Values of *β-Actin,NeuroD1* and *DLG3* in the Hippocampus of Wistar rats at Adulthood

Dose (mg/kg)	Mean±SEM			*t* - value	*p*-value
	***β-Actin**	****NeuroD1**	****DLG3**		
Normal saline	24.58 ± 0.01	25.39 ± 0.05	23.27±0.01	-14.99	<0.001
200	19.99 ± 0.03	24.33 ± 0.11	22.30±0.08		
400	20.41 ± 0.19	23.72 ± 0.06	22.10±0.03	-18.15	<0.001
800	21.89 ± 0.05	25.07 ± 0.02	22.88±0.02	-6.03	<0.001

* House – Keeping Gene, **Gene of Interest, *NeuroD1*: Neurogenic Differentiation 1, *DLG3*: Disc large homolog 3

Table 4.18. shows descriptive statistics of fold changes in the expressions of*NeuroD1* and *DLG3* genes from the hippocampus of Wistar rats treated with graded doses of hyoscyamine fraction at adulthood. In both cases, the expression of genes in the control samples was higher compared to the treated groups. The mean fold changes increased in a dose-dependent manner with the least fold changes observed in the samples of the group that received the least,200 mg/kg, as 0.09 ± 0.01 and 0.08 ± 0.01 for the *NeuroD1* and *DLG3* respectively. The highest means were however observed in the sample of the group that received the highest dose, 800 mg/kg, as 0.20 ± 0.01 for two of the genes.

Table 4.18. Descriptive Statistics for the Fold Changes in the Expressions of*NeuroD1* and *DLG3* gene in the Hippocampus of Wistar Rats at Adulthood

Gene	Dose (mg/kg)	Mean ± SEM	Min.	Max.
NeuroD1	Normal saline	1.00 ± 0.04	0.88	1.10
	200	0.09 ± 0.01	0.08	0.11
	400	0.18 ± 0.02	0.11	0.24
	800	0.20 ± 0.01	0.17	0.21
DLG3	Normal saline	1.00 ± 0.01	0.99	1.04
	200	0.08 ± 0.01	0.07	0.09
	400	0.12 ± 0.02	0.08	0.17
	800	0.20 ± 0.01	0.18	0.23

* House – Keeping Gene, **Gene of Interest, *NeuroD1*: Neurogenic Differentiation 1, *DLG3*: Disc large homolog 3

Table 4.19 shows comparisons for gene expression of fold changes of Neurogenic Differentiation (*NeuroD1*) and Disc Large Homolog 3 (*DLG3*) genes in the hippocampus of the Wistar rats treated with graded doses of hyoscyamine fractioninadulthood. Statistically significant downregulation of the two genes [F (3, 16) = 374.70, p <0.001] was observed between the control and treated groups. The post-hoc multiple comparisons also show that the genes down-regulated significantly (p <0.05) within the treated groups of *NeuroD1* gene, except for between the medium (400 mg/kg) and highest (800 mg/kg) doses (p = 0.710). A significant [F (3, 16) = 1625.34, p <0.001] downregulation in the expression of the *DLG3* gene was also observed between groups in the treated groups when compared to the control. Post-hoc multiple comparisons also show that the genes down-regulated significantly (p <0.05) within all the treated groups of the *DLG3* gene.

Table 4.19. Comparisons of Fold Changes in the Expression of *NeuroD*1and*DLG3* Genes in the Hippocampus of Wistar rats at Adulthood

Gene	Dose (mg/kg)	Group	Mean±SEM	*t* - value	*p*-value
NeuroD1	Normal saline	Control	1.00 ± 0.04	11.06	<0.001
	200 mg	Treated	0.09 ± 0.01		
	400 mg	Treated	0.18 ± 0.02	17.45	<0.001
	800 mg	Treated	0.20 ± 0.01	3.39	0.004
DLG3	Normal saline	Control	1.00 ± 0.01	60.36	<0.001
	200 mg	Treated	0.08 ± 0.01		
	400 mg	Treated	0.13 ± 0.02	57.24	<0.001
	800 mg	Treated	0.20 ± 0.01	52.29	<0.001

NeuroD1: Neurogenic Differentiation 1, *DLG3*: Disc large homolog 3

Table 4.20 shows an estimation of neuronal number for the dentate gyrus and hippocampal area CA1 and CA3 in Wistar treated with graded doses of hyoscyamine fraction at gestation, lactation and adulthood and their respective controls. The neuronal estimate of the DG, CA1 and CA3 counts were 4.042×10^6, 8.084×10^6cellsand 2.425×10^6cellsrespectively. The highest estimated neuronal DG counts were 7.575× 106 cellsand found in the adult groups treated with the 400 mg/kg of the hyoscyamine fraction. The least was 2.425 × 106 cellswhich were recorded in the groups treated at gestation, lactation, and adolescence respectively. The highest number of the CA1 area count was 8.084 × 106 cells, which was observed in control and treated groups of gestation and lactation respectively. The least count was 1.617×10^6cellsand was recorded in the groups treated at lactation and that treated with the highest dose in adulthood. The least count observed was 0.808×10^6cellsin the group treated at adulthood for the CA3 count whereas, the highest observed was 3.233×10^6cellsand was recorded in the control groups treated at lactation adolescence.

Table 4.20. Estimation of Neuronal Numbers in the Dentate gyrus, and Hippocampus Subfields (CA1 and CA3)of Wistar rats

Stage	**$\sum Q_D$-**	**$\sum Q_1$-**	**$\sum Q_3$-**	**a(frame) µm^2**	**X - Y step (µm)**	**1/asf**	**1/ssf**	**(DG × 10^6)**	**(CA1 × 10^6)**	**CA3 (× 10^6)**
Gestation	600	1200	360	10^4	367.05	13.47	50	4.042	8.084	2.425
(200 mg/kg)	360	240	240	10^4	367.05	13.47	50	2.425	1.617	1.617
Lactation	720	600	480	10^4	367.05	13.47	50	4.850	4.042	3.233
(400mg/kg)	600	600	120	10^4	367.05	13.47	50	4.042	4.042	0.808
	360	600	360	10^4	367.05	13.47	50	2.425	4.042	2.425
	720	1200	360	10^4	367.05	13.47	50	4.850	8.084	2.425
Adolescence	720	600	480	10^4	367.05	13.47	50	4.850	4.042	3.233
(800 mg/kg)	720	480	360	10^4	367.05	13.47	50	4.850	3.233	2.425
	360	600	360	10^4	367.05	13.47	50	2.425	4.042	2.425
	960	840	360	10^4	367.05	13.47	50	6.467	5.658	2.425
Adulthood	720	480	240	10^4	367.05	13.47	50	4.850	3.233	1.617
(mg/kg)	1080	360	600	10^4	367.05	13.47	50	7.275	2.425	4.042
	960	480	360	10^4	367.05	13.47	50	6.467	3.233	2.425
	600	240	360	10^4	367.05	13.47	50	4.042	1.617	2.425

CA1: Cornu Ammonis 1, CA3: Cornu Ammonis 3, DG: Dentate gyrus

Table 4.21 shows descriptive statistics estimation of the hippocampus areas CA1 and CA3 and dentate gyrus (DG) neuronal numbers in control and groups treated with graded doses of hyoscyamine fraction at gestation, lactation and adulthood respectively. The mean ± SEM neuronal numbers of the DG and CA3 in the control and treated groups of the gestational exposure were higher, $4.042 \times 10^6 \pm 0.224 \times 10^6$, $24.25 \times 10^6 \pm 0.19 \times 10^6$ and $2.424 \times 10^6 \pm 0.173 \times 10^6$, $1617 \times 10^6 \pm 0.187 \times 10^6$cellsrespectively when compared to that of CA1, $8.08 \times 10^6 \pm 0.248 \times 10^6$, $1.617 \times 10^6 \pm 0.211 \times 106$ cells. At lactation, however, the mean neuronal numbers of the DG were higher in the control, $4.850 \times 10^6 \pm 0.242 \times 106$ cellswhen compared to the treated $4.042 \times 10^6 \pm 0.296 \times 10^6$. After the animals reached adulthood, the neuronal number of controls reduced to $2.425 \times 10^6 \pm 0.234 \times 106$ cellsagainst that of the treated $4.850 \times 10^6 \pm 0.375 \times 106$ cells.The neuronal numbers of the CA1 were virtually the same in the control and the treated group at lactation, but as the animals reached adulthood, the mean neuronal CA1 number of the treated almost doubled $0.8084 \times 10^6 \pm 0.244 \times 10^6$ cells. The mean neuronal numbers in the hippocampal CA3 region was higher in the control group at lactation, $3.233 \times 10^6 \pm 0.546 \times 10^6$cellswhen compared to the treated, $0.808 \times 10^6 \pm 0.446 \times 106$ cells. But in adulthood, both control and treated virtually remained the same, $2.425 \times 10^6 \pm 0.155 \times 10^6$ and $2.425 \times 10^6 \pm 0.226 \times 10^6$cellsrespectively. The adolescence exposure to hyoscyamine fraction gave an equal neuronal number in the DG region of the hippocampus in both the control, $4.851 \times 10^6 \pm 0.242 \times 10^6$ cells and treated $4.850 \times 10^6 \pm 0.073 \times 10^6$ cells groups respectively. The number, however, was higher in the treated group as the animal reached adulthood. For the CA1 region, the control showed a high number of neuronal numbers, $4.041 \times 10^6 \pm 0.442 \times 10^6$ cellswhen compared to the

treated group, $3.234 \times 10^6 \pm 0.400 \times 10^6$ cells in the early stage. On reaching adulthood, the number of the treated group increased as $5.658 \times 10^6 \pm 0.119 \times 10^6$ cells and $4.042 \times 10^6 \pm 0.117 \times 106$ cells in the control. The CA3 region was only higher in the control group of the animals, $3.233 \times 10^6 \pm 0.546 \times 10^6$ cells when compared to the treated, $2.425 \times 10^6 \pm 0.102 \times 10^6$ cellsat the early adolescent stage. As the animals reached adulthood, the neuronal numbers decreased and became virtually equal. The neuronal of the DG areas of the animals treated with the hyoscyamine fraction at adulthood increased in all the groups, $7.275 \times 10^6 \pm 0.296 \times 10^6$ and $6.467 \times 10^6 \pm 0.114 \times 106$ cells compared to the control, $4.851 \times 10^6 \pm 0.171 \times 10^6$cellsexcept for the highest dose $4.041 \times 10^6 \pm 0.314 \times 10^6$ cells, where the number decreased. The mean neuronal numbers in the treated groups CA1 region reduced when compared to the control, $3.233 \times 10^6 \pm 0.117 \times 10^6$ cells in the lowest and highest doses as $2.425 \times 10^6 \pm 0.162 \times 10^6$ and $1.617 \times 10^6 \pm 0.146 \times 10^6$cellsrespectively. However, the numbers remained almost the same as control, $3.233 \times 10^6 \pm 0.295 \times 10^6$ cells in medium doses. For the CA3 region, the treated groups had a mean neuronal number $4.042\times 10^6 \pm 0.216 \times 10^6$, $2.425 \times 10^6 \pm 0.101 \times 10^6$ and $2.425 \times 10^6 \pm 0.063 \times 10^6$cellsrespectively when compared to the control, $1.617 \times 10^6 \pm 0.236 \times 10^6$ cells.

Table 4.21. Descriptive Statistics for the Estimation of Neurons in the HippocampusSubfields(CA1 and CA3) and Dentate gyrus in Wistar rats

Stage	Region	Dose (mg/kg)		Group	Mean ± SEM ($\times 10^6$)	Min. ($\times 10^6$)	Max. ($\times 10^6$)
Gestation	DG	200	After	Control	4.042 ± 0.224	4.041	4.043
				Treated	2.425 ± 0.190	2.424	2.426
	CA1		After	Control	8.084 ± 0.248	8.084	8.085
				Treated	1.617 ± 0.211	1.617	1.618
	CA3		After	Control	2.425 ± 0.173	2.424	2.425
				Treated	1.617 ± 0.187	1.616	1.617
Lactation	DG	400	At	Control	4.851 ± 0.242	4.850	4.851
				Treated	4.042 ± 0.296	4.041	4.043
			After	Control	2.425 ± 0.234	2.425	2.426
				Treated	4.850 ± 0.375	4.849	4.851
	CA1		At	Control	4.041 ± 0.442	4.040	4.042
				Treated	4.042 ± 0.273	4.042	4.043
			After	Control	4.042 ± 0.117	4.041	4.042
				Treated	8.084 ± 0.244	8.083	8.084
	CA3		At	Control	3.233 ± 0.546	3.232	3.235
				Treated	0.808 ±0.446	0.807	0.809
			After	Control	2.425 ± 0.155	2.425	2.426
				Treated	2.425 ± 0.226	2.424	2.425
Adolescence	DG	800	At	Control	4.851 ± 0.242	4.850	4.851
				Treated	4.850 ± 0.073	4.850	4.850
			After	Control	2.425 ± 0.234	2.425	2.426
				Treated	6.467 ± 0.145	6.467	6.468
	CA1		At	Control	4.041 ± 0.442	4.040	4.042
				Treated	3.234 ± 0.400	3.233	3.235
			After	Control	4.042 ± 0.117	4.041	4.042
				Treated	5.658 ± 0.119	5.658	5.659
	CA3		At	Control	3.233 ± 0.546	3.232	3.235
				Treated	2.425 ± 0.102	2.425	2.425
			After	Control	2.425 ± 0.155	2.425	2.426
				Treated	2.425 ± 0.218	2.424	2.425
Adulthood	DG	N. saline	At	Control	4.851 ± 0.171	4.850	4.851
		200		Treated	7.275 ± 0.296	7.274	7.276
		400		Treated	6.467 ± 0.114	6.467	6.468
		800		Treated	4.041 ± 0.314	4.040	4.042
	CA1	N. saline	At	Control	3.233 ± 0.117	3.233	3.234
		200		Treated	2.425 ± 0.162	2.425	2.426
		400		Treated	3.233 ± 0.295	3.232	3.234
		800		Treated	1.617 ± 0.146	1.616	1.617
	CA3	N. saline	At	Control	1.617 ± 0.236	1.616	1.618
		200		Treated	4.042 ± 0.216	4.041	4.043
		400		Treated	2.425 ± 0.101	2.425	2.425
		800		Treated	2.425 ± 0.063	2.425	2.425

CA1: Cornu Ammonis 1, CA3: Cornu Ammonis 3, DG: Dentate gyrus, HC: Hippocampus

Table 4.22.; shows results of comparisons for the estimation of neuronal numbers in the dentate gyrus, and hippocampus areasCA1 and CA3 in the Wistar rats treated with graded doses of hyoscyamine fraction at gestation, lactation and adulthood respectively. Statistically significant reduction of neuronal numbers in DG and CA3 regions were observed [F (1, 9) = 3.0×10^7, p <0. 0.001] and CA3 = [F (1, 9) = 1.0×10^7, p <0. 0.001] respectively in the prenatally exposed group when compared to the respective controls. The number in the CA1 region however, significantly increased CA1 = [F (1, 9) = 3.9×10^8, p <0. 0.001] in the treated group when compared to the control. No statistically significant difference was observed in the DG area between the control and treated groups in lactation, however, a significant decrease in the neuronal number [F (2, 14) = 4.3×10^7, p <0. 0.001] of the region was observed in the DG area of the animals treated when the animals reached adulthood. For the CA1 and CA3 regions of the hippocampus, a significant decrease in the number of the regional cells was observed as [F (2, 14) = 1.5×10^7, p <0. 0.001] and [F (2, 14) = 9.0×10^7, p <0. 0.001] respectively compared to the respective controls. The neuronal numbers of the DG and CA3 regions in the group treated in adolescence decreased significantly DG = [F (2, 14) = 6.4×10^7, p <0. 0.001], CA3 = [F (2, 14) = 1.5×10^8, p <0. 0.001] in comparison with their respective controls. As the animals reached adulthood, the DG and CA1 neuronal numbers of the treated groups significantly increased [F (2, 14) = 6.4×10^7, p <0. 0.001]. The neuronal numbers of the treated group at the adolescence for the CA1increased significantly [F (2, 14) = 9.0×10^6, p <0. 0.034] however, the CA3 neuronal count as the animals reached adulthood were not significant from their control F(2, 14) = -1.06, p = 0.310]. There was a statistically significant increase in the DG and CA3 neuronal numbers in all the animal

groups treated at adulthood as [F (3, 19) = 3.8×10^7, p <0. 0.001] and [F (3, 19) = 3.5×10^7, p <0. 0.001] respectively except for the group treated with the highest dose, where the DG cells significantly decreased. A significant decrease in the neuronal number of the CA1 was also observed in the group CA1= [F (3, 19) = 1.6×10^7, p <0. 0.001] except for the group treated with medium-dose where the numbers significantly decreased.

Table 4.22. Comparisons of Neuronal Numbers in the HippocampusAreas (CA1 and CA3)and Dentate gyrus, in Wistar rats

Stage of Treatment	Region	Dose (mg/kg)		Group (n=6)	Mean ± SEM ($\times 10^6$)	*t* – value	*p* -value
Gestation	DG	200	After	Control	4.042±0.224	-5508.50	<0. 0.001
				Treated	2.425±0.190		
	CA1	200	After	Control	8.084±0.248	-19850.19	<0. 0.001
				Treated	1.617±0.211		
	CA3	200	After	Control	2.425±0.173	-3172.41	<0. 0.001
				Treated	1.617±0.187		
Lactation	DG	400	At	Control	4.851±0.242	-1.46	0.170
				Treated	4.850±0.073		
			After	Control	2.425±0.234	6738.43	<0. 0.001
				Treated	4.850±0.375		
	CA1	400	At	Control	4.041±0.442	-1509.00	<0. 0.001
				Treated	3.234±0.400		
			After	Control	4.042±0.117	17201.50	<0. 0.001
				Treated	8.084±0.244		
	CA3	400	At	Control	3.233±0.546	-1389.77	<0. 0.001
				Treated	2.425±0.102		
			After	Control	2.425±0.155	-1.95	0.075
				Treated	2.425±0.226		
Adolescence	DG	800	At	Control	4.851±0.242	-2544.11	<0. 0.001
				Treated	4.042±0.296		
			After	Control	2.425±0.234	11232.97	<0. 0.001
				Treated	6.467±0.145		
	CA1	800	At	Control	4.041±0.442	2.39	0.034
				Treated	4.042±0.273		
			After	Control	4.042±0.117	6880.87	<0. 0.001
				Treated	5.658±0.119		
	CA3	800	At	Control	3.233±0.546	-4170.15	<0. 0.001
				Treated	0.808±0.446		
			After	Control	2.425±0.155	-1.06	0.310
				Treated	2.425±0.218		
Adulthood	DG	N. Saline	At	Control	4.851±0.171	7171.18	< 0.001
		200	At	Treated	7.275±0.296		
		400	At	Treated	6.467±0.114	4782.27	< 0.001
		800	At	Treated	4.041±0.314	-2395.08	< 0.001
	CA1	N. Saline	At	Control	3.233±0.117	-2967.60	< 0.001
		200	At	Treated	2.425±0.162		
		400	At	Treated	3.233±0.295	-0.54	0.594
		800	At	Treated	1.617±0.146	-5936.26	< 0.001
	CA3	N. Saline	At	Control	1.617±0.236	10043.19	< 0.001
		200	At	Treated	4.042±0.216		
		400	At	Treated	2.425±0.101	3346.53	< 0.001
		800	At	Treated	2.425±0.063	3346.82	< 0.001

CA1: Cornu Ammonis 1, CA3: Cornu Ammonis 3, DG: Dentate gyrus, HC: Hippocampus

Table 4.23 provides descriptive statistics for the estimation of the hippocampus and dentate gyrus thicknesses in Wistar rats treated with hyoscyamine fractionof *D. stramonium* seeds in gestation, lactation and adulthood respectively. The mean thickness for the dentate gyrus and hippocampus of the animals treated at gestation and measured at birth in both control and treated groups were 0.014 ± 0.001, 0.018 ± 0.007and 0.014 ± 0.001, 0.022 ± 0.001 mm respectively. The mean dentate gyrus and hippocampal thickness as the animals reached adulthood were 0.137 ± 0.025, 102 ± 0.017 and 0.0120 ± 0.011, 0.115 ± 0.016 mm respectively. The mean thickness of dentate gyrus and hippocampus at and after lactation for the controls and treated groups were 0.142 ± 0.023, 0.124 ± 0.019 and 0.150 ± 0.017, 0.118 ± 0.013 mm respectively. Although the mean thickness of dentate gyrus and hippocampus decreased in both control and treated groups, the mean thickness for the dentate gyrus and hippocampus after reaching adulthood for the groups were 0.092 ± 0.007, 0.096 ± 0.014and 0.150 ± 0.012, 0.135 ± 0.022 mm respectively. Generally, there was an increase in the dentate gyrus and hippocampal thickness when compared to control for the group of the animals treated with various doses of the hyoscyamine fraction at adulthood. The mean dentate gyri were 0.146 ± 0.022, 0.129 ± 0.015 and 0.169 ± 0.027 mm for the low, medium and high doses respectively. Equally, the mean hippocampus thicknesses observed in the animal's group were 0.141 ± 0.020, 0.149 ± 0.020 and 0.167 ± 0.023 mm for the low, medium and high doses respectively.

Table 4.23.Descriptive Statistics Hippocampus and Dentate Gyrus Thickness in Wistar rats

Stage of Exposure	Region	Dose (mg/kg)		Group	Mean ± SEM (mm)	Min.	Max.
Gestation	DG	200	At	Control (n = 6)	0.014 ± 0.001	0.009	0.018
				Treated (n = 6)	0.014 ± 0.001	0.010	0.017
			After	Control (n = 6)	0.137 ± 0.025	0.039	0.248
				Treated (n = 6)	0.120 ± 0.011	0.071	0.156
	HC		At	Control (n = 6)	0.018 ± 0.007	0.015	0.021
				Treated (n = 6)	0.022 ± 0.001	0.018	0.024
			After	Control (n = 6)	0.102 ± 0.017	0.026	0.184
				Treated (n = 6)	0.115 ± 0.016	0.039	0.169
Lactation	DG	400	At	Control (n = 6)	0.142 ± 0.023	0.054	0.225
				Treated (n = 6)	0.124 ± 0.019	0.047	0.195
			After	Control (n = 6)	0.092 ± 0.007	0.061	0.113
				Treated (n = 6)	0.113 ± 0.016	0.052	0.166
	HC		At	Control (n = 6)	0.150 ± 0.017	0.055	0.201
				Treated (n = 6)	0.118 ± 0.013	0.032	0.154
			After	Control (n = 6)	0.150 ± 0.012	0.093	0.177
				Treated (n = 6)	0.123 ± 0.017	0.026	0.166
Adolescence	DG	800	At	Control (n = 6)	0.150 ± 0.017	0.055	0.201
				Treated (n = 6)	0.136 ± 0.026	0.032	0.264
			After	Control (n = 6)	0.092 ± 0.007	0.061	0.113
				Treated (n = 6)	0.096 ± 0.014	0.054	0.153
	HC		At	Control (n = 6)	0.150 ± 0.017	0.055	0.201
				Treated (n = 6)	0.135 ± 0.023	0.035	0.199
			After	Control (n = 6)	0.150 ± 0.012	0.093	0.177
				Treated (n = 6)	0.135 ± 0.022	0.026	0.218
Adulthood	DG	N. saline	At	Control (n = 6)	0.125 ± 0.019	0.048	0.197
		200		Treated (n = 6)	0.146 ± 0.022	0.064	0.237
		400		Treated (n = 6)	0.129 ± 0.015	0.066	0.183
		800		Treated (n = 6)	0.169 ± 0.027	0.065	0.267
	HC	N. saline	At	Control (n = 6)	0.139 ± 0.016	0.042	0.174
		200		Treated (n = 6)	0.141 ± 0.020	0.033	0.201
		400		Treated (n = 6)	0.149 ± 0.020	0.040	0.220
		800		Treated (n = 6)	0.167 ± 0.023	0.040	0.252

DG: Dentate gyrus, HC: Hippocampus

Table 4.24 shows comparisons test results for hippocampus thickness in Wistar rats treated with hyoscyamine fractionof *D. stramonium* seeds in gestation, lactation, adolescence and adulthood respectively. No statistically significant changes in thickness of the dentate gyrus (DG) was observed at birth [F (1, 15) = 0.29, p = 0.598] or adulthood [F (1, 15) = 0.36, p = 0.561] in all the stages of the treatment except for the group treated prenatally where, significant increase in the hippocampal thickness was observed in treated group [F (1, 15) = 11.36, p = 0.005] at birth, but not when the animals reached adulthood[F (1, 15) = 0.29, p = 0.601]. For the group treated at lactation, no significant changes were observed at both early and later (adulthood stages of development for both dentate gyrus [F (2, 23) = 0.16, p = 0.850] and hippocampus [F (1, 15) = 0.80, p = 0.464] respectively. Similar observations were made for the animals treated at both adolescence and adulthood stages where no significant differences observed between the control and treated groups in the mean thickness of the DG, [F (2, 23) = 0.79, p = 0.469], DG = [F (3, 28) = 0.87, p = 0.467] and HC, [F (2, 23) = 0.63, p = 0.542], HC = [F (3, 28) = 0.42, p = 0.741] respectively.

Table 4.24. Comparisons of Thickness in Dentate gyrus and Hippocampusin Wistar Rats

Stage of Treatment	Region	Dose (mg/kg)		Group (n = 6)	Mean ± SEM (mm)	*t* – value	*p*-value
Gestation	DG	200	At	Control	0.014± 0.001	-0.54	0.598
				Treated	0.014 ± 0.001		
			After	Control	0.137 ± 0.025	0.53	0.601
				Treated	0.120 ± 0.011		
	HC		At	Control	0.018 ± 0.007	3.37	0.005
				Treated	0.022 ± 0.001		
			After	Control	0.102 ± 0.017	-0.60	0.561
				Treated	0.115 ± 0.016		
Lactation	DG	400	At	Control	0.142 ± 0.023	-0.56	0.580
				Treated	0.124 ± 0.019		
			After	Control	0.092 ± 0.007	1.18	0.251
				Treated	0.113 ± 0.016		
	HC		At	Control	0.150 ± 0.017	-1.26	0.221
				Treated	0.118 ± 0.013		
			After	Control	0.150 ± 0.012	-1.26	0.275
				Treated	0.123 ± 0.017		
Adolescence	DG	800	At	Control	0.142 ± 0.023	-0.19	0.854
				Treated	0.136 ± 0.026		
			After	Control	0.092 ± 0.007	0.23	0.822
				Treated	0.096 ± 0.014		
	HC		At	Control	0.150 ± 0.017	-0.58	0.568
				Treated	0.135 ± 0.023		
			After	Control	0.150 ± 0.012	-0.63	0.533
				Treated	0.135 ± 0.022		
Adulthood	DG	N. saline	At	Control	0.125 ± 0.019		
		200	At	Treated	0.146 ± 0.022	0.70	0.488
		400	At	Treated	0.129 ± 0.015	0.15	0.881
		800	At	Treated	0.169 ± 0.027	1.46	0.155
	HC	N. saline	At	Control	0.139 ± 0.016		
		200	At	Treated	0.141 ± 0.020	0.05	0.957
		400	At	Treated	0.149 ± 0.020	0.34	0.735
		800	At	Treated	0.167 ± 0.023	1.00	0.327

DG: Dentate gyrus, HC: Hippocampus

Table 4.25. shows descriptive statistics for the estimation of the surface area for the hippocampus and dentate gyrus in Wistar rats treated with 200, 400 and 800 mg/kg doses of hyoscyamine fraction at gestation, lactation and adulthood respectively. The mean areas of the dentate gyrus at birth were 0.002 ± 0.001, 0.003 ± 0.001 $mm^2$for the control and treated groups respectively. The mean surface areas for the dentate gyrus at birth and on reaching adulthood were 0.002 ± 0.001, 0.058 ± 0.006 and 0.003 ± 0.001, 0.055 ± 0.001 $mm^2$for the control and treated groups respectively. The surface areas for the hippocampus at birth and on reaching adulthood were equally 0.005 ± 0.001, 0.115 ± 0.017 and 0.007 ± 0.001, 0.103 ± 0.001 $mm^2$for the groups respectively. At lactation, the mean surface area for the dentate gyrus was 0.061 ± 0.008, 0.048 ± 0.001, and 0.059 ± 0.002, 0.055 ± 0.002 $mm^2$for the control and treated groups at and on reaching adulthood, whereas or the hippocampus were 0.107 ± 0.008, 0.178 ± 0.002 and 0.090 ± 0.003, 0.097 ± 0.002 $mm^2$for the groups respectively. The mean dentate gyrus and hippocampus surface areas in the group treated at the adolescence 0.061 ± 0.008, 0.107 ± 0.008 and 0.055 ± 0.001, 0.134 ± 0.001 $mm^2$for the controls and the treated groups respectively. The areas after the animals reached adulthood were 0.048 ± 0.001, 0.048 ± 0.001 and 0.134 ± 0.001, 0.100 ± 0.006mm^2 for control and treated groups in the dentate gyrus and hippocampus respectively. For the groups treated at the adulthood, the mean hippocampus surface areas were 0.136 ± 0.001, 0.127 ± 0.015 and 0.162 ± 0.003mm^2 for the lower, medium and higher doses whereas, 0.078 ± 0.001, 0.076 ± 0.003 and 0.091 ± 0.003mm^2 for the dentate gyrus respectively. The mean control surface areas in both

cases, however, were 0.136 ± 0.010mm^2, for the hippocampus and 0.060 ± 0.002 mm^{2}for the dentate gyrus respectively.

Table 4.25.Descriptive Statistics of Surface Areasin the Hippocampus and Dentate gyrus in Wistar Rats

Stage of treatment	Region	Group	Group (n = 6)	Mean ± SEM (mm^2)	Min.	Max.
Gestation	DG	At	Control	0.002 ± 0.001	0.002	0.003
			Treated	0.003 ± 0.001	0.002	0.003
		After	Control	0.058 ± 0.006	0.044	0.072
			Treated	0.055 ± 0.001	0.055	0.055
	HC	At	Control	0.005 ± 0.001	0.005	0.005
			Treated	0.007 ± 0.001	0.007	0.008
		After	Control	0.115 ± 0.017	0.073	0.156
			Treated	0.103 ± 0.001	0.103	0.103
Lactation	DG	At	Control	0.061 ± 0.008	0.042	0.080
			Treated	0.059 ± 0.002	0.052	0.064
		After	Control	0.048 ± 0.001	0.048	0.048
			Treated	0.055 ± 0.002	0.049	0.060
	HC	At	Control	0.107 ± 0.008	0.089	0.126
			Treated	0.097 ± 0.002	0.092	0.101
		After	Control	0.178 ± 0.002	0.172	0.184
			Treated	0.090 ± 0.003	0.083	0.098
Adolescence	DG	At	Control	0.061 ± 0.008	0.042	0.080
			Treated	0.055 ± 0.001	0.055	0.055
		After	Control	0.048 ± 0.001	0.048	0.048
			Treated	0.039 ± 0.007	0.029	0.058
	HC	At	Control	0.107 ± 0.008	0.089	0.126
			Treated	0.134 ± 0.001	0.134	0.134
		After	Control	0.048 ± 0.001	0.048	0.048
			Treated	0.100 ± 0.006	0.088	0.116
Adulthood	DG	N. saline	Control	0.060 ± 0.002	0.054	0.067
		200	Treated	0.078 ± 0.001	0.077	0.079
		400	Treated	0.076 ± 0.003	0.067	0.081
		800	Treated	0.091 ± 0.003	0.083	0.099
	HC	N. saline	Control	0.136 ± 0.010	0.123	0.166
		200	Treated	0.136 ± 0.001	0.132	0.139
		400	Treated	0.127 ± 0.015	0.086	0.157
		800	Treated	0.162 ± 0.003	0.155	0.169

DG: Dentate gyrus, HC: Hippocampus

Table 4.26 shows comparisons for the estimation of the hippocampus and dentate gyrus surface areas in Wistar rats treated with hyoscyamine fractionof *D. stramonium* seeds in gestation, lactation and adulthood respectively. There were no statistically significant differences in the surface areas observed in both control and treated groups prenatally treated both at birth and after reaching adulthood for the dentate gyrus (DG), [F (1, 7) = 3.52, p = 0.110] and [F (1, 7) = -0.51, p = 0.628] respectively. However, a significant increase in the surface area was observed in the hippocampus at birth [F (1, 7) = 74.95, p <0.001], but not at adulthood [F (1, 7) = 4.70, p = 0.519] between the control and treated groups respectively. A similar trend was observed in the group treated at lactation, in that no statistically significant difference observed between the surface areas of the dentate gyrus (DG), [F (2, 11) = 3.62, p = 0.070] of the control and treated groups both during and after lactation (adulthood). However, a significant decrease in the hippocampal surface areas (HC), [F (2, 11) = 136.69, p <0.001] were observed after the animals reached adulthood. At adolescence, no statistically significant differences were observed in the surface areas of the dentate gyrus both during adolescence and after reaching adulthood, [F (2, 11) = -0.51, p = 0.628] of the control and treated groups. However, a significant increase was observed in the hippocampus surface areas (HC), [F (2, 11) = 18.51, p = 0.001] both at adolescence and after reaching adulthood between the control and treated groups. For the groups treated in adulthood, a significant increase in the dentate gyrus surface areas, DG = [F (3, 15) = 23.89, p <0. 0.001]; was observed in all

the treated groups when compared to control. The hippocampal surface area, however, showed no significant difference in all the treated groups, HC = [F (3, 15) = 2.70, p = 0.092] when compared to control.

Table 4.26. Comparisons of Surface Areas in the Hippocampus and Dentate gyrus in Wistar rats

Stage of Treatment	Region	Dose (mg/kg)		Group (n = 6)	Mean ± SEM (mm^2)	*t* – value	*p*-value
Gestation	DG	200	At	Control	0.002 ± 0.001	1.88	0.110
				Treated	0.003 ± 0.001		
			After	Control	0.058 ± 0.006	0.51	0.615
				Treated	0.055 ± 0.001		
	HC	200	At	Control	0.005 ± 0.001	8.66	< 0.001
				Treated	0.007 ± 0.001		
			After	Control	0.115 ± 0.017	-0.68	0.519
				Treated	0.103 ± 0.001		
Lactation	DG	400	At	Control	0.061 ± 0.008	-0.39	0.707
				Treated	0.059 ± 0.002		
			After	Control	0.048 ± 0.001	1.18	0.267
				Treated	0.055 ± 0.002		
	HC	400	At	Control	0.107 ± 0.008	-1.69	0.125
				Treated	0.097 ± 0.002		
			After	Control	0.178 ± 0.002	-15.07	< 0.001
				Treated	0.090 ± 0.003		
Adolescence	DG	800	At	Control	0.061 ± 0.008	-1.00	0.342
				Treated	0.055 ± 0.001		
			After	Control	0.048 ± 0.001	-1.50	0.167
				Treated	0.039 ± 0.007		
	HC	800	At	Control	0.107 ± 0.008	4.21	0.002
				Treated	0.134 ± 0.001		
			After	Control	0.178 ± 0.002	-13.42	< 0.001
				Treated	0.097 ± 0.002		
Adulthood	DG	N. saline	At	Control	0.060 ± 0.002		
		200	At	Treated	0.078 ± 0.001	4.94	< 0.001
		400	At	Treated	0.076 ± 0.003	4.31	0.001
		800	At	Treated	0.091 ± 0.003	8.42	< 0.001
	HC	N.	At	Control	0.136 ± 0.010		
		200	At	Treated	0.136 ± 0.001	-0.05	0.958
		400	At	Treated	0.127 ± 0.015	-0.69	0.506
		800	At	Treated	0.162 ± 0.003	1.99	0.069

DG: Dentate gyrus, HC: Hippocampus, N. saline: Normal saline

Table 4.27, showed the descriptive statistics of hippocampus volume estimate in Wistar rats treated with hyoscyamine fraction of *D. stramonium* seeds in gestation, lactation, adolescence and adulthood respectively. The mean hippocampus volume in the treated group was higher in the prenatally treated group (07.15 ± 0.04 mm^3) in comparison with the control (05.98 ± 0.08 mm^3). The trend persisted between the groups even in adulthood. A similar observation was made in the animal groups treated with 400 mg/kg of the hyoscyamine fraction during lactation with the control group, 37.39 ± 0.15 and the treated, 42.96 ± 0.20 mm^3. However, when the volume was estimated at adulthood, the mean volume of the treated group was lower than (26.64 ± 0.21 mm^3) the control (42.02 ± 0.08 mm^3). The pattern in the adolescent group, however, showed a decrease in the mean hippocampus volume in the treated group as; 30.52 ± 0.13 mm^3, 30.97 ± 0.19 mm^3 at adolescence and when reaching adulthood, when compared to their controls, as 37.39 ± 0.15 mm^3 and 42.02 ± 0.08 mm^3 respectively. The mean volume estimate of the treated groups in the adulthood at low (46.95 ± 0.16mm^3) and medium (47.66 ± 0.28 mm^3) doses exposure was slightly lower when compared to the control group (47.82 ± 0.09 mm^3), except for the highest dose where the mean volume was higher, 59.85 ± 0.33 mm^3 when compared to the control group.

Table 4.27. Descriptive Statisticsof Hippocampus volume in Wistar Rats

Stage	Dose (mg/kg)		Group (6)	Mean ± SEM (mm^3)	Min.	Max.
Gestation	200	At	Control	05.98 ± 0.08	05.73	06.20
			Treated	07.15 ± 0.04	07.07	07.27
		After	Control	26.57 ± 0.05	26.37	26.67
			Treated	34.59 ± 0.12	34.17	34.84
Lactation	400	At	Control	37.39 ± 0.15	37.03	37.85
			Treated	42.96 ± 0.20	42.53	43.57
		After	Control	42.02 ± 0.08	41.87	42.22
			Treated	26.64 ± 0.21	26.00	27.26
Adolescence	800	At	Control	37.39 ± 0.15	37.03	37.85
			Treated	30.52 ± 0.13	30.14	30.86
		After	Control	42.02 ± 0.08	41.87	42.22
			Treated	30.97 ± 0.19	30.29	31.34
Adulthood	N.saline	At	Control	47.82 ± 0.09	47.67	48.12
	200		Treated	46.95 ± 0.16	46.58	47.41
	400		Treated	47.66 ± 0.28	46.82	48.37
	800		Treated	59.85 ± 0.33	58.66	60.65

Table 4.28., shows a comparison test for a hippocampus volume estimate in the Wistar rats treated with hyoscyamine fraction at gestation, lactation, adolescence and adulthood stages. No significant difference (p = 0.088) in the mean hippocampus volume between the control and treated groups at gestation groups. However, a significant difference was observed (p< 0.001) when the group volume was estimated after reaching adulthood. A similar observation was made in the lactation group, where (p = 0.924, p< 0.001) at and after lactation respectively. For the adolescence group, a significant difference was observed (p< 0.001) however, as the group reached adulthood, no significant difference was observed p = 0.072. For the groups treated at adulthood, also no statistically significant difference (p> 0.05) was observed at low and medium doses exposure except for the highest dose (p< 0.001) where a significant increase in the hippocampus volume was observed.

Table 4.28.Comparison of Hippocampus Volume in Wistar rats

Stage of treatment	Dose (mg/kg)		Group	Mean ± SEM (mm^3)	CE	*p*-value
Gestation	200	At	Control	06.20 ± 0.17	0.03	0.088
			Treated	07.07 ± 0.17	0.02	
		After	Control	26.62 ± 0.30	0.01	< 0.001
			Treated	34.60 ± 0.39	0.01	
Lactation	400	At	Control	37.60 ± 0.39	0.01	0.924
			Treated	42.78 ± 0.43	0.01	
		After	Control	42.19 ± 0.41	0.01	< 0.001
			Treated	26.50 ± 0.40	0.02	
Adolescence	800	At	Control	37.60 ± 0.39	0.01	< 0.001
			Treated	30.61 ± 0.33	0.01	
		After	Control	42.19 ± 0.41	0.01	0.072
			Treated	31.20 ± 0.43	0.01	
Adulthood	Control	At	Control	47.68 ± 0.49	0.01	
	200	At	Treated	46.93 ± 0.49	0.01	0.571
	400	At	Treated	47.44 ± 0.48	0.01	0.858
	800	At	Treated	60.00 ± 0.56	0.01	< 0.001

Plate XXIII showed a photomicrograph of Cornus Ammonis 1 (CA1) area of hippocampus in Wistar rat pups treated prenatally with equivalent bodyweightnormal saline from GD 15 – 20. Normal histology of CA1 area was observed featuring evenly spread immature neuronal and neuroglial cells in the stratum oriens (SO), stratum radiatum (SR) and pyramidal cell layer of the control group. Severalcell death or apoptosis (white arrows) and pyknotic cells (white arrowheads) which probably suggested high mitotic activities patterning to cellular trimming and neuronal plasticity for the differentiation, when compared to the treated group in the plate were, a significant amount of mesenchymal tissues was observed probably suggesting delayed differentiation and migration. The plate also shows patches of neuroglial cells (red arrows).

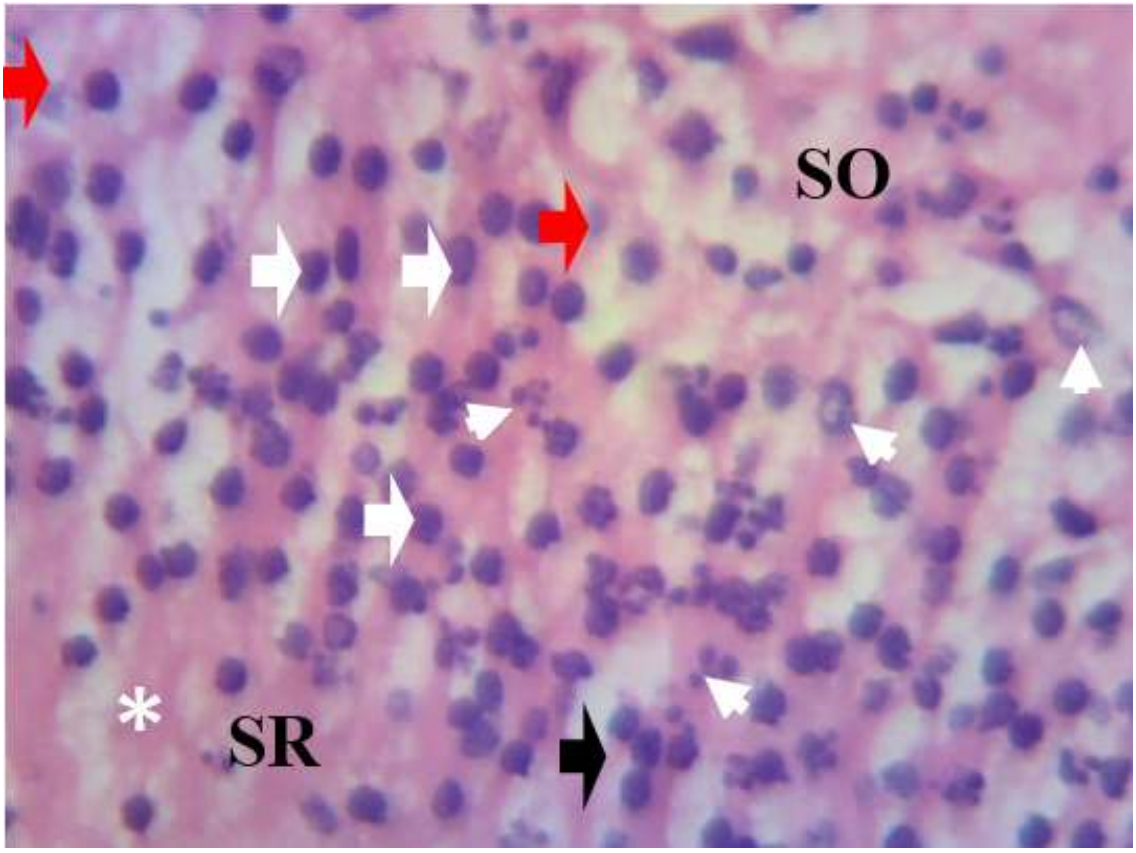


Plate XXIII: Photomicrograph of Cornu Ammonis (CA1) region of the hippocampus in a control group of Wistar rat pups at PND 1 after being treated prenatally with 200 mg/kgof normal saline from GD 15 – 20 (H and E, x 400).

SO = stratum oriens, SR = stratum radiatum, ✚ = mesenchymal tissues, ⇨ = Necrotic cells, ➞ = poorly differentiated neuronal cells, ➔ = Neuroglial cells, △ = pyknotic cells.

Plate XXIV showed a photomicrograph of Cornus Ammonis 1 (CA1) area of hippocampus in Wistar rat pups treated prenatally with 200 mg/kg of hyoscyamine fraction of *D. stramonium*seeds from GD 15 – 20.Comparatively, the number of the cells was slightly higher, densely packed and thicker in the treated groups when compared to the control. Equally, the SO showed a slight density in the control group compared to the treated group, where the region showed thinner and abundant migrating cells toward the cortical margin.

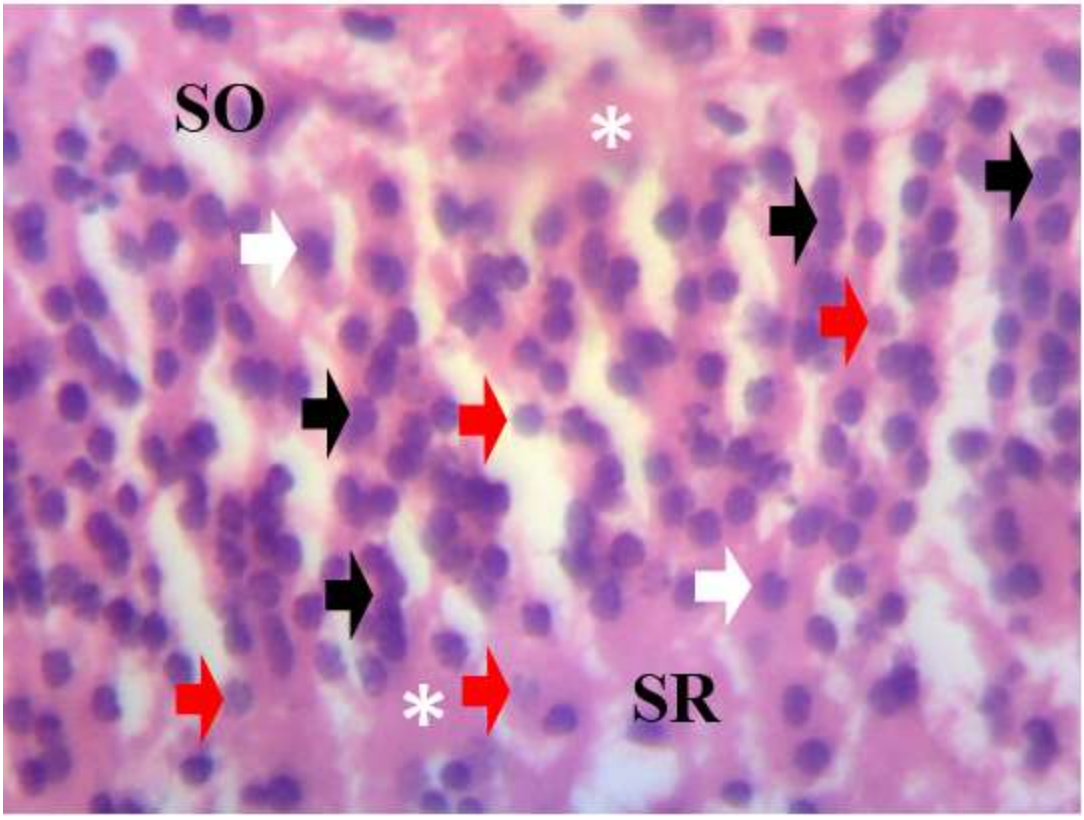


Plate XXIV: Photomicrograph of Cornu Ammonis (CA1) region of the hippocampus in of Wistar rat pups at PND 1 after being treated prenatally with 200 mg/kg hyoscyamine fraction of *D. stramonium*seeds from GD 15 – 20. (H and E, x 400).

SO = stratum oriens, SR = stratum radiatum, ✠ = mesenchymal tissues, ⇨ = Necrotic cells, ➞ = poorly differentiated neuronal cells, ➞ = Neuroglial cells.

In plate XXV, a photomicrograph showed Cornus Ammonis 3 (CA3) area of hippocampus in the control Wistar rats treated prenatally with 200 mg/kg normal saline from GD 15 – 20. Showed more organized and evenly distributed immature neuronal and neuroglial cells which space out. The regional organization of the CA3 was more prominent in the control group compared to the treated group. Equally, the SO region was slightly spacious in the control than the treated group.

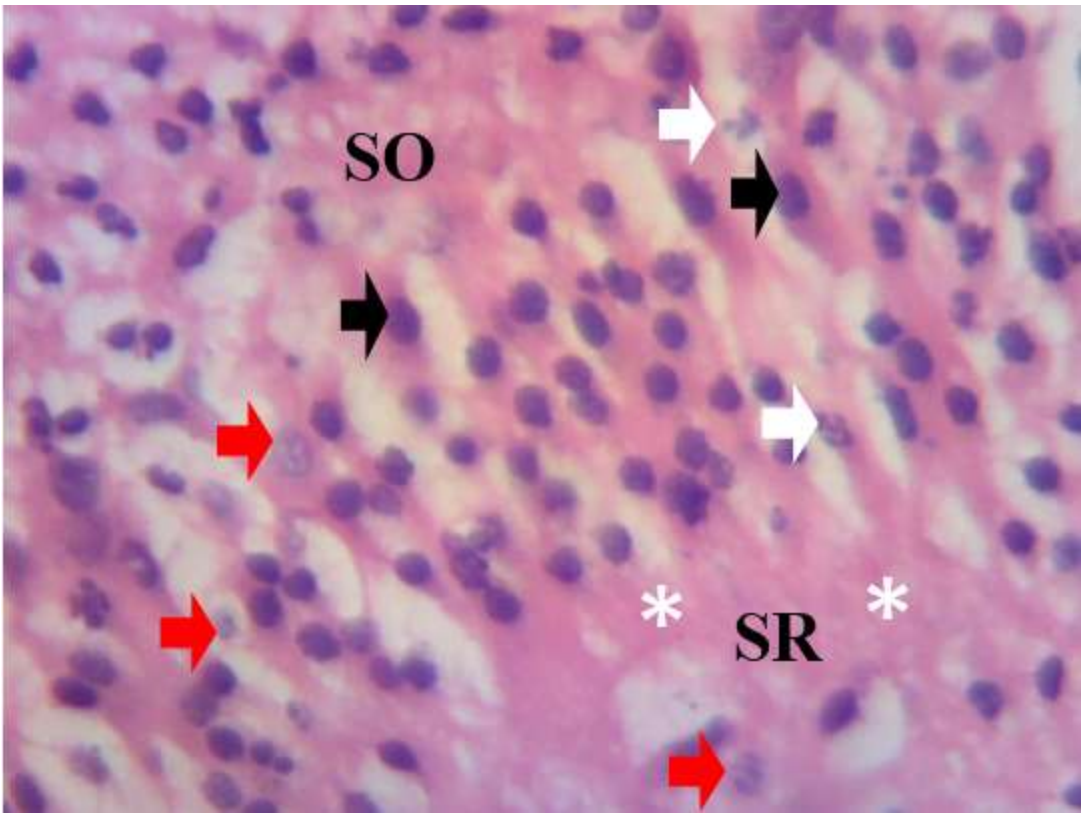


Plate XXV: Photomicrograph of Cornu Ammonis (CA3) region of the hippocampus in a control group of Wistar rat pups at PND 1 after being treated prenatally with 200 mg/kg normal saline from GD 15 – 20. (H and E, x 400).

SO = stratum oriens, SR = stratum radiatum, ✚ = mesenchymal tissues, ⇨ = Necrotic cells, ➞ = poorly differentiated neuronal cells, ➔ = Neuroglial cells.

In plate XXVI, a photomicrograph showed Cornus Ammonis 3 (CA3) area of hippocampus in the Wistar rats treated prenatally with 200 mg/kg of hyoscyamine fraction from GD 15 – 20. A significant increase in the number of cells was observed in the CA3 region of the treated group compared to the control. Also, significant amounts of pyknotic cells (white arrows) and apoptotic cells (white arrowheads) amidst the immature neurons (black arrows) and neuroglial cells (red arrows) were more visible in the treated compared to the control group. It also shows the populationof more densely slightly shrunken, hyperchromic and poorly developed neuronal cells. Also, significant amounts of pyknotic cells (white arrows) and apoptotic cells (white arrowheads) amidst the immature neurons (black arrows) and neuroglial cells (red arrows), that were hyperchromic were more visible in the treated compared to the control group.

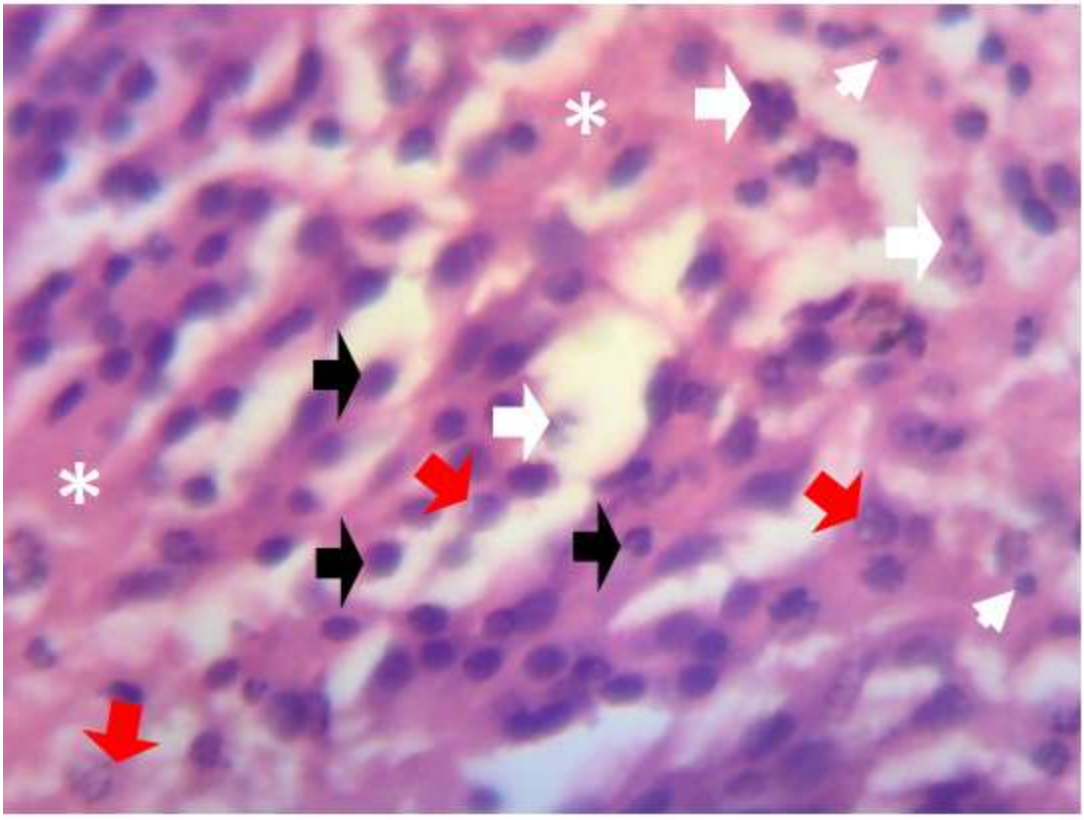


Plate XXVI: Photomicrograph of Cornu Ammonis (CA3) region of the hippocampus in Wistar rat pups at PND 1 after being treated prenatally with 200 mg/kg hyoscyamine fraction of *D. stramonium*seeds from GD 15 – 20 (H and E, x 400).

✱ = mesenchymal tissues, ⇨ = Necrotic cells, ➞ = poorly differentiated neuronal cells, ➞ = neuroglial cells, △ = pyknotic cell.

Plate XXVII showed a photomicrograph of dentate gyrus (DG) area of hippocampus in control Wistar rat pups treated prenatally with 200 mg/kg normal saline GD 15-20. A sparsely populated but relatively organized DG region was observed in the control compared to the densely populated (hyperplasic) but relatively organized DG region in the treated group. The mesenchymal tissues were relatively fewer, consisted of sparsely populated immature neuronal cells when compared to the treated group.

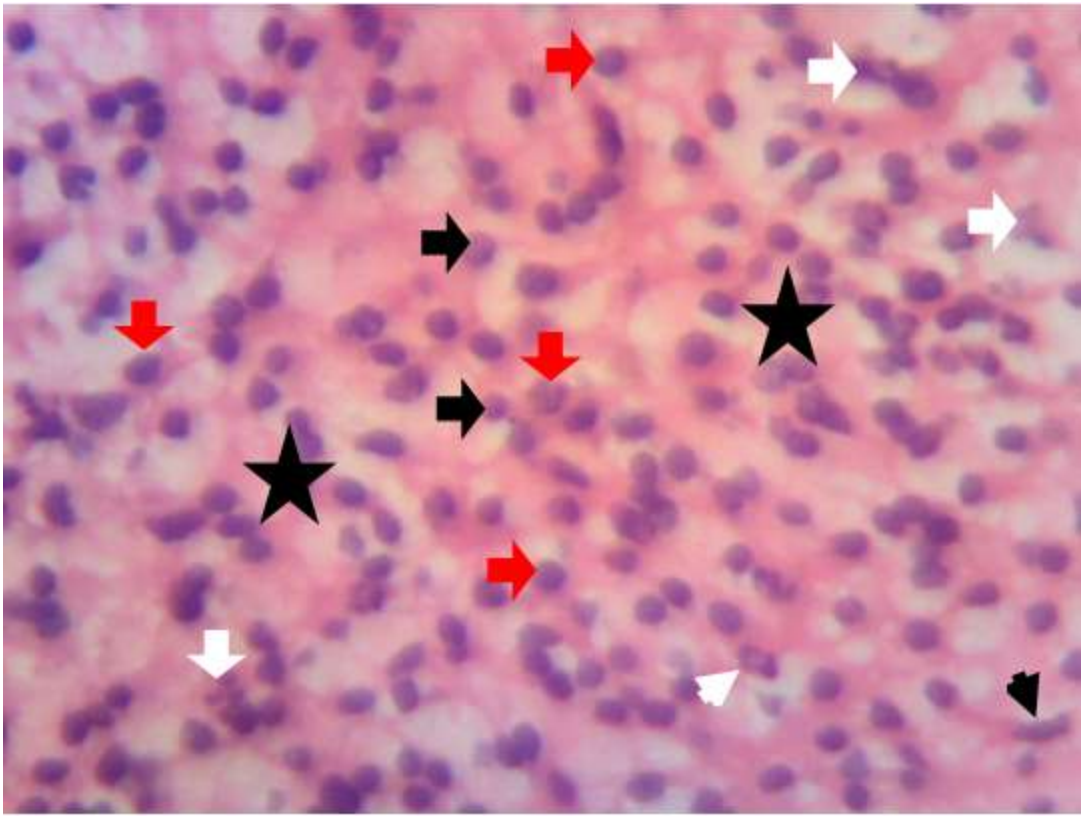


Plate XXVII: Photomicrograph of Dentate gyrus (DG) region of the hippocampus in a control group of Wistar rat pups at PND 1 after being treated prenatallywith 200 mg/kg normal saline from GD 15 – 20. (H and E, x 400).

⇨ = poorly differentiated granule cells, ➞ = Pyramidal cells, ◄ = Microglia, △ = pyknotic cells, ➔ = granule cells, ★ = densely populated granule cells.

Plate XXVIII showed a photomicrograph of dentate gyrus (DG) area of hippocampus in control Wistar rat pups treated prenatally with 200 mg/kg of hyoscyamine fraction of *D.stramonium*from GD 15-20 were densely populated with irregularly organized DG region was observed compared to the control group. The mesenchymal tissues were relatively more compared to the control group with densely populated immature neuronal featuring necrotic (white arrowheads) and neuroglial cells (red arrows).

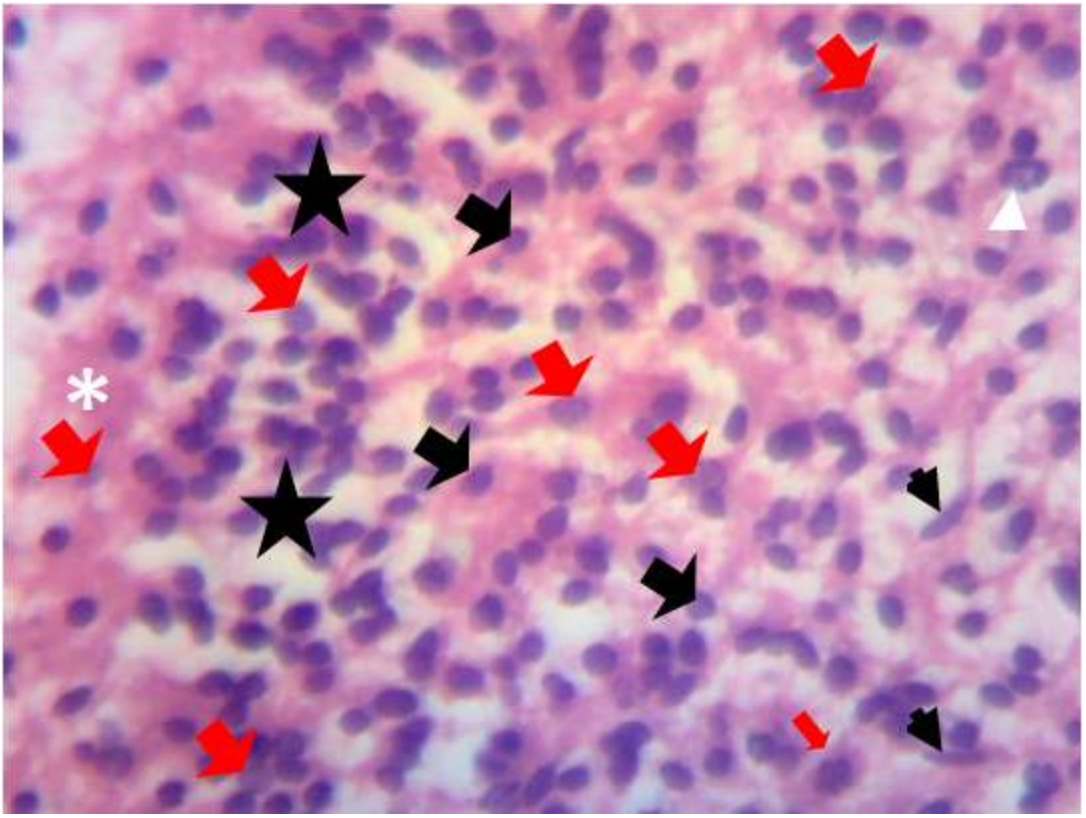


Plate XXVIII: Photomicrograph of Dentate gyrus (DG) of the hippocampus in Wistar rat pups at PND 1 after being prenatally treated with 200 mg/kg hyoscyamine fraction*D. stramonium*seeds from GD 15 – 20 (H and E, x 400).

✱ = mesenchymal tissues, ⇨ = poorly differentiated granule cells, ➞ = Pyramidal cells, ◀ = Microglia, △ = pyknotic cells, ➔ = granule cells, ★ = densely populated granule cells.

Plate XXIX shows a photomicrograph of the CA1 region of adult Wistar rats at the 12th week after being treated prenatally with 200 mg/kg normal saline from GD 15 – 20 respectively. The histology of the control shows a normal cytoarchitecture of an organized CA1 region with SO, and SR. Below which is the SR region bearing blood vessels (red arrow) and above was SO showing patches of mature neurons (black arrow).

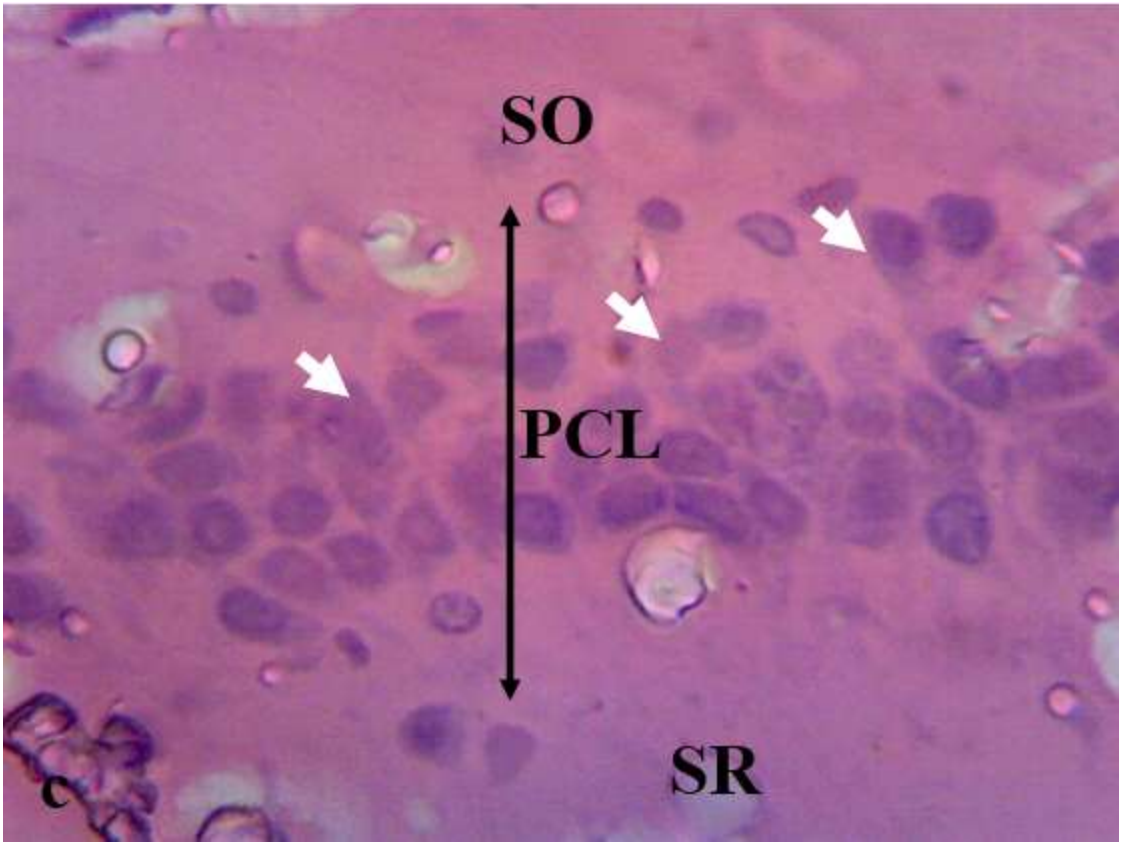


Plate XXIX: Photomicrograph of Cornu Ammonis (CA1) region of the hippocampus in control Wistar rat at PND 85 after treatment with 200 mg/kg normal saline GD 15 – 20(H and E, x 400).

SO = Stratum Oriens, SR = stratum radiatum, PC=pyramidal cells, (H and E, x 400).

Plate XXX shows a photomicrograph of the CA1 region of adult Wistar rats at PND 85 after being treated prenatally with 200 mg/kg of hyoscyamine fraction of *D. stramonium* from GD 15 – 20 respectively.Comparatively, the treated group showed hypochromic (white arrows) but a relatively thin region of the pyramidal cell layers (PCL).The photomicrograph poorlyorganized layer of scanty but hypochromic pyramidal cells (PC) in the region.

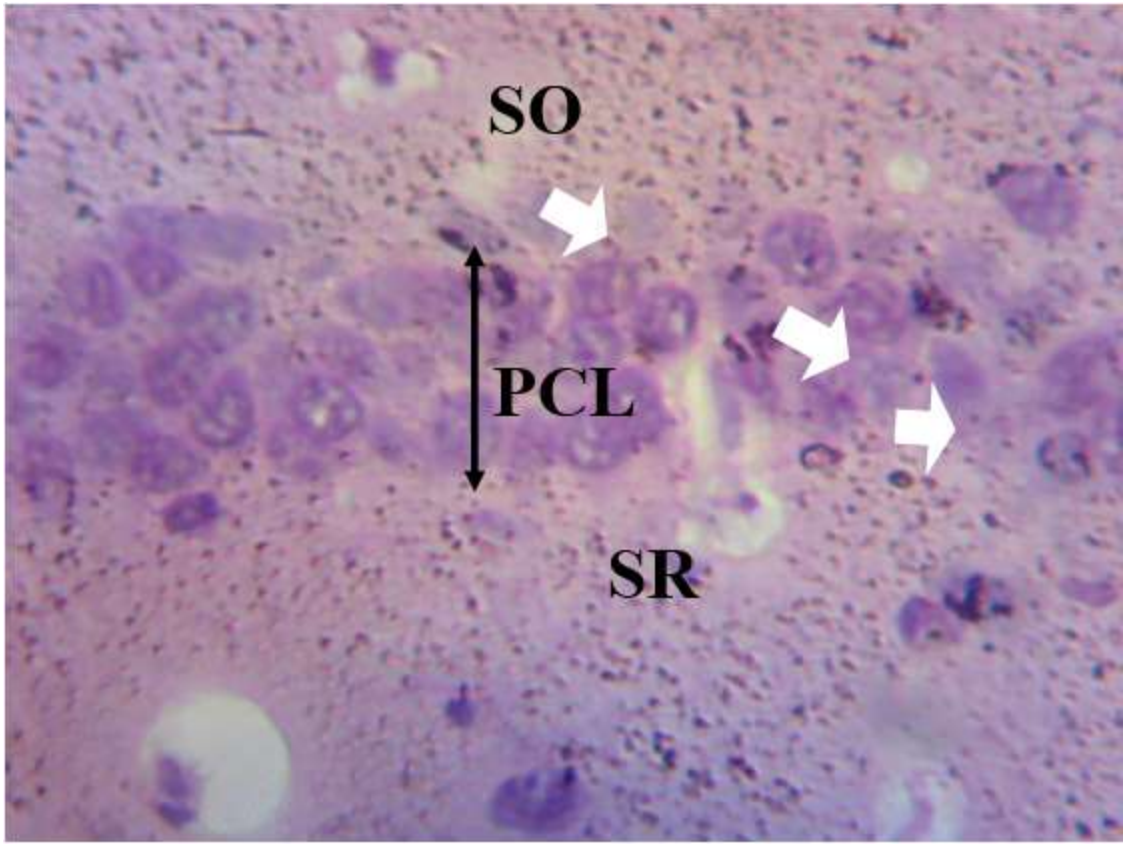


Plate XXX: Photomicrograph of Cornu Ammonis (CA1) region of the hippocampus in Wistar rat at PND 85 week after treated 200 mg/kg hyoscyamine fraction of *D. stramonium*seeds from GD 15 – 20(H and E, x 400).

SO = Stratum Oriens, SR = stratum radiatum, ⇨ = Poorly developed pyramidal cells, (H and E, x 400).

Plate XXXI shows a photomicrograph of the CA3 region of adult Wistar rats at PND 85 after being treated prenatally with200 mg/kg normal saline from GD 15 – 20 as a control. Organized patterns of cellular layering and alignment were observed in the region featuring SO, SR and SLM and patches of blood vessels. The photomicrograph also featured a well-organized pyramidal cell layer (black arrows).

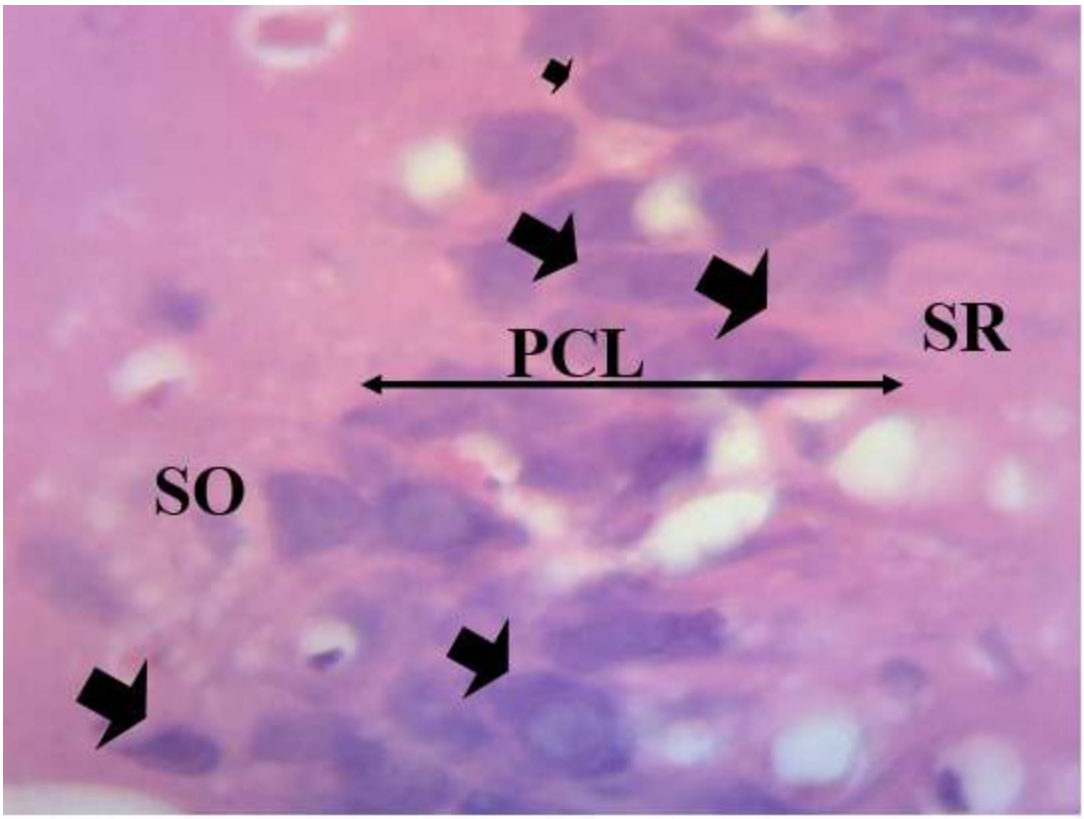


Plate XXXI: Photomicrograph of Cornu Ammonis (CA3) region of the hippocampus in control groups of Wistar rat pups at PND 85 after treatment with 200 mg/kg normal saline from GD 15 – 20(H and E, x 400).

PCL = Pyramidal cell layer, SO = stratum oriens, SR = stratum radiatum, ➞ = normal pyramidal cells.

Plate XXXII shows a photomicrograph of the CA3 region of adult Wistar rats at PND 85 after being treated prenatally with 200 mg/kg of hyoscyamine fraction of *D. stramonium*from GD 15 – 20. The pyramidal cell layer (PCL) showed relatively misaligned and poorly developed neurons (white arrows).On the other hand, showed the same region but in the treated group, where relatively scanty and poorly developed neuronal cells (white arrows) were observed. Similarly, the SO, SR and SLM regions developed, however, comparatively thinner compared to the control.

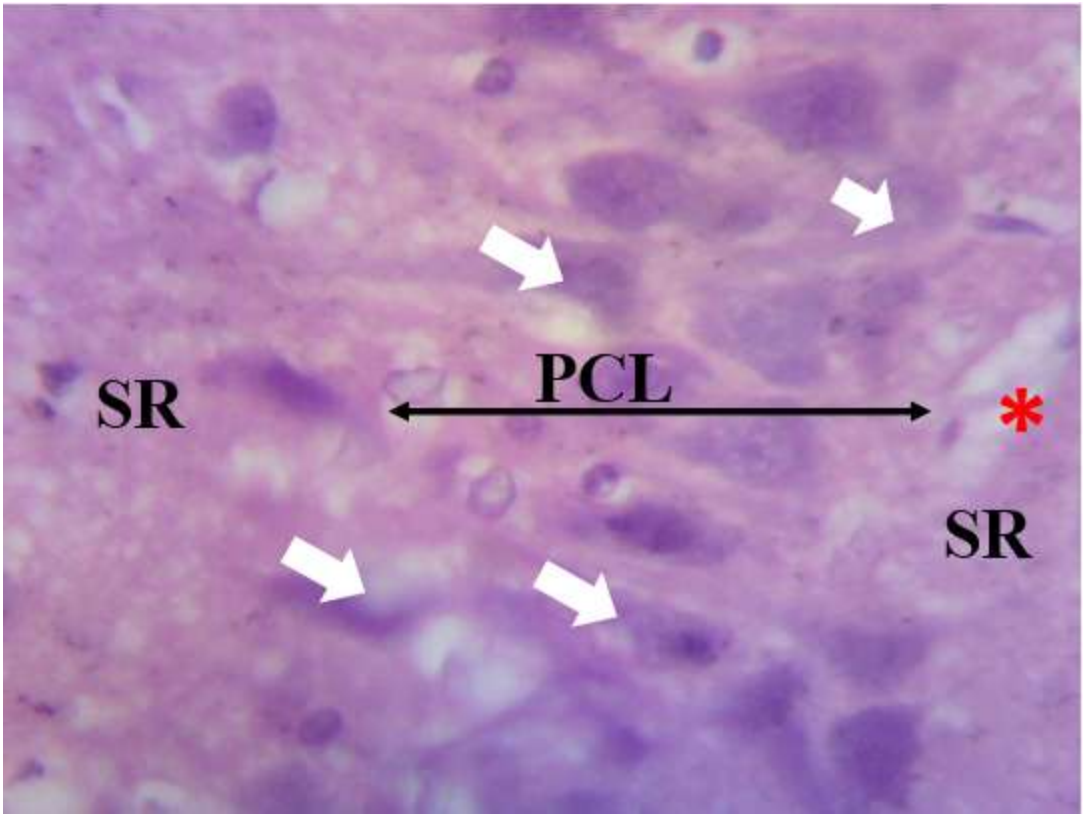


Plate XXXII: Photomicrograph of Cornu Ammonis (CA3) region of the hippocampus in Wistar rat pups at PND 85 after treatment with 200 mg/kghyoscyamine fraction of *D. stramonium* seeds from GD 15 – 20(HandE, x 400).

PCL = Pyramidal cell layer, SO = stratum oriens, SR = stratum radiatum, ⇨ = Poorly developed pyramidal cells * = blood vessels

Plate XXXIII showed a photomicrograph of the dentate gyrus in a control group of adult Wistar rat PND 85 that was treated prenatally with 200 mg/kg normal saline from GD 15 – 20. The plate shows a normal granular cell layer (GCL) of the DG area below of which the subventricular zone (SVZ) and polymorphic layer featuring immature cells. The area also featured densely packed stained granular cells.

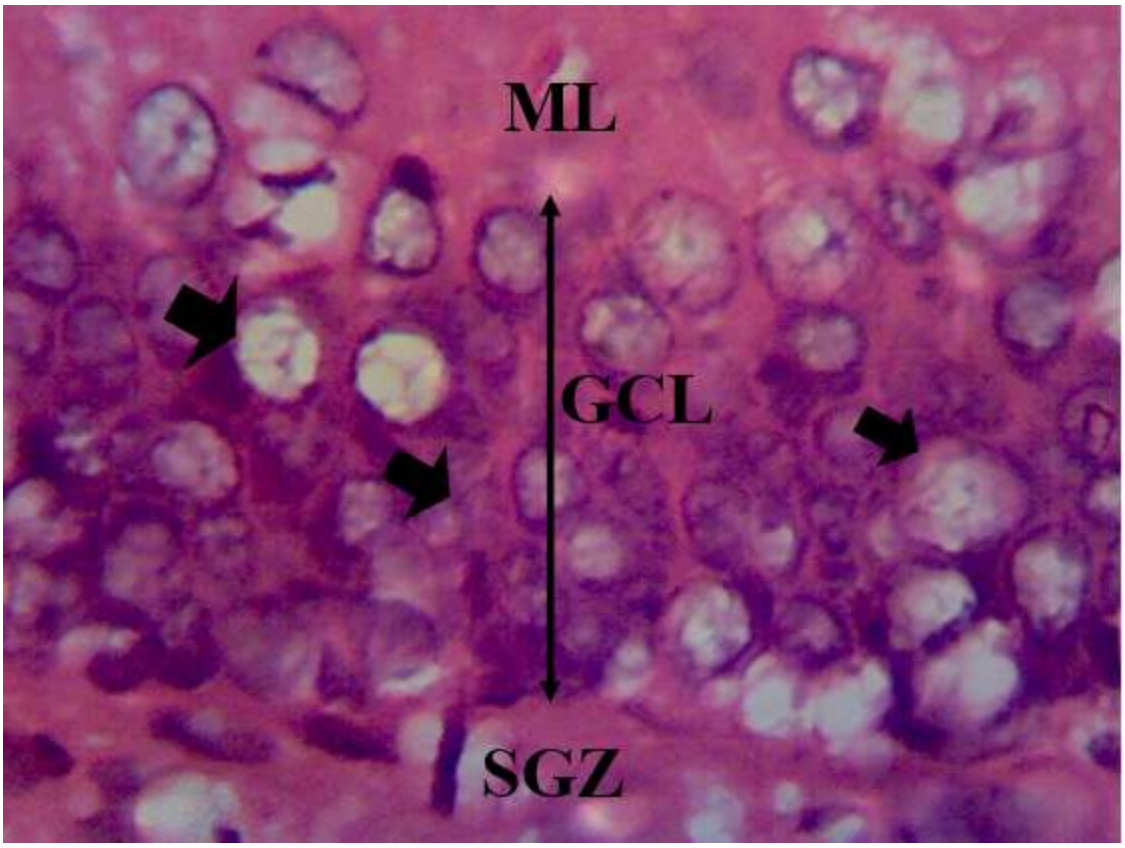


Plate XXXIII: Photomicrograph of Dentate gyrus (DG) region of the hippocampus in control Wistar rat at PND 85 after treated prenatally with200 mg/kgnormal salinefrom GD 15 – 20 (HandE, x 400).

GCL = granular cell layer, SGZ = subgranular zone, ML = Molecular layer, ⟶ = granular cells, (H and E, x 400).

Plate XXXIV showed a photomicrograph of the dentate gyrus inof adult Wistar rat at PND 85 that was treated prenatally with 200 mg/kg of hyoscyamine fraction of *D. stramonium* from GD 15 – 20. The plate showed a slightly denser layer of the granule cell (GC). Above was the molecular layer (ML) and below, SVZ and PM layer.The plate shows a relatively hyperchromic (black arrowhead), vacuolated (black asterisk) and pyknotic cells (white arrowhead), necrotic cells (red asterisk) when compared to the control group.

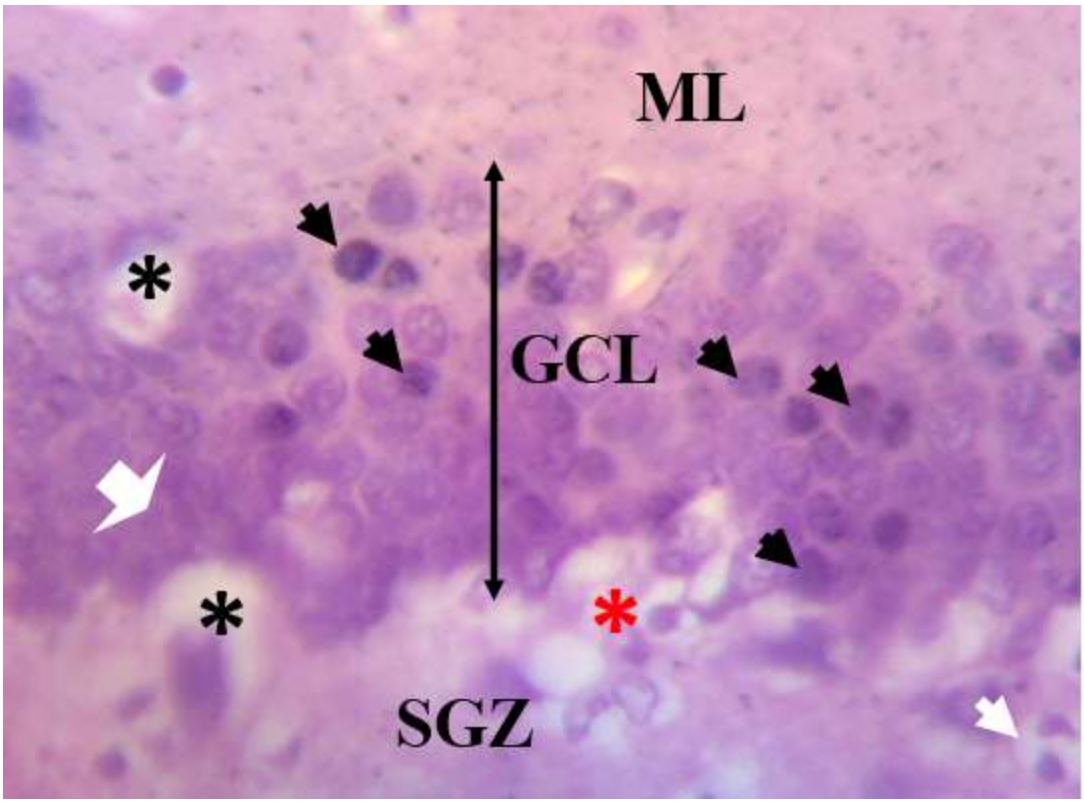


Plate XXXIV: A photomicrograph of Dentate gyrus (DG) region of the hippocampus in Wistar rat at PND 85 after treated prenatally with200 mg/kg hyoscyamine fraction of *D. stramonium*seeds from GD 15 – 20 (HandE, x 400).

GCL = granular cell layer, SGZ = subgranular zone, ML = Molecular layer, ✱ = necrotic cells, ◀ = hyperchromic cells, * = Karyorrhectic cells, △ = pyknotic cells. (H and E, x400).

Plate XXXV presented the CA1 region of the hippocampus in Wistar rat at PND 42 week after being breastfed by a dam that received 400 mg/kg normal saline at lactation withfrom the PND 21 – 42. The region showed a relatively denser and more organized CA1 region featuring well developed SO, below which is a stratum radiatum (SR). A significant number of well – developed pyramidal cell (black arrows) in the PCL region was also observed compared to the treated group.

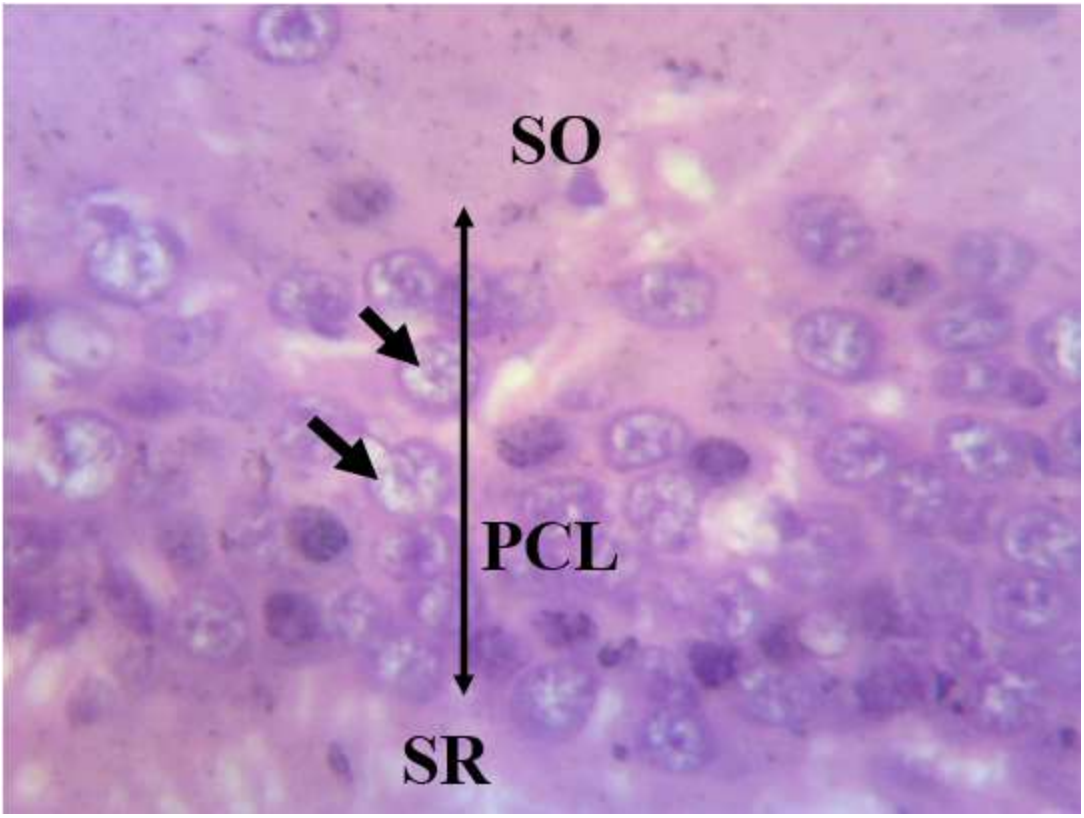


Plate XXXV: A photomicrograph of Cornu Ammonis (CA1) region of the hippocampus in control Wistar rat group at PND 42 after being breastfed by a dam that received 400 mg/kg normal saline fromPND 21 – 42(H and E, x400).

PCL = pyramidal cell layer, SO = stratum oriens, SR = stratum radiatum, ➞ = pyramidal cells, (H and E, x400).

Plate XXXVI presented the CA1 region of the hippocampus in Wistar rat at PND 42 after being breastfed by a dam that received 400 mg/kg of hyoscyamine fraction of *D. stramonium*at lactation from PND 21 – 42. The cells showed poorly developed (white arrows), cytoplasmic vacuolated (black asterisk) and pyknotic pyramidal cells (arrowhead) that were hypochromic and less dense (white arrows) when compared to the control group.

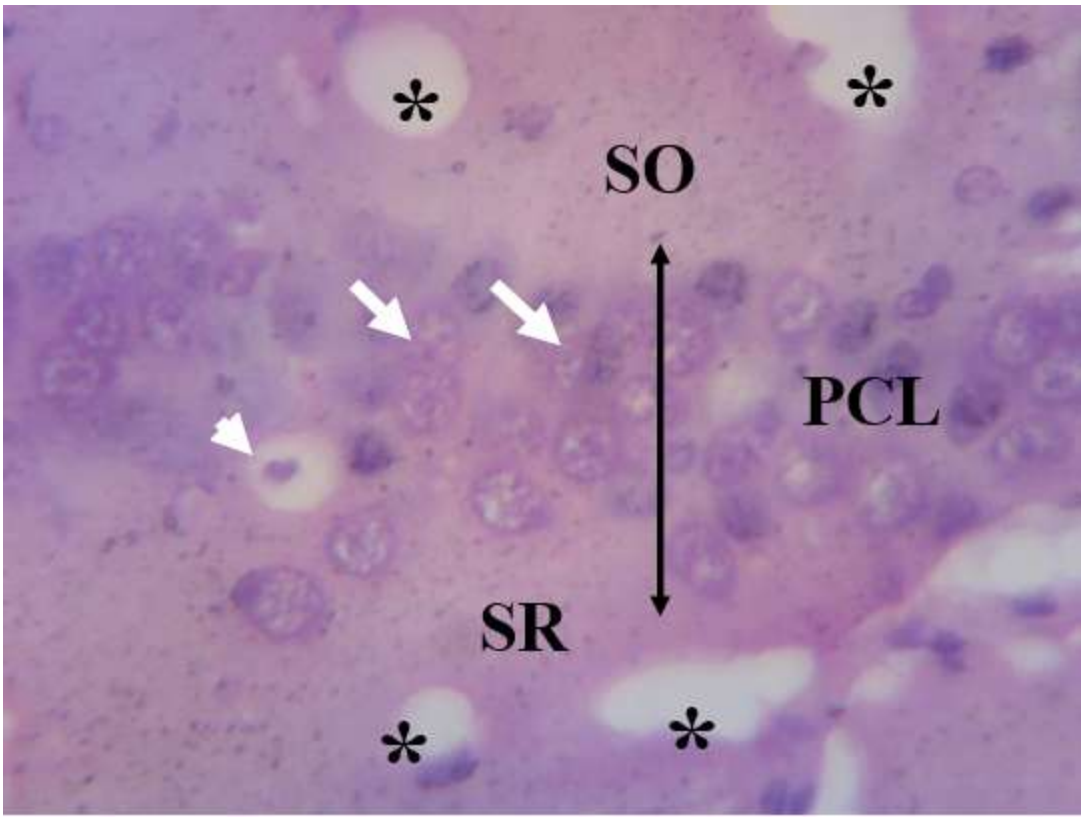


Plate XXXVIphotomicrograph of Cornu Ammonis (CA1) region of the hippocampus in Wistar rat at PND 42 after being breastfed by a dam that received 400 mg/kg hyoscyamine fraction of *D. stramonium*at lactation fromPND 21 – 42(H and E, x400).

PCL = pyramidal cell layer, SO = stratum oriens, SR = stratum radiatum, ⟶ = pyramidal cells, , ⇨ =poorly developed pyramidal cells, * = cytoplasmic vacuolated cells, △ = pyknotic cells. (H and E, x400).

Plate XXXVII was the CA3 region of the hippocampus at PND 42after being breastfed by a dam that received 400 mg/kg normal saline at lactation from the PND 21 – 42. The region showed a clear outline of normal,developed but slightly hyperchromic neurons (black arrows) and a few numbers of oligodendrocytes surrounded by haloes (red arrows) in comparison to the treated group.

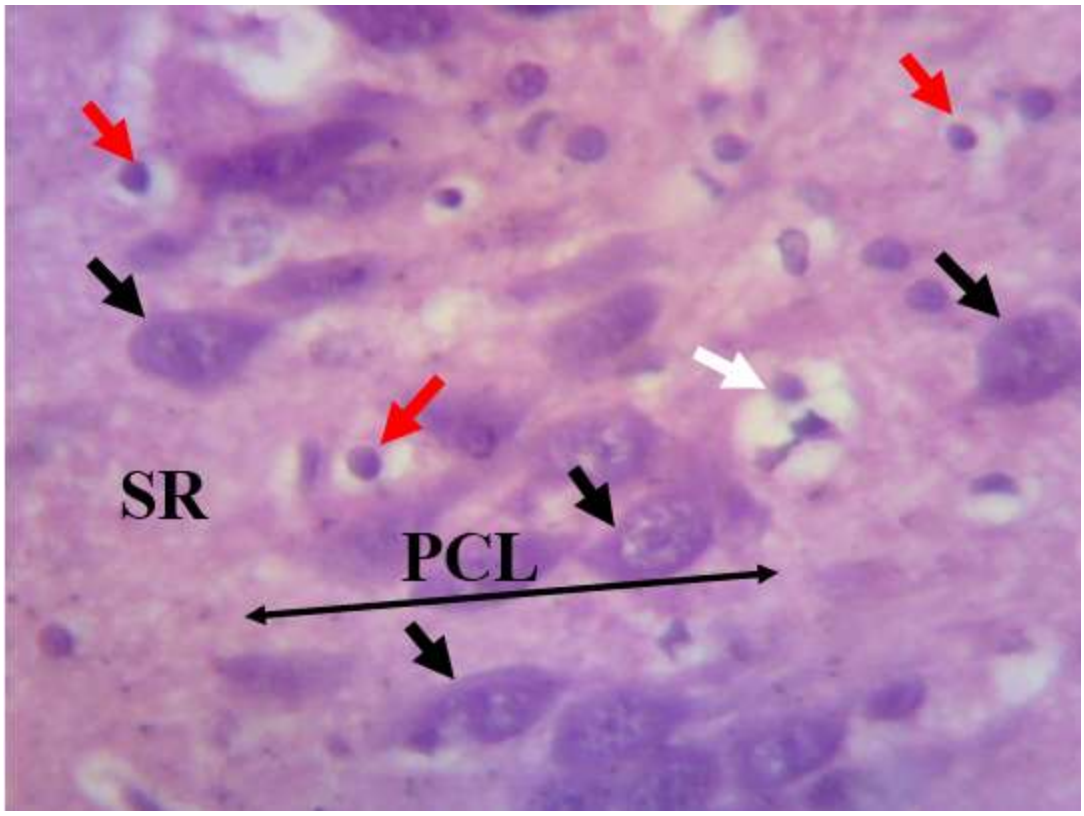


Plate XXXVII: A photomicrograph of Cornu Ammonis (CA3) region of the hippocampus in a control group of Wistar rat at PND 42 after being breastfed by a dam that received 400 mg/kg normal saline from PND 21 – 42 (H and E, x400).

PCL = pyramidal cell layer, ⇨necrotic pyramidal cells area, ➞ = pyramidal cells, ➔ = oligodendrocytes.

Plate XXXVIII was the CA3 region of the hippocampus at PND 42after being breastfed by a dam that received 400 mg/kg of hyoscyamine fraction of *D. stramonium*at lactation from the PND 21 – 42. The pyramidal cells in the region were marked by a hypochromic PCL with scanty and poorly developed or degenerating neurons (white arrow).

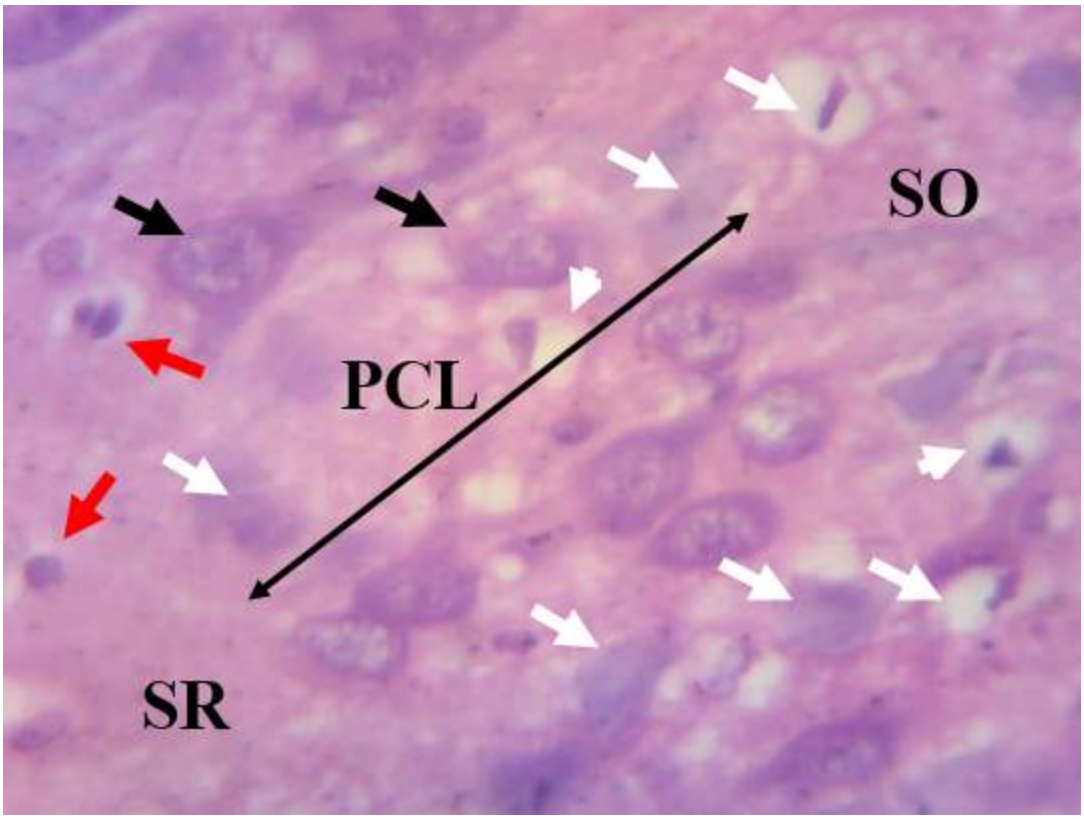


Plate XXXVIII: A photomicrograph of Cornu Ammonis (CA3) region of the hippocampus in Wistar rat at PND 42 after being breastfed by a dam that received 400 mg/kg hyoscyamine fraction of *D. stramonium*at lactation from PND 21 – 42 (H and E, x400).

PCL = pyramidal cell layer, SO = Stratum Oriens, SR = stratum radiatum, ⇨necrotic pyramidal cells area, → = pyramidal cells, △ = pyknotic cells➔ = oligodendrocytes.

Plate XXXIX shows a DG region of the hippocampus at PND 42after being breastfed by a dam that received 400 mg/kg normal saline at lactation from the PND 21 – 42. The plate showed an organized granular cell layer (GCL) region with molecular and active SGZ when compared to the treated. An array of densely packed but highly organized DG featuring oligodendrocytesin the SGZ regionwas also observed.

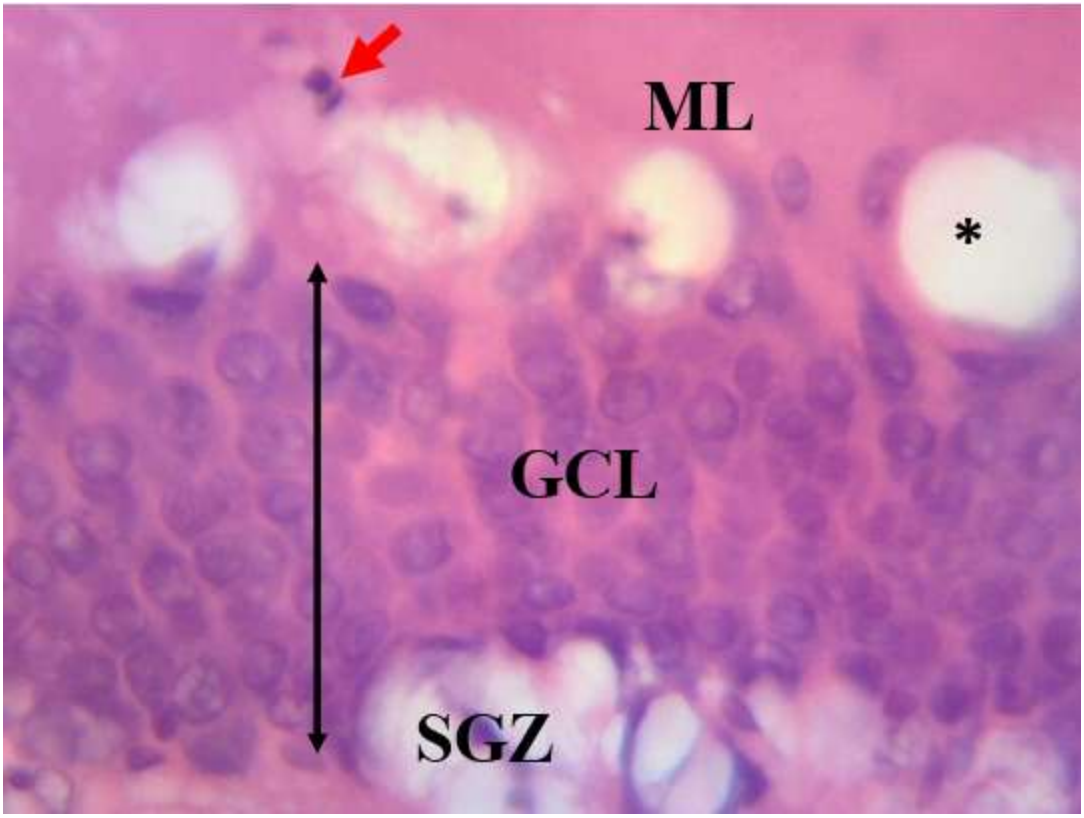


Plate XXXIX: A photomicrograph of Dentate gyrus (DG) region of the hippocampus in control groups of Wistar rats at PND 42 after being breastfed by a dam that received 400 mg/kg normal saline in lactation from PND 21 – 42(H and E, x 400).

GCL = granular cell layer, ML = Molecular layer, SGZ = subgranular zone * = cytoplasmic vacuolated cells, ➔ = oligodendrocytes.

Plate XL shows a DG region of the hippocampus at PND 42 after breastfed by a dam that received 400 mg/kg of hyoscyamine fraction of *D. stramonium*at lactation from the PND 21 – 42. The plate showed a relatively misaligned GCL with relatively hyperchromatic cells (black arrowheads) in which the cytoplasm was vacuolated (black asterisk) in addition to the patches of oligodendrocytes.

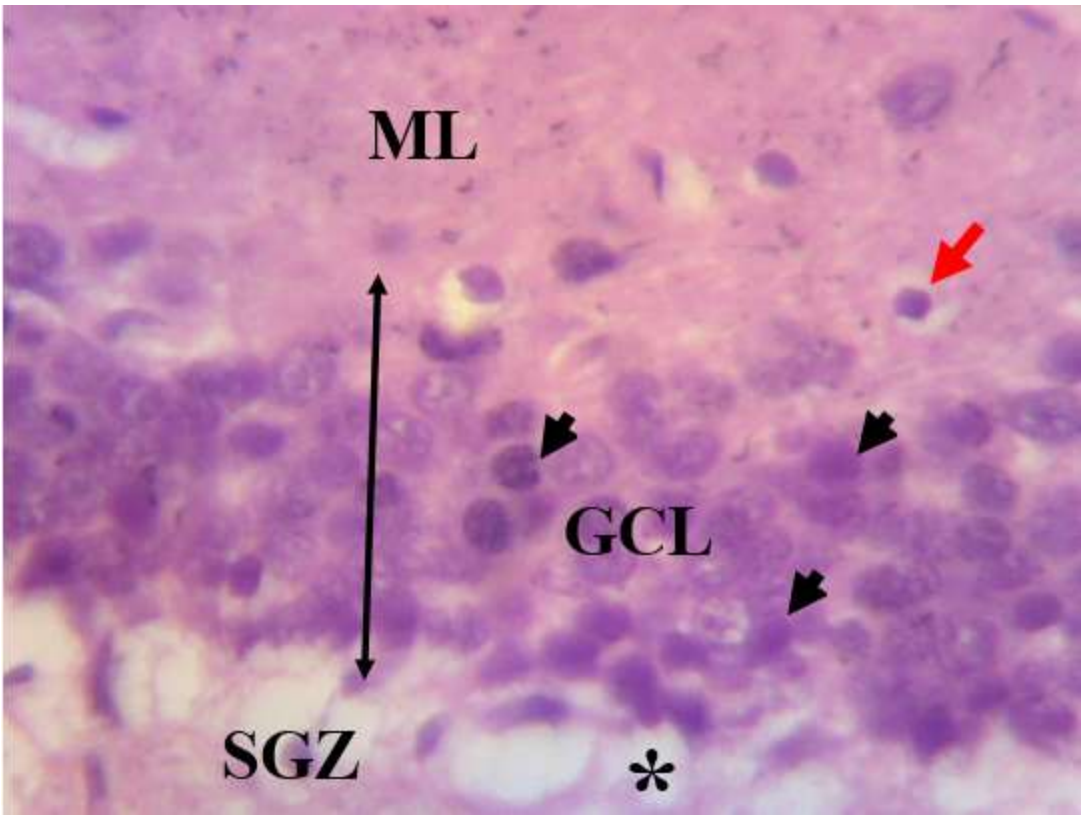


Plate XL: A photomicrograph of Dentate gyrus (DG) region of the hippocampus in Wistar rats at PND 42 after being breastfed by a dam that received 400 mg/kghyoscyamine fraction of *D. stramonium*at lactation from the PND 21 – 42 from PND 21 – 42(H and E, x 400).

GCL = granular cell layer, ML = Molecular layer, SGZ = subgranular zone ◀= hyperchromic granular cells, * = cytoplasmic vacuolated cell, ➔ = oligodendrocytes.

Plate XLI featured a photomicrograph of the CA1 region in adult Wistar rats at PND 85 after being breastfed by a dam that received 400 mg/kg normal saline from PND 21 – 42. The region shows normal histology of the SO, SR and PCL with slight patches of oligodendrocytes than the treated.

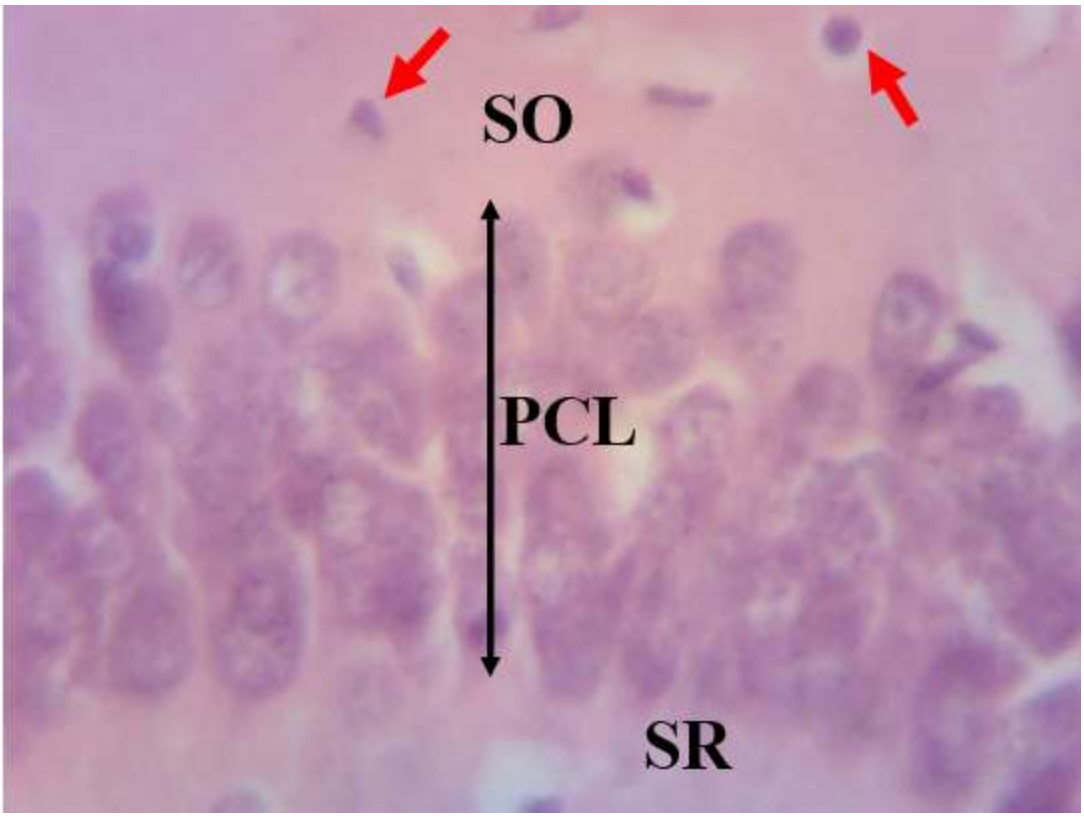


Plate XLI: A photomicrograph of Cornu Ammonis (CA1) region of the hippocampus in a control groupof adult Wistar rats at PND 85 after being breastfed by a dam that received 400 mg/kg normal saline from PND 21 – 42(H and E, x 400).

PCL = Pyramidal Cell Layer, SO = stratum oriens, SR = stratum radiatum, △ = pyknotic pyramidal cells, ◀ = hyperchromic granular cells → = oligodendrocytes.

Plate XLII featured a photomicrograph of CA1 region in adult Wistar rats at PND 85 after being breastfed by a dam that received with 400 mg/kg hyoscyamine fraction of *D. stramonium*seedsfrom PND 21 – 42. The SO, SR and PCL regions show normal histology, only that slight patches of smaller but hyperchromic nuclei (black arrows) and pyknotic cells (white arrowhead) were observed in the treated group compared to the control.

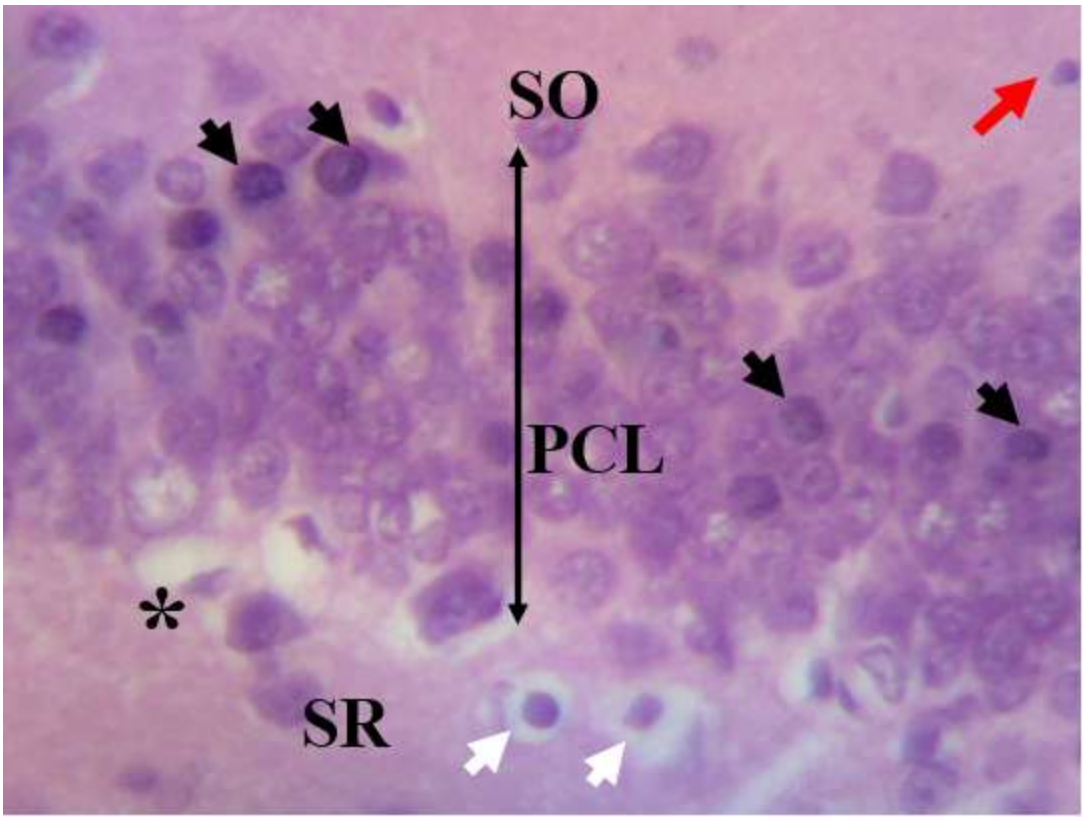


Plate XLII: A photomicrograph of Cornu Ammonis (CA1) region of the hippocampus in Wistar rats at PND 85 after being breastfed by a dam that received 400 mg/kg hyoscyamine fraction of *D. stramonium*seeds from PND 21 – 42(H and E, x 400).

PCL = Pyramidal Cell Layer, SO = stratum oriens, SR = stratum radiatum, △ = pyknotic pyramidal cells, ◄ = hyperchromic granular cells ➔ = oligodendrocytes, ✱= cytoplasmic vacuolated cell.

Plate XLIII was a photomicrograph of the CA3 regions in adult Wistar rats at PND 85 after being breastfed by a dam that received 400 mg/kg normal saline from the PND 21 – 42. There were a normal, SR and SLM regions PCL region that was observably denser with abundant pyramidal cells when compared to the treated group.

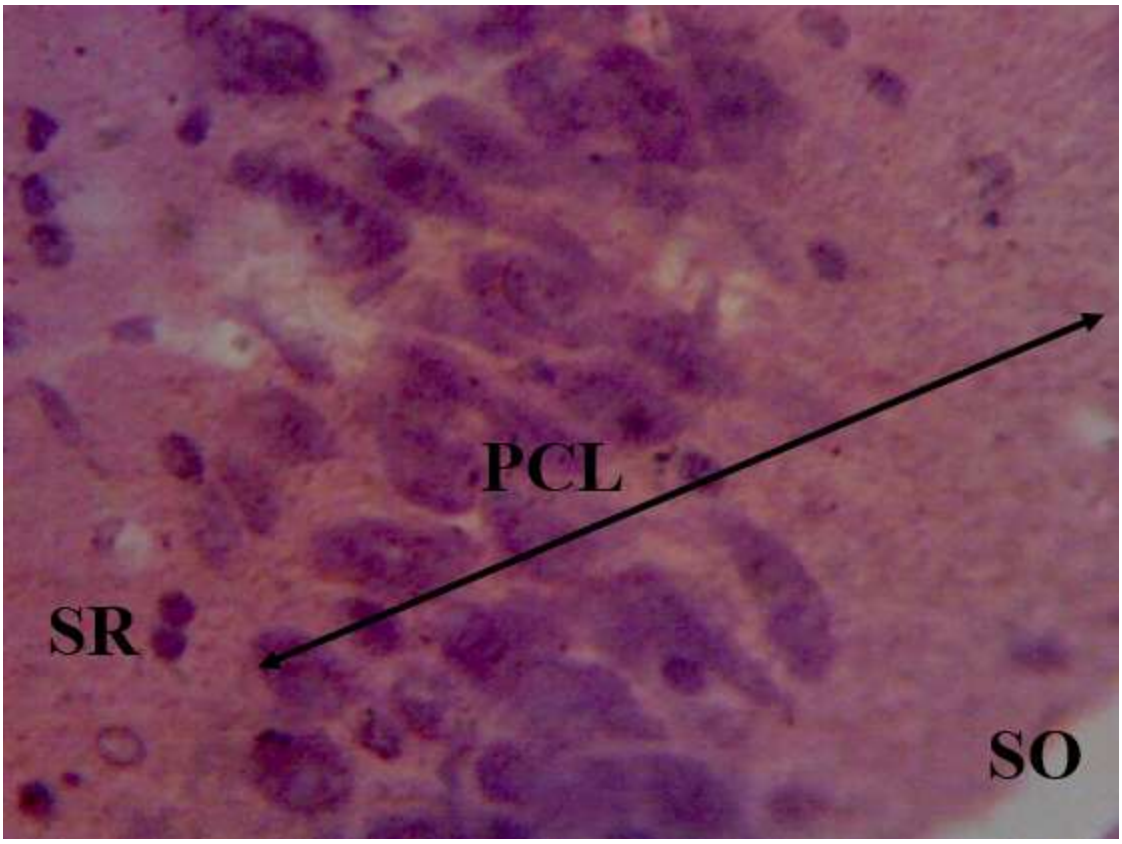


Plate XLIII: A photomicrograph of Cornu Ammonis (CA3) region of the hippocampus in the control group of adult Wistar rats at PND 85 (adulthood) after being breastfed by a dam that received 400 mg/kg normal saline from PND 21 – 42(H and E, x 400).

PCL = pyramidal cell layer, SO = stratum oriens, SR = stratum radiatum.

Plate XLIV, was a photomicrograph of the CA3 regions in adult Wistar rats at PND 85 after being breastfed by a dam that received 400 mg/kg hyoscyamine fraction of *D. stramonium*seeds from the PND 21 – 42. The SO, SR and SLM regions featured normal histologywith pyknotic cells (white arrowheads) and patches of oligodendrocytes (red arrows) when compared to the control group.

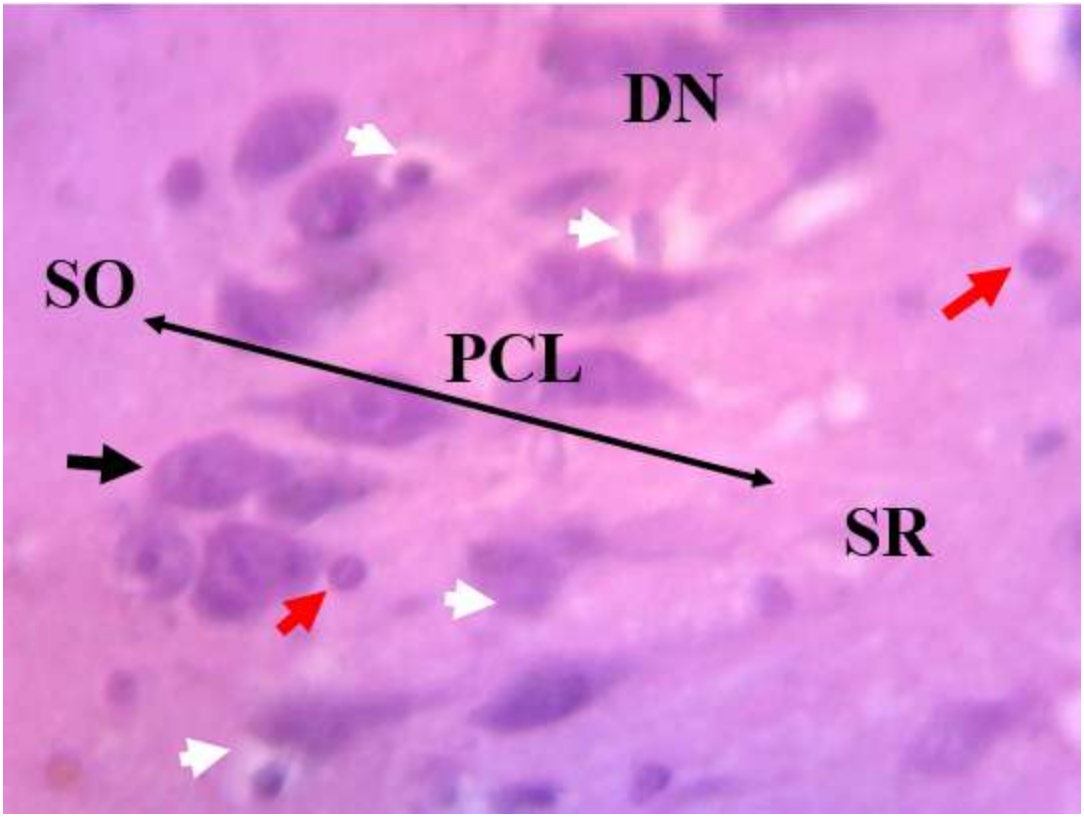


Plate XLIV: A photomicrographof Cornu Ammonis (CA3) region of the hippocampus in adult Wistar rats at PND 85 (adulthood) after being breastfed by a dam that received 400 mg/kg hyoscyamine fraction of *D. stramonium*seedsfrom PND 21 – 42 (H and E, x400).

Plate XLV shows the DG regions of the control adult Wistar rats at PND 85 after being breastfed by a dam that received 400 mg/kg normal saline at lactation from PND 21 – 42.The plate of the control group showed normal histology of the GCL featuring granule cells, molecular layer (ML), subventricular zone (SVZ).

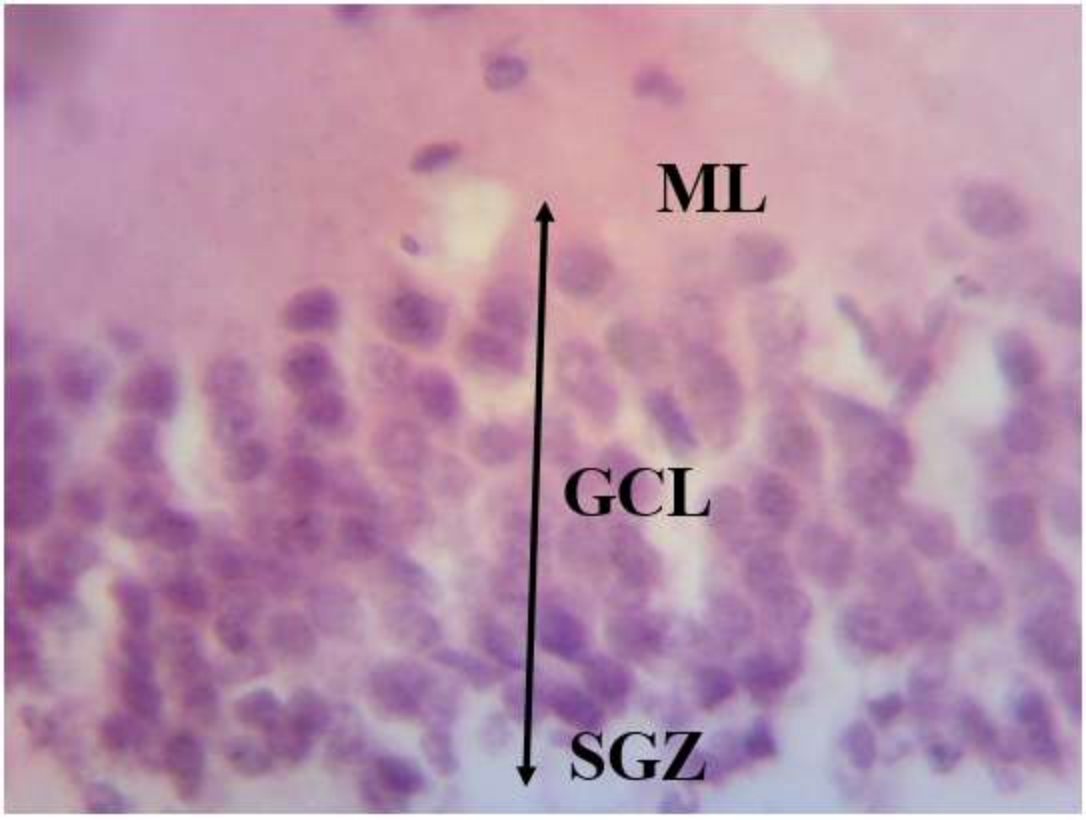


Plate XLV: A photomicrograph of Dentate gyrus (DG) area of hippocampus in control adult group of Wistar rat pups at PND 85 week (adulthood) after being breastfed by a dam that received 400 mg/kg normal saline at lactationfrom the PND 21 – 42(H and E, x400).

GCL = Granular Cell Layer, ML = Molecular layer, PM = polymorphic Layer, SGZ = subgranular zone,

Plate XLVI shows the DG regions of the adult Wistar rats at PND 85 after being breastfed by a dam that received 400 mg/kg hyoscyamine fraction of *D. stramonium*seeds in lactation from PND 21 – 42. There were no observable histological differences between the control and treated groups in the cellular arrangement of granule cell layers except that the GCL region in the treated group stained darkly granular cells and showed patches of hyperchromic granular (black arrowheads) and pyknotic (white arrowheads) cells.

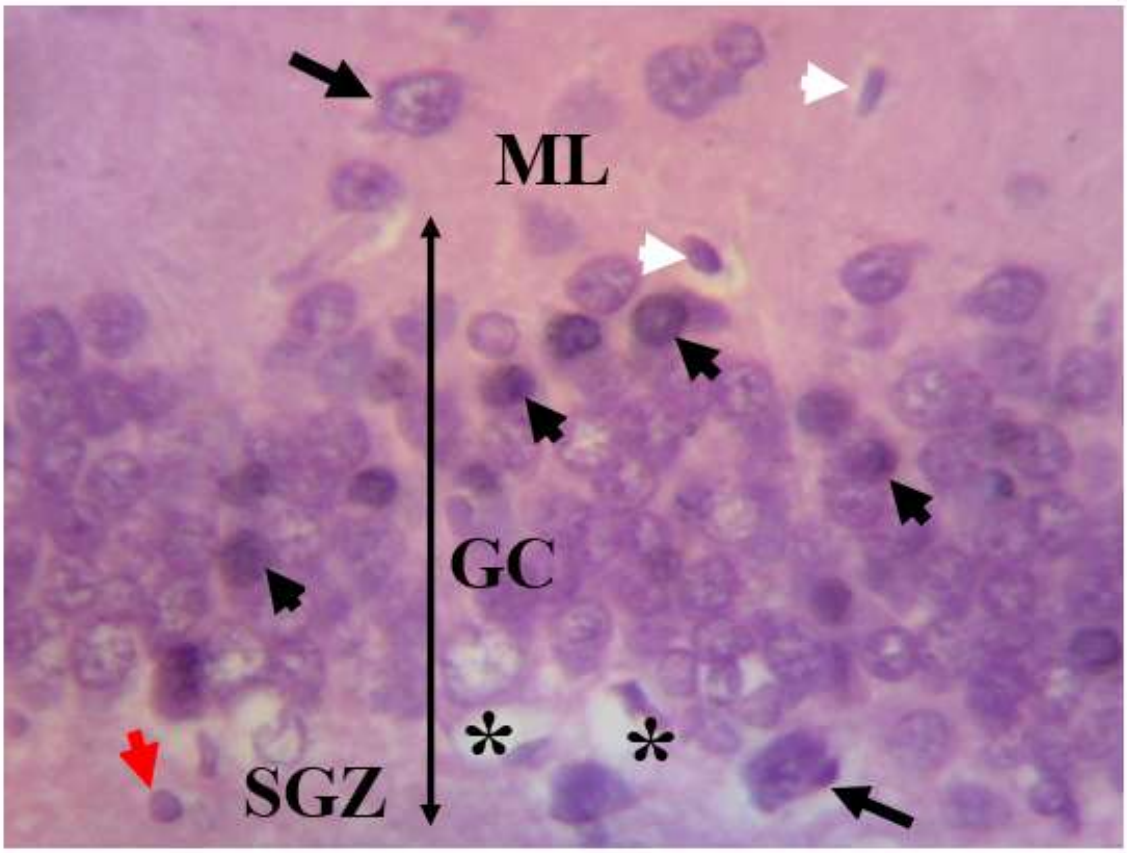


Plate XLVI: A photomicrograph of Dentate gyrus (DG) area of hippocampus in adult Wistar rat at PND 85 after being breastfed by a dam that received 400 mg/kghyoscyamine fraction of *D. stramonium*seeds from PND 21 – 42(H and E, x 400).

GCL = Granular Cell Layer, ML = Molecular layer, PM = polymorphic Layer, SGZ = subgranular zone, △ = pyknotic pyramidal cells, ◄ = hyperchromic granular cells, ➞ = karyolytic neurons, ➞ = oligodendrocytes, *= cytoplasmic vacuolation.

Plate XLVII showed the photomicrograph of the CA1 region of the hippocampus ina control group of adolescent Wistar rats at PND 49 after being treated with 800 mg/kg normal saline from PND 28 – 49. The histology of the region showed normal PCL with the cells and the SO and SLM.

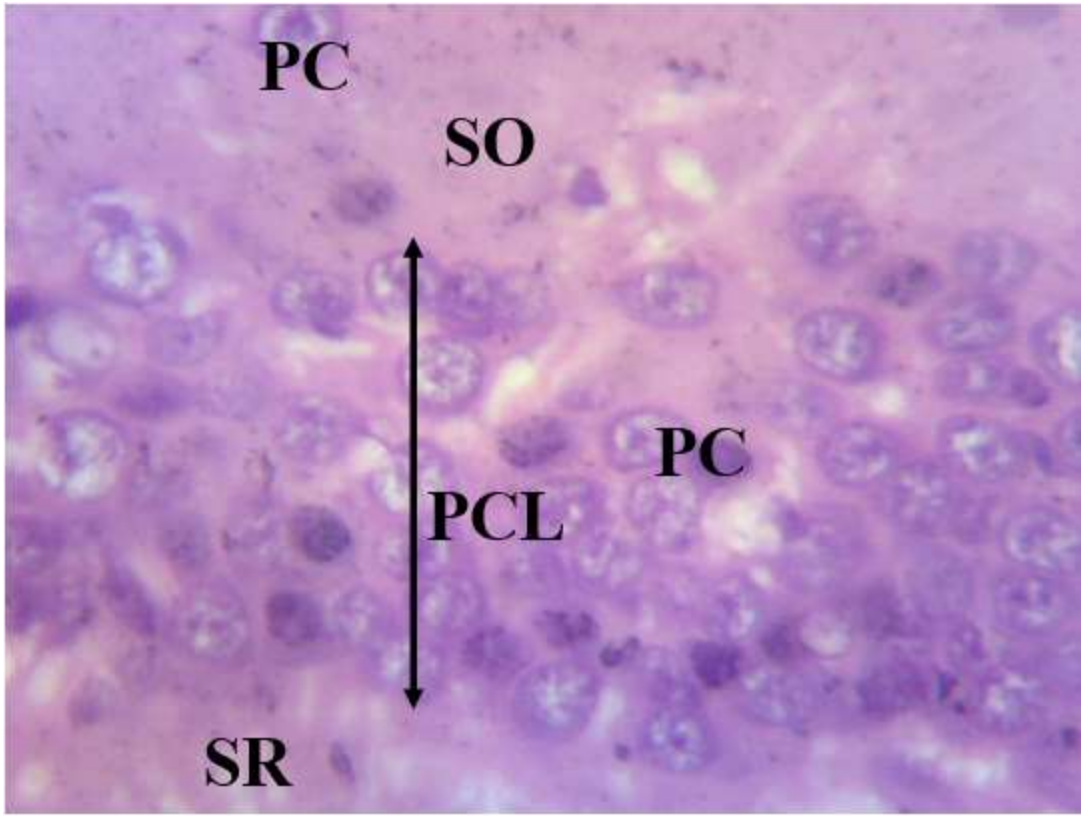


Plate XLVII: Photomicrograph of Cornu Ammonis (CA1) region of the hippocampus in control the group of adolescent Wistar rat at PND49 after being treated 800 mg/kg normal salinefrom PND 28 – 49(H and E, x400).

PCL = Pyramidal Cell Layer, SO = Stratum Oriens, SR = stratum radiatum.

Plate XLVIII showed the photomicrograph of the CA1 region of the hippocampus in an adolescent group of Wistar rat at PND 49 after being treated with 800 mg/kg of hyoscyamine fraction of *D. stramonium*from PND 28 – 49.The area in theregion, however, showed highly cytoplasmic vacuolated neuronal cells (black asterisk) in the SO, SR and SLM regions which were fewer and slightly when compared to the control.

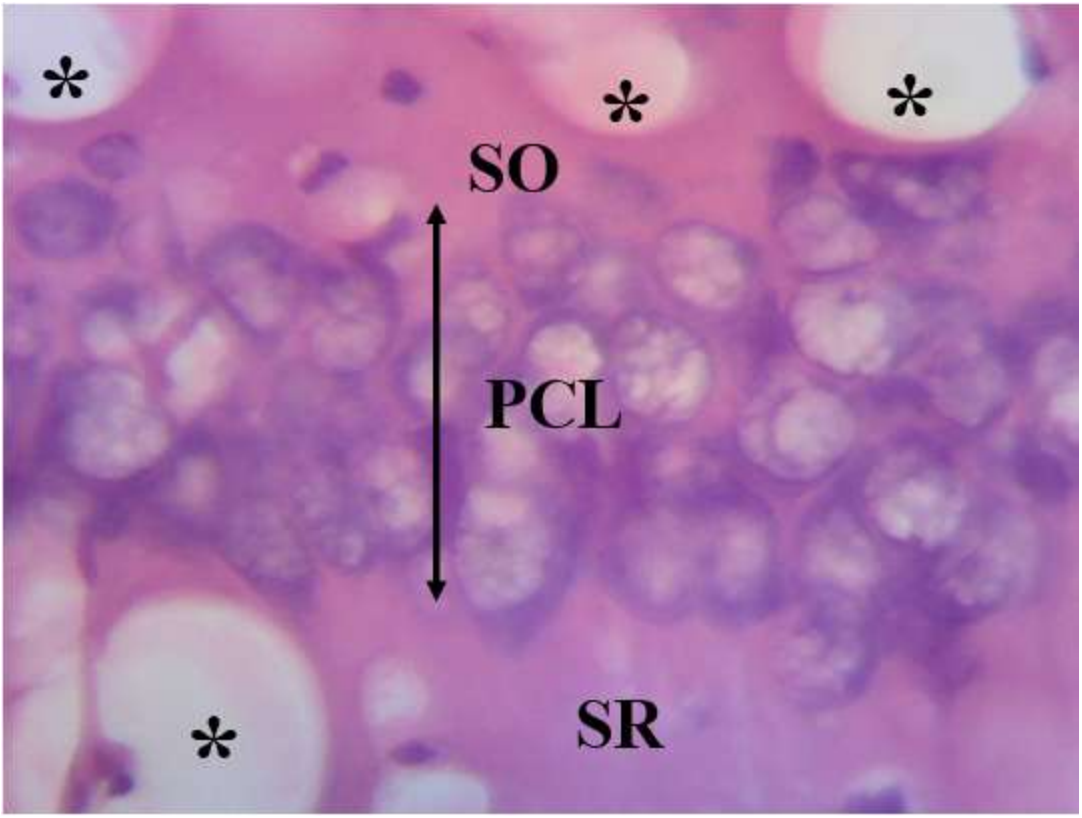


Plate XLVIII:A photomicrograph of Cornu Ammonis (CA1) region of the hippocampus in adolescent Wistar rat at PND 49 after being treated with 800 mg/kg of hyoscyamine fraction of *D. stramonium*seeds from PND 28 – 49(H and E, x 400).

PCL = Pyramidal Cell Layer, SO = Stratum Oriens, SR = stratum radiatum, * = cytoplasmic vacuolation.

Plate XLIX showed a photomicrograph of normal CA3 region in thehippocampus of adolescent Wistar ratat PND 49 after being treated with 800 mg/kg normal salinefrom PND 28 – 49. A well-developed PCL region featuring pyramidal cells (PC) and oligodendrocytes (red arrows)was observed.

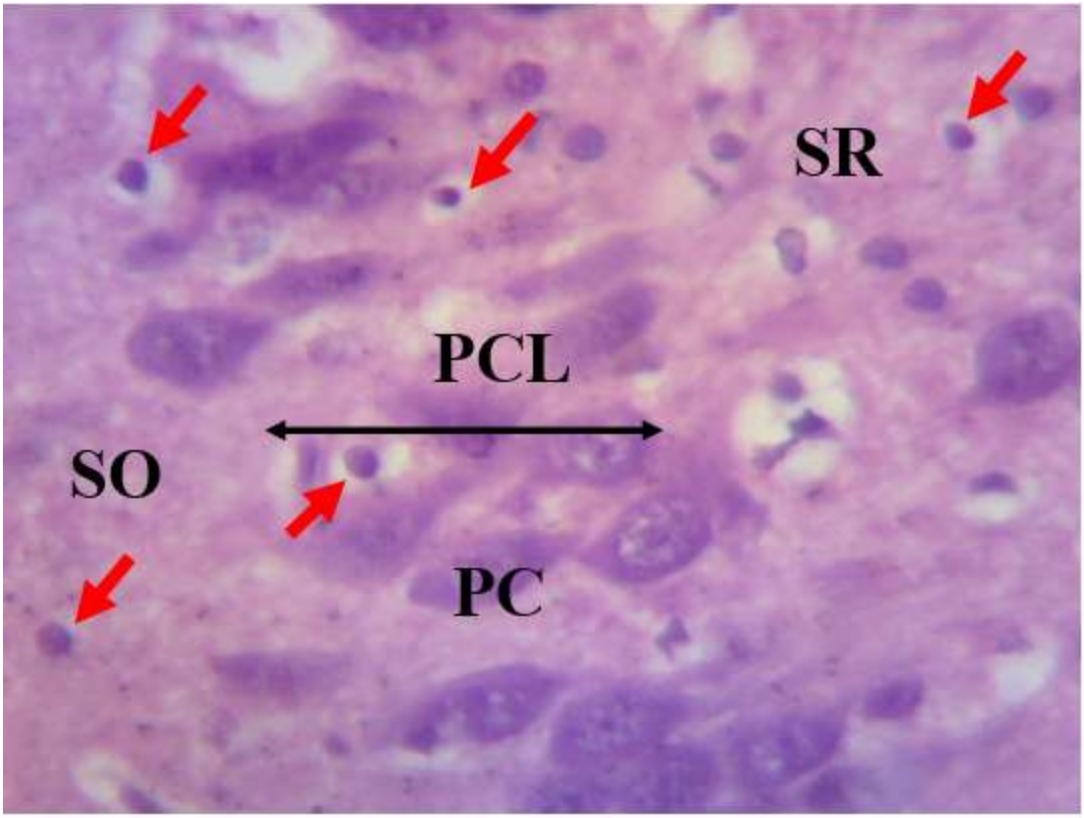


Plate XLIX: A photomicrograph of Cornu Ammonis (CA3) region of the hippocampus in the control group of adolescent Wistar rat at PND 49 after treated with 800 mg/kg normal salinefrom PND 28 – 49 (H and E, x400).

PCL = pyramidal cell layer, PC = pyramidal cell, SO = Stratum Oriens, SR = stratum radiatum, ➔ = oligodendrocytes.

Plate Lshowed a photomicrograph of normal CA3 region in the hippocampus of adolescent Wistar rat at PND 49 after being treated with 800 mg/kg hyoscyamine fraction of *D. stramonium*from PND 28 – 49. Significant cytoplasmic vacuolation (black asterisk) with poorly developed pyramidal cells (black arrows) that were scattered were also observed.

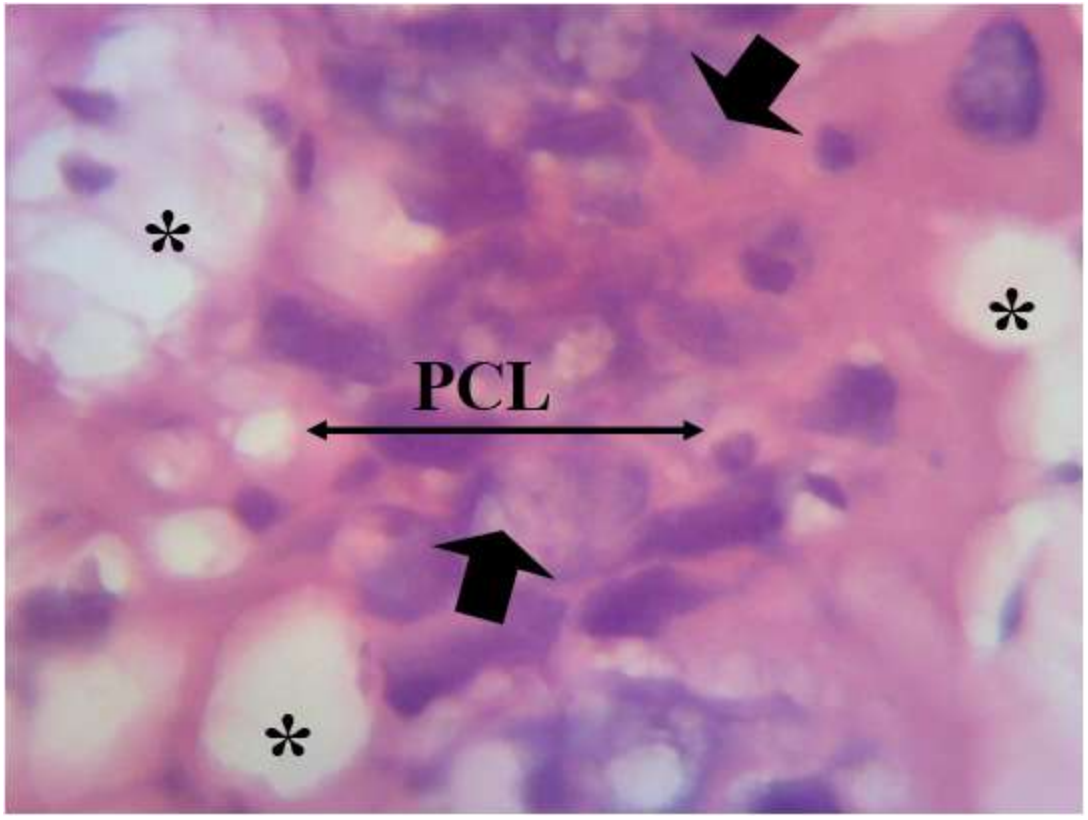


Plate L: A photomicrograph of Cornu Ammonis (CA3) region of the hippocampus in adolescent Wistar rat at PND 49 after being treated with 800 mg/k hyoscyamine fraction of *D. stramonium*seeds from PND 28 – 49 (H and E, x400).

PCL = pyramidal cell layer, * = cytoplasmic vacuolation → = poorly developed pyramidal cells area.

Plate LI showed a photomicrograph of the dentate gyrus (DG) region of the hippocampus in adolescent Wistar rats at PND 49after being treated with 800 mg/kgnormal saline from PND 28 – 49.There wasa well-organized dense layer of the granule cellsfeaturing mild cytoplasmic vacuolation (black asterisk)in the region when compared to the treated group.

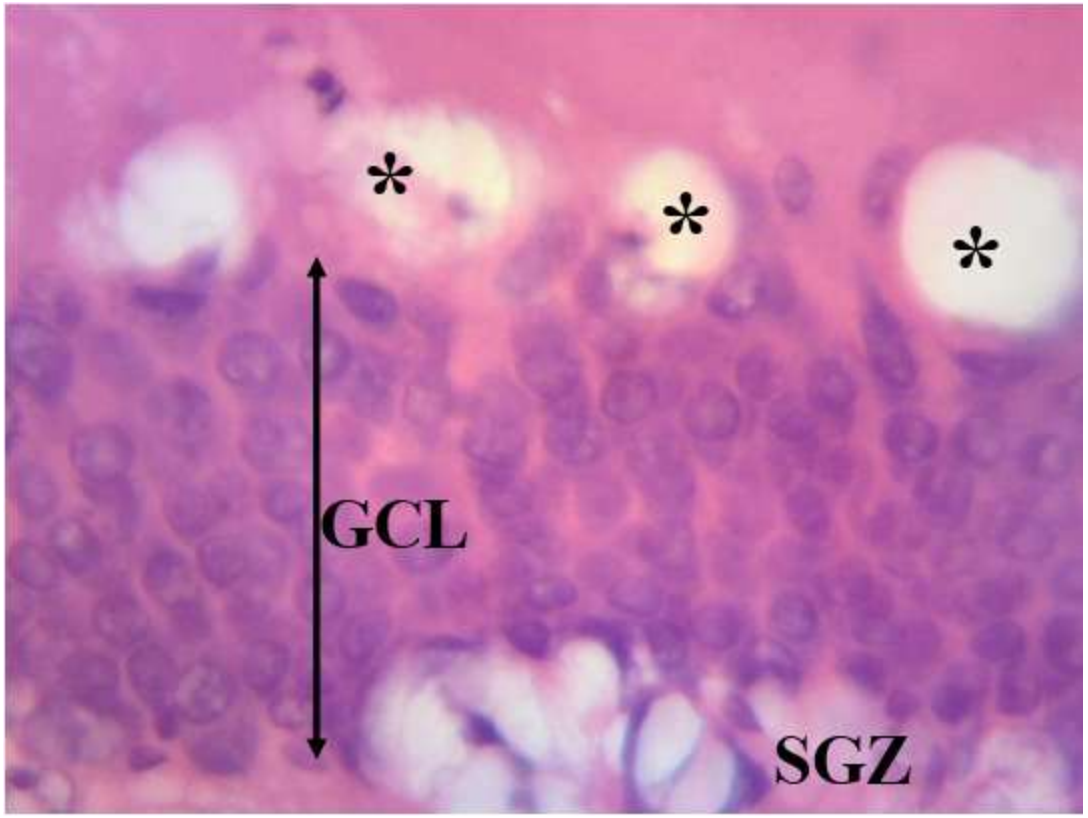


Plate LI: A photomicrograph of the Dentate gyrus (DG) region of the hippocampus in the control group of adolescent Wistar rat at PND 49 after being treated with 800 mg/kg normal saline from PND 28 – 49(H and E, x400).

GCL = Granular Cell Layer, ML = Molecular layer, SGZ = subgranular zone, * = cytoplasmic vacuolation.

Plate LII showed a photomicrograph of the dentate gyrus (DG) region of the hippocampus in adolescent Wistar rats at PND 49 after being treated with 800 mg/kg hyoscyamine fraction of *D. stramonium*from PND 28 – 49. A relatively slightly denser and loosely arranged poorly developed granular cells (white arrows), featuring the ghost cells (black arrows) was observed in the region.

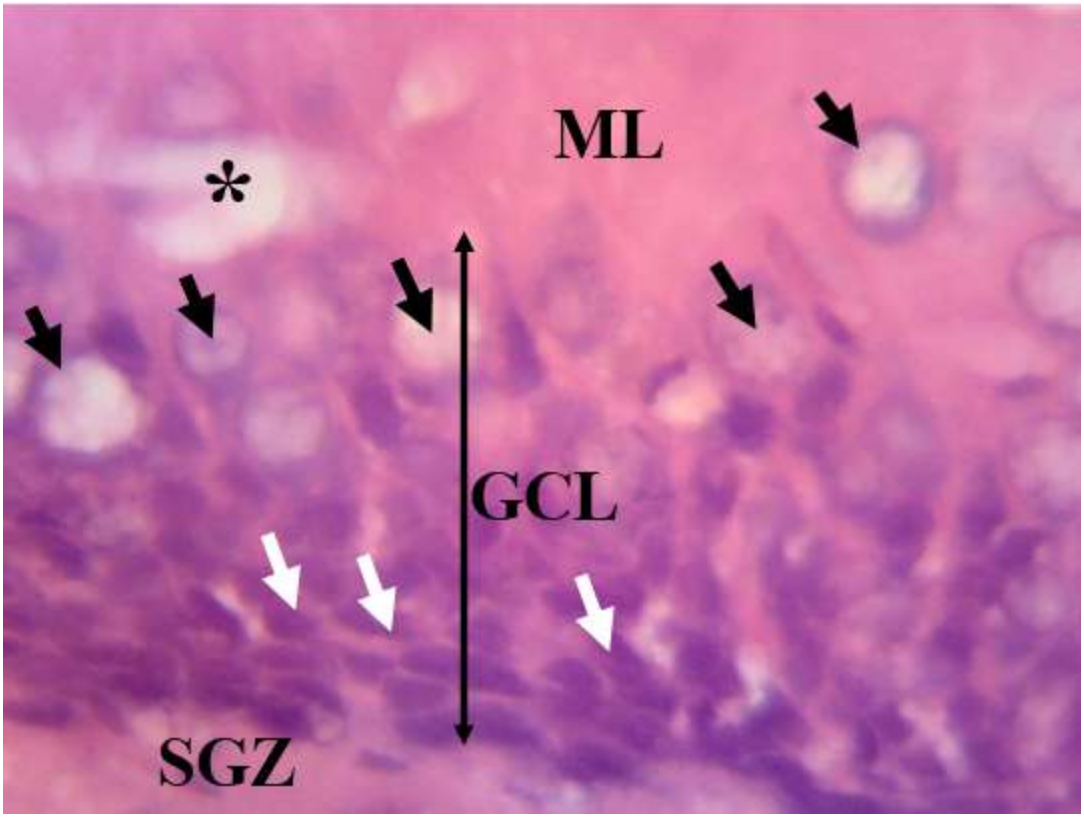


Plate LII: A photomicrograph of Dentate gyrus (DG) region of the hippocampus in the adolescent Wistar rat at PND 49 after being treated with 800 mg/kg hyoscyamine fraction of *D. stramonium*seeds from PND 28 – 49(H and E, x400).

GCL = Granular Cell Layer, ML = Molecular layer, SGZ = subgranular zone, * = cytoplasmic vacuolation, ➞ = ghost cells, ⇨ poorly developed granule cells.

Plate LIII showed a photomicrograph of the CA1 region of the hippocampus in a control group of adult Wistar rat at PND 85 after being treated orally with 800 mg/kg normal saline at the adolescence from the PND 28 – 49. The region showed normal histology of SO, SR, PCL and SLM and relativelynormal pyramidal cell densityin the PCL.

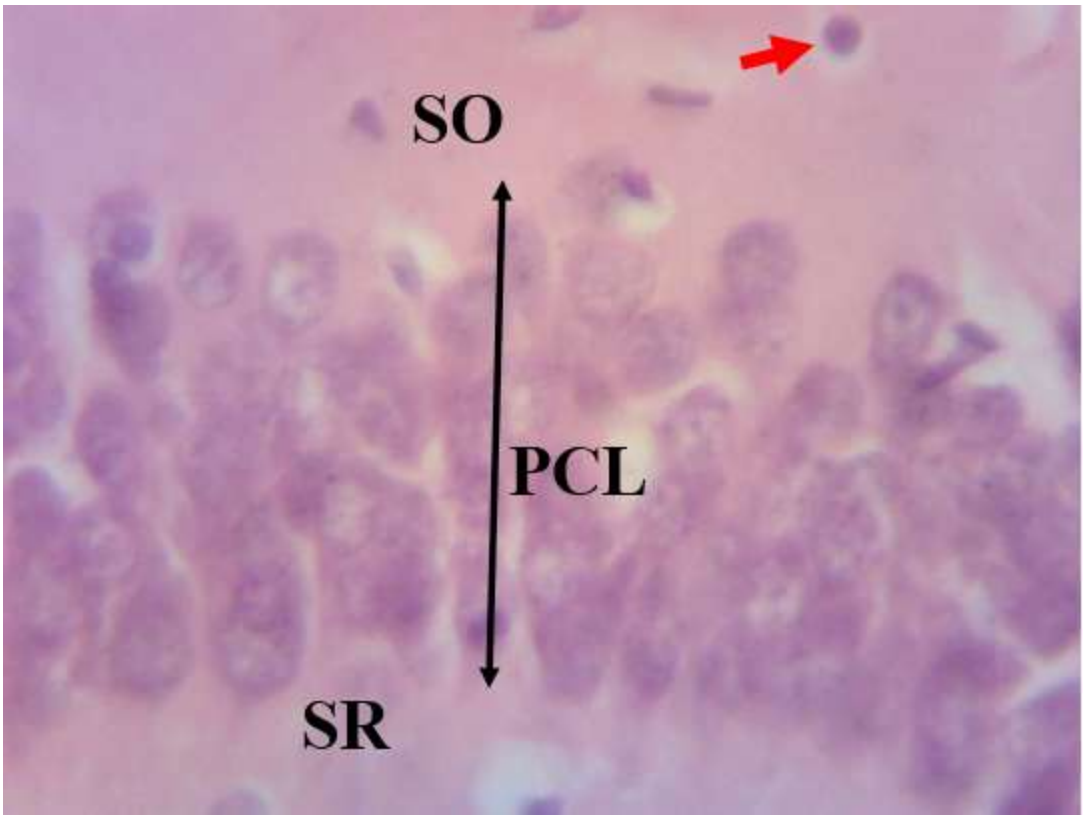


Plate LIII: A photomicrograph of Cornu Ammonis (CA1) region of the hippocampus in a control group of adolescent Wistar rat at PND 85 (adulthood) after being treated with 800 mg/kg normal salinefrom PND 28 – 49 (H and E, x400).

PCL = pyramidal cell layer, SO = Stratum Oriens, SR = stratum radiatum, → = oligodendrocytes.

Plate LIV showed photomicrograph of CA1 region of the hippocampus in adult Wistar rats at PND 85 after being treated orally with 800 mg/kg hyoscyamine fractionof *D. stramonium*at the adolescence from the PND 28 – 49. The SR and SLM regions of the treated group showed significant cytoplasmic vacuolations (black asterisks) with scattered, partly hyperchromic (black arrows) and apoptotic (black arrowhead) cells.

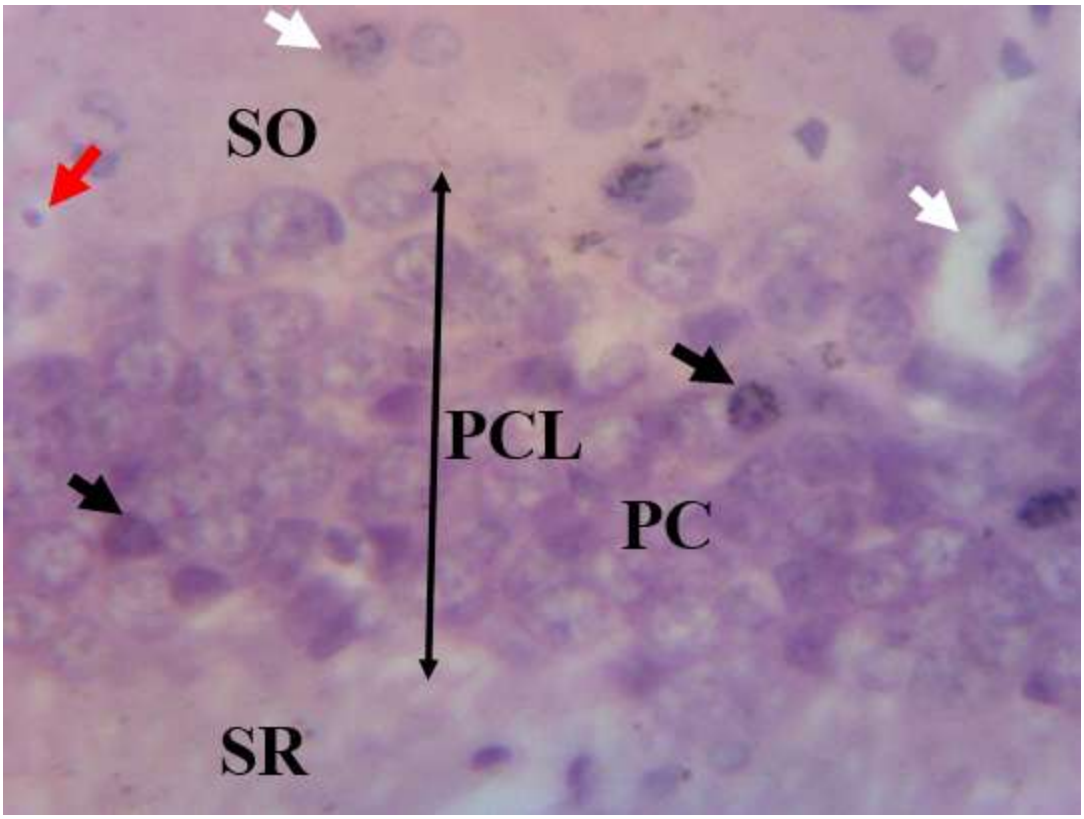


Plate LIV: A photomicrograph of Cornu Ammonis (CA1) region of the hippocampus in a treated group of adolescentWistar rat at PND 85 (adulthood) after being treated with 800 mg/kg hyoscyamine fraction of *D. stramonium*seedsfrom PND 28 – 49 (H and E, x400).

PCL = pyramidal cell layer, SO = Stratum Oriens, SR = stratum radiatum, PC = pyramidal cell, ➔ = oligodendrocytes, ➔ = hyperchromic cells, ⇨ = necrotic cells.

Plate LV showed a photomicrograph of CA3 of the hippocampus in adult Wistar rats at PND 85 after being treated orally 800 mg/kg normal saline from the PND 28 – 49.There was normal histology of the SO, SR, PCL and SLM regions with relatively denser, abundant pyramidal cells and a more organized PCL than the treated group.

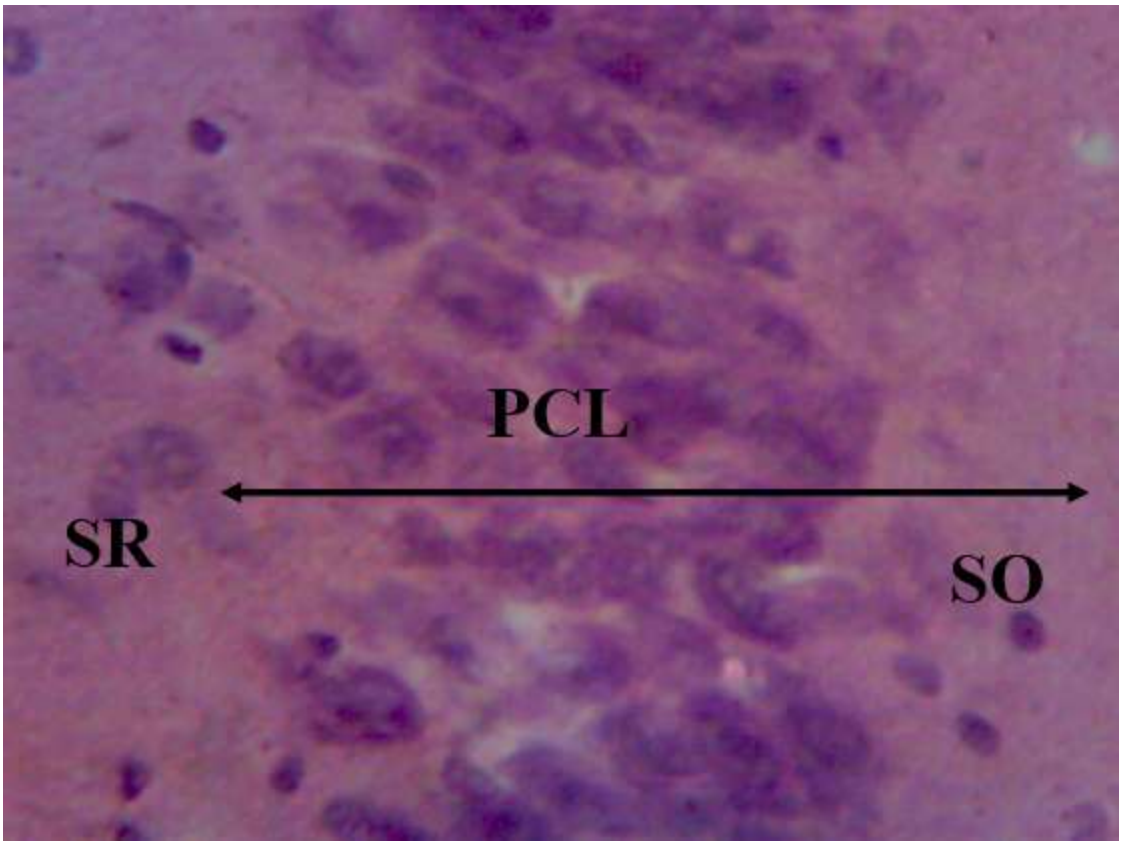


Plate LV: A photomicrograph of Cornu Ammonis (CA3) region of the hippocampus in a control group of adolescent Wistar rat at PND 85 (adulthood) after being treated 800 mg/kg normal saline from the PND 28 – 49(H and E, x400).

PCL = pyramidal cell layer, SR = stratum radiatum, SR= Stratum oriens

Plate LVI showed a photomicrograph of CA3 of the hippocampus in adult Wistar rats at PND 85 after being treated orally with 800 mg/kg hyoscyamine fraction of *D. stramonium* at adolescence from the PND 28 – 49. The plate showed mild cytoplasmic cellular vacuolations (black asterisk) in the control group compared to the treated groupwhere scanty pyramidal cells (PC), cytoplasmic vacuolations (black asterisk), and patches of scattered oligodendrocytes (red arrows) were observed.

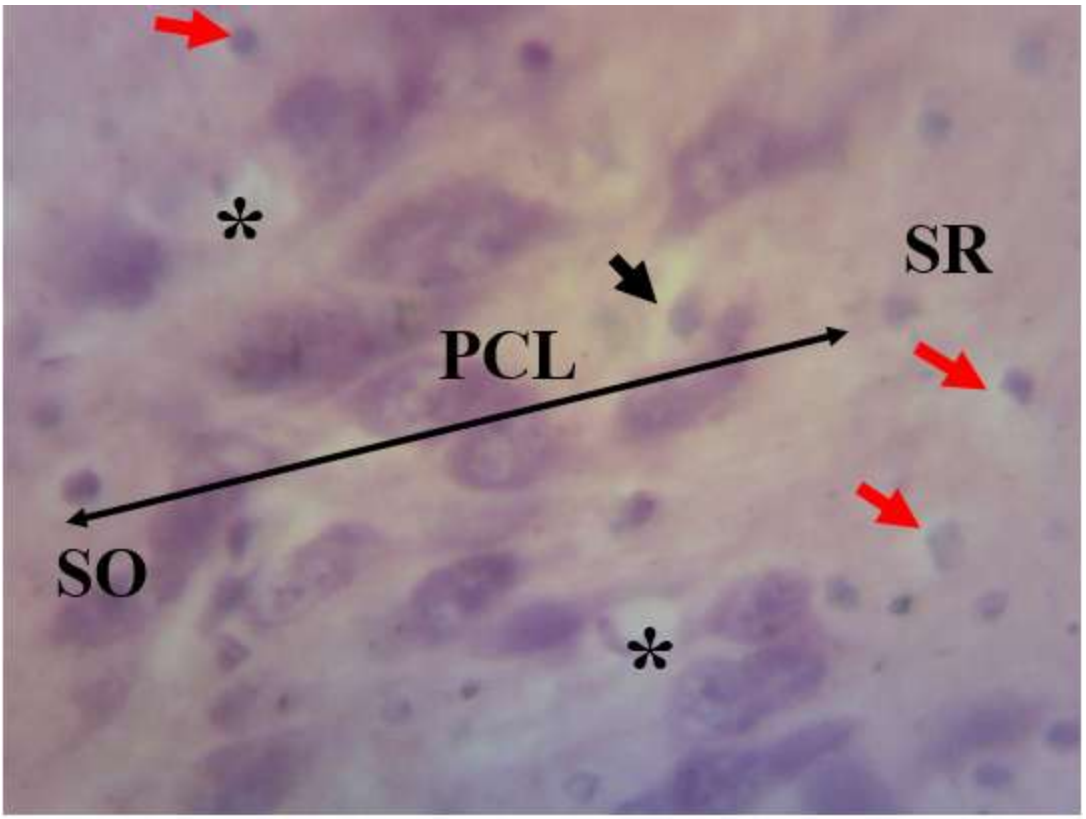


Plate LVI: A photomicrograph of Cornu Ammonis (CA3) region of the hippocampus in treated group of Wistar rat at PND 85 (adulthood) after being treated with 800 mg/kg hyoscyamine fraction of *D. stramonium*seeds from PND 28 – 49. (H and E, x400).

PCL = pyramidal cell layer, SO = Stratum Oriens, SR = stratum radiatum, * = cytolasmic vacuolation, → = oligodendrocytes.

Plate LVII shows the photomicrograph of the DG region of the hippocampus in a control group of adult Wistar rats at PND 85 after being treated orally with 800 mg/kg normal saline at the adolescence from PND 28 – 49. The plate showed normal histology of the region featuring ML, SGZ, and clearly defined GCL regions.

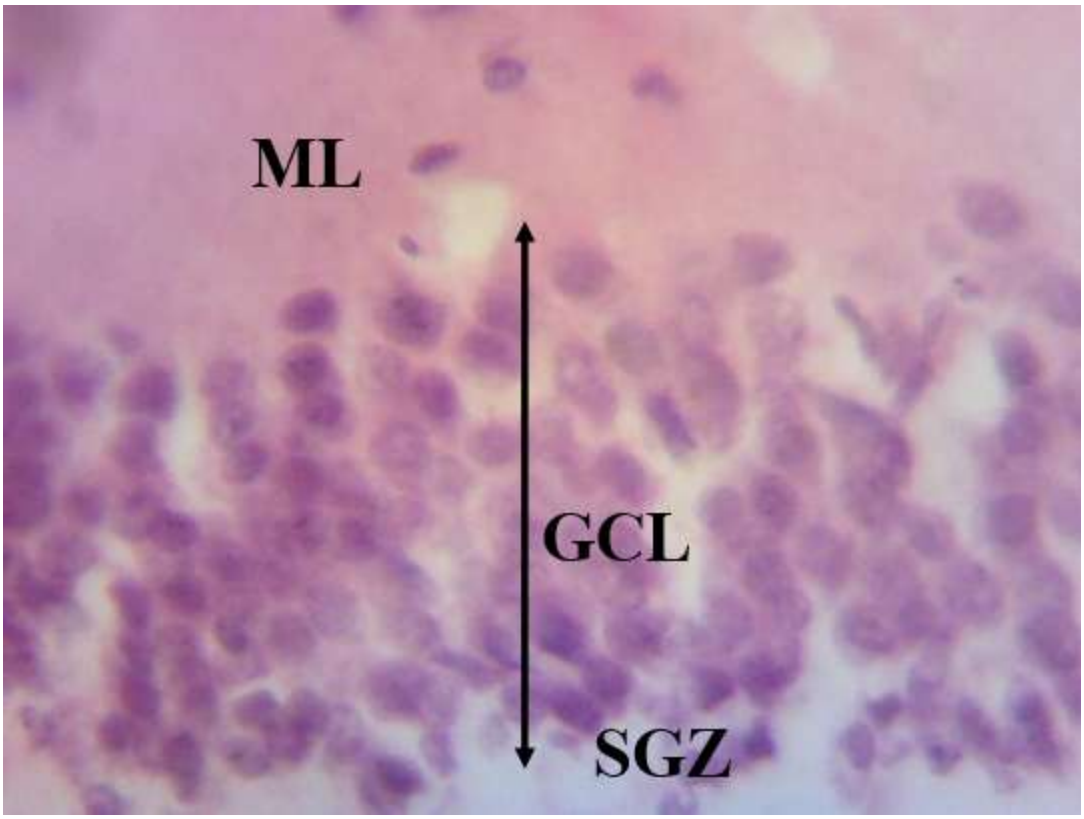


Plate LVII: A photomicrograph of Dentate gyrus (DG) region of the hippocampus in a control group of adolescent Wistar rat at PND 85 (adulthood) after being treated with 800 mg/kg normal saline from the PND 28 – 49.(H and E, x400).

GCL = Granular Cell Layer, ML = Molecular layer, SGZ = subgranular zone.

Plate LVIII shows the photomicrograph of the DG region of the hippocampus in adult Wistar rats at PND 85 after being treated orally to 800 mg/kg hyoscyamine fraction at adolescence from the PND 28 – 49. There was more hyperchromic, but normal ML, GCL, SGZ and clearly defined PM regions as in the control.

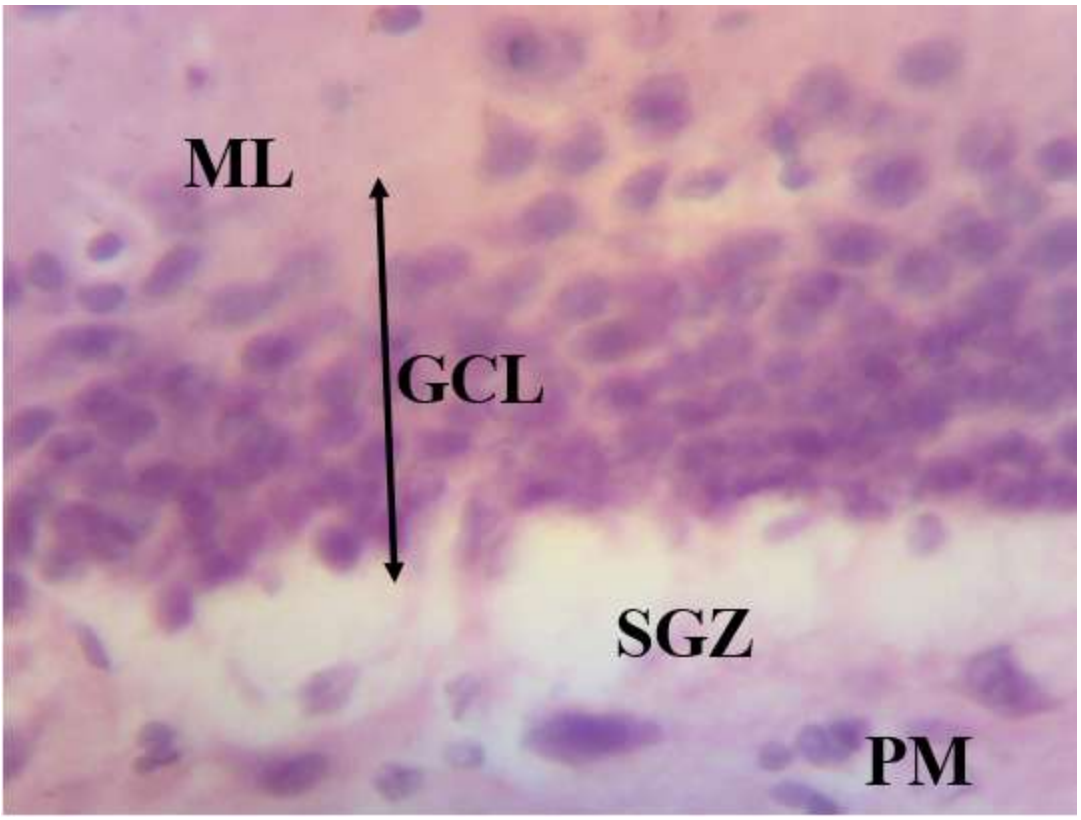


Plate LVIII:A photomicrograph of Dentate gyrus (DG) region of the hippocampus in treated group of adolescent Wistar rat at PND 85 (adulthood) after treatment with 800 mg/kg hyoscyamine fraction of *D. stramonium*seeds from the PND 28 – 49.(H and E, x400).

GCL = Granular Cell Layer, ML = Molecular layer, PM = polymorphic Layer, SGZ = subgranular zone.

Plate LVIX showed a photomicrograph of the CA1 region of the hippocampus in a control group of adult Wistar rats at PND 85 after being treated with orally 800 mg/kg normal saline for three weeks, from PND 56 – 77. The plate showed normal histology of the SO, SR, PCL, and SLM regions when compared to the treated groups.

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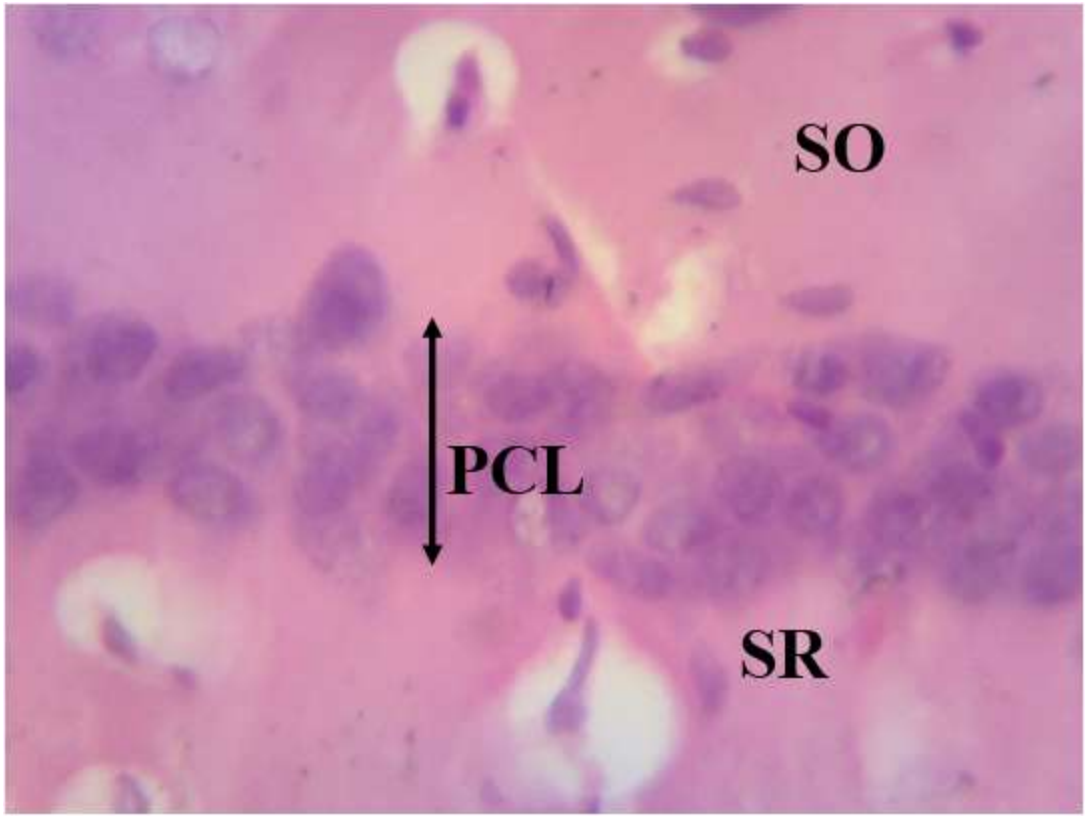


Plate LIX: A photomicrograph of Cornu Ammonis (CA1) of the hippocampus in a control group of Adult Wistar rats at PND 85 after being treated with 200 mg/kg normal salinefrom the PND 56 – 77(H and E, x400).

PCL = pyramidal cell layer, SO = stratum oriens, SR = stratum radiatum.

Plate LX provided the photomicrograph of the CA1 region of the hippocampus in adult Wistar rats at PND 85 after being treated orally with 200mg/kg hyoscyamine fraction respectively for three weeks, from PND 56 – 77. The plate showed hypochromic pyramidal cells. Equally, the SO, SR, and PCL, regions were marked by cytoplasmic vacuolations (black asterisk), and necrotic cells (black arrows).

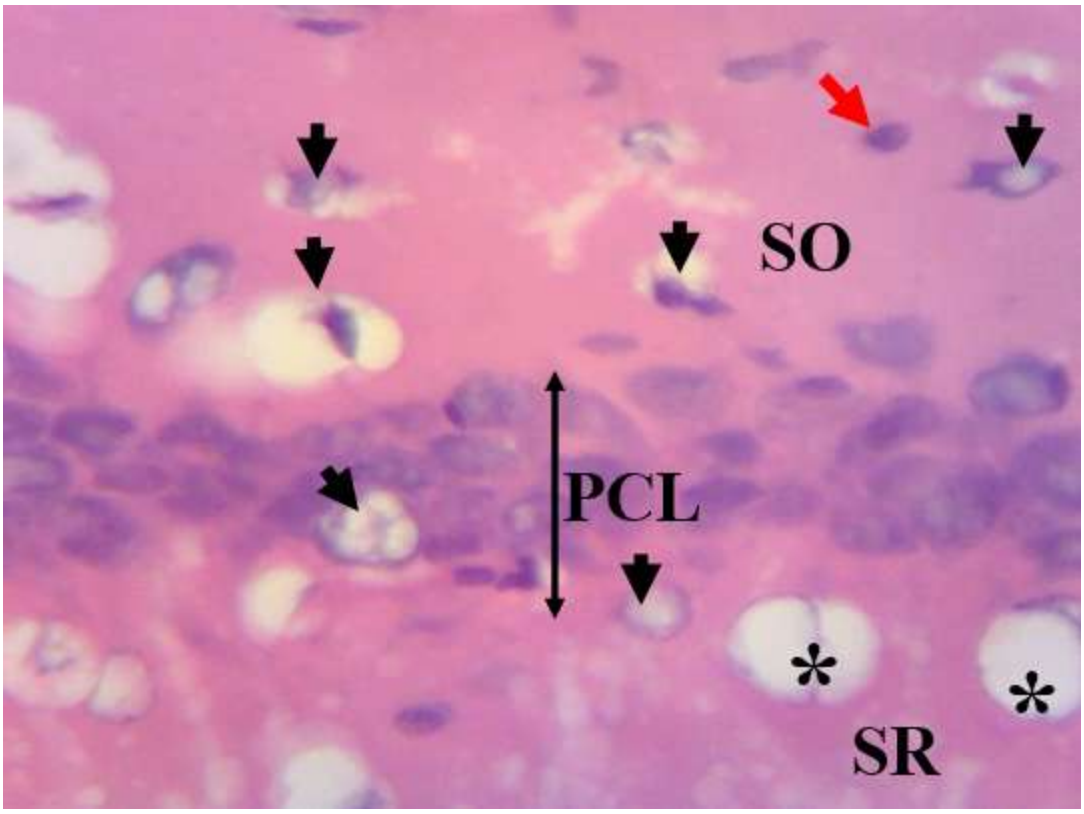


Plate LX:A photomicrograph of CA1 region of the hippocampus adult Wistar rats at PND 85 after being treated 200mg/kg hyoscyamine fractionof *D. stramonium* seeds for three weeks, from PND 56 – 77.

PCL = pyramidal cell layer, SO = stratum oriens, SR = stratum radiatum, * = cytoplasmic vacuolation, ➞ = necrotic cells, ➞ = oligodendrocyte.

Plate LXI showed a photomicrograph of the CA1 region of the hippocampus in adult Wistar rats at PND 85 after being treated with 400 mg/kg of hyoscyamine fraction of *D. stramonium*seeds for three weeks from the PND 56 – 77. The plate showed normal SO, SR and PCL regions with slightly hypochromic pyramidal cells with necrotic cell (black arrows).

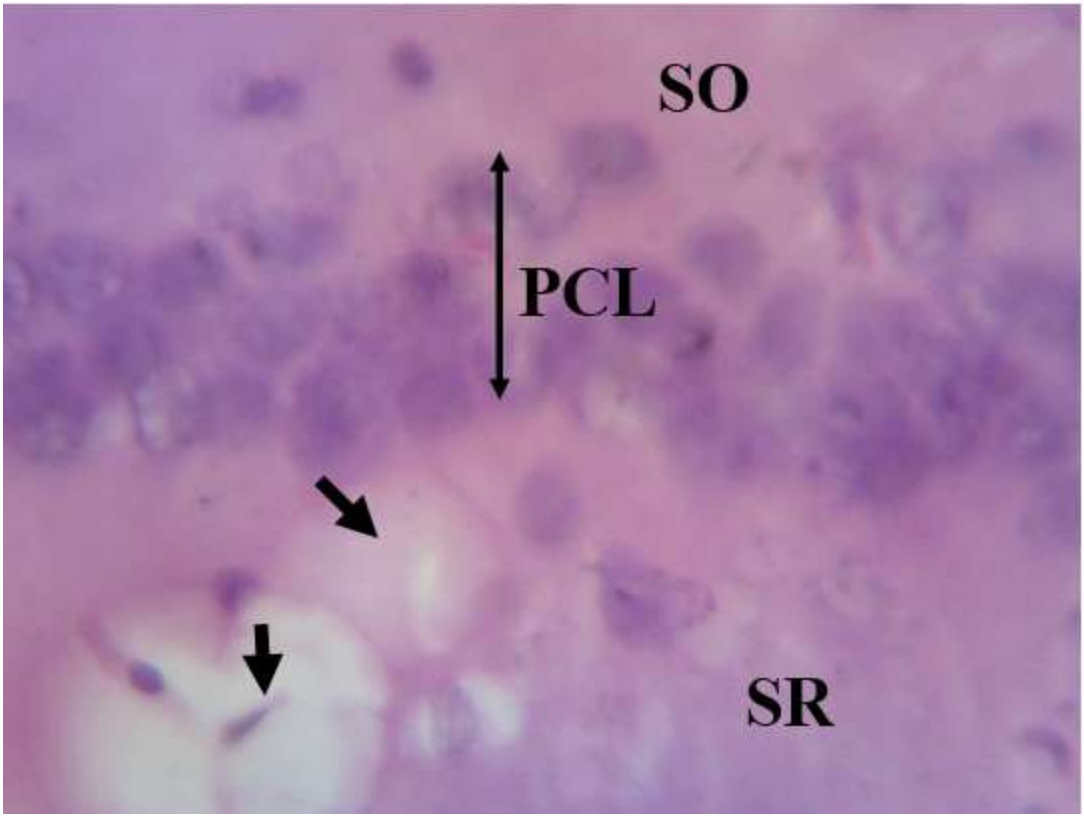


Plate LXI:A photomicrograph of CA1 region of the hippocampus adult Wistar rats at PND 85 after being treated orally with 400 mg/kg hyoscyamine fraction for three weeks, from PND 56 – 77.

PCL = pyramidal cell layer, SO = stratum oriens, SR = stratum radiatum, ➞ = necrotic cells.

Plate LXII showed a photomicrograph of the CA1 region of the hippocampus in adult Wistar rats at PND 85 after being treated with 800 mg/kg of hyoscyamine fraction of *D. stramonium*seeds for three weeks from the PND 56 – 77. The plate showed normal SO, SR and PCL regions with slightly hypochromic pyramidal cells with patches of hypertrophiedghost (black arrow-heads) and necrotic cells (black arrows).

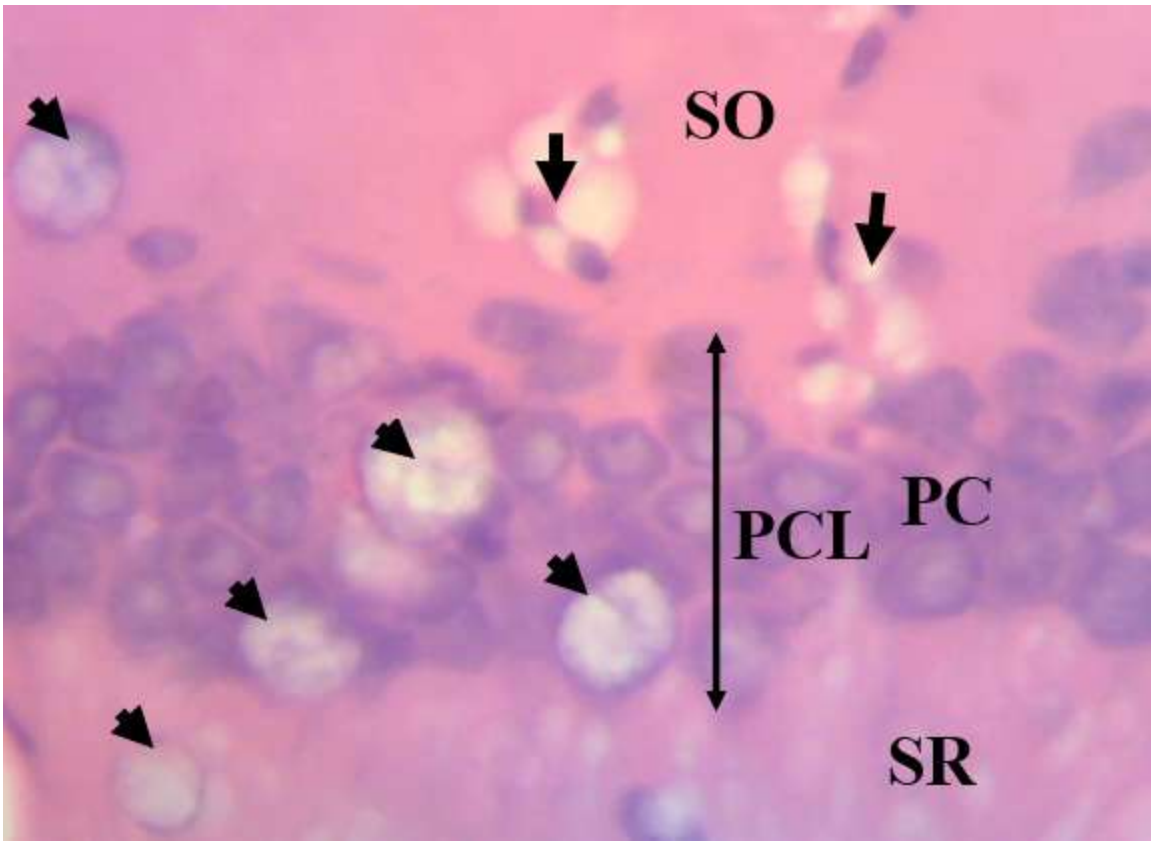


Plate LXII:A photomicrograph of CA1 region of the hippocampus in adult Wistar rats at PND 85 week after being treated with orally 800 mg/kg hyoscyamine fraction for three weeks, from PND 56 – 77.

PC= Pyramidal cell, PCL = pyramidal cell layer, SO = stratum oriens, SR = stratum radiatum, ⟶ = necrotic cells, ◀ = hypertrophied pyramidal cells.

Plate LXIII showed a photomicrograph of the CA3 region of the hippocampus in a control group of adult Wistar rats at PND 85 after being treated orally with normal saline for three weeks, from PND 56 – 77.There was normal histology of the SO, SR and PC in the region with traces of mossy fibres within the neuropil.

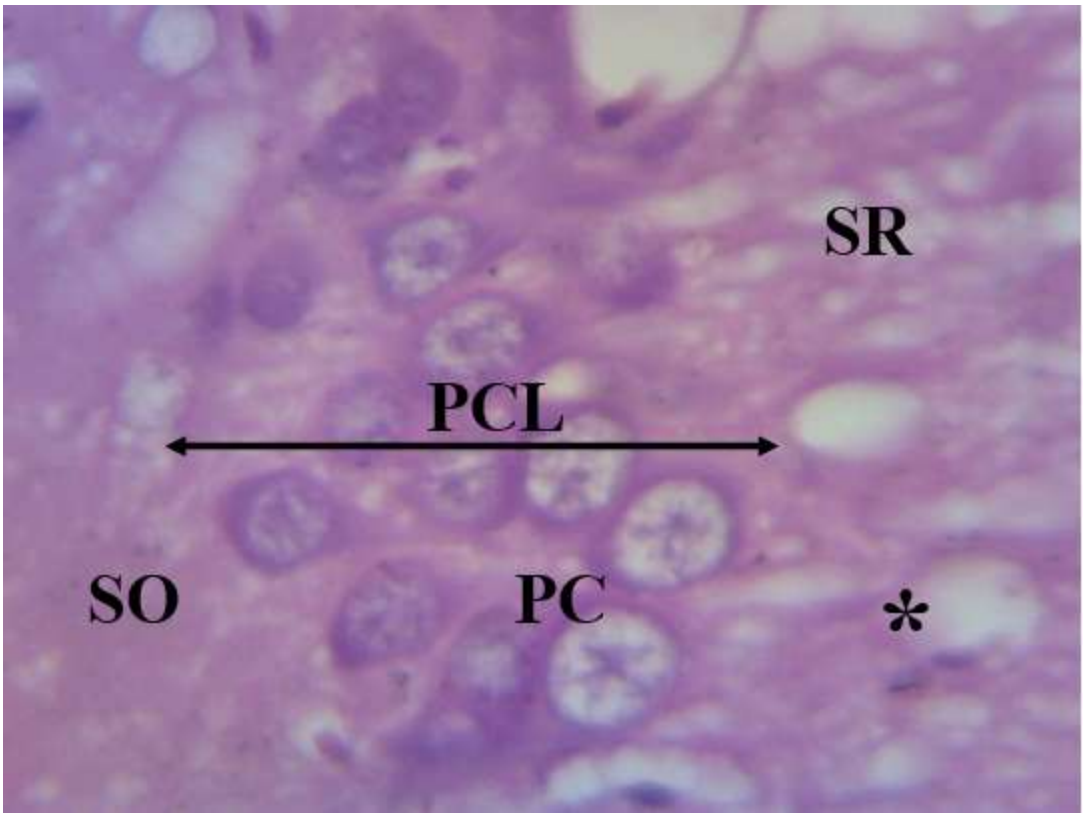


Plate LXIII:A photomicrograph of Cornu Ammonis (CA3) of the hippocampus in a control group of adult Wistar rat at PND 85 after being treated with 200 mg/kgnormal salinefrom the PND 56 – 77(H and E, x400).

PCL = pyramidal cell layer, SO = stratum oriens, SR = stratum radiatum, , PC= pyramidal cells, * = cytoplasmic vacuolation.

Plate LXIV showed a photomicrograph of the CA3 region of the hippocampus inadult Wistar rats at PND 85 after being treated orally with 200 mg/kg of hyoscyamine fraction of *D. stramonium* for three weeks, from PND 56 – 77. The PCL regions showed scanty, hypochromic, pyknotic (black arrowheads), cytoplasmic vacuolation (black asterisk) and necrotic (black arrowheads)cells.

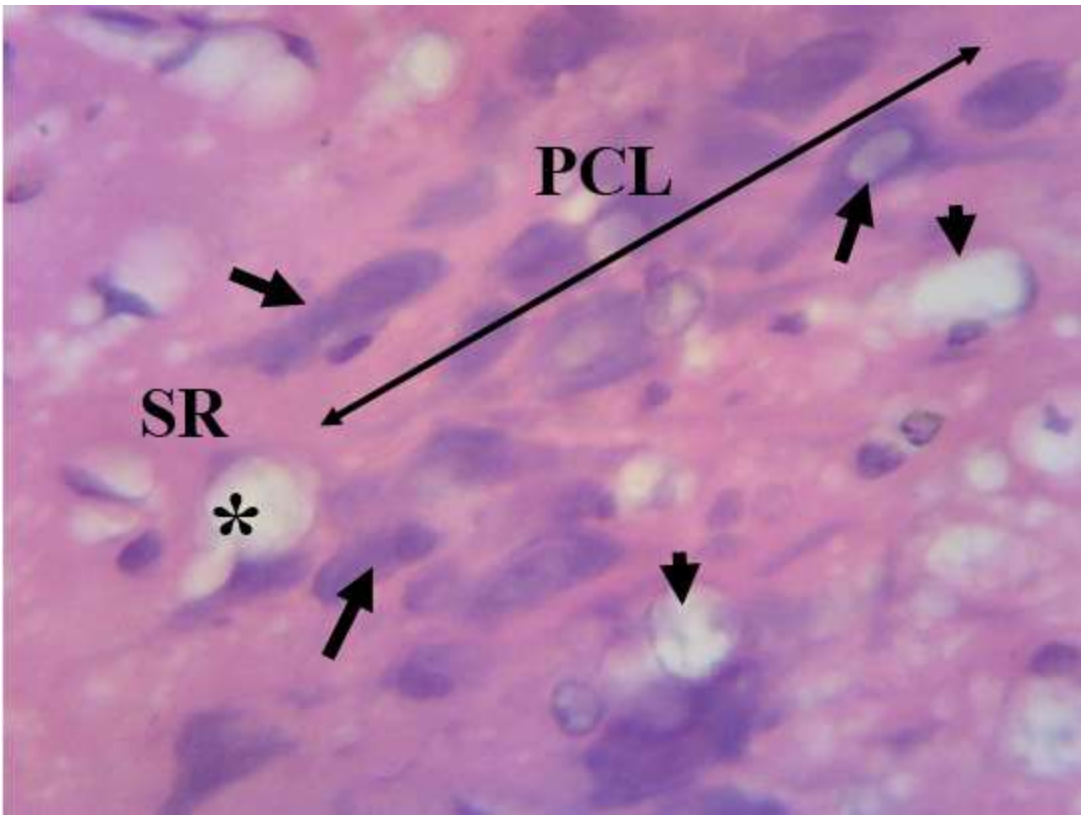


Plate LXIV:A photomicrograph of Cornu Ammonis (CA3) of the hippocampus in Adult Wistar rats at PND 85 after being treated with 200 mg/kg hyoscyamine fraction of *D. stramonium* seedsfor three weeks, from the PND 56 – 77(H and E, x400).

PCL = pyramidal cell layer, SO = stratum oriens, SR = stratum radiatum, SLM = stratum lacunosum moleculare, PCL = pyramidal cells layer, ⟶ = necrotic neuronal cells, ◀ = pyknotic neuronal cells, * = cytoplasmic vacuolation.

Plate LXV showed a photomicrograph of the CA3 region of the hippocampus inadult Wistar rats at PND 85 after being treated orally with 400 mg/kg of hyoscyamine fraction of *D. stramonium* for three weeks, from PND 56 – 77. The PCL regions showed scanty, hypochromic, cytoplasmic vacuolation (black asterisk) and necrotic (black arrowheads)cells.

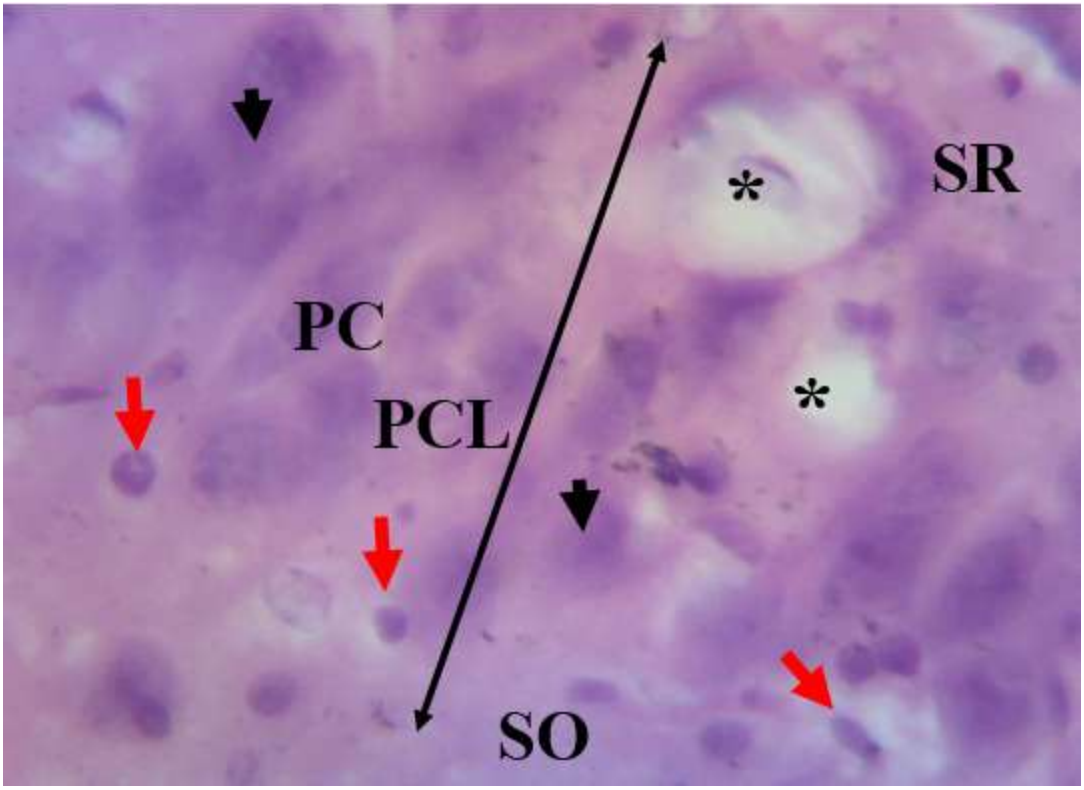


Plate LXV: A photomicrograph of Cornu Ammonis (CA3) of the hippocampus in Adult Wistar rats at PND 85 after being treated with 400 mg/kg hyoscyamine fraction of *D. stramonium*seeds for three weeks, from the PND 56 – 77(H and E, x400).

PCL = pyramidal cell layer, SO = stratum oriens, SR = stratum radiatum, PC = pyramidal cells, ➞ = necrotic neuronal cells, * = cytoplasmic vacuolation, ➔ = oligodendrocytes.

Plate LXVI showed a photomicrograph of the CA3 region of the hippocampus inadult Wistar rats at PND 85 after being treated orally with 800 mg/kg of hyoscyamine fraction of *D. stramonium* for three weeks, from PND 56 – 77. The PCL regions showed scanty, hypochromic, hypertrophied pyramidal cells (black arrows), cytoplasmic vacuolation (black asterisk) and necrotic (black arrowheads)cells.

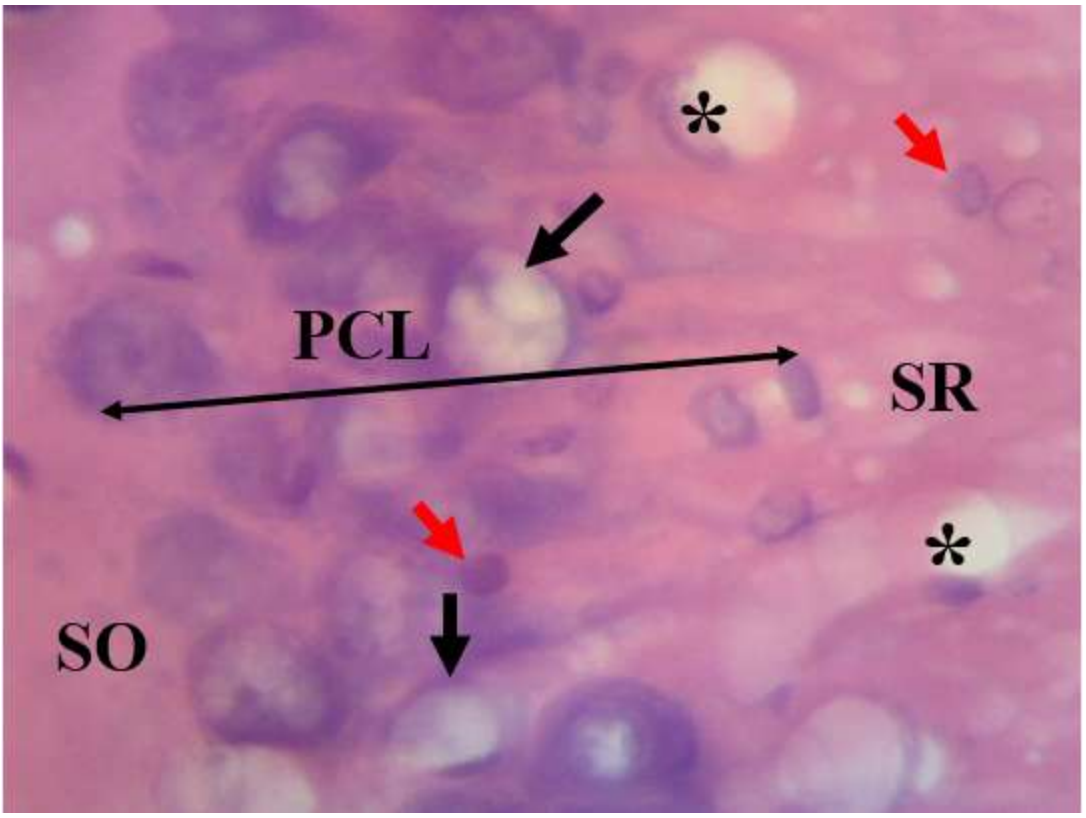


Plate LXVI: A photomicrograph of Cornu Ammonis (CA3) of the hippocampus in Adult Wistar rats at PND 85 after being treated with 800 mg/kg hyoscyamine fraction of *D. stramonium* for three weeks, from the PND 56 – 77 (H and E, x400).

PCL = pyramidal cell layer, SO = stratum oriens, SR = stratum radiatum, ➞ = necrotic neuronal cells, * = cytoplasmic vacuolation, ➞ = oligodendrocytes.

Plate LXVII showed a photomicrograph of the Dentate gyrus (DG) of the hippocampus in a control group of adult Wistar rats at PND 85 after being treated with normal saline for three weeks, from the PND 56 – 77. TheDG area showed normal histology of organized GCL, ML, SGZ and PM compared to the treated group.

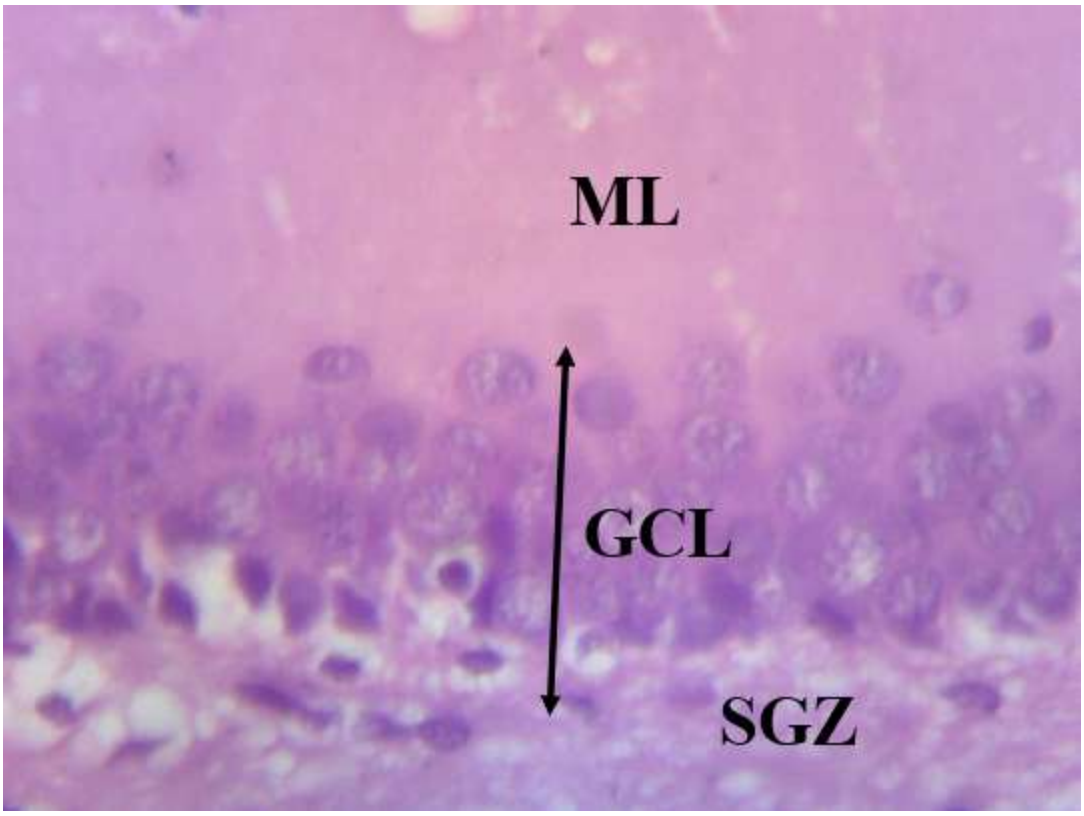


Plate LXVII:A photomicrograph of Dentate gyrus (DG) of the hippocampus in a control group ofadult Wistar rats at PND 85 after being treated with 200 mg/kgnormal saline for three weeks,from the PND 56 – 77(H and E, x400).

GCL = granular cell layer, ML = molecular layer, SGZ = subgranular zone,

Plate LXVIII showed a photomicrograph of Dentate gyrus (DG) of the hippocampus in a control group of adult Wistar rats at PND 85 after being treated with 200 mg/kg hyoscyamine fraction of *D. stramonium*seeds for three weeks, from the PND 56 – 77. The ML and SGZ and GCL regions weredenser with more hyperchromic granule cells(black arrows)and cytoplasmic vacuolations (black asterisk) when compared to the control group.

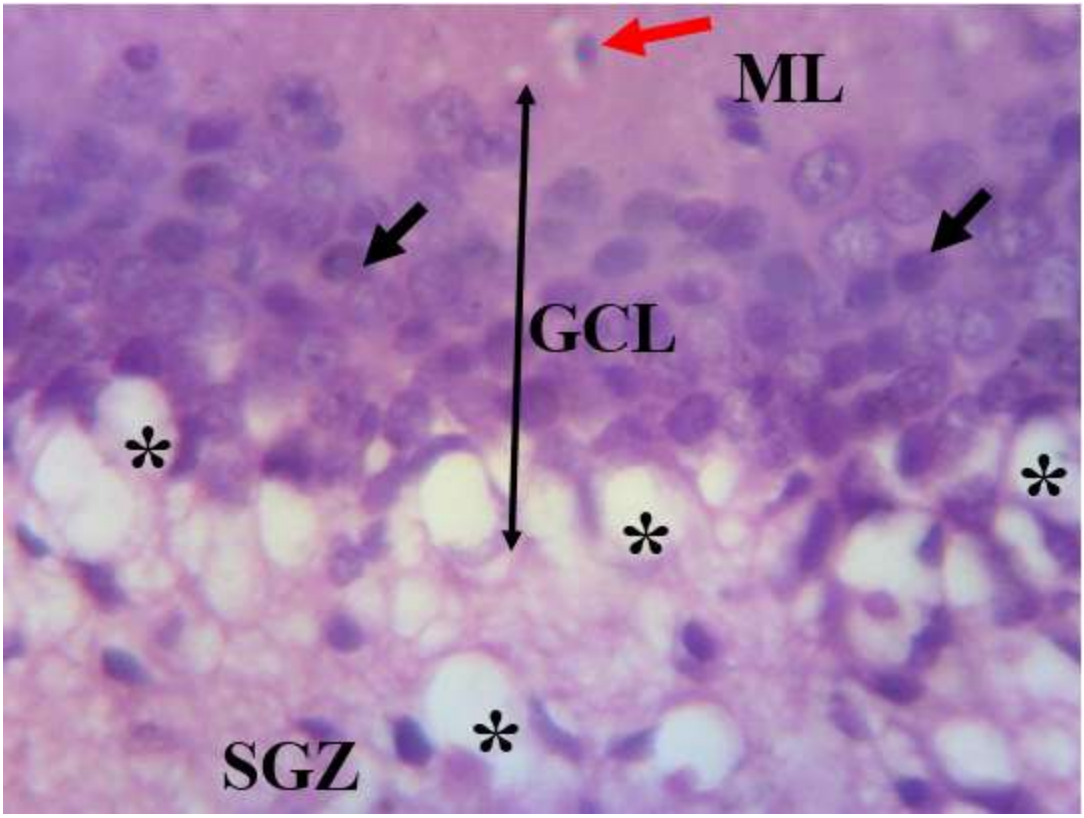


Plate LXVIII:A photomicrograph of Dentate gyrus (DG) of the hippocampus in adult Wistar rats at PND 85 after being treated with 200 mg/kg hyoscyamine fraction of *D. stramonium*seeds for three weeks, from the PND 56 – 77.(H and E, x400).

GCL = granular cell layer, ML = molecular layer, SGZ = subgranular zone, ➞ = hyperchromic granule cells, *= cytoplasmic vacuolation, ➞ = oligodendrocyte.

Plate LXIX showed a photomicrograph of the Dentate gyrus (DG) of the hippocampus in a control group of adult Wistar rats at PND 85 after being treated with 400 mg/kg hyoscyamine fraction of *D. stramonium*seeds for three weeks, from the PND 56 – 77. The plate shows normal histology of the ML and SGZ and GCL regions except that the granule cells were denser with more hyperchromic granule cells(black arrows), while the ML was featured by patches of cytoplasmic vacuolations (black asterisk) when compared to the control group.

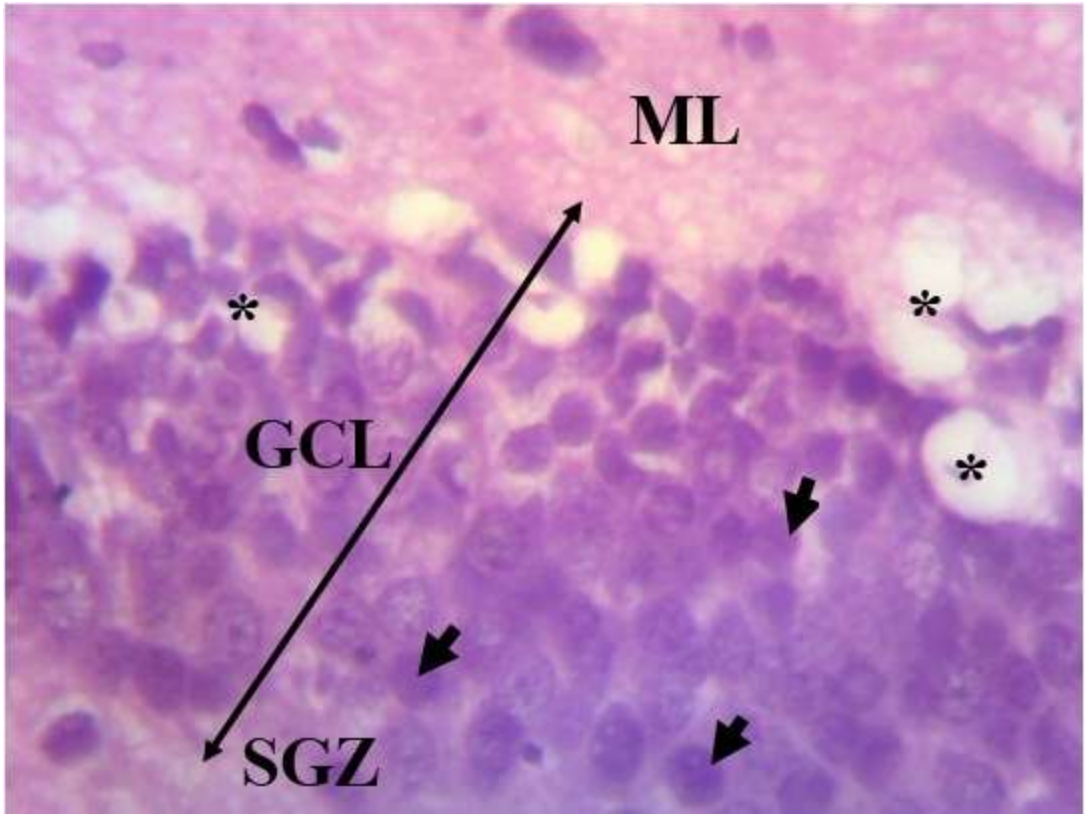


Plate LXIX: A photomicrograph of Dentate gyrus (DG) of the hippocampus in adult Wistar rats at PND 85 after being treated with 400 mg/kg hyoscyamine fraction of *D. stramonium*seeds for three weeks, from the PND 56 – 77. (H and E, x400).

GCL = granular cell layer, ML = molecular layer, SGZ = subgranular zone, ⟶ = hyperchromic granule cells, * = cytoplasmic vacuolation.

Plate LXX showed a photomicrograph of Dentate gyrus (DG) of the hippocampus in a control group of adult Wistar rats at PND 85 after being treated with 800 mg/kg hyoscyamine fraction of *D. stramonium*seeds for three weeks, from the PND 56 – 77. The plate shows normal histology of the ML and SGZ and GCL regions except for patches of necrotic (black arrows) and cytoplasmic vacuolated (black asterisk) cells when compared to the control group.

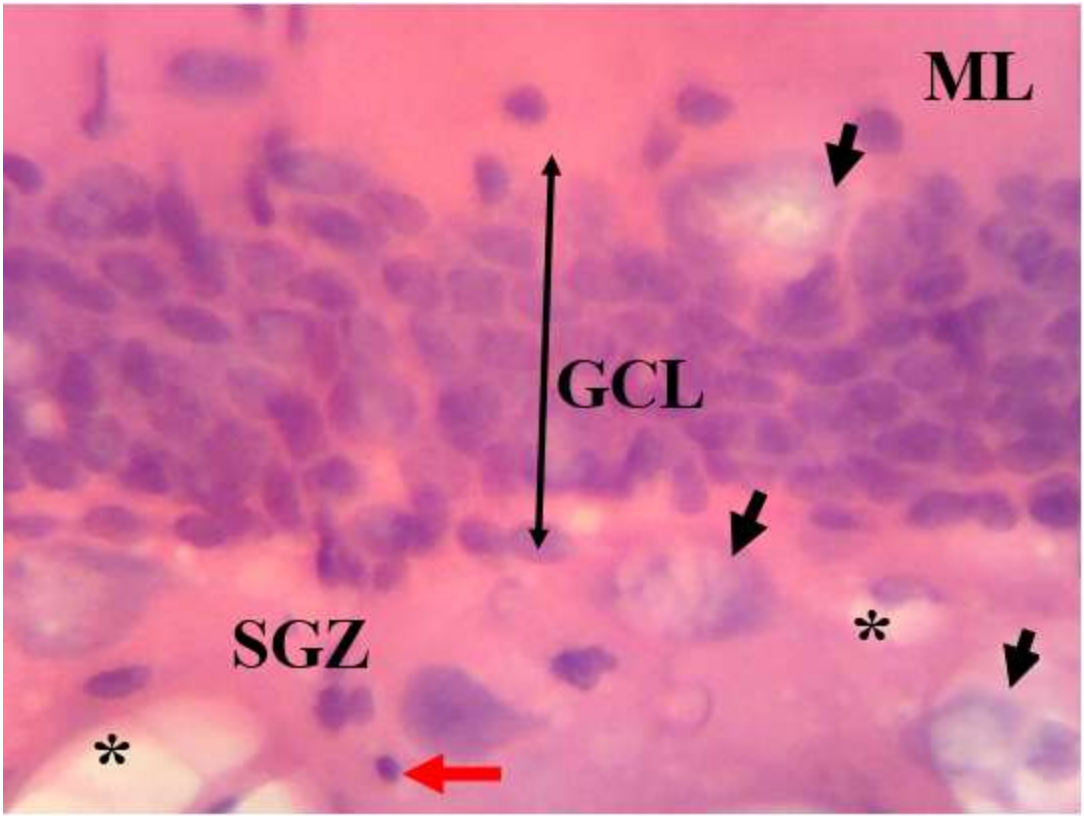


Plate LXX:A photomicrograph of Dentate gyrus (DG) of the hippocampus in adult Wistar rats at PND 85 after being treated with 800 mg/kg hyoscyamine fraction of *D. stramonium*seeds for three weeks, from the PND 56 – 77. (H and E, x400).

GCL = granular cell layer, ML = molecular layer, SGZ = subgranular zone, ➔ = oligodendrocyte, ➞ = necroticgranule cells, * = cytoplasmic vacuolation.

CHAPTER FIVE

5.0. DISCUSSION

The findings of this study revealed the presenceof alkaloids in the ethanol crude extract of the seeds of *D. stramonium.* The preliminary phytochemicalscreening observed that the seeds show the presence ofsaponins, cardiac glycosides, carbohydrates, and steroids.Surprisingly, tannins and flavonoids were absent in the phytoconstituents present. However, phytochemical screening of *D. stramonium*seeds revealed the presence ofsaponins, tannins, steroids, alkaloids, flavonoids, phenolsand glycosides(Ivancheva *et al.,* 2006; Shagal *et al.,* 2012; Carpa*et al.,* 2017). Likely, environmental factors such as plant species, rainfall, altitude, soil composition, soil salinity, pH in addition to the phenological stage, extraction and fractionation method play an important role in the differences seen in the results observed(Afsharypuor *et al.*, 1995; Shonle and Bergelson, 2000; Miraldi *et al.*,2001; Berkov and Zayed, 2004).

*D. stramonium*is known to contain highly toxic tropane alkaloids, including the pharmacologically active compounds atropine and scopolamine (Dugan *et al.,* 1989; Desachy *et al.,* 1997). Tropane alkaloids are easily separated and detected TLC (Papadoyannis, 1995; Bruneton, 1999;Steenkamp *et al.,* 2004). The TLC chromatogram of the present study shows an orange colour spot of alkaloidin both the sample and standard powder. However, the hyoscyamine size of the hyoscyamine sample spot was relatively smaller when compared to that of the standard powder. A similar study by Carpa*et al. (*2017) reported an orange colourationin the chromatogram plateof the tropane atropine and scopolamine alkaloids for the standard atropine sulfate and scopolaminehydrobromidewith position and size that resembles the standards ofatropine

and scopolamine chromatogram. This proved the presence of hyoscyamine alkaloid in the content of the crude extract obtained.

HPLC remains one of the most commonly used techniques to analyze alkaloids (Steenkamp *et al.,* 2004).The present study observeda relatively small amount of the hyoscyamine, the lower limit of detection and quantificationin the sample fraction.This was not a surprise as commercial production of alkaloids was generally considered to yield low,especially for hyoscyamine (Matsuura *et al.,* 2018). In a related study, Tayoub *et al.* (2016)quantified hyoscyamine in *Datura innoxia*and reporteda relatively even lower amount of the compound. It was generally however reported that the main alkaloid in seeds of*D. stramonium*was the atropine (List *et al.*,1979; Klein-Schwartz and Oderda, 1984; Dugan *et al.,* 1989; Miraldi *et al.,* 2001), although scopolamine is present in significant amounts(Miraldi *et al.*, 2001), and that strong evidence indicated that the hyoscyamine-scopolamineracemization occurs in the species of *Datura*.This probably could have attributed to the lower yield of the hyoscyamine in the fractionated sample.

There was a similarity in the pattern size, shape and retention factor value (Rf-value) of the hyoscyamine fraction and that of the standard powder.Some pieces of literaturehave already reported that atropine, hyoscyamine and scopolamine were the tropane alkaloids found in all species of the genus *Datura*, however, their concentrations varied depending on species and on the part of the plant (Duez *et al.*, 1985; Ren-sheng *et al.*, 1985 and Duke, 1992). Also, the quantitative analysis of hyoscyamine reported that the seeds of *D. stramonium*contained a significantly low amount of hyoscyamine of the total alkaloid contents of the extract (Afsharypuour *et al.,* 1995). Similarly, in a related study that

quantified the amount of the hyoscyamine although, in a plant species, *Hyoscyamus reticulatus* found the seeds contained a higher quantity of the hyoscyamine compared to the rest of the plant species (Doerk *et al.,* 1989). By the virtues of the shape, size and values observed, this has indicated that the sample contains hyoscyamine although not in the purest quality.

Ingestion of hyoscyamine fractionresulted in both central and peripheral muscarinic symptoms in the rats fed with graded doses of the fraction.However, no mortality was recorded as a result of ingestion both during toxicity testing and the experiment itself.A related study by Babalola*et al.* (2015),reported that the median toxic dose of *D. stramonium*fed orally in dogs was at the safety margin as considered Centre forDisease Control (CDC) the United State of America, states. However, there was no published literature to comparethe present study in Wistar rats. Considering the foregoing it could be assumed that oral ingestionof *D. stramonium* seeds might equallyhave a high safety margin in Wistar rats.The clinical symptoms observed might probably related to the anticholinergic properties of tropane alkaloids which competes and irreversibly inhibits acetylcholine on muscarinic receptors, thereby causing both central and peripheral nervous system manifestations (Hanna *et al.,* 1992). The central nervous system features include restlessness (hyperactivity), laboured breathing and delirium, while the peripheral symptoms observed include laboured breathing (dyspnea), piloerection, abdominal cramps, stooling (diarrhoea), and urination. Similar observations were reported in patients involved in *D. stramonium*poisoning (Ramirez *et al.,* 1999).

The assessment of physical growth indices of the pre-weaning development in the present study did not show a significant difference in the eye-opening, hair growth, teeth eruption and bilateral ear detachment between the control and treated groups.This has contradicted the report that exposure of the foetusto *D. stramonium* when a mother uses it for asthma could ultimatelyresult in permanent damage to the foetus (Pretorius and Marx, 2006),also that subcutaneous injection of atropine resulted in malformed fetuses was observed (Yu *et al.*, 1988). But in a separate study no adverse effects on prenatal viability, no evidence of teratogenesis, and only a marginal reduction in fetal body weight was observed in mice treated at doses of 450 and 900 mg/kg body weight per day of tropane alkaloid (NTP,1987a). Although no direct relationship between first-trimester use of atropine and birth defects was found, a study reported an increase in birth defects in the offspring of mothers who had taken belladonna alkaloids (Miodini *et al.*, 1999). Also, Ishola and Adeniyi (2013), reported that no physical malformation observed in Wistar rats treated prenatally with ethanolic leaves extract of *Datura metel*. The discrepancies observed probably resulted from the environmental factors such as plant species, rainfall, altitude, soil composition, soil salinity, pH in addition to the phenological stage, extraction and fractionation method used (Afsharypuor *et al.*, 1995; Shonle and Bergelson, 2000; Miraldi *et al.*,2001; Berkov and Zayed, 2004). Although single-dose was used, this has suggested that prenatal exposure of hyoscyamine fraction of *D. stramonium* does not cause physical malformationin the development of Wistar rats.

No statistically significant difference in the bodyweights of the prenatally treated Wistar rat pupswas observed when compared to the control group at the beginning of the first two weeks of postnatal development. Surprisingly, the trend changed from the weaning

period of the3rd week to the adulthood period of the 12th week in which, a statistically significant decrease in the bodyweight of the treated group was observed when compared to the control. Although no published data on the effect of prenatal exposure of hyoscyamine fraction of *D. stramonium* on the bodyweight of Wistar rat pups, pieces of literature reported a significant reduction in the bodyweight (Kurzbaum *et al.*,2001; Hilaly *et al.,* 2004; Pretorius and Marx 2006) in the Wistar rat pups treated with *D. stramonium* leaves postnatally.

There wasa statistically significant delay in response to surface righting and cliff avoidance reflexes in the prenatally treated group when compared to the control at PND 5. However, for the rest of the test days, no difference was observed between the two groups. Surprisingly, the response in negative geotaxisreflex was not significant from the control at PND 5, however, a statistically significant delay in response was observed in the treated group when the animals were getting older at PND 35. Although no published literature available that reported the effect of prenatal exposure of hyoscyamine fraction of *D. stramonium* seeds on the functional battery test, it was however reported that newborns are particularly vulnerable to atropinic effects,such as hyperexcitability, suction disorders, constipation,convulsion or tachycardia, when the pregnant women are exposed at the end of the pregnancy (Webster, 1973; Briggs*et al*., 2011). It was also reported that rodents exposed prenatallyto antipsychotic drugs show deficits in learningand memory acquisition and retention (Costa*et al*., 2004), although the potential long-term neurodevelopmental effects of thesedrugs have been investigated with inconclusive results (Previti*et al*., 2014).

The triple horizontal bars motor coordination indicated no significant difference in the motor ability between the control and treated groups especially at the prenatal and lactational stages as indicated by the fall-off time. However,a significant difference was observed in the group treated at the adolescence stage, and in the group that received medium-dose at adulthood. In a related study by Dugan *et al*. (1989), for Sprague Dawley rats weanlings treated for ninety days with *D. stramonium*seeds that contained a reasonable quantity of scopolamine and atropine, no behavioural differences between groups were observed but a statisticallysignificant and dose-dependent growth inhibition occurred in all groups. The principal effects ofexposure to *Datura*were decreased body weight gain, serum albumin and serum calcium, increasedliver and testes weights, and elevated serum alkaline phosphatase and blood urea nitrogen levels,with female rats showing more marked responses than males.It was also reported that oral absorption of the anticholinergic agents, such as atropine and glycopyrrolate was poor as a trace or no amount was reported to be found in breast milk (Hale, 1999). The statistically significant observation made might signify that though, hyoscyamine fraction of *D. stramonium*could be toxic and affect the psychomotor activities of the rats, but it may not cause long-term and adverse neurotoxicity.

The significant difference observed in the fall-off time in the treated group of the adolescence stage might result from ontogenetic variations in drug response, age-related differences in pharmacokinetics, drug metabolism and excretion rates (Hein, 1987) when compared to prenatal and lactational exposed groups. In a related study, adolescent rats found to be more sensitive than adult animals for the cataleptic effect of haloperidol (Spear, 1980;Campbell, 1988) or hyperlocomotion induced by morphine (Spear, 1982).

The hippocampus is one of the most studied areas of the brainby neuroscientists, especially in the field investigating epileptogenesis,learning, and memory processing. Neuroanatomically,the hippocampus is composed of the cornu ammonis (CA) andthe dentate gyrus. The dentate gyrus receives afferent input fromthe entorhinal cortex and sends efferents to the hippocampalpyramidal cells. The CA is subdivided into regionsCA1, CA2, and CA3 (Rao, 2013).Cornu Ammonis1 (CA1) is the zone that is most sensitive to variousinsults (Treuting*et al.,* 2012). On the other hand, tropane alkaloids (TA) is believed to alterneurogenesis, but little knowledge is known as to themechanisms of their actions(Joels *et al.*, 2004; Joosen,2009).

Novel object recognition (NOR) tests conducted showed a significant difference in the discrimination time between a novel and familiar object in the control group compared to the treated in all the developmental stages (that is, gestational, lactation and adulthood stages). However, novelty preferences and discrimination (DI) indexes were not significant at the lactational stage and only at the lowest dose of exposure during adulthood. Tropane is believed to alter neurogenesis (Joels *et al.*, 2004; Joosen, 2009), but little knowledge is known as to the mechanisms of its actions. However, it was thought to exert its activities by either involved in the destruction of the neural precursor cells thus limiting their survival rate. Also, scopolamine as a tropane alkaloid drug that produces a competitive antagonist at muscarinic acetylcholine receptors (mAChRs) by interfering with the cholinergic transmission, causes impairment in learning and short-term memory in rodents and humans (Iversen, 1997). In a related study atropine have been shown to have an affinity for rapidly dividing cells (Shiu *et al.,* 2007;Chia-hua *et*

al., 2007, 2008; Li *et al.*, 2009; Lingmei *et al.*, 2010, 2011; Xia et. al., 2011; Xia *et al.*, 2012). A separate study by Olawepo*et al.*(2017), showed that a memory test conducted on adolescent rats when atropine was administered demonstrated a deficit in cognition, particularly recognition memory in animals as compared to the control from the novel object recognition test (NORT) result. The findings of Joels *et al.*(2004) and Joosen *et al.*(2009) earlier reported also shows that atropine impairs learning abilities and neurodegeneration. Moreover, the results of the NOR paradigm are influenced by both hippocampal and cortical lesions (Buckmaster *et al.*, 2004; Clark *et al.*, 2000). It is widely accepted that in both the monkey and the rat brain, the perirhinal cortex plays an important role in object recognition memory (Aggleton *et al.*, 2010), that is, the ability to evaluate a previously encountered item as familiar depends on the integrity of the medial temporal lobe (Hammond *et al.*, 2004). This brain structure plays an important role in the recognition of memory formation, and when some damage exists, the performance in recognition memory tasks is impaired (Albasser *et al.*, 2009). Studies with primates and rodents have shown that for visual object recognition memory, the parahippocampal regions of the temporal lobe (namely the perirhinal, entorhinal, and inferior temporal cortices) are very important (Hammond *et al.*, 2004).

Lactational drug exposure of *Atropa* decreases the production of breast milk due to its anticholinergic properties and the that it is secreted in breast milk (Barrager *et al.*, 2002). However, it was not reported in the scientific literature as either being safe or causing harm to the nursing infant (Mills, 2006).

In adulthood, significant memory impairment observed during the NOR test in the least – dose between the control and treated rats was probably a result of non – monotonic dose-response or hormesis of the animals to hyoscyamine fraction. Hormesis is induced by physical and chemical stressors including phenolic compounds (Boyd *et al.,* 1997; Sinclair *et al.,* 1999; Okolo *et al.,* 2007; Zaki *et al.,* 2008; Nweke and Okpokwasili, 2010a,b; Nweke *et al.,* 2014, 2015), perfluorinated carboxylic acids (Mulkiewicz *et al.,* 2007), mycotoxins (Li *et al.,* 2014; Wang *et al.,* 2014), bacteriocins (Murado and Vázquez, 2010), antibiotics (Welch *et al.,* 1946; Randall *et al.,* 1947, Linares *et al.,* 2006; Migliore *et al.,* 2010, 2013), herbicides (Cedergreen 2008a,b; Cedergreen *et al.,* 2009; Belz and Cedergreen, 2010; Cedergreen and Olesen, 2010; Belz *et al.,* 2011; Belz and Leberle, 2012; Nweke *et al.,* 2016), Wastewater (Hoffmann and Christofi, 2001; Nwanyanwu and Abu, 2010), heavy metals (Christofi *et al.,* 2002; Rodea- Palomares *et al.,* 2009; Shen *et al.,* 2009) and ionic liquids (Cho *et al.,* 2007; 2008, Wang *et al.,* 2011) either as an individual or as mixtures. In a separate study, two African medicinally used *Datura* species namely *Daturametel* and *innoxia* were reported as metallophytes and accumulator of heavy ions such as cobalt (Co) and Nickel (Ni) respectively. As such recommended as a phyto-monitor (Bhattacharjee *et al.,* 2004), and metal tolerant species (Kelly *et al.,* 2002) respectively. The low – dose-response observed was probably due to the accumulation of the heavy metal observed.

Morris water maze (MWM) is a neurobehavioural paradigm use to test spatial learning and memory (Morris *et al.,* 1982). It is also commonly used to assess hippocampus-dependent spatial memory (Vorhees and Williams, 2006). The present study observed a significant decrease in spatial learning and memory task in the test days following five

days of consecutive training in the hyoscyamine fraction treated rats compared to their controls at prenatal, and adulthood exposures. However, no statistically significant decrease in the task was observedat the lactational and adolescence stages of exposure. Literature reported that tropane alkaloids, such as hyoscyamine fraction are competitive antagonists at muscarinic acetylcholine receptors (mAChRs) which interferes with the cholinergic transmission, leading to impairment of learning and short-term memory in rodents and humans (Iversen, 1997). Therefore, the prenatally exposed Wistar rat groups who letter manifested the spatial learning and memory impairments in adulthood indicated possible interference in the neurogenesis of the hippocampus and its lasting effect. In a related study, Olawepo*et al. (*2017) reported a deficit in spatial memory using Y-maze following oral ingestion of graded doses of *D.metel* in Wistar rat pups.Similar observations were made using Y-maze, results showed clear indications of atropine long-lasting effect on the brain (Brown *et al.,* 2000; Spear 2002; Squeglia *et al.,* 2009). The deficit in spatial memory was also reported inscopolamine-induced memory impairment (Zhang *et al.,* 2008; Han *et al.,* 2012). Zhang *et al. (*2008) also reported increased latency times in the spatial acquisition phase in mice treated with graded doses of scopolamine intraperitoneally. Han *et al. (*2012) also reported learning and memory deficits in mice after treatment with scopolamine. Scopolamine administration not only induces dysregulation of the cholinergic system and memory circuits in the brain but also decreases the expression of cAMP-response element-binding protein (CREB) and brain-derived neurotrophic factor (BDNF) in the central nervous system (CNS). BDNF is responsible for synaptic plasticity and memory performance and is coupled to CREB

activation. CREB is closely related to hippocampal learning and memory (Tyler *et al.*, 2002).

The Morris water maze spatial learning and memory task showed no statistically significant difference in the adolescent treated rats when compared to the control. This corroborated the result of Olawepo *et al.* (2017), on spatial learning and memory task using Y-maze where it was reported that atropine treated Wistar rats at adolescence showed no statistically significant in right and wrong decisionwhen compared to the control. Adolescence is a period of transition of physical and psychological development(Christie and Viner, 2005), and it heralded the vulnerability of stress and in some situations, opportunities to alleviate the adverse effects of events experienced earlier during developmental processes (Dahl, 2004). Major activities going on in the adolescent brain involved synaptic pruning (Yakovlev and Lecours, 1967; Innocenti, 1995; Giedd *et al.*, 1999, 2004; Winters, 2008).

A significant decrease in the amount of Pb was observed in the lactation and adolescent treated groups when compared to thecontrol. However, a statistically significant increase in the amount of Ni was observed in the group treated at lactation.No statistically significant difference in the amount of Cu and Zn was observed in either lactation or adolescent treated groups, nor of Ni in the adolescent treated group. A study on the seeds of *D. stramonium*revealed that as a metallophyte which canaccumulate heavy metals when grown under natural conditions (Bhattacharjee *et al.*, 2004). It contains variable amounts of trace elements such as Zn, Cu, Pb, Ni and Cr (Oseni *et al*., 2011). Trace elements are quintessential to maintain various biochemical and physiological functions

in living organisms when in very low concentrations; however, they become noxious when they exceed certain threshold concentrations (Jaishankar *et al.,* 2014). The observed variations in the mean concentrations of these elements might not be a surprise since the extent to which these elements are released into breast milk might naturally vary. For instance, the concentration of Pb has been shown to almost always be higher than the concentration of Hg and Cd in the same cohort of breast milk (García-Esquinas *et al.,* 2011). At the adolescence stage, however, the amounts of Niand Pbsignificantly decreased compared to their controls.

The quantity of Ni and Pb in the adult treated group at low and high doses increased significantly when compared tothe control group. However, only Ni increased significantly in the medium dose.In a similar study, it was, reported that toxicity of trace elements depends primarily on the degree of contamination, but also on the species and age of the organism, the route of introduction of the body, the form of the chemical, the type of interaction with other metals, or the physiological condition of the body (Niesiobędzka *et al.*, 2005). Generally, it was observed that the amount of Cu and Zn ions remained statistically not significant in all the stages of the treatments when compared to their respective controls. It has been reported that copper, zinc, chromium and iron are trace elements or elements required for the proper growth, development and functioning of living organisms (Bogda, 1973).

The expression of Disc-large homolog 3 (*DLG3*) was significantly downregulated in this study in the treated groupwhen compared to the control in a hormetic response manner. *DLG*3is a gene that encodes the synapse-associated protein 102 (SAP102) and is located

at Xq13.1. It was first identified as a component of glutamatergic synapses through its binding to NMDA receptors (Muller *et al.,* 1996). SAP102 associates with GluN2 subunits of NMDA receptors, particularly GluN2B which is the dominant subunit in cortical neurons during early development (Sans *et al.,* 2000). It was reported that SAP102 is relatively highly expressed early in development (Sans *et al.*, 2000) compared to the rest of MAGUK proteins, suggesting its specific role in synaptic development. In the present study, significant down-regulation in the expression of the *DLG3* gene was observed compared to the control, however, in a hormetic-response manner. Consistent with an important role for SAP102 in synaptic development and plasticity, adult mice lacking SAP102 show impaired hippocampus-dependent spatial learning and altered synaptic plasticity but normal hippocampal synaptic transmission (Cuthbert *et al.*, 2007). This probably has explained the observations recorded in the Morris Water Maze and novel objects recognition tests conducted on the animals. Repeated exposure to exogenous substances such ashyoscyamine fractionmight have resulted in ligand-induced desensitization or internalization of that receptor, which typically could lead to addictions. This probably was the cause of the hormetic response patterns of the doses administered, partly it could be as a result of the presence of the identified trace elements.The lead was reported to interfere with the release ofneurotransmitters (Dart *et al.*, 2004; Needlemann, 2004).This interference leads to disruption of communication between cells. Lead usuallyinterferes with the neurotransmitter glutamate which isimportant for many functions, like learning. It operatesby blocking NMDA receptors. The blocking of such receptors was thought to be the main target of lead toxicity. In a different study, it was found that in addition to inhibition of the NMDAreceptor, lead

exposure also decreased the amount of genefor this receptor in part of the brain (Kosnett, 2005). The leadwas also found to be involved in apoptosis of brain cells inanimal studies (Nedlemann, 2004).

The expression of Neurogenic Differentiation1 (*NeuroD1*) downregulated significantlyin the treated groups when compared to the control in a monotonic-dose response manner. Thus, by down-regulating *NeuroD1* activity, hyoscyamine fraction probably impaired the differentiation of newborn glial cells, leading to a reduction in neuroblasts and immature neurons formation. In a related study, it was found that the activity of a transcriptional factor, neurogenic differentiation 1 (*NeuroD1*) could be down-regulated by exogenous substances such as morphine (Zhang *et al.,* 2016) and carbofuran exposures (Mishra *et al.,* 2012). Several studies have suggested that newly generated cells in the dentate gyrus play an important role in hippocampal functions because the proliferating newly born cells differentiate into mature neurons, establish synapses with existing hippocampal circuitry, and regulate hippocampus-dependent learning and memory processes (Laplagne *et al.,* 2006; Snyder *et al.,* 2001; Toni *et al.,* 2008; van Praag *et al.,* 2002; Zhao *et al.,* 2008).

Stereological studies of the hippocampus subfields (CA1 and CA3) and DG regions showed statisticallysignificant differencesin values in the neuronal cell numbers of the regions in the treated groups when compared to the controls in the present study. For the prenatally treated group, a statistically significant decrease in the neuronal cells of the hippocampal regions including the DG was observed in the treated group when compared to the control. For the group treated at lactation, a significant decrease in the neuronal

cells number of the CA1 and CA3 regions in the treated group at the end of the periodcompared to the control, however, the neuronal cells number in theDG and CA1 regions significantly increased after the animals reached adulthood. Similarly, a significant decrease in the neuronal cell numbers of the DG regions of the hippocampus was observed however, the cells in the region increased statistically as the rats reached adulthood. The CA1 and CA3 regions decreased statistically at the end of the treatment, however, the CA1 region increased statistically after the animals reached adulthood except for the neuronal cells of the CA3 region which showed no statistically significant difference from the control group. Oral ingestion of the hyoscyamine fractionof *D. stramonium*showed statistically significant differences in the neuronal cell numbers of the hippocampus subfields including the DG region in the groups treated at adulthood with graded doses of the fraction. Statistically significant increase in theneuronal cell numbers of the DG and CA3 regions of the hippocampus although not dose-related when compared to the control group. The neuronal cell numbers of the CA1 region, however, decreased significantly in the treated group also in a somewhat dose-related manner when compared to the control group.In a related study, Falluel-Morel *et al*. (2007) reported that exposure to MeHg during the perinatal period induced apoptotic cell death in the hippocampus, whichled to structural and functional deficits at a later stage. The investigation reported a marked decrease (29% and 22%) in the number of cells in the GCL and hilus respectively following exposure to MeHg. Also, a non-significant change in the number of granule cells (DGEC, DGEN) of Wistar rats was reported followingtreatment with sodium Arsenite (Kaler*et al*., 2013). A significant reduction in the neuronal number in CA1, CA3, and area dentatae (rat hippocampus) after

subcutaneous and dermal exposure to chlorpyrifos has been reported earlier (Roy*et al*., 2005).Neuronal cell death could be the underlying basis for the deficit in neuronal numbers in these regions. Equally, quantitative analysis revealed a significant difference in packing density of cells in the CA1 region of experimental rats treated with sodium arsenite as against the control. This suggests more vulnerability of the CA1 region to insults because of high intrinsic superoxide and endogenous reactive oxygen species (ROS) production in CA1 than CA3 region (Wilde*et al*., 2002; Wang*et al*., 2005). So, for those under the effect of hyoscyamine fraction of *D. stramonium*seeds which produces excess oxidative stress that leads to depletion of antioxidant in the CA1 region, cell death ensues. Similar observations were reported following exposure to alcohol on the hippocampus (Tran and Kelly, 2003; Miki*et al*., 2004). Mitochondrial permeability transition pore of CA1 region is more sensitive to calcium homeostasis and leads to active production of ROS (Mattiasson*et al*., 2003), so that vulnerability of CA1 for cerebral ischemia and neurodegenerativedisease is more in comparison to CA3 region. The statistically significant increase in the neuronal cell numbers probably suggested that the neuronal cell loss observed immediately after the treatment might be reversible.

No statistically significant difference in the hippocampus thickness neither that of the DG in all the major developmental stages except for the hippocampus of the prenatally treated group after reaching adulthood which showed a statistically significant increase in thickness. A similar observation was made on the pyramidal cell layer of the hippocampus where a significant reduction in thickness with somewhat reduced and

disrupted cells in experimental animals exposed to sodium arsenite during the PND 4 – 11 (Kaler*et al.*, 2013).

No statistically significant increase in hippocampus and DG areas of all the animals exposed to the hyoscyamine fraction of *D. stramonium* was observed during the major development timelines except for the prenatally exposed group where the hippocampus area was found to increase statistically when compared to that of the control at birth. However, in the group treated through lactation, a statistically significant decrease in the hippocampus area was made only after the animals reached adulthood. For the group treated at adolescence, only the hippocampus areas of the animals treated differed significantly from that of the controlwhen the animals reached adulthood. However, for the group treated at adulthood, only the DG area of the treated groups indicated a significant increase from the control in a somewhat dose-dependent manner.In a related study on the effects of neonatal exposure to sodium arsenite, an increase in nuclear area of the pyramidal cells (CA1, CA3) and granule cells (DGEC, DGEN) was reported (Kaler*et al.*, 2013). An increase in the soma size of pyramidal cells (CA1 region) and granule cells (DG) was also reported by Isgor *et al*. (2004) following chronic stress. An increase in neuronal nuclear size (reflecting an increase in cell size) could be linked to an increase in organelle size (hypertrophy).Proper functioning of the sodium-potassium adenosine triphosphatase (Na-K ATPase) pump is one of the important requisites for the maintenance of intracellular fluid/substance balance (Kaler *et al.*, 2013).Exposure to arsenic during the early life postnatal period was reported to result in the malalignment,

increase in nuclear area and decrease in cell density of the pyramidal cells (CA) and granule cells (DG) in experimental animals (Kaler *et al.*, 2013).

The volumes of the hippocampus were found to differ significantly in the treated groups in allthe developmental stages. A statistically significant increase in the volume of the hippocampus was observed in the prenatally treated group when the animals reached adulthood. However, inthe lactation treated group, a statistically significant decrease in the volume was observed after the animals reached adulthood. But the group treated at adolescence, the volume decreased statistically only at the end of the treatment. For the group treated at adulthood, a statistically significant increase in the hippocampus volume was observed only at high dose exposure.Somewhat similar observations were reported in neonatal rats exposed to formaldehyde (Sarsilmaz*et al*., 2007), where investigators noticed a significant decrease in volume of a pyramidal cell layer in exposed animals (at PND 30 and PND 90). The lack of significant difference observed in the groups exposed during lactation might probably be linked to the first – pass effect and a long period of break observed from the weaning period to adulthood.

There were observable changes in the histology of the control and treated hyoscyamine fraction of *D. stramonium*treated groups. Mild hyperplasia with densely compacted and thicker pyramidal cells layer, apoptotic and pyknotic cells, a significant amount of mesenchymal and patches of neuroglial cellswere observed in the CA1 and CA3 hippocampal subfields including the DG of the treated group as compared to the control at *in utero* exposure. As the animals reached adulthood, hypochromicpyramidal cells with

sparsely organized CA1, relatively misaligned scanty and poorly developed neurons CA3 pyramidal cell, and relatively hyperchromicwith vacuolation, pyknotic cells and necrotic cells were observed in the DG region of the treated group compared to the control. Whereas, at lactation, hyperchromic nuclei, patches of oligodendrocytes and pyknotic cells were also observed in the hippocampal subfields of the treated compared to the control group. But when they reach adulthood, several vacuolated and slightly misaligned pyramidal cells were observed in the areas of the treated hippocampus of the rats when compared to the control group. The slightly thicken and hyperchromic cells observed in the treated compared to the control could have resulted fromthe altered ratio between the neurons and neuroglia which might be a result of increased production of neuroglial cells or it might be a result of degeneration or apoptosis of the neurons. Stem cells are capable of proliferation, self-renewal and production of differentiated functional progeny that is characteristic of the organ from which they were derived (Hall and Watt, 1989). The proliferation and differentiation of neural stem cells are under the control of various intracellular and extracellular signalling molecules and growth factors (Weissmann, 2000; Chan *et al.*, 2002). The exogenous small molecules that can mimic the activity of the growth factors could, in principle, induce neurogenesis or gliogenesis *in situ*, in the brain and the endogenous neural stem cells (Dai and Holland, 2003; Connor and Dragunow, 1998; Kruger and Morrison, 2002). The hyperchromic neuronal and neuroglial cells observed were probably resulted due to the intensive biosynthesis of proteins forthe own needs of neurons, which could be a way of adaptation of thebrain hippocampal neurons to compensate the death as a resultof antenatal exposure to the hyoscyamine fraction.The passage of psychotropic medications through breast milk

depends upon several factors including administration route, absorption rate, half-life, and time of peak serum concentration, dissociation constant, the volume of distribution, molecular size, degree of ionization, pH of plasma and milk, the solubility of drugs in water and lipids, and greater binding to plasma protein than to milk protein (Gentile, 2004). The higher lipid content of hindmilk (the milk ejected during the second half of a feeding) makes it likely that the second half will have a higher concentration of maternal medication than the first half (fore-milk) (Burt, 2001).

Significant vacuolated cytoplasm, partiallyhyperchromicand apoptotic cells were observed in the treated group but when allowed to reach adulthood, scattered oligodendrocytes, degenerating, poorly developed and scanty pyramidal cells which were slightly hyperchromic in the treated group than the control of adolescent Wistar rats. At adulthood, hypochromic and vacuolated, slightly disoriented, karyolitic and necrotic, relatively degenerating, ruptured blood vessels, scanty oligodendrocytes were observed in the treated group when compared to the control. Adolescents are the most prevalent age group forpoisoning with *D. stramonium*intentional abuseis common by eating seeds, drinking tea or smokingcigarettes (Amini*et al.,* 2012).In a related study, Ekanem *et al.* (2016) indicated that *D. stramonium*extractwas found to cause atrophy of the axons and fibres,cytoplasmic vacuolation, cell necrosis and cell losses as comparedto the controls. It was also inconsistent with thestudy carried out by Bihaqi *et al. (*2012) which reported that the histology of the cerebral cortex examined under a light microscope showed an increased neuronal loss, ghost cells, haemorrhage and vacuolated cytoplasm on treated rats that received daily intraperitoneal administration of scopolamine. Maryam *et al. (*2013) showed in their work that Vinca alkaloid, which has medicinal value for the

treatment of certain diseases including the treatment regimens for testicular carcinoma, was found to be neurotoxic at higher doses. The primary pathologic effect observed was related to axonal degeneration and a decrease in axonal transport. Adekomi *et al. (*2011) found from their studies that exposing Sprague Dawley rats to the smoke of *D. stramonium* leaves induced histological changes in certain organs of the body compared to the control rats. Fowler (2014), using histological analysis combined with stereological technique, demonstratedthat the prefrontal cortex (PFC) which is part of the FC is vulnerable to chronic, alcohol-induced oxidativestress and neuronal cell death. Excessive oxidative stress and subsequent DNA damage can be responsiblefor neuronal apoptosis and neuronal dysfunction associated with different neurological pathologies (Jacintho and Kovacic, 2003; Ramalingam and Kim, 2012; Choi *et al.*, 2012).

CHAPTER SIX
6.0. CONCLUSION AND RECOMMENDATIONS

6.1. CONCLUSION

Hyoscyamine fraction of *D. stramonium*is an orange colour spot of the alkaloid which is identifiable on a thin layer chromatogram plate. It is a toxic substance with both central and peripheral symptoms. Prenatal ingestion of hyoscyamine fraction does not cause teratogenicityor alters physical body growth, it, however, decreasedbodyweight in Wistar rats treated prenatally. It can also mildly affect the dynamic tests of sensorimotor development; negative geotaxis, surface righting reflexes butsignificantly affects both visual, spatial memory and learning assessments in the animals exposed prenatally and postnatally. Being an alkaloid of metallophyte plant, hyoscyamine fractioncan alter the hippocampal trace elements balance, thereby causing fluctuation of elements like Cu, Ni,

Pband Zn. It can also enhance the downregulation in the expressions of the Disc-large homolog 3 (*DLG3*) and Neuro-differentiation1 (*NeuroD1)* genes,a significant reduction in the neuronal numbers of the DG and CA3 regions of the hippocampus when ingested prenatally and at adolescence in rats. Its impact on the histology of the hippocampus was toxic, as it caused cellular apoptosis, vacuolations, pyknosis, karyorrhexis, hyperchromatic, and necrosis in the hippocampal subfields and DG of the hippocampus following both prenatal and postnatal exposure of the different doses of the hyoscyamine fraction.

6.2. RECOMMENDATIONS

i. Further research shouldvary the dose for exposure to hyoscyamine fraction of *D. stramonium* seeds to see the effects of prenatal exposure at a different dosage.

ii. Further research should be carried out on gender differences as regards prenatal, lactation, adolescence and adulthood exposure to hyoscyamine fraction of *D. stramonium* seeds concerning neurobehavioural,biochemical and stereological analyses.

iii. DNA sequencing should be carried out on the gene segments mostly affected by the downregulation in the expressions of both *NeuroD1* and *DLG3* genes.

iv. The RT-PCR should be carried out to evaluate the activities of *NeuroD1* and *DLG3* genes at prenatal, lactational and adolescence stages of exposure to hyoscyamine fraction of*D. stramonium* seeds.

v. For a better and more accurate result, more housekeeping genes should be used for the gene expression study.

vi. Further research should consider the use of vibratomes, motorized stage, confocal microscopeand stereology software such as *Visiopharm* for better accuracy.

vii. Further research should include immunohistochemistry stains such as Ki67, Acridine orange/Annexin V stains, to demonstrate histologically cellular proliferation, differentiation, and apoptosis in the developing neuronal cells.

6.3. CONTRIBUTION TO KNOWLEDGE

The study provides;

i. a baseline data for the amount of the hyoscyamine in the crude ethanol extract of *D. stramonium* as 11.035 mg/µl at the limit of detection (LOD) and limit of quantification (LOQ) of 0.75 and 2.26 mg/µl respectively. Also, generated a regression equation for quantification of hyoscyamine in the fraction of crude ethanol extract of *D. stramonium*seeds was generated as y = 28.34x - 253.63. Based on the equation, y = the peak area of the hyoscyamine fraction whereas, x = the quantity of the hyoscyamine in the sample.

ii. that prenatal exposure to hyoscyamine fraction of *D. stramonium* seeds treated decreased bodyweightin bothyoung and adult Wistar rats.

iii. that ingestion of hyoscyamine fraction of *D. stramonium* seedsimpaired recognition ability and spatial learning and memory of Wistar rats treated inprenatal and adulthood stages.

iv. that quantities of trace/heavy elements in the hippocampuswerealtered with exposure/ingestion ofhyoscyamine fraction of *D. stramonium* seeds administration.

v. that ingestion of hyoscyamine fractionof *D. stramonium* seeds downregulated the expression of Neurogenic differentiation 1 (*NeuroD1*) and Disc large homolog 3 (*DLG3*) genes cyclic threshold values and fold changes.

vi. ingestion or exposure to hyoscyamine fraction of *D. stramonium* seeds treated groups in the major developmental stages impaired stereological indices (neuronal numbers, thickness, surface areas and volumes) of the hippocampus and dentate gyrus of Wistar rats.

vii. that both pre-and postnatal ingestion of hyoscyamine fraction of *D. stramonium* seeds caused neuronal lesions in the histology of the hippocampus and dentate gyrus.

REFERENCES

Abercrombie, M.(1946).Estimationofnuclearpopulationfrommicrotome sections. *Anatomical Records.* 94, 239–247.doi:10.1002/ar.1090940210

Adams, J. (1986). Methods in *Behavioural* Teratology. In: *Handbook of Behavioral Teratology.* Riley, E.P., Vorhees, C.V. (eds.), New York Plenum Press, pp. 67-100.

Adekomi, D., Musa, A., Tijani, A., Adeniyi, T. and Usman, B. (2011). Exposure to Smoke Extract of *Daturastramonium* Leaf: Some of its Effects on the Heart, Liver, Lungs, Kidneys and Testes of Male Sprague Dawley Rats. *Journal of Pharmacognosy and Phytotherapy*, [online] 3, pp.67-75. Available at: http://www.academicjournals.org/jpp [Accessed 26 Nov. 2019].

Adriani, W. and Laviola, G. (2004). Windows of vulnerability to psychopathology and therapeutic strategy in the adolescent rodent model. *Behavioural Pharmacology*, 15(5), pp.341-352.

Adriani, W., Granstrem, O., Macri, S., Izykenova, G., Dambinova, S., and Laviola, G. (2004). P1 Behavioral and Neurochemical Vulnerability During Adolescence in Mice: Studies with Nicotine. *Behavioural Pharmacology*, *15*(5), A8. doi: 10.1097/00008877-200409000-00041

Afsharypuor, S., Mostajeran, A., and Mokhtary, R. (1995). Variation of Scopolamine and Atropine in Different Parts of *Datura* metel During Development. *Planta Medica*, *61*(04), 383-384. doi: 10.1055/s-2006-958114

Aggleton, J., Albasser, M., Aggleton, D., Poirier, G., and Pearce, J. (2010). Lesions of the rat perirhinal cortex spare the acquisition of a complex configural visual discrimination yet impair object recognition. *Behavioural Neuroscience*, *124*(1), 55-68. doi: 10.1037/a0018320

Ajasa, M.A., Bello, O.M., Ibrahim, O.M., Ogunwande, A.I., Olawore, O.N., 2004. Heavy trace metals and macronutrients status in herbal plants of Nigeria. Food Chemistry 85, 67–71.

Akal, Z.Ü., Gürkan, S., Alpsoy, L. and Yıldız, A. (2014). Evaluation of Cytotoxic and Genotoxic Effects of *Datura stramonium*Extracts on Cultured Human Lymphocytes. Med Aromat Plants 3: 170. doi: 10.4172/2167-0412.1000170.

Alba, M. and Salvatori, R. (2004). A Mouse with Targeted Ablation of the Growth Hormone-Releasing Hormone Gene: A New Model of Isolated Growth Hormone Deficiency. *Endocrinology*, 145(9), pp.4134-4143.

Albasser, M., Davies, M., Futter, J., & Aggleton, J. (2009). Magnitude of the object recognition deficit associated with perirhinal cortex damage in rats: Effects of varying the lesion extent and the duration of the sample period. Behavioural Neuroscience, 123(1), 115-124. doi: 10.1037/a0013829

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., Walter, P. (2008): Molecular biology of the cell. 5th eds. *GarlandScience*, NY.

Alleva E, D' Udine B: Early learning capability in rodents: a review (Rattus norvegicus and Mus musculus). Int J Comp Psychol 1987, 107-125.

Altun, M., Bergman, E., Edström, E., Johnson, H. and Ulfhake, B. (2007). Behavioural impairments of the ageing rat. *Physiology and Behavior*, 92(5), pp.911-923.

Alvarez, M., Marconi, P.L. (2011) Genetic transformation for metabolic engineering of tropane alkaloids, InTech, 291-300

Amini, M., Khosrojerdi, H. and Afshari, R. (2012). Acute *stramonium* poisoning in East of Iran: A case series. *Avicenna Journal of Phytomedicine*, 2, pp.86-89.

Andersen, S. (2003). Trajectories of brain development: point of vulnerability or window of opportunity. *Neuroscience and Biobehavioral Reviews*, *27*(1-2), 3-18. doi: 10.1016/s0149-7634(03)00005-8

Ang, S., and Rossant, J. (1994). HNF-3 beta is essential for node and notochord formation in mouse development. *Cell*, *78*, 561-74. doi: 10.1016/0092-8674(94)90522-3

Annan, K., Kojo, A., Asare, C., Asare-Nkansah, S., Bayor, M., 2010. Profile of heavy metals in some medicinal plants from Ghana commonly used as components of herbal formulations. Pharmacognosy Research 2, 41–44.

Aschner, M. and Costa, L. (2005). *The role of glia in neurotoxicity*. Boca Raton, FL: CRC Press.

Assémat, E., Bazellières, E., Pallesi-Pocachard, E., Le Bivic, A., and Massey-Harroche, D. (2008). Polarity complex proteins. *Biochim Biophys Acta, 1778*, 614-30. doi: 10.1016/j.bbamem.2007.08.029

Ayuba, V.O., Ojobe, J.O., Ayuba, S.A. (2011). Phytochemical and proximate composition of innoxia leaf, seed, stem, pod and root. *Journal of Medicinal Plants Research*, 5(14):2952-55.

Babalola, S., Suleiman, M., Hassan, A. and Adawa, D. (2015). Evaluation of *Datura* Metel L Seed Extract as a Sedative/Hypnotic: A Preliminary Study. *Journal of Veterinary Advances*, 5(4), p.857.

Babalola, S.A. (2014). *Datura* Metel L: Analgesic or Hallucinogen? "Sharo" Perspective Middle-East *Journal of Scientific Research* 21(6):993-97.

Balachandran, P., and Govindarajan, R. (2005). Cancer—an ayurvedic perspective. *Pharmacological Research, 51*(1), 19-30. doi: 10.1016/j.phrs.2004.04.010

Balme, K., Roberts, J.C., Glasstone, M., Curling, L., Mann, M.D., 2012. The changing trends of childhood poisoning at a tertiary children's hospital in South Africa. South African Medical Journal 102, 142–146.

Balsevich, G., Poon, A., Goldowitz, D. and Wilking, J. (2014). The effects of pre-and post-natal nicotine exposure and genetic background on the striatum and behavioural phenotypes in the mouse. *Behavioural Brain Research*, 266, pp.7-18.

Bania, T., Chu, J., Bailes, D., and O'Neill, M. (2004). Jimson Weed Extract as a Protective Agent in Severe Organophosphate Toxicity. *Academic Emergency Medicine*, *11*(4), 335-338. doi:10.1197/j.aem.2003.12.002

Barnes, C. (1979). Memory deficits associated with senescence: A neurophysiological and behavioural study in the rat. *Journal of Comparative and Physiological Psychology*, 93(1), pp.74-104.

Barone, S., Stanton, M. and Mundy, W. (1995). Neurotoxic effects of neonatal triethyltin (TET) exposure are exacerbated with ageing. *Neurobiology of Aging*, 16(5), pp.723-735.

Barrager, E., Veltmann, J., Schauss, A., and Schiller, R. (2002). A Multicentered, Open-Label Trial on the Safety and Efficacy of Methylsulfonylmethane in the Treatment of Seasonal Allergic Rhinitis. *The Journal of Alternative and Complementary Medicine*, *8*(2), 167-173. doi: 10.1089/107555302317371451

Basha, M., Wei, W. and Bakheet, S. (2005). The Fetal Basis of Amyloidogenesis: Exposure to Lead and Latent Overexpression of Amyloid Precursor Protein and -Amyloid in the Aging Brain. *Journal of Neuroscience*, 25(4), pp.823-829.

Basha, R. and Reddy, G. (2010). "Developmental exposure to lead and late-life abnormalities of the nervous system". *Indian Journal of Experimental Biology*, 48(7), pp.636–641.

Basu, N.K. and Lal, S.B. (1947). Investigations on Indian medicinal plants. Q. *Journal of Pharmacy and Pharmacology*, 20: 38.

Bayer, S., Altman, J. and Russo, R. (1993). Timetables of neurogenesis in the human brain based on experimentally determined patterns in the rat". *Neurotoxicology*, 14(1), pp.83–144.

Behçet, A. (2014). The source-synthesis-history and use of atropine, *The Journal of Academic Emergency Medicine*, **13**:2-3

Belz, R., and Cedergreen, N. (2010). Parthenin hormesis in plants depends on growth conditions. *Environmental and Experimental Botany*, *69*(3), 293-301. doi: 10.1016/j.envexpbot.2010.04.010

Belz, R., and Leberle, C. (2012). Low dose responses of different glyphosate formulations on plants. In *Proceedings of 25th German Conference on Weed Biology and Weed Control*. Braunschweig, Germany. Retrieved from http://dx.doi.org/10.5073/ jka.2012.434.052.

Belz, r., Cedergreen, N., and Duke, S. (2011). Herbicide hormesis - can it be useful in crop production. *Weed Research*, *51*(4), 321-332. doi: 10.1111/j.1365-3180.2011. 00862.x

Berkov S., Zayed R., (2004): Comparison of tropane alkaloid spectra between *Datura inoxia*grown in Egypt and Bulgaria. *Z. Naturforsch*., 59: 184-186.

Berta, P., Hawkins, J., Sinclair, A., Taylor, A., Griffiths, B., Goodfellow, P. and Fellous, M. (1990). Genetic evidence equating SRY and the testis-determining factor. *Nature*, 348(6300), pp.448-450.

Bhattacharjee, S., Kar, S., and Chakravarty, S. (2004). Mineral Compositions of *Datura*: A Traditional Tropical Medicinal Plant. *Communications In Soil Science And Plant Analysis*, *35*(7-8), 937-946. doi: 10.1081/css-120030565

Bihaqi, S., Sharma, M., Singh, A. and Tiwari, M. (2012). Neuroprotective role of Convolvulus pluricaulis on aluminium induced neurotoxicity in rat brain. *Journal of Ethnopharmacology*, 124(3), pp.409-415.

Blaney, C., Gunn, R., Stover, K. and Brown, R. (2013). Maternal genotype influences the behavioural development of 3×Tg-AD mouse pups. *Behavioural Brain Research*, 252, pp.40-48.

Bodden, C., Richter, S., Schreiber, R., Kloke, V., GerÃŸ, J., Palme, R., Lesch, K., Lewejohann, L., Kaiser, S. and Sachser, N. (2015). Benefits of adversity?! How life history affects the behavioural profile of mice varying in serotonin transporter genotype. *Frontiers in Behavioral Neuroscience*, 9.

Bogda, A. (1973). Mineralogical and micromorphological studies of weathering products of some igneous parent rocks of soils occurring in the Sudetes. *Rocz. Glebozn*, *24*(2), 85-132.

Boldrini, M., Hen, R., Underwood, M.D., Rosoklija, G.B., Dwork, A.J., Mann, J.J. and Arango, V. (2012) Hippocampal angiogenesis and progenitor cell proliferation are increased with antidepressant use in major depression. Biol. Psychiatry, 72, 562–571.

Boumba, A., Mitselou, A., and Vougiouklakis, T. (2004). Fatal poisoning from ingestion of *Daturastramonium* seeds. *Veterinary and Human Toxicology*, *46*, 81-82.

Bouzidi, A., Mahdeb, N., and Kara, N. (2011). Toxicity studies of alkaloids of seeds of *Daturastramonium* and synthesis alkaloids in male rats. *Journal of Medicinal Plant Research*, *5*(15), 3421-3431.

Boyd, E., Meharg, A., Wright, J., and Killham, K. (1997). Assessment of toxicological interactions of benzene and its primary degradation products (catechol and phenol) using alux-modified bacterial bioassay. *Environmental Toxicology and Chemistry*, *16*(5), 849-856. doi: 10.1002/etc.5620160503

Braun, S.M. and Jessberger, S. (2014) Adult neurogenesis: mechanisms and functional significance. Development, 141, 1983–1986.

Brennan, M., Dallob, A. and Friedman, E. (1981). Involvement of hippocampal serotonergic activity in age-related changes in exploratory behaviour. *Neurobiology of Aging*, 2(3), pp.199-203.

Briggs., G., Freeman, R. and Yaffe, S. (2011). *Drugs in Pregnancy and Lactation: A Reference Guide to Fetal and Neonatal Risk*. Philadelphia: Lippincott, Williams and Wilkins.

Bronders, R., Brandy, S. and Yehuda, S. (1989). The use of MWM in the study of memory and learning. *Journal of Neuroscience*, 48, pp.29-62.

Bronoosh, P., Tanideh, N., Noorafshan, A., Andisheh Tadbir, A., Aalipanah, M., Kamali, F., *et al. (*2015). Effects of low-intensity pulsed ultrasound on the

healing of mandibular bone defects: an experimental study in rabbits. *Int. J. Oral. Maxillofac. Surg.* 44, 277– 4.doi:10.1016/j.ijom.2014. 09.020.

Brown, J.H. and Taylor, P. (2006). Goodman and Gilman's The Pharmacological Basis of Therapeutics, J.S.L.a.K.L.P. L.L. Brunton, McGraw-Hill Med: New York. p. 183-200.

Brown, S., Tapert, S., Granholm, E., and Delis, D. (2000). Neurocognitive Functioning of Adolescents: Effects of Protracted Alcohol Use. *Alcoholism: Clinical and Experimental Research*, *24*(2), 164-171. doi: 10.1097/00000374-200002000-00005

Bruel-Jungerman, E., Laroche, S. and Rampon, C. (2005) New *Neuron*s in the dentate gyrus are involved in the expression of enhanced long-term memory following environmental enrichment. Eur. *Journal of Neuroscience.*, 21, 513–521.

Bruneton, J. (1993). Pharmacognosie, phytochimie, plantes médicinales. Paris: Lavoisier.

Buckmaster, C. (2004). Entorhinal Cortex Lesions Disrupt the Relational Organization of Memory in Monkeys. *Journal of Neuroscience*, *24*(44), 9811-9825. doi: 10.1523/jneurosci.1532-04.2004

Burt, V., Suri, R., Altshuler, L., Stowe, Z., Hendrick, V. and Muntean, E. (2001). The Use of Psychotropic Medications During Breast-Feeding. *American Journal of Psychiatry*, 158(7), pp.1001-1009.

Busia, K. (2005). A medical provision in Africa -- past and present. *Phytotherapy Research*, 19: 919-923.

Busnel, M., Granier-Deferre, C. and Lecanuet, J. (1992). Fetal Audition. *Annals of the New York Academy of Sciences*, 662(1 Developmental), pp.118-134.

Byers, S.L., Wiles, M.V., Dunn, S.L. and Taft, R.A. (2012). Mouse Estrous Cycle Identification Tool and Images. *PLoS ONE* 7(4): e35538. doi:10.1371/ journal.pone.0035538.

Campbell, A., Baldessarini, R., and Teicher, M. (1988). Decreasing sensitivity to neuroleptic agents in developing rats; evidence for a pharmacodynamic factor. *Psychopharmacology*, *94*(1), 46-51. doi: 10.1007/bf00735879.

Carpa, R., Dumitru, D., Burtescu, R., Maior, M., Dobrotă, C., and Olah, N. (2017). Biochemical analysis of *Daturastramonium* extract. Studia Universitatis Babeş-Bolyai Biologia, 62(2), 5-19. doi: 10.24193/subbbiol.2017.2.01

Cedergreen, N. (2008). Is the growth stimulation by low doses of glyphosate sustained overtime? *Environmental Pollution*, *156*(3), 1099-1104. doi: 10.1016/j.envpol.2008.04.016

Cedergreen, N., and Olesen, C. (2010). Can glyphosate stimulate photosynthesis? *Pesticide Biochemistry And Physiology*, *96*(3), 140-148. doi: 10.1016/j.pestbp.2009.11.002

Cedergreen, N., Felby, C., Porter, J., and Streibig, J. (2009). Chemical stress can increase crop yield. *Field Crops Research*, *114*(1), 54-57. doi: 10.1016/j.fcr.2009.07.003

Champagne, F. and Curley, J. (2009). Epigenetic mechanisms mediating the long-term effects of maternal care on development. *Neuroscience and Biobehavioral Reviews*, 33(4), pp.593-600.

Chan, R. (2002). Estrogen-Like Activity of Ginsenoside Rg1 Derived from Panax notoginseng. *Journal of Clinical Endocrinology and Metabolism*, 87(8), pp.3691-3695.

Chan, S. and Rovet, J. (2003). Thyroid Hormones in Fetal Central Nervous System Development. *Fetal and Maternal Medicine Review*, 14(3), pp.177-208.

Chandra, S. (2012) *Biotechnology for medicinal plants*, Micropropagation and Improvement, Springer, pp 464

Cheal, M. (1986). The gerbil: A unique model for research on ageing. *Experimental Aging Research*, 12(1), pp.3-21.

Chehab, F. (1997). Early Onset of Reproductive Function in Normal Female Mice Treated with Leptin. *Science*, 275(5296), pp.88-90.

Chen, Q., Panksepp, J. and Lahvis, G. (2009). Empathy Is Moderated by Genetic Background in Mice. *PLoS ONE*, 4(2), p.e4387.

Chia-Hua, L., Shiu, L., Chang, L., Sheu, H., & Kuo, K. (2007). Solamargine upregulation of Fas, downregulation of HER2, and enhancement of cytotoxicity using epirubicin in NSCLC cells. Molecular Nutrition & Food Research, 51(8), 999-1005. doi: 10.1002/mnfr.200700044

Chia-Hua, L., Shiu, L., Chang, L., Sheu, H., Tsai, E., & Kuo, K. (2008). Solamargine Enhances HER2 Expression and Increases the Susceptibility of Human Lung Cancer H661 and H69 Cells to Trastuzumab and Epirubicin. Chemical Research In Toxicology, 21(2), 393-399. doi: 10.1021/tx700310x

Cho, C., Jeon, Y., Pham, T., Vijayaraghavan, K., and Yun, Y. (2008). The ecotoxicity of ionic liquids and traditional organic solvents on microalga Selenastrum capricornutum. *Ecotoxicology And Environmental Safety*, *71*(1), 166-171. doi: 10.1016/j.ecoenv.2007.07.001

Cho, C., Pham, T., Jeon, Y., Vijayaraghavan, K., Choe, W., and Yun, Y. (2007). Toxicity of imidazolium salt with anion bromide to a phytoplankton Selenastrum

capricornutum: Effect of alkylchain length. *Chemosphere*, *69*(6), 1003-1007. doi: 10.1016/j.chemosphere.2007.06.023

Choi, D., Cristóvão, A., Guhathakurta, S., Lee, J., Joh, T., Beal, M. and Kim, Y. (2012). NADPH Oxidase 1-Mediated Oxidative Stress Leads to Dopamine Neuron Death in Parkinson's Disease. *Antioxidants and Redox Signaling*, 16(10), pp.1033-1045.

Christie, D., and Viner, R. (2005). Adolescent development. *BMJ*, *330*(7486), 301-304.

Christofi, N., Hoffmann, C., and Tosh, L. (2002). Hormesis Responses of Free and Immobilized Light-Emitting Bacteria. *Ecotoxicology and Environmental Safety*, *52*(3), 227-231. doi: 10.1006/eesa.2002.2203

Clark, R., Zola, S., and Squire, L. (2000). Impaired Recognition Memory in Rats after Damage to the Hippocampus. *The Journal of Neuroscience*, *20*(23), 8853-8860. doi: 10.1523/jneurosci.20-23-08853.2000

Connor, B. and Dragunow, M. (1998). The role of neuronal growth factors in neurodegenerativedisorders of the human brain. *Brain Research Reviews*, 27(1), pp.1-39.

Copp, A. J., and Greene, N. D. (2013). Neural tube defects—Disorders ofneurulation and related embryonic processes. Wiley Interdiscip Rev DevBiol 2, 213–27.

Copp, A., Greene, N. and Murdoch, J. (2003). The genetic basis of mammalian neurulation. *Nature Reviews Genetics*, 4(10), pp.784-793.

Cory-Slechta, D., Thiruchelvam, M., Richfield, E., Barlow, B. and Brooks, A. (2005). Developmental pesticide exposures and Parkinson's disease phenotype. *Birth Defects Research Part A: Clinical and Molecular Teratology*, 73(3), pp.136-139.

Costa, L. (1998). Neurotoxicity Testing: A Discussion of in Vitro Alternatives. *Environmental Health Perspectives*, 106, p.505.

Costa, L. and Giornado, G. (2007). Developmental neurotoxicity of polybrominated diphenyl ether (PBDE) flame retardants. *NeuroToxicology*, 28(6), pp.1047-1067.

Costa, L., Aschner, M., Vitalone, A., Syversen, T. and Soldin, O. (2004). "Developmental neuropathology of environmental agents". *Annual Review of Pharmacology and Toxicology*, 44, pp.87–110.

Costa, L., Steardo, L. and Cuomo, V. (2004). Structural Effects and Neurofunctional Sequelae of Developmental Exposure to Psychotherapeutic Drugs: Experimental and Clinical Aspects. *Pharmacological Reviews*, 56(1), pp.103-147.

Cox, B., Vollmer, M., Tamplin, O., Lu, M., Biechele, S., and Gertsenstein, M. *et al.* (2010). Phenotypic annotation of the mouse X chromosome.

Cratty, M., Ward, H., Johnson, E., Azzaro, A. and Birkle, D. (1995). Prenatal stress increases corticotropin-releasing factor (CRF) content and releases in rat amygdala mince. *Brain Research*, 675(1-2), pp.297-302.

Crofton, K. (2008). Thyroid disrupting chemicals: mechanisms and mixtures. *International Journal of Andrology*, 31(2), pp.209-223.

Cruz-Orive, L.M. (2004). The precision of the fraction at or from Cavalieri designs. *J. Microsc.* 213, 205–211.doi:10.1111/j.1365-2818.2004. 01291.x

Cruz-Orive, L.M., and Weibel, E.R. (1981). Sampling designs for stereology. *J. Microsc.* 122, 235–257.doi:10.1111/j.1365-2818. 1981.tb01265. x.

Cuthbert, P., Stanford, L., Coba, M., Ainge, J., Fink, A., and Opazo, P. *et al.* (2007). Synapse-Associated Protein 102/*Dlg*h3 Couples the NMDA Receptor to Specific Plasticity Pathways and Learning Strategies. *Journal of Neuroscience*, *27*(10), 2673-2682. doi: 10.1523/jneurosci.4457-06.2007

Dahl, R. (2004). Adolescent Brain Development: A Period of Vulnerabilities and Opportunities. Keynote Address. *Annals of the New York Academy of Sciences, 1021*(1), 1-22. doi: 10.1196/annals.1308.001

Dai, C. and Holland, E. (2003). Astrocyte Differentiation States and Glioma Formation. *The Cancer Journal*, 9(2), pp.72-81.

Dargan, P.I., Gawarammana, I.B., Archer, J.R.H., House, I.V., Shaw, D., Wood, D., 2008. Heavy metal poisoning from Ayurvedic traditional medicines: an emerging problem? International Journal of Environment and Health 2, 463–472.

Dart, R., Hurlbut, K., & Boyer-Hassen, L. (2004). Lead. In: Dart RC, editor. Medical Toxicology (3rd ed.). Lippincott Williams and Wilkins.

Das, B.K., and Mukherjee, S.C. (2003). Toxicity of cypermethrin in Labeo rohita fingerlings: biochemical, enzymatic and haematological consequences. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, *134*(1), 109-121. doi: 10.1016/s1532-0456(02)00219-3

Das, S., Kumar, P., and Basu, S. (2012). Phytoconstituents and Therapeutic Potentials of *DaturaStramonium* Linn. *Journal of Drug Delivery and Therapeutics*, *2*(3). doi: 10.22270/jddt. v2i3.141

David, D.J., Samuels, B.A., Rainer, Q., Wang, J.W., Marsteller, D., Mendez, I., Drew, M., Craig, D.A. *et al.* (2009) Neurogenesis-dependent and –independent effects of fluoxetine in an animal model of anxiety/depression. *Neuron*, 62, 479–493.

Deacon, R. (2013). Measuring Motor Coordination in Mice. Journal Of Visualized Experiments, (75). doi: 10.3791/2609

Denholm, J., 2010. Complementary medicine and heavy metal toxicity in Australia. WebmedCentral 1, 1–6.

Desachy, A., Frois, B., Vignon, P., Roustan, J., & Gay, R. (1997). Uncommon poisoning by Datura *stramonium*; two case reports. Reanim. Urg., 6(1), 51-53.

Devi, M.R, Meenakshi, B., Paul, S.B, and Sharma, G.D. (2011). Neurotoxic and medicinal properties of *Daturastramonium* L.-Review. *Assam University Journal of Science and Technology: Biological and Environmental Sciences*, *7*(1), 139-144.

Diederichs, N., Feiter, U., Wynberg, R., 2006. Production of traditional medicines: technologies, standards and regulatory issues. In: Diederichs, N. (Ed.), Commercialising Medicinal Plants—A Southern African Guide. Sun Press, Stellenbosch, pp. 155–166.

Diker, D., Markovitz, D., Rothman, M., and Sendovski, U. (2007). Coma as a presenting sign of *Daturastramonium* seed tea poisoning. *European Journal of Internal Medicine*, *18*(4), 336-338. doi: 10.1016/j.ejim.2006.09.035

Djilani, A., Legseir, B., Soulimani, R., Dicko, A., and Younos, C. (2006). New extraction technique for alkaloids. Journal Of The Brazilian Chemical Society, 17(3), 518-520. doi: 10.1590/s0103-50532006000300013

Dobrossy, M.D., Drapeau, E., Aurousseau, C., Le Moal, M., Piazza, P.V. and Abrous, D.N. (2003) Differential effects of learning on neurogenesis: learning increases or decreases the number of newly born cells depending on their birth date. *Molecular Psychiatry*, 8, 974–982.

Doerk, K., Jonkova, I., Witte, L., and Alfermann, A. (1989). Synthesis of Tropane Alkaloids in Hairy Root Cultures of *Datura*and Hyoscyamus Species. *Planta Medica*, *55*(07), 688-689. doi: 10.1055/s-2006-962304

Drickamer, L. and Murphy, R. (1978). Female mouse maturation: Effects of excreted and bladder urine from juvenile and adult males. *Developmental Psychobiology*, 11(1), pp.63-72.

Dubrovsky, J.G., Rost, T.L. (2012) *Pericycle*, Wiley online Library, eLS

Duez, P., Chamart, S., Hanocq, M., Molle, L., Vanhaelen, M., and Vanhaelen-Fastré, R. (1985). Comparison between thin-layer chromatography—densitometry and high-performance liquid chromatography for the determination of hyoscyamine and hyoscine in leaves, fruit and seeds of *Datura* (*Datura* spp.). *Journal of Chromatography A*, *329*, 415-421. doi: 10.1016/s0021-9673(01)81949-x

Dugan G.M., Gubmann M.R., Friedman M., (1989): Toxicological evaluation of Jimson weed (*Daturastramonium*) seed. Food Chem. Toxicol., 27: 501-510.

Dugan, G., Gumbmann, M., & Friedman, M. (1989). Toxicological evaluation of jimson weed (Datura *stramonium*) seed. Food And Chemical Toxicology, 27(8), 501-510. doi: 10.1016/0278-6915(89)90045-8

Dugan, G., Gumbmann, M., & Friedman, M. (1989). Toxicological evaluation of jimson weed (Datura *stramonium*) seed. Food And Chemical Toxicology, 27(8), 501-510. doi: 10.1016/0278-6915(89)90045-8

Duke, J. (1992). *Handbook of phytochemical constituents of GRAS herbs and other economic plants* (p. 654.). Boca Raton, FL 33431: CRC Press, Inc.

Dupret, D., Fabre, A., Dobrossy, M.D., Panatier, A., Rodriguez, J.J., Lamarque, S., Lemaire, V., Oliet, S.H. *et al.* (2007) Spatial learning depends on both the addition and removal of new hippocampal Neurons. *PLoS Biology*, 5, e214.

Duruibe, J.O., Ogwuegbu, M.O.C., Egwurugwu, J.N., 2007. Heavy metal pollution and human bio-toxic effects. International Journal of Physical Sciences 2, 112–118.

Ebrahim, A.M., Eltayeb, M.H., Khalid, H., Mohamed, H., Abdalla, W., Grill, P., Micalke, B., 2012. Study on selected trace elements and heavy metals in some popular medicinal plants from Sudan. Journal of Natural Medicine 66, 671–679.

ECETOC, *Evaluation of the Neurotoxic Potential of Chemicals*, European Center for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium, 1992.

Ehret, G. and Bernecker, C. (1986). Low-frequency sound communication by mouse pups (Mus musculus): wriggling calls release maternal behaviour. *Animal Behaviour*, 34(3), pp.821-830.

Eisch, A., & Petrik, D. (2012). Depression and Hippocampal Neurogenesis: A Road to Remission?. Science, 338(6103), 72-75. doi: 10.1126/science.1222941

Ekanem, P., Ekanem, R. and Gaim, K. (2016). Histological Patterns of neurodegeneration of Frontal Cortex Neurons in *Daturastramonium* Treated Wistar Rats. *Journal of Behavioral and Brain Science*, 06(02), pp.85-92.

Elias, P., Elias, M. and Eleftheriou, B. (1975). Emotionality, Exploratory Behaviour, and Locomotion in Ageing Inbred Strains of Mice. *Gerontology*, 21(1), pp.46-55.

Elwood, R. and Keeling, F. (1982). Temporal organization of ultrasonic vocalizations in infant mice. *Developmental Psychobiology*, 15(3), pp.221-227.

Ennaceur A, Delacour J (1988) A new one-trial test for neurobiologicalstudies of memory in rats. 1. Behavioral data. BehavBrain Res 31:47–59

Ernst, E., 2002. Toxic heavy metals and undeclared drugs in Asian herbal medicines. Trends in Pharmacological Sciences 23, 136–139.

Ertekin, V., Selimoğlu, M., and Altinkaynak, S.A. (2005). A combination of unusual presentations of *Daturastramonium* intoxication in a child: Rhabdomyolysis and fulminant hepatitis. *The Journal of Emergency Medicine, 28*(2), 227-228. doi: 10.1016/j.jemermed.2004.11.006

Evans, W. (2014). *Trease and Evans' Pharmacognosy*. St. Louis: Elsevier Health Sciences UK.

Facchini, P.J. (2001) Alkaloid biosynthesis in plants: biochemistry, cell biology, molecular biology and metabolic engineering applications, *Annual review of plant physiology and plant molecular biology*, **52**, 29-66.

Fahlström, A., Yu, Q. and Ulfhake, B. (2011). Behavioural changes in ageing female C57BL/6 mice. *Neurobiology of Aging*, 32(10), pp.1868-1880.

Falluel-Morel, A., Sokolowski, K., Sisti, H., Zhou, X., Shors, T., and DiCicco-Bloom, E. (2007). Developmental mercury exposure elicits acute hippocampal cell death, reductions in neurogenesis, and severe learning deficits during puberty. *Journal Of Neurochemistry*, *103*(5), 1968-1981. doi: 10.1111/j.1471-4159.2007.04882.x

Fan, L., Tien, L., Zheng, B., Pang, Y., Lin, R., Simpson, K., Ma, T., Rhodes, P. and Cai, Z. (2011). Dopaminergic *Neuron*al injury in the adult rat brain following neonatal exposure to lipopolysaccharide and the silent neurotoxicity. *Brain, Behavior, and Immunity*, 25(2), pp.286-297.

Floderus, S. (1944).Studies on the human pituitary gland with special regard to quantitative micromorphologicalrelationships. *Acta. Pathol.Microbial.Scand.* 53(Suppl.), 1–176.

Flora, S., Mittal, M., and Mehta, A. (2009). Heavy metal-induced oxidative stress and its reversal by chelation therapy. *Ind. J. Med. Res.*, *128*, 501–523.

Flurkey, K., Brandvain, Y., Klebanov, S., Austad, S., Miller, R., Yuan, R. and Harrison, D. (2007). PohnB6F1: A Cross of Wild and Domestic Mice That Is a New Model of Extended Female Reproductive Life Span. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, 62(11), pp.1187-1198.

Fotuhi, M., Do, D. and Jack, C. (2012) Modifiable factors that alter the size of the hippocampus with ageing. Nat. Rev. Neurol., 8, 189–202.

Fox, W.M. (1965). Reflex-ontogeny and *behavioural* development of the mouse. *Animal Behaviour*, 13:234–41. [PubMed: 5835840].

Freye, E. (2010). Toxicity of *Daturastramonium*: Pharmacology and Abuse of Cocaine, Amphetamines, Ecstacy and Related Designer Drugs. *Springer, Netherlands*, (217-218).

Friedman, M. and Levin, C.E. (1989). The composition of Jimson Weed (*Daturastramonium*) seeds. *Journal of Agriculture Food and Chemistry,* 37: 998-1005.

Gallavan, R.H. Jr., Holson, J.F., Stump, D.G., Knapp, J.F. and Reynolds, V.L. (1999). Interpreting the toxicologic significance of alterations in the anogenital distance: potential for confounding effects of progeny body weights. *Reproductive Toxicology,* 13:383-390.

Gao, Z., Ure, K., Ables, J., Lagace, D., Nave, K., and Goebbels, S. *et al.* (2009). *NeuroD*1 is essential for the survival and maturation of adult-born neurons. *Nature Neuroscience*, *12*(9), 1090-1092. doi: 10.1038/nn.2385

García-Esquinas, E., Pérez-Gómez, B., Fernández, M., Pérez-Meixeira, A., Gil, E., and Paz, C. *et al.* (2011). Mercury, lead and cadmium in human milk in relation to diet, lifestyle habits and sociodemographic variables in Madrid (Spain). *Chemosphere*, *85*(2), 268-276. doi: 10.1016/j.chemosphere.2011.05.029

Gardoni, F. (2008). MAGUK proteins: New targets for pharmacological intervention in the glutamatergic synapse.

Garratt, M., Stockley, P., Armstrong, S., Beynon, R. and Hurst, J. (2011). The scent of senescence: sexual signalling and female preference in house mice. *Journal of Evolutionary Biology*, 24(11), pp.2398-2409.

Garvey, G.J., Hahn, G., Lee, R.V., Harbison, R.D., 2001. Heavy metal hazards of Asian traditional remedies. International Journal of Environmental Health Research 11, 63–71.

Gary, I., Stafford, A., Anna, K., Jager, B., and Johannes, V.S. (2005). Activity of traditional South African sedative and potentially CNS acting plants in the GABA-benzodiazepine receptor assay. *Journal of Ethnopharmacology*, *100*, 210-215.

Gee, J., Hedge, J. and Moser, V. (2008). Lack of Alterations in Thyroid Hormones Following Exposure to Polybrominated Diphenyl Ether 47 during a Period of Rapid Brain Development in Mice. *Drug and Chemical Toxicology,* 31(2), pp.245-254.

Gentile, S. (2004). Clinical Utilization of Atypical Antipsychotics in Pregnancy and Lactation. *Annals of Pharmacotherapy*, 38(7-8), pp.1265-1271.

Giadado, A., Zainab, A., Hadiza, M.U., Serah, D., Anas, H., and Milala, M.A. (2007). Toxicity studies of ethanol extract of the leaves of *Daturastramonium* in rats. *African Journal of Biotechnology, 6*(8), 1012-1015.

Giedd, J. (2004). Structural Magnetic Resonance Imaging of the Adolescent Brain. *Annals of The New York Academy of Sciences*, *1021*(1), 77-85. doi: 10.1196/annals.1308.009

Giedd, J., Blumenthal, J., Jeffries, N., Castellanos, F., Liu, H., and Zijdenbos, A. *et al.* (1999). Brain development during childhood and adolescence: a longitudinal MRI study. *Nature Neuroscience*, *2*(10), 861-863. doi: 10.1038/13158

Gilbert, M. (2011). Impact of Low-Level Thyroid Hormone Disruption Induced by Propylthiouracil on Brain Development and Function. *Toxicological Sciences*, 124(2), pp.432-445.

Godfrey, K. and Barker, D. (2001). Fetal programming and adult health. *Public Health Nutrition*, 4(2b), pp.611-624.

Graham, E., Moss, J., Burton, N., Roochun, Y., Armit, C., Richardson, L., andBaldock, R. (2015). The atlas of mouse development eHistology resource.Development 142, 1909–11.

Grandjean, P. (2008). Late Insights into Early Origins of Disease. *Basic and Clinical Pharmacology and Toxicology*, 102(2), pp.94-99.

Grandjean, P. and Landrigan, P. (2006). Developmental neurotoxicity of industrial chemicals. *The Lancet*, 368(9553), pp.2167-2178.

Gray, L.E. Jr., Ostby, J., Furr, J., Price, M., Veeramachaneni, D.N. and Parks, L. (2000) Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. *Toxicological Sciences*, 58:350-365.

Groó, Z., Szenczi, P., Bánszegi, O. and Altbäcker, V. (2012). Natal dispersal in two mice species with contrasting social systems. *Behavioural Ecology and Sociobiology*, 67(2), pp.235-242.

Guarrera, P.M. (1999). Traditional antihelmintic, antiparasitic and repellent uses of plants in Central Italy. *Assam University Journal of Science and Technology: Biological and Environmental Sciences*, *68*(1-3), 183-192. doi: 10.1016/s0378-8741(99)00089-6

Gundersen, H. (1977). Notes on the estimation of the numerical density of arbitrary profiles: the edge effect. Journal Of Microscopy, 111(2), 219-223. doi: 10.1111/j.1365-2818.1977.tb00062.x

Gundersen, H. and Jensen, E. (1987). The efficiency of systematic sampling in stereology and its prediction*. *Journal of Microscopy*, 147(3), pp.229-263.

Gundersen, H., and Jensen, E. (1987). The efficiency of systematic sampling in stereology and its prediction*. *Journal of Microscopy*, *147*(3), 229-263. doi: 10.1111/j.1365-2818.1987.tb02837.x

Gupta, P.D. (2008). *The herb, habitat, morphology and pharmacognosy of most important popular Indian medicinal plant* (1st ed., p. 185). Madhya Pradesh: Printwell Offset Publisher.

Hacker A, Capel B, Goodfellow P, Lovellbadge R: Expression of SRY themouse sex-determining gene. Development 1995, 121:1603-1614.

Haddow, J., Palomaki, G., Allan, W., Williams, J., Knight, G., Gagnon, J., O'Heir, C., Mitchell, M., Hermos, R., Waisbren, S., Faix, J. and Klein, R. (1999). Maternal Thyroid Deficiency During Pregnancy and Subsequent Neuropsychological Development of the Child. *New England Journal of Medicine*, 341(8), pp.549-555.

Hagihara, H., Toyama, K., Yamasaki, N. and Miyakawa, T. (2009). Dissection of Hippocampal Dentate Gyrus from Adult Mouse. *Journal of Visualized Experiments*, (33).

Hahn-Deinstrop, E. (2007). *Applied thin-layer chromatography*. 2nd ed. Weinheim: Wiley-VCH.

Hale, T. (1999). Anaesthetic Medications in Breastfeeding Mothers. Journal Of Human Lactation, 15(3), 185-194. doi: 10.1177/089033449901500302

Hall, P. and Watt, F. (1989). Stem cells the generation and maintenance of cellular diversity. *Development*, 106, pp.619-633.

Hammond, R. (2004). On the delay-dependent involvement of the hippocampus in object recognition memory. *Neurobiology of Learning And Memory*, *82*(1), 26-34. doi: 10.1016/j.nlm.2004.03.005

Han, R., Zhang, R., Chang, M., Peng, Y., Wang, P., and Hu, S. *et al.* (2012). Reversal of scopolamine-induced spatial and recognition memory deficits in mice by novel multifunctional dimers bis-cognitins. *Brain Research*, *1470*, 59-68. doi: 10.1016/j.brainres.2012.06.015

Hanna, J., Schmidley, J., and Braselton, W. (1992). *Datura* Delirium. *Clinical Neuropharmacology*, *15*(2), 109-113. doi: 10.1097/00002826-199204000-00004.

Hanson, G. (2008). The toxicity of plants in equines: A modern three-point approach to disseminating information. A thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Rangeland ecology and management in the college of graduate studies. University of Idaho, 83-89.

Hanson, N.D., Owens, M.J. and Nemeroff, C.B. (2011) Depression, antidepressants, and neurogenesis: a critical reappraisal. *Neuropsychopharmacology*, 36, 2589–2602.

Harding, A.J., Halliday, G.M., and Cullen, K. (1993).Practical considerations for the use of the optical dissector in estimating the neuronal number. *J. Neurosci. Methods* 51, 83–89.doi:10.1016/0165-0270(94)90028-0.

Hashimoto, T., Hayashi, A., Amano, Y., Kohno, J., Iwanari, H., Usudal, S., Yamada, Y. (1991) Hyoscyamine 6ß-hydroxylase, an enzyme involved in tropane alkaloid biosynthesis, is localized at the pericycle of the root, *The Journal of Biological Chemistry*, **266**, 7, 4648-4653

Hashmi, D.S., Ismail, S., Shaikh, G.H., 2007. Assessment of the level of trace metals in commonly edible vegetables locally available in the markets of Karachi city. Pakistan Journal of Botany 39, 747–751.

Hefner, K. and Holmes, A. (2007). Ontogeny of fear-, anxiety- and depression-related behaviour across adolescence in C57BL/6J mice. *Behavioural Brain Research*, 176(2), pp.210-215.

Hein, K. (1987). The use of therapeutics in adolescence. *Journal of Adolescent Health Care*, *8*(1), 8-35. doi: 10.1016/0197-0070(87)90244-0

Helrich, K. (1990). *Official methods of analysis of the Association of Official Analytical Chemists* (15th ed.). Arlington, VA: The Association.

Henderson, L. (1995). *Plant Invaders of Southern Africa* p: 16.

Henderson, N. (1981). A fit mouse is a hoppy mouse: Jumping behaviour in 15-day-oldus musculus. Developmental Psychobiology, 14(5), 459-472. doi: 10.1002/dev.420140508

Hilaly, E.I., Israili, Z.H., and Lyoussi, B. (2004). Acute and chronic toxicological studies of Ajugaivan in experimental animals. *Journal of Ethnopharmacology*, 91: 43-50.

Hoffmann, C., and Christofi, N. (2001). Testing the toxicity of influents to activated sludge plants with the *VibrioFisheri* bioassay utilising a sludge matrix. *Environmental Toxicology*, *16*(5), 422-427. doi: 10.1002/tox.10000

Hurst, J. (1990). Urine marking in populations of wild house mice Mus domesticus rutty. I. Communication between males. *Animal Behaviour*, 40(2), pp.209-222.

Hurst, J. (1993). The priming effects of urine substrate marks on interactions between male house mice, Mus musculus domesticus Schwarz and Schwarz. *Animal Behaviour*, 45(1), pp.55-81.

Ibeto, C.N., Okoye, C.O.B., 2010. High levels of heavy metals in the blood of the urban population in Nigeria. Research Journal of Environmental Sciences 4, 371–382.

Ikonomidou, C., Bittigau, P., Koch, C., Genz, K., Hoerster, F., Felderhoff-Mueser, U., Tenkova, T., Dikranian, K. and Olney, J. (2001). Neurotransmitters and apoptosis in the developing brain11Abbreviations: GABAA, γ-aminobutyric acid; NMDA; N-methyl-d-aspartate; PCP; phencyclidine; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling. *Biochemical Pharmacology*, 62(4), pp.401-405.

Indra, K.V. (2012) *Cell Culture and Somatic Cell Genetics of Plants*, Vol. 4 Cell culture in phytochemistry, Elsevier, pp 330

Innocenti, G. (1995). Exuberant development of connections, and its possible permissive role in cortical evolution. *Trends in Neurosciences*, *18*(9), 397-402. doi: 10.1016/0166-2236(95)93936-r

Iranbakhsh, A., Ali Oshanghi, M., Majd, A. (2006) Distribution of atropine and scopolamine in different organs and stages of development in *Datura stramonium*L. (Solanaceae). Structure and ultrastructure of biosynthesizing cells, *Biological Association Cracoviensia Botanical Series*, **48**,1: 13–18

Isgor, C., Kabbaj, M., Akil, H., and Watson, S. (2004). Delayed effects of chronic variable stress during the peripubertal-juvenile period on hippocampal morphology and on cognitive and stress axis functions in rats. *Hippocampus*, *14*(5), 636-648. doi: 10.1002/hipo.10207

Ishola, A.O. and Adeniyi, P.A. (2013). Retardedhippocampal development followingprenatal exposure to ethanolic leafextract of *Datura metel* leaves in Wistarrats. *Nigerian Medical Journal*; 54 (6): 413-416.

Ivancheva, S., Nikolova, M., and Tsvetkova, R. (2006). *Pharmacological activities and biologically active compounds of Bulgarian medicinal plants. In: Inperato F, editor. Phytochemistry:* (pp. 87-103). Kerala: Signpost: Advances in research.

Iversen, S. (1997). Behavioural evaluation of cholinergic drugs. *Life Sciences, 60*(13-14), 1145-1152. doi: 10.1016/s0024-3205(97)00059-3

Jablonski, S., Schreiber, W., Westbrook, S., Brennan, L. and Stanton, M. (2013). Determinants of novel object and location recognition during development. *Behavioural Brain Research*, 256, pp.140-150.

Jacintho, J. and Kovacic, P. (2003). Neurotransmission and Neurotoxicity by Nitric Oxide, Catecholamines, and Glutamate: Unifying Themes of Reactive Oxygen Species and Electron Transfer. *Current Medicinal Chemistry*, 10(24), pp.2693-2703.

Jackson, P. and Blythe, D. (2008). Immunohistochemical techniques. In: John D Bancroft, Marilyn Gamble. Theory and practice of immunohistological techniques. p. 433-472.

Jacobowitz, D. M., and Abbot, L. C. (Eds.) (1998). Chemoarchitectonic Atlasof the Developing Mouse Brain, p. 304. CRC Press, Boca Raton, FL.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B., and Beeregowda, K. (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary Toxicology*, *7*(2), 60-72. doi: 10.2478/intox-2014-0009

Jarald, E., and Edwin, S. (2007). *Textbook of Pharmacognosy and phytochemistry* (1st ed., p. 224). New Dehli: CBS Publisher and Distributors.

Jean-Faucher, C., Berger, M., de Turckheim, M., Veyssiere, G. and Jean, C. (1978). Developmental Patterns of Plasma And Testicular Testosterone in Mice From Birth to Adulthood. *Acta Endocrinologica*, 89(4), pp.780-788.

Joëls, M., Karst, H., Alfarez, D., Heine, V., Qin, Y., and Riel, E. *et al. (*2004). Effects of Chronic Stress on Structure and Cell Function in Rat Hippocampus and Hypothalamus. *Stress*, *7*(4), 221-231. doi: 10.1080/10253890500070005

Johnson, C. and Wilbrecht, L. (2011). Juvenile mice show greater flexibility in multiple-choice reversal learning than adults. *Developmental Cognitive Neuroscience*, 1(4), pp.540-551.

Johnson, E., & Deckwerth, T. (1993). Molecular Mechanisms of Developmental Neuronal Death. Annual Review Of Neuroscience, 16(1), 31-46. doi: 10.1146/annurev.ne.16.030193.000335

Joosen, M., Jousma, E., van den Boom, T., Kuijpers, W., Smit, A., Lucassen, P., and van Helden, H. (2009). Long-term cognitive deficits accompanied by reduced neurogenesis after soman poisoning. *Neurotoxicology*, *30*(1), 72-80. doi: 10.1016/j.neuro.2008.11.010

Joshi, P., Wicks, A.C. and Munshi, S.K. (2003). Recurrent autumnal psychosis. *Postgraduates Medical Journal*, 79: 239-240.

Joyal, C., Beaudin, S. and Lalonde, R. (2000). Longitudinal Age-Related Changes In Motor Activities And Spatial Orientation In CD-1 Mice. *Archives of Physiology and Biochemistry*, 108(3), pp.248-256.

Kaler, S., Dhar, P., Bhattacharya, A., and Mehra, R. (2013). Preliminary morphological and immunohistochemical changes in rat hippocampus following postnatal exposure to sodium arsenite. *Toxicology International*, *20*(2), 160. doi: 10.4103/0971-6580.117259.

Kamath, S., Pemiah, B., Sekar, R., Krishnaswamy, S., Sethuraman, S., Krishnan, U., 2012. Mercury-based traditional herbo-metallic preparations: a toxicological perspective. Archives of Toxicology 1–8.

Kandoth, K.P., Singh, S., Jayabaskaran, J. (2010) Expression of hyoscyamine 6β-hydroxylase in root pericycle cells and accumulation of its product scopolamine in leaf and stem tissues of *Datura metel* L., *Plant Science*,**178**, 2: 202-206

Kanegae, T., Kajiya, H., Amano, Y., Hashimoto, T., Yamada, Y. (1994) Species-dependent expression of the hyoscyamine 6β-hydroxylase gene in the pericycle, *Plant Physiology*, **105**, 483-490

Kaplan, S., Odaci, E., Canan, S., Onger, E., Aslan, H., and Unal, B. (2012). The disector counting technique. *NeuroQuantology* 10, 44–53.doi:10.14704/nq. 2012.10.1.425.

Kasture, A.V., Wadodkar S. G. (2008). *Pharmaceutical Chemistry* I, Pregati Books Pvt. Ltd., pp 215

Kaufman, M. H. (Ed.) (1992). The Atlas of Mouse Development, p. 525. Academic Press, San Diego, CA.

Keene DE, Suescun MO, Bostwick MG, Chandrashekar V, Bartke A, Kopchick JJ: Puberty is delayed in male growth hormone receptor genedisrupted mice. J Androl 2002, 23:661-668

Kelly, R., Andrews, J., and DeWitt, J. (2002). An X-ray absorption spectroscopic investigation of the nature of the zinc complex accumulated in *Datura* innoxia plant tissue culture. *Microchemical Journal*, *71*(2-3), 231-245. doi: 10.1016/s0026-265x(02)00015-2

Kennard, J. (2011). Age sensitivity of behavioural tests and brain substrates of normal ageing in mice. *Frontiers in Aging Neuroscience*, 3.

Keuker, J., Vollmann-Honsdorf, G.K., and Fuchs, E. (2001). How to use the optical fractionator: an example based on the estimation of neurons in the hippocampal CA1andCA3regionsoftreeshrews. *Brain Res. Brain Res.Protoc.* 7, 211–221.doi:10.1016/s1385-299x(01)00064-2.

Key, M. (2009). Immunohistochemistry staining methods, Immunohistochemical staining methods.5th edition, Dako, California.

Khalequzzaman, M., and Islam, M.N. (1992). Pesticidal action of *Daturametel*Linn. Leaf extracts on *Tribolium castaneum*(Hbst). *Bangladesh Journal of Zoology*, 20(2): 223-29.

Khandare, K.R., and Salve, S.B. (2011). Management of wilt of pigeon pea (Cajanus cajan L.) through biopesticide (leaf extracts). *International Reference Research Journal, 2*(18), 21-22.

Kim, W.Y., Fritzsch, B., Serls, A., Bakel, L.A., Huang, E.J., Reichardt, L.F., *et al.* (2001). *NeuroD*-null mice are deaf due to a severe loss of the inner ear sensory neurons during development. *Development*, 128:417–26.

Kinder, S., Tsang, T., Wakamiya, M., Sasaki, H., Behringer, R., and Nagy, A. (2001). The organizer of the mouse gastrula is composed of a dynamic population of progenitor cells for the axial mesoderm. *Development*, *128*, 3623-3634.

Kinzel, D., Boldt, K., Davis, E., Burtscher, I., Trümbach, D., and Diplas, B. *et al.* (2010). Pitchfork Regulates Primary Cilia Disassembly and Left-Right Asymmetry. *Developmental Cell*, *19*(1), 66-77. doi: 10.1016/j.devcel.2010.06.005

Kirtikar, J.D., and Basu, B.D. (1994). *Indian medicinal plants* (pp. 1229-1231). Allahabad: Lalit Mohan Basu.

Klein-Schwartz W., Oderda G.M., (1984): Jimsonweed intoxication in adolescents and young adults. Am. J. Dis. Child., 138: 737-739.

Kokate, C. (1999). *"Practical pharmacognosy"*. 4th ed. New Delhi, India: Vallabh Prakashan Publication.

Kosnett, M. (2005). Lead. In: Brent J, editor. Critical Care Toxicology: Diagnosis and Management of the Critically Poisoned Patient. Gulf Professional Publishing.

Kotrschal, A., Ilmonen, P. and Penn, D. (2007). Stress impacts telomere dynamics. *Biology Letters*, 3(2), pp.128-130.

Krishnamurthy, H., Suresh Babu, P., Morales, C. and Sairam, M. (2001). Delay in Sexual Maturity of the Follicle-Stimulating Hormone Receptor Knockout Male Mouse1. *Biology of Reproduction*, 65(2), pp.522-531.

Kruger, G. and Morrison, S. (2002). Brain Repair by Endogenous Progenitors. *Cell*, 110(4), pp.399-402.

Kumar, A. (2009). *A textbook of practical botany* II, Seventh edition, Rastogi Publications, India, pp 646

Kumral, N., Çobanoğlu, S., and Yalcin, C. (2009). Acaricidal, repellent and oviposition deterrent activities of *Daturastramonium* L. against adult Tetranychus urticae (Koch). *Journal of Pest Science,83*(2), 173-180. doi: 10.1007/s10340-009-0284-7

Kurzbaum, A., Simsolo, C., and Blum, A. (2001). Toxic delirium due to *Daturastramonium*. Isr. Med. Assoc. J, 3: 538-539.

Kuwabara, T., Hsieh, J., Muotri, A., Yeo, G., Warashina, M., and Lie, D. *et al.* (2009). Wnt-mediated activation of *NeuroD*1 and retro-elements during adult neurogenesis. *Nature Neuroscience*, *12*(9), 1097-1105. doi: 10.1038/nn.2360

Landrigan, P., Sonawane, B., Butler, R., Trasande, L., Callan, R. and Droller, D. (2005). Early Environmental Origins of Neurodegenerative Disease in Later Life. *Environmental Health Perspectives*, 113(9), pp.1230-1233.

Laplagne, D., Espósito, M., Piatti, V., Morgenstern, N., Zhao, C., and van Praag, H. *et al.* (2006). Functional Convergence of Neurons Generated in the Developing and Adult Hippocampus. *Plos Biology*, *4*(12), e409. doi: 10.1371/journal.pbio.0040409

Latham, N., & Mason, G. (2004). From house mouse to mouse house: the behavioural biology of free-living Mus musculus and its implications in the laboratory. Applied Animal Behaviour Science, 86(3-4), 261-289. doi: 10.1016/j.applanim.2004.02.006

Laviola, G., Macrì, S., Morley-Fletcher, S., and Adriani, W. (2003). Risk-taking behaviour in adolescent mice: psychobiological determinants and early epigenetic influence. *Neuroscience and Biobehavioral Reviews*, *27*(1-2), 19-31. doi: 10.1016/s0149-7634(03)00006-x

Le Roy, I., Carlier, M. and Roubertoux, P. (2001). Sensory and motor development in mice: genes, environment and their interactions. *Behavioural Brain Research*, 125(1-2), pp.57-64.

Lee, J., Hollenberg, S., Snider, L., Turner, D., Lipnick, N., and Weintraub, H. (1995). Conversion of Xenopus ectoderm into neurons by *NeuroD*, a basic helix-loop-helix protein. *Science*, *268*(5212), 836-844. doi: 10.1126/science.7754368

Lee, M. and Rabe, A. (1992). Premature decline in morris water maze performance of ageing microcephalic rats. *Neurotoxicology and Teratology*, 14(6), pp.383-392.

Lewejohann, L., Zipser, B. and Sachser, N. (2011). "Personality" in laboratory mice used for biomedical research: A way of understanding variability. *Developmental Psychobiology*, 53(6), pp.624-630.

Lhotellier L, Cohensalmon C: Senescence of motor behaviour in mice - contribution of genetic methods. Cah Psychol Cogn 1991, 11:27-53.

Li, S., Liang, C., Chang, L., Sheu, H., Tsai, E., & Kuo, K. (2008). Solamargine induces apoptosis and enhances susceptibility to trastuzumab and epirubicin in breast cancer cells with low or high expression levels of HER2/neu. Bioscience Reports, 29(1), 35-45. doi: 10.1042/bsr20080028

Li, Y., Zhang, B., He, X., Cheng, W., Xu, W., and Luo, Y. *et al.* (2014). Analysis of Individual and Combined Effects of Ochratoxin A and Zearalenone on HepG2 and KK-1 Cells with Mathematical Models. *Toxins*, *6*(4), 1177-1192. doi: 10.3390/toxins6041177

Lillie, R., and Fullmer, H. (1976). *Histopathologic technic and practical histochemistry*. New York [usw.], Düsseldorf: Mc-Graw-Hill.

Linares, J., Gustafsson, I., Baquero, F., and Martinez, J. (2006). Antibiotics as intermicrobial signalling agents instead of weapons. *Proceedings of the National Academy of Sciences*, *103*(51), 19484-19489. doi: 10.1073/pnas.0608949103

Ling, Z., Gayle, D., Ma, S., Lipton, J., Tong, C., Hong, J. and Carvey, P. (2001). In utero bacterial endotoxin exposure causes loss of tyrosine hydroxylase neurons in the postnatal rat midbrain. *Movement Disorders*, 17(1), pp.116-124.

Lingmei, S., Zhao, Y., Li, X., Yuan, H., Cheng, A., & Lou, H. (2010). A lysosomal–mitochondrial death pathway is induced by solamargine in human K562 leukaemia cells. Toxicology In Vitro, 24(6), 1504-1511. doi: 10.1016/j.tiv.2010.07.013

Liscovitch, N. and Chechik, G. (2013). Specialization of Gene Expression during Mouse Brain Development. *PLoS Computational Biology*, 9(9), p.e1003185.

List G.R., Spencer G.F., Hunt W.H., (1979). Toxic weed seed contaminants in soybean processing. J. Am. Oil Chem. Soc., 56: 706-710.

List, G., Spencer, G., & Hunt, W. (1979). Toxic weed seed contaminants in soybean processing. Journal Of The American Oil Chemists' Society, 56(8), 706-710. doi: 10.1007/bf02663046

Liu, M., Pleasure, S., Collins, A., Noebels, J., Naya, F., Tsai, M., and Lowenstein, D. (2000). Loss of BETA2/*NeuroD* leads to malformation of the dentate gyrus and epilepsy. *Proceedings of the National Academy of Sciences*, *97*(2), 865-870. doi: 10.1073/pnas.97.2.865

Liu, S., Li, L., Shen, W., Shen, X., Yang, G., Zhou, W. (2013). Scopolamine detoxification technique for heroin dependence: a randomized trial, *Neuroscience and Biobehavioral Review*s, **34**: 1307 – 1350.

Livak, K. and Schmittgen, T. (2001). Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. *Methods*, 25(4), pp.402-408.

Logan, T.J., Goins, L.E., Lindsay, B.J., 1997. Field assessment of trace element uptake by six vegetables from N-Viro soil. Water and Environmental Research 69, 28–33.

Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicity*. 53: 275 – 287.

Mace, M. (1963). Histochemical Localization of Phenols in Healthy and Diseased Banana Roots. *Physiologia Plantarum*, 16(4), pp.915-925.

MacKnight, C. (2001). Clinical Implications of Bovine Spongiform Encephalopathy. *Clinical Infectious Diseases*, 32(12), pp.1726-1731.

Macrì, S., Adriani, W., Chiarotti, F. and Laviola, G. (2002). Risk-taking during exploration of a plus-maze is greater in adolescent than in juvenile or adult mice. *Animal Behaviour*, 64(4), pp.541-546.

Mahar, I., Bambico, F.R., Mechawar, N. and Nobrega, J.N. (2014) Stress, serotonin, and hippocampal neurogenesis in relation to depression and antidepressant effects. *Neurosciences and Biobehavioral Reviews*, 38, 173–192.

Maharia, R.S., Dutta, R.K., Acharya, R., Reddy, A.V.R., 2010. Journal of Environmental Science and Health, Part B: Pesticides, Food Contaminants and Agricultural Wastes 45, 174–181.

Makris, S., Raffaele, K., Allen, S., Bowers, W., Hass, U., Alleva, E., Calamandrei, G., Sheets, L., Amcoff, P., Delrue, N. and Crofton, K. (2009). A Retrospective Performance Assessment of the Developmental Neurotoxicity Study in Support of OECD Test Guideline 426. *Environmental Health Perspectives*, 117(1), pp.17-25.

Mann, J. (2008). Belladonna, broomsticks and brain chemistry, Royal Society of Chemistry

Mapanda, F., Mangwayana, E.N., Nyamangara, J., Giller, K.E., 2007. Uptake of heavy metals by vegetables irrigated using wastewater and the subsequent risks in Harare, Zimbabwe. Physics and Chemistry of the Earth 32, 1399–1405.

Marcondes, F. K., Bianchi, F. J., and Tanno, A. P. (2002). Determination of the Estrous Cycle Phases of Rats: Some Helpful Considerations. *Brazilian Journal of Biology, 62*(4A): 609-614.

Marcovecchio, J.E., Botté, S.E., Freije, R.H., 2007. Heavy metals, major metals, trace elements, In: Nollet, L.M.L. (Ed.), Handbook of water analysis, 2nd ed. CRC Press, Boca Raton, pp. 275–311.

Marquette, B., & Schneider, C. (1991). A longitudinal investigation of the effects of experience on the behaviour of ageing mice. Growth Develop Aging, 55(35-42).

Maryam, M., Rusea, G., Yong, S. and Mohd, N. (2013). Vinca Alkaloids. *International Journal of Preventive Medicine*, 4, pp.1231-1235.

Masuo, Y. and Ishido, M. (2011). Neurotoxicity of Endocrine Disruptors: Possible Involvement in Brain Development and Neurodegeneration. *Journal of Toxicology and Environmental Health, Part B*, 14(5-7), pp.346-369.

Matsuura, H., Malik, S., de Costa, F., Yousefzadi, M., Mirjalili, M., Arroo, R., Bhambra, A., Strnad, M., Bonfill, M. and Fett-Neto, A. (2018). Specialized Plant Metabolism Characteristics and Impact on Target Molecule Biotechnological Production. *Molecular Biotechnology*, 60(2), pp.169-183.

Mattiasson, G., Friberg, H., Hansson, M., Elmér, E., and Wieloch, T. (2003). Flow cytometric analysis of mitochondria from CA1 and CA3 regions of rat hippocampus reveals differences in permeability transition pore activation. *Journal Of Neurochemistry*, *87*(2), 532-544. doi: 10.1046/j.1471-4159.2003.02026.x

Mayhew, T.M. (2014). Quantitative immunocytochemistry at the ultrastructural level: a stereology-based approach to molecular nanomorphomics. *Cell Tissue Res.* 11, 43–59.doi:10.1007/s00441-014-2038-y.

Michelle, C., Cora, Linda, K., and Greg, T. (2015). Vaginal Cytology of the Laboratory Rat and Mouse: Review and Criteria for the Staging of the Estrous Cycle Using Stained Vaginal Smears. *Toxicologic Pathology*, 43: 776-793.

Migliore, L., Godeas, F., De Filippis, S., Mantovi, P., Barchi, D., and Testa, C. *et al.* (2010). Hormetic effect(s) of tetracyclines as an environmental contaminant on Zea mays. *Environmental Pollution*, *158*(1), 129-134. doi: 10.1016/j.envpol.2009.07.039

Migliore, L., Rotini, A., and Thaller, M. (2013). Low Doses of Tetracycline Trigger the E. coli Growth: A Case of Hormetic Response. *Dose-Response*, *11*(4), dose-response.1. doi: 10.2203/dose-response.13-002.migliore

Miki, T., Harris, S., Wilce, P., Takeuchi, Y., and Bedi, K. (2004). Effects of age and alcohol exposure during early life on pyramidal cell numbers in the CA1-CA3 region of the rat hippocampus. *Hippocampus*, *14*(1), 124-134. doi: 10.1002/hipo.10155

Mills, E., and Saunders, P. (2006). *Herbal medicines in pregnancy and lactation*. London: Taylor and Francis.

Ming, G.L. and Song, H. (2011) Adult neurogenesis in the mammalian brain: significant answers and significant questions. *Neuron*, 70, 687–702.

Miodini, P., Fioravanti, L., Fronzo, G., and Cappelletti, V. (1999). The two phyto-oestrogens genistein and quercetin exert different effects on oestrogen receptor function. *British Journal Of Cancer*, *80*(8), 1150-1155. doi: 10.1038/sj.bjc.6690479

Miodovnik, A. (2011). Environmental Neurotoxicants and Developing Brain. *Mount Sinai Journal of Medicine: A Journal of Translational and Personalized Medicine*, 78(1), pp.58-77.

Miraldi E., Masti A., Ferri S., Barni Comparini I., (2001): Distribution of hyoscyamine andscopolamine in *Datura stramonium*. *Fitoterapia*., 72: 644-648.

Mishra, D., Tiwari, S., Agarwal, S., Sharma, V., and Chaturvedi, R. (2012). Prenatal Carbofuran Exposure Inhibits Hippocampal Neurogenesis and Causes Learning and Memory Deficits in offspring. *Toxicological Sciences*, *127*(1), 84-100. doi: 10.1093/toxsci/kfs004

Miyata, T., Maeda, T., and Lee, J. (1999). *NeuroD* is required for differentiation of the granule cells in the cerebellum and hippocampus. *Genes and Development*, *13*(13), 1647-1652. doi: 10.1101/gad.13.13.1647

Molenhuis, R., de Visser, L., Bruining, H. and Kas, M. (2014). Enhancing the value of psychiatric mouse models; differential expression of developmental behavioural and cognitive profiles in four inbred strains of mice. *European Neuropsychopharmacology*, 24(6), pp.945-954.

Moore, E., Linsenbardt, D., Melón, L. and Boehm, S. (2011). Ontogenetic differences in adolescent and adult C57BL/6J and DBA/2J mice: Anxiety-like, locomotor, and consummatory behaviours. *Developmental Psychobiology*, 53(2), pp.141-156.

Morris, R. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. *Journal of Neuroscience Methods*, 11:47-60.

Morris, R., Garrud, P., Rawlins, J., and O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature*, *297*(5868), 681-683. doi: 10.1038/297681a0

Morrow, E.M.,Furukawa, T., Lee, J.E. and Cepko, C.L. (1999). *NeuroD* regulatesmultiple functions in the developing neural retina in a rodent.*Development*,126:23–36.

Moser, V.C. (1999). Neuro*behavioural* screening in rodents. *Current Protocols in Toxicology*, 11:2.1–2.16.

Motz, B.A. (2005). Alberts JR. The validity and utility of geotaxis in young rodents. *Neurotoxicology and Teratology*, 27:529–33. [PubMed: 16040231].

Mulkiewicz, E., Jastorff, B., Składanowski, A., Kleszczyński, K., and Stepnowski, P. (2007). Evaluation of the acute toxicity of perfluorinated carboxylic acids using eukaryotic cell lines, bacteria and enzymatic assays. *Environmental Toxicology and Pharmacology*, *23*(3), 279-285. doi: 10.1016/j.etap.2006.11.002

Müller, B., Kistner, U., Kindler, S., Chung, W., Kuhlendahl, S., and Fenster, S. *et al.* (1996). SAP102, a Novel Postsynaptic Protein That Interacts with NMDA Receptor Complexes In Vivo. *Neuron*, *17*(2), 255-265. doi: 10.1016/s0896-6273(00)80157-9

Murado, M., and Vázquez, J. (2010). Basic toxicodynamic features of some antimicrobial agents on microbial growth: a dynamic mathematical model and its implications on hormesis. *BMC Microbiology*, *10*, 220. doi: dx. doi.org/10.1186/1471- 2180-10-220

Mutschler, E., Geisslinger, H., Kroemer, H.K., Ruth, P., Schäfer-Korting, M. (2008): Mutschler- Arzneimittelwirkungen. Lehrbuch der Pharmakologie und Toxikologie. 9th edit., WVG, Stuttgart, Schmeller, T., Latz-Brüning, B. and Wink, M. (1997): Biochemical activities of berberine, palmatine and sanguinarine mediating chemical defence against microorganisms and herbivores. *Phytochemistry* 44, 257-266.

Nadkarni, K.M., and Nadkarni, A.K. (1996). *Indian medical matter* (p. 435). Bombay: Popular Prakashan.

Nakamura, K., Itoh, K., Dai, H., Han, L., Wang, X., Kato, S., Sugimoto, T. and Fushiki, S. (2012). Prenatal and lactational exposure to low doses of bisphenol A alters adult mice behaviour. *Brain and Development*, 34(1), pp.57-63.

Nakamura, K., Itoh, K., Sugimoto, T. and Fushiki, S. (2007). Prenatal exposure to bisphenol A affects adult murine neocortical structure. *Neuroscience Letters*, 420(2), pp.100-105.

Narendhirakannan, R., Subramanian, S., Kandaswamy, M., 2005. Mineral content of some medicinal plants used in the treatment of diabetes mellitus. Biological Trace Element Research 103, 109–115.

Needleman, H. (2004). Lead Poisoning. Annual Review Of Medicine, 55(1), 209-222. doi: 10.1146/annurev.med.55.091902.103653

Nelson, J., Karelus, K., Felicio, L. and Johnson, T. (1990). Genetic Influences on the Timing of Puberty in Mice1. *Biology of Reproduction*, 42(4), pp.649-655.

Newland, M. and Rasmussen, E. (2000). Ageing unmasks the adverse effects of gestational exposure to methylmercury in rats. *Neurotoxicology and Teratology*, 22(6), pp.819-828.

Nguyen, L., Rigo, J., Rocher, V., Belachew, S., Malgrange, B., Rogister, B., Leprince, P. and Moonen, G. (2001). Neurotransmitters as early signals for central nervous system development. *Cell and Tissue Research*, 305(2), pp.187-202.

Niesiobędzka, K., Wojtkowska, M., and Krajewska, E. (2005). Migration of zinc, lead and cadmium in the soil - vegetation system in an urban environment. IOŚ,

Warsaw. Regulation of the Minister of the Environment on soil quality standards and soil quality standards. *Dziennik Ustaw*, *165*, 1359.

NTP (US National Toxicology Programme), 1987a. Teratologic Evaluation of scopolamine hydrobromide (CAS No. 114-49-8) administered to CD-1 mice on gestational days 6 through 15. NTP Study: TER85107, abstract available online

Nwanyanwu, C., and Abu, G. (2010). In vitro effects of petroleum refinery wastewater on dehydrogenase activity in marine bacterial strains. *Ambiente E Agua - An Interdisciplinary Journal of Applied Science*, *5*(2), 21-29. doi: 10.4136/ambi-agua.133

Nweke, C., Ahumibe, N., and Orji, J. (2014). Toxicity of binary mixtures of formulated glyphosate and phenols to Rhizobium species dehydrogenase activity. *J. Microbiol. Res.*, *4*(4), 161 – 169. doi: http://dx.doi.org/10.5923/j.microbiology.20140404.02

Nweke, C., and Okpokwasili, G. (2010). Inhibition of dehydrogenase activity in petroleum refinery wastewater bacteria by phenolic compounds. *Ambiente E Agua - An Interdisciplinary Journal Of Applied Science*, *5*(1), 6-16. doi: 10.4136/ambi-agua.115

Nweke, C., Ike, C., and Ibegbulem, C. (2016). Toxicity of quaternary mixtures of phenolic compounds and formulated glyphosate to microbial community of river water. *Ecotoxicology and Environmental Contamination*, *11*(1), 63-71. doi: 10.5132/eec.2016.01.09

Nweke, C., Orji, J., and Ahumibe. (2015). Prediction of phenolic compound and formulated glyphosate toxicity in binary mixtures using Rhizobium species dehydrogenase activity. *Adv. Life Sci.*, *5*(2), 27 – 38. doi: http://dx.doi.org/10.5923/j. als.20150502.01 1500

Okolo, J., Nweke, C., Nwabueze, R., Dike, C., and Nwanyanwu, C. (2007). Toxicity of phenolic compounds to oxidoreductases of Acinetobacter species isolated from tropical soil. *Sci. Res. Essays*, *2*(7), 244 – 250.

Okon E.E. (1970). The effect of environmental temperature on the production ofultrasounds by isolated non-handled albino mouse pups. *JournalofZoology*,162:71-83.

Olawepo, A., Ishola, A., Ajao, M., Olayemi, O., and Olayaki, L. (2017). Atropine exposure in adolescence predisposes to adult memory loss in Wistar rats. *International Journal Of Biological And Chemical Sciences*, *11*(5), 1937. doi: 10.4314/ijbcs.v11i5.1

Olowoyo, J.O., Van Heerden, E., Fisher, J.L., Baker, C., 2010. Trace metals in soil and leaves of Jacaranda mimosifolia in Tshwane area, South Africa. Atmospheric Environment 44, 1826–1830.

Ongür D, Price JL. The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans. Cerebral Cortex. 2000; 10(3):206–219. [PubMed: 10731217].

Opler, M., Buka, S., Groeger, J., McKeague, I., Wei, C., Factor-Litvak, P., Bresnahan, M., Graziano, J., Goldstein, J., Seidman, L., Brown, A. and Susser, E. (2008). Prenatal Exposure to Lead, δ-Aminolevulinic Acid, and Schizophrenia: Further Evidence. *Environmental Health Perspectives*, 116(11), pp.1586-1590.

Oseni, O.A., Olarinoye, C.O, and Amoo, I.A. (2011). Studies on chemical compositions and functional properties of thorn apple (*Daturastramonium* L) Solanaceae. *African Journal of Food Science,5*(2), 40-44.

Pandey, M., Debnath, M., Gupta, M., and Chikara, S.K. (2011). Phytomedicine: An ancient approach turning into a future potential source of therapeutics. *Journal of Pharmacognosy and Phytotherapy*, *3*(3), 27-37.

Papadoyannis, I. (1995). Determination of datura alkaloids by using chromatographic techniques: A review. Natural Toxins, 3(4), 310-316. doi: 10.1002/nt.2620030427

Parashuram, M. (2011). Isolation of 11,12,13,17-Tetrahydroxy-(Hydroxymethyl)-10-Nitrodotriacontahydrospiro [Indeno[5,6-A] Hexacene-2,2'-Pyran] =3,6(1H,18bh) Dione and its spectroscopic characterization and biological activities of bimetals from seeds of *Daturastramonium*. *Asian Journal of Biochemical and Pharmaceutical Research*, *3*(1), 501-506.

Parkening, T. (1989). Fertilizing ability of spermatozoa from aged C57BL/6NNia mice. *Reproduction*, 87(2), pp.727-733.

Patel, S. and Hillard, C. (2001). Cannabinoid CB (1) receptor agonists produce cerebellar dysfunction in mice. *Journal of Pharmacology and Experimental Therapeutics*, 297, pp.629-637.

Paxinos, G., Halliday, G., Watson, C., Koutcherov, Y., and Wang, H. (2007).Atlas of the Developing Mouse Brain at E17.5, P0, and P6, p. 375. academic press (Elsevier), San Diego, CA.

Pocock, M., Hauffe, H. and Searle, J. (2005). Dispersal in house mice. *Biological Journal of the Linnean Society*, 84(3), pp.565-583.

Prather, R. and First, N. (1988). A Review of Early Mouse Embryogenesis and its Applications to Domestic Species. *Journal of Animal Science*, 66(10), p.2626.

Preissel, U. and Preissel, H. (2005). *Brugmansia and Datura*. Willowdale, Ont.: Firefly.

Pretorius, E., and Marx, J. (2006). *Daturastramonium* in asthma treatment and possible effects on prenatal development. *Environmental Toxicology And Pharmacology*, *21*(3), 331-337. doi: 10.1016/j.etap.2005.10.006

Previti, G., Pawlby, S., Chowdhury, S., Aguglia, E. and Pariante, C. (2014). Neurodevelopmentaloutcome for offspring of women treated for antenatal depression: a systematic review. *Archives of Women's Mental Health*, 17(6), pp.471-483.

Price, JL. Prefrontal cortex. In: Moldin, SO.; Rubenstein, JLR., editors. Understanding Autism. CRC Press; Boca Raton: 2006

Puelles, L., Kuwana, E., Puelles, E., Bulfone, A., Shimamura, K., and Keleher, J. *et al.* (2000). Pallial and subpallial derivatives in the embryonic chick and mouse telencephalon, traced by the expression of the genes Dlx-2, Emx-1, Nkx-2.1, Pax-6, and Tbr-1. *The Journal Of Comparative Neurology*, *424*(3), 409-438. doi: 10.1002/1096-9861(20000828)424:3<409: aid-cne3>3.0.co;2-7

Qing, H., He, G., Ly, P.T., Fox, C.J., Staufenbiel, M., Cai, F., Zhang, Z., Wei, S., Sun, X., Chen, C.H., Zhou, W., Wang, K. and Song, W. (2008). Valproic acid inhibits Abeta production, neuritic plaque formation, and behavioural deficits in Alzheimer's disease mouse models. *Journal of Experimental Medicine,* (205): 2781-2789.

Qiu, X., Dowling, A., Marino, J., Faulkner, L., Bryant, B., Brüning, J., Elias, C. and Hill, J. (2013). Delayed Puberty but Normal Fertility in Mice with Selective Deletion of Insulin Receptors FromKiss1Cells. *Endocrinology*, 154(3), pp.1337-1348.

Ramalingam, M. and Kim, S. (2012). Reactive oxygen/nitrogen species and their functional correlations in neurodegenerativediseases. *Journal of Neural Transmission*, 119(8), pp.891-910.

Ramirez, M., Rivera, E., and Ereu, C. (1999). Fifteen cases of atropine poisoning after honey ingestion. *Veterinary and Human Toxicology*, *41*, 19-20.

Ramón y Cajal , S. (1995). *Histology of the nervous system of man and vertebrates*. New York: Oxford Univ. Press.

Randall, W., Price, C., and Welch, H. (1947). Demonstration of Hormesis (Increase in Fatality Rate) by Penicillin. *American Journal of Public Health and The Nation's Health*, *37*(4), 421-425. doi: 10.2105/ajph.37.4.421

Rao, D., Little, P. and Sills, R. (2013). Subsite Awareness in Neuropathology Evaluation of the National Toxicology Program (NTP) Studies. *Toxicologic Pathology*, 42(3), pp.487-509.

Ren-sheng, X., Giaozhen, Z., and Yuyuan, X. (1985). Drugs acting on the nervous system. *Ethnopharmacology*, *14*, 223-253.

Reuhl, K. (1991). Delayed expression of neurotoxicity: the problem of silent damage". *Neurotoxicology*, 12(3), pp.341–346.

Richardson, J., Caudle, W., Wang, M., Dean, E., Pennell, K. and Miller, G. (2006). Developmental exposure to the pesticide dieldrin alters the dopamine system and increases neurotoxicity in an animal model of Parkinson's disease. *The FASEB Journal*, 20(10), pp.1695-1697.

Roberts, M.F., Wink, M. (eds.) (2013) *Alkaloids: biochemistry, ecology and medicinal applications*, Springer Science and Business Media, New York, pp 482

Rodea-Palomares, I., González-García, C., Leganés, F., and Fernández-Piñas, F. (2009). Effect of pH, EDTA, and Anions on Heavy Metal Toxicity Toward a Bioluminescent Cyanobacterial Bioreporter. *Archives of Environmental Contamination and Toxicology, 57*(3), 477-487. doi: 10.1007/s00244-008-9280-9

Rödel, H., Hudson, R., Rammler, L., Sänger, N., Schwarz, L. and Machnik, P. (2011). Lactation does not alter the long-term stability of individual differences in the behaviour of laboratory mice on the elevated plus-maze. *Journal of Ethology*, 30(2), pp.263-270.

Rodier, P. (1994). Vulnerable Periods and Processes during Central Nervous System Development. *Environmental Health Perspectives,* 102, pp.121 - 124.

Rodier, P. (1995). Developing Brain as a Target of Toxicity. *Environmental Health Perspectives*, 103, pp.73 - 76.

Romeo, R., Richardson, H. and Sisk, C. (2002). Puberty and the maturation of the male brain and sexual behaviour: recasting a behavioural potential. *Neuroscience and Biobehavioral Reviews*, 26(3), pp.381-391.

Rose, R. D. and Rohrlich, D. (1987). Counting sectioned cells via mathematical reconstruction. *J. Comp. Neurol.* 263, 365–386.doi:10.1002/ one.902630305.

Rosell, J., de la Fuente, L., Carbajo, M., and Fernández, X. (2020). Reproductive Diseases in Farmed Rabbit Does. Animals, 10(10), 1873. doi: 10.3390/ani10101873

Roy, T., Sharma, V., Seidler, F., and Slotkin, T. (2005). Quantitative morphological assessment reveals neuronal and glial deficits in the hippocampus after a brief subtoxic exposure to chlorpyrifos in neonatal rats. *Developmental Brain Research*, *155*(1), 71-80. doi: 10.1016/j.devbrainres.2004.12.004

Rubenstein, J. (2010). Annual Research Review: Development of the cerebral cortex: implications for neurodevelopmentaldisorders. *Journal of Child Psychology and Psychiatry*, 52(4), pp.339-355.

Rubenstein, J. L., Shimamura, K., Martinez, S., and Puelles, L. (1998). Regionalizationof the prosencephalic neural plate. Annu Rev Neurosci 21, 445–77.

Salamah, N., and Ningsih, D. (2017). Total alkaloid content in various fractions of Tabernaemonata sphaerocarpa Bl. (Jembirit) leaves. IOP Conference Series: Materials Science and Engineering, 1(01), 259. doi: 10.1088/1757-899x/259/1/012017

Sales, G. and Skinner, N. (2009). The effect of ambient temperature on body temperature and ultrasonic behaviour in litters of albino laboratory mice deprived of their mothers. *Journal of Zoology*, 187(2), pp.265-281.

Sans, N., Petralia, R., Wang, Y., Blahos, J., Hell, J., and Wenthold, R. (2000). A Developmental Change in NMDA Receptor-Associated Proteins at Hippocampal Synapses. *The Journal of Neuroscience*, *20*(3), 1260-1271. doi: 10.1523/jneurosci.20-03-01260.2000

Sans, N., Prybylowski, K., Petralia, R., Chang, K., Wang, Y., and Racca, C. *et al.* (2003). NMDA receptor trafficking through an interaction between PDZ proteins and the exocyst complex. *Nature Cell Biology*, *5*(6), 520-530. doi: 10.1038/ncb990

Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., Weisstaub, N., Lee, J. *et al.* (2003) Requirement of hippocampal neurogenesis for the *behavioural* effects of antidepressants. *Science,* 301, 805–809.

Saper, R.B., Phillips, R.S., Sehgal, A., Khouri, N., Davis, R.B., Paquin, J., Thuppil, V., Stefanos, N., Kales, S.N., 2008. Lead, mercury, and arsenic in the US- and Indian-manufactured ayurvedic medicines sold via the internet. JAMA: The Journal of the American Medical Association 300, 915–923.

Sarma, H., Deka, S., Deka, H., Saikia, R.R., 2012. Accumulation of heavy metals in selected medicinal plants. Reviews of Environmental Contamination and Toxicology 214, 63–86.

Sarsilmaz, M., Kaplan, S., Songur, A., Colakoglu, S., Aslan, H., and Tunc, A. *et al.* (2007). Effects of postnatal formaldehyde exposure on pyramidal cell number, the volume of a cell layer in hippocampus and hemisphere in the rat: A stereological study. *Brain Research, 1145*, 157-167. doi: 10.1016/j.brainres.2007.01.139

Schambra, U. B. (Ed.) (2008). Prenatal Mouse Brain Atlas. 1st ed. (p. 530).Springer, New York. doi:10.1007/978-0-387-47093-1.

Schmelzer, G.H., Gurib-Fakim, A. (2008) *Medicinal plants*, vol 1, Prota Foundation, pp. 791

Schmidt, M., Schmidt, M., Czisch, M., Schmidt, M., Czisch, M., Sterlemann, V., Reinel, C., Sämann, P. and Müller, M. (2009). Chronic social stress during adolescence in mice alters fat distribution in late life: Prevention by antidepressant treatment. *Stress*, 12(1), pp.89-94.

Schmidt, M., Sterlemann, V., Ganea, K., Liebl, C., Alam, S., Harbich, D., Greetfeld, M., Uhr, M., Holsboer, F. and Müller, M. (2007). Persistent neuroendocrine and behavioural effects of a novel, etiologically relevant mouse paradigm for chronic social stress during adolescence. *Psychoneuroendocrinology*, 32(5), pp.417-429.

Schulman, M.L. and Bolton, L.A. (1998). *Datura* seed intoxication in two horses. *Journal of South African Veterinary Association*, 69: 27-29.

Schwab, M., Bartholomae, A., Heimrich, B., Feldmeyer, D., Druffel-Augustin, S., and Goebbels, S. *et al.* (2000). Neuronal Basic Helix-Loop-Helix Proteins (NEX and BETA2/Neuro D) Regulate Terminal Granule Cell Differentiation in the Hippocampus. *The Journal Of Neuroscience*, *20*(10), 3714-3724. doi: 10.1523/jneurosci.20-10-03714.2000.

Seckl, J. (2004). Prenatal glucocorticoids and long-term programming. *European Journal of Endocrinology*, 151: pp. U49-U62.

Senthil, K., C., Moorthi, C., Prabhu, P.C., Benoto Jonson, B., Venkatnarayan, R., 2011. Standardization of anti-arthritic herbo-mineral preparation. Research Journal of Pharmaceutical, Biological and Chemical Sciences 2, 679–684.

Sewram, V., Street, R.A., Myers, J., Gqaleni, N., Connolly, C., 2010. The determination of hexavalent chrome content in South African traditional medicines used as enemas. Technical Report. South African Medical Research Council.

Shagal, M.H., Modibbo, U.U., and Liman, A.B. (2012). The pharmacological justification for the ethnomedical use of *Daturastramonium* stem-bark extract in the treatment of diseases caused by some pathogenic bacteria. *International Research Journal of Pharmacy and Pharmacology*, *2*(1), 16-19.

Sharma, A., Patel, V.K., and Chaturvedi, A.N. (2009). Vibriocidal activity of certain medicinal plants used in Indian folklore medicine by tribals of Mahakoshal region of central India. *Indian Journal of Pharmacology*, *41*(3), 129. doi: 10.4103/0253-7613.55212

Sharma, M., and Sharma, S. (2010). Phytochemical, preliminary pharmacognostical and antimicrobial evaluation of combined crude aqueous extract. *International Journal of Microbiology Research*, *1*(3), 166-170.

Shaw, D., 1998. Risks or remedies? Safety aspects of herbal remedies in the UK. Journal of the Royal Society of Medicine 91, 294–296.

Shen, K., Shen, C., Lu, Y., Tang, X., Zhang, C., and Chen, X. *et al.* (2009). Hormesis response of marine and freshwater luminescent bacteria to metal exposure. *Biological Research*, *42*(2). doi: 10.4067/s0716-97602009000200006

Shiu, L., Chang, L., Liang, C., Huang, Y., Sheu, H., & Kuo, K. (2007). Solamargine induces apoptosis and sensitizes breast cancer cells to cisplatin. Food And Chemical Toxicology, 45(11), 2155-2164. doi: 10.1016/j.fct.2007.05.009

Shonle I., Bergelson J., (2000): Evolutionary ecology of the tropane alkaloids of *Daturastramonium*L. (*Solanaceae*). *Evolution*, 54(3): 778-788.

Sinclair, G., Paton, G., Meharg, A., and Killham, K. (1999). Lux-biosensor assessment of pH effects on microbial sorption and toxicity of chlorophenols. *FEMS Microbiology Letters*, *174*(2), 273-278. doi: 10.1111/j.1574-6968.1999.tb13579.x

Singh, V., Garg, A.N., 1997. Availability of essential trace elements in ayurvedic Indian medicinal herbs using instrumental neutron activation analysis. Applied Radiation and Isotopes 48, 97–101.

Sisk, C. and Zehr, J. (2005). Pubertal hormones organize the adolescent brain and behaviour. *Frontiers in Neuroendocrinology*, 26(3-4), pp.163-174.

Smith, E. (1991). The influence of nutrition and postpartum mating on weaning and subsequent play behaviour of hooded rats. *Animal Behaviour*, 41(3), pp.513-524.

Smotherman, W. and Robinson, S. (1985). The rat fetus in its environment: Behavioural adjustments to the novel, familiar, aversive, and conditioned stimuli presented in utero. *Behavioural Neuroscience*, 99(3), pp.521-530.

Snyder, J., Kee, N., and Wojtowicz, J. (2001). Effects of Adult Neurogenesis on Synaptic Plasticity in the Rat Dentate Gyrus. *Journal of Neurophysiology*, *85*(6), 2423-2431. doi: 10.1152/jn.2001.85.6.2423

Sorsby, A., Koller, P., Attfield, M., Davey, J., & Lucas, D. (1954). Retinal dystrophy in the mouse: Histological and genetic aspects. Journal Of Experimental Zoology, 125(2), 171-197. doi: 10.1002/jez.1401250202

Spear, L. (2000). The adolescent brain and age-related behavioural manifestations. *Neuroscience and Biobehavioral Reviews*, 24(4), pp.417-463.

Spear, L. (2002). Alcohol's effects on adolescents. *Alcohol Health and Research World*, *26*(4), 287-291.

Spear, L., Horowitz, G., and Lipovsky, J. (1982). Altered behavioural responsivity to morphine during the preadolescent period in rats. *Behavioural Brain Research*, *4*(3), 279-288. doi: 10.1016/0166-4328(82)90005-5

Spear, L., Shalaby, I., and Brick, J. (1980). Chronic administration of haloperidol during development: Behavioral and psychopharmacological effects. *Psychopharmacology*, *70*(1), 47-58. doi: 10.1007/bf00432369

Sprott, R. (1975). Behavioural characteristics of C57BL/6J, DBA/2J, and B6D2F1mice are potentially useful for gerontological research. *Experimental Aging Research*, 1(2), pp.313-323.

Squeglia, L., Jacobus, J., and Tapert, S. (2009). The Influence of Substance Use on Adolescent Brain Development. *Clinical EEG and Neuroscience, 40*(1), 31-38. doi: 10.1177/155005940904000110

Sreenivasa, S., Vinay, K., Mohan, N. R. (2012) Phytochemical analysis, the antibacterial and antioxidant activity of leaf extract of *Datura stramonium*, *International Journal of Science Research*, **1**, 83-86

Steenkamp, P.A., Harding, N.M., Van Heerden, F.R. and Van WYk, B.E. (2004). Fatal *Datura* Poisoning: Identification of atropine and scopolamine by high-performance liquid chromatography/ photodiode array/mass spectrometry. *Journal of Forensic Science International.* 145:31-39.

Steenkamp, V., Stewart, M.J., Curowska, E., Zuckerman, M., 2002. A severe case of multiple metal poisoning in a child treated with traditionalmedicine. Forensic Science International 128, 123–126.

Steenkamp, V., Von Arb, M., Stewart, M.J., 2000. Metal concentrations in plants and urine from patients treated with traditional remedies. Forensic Science International 114, 89–95.

Sterio, D.C.(1984).The unbiased estimation of the number and size of arbitrary particles using the disector. *J. Microsc.* 134, 127–136.doi:10.1111/j.1365-2818. 1984.tb02501.x

Sterlemann, V., Rammes, G., Wolf, M., Liebl, C., Ganea, K., Müller, M. and Schmidt, M. (2009). Chronic social stress during adolescence induces cognitive impairment in aged mice. *Hippocampus*, pp.540-549.

Stewart, M.J., Moar, J.J., Steenkamp, P., Kokot, M., 1999. Findings in fatal cases of poisoning attributed to traditional remedies in South Africa. Forensic Science International 101, 177–183.

Stone, E., & Quartermain, D. (1997). Greater Behavioral Effects of Stress in Immature as Compared to Mature Male Mice. Physiology & Behavior, 63(1), 143-145. doi: 10.1016/s0031-9384(97)00366-1

Stump, D., Beck, M., Radovsky, A., Garman, R., Freshwater, L., Sheets, L., Marty, M., Waechter, J., Dimond, S., Van Miller, J., Shiotsuka, R., Beyer, D., Chappelle, A. and Hentges, S. (2010). Developmental Neurotoxicity Study of Dietary Bisphenol A in Sprague-Dawley Rats. *Toxicological Sciences*, 115(1), pp.167-182.

Swan, S. (2000). Intrauterine exposure to diethylstilbestrol: Long-term effects in humans. *All Purpose Medical Information System (APMIS)*, 108(12), pp.793-804.

Swanson LJ, Desjardins C, Turek FW: Aging of the reproductive system in the male hamster: behavioural and endocrine patterns. *BiologyandReproduction* 1982, 26:791-799.

Swathi, S., Murugananthan, G., Ghosh, S.K., and Pradeep, A.S. (2012). Larvicidal and repellent activities of ethanolic extract of *Daturastramonium* leaves against mosquitoes. *International Journal of Pharmacognosy and Phytochemical Research*, *4*(1), 25-27.

Sweta, V.R., Lakshmi, T. (2015) Pharmacological profile of tropane alkaloids, *Journal of Chemical and Pharmaceutical Research*, **7** (5): 117 – 119

Szenczi, P., Bánszegi, O., Groó, Z. and Altbäcker, V. (2012). Development of the Social Behaviour of Two Mice Species with Contrasting Social Systems. *Aggressive Behavior*, 38(4), pp.288-297.

Szyf, M., Weaver, I. and Meaney, M. (2007). Maternal care, the epigenome and phenotypic differences in behaviour. *Reproductive Toxicology*, 24(1), pp.9-19.

Taha, S.A., and Mahdi, A.W. (1984). *Datura* intoxication in Riyadh. *Transactions of The Royal Society of Tropical Medicine And Hygiene*, *78*(1), 134-135. doi: 10.1016/0035-9203(84)90196-2

Takhi, D., Ouinten, M., and Yousfi, M. (2011). Study of Antimicrobial Activity of Secondary Metabolites Extracted From spontaneous Plants from the Area of Laghouat, Algeria. *Advances in Environmental Biology*, *5*(2), 469-476.

Tarín, J., Gómez-Piquer, V., García-Palomares, S., García-Pérez, M. and Cano, A. (2014). The absence of long-term effects of reproduction on longevity in the mouse model. *Reproductive Biology and Endocrinology*, 12(1), p.84.

Tarpey, P., Parnau, J., Blow, M., Woffendin, H., Bignell, G., and Cox, C. *et al.* (2004). Mutations in the *DLG*3 Gene Cause Nonsyndromic X-Linked Mental Retardation. *The American Journal of Human Genetics*, *75*(2), 318-324. doi: 10.1086/422703

Tashiro, A., Makino, H. and Gage, F.H. (2007) Experience-specific functional modification of the dentate gyrus through adult neurogenesis: a critical period during an immature stage. *Journal of Neuroscience*, 27, 3252–3259.

Tayoub, G., Sulaiman, H. and Alorfi, M. (2016). Quantitative identification of Atropine and Scopolamine in wild *Datura* (*Datura* metel) using HPLC. *Journal of Natural Products*, [online] 9, pp.21-28. Available at: http://www.JournalofNaturalProducts.com [Accessed 19 Jan. 2020].

Terranova, M., Laviola, G., de Acetis, L. and Alleva, E. (1998). A description of the ontogeny of mouse agonistic behaviour. *Journal of Comparative Psychology*, 112(1), pp.3-12.

Theiler, K (1972). The house mouse - Development and normal stages from fertilization to 4 weeks of age Berlin, Heidelberg, New York: Springer.

Theiler, K. (1989). The House Mouse: Atlas of Embryonic Development, p.178. Springer, New York. Accessed February 1, 2017. http://www.emouseatlas.org/emap/ema/theiler_stages/house_mouse/book.html.

Thifault, S., Lalonde, R., Sanon, N. and Hamet, P. (2001). Longitudinal analysis of motor activity and coordination, anxiety, and spatial learning in mice with altered blood pressure. *Brain Research*, 910(1-2), pp.99-105.

Toni, N., Laplagne, D., Zhao, C., Lombardi, G., Ribak, C., Gage, F., and Schinder, A. (2008). Neurons born in the adult dentate gyrus form functional synapses with target cells. *Nature Neuroscience*, *11*(8), 901-907. doi: 10.1038/nn.2156

Tran, T., and Kelly, S. (2003). Critical periods for ethanol-induced cell loss in the hippocampal formation. *Neurotoxicology And Teratology*, *25*(5), 519-528. doi: 10.1016/s0892-0362(03)00074-6

Traweger, A., Wiggin, G., Taylor, L., Tate, S., Metalnikov, P., and Pawson, T. (2008). Protein phosphatase 1 regulates the phosphorylation state of the polarity scaffold Par-3. *Proceedings Of The National Academy Of Sciences*, *105*(30), 10402-10407. doi: 10.1073/pnas.080410210.

Treuting, M., Dintzis, M., Frevert, W. and Liggitt, D. (2019). *Comparative anatomy and histology of a mouse and human atlas*. 7th ed. Massachusetts: Academic Press, pp.356–358.

Tschanz, S.A., Burri, P.H. and Weibel, R., (2011). A simple tool for stereological assessment of digital images: the STEPanizer. *JournalofMicroscopy*. 243, 47–59. https://doi.org/10.1111/j

Tsuda, M., Yamaguchi, N. and Ogawa, S. (2011). Early life stress disrupts the peripubertal development of aggression in male mice. *NeuroReport*, 22(6), pp.259-263.

Tyler, W., Alonso, M., Bramham, C., and Pozzo-Miller, L. (2002). From Acquisition to Consolidation: On the Role of Brain-Derived Neurotrophic Factor Signaling in Hippocampal-Dependent Learning. *Learning and Memory, 9*(5), 224-237. doi: 10.1101/lm.51202

Usha, K., Singh, B., Praseetha, P., Deepa, N., Agarwal, D.K., Agarwal, R., and Nagaraja, A. (2009). Antifungal activity of *Daturastramonium*, Calotropis gigantea and Azadirachta indica against Fusarium mangiferae and floral malformation in mango. *European Journal of Plant Pathology*, *124*(4), 637-657. doi: 10.1007/s10658-009-9450-2.

Van Den Hazel, P., Zuurbier, M., Babisch, W., Bartonova, A., Bistrup, M., Bolte, G., Busby, C., Butter, M., Ceccatelli, S., Fucic, A., Hanke, W., Johansson, C., Kohlhuber, M., Leijs, M., Lundqvist, C., Moshammer, H., Naginiene, R., Preece, A., Ronchetti, R., Salines, G., Saunders, M., Schoeters, G., Stilianakis, N., Ten Tusscher, G. and Koppe, J. (2006). Today's epidemics in children: Possible relations to environmental pollution and suggested preventive measures. *Acta Paediatrica*, 95(0), pp.18-25.

van Praag, H., Christie, B.R., Sejnowski, T.J. and Gage, F.H. (1999b) Running enhances neurogenesis, learning, and long-term potentiation in mice. *Proceedings of the National Academy of Science*USA, 96, 13427–13431.

van Praag, H., Kempermann, G. and Gage, F.H. (1999a) Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nature Neuroscience*, 2, 266–270.

van Praag, H., Schinder, A., Christie, B., Toni, N., Palmer, T., and Gage, F. (2002). Functional neurogenesis in the adult hippocampus. *Nature*, *415*(6875), 1030-1034. doi: 10.1038/4151030a

Van Campenhout, C., Eitelhuber, A., Gloeckner, C., Giallonardo, P., Gegg, M., and Oller, H. *et al.* (2011). *DLG*3 Trafficking and Apical Tight Junction Formation are Regulated by Nedd4 and Nedd4-2 E3 Ubiquitin Ligases. *Developmental Cell*, *21*(3), 479-491. doi: 10.1016/j.devcel.2011.08.003

Vandenbergh, J. (1971). The Influence of the Social Environment on Sexual Maturation in Male Mice. *Reproduction*, 24(3), pp.383-390.

Vearrier, D., & Greenberg, M. (2010). Anticholinergic delirium following Datura *stramonium* ingestion: Implications for the Internet age. *Journal Of Emergencies, Trauma, And Shock*, 3(3), 303. doi: 10.4103/0974-2700.66565

Vélez, D., Devesa, V., Súñer, M.A., Montoro, R., 2004. Metal contamination in food, In Nollet, L.M.L. (Ed.), Handbook of Food Analysis, 2nd ed. Marcel Dekker, New York, pp. 1485–1511.

Viberg, H., Fredriksson, A., Buratovic, S. and Eriksson, P. (2011). Dose-dependent behavioural disturbances after a single neonatal Bisphenol A dose. *Toxicology*, 290(2-3), pp.187-194.

Vitalone, A., Catalani, A., Cinque, C., Fattori, V., Matteucci, P., Zuena, A. and Costa, L. (2010). Long-term effects of developmental exposure to low doses of PCB 126 and methylmercury. *Toxicology Letters*, 197(1), pp.38-45.

vom Saal, F. and Bronson, F. (1980). Sexual characteristics of adult female mice are correlated with their blood testosterone levels during prenatal development. *Science*, 208(4444), pp.597-599.

Vorhees, C., and Williams, M. (2006). Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nature Protocols, 1*(2), 848-858. doi: 10.1038/nprot.2006.116

Wagner, H. (1993). *"Pharmazeutische Biologic"*. 5th ed. Stuttgart, Germany: Gustav Fisher, Vwelag, pp. AUFI.15 BN 3-437-20 498-X.

Wahlsten, D. (1974). A developmental time scale for postnatal changes in brain and behaviour of B6D2F2 mice. *Brain Research*, 72(2), pp.251-264.

Wahlström, G. and Nordberg, A. (1992). Atropine as an inhibitor of voluntary ethanol intake in male rats. *Alcohol,* 27: 381-391.

Walker, C. and Byers, J. (1991). Heritability of locomotor play in house mice, Mus domesticus. *Animal Behaviour*, 42(6), pp.891-897.

Wang, H., Wang, J., Zheng, B., Li, S., Zhang, Y., Li, F., and Zheng, N. (2014). Cytotoxicity induced by ochratoxin A, zearalenone, and α-zearalenol: Effects of individual and combined treatment. *Food and Chemical Toxicology, 71*, 217-224. doi: 10.1016/j.fct.2014.05.032

Wang, L., Liu, S., Yuan, J., and Liu, H. (2011). Remarkable hormesis induced by 1-ethyl-3-methyl imidazolium tetrafluoroborate on Vibrio qinghaiensis sp.-Q67. *Chemosphere*, *84*(10), 1440-1445. doi: 10.1016/j.chemosphere.2011.04.049

Wang, X., Pal, R., Chen, X., Limpeanchob, N., Kumar, K., and Michaelis, E. (2005). High intrinsic oxidative stress may underlie the selective vulnerability of the hippocampal CA1 region. *Molecular Brain Research, 140*(1-2), 120-126. doi: 10.1016/j.molbrainres.2005.07.018

Webb, S., Monk, C. and Nelson, C. (2001). Mechanisms of Postnatal Neurobiological Development: Implications for Human Development. *Developmental Neuropsychology*, 19(2), pp.147-171.

Webster, P. (1973). Withdrawal Symptoms In Neonates Associated With Maternal Antidepressant Therapy. *The Lancet*, 302(7824), pp.318-319.

Weibel, E.R. (1969). Stereological principle for morphometry in electron microscopic cytology. *Int. Rev. Cytol.* 36, 235–302.doi:10.1016/s0074- 7696(08)61637-x.

Weiss, B. (1991). "Cancer and the dynamics of Neurodegenerative processes," *Neurotoxicology*, 12(3), pp.379–386.

Weiss, B., Clarkson, T. and Simon, W. (2002). Silent latency periods in methylmercury poisoning and Neurodegenerative disease. *Environmental Health Perspectives*, 110(suppl 5), pp.851-854.

Weissmann, I. (2000). Stem cells units of development, units of regeneration, and units in evolution. *Cell*, 100, pp.157-168.

Weitz, G. (2003). Love and death in Wagner's Tristan und Isolde--an epic anticholinergic crisis. *British Medical Journal*, 327: 1469-1471.

Welch, H., Price, C., and Randall, W. (1946). Increase in a fatality rate of *E. typhosa* for white mice by streptomycin*. *Journal ofThe American Pharmaceutical Association (Scientific Ed.)*, *35*(5), 155-158. doi: 10.1002/jps.3030350505

West, M.J. (2012b). ''Systematicversusrandomsamplinginstereologicalstudies,'' in *Basic Stereology for Biologists and Neuroscientists*, ed. D. Crotty (New York, NY: Cold Spring Harbor Laboratory Press),109–114.

West, M.J.(2012a). ''Introduction to stereology,'' in *Basic Stereology for Biologists and Neuroscientists*, ed. D. Crotty (New York, NY: Cold Spring Harbor Laboratory Press), 1–14.

West, M.J., Slomianka,L.,and Gundersen, H.J.G. (1991). Unbiased stereological estimation of the total number of neurons in the subdivisions of the rat hippocampus using the optical fractionator. *Anat. Rec.* 231, 482–497.doi:10. 1002/ar.1092310411.

White, L., Cory-Slechta, D., Gilbert, M., Tiffany-Castiglioni, E., Zawia, N., Virgolini, M., Rossi-George, A., Lasley, S., Qian, Y. and Basha, M. (2007). New and evolving concepts in the neurotoxicology of lead☆. *Toxicology and Applied Pharmacology*, 225(1), pp.1-27.

WHO, 1998. Quality control methods for medicinal plant materials. Geneva.

WHO, 2005. National policy on traditional medicine and regulations of herbal medicines. Geneva.

Wilde, G., Pringle, A., Wright, P., and Iannotti, F. (2002). Differential Vulnerability of the CA1 and CA3 Subfields of the Hippocampus to Superoxide and Hydroxyl Radicals In Vitro. *Journal of Neurochemistry*, *69*(2), 883-886. doi: 10.1046/j.1471-4159.1997.69020883.x

Williams, E. and Scott, J. (1954). The Development of Social Behaviour Patterns in the Mouse, in Relation to Natural Periods 1). *Behaviour*, 6(1), pp.35-64.

Wink, M (1993a): Allelochemical properties and the raison d'être of alkaloids. In The Alkaloids, G. Cordell, (ed.), *Academic Press*, vol. 43, 1-118.

Wink, M. (2000): Interference of alkaloids with neuroreceptors and ion channels. *Bioactive Natural Products* 11, 3-129.

Wink, M. (2003a): Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. *Phytochemistry* 64, 3-19.

Wink, M. and Van Wyk, B.E. (2008): Mind-altering and poisonous plants of the world. BRIZA, Pretoria (SA).

Winters, K. (2008). *Adolescent Brain Development and Drug Abuse* [Ebook]. Mentor Foundation Online Book.

Wittingham, D. and Wood, M. (1983). *Reproductive Physiology*. New York: Academic Press, pp.137-164.

Wolf, F., and Geisel, T. (1998). Spontaneous pinwheel annihilation during visual development. *Nature*, *395*(6697), 73-78. doi: 10.1038/25736

Woods, R., Mills, P.B., Knobel, G.J., Hurlow, W.E., Stokol, J.M., 1990. Acute dichromate poisoning after use of traditional purgatives. A report of 7 cases. South African Medical Journal 77, 640–642.

Wu, J., Basha, M., Brock, B., Cox, D., Cardozo-Pelaez, F., McPherson, C., Harry, J., Rice, D., Maloney, B., Chen, D., Lahiri, D. and Zawia, N. (2008). Alzheimer's Disease (AD)-like Pathology in Aged Monkeys after Infantile Exposure to Environmental Metal Lead (Pb): Evidence for a Developmental Origin and Environmental Link for AD. *Journal of Neuroscience*, 28(1), pp.3-9.

Xia, D., Zhu, F., Li, M., & Gao, S. (2012). Induction of apoptosis in human hepatoma SMMC-7721 cells by solamargine from Solanum nigrum L. *Journalof Ethnopharmacology*, 139(2), 599-604. doi: 10.1016/j.jep.2011.11.058

Xia, L., Ying, Z., Mei, J., Shan-Shan, L., Min, C., & Hong-Xiang, L. (2011). Induction of actin disruption and downregulation of P-glycoprotein expression by solamargine in multidrug-resistant K562/AO2 cells. Chinese Medicine Journal (English), 124(13), 2038-2044.

Yakovlev, P., and Lecours, A. (1967). *1967. The myelogenetic cycles of regional maturation of the brain. In Regional Development of the Brain in Early Life, Minkowski A (ed)* (pp. 3-70). Boston: Blackwell Scientific.

Yang, Y., Dang, Y., Li, Q., Lu, J., Li, X., Wang, Y. (2014) Complete chloroplast genome sequence of poisonous and medicinal plant *Datura stramonium*: organizations and implications for genetic engineering, *Plos One*, **10** (2)

Yasuma, A., and Ichikawa (1953). "Ninhydrin-Schiff and alloxan- Schiff staining. A new histochemical staining method for proteins". *Journal of Laboratory and Clinical Medicine*, 41, pp.296-299.

Yu, J., Yang, Y., Wang, W., Xiong, G., and Chen, M. (1988). Mutagenicity and teratogenicity of chlorpromazine and scopolamine. *Chinese Medical Journal*, *101*(339-345).

Yuan, R., Meng, Q., Nautiyal, J., Flurkey, K., Tsaih, S., Krier, R., Parker, M., Harrison, D. and Paigen, B. (2012). Genetic coregulation of the age of female sexual maturation and lifespan through circulating IGF1 among inbred mouse strains. *Proceedings of the National Academy of Sciences*, 109(21), pp.8224-8229.

Yuan, R., Peters, L., and Paigen, B. (2011). Mice as a Mammalian Model for Research on the Genetics of Ageing. *ILAR Journal*, 52(1), pp.4-15.

Zaki, S., Abd-El-Haleem, D., Abulhamd, A., Elbery, H., and AbuElreesh, G. (2008). Influence of phenolics on the sensitivity of free and immobilized bioluminescent Acinetobacter bacterium. *Microbiological Research*, *163*(3), 277-285. doi: 10.1016/j.micres.2006.07.006

Zanni, G., van Esch, H., Bensalem, A., Saillour, Y., Poirier, K.,Castelnau, L., Ropers, H.H., de Brouwer, A.P., Laumonnier, F.,Fryns, J.P. *et al. (*2010) A novel mutation in the *DLG*3 geneencoding the synapse-associated protein 102 (SAP102)causes non-syndromic mental retardation. Neurogenetics, 11,251–255.

Zawia, N. and Basha, M. (2005). Environmental Risk Factors and the Developmental Basis for Alzheimer's Disease. *Reviews in the Neurosciences*, 16(4).

Zhang, W., Song, M., Cui, Y., Wang, H., Zhu, L., and Niu, Y. *et al. (*2008). Differential neuropsychopharmacological influences of naturally occurring tropane alkaloids anisodamine versus scopolamine. *Neuroscience Letters*, *443*(3), 241-245. doi: 10.1016/j.neulet.2008.07.048

Zhang, Y., Loh, H., and Law, P. (2016). Effect of Opioid on Adult Hippocampal Neurogenesis. *The Scientific World Journal*, *2016*, 1-7. doi: 10.1155/2016/2601264

Zhang, Z., Anderson, D., Lavine, L., and Mantel, N. (1990). Patterns of Acquiring Parkinsonism-Dementia Complex on Guam. *Archives of Neurology*, 47(9), pp.1019 - 1024.

Zhao, C., Deng, W., and Gage, F. (2008). Mechanisms and Functional Implications of Adult Neurogenesis. *Cell*, *132*(4), 645-660. doi: 10.1016/j.cell.2008.01.033.

Zhou, T., Taylor, M., De Vito, M. and Crofton, K. (2002). Developmental Exposure to Brominated Diphenyl Ethers Results in Thyroid Hormone Disruption. *Toxicological Sciences,* 66(1), pp.105-116.

Zoeller, R. and Crofton, K. (2005). Mode of Action: Developmental Thyroid Hormone Insufficiency—Neurological Abnormalities Resulting from Exposure to Propylthiouracil. *Critical Reviews in Toxicology,* 35(8-9), pp.771-781.

Zohn, I. E. (2012). Mouse as a model for multifactorial inheritance of neuraltube defects. Birth Defects Res C Embryo Today 96, 193–205.

Zohn, I. E., and Sarkar, A. A. (2008). Modelling neural tube defects in themouse. Curr Top Dev Biol 84, 1–35.

Zuo, X., Guo, W. and Lipschutz, J. (2009). The Exocyst Protein Sec10 Is Necessary for Primary Ciliogenesis and Cystogenesis In Vitro. *Molecular Biology of the Cell*, 20(10), pp.2522-2529.

APPENDIX – I:Ethical Approval

AHMADU BELLO UNIVERSITY, ZARIA
DIRECTORATE OF ACADEMIC PLANNING & MONITORING
Vice Chancellor *Prof. Ibrahim Garba, B.Sc. (Hons) Geology, M.Sc. (Mineral Exploration) A.B.U., Ph.D Geology (London), D.I.C., FNMGS*
Director: Prof. M.F. Ishiyaku, B.Sc. (Hons) Botany (ABU), M.Sc. Plant Breeding (Ibadan), Ph.D Agriculture (University of Reading, U.K.), MASN, MBSN
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Appl No.: ABUCAUC/2018/Human Anatomy/042
Approval No: ABUCAUC/2018/042

3rd October, 2018

Dr. S. A. Musa
Department of Human Anatomy,
Faculty of Basic Medical Sciences,
College of Health Sciences,
Ahmadu Bello University,
Zaria.

Sir,

APPROVAL OF RESEARCH STUDY 'EVALUATION OF INGESTION OF HYOSCYAMINE FRACTIONATE OF DATURA STRAMONIUM SEEDS ON CEREBRUM DEVELOPMENT IN WISTAR RATS *(RATTUS NORVEGICUS)*

This is to convey the approval of the ABUCAUC to you for the aforestated study domiciled in the Department of Human Anatomy. The approval is predicated on the assumption that you shall maintain and care for the Experimental Animals as approved after the visitation of the Committee.

Monitoring of the Research by spot checks, invitations or any other means the Committee deems fit shall be undertaken at the convenience of the Committee.

This approval can and shall be revoked should a significant breach in the terms and condition of the approval occur. It is hence your responsibility to ensure that the agreed terms are maintained to the end of the Study.

The said approval shall be posted on the ABUCAUC Page on the University's website.
Note upon completion of the research, ethical clearance certificate will be issued.

U.D. Abdullahi
For: Chairman, ABUCAUC.

Cc. Director, DAPM
Director, IC &ICT
Provost, College of Health Sciences
Dean, Faculty of Basic Medical Sciences,
HOD, Human Anatomy,
Prof. Aliyu Mohammed

APPENDIX – II:Physical Observation Register

		Control (PND 0 – 21)																			
SN	**Features**	**1**	**2**	**3**	**4***	**5**	**6[b]**	**7[Δ]**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**
1	**Pigmentation**																					
2	**Ear detachment**																					
3	**Hair growth**																					
4	**Eye-opening**																					
5	**Teeth eruption**																					
		***Inutero* (PND 0 – 21)**																				
SN	**Features**	**1**	**2**	**3**	**4***	**5**	**6[b]**	**7[Δ]**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**
1	**Pigmentation**																					
2	**Ear detachment**																					
3	**Hair growth**																					
4	**Eye-Opening**																					
5	**Teeth eruption**																					

***Surface righting reflex**
[b]Cliff Avoidance/Aversion
[Δ] Negative geotaxis

APPENDIX – III:Bodyweight MeasurementRegister

1. LACTATION

	Group	**Bodyweight (g) (PND 0 – 21)**																			
SN	**Control**	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1																						
2																						
SN	**In – *utero***	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1																						
2																						

2. ADOLESCENCE

	Group	**Bodyweight (g) Weeks during PND 22 – 60**				
SN	**Control**	4^{th}	5^{th}	6^{th}	7^{th}	8^{th}
1						
2						
SN	**In *utero***	4^{th}	5^{th}	6^{th}	7^{th}	8^{th}
1						
2						

3. ADULTHOOD

	Group	**Bodyweight (g) Weeks during PND 60 – 85Adulthood stage)**			
SN	Control	9^{th}	10^{th}	11^{h}	12^{th}
1					
2					
SN	In *utero*	9^{th}	10^{th}	10^{th}	10^{th}
1					
2					

APPENDIX – IV: Hyoscyamine fraction Administration Time Table

		Gestation (GD 15 – 20) Normal saline														
SN	**Control**										**15**	**16**	**17**	**18**	**19**	**20**
1																
2																
		Gestation (GD 15 – 20) 200 mg/kg														
SN	**Treated**										**15**	**16**	**17**	**18**	**19**	**20**
1																
2																

		Lactation (PND 22 –49) Normal saline																		
SN	**Control**														**15**	**16**	**17**	**18**	**19**	**20**	**21**
1																					
2																					
		(PND 22 – 49) 400 mg/kg																			
SN	**Treated**														**15**	**16**	**17**	**18**	**19**	**20**	**21**
1																					
2																					

	Group	**Adolescence (PND 22 – 49) 800 mg/kg**			
SN	**Control**	**4th**	**5th**	**6th**	**7th**
1					
2					
SN	**Treated**	**4th**	**5th**	**6th**	**7th**
1					
2					

	Group	**Adulthood (PND 56 – 84)**			
SN	**Control**	**9th**	**10th**	**11th**	**12th**
1					
2					
SN	**Treated**	**9th**	**10th**	**11th**	**12th**
1	200 mg/kg				
2	400 mg/kg				
3	800 mg/kg				

APPENDIX – V: Functional Battery Tests Register

1. Surface Righting Reflex

Day	SN	Time(s)
4	1	
	2	
	3	
	4	
	5	

2. Cliff Avoidance

		Trials Time taken to turn 180^0 (s)	
Day	**SN**	**1**	**2**
6	1		
	2		
	3		
	4		
	5		

3. Negative Geotaxis

		Time taken to turn 180^0 Away (s)		
Day	**SN**	**1^{st} Trial**	**2^{nd} Trial**	**3^{rd} Trial**
7	1			
	2			
	3			
	4			
	5			

APPENDIX –VI: Research Design

