EFFECTS OF PRENATAL ADMINISTRATION OF MONOSODIUM GLUTAMATE ON THE DEVELOPING CEREBRUM OF WISTAR RATS

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MUHAMMAD, Abdulhakeem Miko

AUGUST, 2015

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AUGUST, 2015.

DECLARATION

I hereby declare that the research work reported in this dissertation entitled "**Effects of Prenatal Administration of Monosodium glutamate on the Developing Cerebrum of Wistar rats.**" was carried by me in the Department of Human Anatomy, Faculty of Medicine, A.B.U, Zaria, under the supervision of Dr. James.O Nzalak and Dr. Aliyu Mohammed. The information derived from the literatures have been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree in any institution.

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Muhammad, Abdulhakeem Miko **Date**

CERTIFICATION

This dissertation entitled "**Effects of Prenatal Administration of Monosodium glutamate on the Developing Cerebrum of Wistar rats**" by **Muhammad Abdulhakeem Miko** meet the regulation governing the award of Master of Science (M.Sc.) degree in Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria, under the supervision of **Dr. James.O. Nzalak** and **Dr. Aliyu Mohammed.** It is therefore approved for its contribution to knowledge and literary presentation.

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DEDICATION

This research work is dedicated to Allah (S.W.A) for giving me the strength and health to carry out successfully the research reported in this thesis and also to my late Father, Alhaji Muhammad Miko, my mother Hajiya Amina Muhammad and my lovely wife Raleeya Yahya Zimit.

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LIST OF ABBREVIATION AND SYMBOLS

ANS	Autonomic Nervous System
ABC	Avidin Biotin Complex Method
BW	Brain Weight
CA	Cytoarchitecture
CC	Clumped Cells
CC	Cerebral Cortex
CC	Cornified Cell
CNS	Central Nervous System
CP	Cell Proliferation
CR	Caudal Rhombencephalon
CRL	Crown Rump Length
CRS	Chinese Restaurant Syndrome
CSD	Cortical Spreading Depression
CW	Cerebral Weight
DC	Degenerated Cells
DN	Disintegrated Nuclei
DPC	Degenerated Pyramidal Cell
DNE	Developmental Neurotoxic Effects
DV	Dorsal Ventricle
EAAR	Excitatory Amino Acid Receptors
EC	Estrous Cycle
ED	Embryonic Day
EEG	Electroencephalography
EGL	External Granular Layer
EPL	External Pyramidal Layer
FDA	Food Drug Administration
FW	Feotal Weight

GABA	Gamma Amino Butyric Acid
GD	Gestational Day
GDN	Glucose Oxidase- DAB- Nickel
GFAP	Glial Fibrillary Acidic Protein
GN	Granular Nuclei
GS	Generally Safe
H and E	Haematoxylin and Eosin
HW	Head Weight
IGL	Internal Granular Layer
IPL	Internal Pyramidal Layer
LA	Laboratory Animals
LC	Leukocyte Cell
LDA	Loss of Dendritic Arborization
LD50	Lethal Dose
LJ	Lamina Japonica
MDN	Mesencephalic Dopaminergic Nuclei
ML	Molecular Layer
ML	Multiform Layer
MSG	Monosodium Glutamate
MZ	Marginal Zone
NC	Nucleated Cell
ND	Nuclei Displacement
NL	Nuclei Loss
NM	Neuronal Migration
NMDA-R	N- methyl D-asparte Receptors
OGT	Oxidative Glutamate Toxicity
OS	Oxidative Stress
PC	Pyramidal Cells
PM	Precordal Mesoderm

PND	Postnatal Day
ROS	Reactive Oxygen Specie
SC	Stellate Cells
TS	Toxicological Studies
VC	Vacoulation Cell

ABSTRACT

Effects of a flavor enhancer, monosodium glutamate (MSG) was evaluated in female albino rats during the gestational period. Thirty two rats were divided into 4 equal groups (each having 8 rats), Group I, control; Group II, treated with 6 g/kg/day of MSG, Group III, treated group with 8 g/kg/day and Group IV received 10 g/kg/day of MSG. Different doses of MSG (6 g/kg, 8 g/kg and 10 g/kg body weight) were administered orally from 7 -15th day of gestational period on completion of the treatment period. The Dams were allowed to deliver their pups. Sixty four pups were used for histology and the remaining were used for motor activity. Morphometric results of the fetal weight ,crown rump length, head of the pups, brain weight, weight of the cerebral cortex, cerebral width (narrow area) and cerebral width (broad area) of the developing cerebral cortex showed that high dose group rats demonstrated significant reduction in all the parameters when compared to the control. MSG also induced some abnormal changes in gestation such as tincture in the stomach of group III animals, dead of the pups, macrocephaly as well as ulceration in the thoracic region among the group IV animals. Cerebral tissues were obtained and processed to prepare sections stained with H&E and toluidine blue. Histological examination of group II showed degenerative changes present on the stellate cells. However, group III showed pyramidal cells, stellate cells and degenerated pyramidal cells with disintegrated nuclei while group IV showed a lot of vacoulation and clumped cells with some degenerating cells. The glial fibrillary acidic protein (GFAP) was detected immunohistochemically in the developing cerebral cortex of the pups. The result from montoya staircase test showed reduced exploratory motor activity in rats exposed to high concentration of monosodium glutamate that was statistically significant in the second and third week. There was significant motor

exploratory activity impairment due to monosodium glutamate consumption even at low doses. The present study suggested that MSG has neurotoxic effect leading to degenerative changes in neurons and adversely affected the fetal development of the offsprings.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Food additives have been used to keep the quality, texture, consistency, taste, colour, alkalinity or acidity of foods. Humans are daily exposed to these chemical substances in their foods. Monosodium glutamate (MSG) is a food additive widely used as flavour enhancer of many foods like meats, poultry, seafood, snacks, soups and stews (Fuke and Shimizu, 1993). MSG is sodium salt of the amino acid glutamate and provides a flavouring function similar to naturally occurring free glutamate in foods (Yamaguchi and Ninomiya, 2000). Glutamate is a major component of most natural protein foods such as meat, fish, milk and some vegetables and plays an essential role in human metabolism (Filer and Stegink, 1994; Fernstorm and Garattini, 2000; Freeman, 2006). MSG is manufactured industrially by a fermentation process of molasses from sugar cane, sugar beets, starch and corn sugar. The breakdown and change of natural bound glutamate into various free forms of glutamate leads to the production of a white crystalline powder, when present in the free form, it has a flavour enhancing effect in food (Food Standard Australia New Zealand) (FSANZ, 2003). This distinctive taste is known as “Umami”, a word coined by the Japanese to describe the taste imparted by glutamate. Westerners often describe this flavour as savory, broth-like or meaty (Fuke and Shimizu, 1993). The optimal palatability concentration for MSG is between 0.2 and 0.8% with the highest palatable dose for humans being about 60 mg/kg body weight (Yang *et al*., 1997).

Since 1960s, MSG has received great opposition against its use as a flavour enhancer. There is a general belief that it has harmful health effects. The high consumption of MSG, has led to the description of a variety of discomforts described by some non-oriental people after eating at a Chinese restaurant as “Chinese restaurant syndrome” (example flushing, tightness of the chest or difficulty in breathing) after the consumption of Chinese foods (Morseli and Garattini, 1970). There are considerable reports about various adverse effects intake of MSG as food additive (Stevenson, 2000; Hermanussen *et al.,* 2006; Farombi and Onyema, 2006; Ortiz *et al*., 2006; Pavlovic and Cekic, 2006). However, there are claims that there has been suppression of information on the toxicity/safety of MSG (Samuels, 1999). Also, there has not been a general acceptance that MSG could be toxic to the humans. With all the controversies surrounding the safety of MSG, it is still being consumed in large quantity in fast and packaged foods.

Several studies assert that the average consumption of MSG in Chinese adults is upwards of 3.6 grams (Zhou *et al.,* 2003; He *et al*., 2008). However, other studies suggest between 1 and 5 grams (Shi *et al*., 2010). This makes MSG an important part of the human diet. MSG is a salt derivation of the amino acid glutamic acid, or glutamate, that is commonly found in Asian cuisine associated with Chinese restaurants and now frequently found in the Western diet (Shi *et al.,* 2010). Because MSG is both an additive and a chemical in natural foods, exposure to this substance is worldwide.

It exist in atypically high concentration in brain regions that are critical in the mediation of cognitive performance such as cerebral cortex, dentate gyrus of hippocampus and striatum (Park *et al*., 2000) indicating that the amino acid plays an important role in higher cognitive functions including memory which has been linked to activation of excitatory amino acid receptors. This stimulation leads to an enzymatic cascade of events ultimately resulting in cell death (Park *et al*., 2000).

Monosodium glutamate is one of the many food ingredients used by human in homes and food industries. The use of monosodium glutamate in food goes back to the oriental cooks of antiquity, who used seaweed called sea Tangle to make starch. The link between the seaweed (also Laminaria Japonica) is flavour important, and Glutamate, (First isolated in 1866) was discovered by Professor Kikunae Ikeda of the University of Tokyo in 1980. Monosodium glutamate is a fine white crystal, easy to dissolve and very similar to salt and sugar in appearance. It is called "Kafi Zabo in Hausa, Magi Funfun in Yoruba and Magi Ocha in Igbo all in Nigeria. It is widely used in food processing and cooking as a flavor enhancer in soups, sauces and spices blends- as well as in the wide variety of canned and frozen meats, poultry, vegetables and combination dishes (Martin, 2004).

Today MSG is produced in the United States from sugar beet molasses in a fermentation process similar to that used in making yeast. The production of MSG in United State had grown to about 40 million pound per year in 1966. It is now produced in about 15 countries throughout the world. The volume of MSG produced by these countries is about 200, 000 tons per year. It is found in abundance in plants and animal tissues. The human body typically has 4.4 pounds of naturally occurring glutamate in its system. High levels found in mushrooms, peas and tomatoes which help to suggest the effectiveness of their food flavours (Jordan, 2005)

In addition to the multiple symptoms mentioned above, there are five most common symptoms; heartburn, diarrhea, abdominal cramps, unsual thirst and nausea (Kwok, 1968). Since 1968 a reaction was identified after a Korean-American physician wrote a letter to the editor of the New England Journal of Medicine describing an unusual physiological reaction he often experience while dinning in a chinese restaurant which he called as " Chinese restaurant syndrome . In most cases the symptoms go away or are reversed by treatment (Cameron *et al.,* 1976). In rare cases, symptoms can be prolonged and may lead to death. It has been proven that asthma can be precipitated by MSG (Little, 2000). MSG was included among the list of carcinogens (WHO, 2004). Many research works have been done on MSG in relation to its effect on tissues of certain organs, or system such as effect of MSG on some gastrointestinal function (Martins, 2004). Park *et al.* (2000) observed that systemic administration of MSG could impair memory and damage hypothalamic neurons in the adult mice. Ali *et al*. (2000), studied the behavioural effects in rats treatment with sub-neurotoxic doses of MSG which they found that there was no significant change in any of the components of spontaneous locomotory activity, but after apomorphine challenge, a marked decreased in the distance travelled was observed. They concluded that exposure to MSG in early life in rats could lead to subtle behaviour aberrations in late adulthood (Ali *et al*., 2000)

1.2 STATEMENT OF THE RESEARCH PROBLEM

MSG is used widely in West Africa and many put 10-20 g/l in their food. The permissible amount of 5 g/l of foodstuff is mostly exceeded (Martins, 2004). There is limited knowledge about the toxic effects of MSG and particularly as regards to its effects when administered prenatally (Martins, 2004). Considering this fact there is need to evaluate the effect of prenatal exposure to MSG on the developing cerebrum.

There is introduction of many brands of MSG in Nigerian markets with many brand names such as Ajinomoto, vedan, kings, Sandoz, Kawasaki among others. More than 20 brands can be found nowadays in shops in Nigeria (Oska, 2005).

1.3 JUSTIFICATION OF THE STUDY

1. There is evidence that MSG has toxic effects on the nervous system of adult mice and rats but little is known of its prenatal effects despite the fact that it is consumed during pregnancy.
2. This study seeks to provide information on the prenatally administered effects of MSG on the developing cerebrum in rats.
3. The study outcome could serve as a basis on which further studies could be carried out.

1.4 AIM AND OBJECTIVES OF THE STUDY

1.4.1 Aim

The aim of the present study was to study the effect of prenatal administration of monosodium glutamate on the developing cerebrum of Wistar rat and possibly behavioural changes in the dams.

1.4.2 Objectives

The specific objectives of the study were:

1. To determine the morphometrical changes due to MSG administration in the cerebrum of the offsprings.
2. To determine the histological changes due to MSG administration in the neurons and glial cells in the cerebrum of the offsprings of adult Wistar rats using Haematoxylin and Eosin (H and E) and Toluidine blue
3. To determine the effect of MSG on calcium binding neurons in the cerebral b cortex using anti-calbindin D28K immunohistochemically.
4. To evaluate the effect of MSG administration on the motor activity of the Wistar rat using Montoyastaircase test.

1.5 RESEARCH HYPOTHESIS

Prenatal exposure of Wistar rats during pregnancy to monosodium glutamate results in toxic changes in the developing cerebrum and behavioural changes in the offsprings.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 CHEMISTRYOF MONOSODIUM GLUTAMATE (MSG)

1-IUPAC Names: 2-aminopentanedioc acid

2- aminoglutamic acid

1-amino propane 1, 3-dicaboxylix acid

Monosodium glutamate or Vetsin

It is White soluble crystal

Its chemical formula is C5 H8NNaO4

MSG is the sodium salt of the amino acid and glutamic acid. Its chemical formula is C5 H8NNaO4 (Jordan, 2005). It is presented as fine white crystal substance, similar in appearance with sugar or salts. MSG stimulates some specific receptors in the tongue to Umami taste (Olney, 1989).

Umami is one of the fine basic tastes; it's a Burwell word from Japanese. In 1959, MSG was classified as a generally recognized safe substance. In 1986, report by the European committee and scientific for food reaffirmed MSG safety and classified as acceptable daily intake. Studies showed that glutamates in the baby play an important role in normal functioning of the nervous system (Olney, 1989).

During 1980s, questions arose on the role of glutamate in food plays in this function whether or not glutamate in food contributes to certain neurological disorders. It was from then researches were developed and confirmed some neurological disorders at certain high dosage.

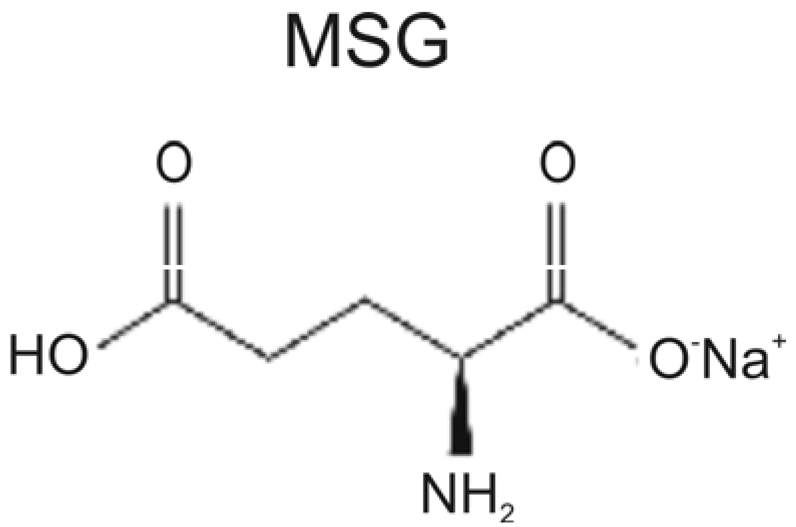

Figure 2.1. *Chemical structure of Monosodium glutamate (MSG) (Jordan, 2005).*

An unknown percentage of the population may react to MSG and develop MSG symptom complex, a condition characterized by one or more of the following symptoms, it is important to identify the areas of the brain and other biological aspects that play a role in MSG consumption. Many studies will suggest that the scarring of the arcuate nucleus of the hypothalamus indirectly contributes to the increase in adiposity and body mass measured in rats and humans, as well as facilitating leptin resistance and the level of food consumption (Pepino *et al.,* 2005; Sasaki *et al.,* 2009). The arcuate nucleus contains neuron bundles that are associated with neuroendocrine functioning, specifically human growth hormones, as well as subject to the effects of leptin and insulin inhibition that is known to affect appetite and the amount of food consumption (Matyskova *et al*., 2007).

Leptin is a protein hormone involved in the regulation of appetite and metabolism.

Leptin is produced mainly by white adipose tissue and its levels are related to the amount of adipose tissue and affects both food consumption and hunger.

Adiposity refers to the amount of fat found in adipose tissue (Matyskova *et al*., 2007).

Asthma is a disease rooted in the inflammation of the lungs that result in obtruction of the air passages as well as bronchospasms of the lungs. Asthma attacks are characterized by wheezing, coughing, and chest tightness (Schwartzstein *et al*., 1987). The hippocampus is a region of the brain and part of the limbic system associated with

memory (Collison *et al*., 2010). Research has concluded that the hippocampus plays an important role in memory, specifically spatial memory (Frieder and Grimm, 1984).

The neurotoxic effect of MSG could be mediated by an oxidative stress process. According to Schubert and Piasecki, (2001), Loo *et al.* (2003) and Shih *et al.* (2006), studies who observed that high levels of extracellular glutamate resulted in the depletion of glutathione and acute concentration dependent efflux of ascorbate from the cells (major cellular antioxidants) leading to a form of cell injury called oxidative glutamate toxicity. Tojo *et al*. (2002) and Audebert *et al*. (2002) reported that this antioxidant deficiency is associated with free radicals and reactive oxygen species accumulation leading to oxidative stress with oxidative damage to mitochondrial DNA which has an important pathogenic role in organ damage. The central nervous system is an important target organ for the actions of MSG, particularly during brain development. Brain maturation in the rat occurs mainly during the lactation period, a stage in which synaptogenesis is intense, corresponding to the synaptogenic period in the human brain, approximately the first year of life (Dobbing, 1968; Morgane *et al.,* 1978).

Animals used in MSG-induced obesity have shown decreased efficiency in tasks testing spatial learning and memory. Research on the effects of MSG on memory and learning is primarily limited to animal studies involving rats, and much of this research focuses on the effects of MSG on spatial learning and memory as a result of the effects of MSG on the hippocampus and its neuronal processing (Bush, 2003). MSG is correlated to a decreased proficiency in spatial learning and memory tasks in rats, although the underlying mechanism causing this deficiency presents several possibilities (Aporn and Virapan, 1984). The glutamatergic system has been shown to be involved in the regulation of brain excitability in both humans (Stagg *et al*., 2011) and laboratory animals (El- Hasser *et al*., 2011). Interestingly, (Lopez- Perez *et al*., 2010) recently demonstrated that MSG-treated rat pups have altered Electroencephalography (EEG) and behavioral reactions, suggesting seizure initiation.

On the other hand, Kaufhold *et al*., (2002), were able to prevent the adverse effects of neonatal MSG treatment with concurrent administration of a selective and highly potent noncompetitive N-methyl-D- aspartate receptors (NMDA-R) antagonist, dizocilpine maleate. In general, MSG has been demonstrated to modulate brain excitability (Lopez-Perez *et al.,* 2010), a process that could influence phenomena such as seizures and Cortical spreading depression (CSD (Guedes and Cavalherio, 1997). Effects on neurotoxicity via the p38 pathway (Torres *et al.,* 2006) or increasing brain glutamate levels after MSG treatment (Lopez-Perez *et al.,* 2010) have been discussed but still require corroboration based on robust experimental evidence.

2.2. MSG IN FOOD

MSG is a salt derivation of the amino acid, glutamic acid, or glutamate, that is commonly found in Asian cuisine associated with Chinese restaurants and now frequently found in the Western diet (Shi *et al*., 2010). Because MSG is both an additive

and a chemical in natural foods, exposure to this substance is worldwide. MSG is commonly found in high fat foods associated with unhealthy eating (Collison *et al.,* 2010). In the last several decades, the public's negative opinion of MSG has led to identification of the presence or absence of MSG by food processor. (Young and Ajami, 2000).

Glutamate is an amino acid that is vital in metabolism specifically involved in the breakdown of protein (Matyskova *et al*., 2007). Glutamate is the most abundant amino acid present in nature (Young and Ajami, 2000). It is part of the intermediary in the metabolism of most living organisms and is found in large amounts in tissues and organs (Ninomiya, 1998). It has been estimated that a 70Kg man has a daily intake of ~28 g of glutamic acid, both derived from his diet and from the breakdown of gut proteins (Garattini, 2000). Glutamate is present in nature both in its free form and bound to peptides and proteins. The free form is responsible for the flavour enhancing properties of MSG, while the bound form does not have any effect on taste (Ninomiya, 1998).

2.2.1 Stability and chemical properties

MSG is sold as a white crystalline material, readily soluble in water. The product is quite stable at room temperature. During processing, MSG remains stable; however, as any other amino acid with nitrogen content, MSG can undergo Maillard reaction. The ionic form of MSG is dependent upon pH, its action being most effective at pH range of 5.5 to 8.0. The only degradation product of MSG identified to date is pyroglutamic acid, which is extremely stable at pH 2.5 to 11.0 (Yamaguchi and Ninomiya, 1998).

2.2.2 Metabolism of MSG

The mechanism of absorption and metabolism of both free and bound glutamate is well understood. Glutamate plays an important role in nutrition and energy metabolism.

Glutamate metabolism involves oxidative deamination, transamination, decarboxylation and amination. In a recent review (Young and Ajami, 2000), the metabolism of MSG was concluded to be “highly compartmented”. This allows the molecule to participate in “a number of distinct and possibly competitive roles, including that of a nutrient, energy-yielding substrate, structural determinant, enzyme regulator and excitatory molecule” (Young and Ajami, 2000). In the central nervous system (CNS), glutamate acts as a precursor of the inhibitory neurotransmitter, gamma-aminobutyric acid (GABA), and it is also the major excitatory neurotransmitter of the CNS. In this context, the metabolism of glutamate is intrinsically related to the availability of vitamin B. As a neurotransmitter, MSG has been a source of controversy for the last fifty years. Neurotoxicity trials in newborn rats and mice have shown permanent lesions in their hypothalamus (De Vries, 1996). This phenomenon has not been observed in higher primates and it is presumably not applicable to humans (Garattini, 2000).

In some animal species, high levels of MSG were known to cause irreversible cell injury such as “necrotizing brain damage in the hypothalamus” (FASEB, 1995) and other disorders such as convulsions, abnormal lipid metabolism and obesity. It is important to note that most of these animals' studies were parenteral (that is; injection rather than consumption) and that MSG was provided at very high levels within a very short period of time (FASEB, 1995).

2.3 DEVELOPMENT OF THE CENTRAL NERVOUS SYSTEM

2.3.1 Pre-differentiation stage

The pre-diffferentiation stage is characterized by germ cells with no distinct morphological differentiation (Suzuki, 1980). For rats, this stage is (the all or nothing period) covers the time from conception to post conceptional day 5. Damage during this period normally has two consequences:

(i) All the cells are killed and no embryo is formed (severe damage) or

(ii) There are enough surviving cells to compensate for the assault and a normal embryo is formed (minimal damage) (Suzuki, 1980)

2.3.2 Embryonic stage

This stage is characterized by the mobilization and organization of cell and tissues groups to form individual organ systems (Suzuki, 1980). The periods of organogenesis are different from one mammalian species to another. In rats, an organogenesis proceeds from gestational day (GD) 6-15, in humans it proceeds from GD20-55, in monkeys it is from GD20-45 and in mice from GD7-16 (Slikker, 1994; Schardein, 2000).

2.3.3. Development of the neural tube

Nerve and glial cells of the nervous system are derived from a specialize region of the ectoderm called the neural plate (Martin, 1985). The excitation and differentiation of the cells of the ectoderm is induced by the notochord and para-axial mesoderm (Moore and Persaud, 1998).The induction is due to an embryonic protein called noggin, which has been identified as a neural inducing agent in amphibians and rodent embryos (Lamb *et al.,* 1993).

The neural plate indents and forms groove from which the lateral lips close to create the neural tube. In rats, the neural tube is formed around gestational day 11 (Martin, 1985). Damage during this early period result in either anencephaly (closure of the neural tube at the rostral level) or spina bifida (caudal portion of the neural tube fails to close).

2.3.4 Start of neurogenesis

The epithelial cells lining the neural tube are called neuroepithelium. The neuroepithelium is the source of all neurons and glial cells of the central nervous system. The nerve cells of the peripheral nervous system (dorsal nerve root ganglion cells and post-ganglionic neurons of the autonomic nervous system) originate from the neural crest cells, a population of cells whose cell bodies lie outside the neural tube (Bayer and Altman, 1995).

The precursors of nerve cells, the neuroblast, divide repeatedly but do not proliferate uniformly along the length of the neural tube. The neuroepithelium appears to be spatiotemporal mosaic where the neuroblasts seem to be committed to generate specific neuron populations according to strict time tables (Moore and Persaud, 1998).

In a strictly time dependent sequence, programmed cell proliferation occurs at specific sites in the neuroepithelium which give rise to the occurrence of three primary brain vesicles called the rostral prosencephalon (primitive forebrain), the mesencephalon (primitive midbrain) and the caudal rhomcephalon (primitive hindbrain). This is known as the three-vesicle stage (Bayer and Altman, 1995; Moore and Persaud, 1998; Singh and Ahluwalia 2003). More programmed cell proliferation leads to an enormous expansion of the primary brain vesicles with further sub-division.

2.3.5 Feotal stage

This stage is characterized by a significant increase in brain size and by enhanced cellular proliferation and differentiation. The prosencephalon divides into (from rostrally to caudally) the telencephalon with the cerebral cortex and basal ganglia, and the diencephalon with the thalamus and hypothalamus. The mesencephalon remains undivided, with the tectum and tegmentum (Bayer and Altman, 1995; Singh and Ahluwalia, 2003). The rhomcephalon divides into the metencephalon with the cerebellum and pons and the myelencephalon with the medulla oblongata. This is known as the five-vesicle stage (Bayer and Altman, 1995; Singh and Ahluwalia, 2003). In the caudal part of the neutral tube programmed cell proliferation form the spinal cord, which contains sensory and motor neurons and many interneurons (Bayer and Altman, 1995; Moore and Persaud, 1998; Singh and Ahluwalia, 2003).

2.3.6 Pre and postnatal stage

This stage is characterized by extensive growth of the brain. In rats, neuronal cell populations are produced up to postnatal day 21, in the rat cerebellum; around half of all the cell population is produced in the postnatal period (Krinkle and Eisenbrandt, 1994). According to Bayer and Altman (1995), central nervous cellular proliferation and

differentiation continues after birth in many species**.**

2.3.7 Impact of neuroteratogen during neurogenesis

According to Bayer and Altman (1995), Bayer *et al*. (1993), loss of precursor neurons due to a neuroteratogen assault will not be compensated for. The surviving precursor neurons produce only the programmed number of neurons during a programmed time window. Should a massive injury causing an entire loss of a certain neuronal population would have further consequences on cell migration, settlement and differentiation of neuronal cell population which have been produced or will be produced after the injury (Bayer *et al.,* 1993; Bayer and Altman, 1995).

Loss of neurons reduced the overall volume of the neuronal population but does not change the density of the structure; this means that cell numbers are primarily observed as change in the size or structures (Rodier and Gramann, 1979; Rodier, 1998). Halls and Das (1978) administered a neuroteratogen agent in rats at gestational day 14 and 15 observed a thinness of the cerebral cortex and a reduction of cortical neurons to about 41% of the controls. Despite, the thinness of the cortex, the six cortical neuronal layers were well stained. This observation is in corcodance with the observations in human infants with mental retardation, where the brain may be small but with no abnormal cytoarchitecture patterns (Suzuki, 1980). Size reduction without an obvious histomorphological recognizable change in the normal cytoarchitecture made Rodier and Gramann (1979) to suggest the use of 'simple morphometry' as a viable tool for evaluating cell loss in the developing brain. As evidenced from the paragraphs above, neurons in the central nervous system are generated according to precise time schedule (Bayer and Altman, 1995; Moore and Persaud, 1998; Singh and Ahluwalia 2003). Bayer and Altman (1995) also postulated that a chronology of neuron production is a pre-requisite for the proper anatomical and functional development of the central nervous system. Therefore, it is important to bear in mind that cell production in the developing central nervous system occurs at specific times in specific regions. When testing for developmental neurotoxic effects it is required that adequate tissues are examined for the exposure time selected.

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2.3.8. Impact during migration and settling of neurons

Once a presumptive neuron turns off cell division in its germinal matrix, it migrates to its predeterrmined location in the brain parenchyma and settles (Rakic, 1971). In the cerebellum, neuroblasts of the neuroepithelium migrate to form a second germinal layer; the external granular layer produces billions of granular cells and interneurons of the cerebellar cortex. The cerebellar cortex is a good site to demonstrate one important pattern of neuronal migration. Neuronal migration is characterized by an astrocyte guided translocation of nerve cells from their primary or secondary germinal layers to their adult locations (Rakic, 1971; Sieviers *et al*., 1994). The physical contact between glial cell migrating neurons is mediated by adhension molecules, which thus facilitate migration of neurons. These cell-cell contacts has been considered as a generally accepted site for neurotoxicant action (Lopachin and Aschner, 1993; Reuhl, 1994).

Bayer *et al*. (1993) assumed that the granular cell ectopias in the cerebellar cortex after x-irradiation were caused by the effect of the neuroteratogen during migration, killing many precursor cells in the external layer. This resulted in a break in the normal steady stream of new granular cells settling in their proper locations. Once an ectopia has been produced by abnormal migration, it becomes a permanent anatomical feature of the brain (Bayer *et al.,* 1993). However, small ectopias are less reliable as a morphological marker for early injury during brain development as there are also reports of a spontaneous occurrence or a disappearance of ectopic cell over time in the brain (Schmel and Rakic, 1979).

2.3.9 Impact on the development of neurotransmitter systems

During neonatal brain growth spurt, a rapid development of all major neurotransmitter systems is observed, especially those that are particularly implicated in the fast transmission of information in the neural networks (Institute of Environment and Health) (IEH, 1996). The process of noradrenergic synaptogenesis is observed as early as gestational day (GD) 14 in rats and reaches adult values in density and distribution at post-gestational day (PD) 14 in the neocortex. Mesencephalic dopaminergic nuclei are shown to give rise to fiber projections GD 14 in the diencephalons, achieve adult topology at PD 12 (neocortex0 and adult densities at PD 35. Sprouting of serotoninergic raphe nuclei axons begins at GD 15 in the pons, and adult levels of terminal field densities are noted at PD 21 in the cortex, hippocampus, septal regions and brainstems (Broening and Silkker, 1998). Another neurotransmitter system that is important in developmental neurotoxicity is the cholinergic system. Its major role in neurotransmission is less direct and more a modulatory one, influencing the excitatory and inhibitory actions of other neurotransmitters (IEH, 1996; Jett, 1998). Such damage may have serious consequences.

2.3.10 The fundamental embryonic layers of cerebral cortex

During embryogenesis, as proliferation in the neuroepithelium thickens the cortical wall, a system of radial glial fibers appears across the radial plane. Postmitotic neurons migrate away from the inner surface of the neural tube along the trajectory set forth by the radial glial fiber system (Rakic 1972). As the first neuronal populations migrate away from the ventricular zone (VZ), a zone of axons appears between the germinative zone and the mantle of postmitotic cells.

This intermediate zone (IZ) consists of pioneer axons laid down by the emigration of the first wave of neurons to become postmitotic away from the ventricular zone (VZ).

Recent studies by Easter and colleagues (1993) demonstrate axon extension at about embryonic day 8.5 as the neuropore is closing, with early axon tracts in place by embryonic day10, the time when sequential neurogenesis and migration begins to set forth the laminar structure of cortical region. At embryonic day 10–12 in mice, the overlying marginal zone consists of Cajal-Retzius cells, perhaps the first generated neuron of cortical regions of brain, neurons that will form the deep layer and subplate neurons (Luskin and Shatz 1985; Shatz *et al.,* 1988).

Within the forebrain, two specific patterns of transcription factor expression set forth the territory—*Otx* and *BF1*. By E8, expression of *Otx* and *BF1* is restricted to the emerging forebrain (Rubenstein *et al*., 1998). Between embryonic days 8– 9.5, the dorsal ventricle (DV) axis is established by expression of bone morphogenic proteins along the dorsal ridges and of Shh in the precordal mesoderm.

Thereafter, territories of transcription factor expression appear (Rubenstein *et al* 1994, Rubenstein and Shimamura 1997). Remarkably, the same transcriptional regulation is often used across the evolutionary scale, from *Caenorhabditis elegans* to mammals.

Neuronal migration occurs after regionalization sets forth a plan for cell fate specification, as a means to generate the cellular architecture specific to a particular brain region.

2.4. ANATOMY OF THE CEREBRUM

2.4.1 The Morphology of the Cerebrum

The cerebrum comprises a large portion of the brain. It lies in front or on top of the brainstem and in humans is the largest and best-developed of the five major divisions of the brain. The cerebral cortex is the largest part of the brain; it is formed by grey matter and cerebral hemisphere on the surface as shown in Figure 2.2. The cortex is convoluted with projections and depression called sulci and gyri. The cerebral cortex is about 1.5 - 4mm thick, and contains nerve cells, fibers, neuroglia and blood vessels. The cerebral hemisphere is supplied by cerebral branches of polygon of Willis that is the anterior, middle and posterior cerebral arteries (Kandel *et al.,* 2000).

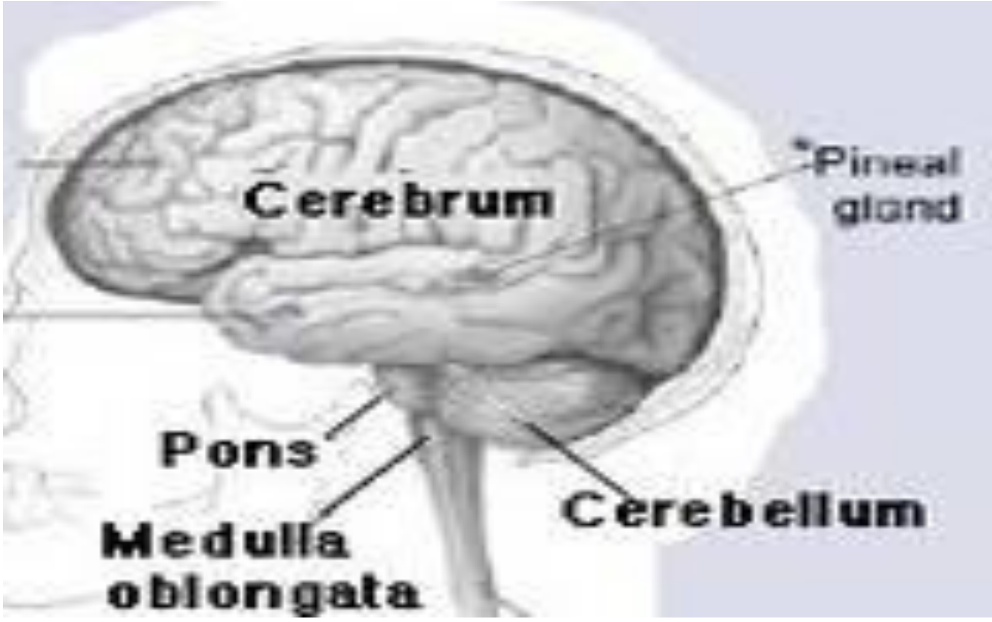

Figure 2.2 The structure of the brain showing the cerebrum (Kandel *et al.,* 2000)

2.4.2 Cellular Layers of the Cerebral Cortex

The neurons of the cerebral cortex vary in size, shapes, lengths, branching patterns and orientation of their processes. These neuronal cells are named based on their specific characteristic feature which demarcates them from each other. The neurons include the following:

The pyramidal cells: They are most abundant cervical neurons in the cortex. All others are referred to as non-pyramidal neurons. About 2/3 (two third) of the cerebral cortex cells are pyramidal cells. Their cell bodies are triangular in shape; apex is directed towards the cortex. Large dendrites arise from the apex while others arise from basal angles. The axis arises from the base of the pyramidal cells. (Kandel *et al.,* 2000). The process of pyramidal cells extends vertically through the entire thickness of the cortex and establish numerous synapses. The axons of pyramidal cells may terminate in different ways. They may travel to the other region like the basal ganglia, the brain stem or the spinal cord. Also they may give collateral fibers that terminate within the cortex and may be short and terminate within the same area in the cortex (Kandel *et al.,* 2000).

The stellate cells: They are small and multipolar and form 1/3 (one third) of the total population of neurons of the cortex under low magnification. They look like granular cells. They are therefore been termed granular neurons (cells) by earlier workers. stellates cell are of various types depending on their bodies and on the pattern of their ramification processes. Their axons are short and end within the cortex and their processes extend chiefly in radical direction within the cortex in some case horizontally. Some stellate cells may be uniform rather than stellates, with one process arising at either end. stellates neurons are classified as spiny and non-spiny neuron. The spiny stellates cells use glutamate as neurotransmitter, while in most non-spiny stellates cells it is gamma amino butyric acid (GABA).The neurons of the cerebral cortex are grouped into six main layers, from outside to inside namely as shown in Figure 2.3, according to Kandel *et al*. (2000), Shipp (2007) and Saladin (2010).

Layer I: the Molecular layer contains few scattered neurons and consists mainly of extensions of apical dendritic tufts of pyramidal neurons and horizontally-oriented axons, as well as glial cells. Some Cajal-Retzius and spiny stellate cells can be found in the molecular layer. Inputs to the apical tufts are thought to be crucial for the ‘‘feedback’’ interactions in the cerebral cortex involved in associative learning and attention (Saladin, 2010). While it was once thought that the input of layer I came from the cortex itself, it is now realized that layer I across the cerebral cortex mantle receives substantial input from ‘‘matrix’’ or M-type thalamus cells in contrast to ‘‘core’’ or C-type that go to layer IV.

Layer II, the external granular layer contains small pyramidal neurons and numerous stellate neurons.

Layer III, the external pyramidal layer contains predominantly small and medium-size pyramidal neurons, as well as non-pyramidal neurons with vertically-oriented intracortical axons. Layers I through III are the main target of interhemispheric corticocortical afferents, and layer III is the principal source of corticocortical efferents (Shipp, 2007).

Layer IV, the internal granular layer contains different types of stellate and pyramidal neurons, and is the main target of thalamocortical afferents from thalamus type C neurons as well as intra-hemispheric corticocortical afferents (Shipp, 2007).

Layer V, the internal pyramidal layer contains large pyramidal neurons such as the Betz cells in the primary motor cortex. It is the principal source of subcortical efferents, as such there are large pyramidal cells which give rise to axons leaving the cortex and running down through the basal ganglia, the brain stem and the spinal cord.

Layer VI, the polymorphic or multiform layer contains few large pyramidal neurons and many small spindle-like pyramidal and multiform neurons. Layer VI sends efferent fibers to the thalamus, establishing a very precise reciprocal interconnection between the cortex and the thalamus. These connections are both excitatory and inhibitory. Neurons send excitatory fibers to neurons in the thalamus and also from collateral to other ones via the thalamic reticular nucleus that inhibit these thalamus neurons or ones adjacent to them. Since the inhibitory output is reduced by cholinergic input to the cerebral cortex, this provides the brainstem with adjustable "gain control for the relay of lemnsical inputs (Shipp, 2007).

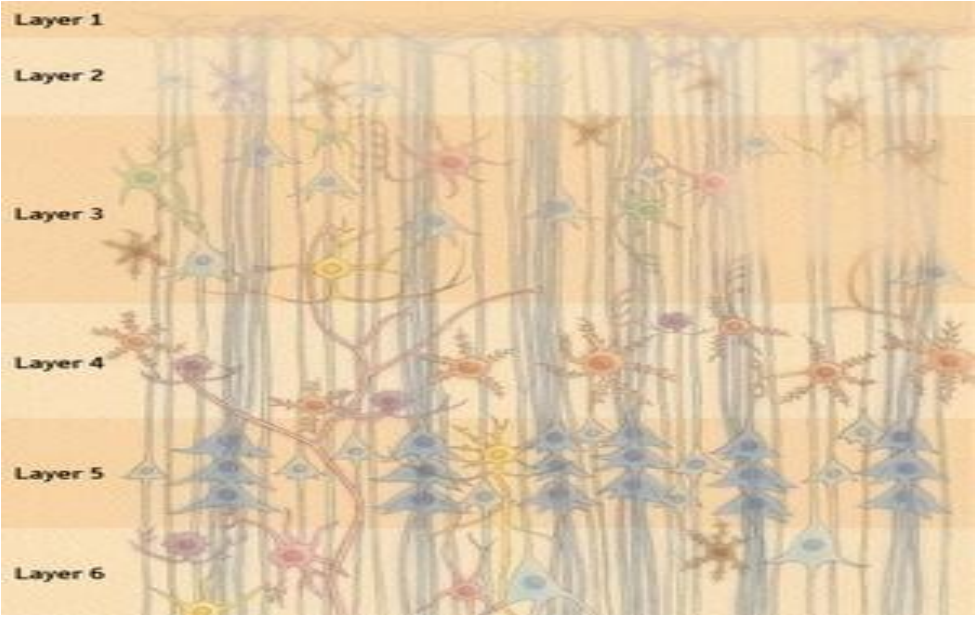

Figure 2.3 The cellular layers of cerebral cortex (kandel *et al.*, 2000).

Variations of cortical structure

The structure of the cerebral cortex shows considerable variations in the region, both in terms of thickness and in prominence of the various laminae. These variations are as follow

I. In the agranular cortex, the external and internal granular laminae are inconspicuous. This type of cortex is refered to as precentral gyrus (area 4) and is therefore to be of typical area.

II. In the granular cortex, the granular cortex is highly developed, while the pyramidal and ganglion layer are poorly developed or absent. This type of cortex is mostly present in sensory area including the post-central gyrus.

2.4.3 Functions Associated With Cerebrum

The cerebrum is the most highly developed part of the human brain and is responsible for determining intelligence**,** motor function, planning and organization**, t**ouch sensation,thinking, perceiving, producing and understanding language. Most information processing occurs in the cerebral cortex. The cerebral cortex is divided into 4 lobes of which each has a specific function as follows (Kandel *et al.,* 2000).

Frontal lobe: is located at the front of the brain and is associated with reasoning motor skills, higher level cognition and expressive language.

Parietal lobe: is located in the middle section of the brain and is associated with processing tactile sensory information such as pressure, touch and pain.

Temporal lobe: is located on the bottom section of the brain. This lobe is also the location of the primary auditory cortex, which is important for interpreting sounds and the language.

Occipital lobe: is located at the posterior of the brain and is associated with interpreting visual stimuli and information.

Motor Function: The cerebrum directs the conscious or volitional motor functions of the body. These functions originate within the primary motor cortex and other frontal lobe motor areas where actions are planned. Upper motor neurons in the primary motor cortex send their axons to the brainstem and spinal cord to synapse on the lower motor neurons, which innervate the muscles. Damage to motor areas of cortex can lead to certain types of motor neuronal diseases (Shipp, 2007). This kind of damage results in loss of muscular power and precision rather than total paralysis. The motor area of classical description is located in the precentral gyrus on the superior lateral surface of the hemisphere and in the anterior part of the precentral lobule; the medial surface. This corresponds to area 4 of Broman and possibly part of area 6, which lies in the pre-central gyrus, the specific region in the area responsible for movement of specific part of the body (Shipp, 2007).

Sensory processing: The primary sensory areas of the cerebral cortex receive and process visual, auditory, somato-sensory, gustatory and olfactory information. Together with association cortical areas, these brain regions synthesize sensory information into our perceptions of the world around us.

Olfaction: The olfactory bulb in most vertebrates is the most anterior portion of the cerebrum, and makes up a relatively large proportion of the telencephalon. However, in humans, this part of the brain is much smaller, and lies underneath the frontal lobe. The olfactory sensory system is unique in the sense that neurons in the olfactory bulb send their axons directly to the olfactory cortex, rather than to the thalamus first. Damage to the olfactory bulb results in the loss of the sense of smell (Kandel *et al*., 2000).

2.5 Estrous Cycle of the Rat

Female rat show a 4-day estrous cycle (Freeman, 1988). A cycle consists of proestrus, eostrus, metesteous (diestous 1) and dioestrus (diestrous 2) phases (Freeman, 1988; Ebling *et al.,* 1978).

Vaginal smears are widely used to identify these phases of the estrus cycle (Ebling *et al.,* 1978). Observation of the cell population in the vaginal smear is the most popular and reliable for identifying the different phases (Freeman, 1988). The populations of the different cell types are controlled by circulating oestrogen, as oestrogen causes a proliferation of vaginal epithelial cells in the proestrous phase and lack of this steroid results in a reduction in their numbers (Freeman, 1988). For mating purposes, a proestrous rat should be transferred to the cage of a male rat and left overnight and the vaginal smear checked the following morning to ensure that successful copulation has occurred (Freeman, 1988). According to Ebling *et al*. (1978) and Freeman (1988), ovulation in the female rat begins at about midnight and is indicative of the receptivity period of the female rat to the male rat.

Nucleated cells are the major population in the smear at the proestrous phase. In the early oestrus, the vaginal smear is full of markedly swollen cornified cells lacking nuclei, whereas at diestrous 1 and diestrous 2, numerous leukocytes appear in addition to the nucleated cells and shrinking cornified cells (Freeman, 1988; Ebling *et al.,* 1978).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

Wistar rats, Monosodium glutamate, Bouin's fluid, Beakers, Specimen bottles, Weighing balance, Nikon digital camera with 12.0 megapixels, 1ml and 5ml needles and syringes and, Dissecting tray. Animal feed, Chloroform and dissecting kit.

3.2 Experimental designed

Thirty two (32) adult Wistar rats of both sexes were obtained from the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The rats were weighed and their weights were between 190 – 230g. The rats were housed in polyester cages with wire gauze covering. The animals were allowed to acclimatize for two (2) weeks in the animal house of the Department of Human Anatomy, Ahmadu Bello University, Zaria. The animals were fed with Grower's mesh brand of the animal feed. The animal feed was prepared in pellet form to reduce spillages and the animals were fed three (3) times daily while clean water was provided in plastic drinking bottle and animals were allowed to feed and drink *ad-bilitum.*

Animals were weighed and randomly divided into four (4) groups with eight animals per group and the administration of the monosodium glutamate lasted for nine (9) consecutive days. This was done orally using 1ml syringe with butterfly rubber that was put on the cap of the syringe to serve as mean of administration.

The LD_{50} of Monosodium glutamate was adopted from Aporn and Virapan (1984) and Bush (2003) as 16,600 g/kg body weight.

3.3 EXPERIMENTAL PROCEDURE

A total of 32 animals, 20 females and 12 males were paired for mating. After 7 days of coupling, 16 female pregnant rats were obtained and taken as the experimental animals. The experimental animals were divided into 4 groups of eight (8) animals per group. Five (5) females to 3 males per group. GI, GII, GIII and GIV respectively. Group I (GI) was used as the control and as such received normal saline, while groups II, III and IV were administered with monosodium glutamate as the treated group.

MSG dissolved in distilled water at dosage of 6 g/10mL/kg body weight was given orally using a syringe to the animals of low dose MSG treated groups (Group II).The medium dose MSG treated groups (Group III) on the other hand received MSG at a dose of 8 g/10mL/kg body weight and the high dose MSG treated groups (Group IV) received 10 g/ 10ML/kg body weight.

The doses were selected after finding out the acute LD_{50} value which was found to be 16,60g/kg (oral, rat) (Bush, 2003). The animals of control (Group I) received normal saline as *ad libitum*. The treatment was given from 7^{th} to 15 th day of gestational period. On the completion of the respective experimental period, all of the pregnant animals were allowed to complete their term and deliver their pups. The pups were sacrificed at days 1, 7, 14 and 21 of gestation humanely by removing the head of the pups and fixed in a bouins fliud (as seen in Table 3.1)

Table 3.1 Animal grouping, treatment given and frequency of administration of monosodium glutamate

S/no	Groups	Concentration	Frequency of administration
1	I	Normal saline	Once daily
2	II	6 g/kg	Once daily
3	III	8 g/kg	Once daily
4	IV	10 g/kg	Once daily

NB: The administration was given nine days from 7 th -15^{th} of gestation (equivalent to prior development of the cerebrum)

3.4 DETERMINATION OF THE OESTROUS CYCLE

Collection of the vaginal smears from the 20 female rats was done using small plastic pipette. Small amount of water was put into the pipette and then inserted into the rat vagina which was gently rubbed against the posterior wall of the vagina to remove some of its contents. The cells are sucked up from the vagina, while a suspended cell was put into a clean grease free microscope slide. Assessment of the population was done with the aid of a celestrone digital light microscope and photomicrographs were taken.

3.4.1 Mating of rats and confirmation of pregnancy

Rats were in the proestrus phase were mated with a male rat in a ratio of 2 females to 1 male (Flagel *et al*., 2002). Positive mating was confirmed by examination of the vaginal plug early in the morning of the day after mating. A clean plastic pipette and normal saline was used, the vaginal lumen was flushed and content aspirated. It was then immediately transferred to clean glass slide and examined under celestrone digital light microscope. The presence of spermatozoa or a brownish plug obtained the following morning confirmed coitus and the sperm positive day was designated as day zero of gestation.

Pregnancy in the rats was confirmed by examining the vaginal cytology from each after 2 days using plastic pipette and distilled water which was pusched at the posterior wall of the vagina cycle implied that the rats regular 4 day cycle has been altered as a result of pregnancy.

3.5 ANIMAL SACRIFICE

At the end of the 9th days of monosodium glutamate administration, the pups were anasthesize humanely using chloroform and the brain tissues were removed by opening through the sutures of the skull using a dissecting kit. The brain tissues were then transferred into specimen bottle, containing Bouin's fluid.

3.6 MORPHOMETRIC STUDIES

The crown-rump length (CRL), the length and the width of the cerebrum of the pups were taken and the average for each group was recorded using ruler and vernier caliper. The crown-rump length was measured as the distance between the peak of the skull and the tip of the tail. The feotal weight of the pups, brain, head and the cerebrum were measured with electronic digital weighing machine (VIC 303).

3.7 Tissue Processing

The tissues were fixed in Bouin's fluid for 48 hrs for proper fixation. The tissues were processed routinely and stained using Heamatoxyline and Eosin technique and special toludiene blue method for histochemical observation and immunohistochemical method was carried out using Avidin biotin method.

3.7.1 H and E Staining Method

The methods of H and E staining technique was carried out by de-waxing the tissue in two changes of xylene for three (3) minutes each, hydrated by passing them through descending grades of alcohol (100%, 95%, 90%, and 70%) for three minutes each, and then stained in Harris haematoxyline for ten minutes, washed in tap water to remove excess stain. The slides were then flooded with acid alcohol for few seconds for differentiation and then washed in tap water again. The slides were then blued in Scott's tap water for five minutes and counter stained with Eosin for three minutes. The sections were rinsed in tap water, and then dehydrated in ascending grades of alcohol, cleared in xylene. Tissues were then mounted with cover slips using a mounting media. Sections of the tissues were viewed under light microscope and photomicrographs were taken using digital Am scope (MD 900) photomicroscope.

3.7.2 Special Stain Using Toluidine Blue Method

The prepared serial sections of cerebrum were de-waxed then transferred to 95% alcohol. The slides were put in alcoholic colophonium solution for 5 minute. The slides were then transferred to two changes of 95% alcohol each for 3 minutes, followed by staining in toluidine blue 0.1% for 30 seconds, and then differentiated in a mixture for 10% analene and 95% alcohol. Clearing in different changes of Cajput oil, and finally mounting in Canada balsam.

3.8 IMMUNOHISTOCHEMICAL STUDIES

3.8.1 Peroxidase linked avidin-biotin complex (Abc) method

Tissues were routinely processed through a graded series of alcohols, cleared in xylene and embedded in paraffin. Sections were obtained and processed for immunohistochemical staining which is used for localization of an intracellular calcium binding protein (CAL D28K). The staining was carried out by the peroxidase linked avidin-biotin complex (ABC) method. Blocking of endogenous peroxidase activity was carried out with 0.08% hydrogen peroxidase (H2O2) in methanol for 10 minutes (Sternberger, 1986).

In order to the block unspecific binding, incubation with (1:10) normal goat serum in 0.1 M PBS, pH 7.2 was performed. Sections were incubated for 16-20 h at 4 °C in mouse anti-calbindin IgG (Abcam). The antibody was diluted to 1:500 in PBS containing 0.25% sodium azide and 2.5% bovine serum albumin. Sections were then incubated in biotinylated sheep anti-mouse IgG (Abcam) followed with streptavidin horseradish peroxidase (thermo scientific), both at a dilution of 1:50 in PBS, for 1 h at room temperature. Sections were washed in PBS for 30 min after each incubation. Sections were then immersed in glucose oxidase-DAB-nickel ammonium sulphate (GDN) substrate (Shu *et al.,* 1988) for 10 minutes, washed in distilled water and counterstained with haematoxylin for 3 minutes and coverslip using DPX mountant. Sections were examined with a DM 750 LEICA digital microscope and photomicrographs were taken.

3.9 NEUROBEHAVIORAL STUDIES

3.9.1 Montoya Staircase Test Method

The Montoya staircase test method was used to study the locomotor function and skills in motor co-ordination. This method helps in determining the effects of a test substance on motor function abilities (Montoya *et al.,* 1990 and 1991). The tasks involved forepaw reaching for food pellets located at various steps along the staircase and the time taking to explore the activity cage (Whishaw *et al.,* 1986 and 2000 ; Abrous *et al.,* 1994; Whishaw and woodward, 1997). The test was carried out as a measure of motor coordination shown by muscle action in climbing and exploring the staircase and motor and locomotor function shown by the independent forelimb ability to reaching out and grasping food pellet in the staircase.

3.9.2 Training Time

At the onset of the test, animals were first trained to recognize that there are food pellets along the wells in Montoya staircase for three consecutive days. The animals were able to familiarize themselves with the Montoya staircase and then the animals were deprived of food for 18 hours in order to facilitate their search for food. During the test, each animal was placed on the foot of the staircase which will enable them to climb the stairs in order to reach out, grasp or retrieve the food pellets and each test session lasted for 5 min, at the end of which the time taken to climb up and down from the basement was recorded and grasping abilities of each animal was also observed as shown in Figure 3.1.

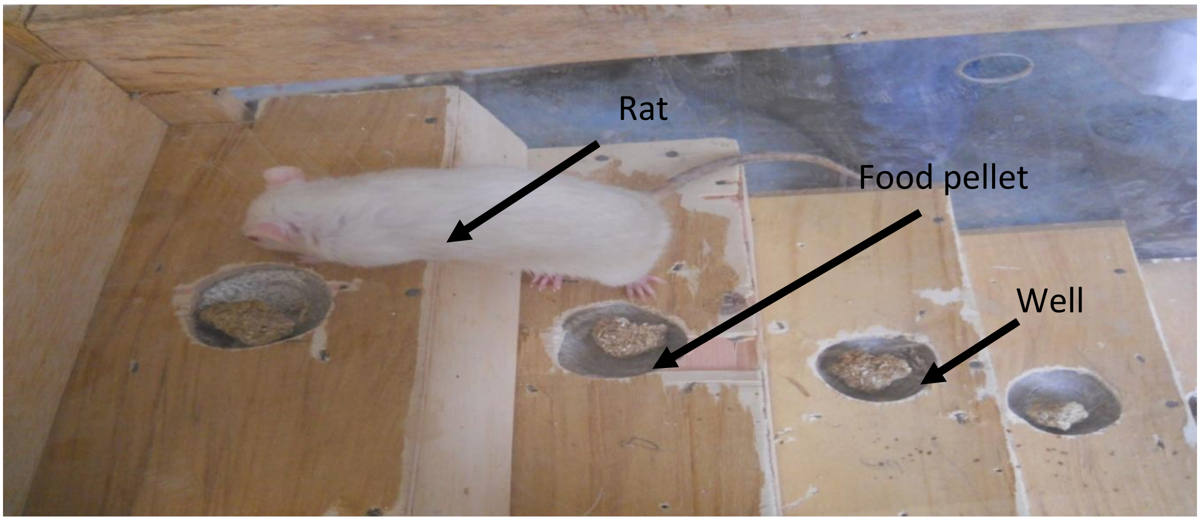

Figure 3.1 Montoya staircase test: Showing wells with food pellets inside and the animal trying to move-up the next step.

3.10 STATISTICAL ANALYSIS

The data obtained were expressed as Mean ± SEM (Standard error of mean). One Way Analysis of Variance (ANOVA) was used to compare the Means difference between and within the groups and a p-value less than 0.05 was considered statistically significant. Statistical analysis was performed using EZanalyze v3.0 and a post hoc test of Turkey was applied. Charts were produced using Microsoft(R) Excel 2007 for windows.

CHAPTER FOUR

4.0 RESULTS

4.1 Physical Observation

No visible physical changes were noticed few hours after administration of normal saline water to the control group, the animals were very active. Malformation such as tincture were observed in animals of group III, serious scratching of the nose was observed among animals of the group after administration and there was apparent reduction in physical activities, mucus secretion from nostril, rising of body hair with frequent urination and watery feces. Dams that received high dose, group IV showed macrocephaly, dead of pups were also observed, ulceration in the thoracic region, watery stool, deep yellow urination, gnawing, exopthalmos, restlessness, decreased physical activity, rising of body hair, less food consumption and mucus secretion were observed.

4.2 MORPHOMETRIC CHANGES

The charts presented on the Figure 4.7- 4.13 showed groups of rats that were given 6 g/kg, 8 g/kg, and 10 g/kg of MSG which demonstrated significant reduction ($p< 0.05$) in all parameters measured when compared to the control.

There were no significant changes in the feotal weight in all the groups in day 1 (5.00 ± 0.00), day 7 showed group 4 was significantly higher (13.75 ± 0.47) when compared to the control (6.75±0.47), day 14 showed group 2 (16.75 ±1.65) and 3 (17.75 ± 0.47) were significantly lower when compared with the control (22.25 ±1.03) and changes observed in day 21 were statistically not-significant

Crown-rump length in day 1 showed that groups 2 (3.25 ± 0.25) and 3 (3.25 ± 0.25) only had significantly lower when compared to the control (5.00± 0.00), day 7 showed groups 2 (7.25 ± 0.25), 3(9.00 ± 0.00) and 4 (9.00 ± 0.00) were significantly higher when compared to the control (3.2500 ± 0.25), in day 14 no significant changes were observed and in day 21

(14.5000±0.50), 3 (14.00±0.00) and 4 (14.00±0.00) were significantly lower when compared to the control (15.25 ±0.25).

No significant changes were observed in the head weight in days 1 (2.00 ±0.00), 7(4.00 ±0.00) and 21 (6.75 ± 0.00), but group 2 day 14 was significantly lower (3.5000 ± 0.28) when compared to the control (5.00 ± 0.00).

Brain weight in day 1 showed groups 2 (0.20 ±0.00) and 3 (0.21±0.00) were significantly lower when compared to the control (0.20 ± 0.10), day 7 showed group 3 (0.55± 0.02) was significantly higher when compared to the control (0.44 ±0.01), day 14 showed group 2 (0.83 ±0.01) was significantly lower when compared to the control (0.90 ± 0.02) and day 21 showed no significance in all the groups.

The cerebral weight in day 1 showed group 2 (0.13±0.00) was significantly higher when compared to the control (0.14±0.00). In day 7 groups 3 (0.40 ± 0.00) and 4 (0.40 ±0 .00) were significantly higher when compared to the control (0.31 ± 0.00). Also in day 14 showed no significant difference in all the groups and in day 21 groups 2 (0.80±0.00) 3 (0.83±0.01) and 4 (.0.80 ± 0.17) were significantly lower when compared to the control (0.90±0.00)

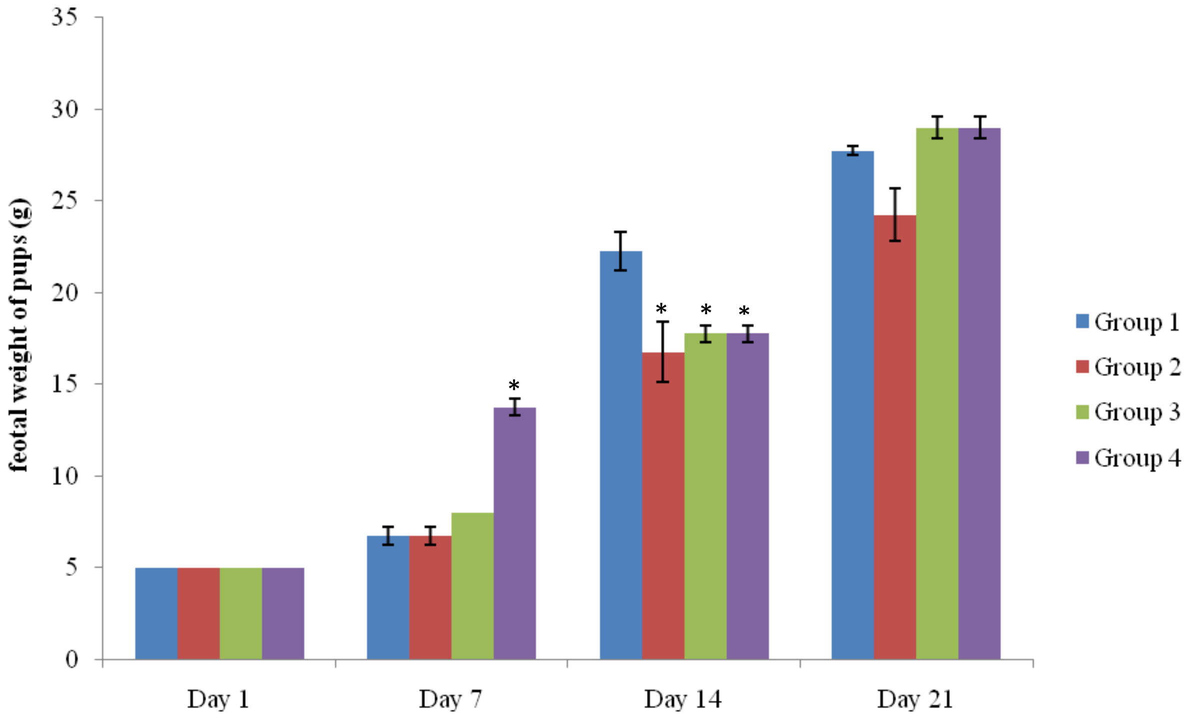

Figure 4.1: Effects of MSG on the feotal weight of the pups of the developing cerebrum of Wistar rat

*indicates significance at $p< 0.05$ when compared to control (Group 1).

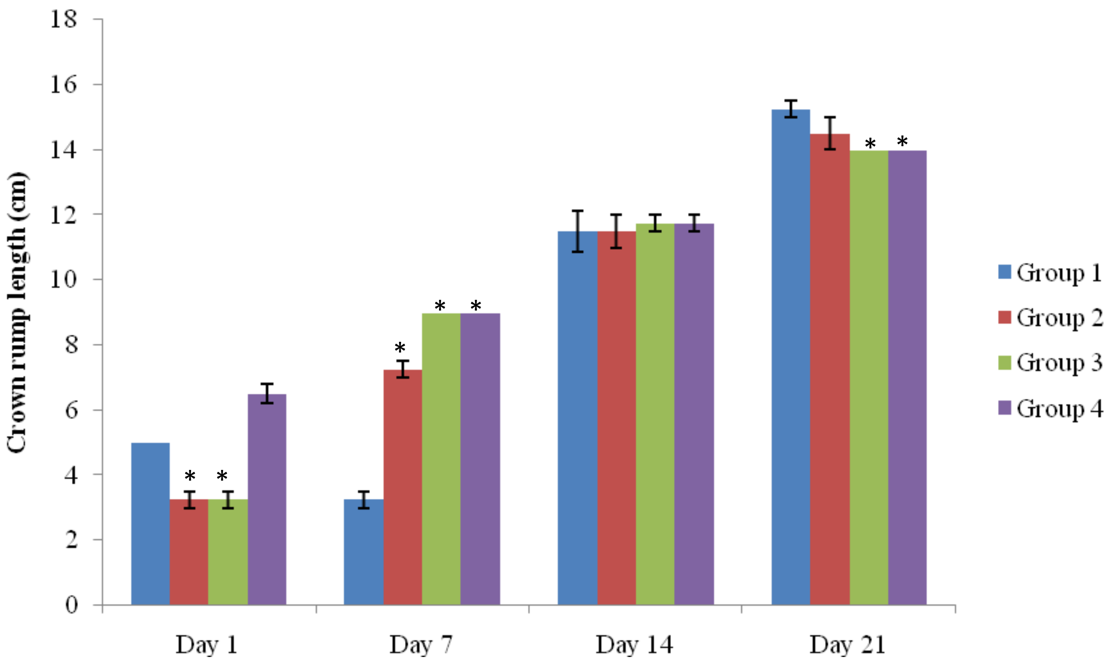

Figure 4.2: Effects of MSG on the crown rump length of the developing cerebrum of Wistar rat

*indicates significance at p< 0.05 when compared to control (Group 1).

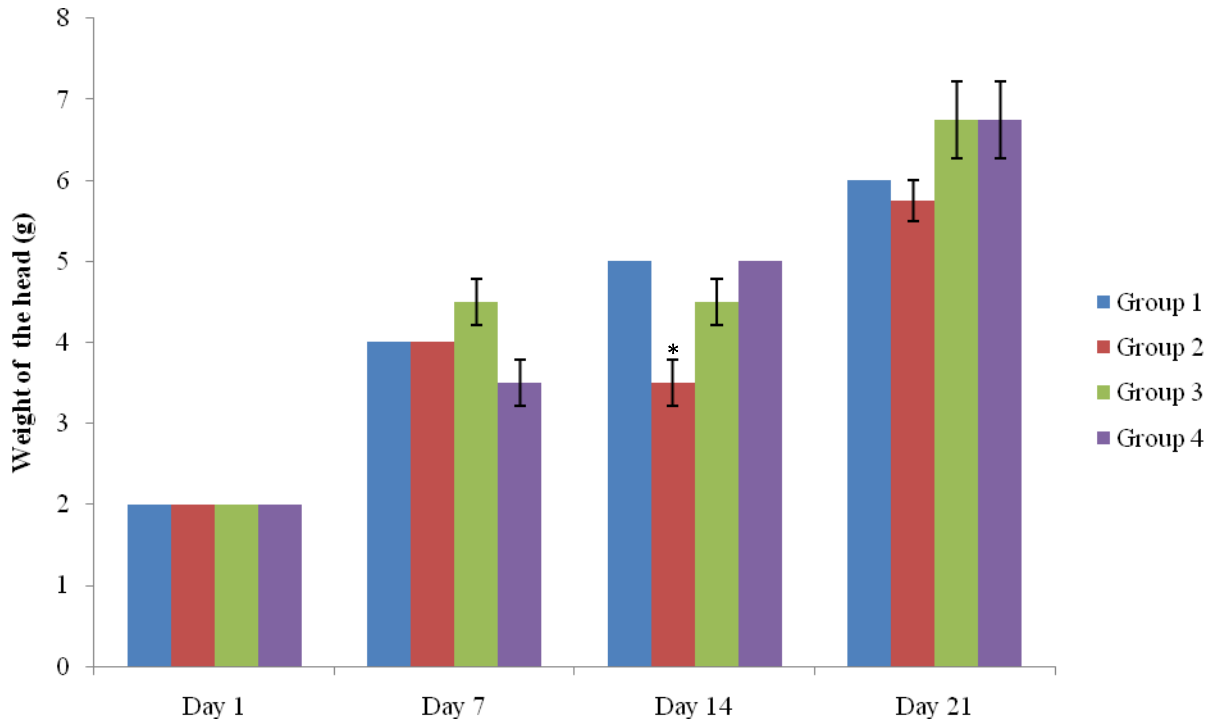

Figure 4.3: Effects of MSG on the weight of the head of the developing cerebrum of Wistar rat

*indicates significance at $p < 0.05$ when compared to control (Group 1).

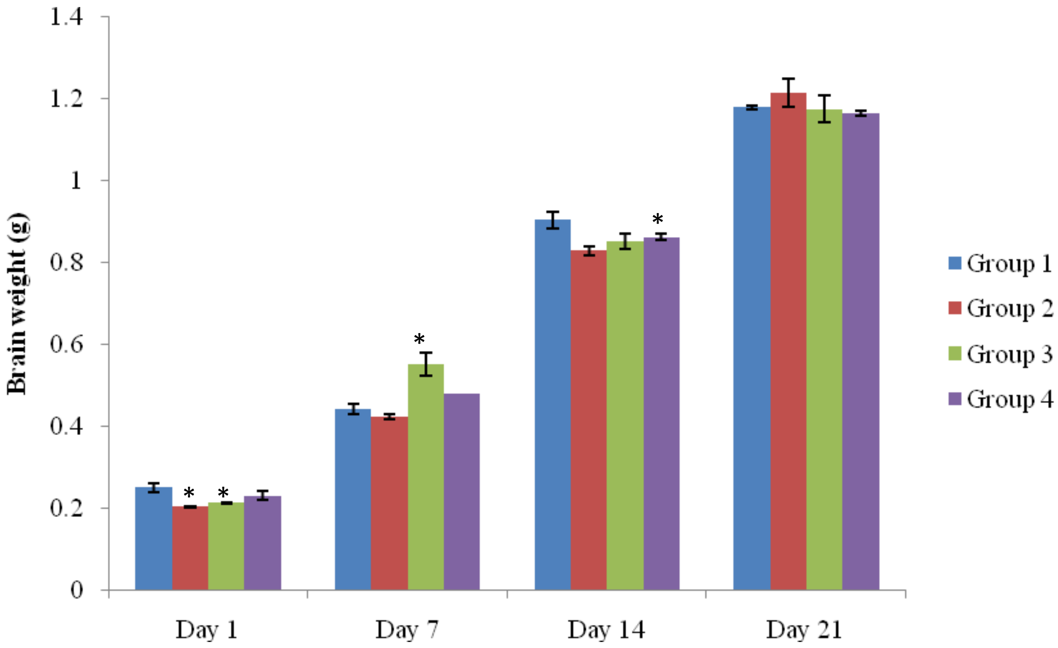

Figure 4.3: Effects of MSG on the brain weight of the developing cerebrum of Wistar rat

* indicates significance at p< 0.05 when compared to control (Group 1).

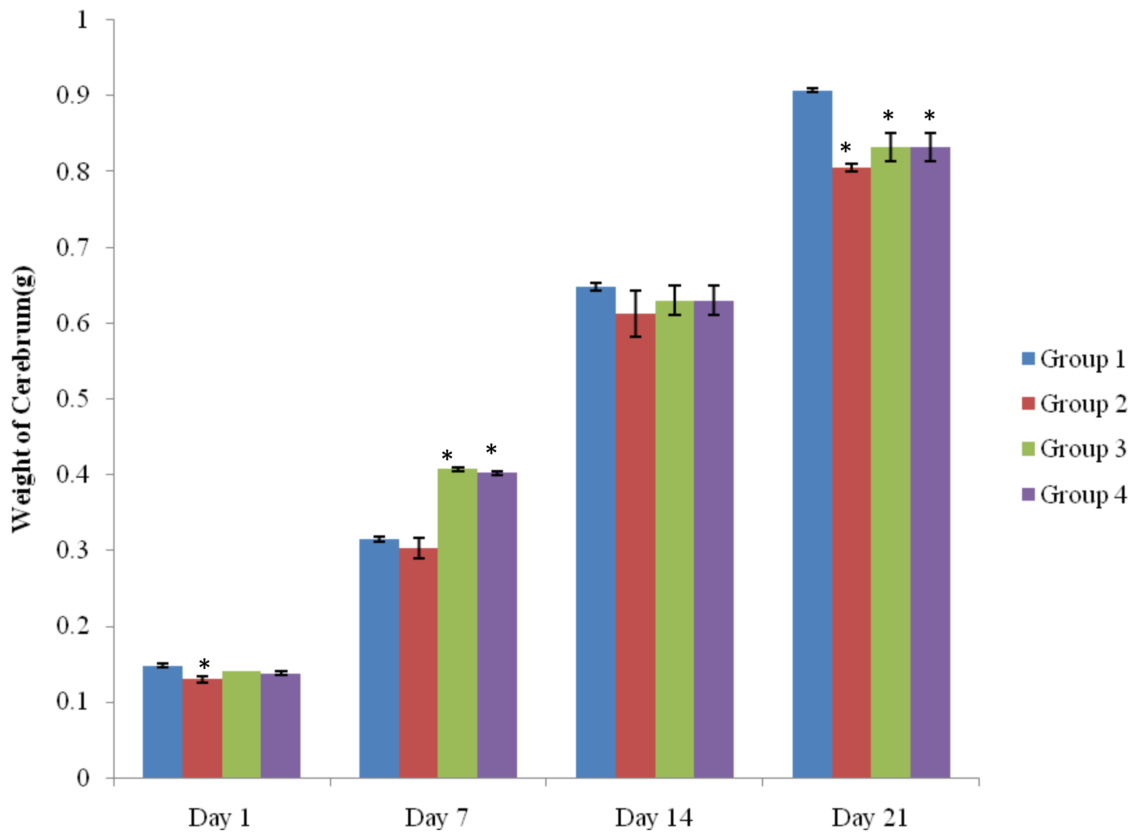

Figure 4.4: Effect of weight of the cerebrum on the developing cerebrum of Wistar rat *indicates significance at p< 0.05 when compared to control (Group 1).

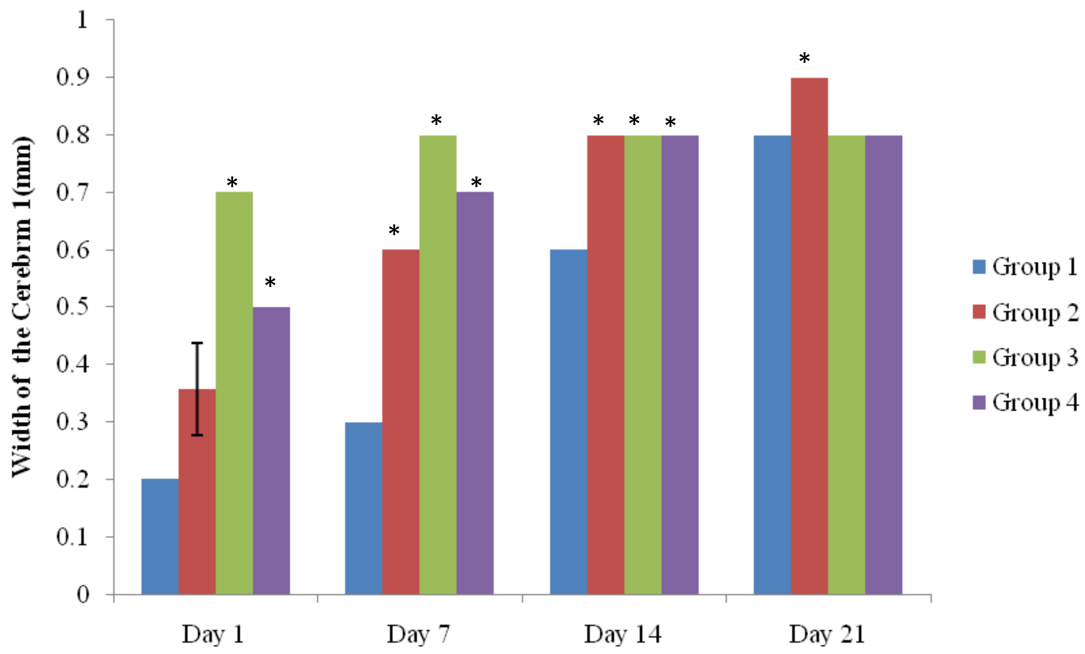

Figure 4.6 Effects of monosodium glutamate on the cerebral width (narrow area) on the developing cerebrum of Wistar rat

*indicates significance at $p< 0.05$ when compared to control (Group 1).

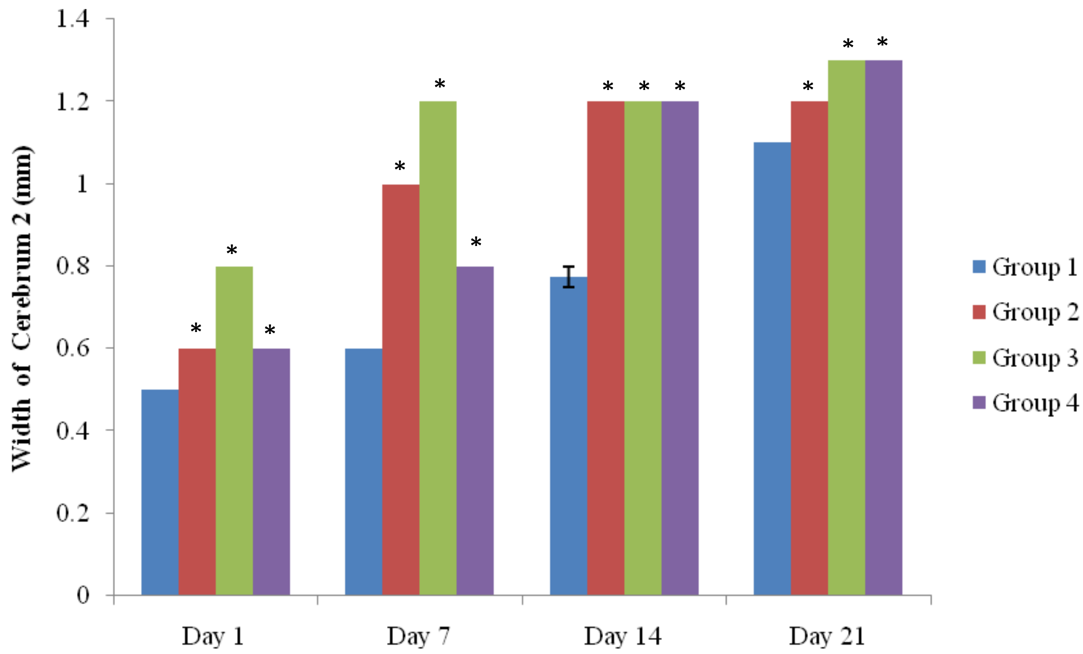

Figure 4.7: Effects of monosodium glutamate on the cerebral width (broad area) on the developing cerebrum of Wistar rat

*indicates significance at $p < 0.05$ when compared to control (Group 1).

4.3 HISTOLOGICAL CHANGES

The results from histological observations showed histological changes in the tissues studied. The results of the histological changes showed that animals in control group had normal histology of the developing cerebral cortex (Plate I), while animals in group II showed the stellate cells in their developing cerebral cortex without any vivid degeneration present on stellate (Plate II). The animals in group III, showed pyramidal cells, stellate cell with some degenerated Pyramidal cells and disintegrated nuclei as in Plate III, while animals in group IV, showed a lot of vacoulation cells, clumped cells with some degenerating cells of the developing Wistar rat as shown in Plate IV.

The cerebral cortex of the animals control group (group I) stained with toluidine blue showed cytoarchitecture of the pyramidal cell, dendrites and with some few granular cell appearing normal as shown in plate V, while group II animals showed nuclei displacement, distintegrated nuclei and loss of dendritic arborization plate VI. The histological observation for the group III animals as shown in plate VII depicts vacuolation and nuclei displacement and loss of neuronal fibers. The same pattern of histological changes was observed in group IV for the pyramidal cells with dendrite cells showing mark of loss of nuclei and vacoulated as shown in plate VIII.

The immunohistochemical studies showed that animals in the control group I had normal positive immunoexpression in pyramidal cells , stellate cells with little glial fibrillary acidic protein as shown in Plate IX, while group II animals showed few glial fibrillary acidic protein, disorientation of the pyramidal cell layer with a normal axon and dendrite looking normal (Plate X). Plate XI showed the cerebral cortex of group III animals, showed mild GFAP immunoexpression of the protein deposit with degenerated pyramidal cells in the cerebral cortex of the developing Wistar rat. Group IV animals showed disorientation of the pyramidal cell layer and degeneration of some pyramidal cells with nuclei displacement and intense GFAP immunoexpression of protein deposits with few cells in cerebral cortex of the developing Wistar rats as shown in Plate XII.

4.4 PHYSICAL OBSERVATIONS OF ANIMALS IN MONTOYA STAIRCASE

The results of physical observation of animals in control group (Group I) showed that the animals were able to use their forepaw in displacing the food pellets along the staircase, grasped some of the food pellets. The animals were able to reach the topmost of the staircase of 6 steps within five minutes. The animals were also observed to have fine movements in

Group I, while Group II animals that were given 6g/kg of monosodium glutamate were able to displace few food pellets and no tremor was observed and the animals were able to move maximum distance and were able to reach the top of the stair at step number 6. There was no loss of balance in animals of group II. Group III animals, there was serious tremor, poor movement and stability and the maximum distance reached was step 4 out of 6 with no attempts to move further. Group IV animals that received the high concentration of monosodium glutamate of 10g/kg, it were observed that animals falls back when trying to move-up to the next step, with maximum distance reached was at step 3 of 6, serious tremor was observed with poor movement and stability and no further attempt to move up for about five minutes, no food pellet was displaced.

4.5 MOTOR ACTIVITY TEST USING MONTOYA STAIRCASE TEST

The results of motor activity test using Montoya staircase method are shown in Figure 4.14. The results showed that there was no significant decrease in the mean time taken for the animals to reach the top of the six stairs throughout the 1^{st} (49.67 ± 9.83), 2^{nd} (48.33 ± 4.26) and 3^{rd} (51.00 ± 5.85) days when compared with the control. The animals in group II that received the minimum concentration (6 g/kg b.w) of monosodium glutamate had the mean time taken to reach the stairs increased in 2^{nd} (126.43 ± 2.59) and 3^{rd} (145.30 ±17.92) days when compared to the control. The results showed that the time taken for the animals to reach the top of the stairs was significantly increased in groups II (125.87 ± 2.43) in the 2^{nd} and 3^{rd} days when compared to the control ($p \geq 0.05$).

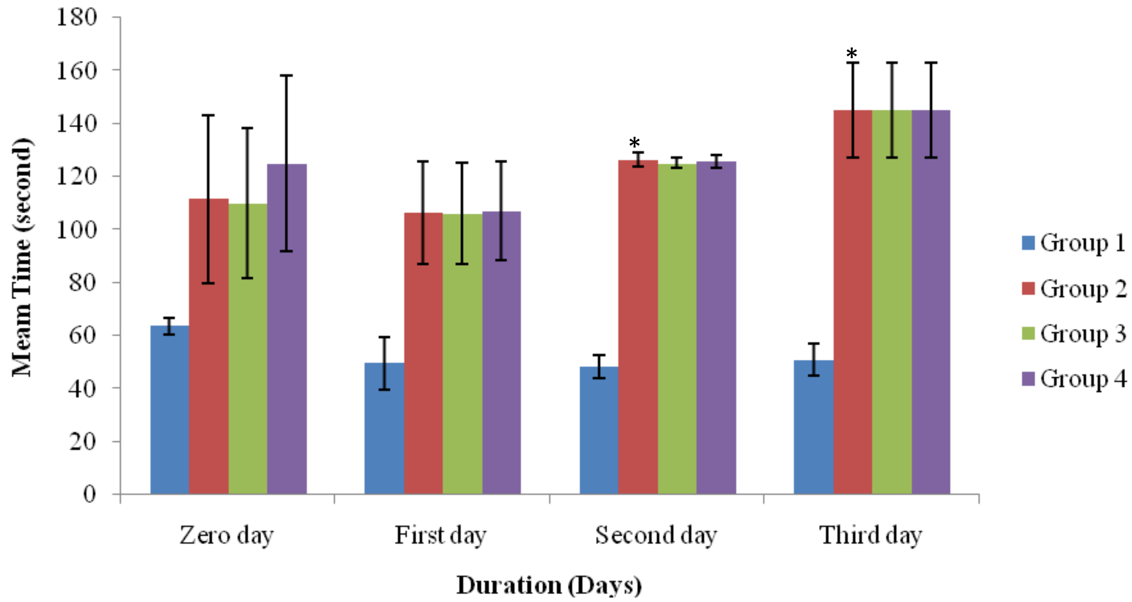

Figure 4.8. Effect of MSG administration in motor activity of adult Wistar rat using Montoya staircase test.

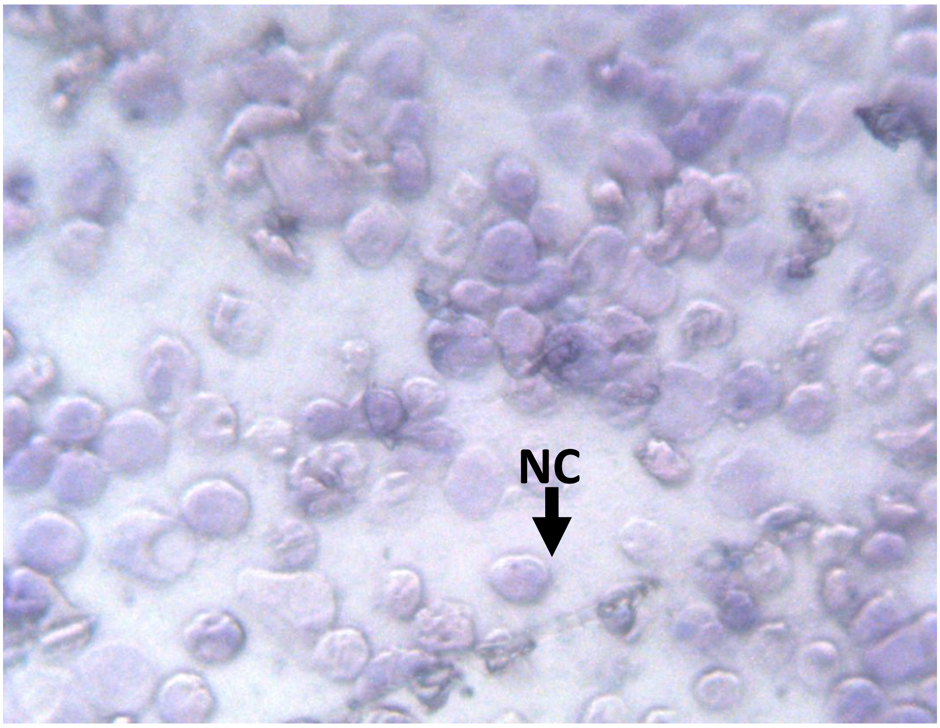

Plate 4.1 A photomicrograph of the proestrous phase, showing numerous nucleated cells (NC) (Giemsa ×250)

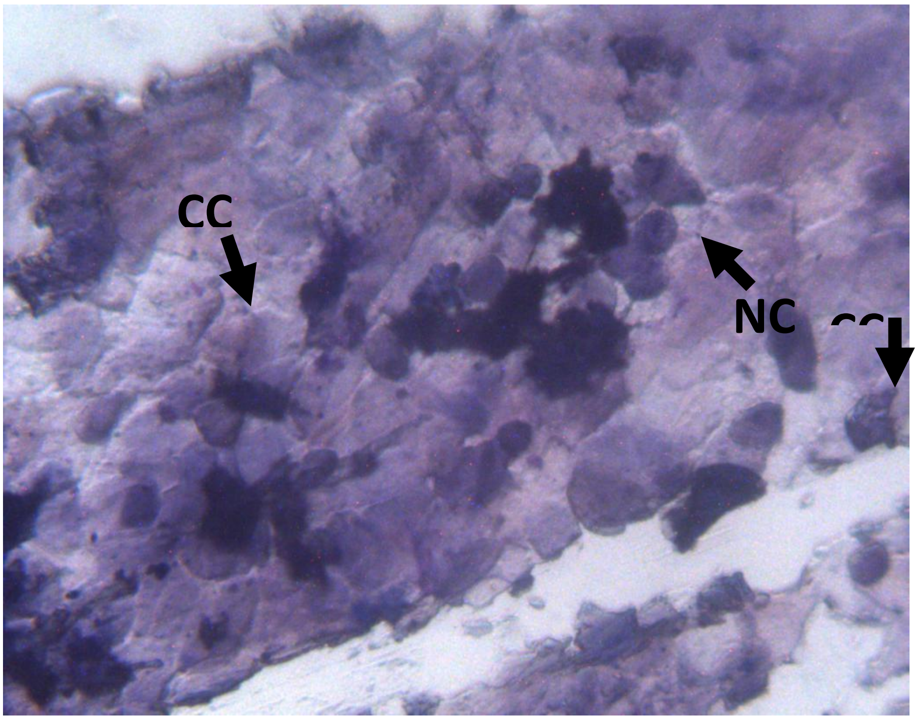

Plate 4.2 A photomicrograph of the estrous phase showing few nucleated cells (NC) and numerous nucleated cornified cells (Giemsa stain ×250)

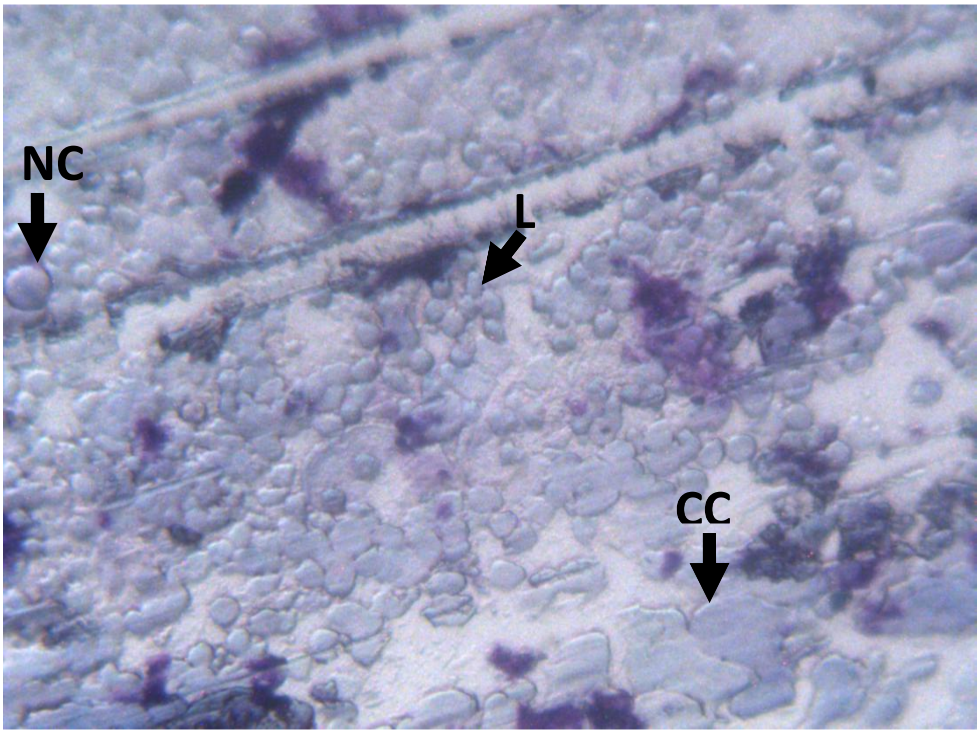

Plate 4.3 A photomicrograph of the metrestrous phase, showing few nucleated cells (NC), cornified cells (CC) and large population of leukocyte cells (L) (Giemsa stain ×250)

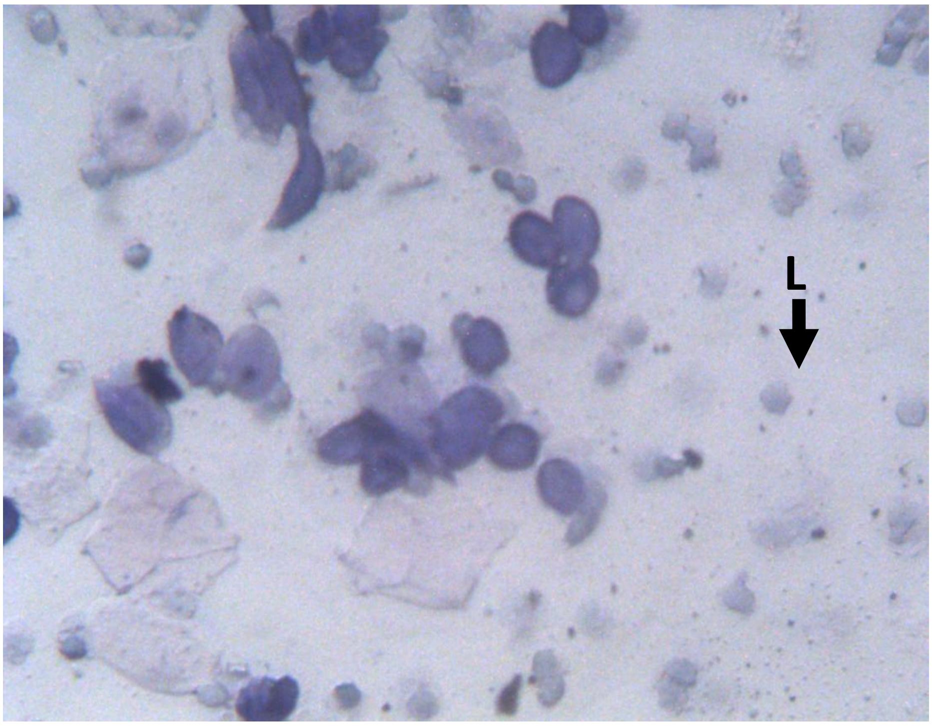

Plate 4.4 A photomicrograph of the metrestrous phase showing a scanty with presence of leukocyte cells (L) (Giemsa stain ×250)

Plate 4.5 Normal pups in the control group day 1 animals

Plate 4.6 Tinge colour in the body of group II pups

Plate 4.7 Dead of pup in group IV animals

Plate 4.8 Dead pups in group IV high dose animals

Plate 4.9 Macrocephaly in group IV animals high dose

Plate 4.10 Ulcer in group IV animals high dose

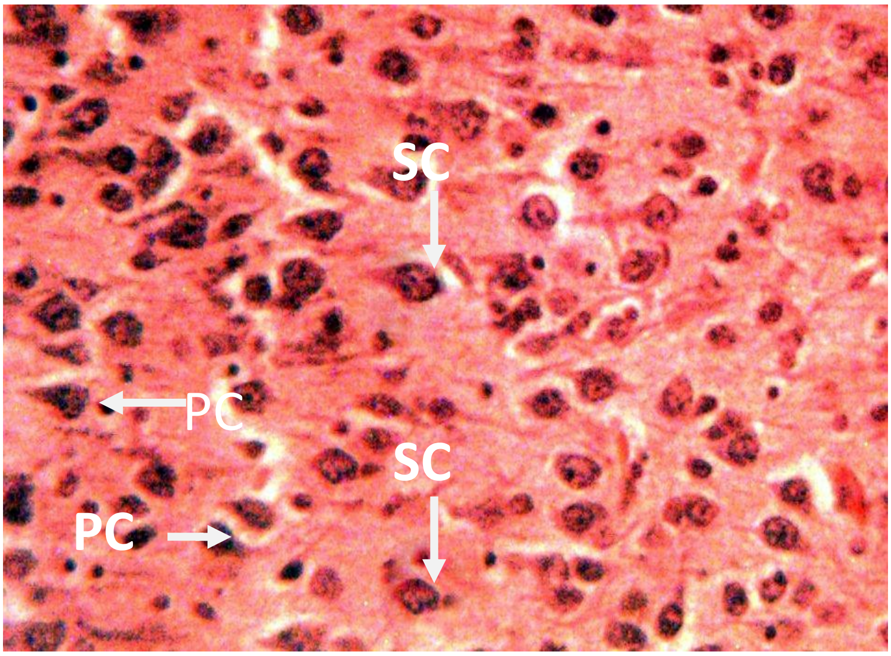

Plate 4.11: A tranverse section of the developing cerebral cortex of the control group (G1) with normal pyramidal cells (PC), stellates cells (SC) (H and E X250).

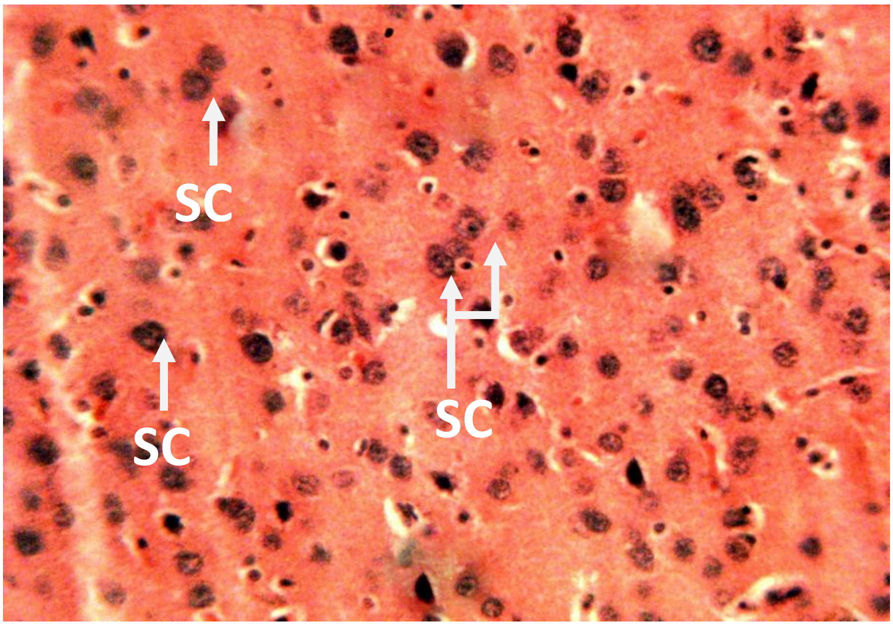

Plate 4.12. A transverse section of the developing cerebral cortex of group II showing the stellate cells (SC) without any vivid degeneration present on stellate cells (H and E X250)

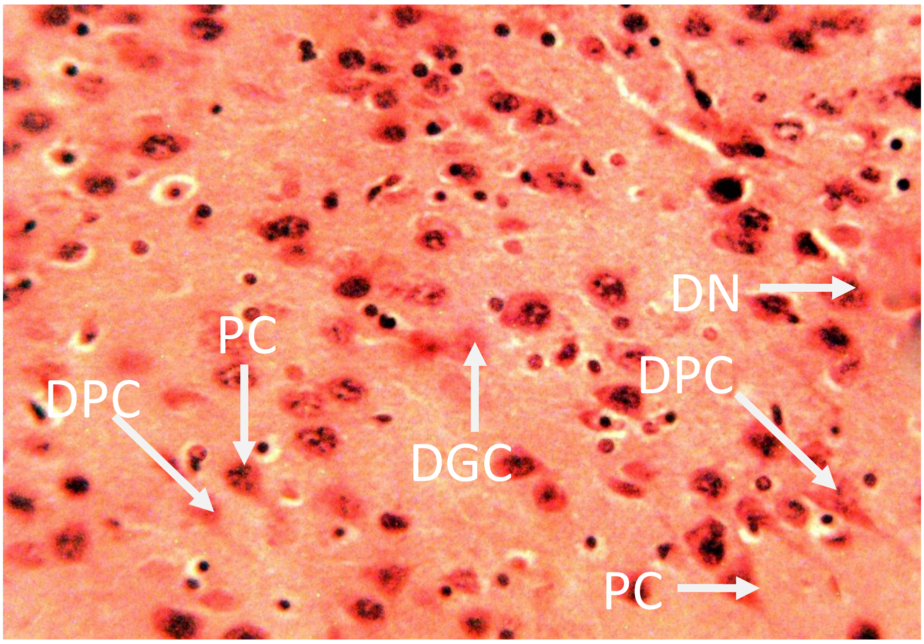

Plate 4.13 A tansverse section of the developing cerebral cortex of group III animals showing pyramidal cell (PC), stellate cells (SC), degenerated pyramidal cells (DPC), and disintegrated nuclei (DN) (H and E X250).

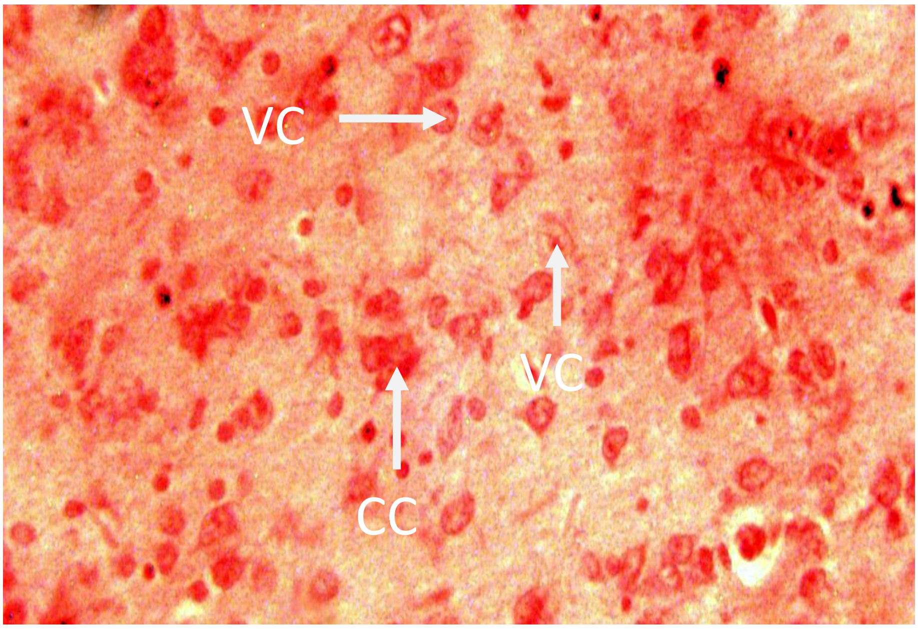

Plate 4.14 A transverse section of the developing cerebral cortex of group IV animals showing a lot of vacuolation cells (VC) and clumped cells (CC) with some degener ating cells of the developing Wistar rat (H & E ×250)

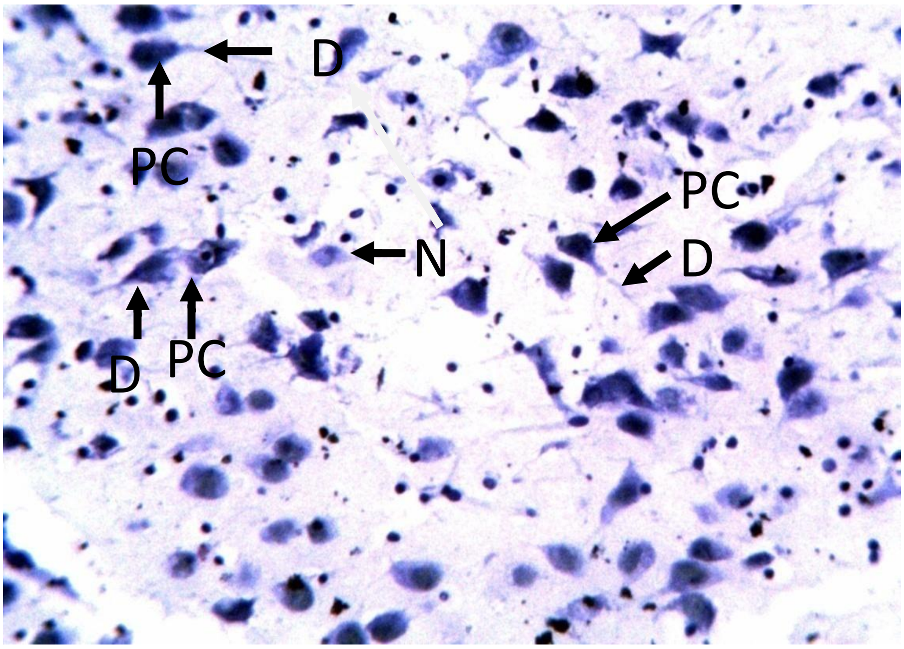

Plate 4.15. A transverse section of developing the cerebral cortex of the control group I animals showing pyramidal cell (PC), normal dendrite (D) and some few granules and nuclei (N) (Toluidine Blue stain ×250)

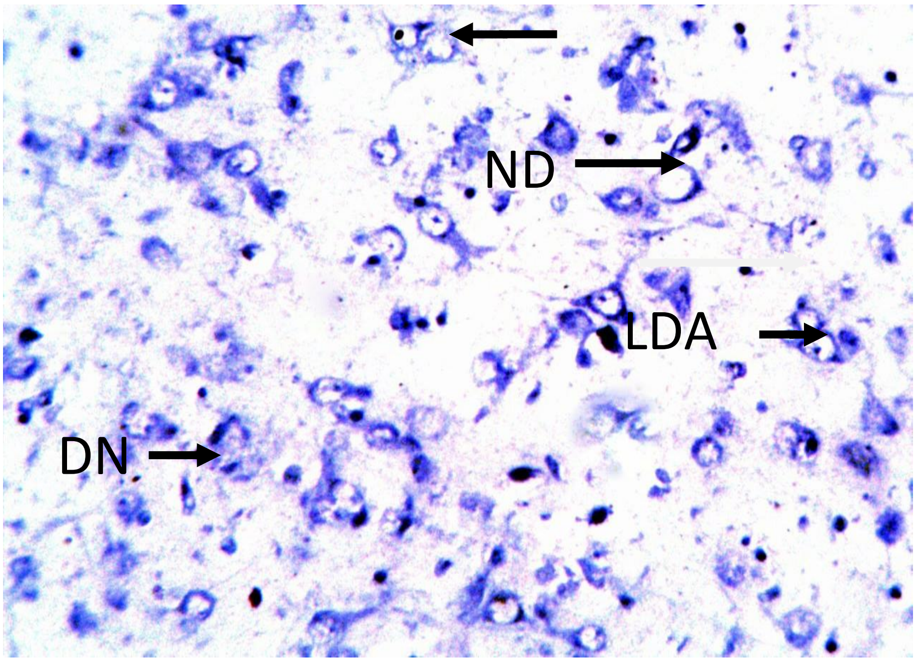

Plate 4.16. A tranverse section of the developing cerebral cortex of the animals in group II showing Nuclei displacement (ND), Disintegrated nuclei (DN), Loss of dendritic arborization (LDA) (Toludiene Blue stain ×250)

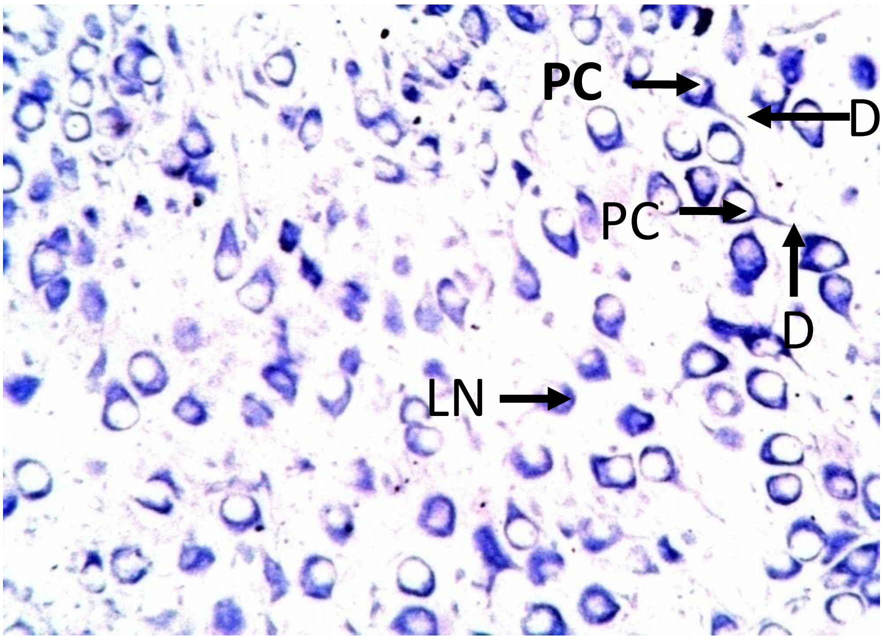

Plate 4.17 A transverse section of the developing cerebral cortex of the group III animals showing pyramidal cell (PC), Dendrite (D), loss of nuclei (LN) and most of the cell display/ are characterized by loss of dendritic arborization and nuclei displacement (Toluidine Blue stain ×250)

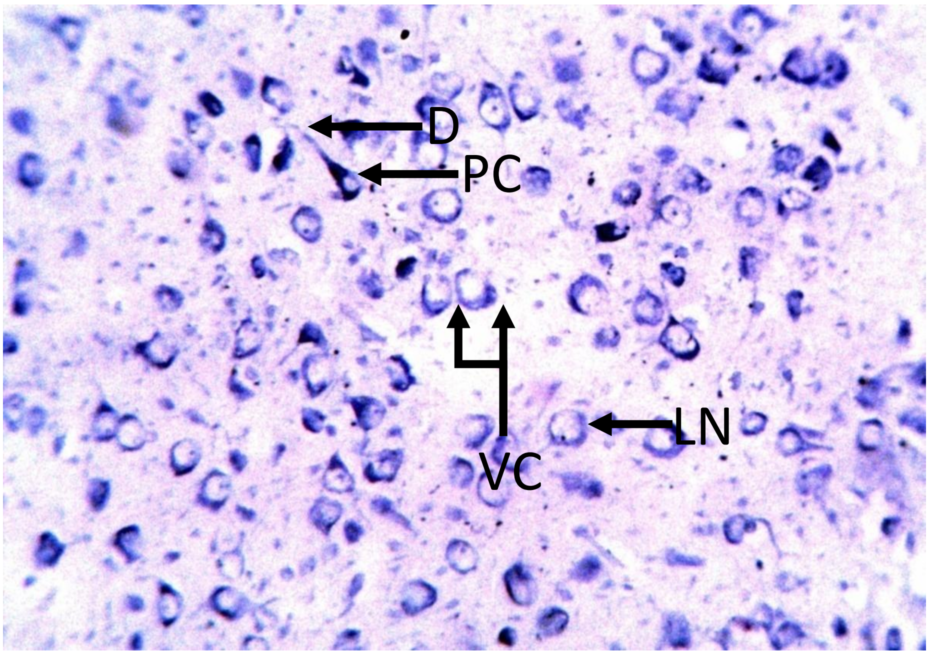

Plate 4.18 A transverse section of developing the cerebral cortex of the animals in group IV showing pyramidal cells (PC), Dendrite (D), loss nuclei (LN), and vacuolated cell (VC) (Toludine stain ×250)

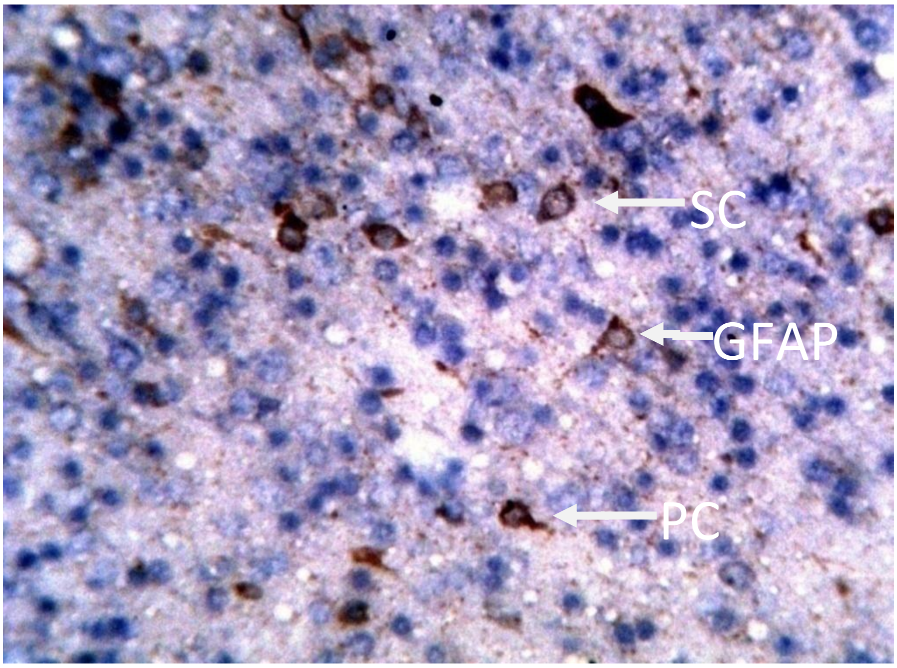

Plate 4.19 A transverse section of the developing cerebral cortex of the

control group I animals showing negative immunoexpression in pyramidal cells (PC), stellate cells (SC), with little glial fibrallary acidic protein deposit (GFAP) (Immunohistochemical stain ×250)

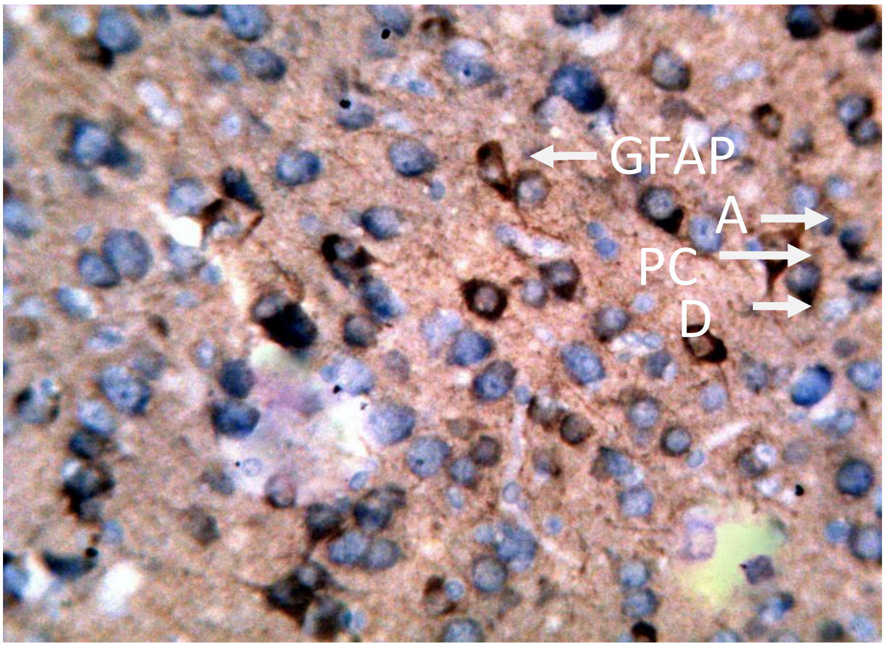

Plate 4.20 A tranverse section of the developing cerebral cortex of the animals in group II showing few glial fibrallary acidic protein (GFAP), Pyramidal cells (PC), Dendrite (D), Axon (A) (Immunohistochemical stain ×250)

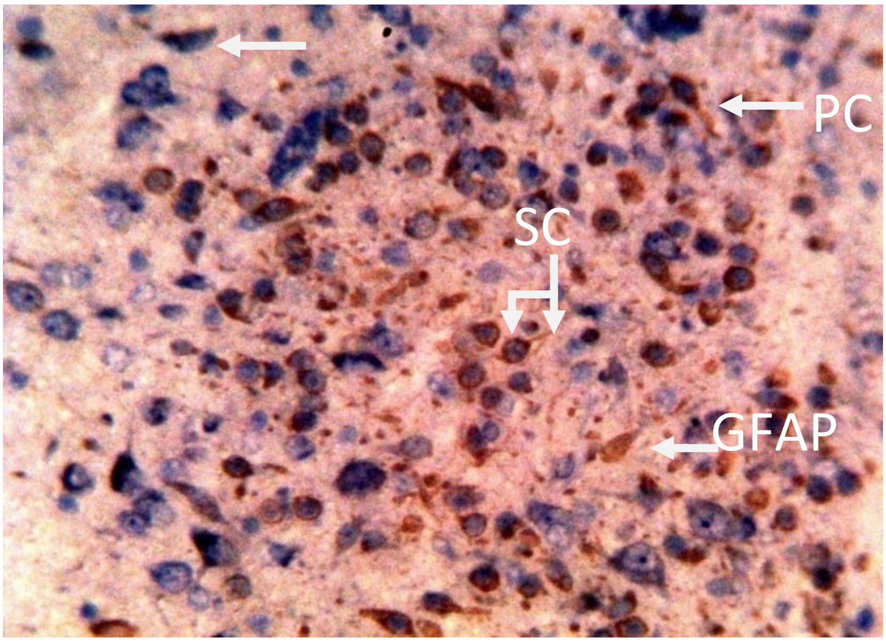

Plate 4.21 A tranverse section of the developing cerebral cortex of the animals in group III showing mild glial fibrillary acidic protein (GFAP), with Degenerated pyramidal cells and Stellate cell (SC) (Immunohistochemical stain ×250)

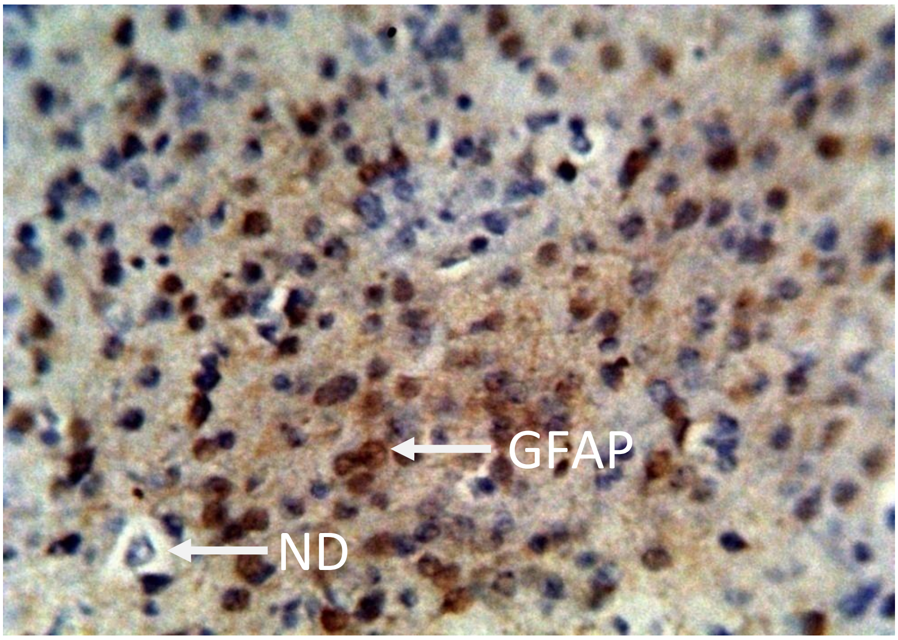

Plate 4.22 A tranverse section of the developing cerebral cortex of the animals in group IV showing intense glial fibrillary acidic protein (GFAP) with few cells, nuclei displacement (ND) (Immunohistochemical stain ×250)

CHAPTER FIVE

5.0 DISCUSSION

The administration of monosodium glutamate did not show any significant difference on the body weight throughout the period of administration even when the highest dose (10 g/kg) of monosodium glutamate that was administered. Morphologic observations indicate tinge colour on the stomach, dead of the pups as well as ulceration in the thoracic region in the treated group. This may be apparently due to the substance administered during development. This agrees with the work of Noback (1967) who reported that as much as 50% cases of congenital malformations and abnormal development involve the nervous system. In experimental animals, malformations of the nervous system mostly occur when the teratogen is administered shortly before or during the closure of the neural groove (Singh *et al*, 1972; Ezurumlu and Killackey, 1982). Several studies have demonstrated a correlation between intrauterine growth retardation and teratogenesis. The parameters measured in the study include: body weight, crown rump length, feotal weight, head weight, cerebrum weight, cerebrum width (narrow area) and cerebrum width (broad area). The p value was observed to be significantly reduced in the high dose group when compared with the control. This may be attributed to the effects of the substance administered to the animals which affects growth during development.

In the present study, the teratogenic potentials of monosodium glutamate have been demonstrated as most of the above named parameters were significantly reduced in the high dose groups when compared with the control and low dose groups. This confirms earlier reports that a teratogenic insult on the developing brain during neurogenic period induces anomalies (Singh *et al,* 1972; Sturrock, 1978; Akpan *et al,* 1992; Igiri *et al,* 1999). The various measurements of the cerebral cortex in the high dose groups were significantly lower than those of the control groups. This indicates that high doses of monosodium glutamate exerted teratogenic effects on the nervous system. This is in line with several reports of developmental toxicity of monosodium glutamate (Xu and Zhang, 1996; WHO, 2002a; WHO, 2002b; Lou *et al,* 2003). The cerebral cortex showed neuronal cell death by clumping of the cortical cells and the loss of pyramidal and stellate cells. Neurodegenerative and histomorphological changes in the cerebral cortex can affect the functional areas of the cerebral cortex which deals with skilled movement, ability to speak, ability to appreciate pains and temperature. This agrees with the work of Gill (2000) and Hughes *et al.* (2004), who observed that MSG plays a key role in neurons cell death. Similar findings were observed by Reistad *et al*. (2007). They observed that glutamate induced death of primary cultures of cerebellar granule cells. Rascher (1981) observed vacuolization of the endoplasmic reticulum, clumping of chromatin and numerous pyknotic nuclei as early as 3 hours following subcutaneous administration of MSG in cingulate cortex of neonatal albino rats

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This neurotoxic effect of MSG is in line with the work of Gill (2000) and Mattson (2008) who reported that sustained high concentrations of MSG, as an excitatory amino acid, in the synaptic cleft region resulted in excessive glutamate receptor activation with persistent depolarization producing metabolic and functional exhaustion of the affected neurons and hence neural necrosis. As regards Glial Fibrillary Acidic Protein (GFAP) immunoexpression in the cytoplasm of astrocytes cell bodies and processes, a significant increase was detected in MSG treated groups III and IV when compared with control group I. This increase is consistent with the findings of Sriram *et al.* (2004), Baydas *et al*. (2006) who reported that any mechanical, chemical or degenerative insults to the brain stimulate astrocyte's proliferation and hypertrophy with increased synthesis of GFAP leading to vigorous astrogliosis. However, this increase in GFAP immunoexpression in the current study was not significant and is associated with presence of apoptotic neurons suggesting a toxic effect of MSG on the astrocytes itself. This finding is in agreement with Pekny *et al.* (1999) who observed that in tissue culture, increase in glutamine levels appear to correlate inversely with GFAP. Also, Chen *et al*. (2000) and Szydlowska *et al.* (2006) reported that an excess of glutamate can cause the death of astrocytes in vitro. The mechanism by which MSG affect astrocyte, was explained by Chen *et al.* (2000), and Re *et al*. (2003) who implicate glutathione depletion and reactive oxygen species accumulation as potential mechanisms of toxic action of glutamate on astrocytes. The results of the present study also demonstrated a significant decrease in motor activity which was shown by the decrease in the time taken to reach the maximum attainable distance in the Montoya staircase method. This could be attributed to degeneration of cells in the motor area of the cerebral cortex. Furthermore, it could be attributed to a decrease in production of neurotransmitter due to degeneration of cortical cells. This agree with the findings of Ibegbu *et al*. (2013) who also observed that degeneration of cortical cells can alter the production of neurotransmitter dopamine which plays a role in motor activity

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CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

It can be concluded from the present study that:

(1) Oral administration of monosodium glutamate consumption has a neurotoxic effect on the developing fetus.

(2) Monosodium glutamate causes impairments of motor function deficits,

(3) Alteration of the histomorphology of the cerebral cortex and cortical cells

(4) The effects of monosodium glutamate exposure in the present study were shown to be time and concentration dependent.

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6.2.1 RECOMMENDATIONS

1. It is recommended that other in parts of the brain such as the olfactory bulb should be used to quantify the amount of monosodium glutamate that could possibly be deposited in the area prenatally
2. Morphometric measurements should be carried out at the postnatal period to ascertain the development of the cerebral cortex.
3. Further studies are recommended on neurochemistry in order to quantify or ascertain the level of neurotransmitter dopamine and GABA depletion and activity as a result of exposure to monosodium glutamate.

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APPENDIX I

Chemical Substance = Monosodium Glutamate C5HN8NNaO_4

LD_{50} of mercury chloride = 16,600 mg/kg body weight

10 g/kg, 8 g/kg and 10 g/kg of the LD_{50} was used as high, medium, and low dose respectively was adopted by Aporn and Virapan 2003

Dose of monosodium glutmatae used

$\frac{16,600}{1000}$ = 16.6 g/kg adopted by Aporn and Virapan 1984 and Bush, 2003

The doses given were adopted from Aporn and Virapan 1984 and Bush, 2003.

Group I animals was used as control and was administered normal saline orally

Group II animals received 6 g/kg of monosodium glutamate

Group III animals received 8 g/kg body weight of monoosdium glutamate

Group IV animals received 10 g/kg body weight of monosodium glutamate.