HISTO-MORPHOLOGICAL, BIOCHEMICAL AND NEUROBEHAVIOURAL EVALUATION OF THE FRONTAL LOBE FOLLOWING CHRONIC ALCOHOL ADMINISTRATION IN WISTAR RATS

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FACULTY OF BASIC MEDICAL SCIENCES

COLLEGE OF HEALTH SCIENCES

AHMADU BELLO UNIVERSITY,

ZARIA

APRIL, 2018

DECLARATION

I declare that the work in this dissertation entitled **Histo-morphological, Biochemical and Neurobehavioural Evaluation of the Frontal Lobe following Chronic Alcohol Administration in Wistar Rats**has been performed by me in the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria.

The information derived from the literature has been duly acknowledged in the and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

AkinyemiAdemola OMONIYI _____ _____

Signature Date

CERTIFICATION

This dissertation entitled **Histo-morphological, Biochemical and Neurobehavioural Evaluation of the Frontal Lobe following Chronic Alcohol Administration in Wistar Rats** by AkinyemiAdemola OMONIYI meets the regulations governing the award of the degree of Masters of the Ahmadu Bello University, and is approved for its Contribution to Knowledge and literary presentation.

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DEDICATION

This dissertation is dedicated to the memory my lovely father MR. OMONIYI TAYELOLU VINCENT and my caring mother, MRS OMONIYI MARY YETUNDE

ACKNOWLEDGEMENTS

All Glory to God Almighty, Abba Father for the Gift of Life; you are the reason am still here today, besides You there is no other.

I sincerely appreciate two special people that I came through into this World, My Loving Late Father Mr. Tayelolu, Vincent Emmanuel Omoniyi and his wonderful wife Mrs Mary YetundeOmoniyi and my caring Siblings for their love and support from the beginning of my life till this moment. My special Thanks to two special people that tutored me during this work, Professor S. S. Adebisi and Dr. S. A. Musa and Family, you play more than a supervisor role in my life and not less than what a father could do, therefore I called you my fathers in Anatomy.

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ABSTRACT

Light to moderate alcohol consumption is associated with improved cardiovascular and brain health. As such, some guidelines for health and wellness advocate drinking one or two alcoholic beverages each day. Conversely, it is widelyaccepted that large amounts of alcohol are detrimental to our health. Alcohol affects many organs of the body including the nervous system. The present study aimed at evaluating the hormetics effect of chronic alcohol consumption on the cognitive, behavioral and brain antioxidant system on adult Wistar Rats.Thirty-two(32) adult Wistar rats of both sexes were divided into 4 experimental groups with 8 animals of both sexes (house separately) in each group: Group1 served as control and was administered distilled water, while Group2 was administered with 0.12g/kg of ethanol, Group3 was administered with 0.16g/kg of ethanol and group4 was administered with 0.24g/kg of ethanol respectively for eleven(11) weeks orally. Cognition was assessed using Morris Water Maze and behavioral activities was assessed using elevatedplus Maze and resident intruder Test before, during and after administration.After administration, the rats were anesthetized lightly with ketamine injection(75mg/kg IP), the brain was removed and part was homogenized in phosphate buffer solution(PBS) for the estimation of Glutathione(GSH), superoxide dismutase(SOD), catalase(CAT), glutathione and malondaldehyde(MDA) and part was fixed in Bouin's fluid for histological studies. One-way analysis of variance, Repeated measure analysis of variance and Friedman test was used to compare the mean value and $p<0.05$.The result indicated that there was no significant alternation of learning and memory in control group of male and female rats while in the administered, consistent significant improved learning and memory was observed in dose dependent (**Group 2**: 37.61±7.34*sec*,15.26±5.57*sec*, 12.74±3.44*sec;* **Group 3**: 28.72±5.52*sec*, 12.20±4.31*sec*, 7.87±2.51*sec* and **Group 4**: 26.64±9.64*sec*, 23.57±1.84*sec*,

8.90±3.05*sec* before, at 5th week and 10th week of administration respectively) over time in male rats while consistent significant improved learning and memory was observed in **Group 2**(17.47±3.06*sec*, 7.55±3.90*sec*, 5.61±2.63*sec*) and Group 3 (31.30±6.83*sec*, 10.77±4.31*sec*) before, at 5th week and 10th week of administration respectively. No significant difference was observed in anxiety like behavior, aggression and brain antioxidant system. Loss of neural processes was observed in Group 4 female.In conclusion, with respect to the present result, daily oral consumption of lose dose of alcohol improve learning and memory in male and female Wistar rats andcould be of health benefit which is in line with mitohormesis concept thereby resulting longevity and could use to delay memory loss associated with aging diseases.

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1.0 INTRODUCTION

1.1 Background of the study

Alcohol is any chemical organic compound in which the hydroxylfunctional group (–OH) is bound to a saturatedcarbon. The term alcohol originally referred to the primary alcohol ethanol (ethyl alcohol), the predominant alcohol in alcoholic beverages. Ethyl alcohol, or ethanol, is an intoxicating ingredient found in beer, wine, and liquor. Alcohol is produce by the fermentation of yeast, sugars, and starches. It is a central nervous system depressant that is rapidly absorbed from the stomach and small intestine into the bloodstream. A standard drink equals 0.6 ounces of pure ethanol, or 12 ounces of beer; 8 ounces of malt liquor; 5 ounces of wine; or 1.5 ounces (a "shot") of 80-proof distilled spirits or liquor (e.g., gin, rum, vodka, or whiskey) (NIAAA, 2010).

Alcohol affects many organs of the body including the nervous system (Adebisi, 2006) and is thought to cause harm partly because of direct damage to DNA caused by its metabolites (*Brooks, 1997)*.

Alcohol affects brain chemistry by altering levels of neurotransmitters. **Neurotransmitters** are chemical messengers that transmit the signals throughout the body that control thought processes, behavior and emotion. Neurotransmitters are either **excitatory**, meaning that they stimulate brain electrical activity, or **inhibitory**, meaning that they decrease brain electrical activity. Alcohol increases the effects of the inhibitory neurotransmitter **GABA** in the brain. GABA causes the sluggish movements and slurred speech that often occur in alcoholics. At the same time, alcohol inhibits the excitatory neurotransmitter **glutamate**. Suppressing this stimulant results in a similar type of physiological slowdown. In addition to increasing the GABA and decreasing the glutamate in the brain, alcohol increases the amount of the chemical **dopamine** in

the brain's reward center, which creates the feeling of pleasure that occurs when someone takes a drink (**Klintsova*et al.*, 2002**)

Alcoholism can affect the brain and behavior in a variety of ways, and multiple factors can influence these effects. A person's susceptibility to alcoholism-related brain damage may be associated with his or her age, gender, drinking history, and nutrition, as well as with the vulnerability of specific brain regions (**Ammendola*et al.*, 2000**).

Ethanol's toxicity is largely caused by its primary metabolite, acetaldehyde (systematically ethanal) (*Fowkes, 1996)* and secondary metabolite, acetic acid (*Maxwellet al., 2010).* Many primary alcohols are metabolized into aldehydes then to carboxylic acids whose toxicities are similar to acetaldehyde and acetic acid. Metabolite toxicity is reduced in rats fed N-acetylcysteine (*Ozaraset al., 2003*) and thiamine.

The frontal lobe, located at the front of the brain, is one of the four major lobes of the cerebral cortex in the mammalian brain. The frontal lobe is located at the front of each cerebral hemisphere and positioned in front of the parietal lobe and above and in front of the temporal lobe. It is separated from the parietal lobe by a space between tissues called the central sulcus, and from the temporal lobe by a deep fold called the lateral sulcus also called the Sylvian fissure. The precentral gyrus, forming the posterior border of the frontal lobe, contains the primary motor cortex, which controls voluntary movements of specific body parts (*Coffeyet al., 1992)*

The frontal lobe contains most of the dopamine-sensitive neurons in the cerebral cortex. The dopamine system is associated with reward, attention, short-term memory tasks, planning, and motivation. Dopamine tends to limit and select sensory information arriving from the thalamus to the forebrain. A report from the National Institute of Mental Health says a gene variant that reduces dopamine activity in the prefrontal cortex is related to poorer performance and inefficient functioning of that brain region during working memory tasks, and to a slightly increased risk for schizophrenia (NIH/NIMH, 2001)

The frontal lobe plays a large role in voluntary movement. It houses the primary motor cortex, which regulates things like walking or punching (*Kimberg and Farah*, 1993).The function of the frontal lobe involves the ability to project future consequences resulting from current actions, the choice between good and bad actions (or better and best), the override and suppression of socially unacceptable responses, and the determination of similarities and differences between things or events (*Kimberg and Farah*, 1993).

The frontal lobe also plays an important part in retaining longer-termmemories, which are not task-based. These are often memories associated with emotions derived from input from the brain's limbic system. The frontal lobe modifies those emotions to generally fit socially acceptable norms (*Kimberg and Farah*, 1993).

Neuropathological studies performed on the brains of deceased patients have revealed decreased neuron density in the frontal cortex of alcoholics (Harper and Matsumoto, 2005). Harper

(1998)established that 15–23% of cortical neurons are selectively lost from the frontal association cortex following chronic alcohol consumption.

The frontal lobes are connected with the other lobes of the brain, and through multiple interconnections, they receive and send fibers to numerous subcortical structures as well (Fuster, 2006). The anterior region of the frontal lobes (prefrontal cortex) plays a kind of executive regulatory role within the brain (Goldberg, 2001). Executive functions (which depend upon many of our cognitive abilities, such as attention, perception, memory, and language) are defined differently by different theorists and researchers. Most agree, however, that executive functions are human qualities, including self-awareness, that allow us to be independent individuals with purpose and foresight about what we will do and how we behave. For example, executive abilities include judgment, problem solving, decision-making, planning, and social conduct, and they allow us to monitor and change behavior flexibly and in accord with internal goals and contextual demands (Oscar-Berman and Marinković, 2007).

An alcoholic who is a highly skilled professional, for example, an engineer or professor, may be perfectly able to perform regular duties such as temporal and spatial orientation, naming things, and calculations. However, this highly skilled person may be frontally impaired and unable to change or control their use of alcohol or drugs, even knowing that this behavior was and is harmful, or make important decisions in urgent situations. Imagine this highly skilled alcoholic subject driving on a road and a soccer ball unexpectedly crosses in front of his or her car, likely followed by a child. Most of us would bring together all faculties needed to evaluate the

situation, the possible consequences, and quickly take action, even if you hurt yourself (Nakamura-Palacios *et al.*, 2014).

Clearly, alcohol affects the brain. Some of these impairments (Difficulty walking blurred vision, slurred speech, slowed reaction times, impaired memory) are detectable after only one or two drinks and quickly resolve when drinking stops (**White, 2003**). On the other hand, a person who drinks heavily over a long period of time may have brain deficits that persist well after he or she achieves sobriety. Exactly how alcohol affects the brain and the likelihood of reversing the impact of heavy drinking on the brain remain hot topics in alcohol research today.

Most of us have witnessed the outward signs of heavy drinking: the stumbling walk, slurred words and memory lapses. People who have been drinking have trouble with their balance, judgment and coordination. They react slowly to stimuli, which is why drinking before driving is so dangerous. All of these physical signs occur because of the way alcohol affects the brain and central nervous system.

1.2 Statement of the Problem

Alcohol and its use is so deeply engrained into our social and cultural environment that it may appear counter-intuitive to have to describe it. However, this over familiarity with alcohol poses in itself an enormous challenge for the promotion of healthier life-styles. The chronic consumption of alcohol is common and the effect is costly. Excessive alcohol use decrease quality of life, academic performance, workplace productivity, and military preparedness; increase crime and criminal justice expenses; increase motor vehicle crashes and fatalities.

The brain, like most body organs, is vulnerable to injury from alcohol consumption, frontal lobe of the cerebrum being the most vulnerable part and because of its function being the main controlling center for executive function of the brain and other associated function (influences emotion, motor function, problem solving, memory, judgment, impulse control, social and sexual control and language), any damage to this part of the brain will affect the well-being of an individual.

These lobes located in the front of the whole brain control our behavior and are responsible for the acts that separate the homo 'thinker' sapiens from the rest of the animal kingdom. The frontal lobes control and inhibit our primal impulses; this inhibition of such impulses prevents us from taking dangerous risks, or behaving in a deviant way, and facilitates our living as a community (Nakamura-Palacios *et al*., 2014)

1.3 Justification of the Study of the Study

Alcohol is part of our society as it is used for social and religious ceremonies. But drinking too much on a single occasion or over time can have serious consequences for our health.

Alcoholism-related cortical changes have been documented throughout the brain, many studies consistently have found the frontal lobes to be more vulnerable to alcohol-related brain damage than other cerebral regions (Dirksen *et al.,* 2006).However, Elias *et al*., (1999) reported that moderate alcohol intake may be beneficial to cognitive activities and cardiovascular diseases from epidemiology research but there is paucity of literatures to backup this report from animal models.

Hence, this study will significantly contribute to the literature on the effect of chronic moderate alcohol intake in animal models.

1.4 Aim and Objectives of the Study

14.1 Aim of the Study

This study was aim at evaluating the effect of chronic alcohol consumption on the frontal lobe of the cerebrum of adult Wistar rats

1.4.2 Objectives of the Study

The objectives of the study were to evaluate the frontal lobe following of chronic alcohol administration;

i. on histo-architecture of frontal lobe.
ii. on cognitive activities, aggression and anxiety using neuro-behavioural tests.
iii. on activities of superoxide dismutase and catalase, glutathione and malnoaldehyde level.

1.5Research Hypothesis

Chronic oral administrationof low dose of alcohol haseffect on the frontal lobe of the Wistar rats.

2.0 LITERATURE REVIEW

2.1 Alcohol Absorption

The alcohol molecule is a small polar molecule with molecular weight of 46.068 g/mol and density of 0.7892g/ml having both lipophilic and hydrophilic characteristics (Ferreira and Willoughby, 2008). The amphipathic qualities of alcohol help to explain its pharmacokinetics within the body. The lipophilic qualities explain how alcohol is absorbed by passive diffusion across the cell membranes without the need for modification. The hydrophilic combined with the polar properties of the alcohol molecule explain how alcohol is completely soluble in water and thus has a similar volume of distribution to total body water (TBW).

Blood alcohol concentration (BAC) is determined by the various factors that affect the rate atwhich alcohol is absorbed (including the amount and concentration of alcohol ingested and the quantity and composition of food in the stomach),distributed, metabolized and excreted from the body (Mumenthaler *et al.*, 1999). Following oral administration absorption and distribution determines the proportion and rate at which orally ingested alcohol reaches the blood and body tissues (bioavailability).

As alcohol is a small water soluble molecule that can cross cell membranes, it is absorbed from both the stomach (20 %) and the upper small intestine (80 %) (Paton, 2005 and Norberg *et al.*, 2003). The rate of absorption varies significantly in both intra-individual and inter-individual comparisons even after standardised conditions (Fraser, 1997). This suggests that intra-individual variability is due to variation in gastrointestinal function (gastric emptying, intestinal transit time, and portal blood flow). The rate of gastric emptying has a significant impact on the speed at which alcohol is absorbed, because alcohol isabsorbed much faster from the small intestine, than it is from the stomach (Fraser, 1997). Factors that affect alcohol availability and gastric emptying

will greatly influence the rate of absorption. For example, theconsumption of alcohol with food inhibits absorption because approximately 20% of the ingested alcohol isoxidised before it can be absorbed (Paton, 2005 and Norberg *et al.*, 2003). The speed ofabsorption is also influence by variation in portal blood flow because alcohol crosses the biologicalmembrane by passive diffusion (Paton, 2005; Ferreira and Willoughby, 2008) thus good blood flow will maintain the concentration gradient andpromote absorption. Any stimulation of the sympathetic nervous system (e.g. emotional state orexercise) will reduce portal blood flow and gastric motility thus decreasing alcohol absorption. The typeof drink consumed also plays a role. Drinks with alcohol content between 20-30% are absorbed quickest (Paton, 2005). Whereas drinks with a higher alcohol content are absorbed more slowly, because an alcohol content over 30% irritates the gastric mucosa increasing mucus secretion and decreasing gastric emptying. Thus, drinks with an alcohol content above 30% can cause a faster rise in BAC if served diluted with a mixer, than if they are serve without dilution. This is especially true if the mixer is a carbonated drink as this can also increase the rate of absorption (Paton, 2005).

2.2 Alcohol Distribution

The bioavailability of alcohol is reduced by first pass metabolism (FPM). Oxidation of alcohol by gastric alcohol dehydrogenase (ADH) in the gastric mucosa accounts for a small proportion of FPM, but the majority occurs via oxidation by ADH in the liver hepatocytes (Ferreira and Willoughby, 2008; Vonghia, *et al.*, 2008; Paton, 2005; Fraser, 1997). The proportion of alcohol that is absorbed, and escapes FPM enters the systemic circulation and is rapidly distributed throughout the body tissues via the blood plasma until an equilibrium between the BAC and tissue concentration is reached (Ferreira and Willoughby, 2008). The time until equilibrium is

dependent upon the permeability (water content), rate of blood flow and mass of the tissue (Mumenthaler, *et al.,* 1999), but is generally achieve within 1-2 hours (Ferreira and Willoughby, 2008). The same amount of alcohol absorbed can affect different people in different ways (Paton, 2005). Differences in TBW will influence alcohol pharmacokinetics because it determines the volume of distribution available for alcohol distribution within the body. Alcohol is preferentially distributed in tissues with higher water contents and a good blood supply (e.g. brain and skeletal muscle). Body composition is therefore an important consideration in pharmacokinetic studies (Jones, 2007) because both body size and composition will have a significant impact on the volume of distribution. Females generally have a proportionately smaller lean body mass and a smaller blood volume (Paton, 2005 and Mumenthaler, *et al.,* 1999). The result is a lower volume of distribution and higher BAC when females ingest the same amount of alcohol as men (Mumenthaler, *et al.,* 1999). It has also been suggested that higher BAC may be due to lower FPM by gastric ADH in the gastric mucosa of females (Vogel-Sprott, 1992). This would increase the bioavailability of alcohol resulting in increased BAC.

However, the ability of gastric ADH to metabolizesignificant amounts of alcohol has been questionedbecause its activity is 100 times lower than hepatic ADH (Fraser, 1997) and more recent studies have failed tosupport this finding (Yin, *et al.,* 1997).

2.3 Alcohol Metabolism

The process by which a substance is metabolize before entering the general circulation is called first pass metabolism (FPM). Some of the alcohol which is ingested orally does not enter the systemic circulation but may be oxidized in the stomach by ADH isoforms such as σADH and class I and class III ADH.This first pass metabolism could modulate alcohol toxicity since its

efficiency determinesthe bioavailability of alcohol. Ethanol is rapidly pass into the duodenum from the stomachin the fasted state. This will minimize first pass metabolism and thereby play a role in thehigher blood alcohol concentrations observed in the fasted versus the fed state (Arthur, 2012).

The major enzyme system(s) responsible for the oxidation of ethanol, alcohol dehydrogenase, and to a lesser extent, the cytochrome -dependent ethanol-oxidizing system, are present to the largest extent in the liver. In the first step of hepatic FPM of alcohol, an oxidative enzyme called alcohol dehydrogenase (ADH) converts alcohol into acetaldehyde (Acetaldehyde is subsequently oxidizing to acetate by an enzyme other than ADH. Acetate can be metabolizing further and eliminated from the body or incorporated into various physiological substances) (Martin*et al.*, 1999).

Hepatic FPM is most significant with low to moderate alcohol doses of about 0.4grams per kilogram of body weight (g/kg), which is equivalent to about two standard drinks (A standard drink contains approximately 13.5grams of alcohol (Eckardt *et al.*, 1998) and is defined as 12 ounces of beer, 5 ounces of wine, or 1.5 ounces of distilled spirits.) for a person weighing 70 kg (approximately 150 pounds). The extent of FPM in the liver depends on the rate of absorption of alcohol from the GI tract. If absorption is fast, the quantity of alcohol reaching the liver can exceed the metabolic capacity of available ADH. This allows a greater proportion of alcohol to escape FPM and reach the general circulation, resulting in a higher peak BAC. Conversely, slow absorption leads to a higher FPM and a lower peak BAC. For example, the presence of food in the stomach leads to slower alcohol absorption, hence more efficient FPM and a lower peak BAC. The time from last drink to peak BAC usually ranges from 30 to 90 minutes (Hardman and Limbird, 1995). Some FPM may occur in the GI tract (mostly in the stomach) as well as in the

liver (Yin *et al.,* 1997). Although earlier research reported decreasedgastric ADH activity in women compared to men (Frezza *et al.,* 1990), the relative contribution of gastric ADH activity to overall FPM in both genders is controversial. One study found the activity of gastric ADH in the rat to be approximately 14 percent that of hepatic ADH (Lee *et al.*, 1992).

Alcohol also is metabolized in non-liver (i.e., extrahepatic) tissues that do not contain ADH, such as the brain, by the enzymes cytochrome and catalase. In general, alcohol metabolism is achieved by both oxidative pathways, which either add oxygen or remove hydrogen (through pathways involving ADH, cytochrome, and catalase enzymes), and non-oxidative pathways (Samir, 2006).

2.4 Alcohol Elimination

Alcohol elimination was originally believed to be a zero-order process, meaning that alcohol was removed from the body at a constant rate, independent of the concentration of alcohol. Since the Km of most ADH isozymes for ethanol is low (about 1 mM), ADH is saturated at low concentrations of alcohol, hence, the overall elimination process proceeds at maximal velocity and is independent of the alcohol concentration. However, linearity is not observed at low alcohol concentration since ADH is no longer saturated with ethanol (Arthur, 2012). Alcohol Elimination of absorbed ethanol occurs primarily through metabolism (95–98%), with small fractions of the administered dose being excrete unchanged in the breath (0.7%), sweat (0.1%), and urine (0.3%) (Holford, 1987). Alcohol can be oxidized by ADH in various organs, including the stomach and small intestine. Quantitatively, most alcohol metabolism occurs in the liver. Between 90% and 98% of alcohol that enters the body is eventually completely oxidize. The rate of alcohol metabolism is related to blood alcohol concentration (BAC). At BACs below

approximately 0.02% (A BAC of 0.10 percent (or less, depending on theState) is the legal limit for operating a motor vehicle (Martin, 1999)),the rate of alcohol metabolism isexponential. At higher BACs, the functionalcapacity of the ADH systembecomes saturated, and the alcoholelimination rate remains relatively constant between approximately 0.020% and 0.065% BAC.The rate of elimination of larger doses of alcohol increases as BAC increases (Holford, 1987). There is a 3 – 4 folds' variability in the rate of alcohol elimination by humans because of various genetic and environmental factors described below.

i. **Sex:** There is a faster rate of alcohol elimination by women when rates are corrected for lean body mass. Since women have smaller body size and therefore smaller lean body mass,ethanol elimination per unit lean body mass is higher in women. Men and women generallyhave similar alcohol elimination rates when results are express as gram per hour or g per literliver volume. Because of the first pass metabolism by the stomach, it is possible that a given oral dose of alcohol will produce a higher blood ethanol concentration in females thanmales (Cole-Harding and Wilson, 1987 andKwo *et al.*, 1998).

ii. **Age:** Very young animals have low alcohol elimination rates because ADH (and CYP2E1) are not fully express. Fetal liver eliminates alcohol very poorly which may have consequences for fetal alcohol syndrome. There may be a small decline in alcohol elimination with aging,perhaps due to decreased liver mass, or body water content (Arthur, 2012).

iii. **Race:** Elimination of alcohol is reported to be somewhat higher in subjects expressing the beta-3 classI ADH isoforms compared with individuals who only express the beta 1 isoform. Some studies, but not all, suggest an increased rate of

alcoholelimination by Native Americans compared to Caucasians. Rates of alcohol elimination byChinese are similar to those of Caucasians. Liver mass may explain ethnic and genderdifferences in alcohol elimination rates. More research on possible population differences inalcohol elimination is required (Reed *et al.*, 1976 and Bennion and Li, 1976).

iv. **Food**: Alcohol metabolism is higher in the fed nutritional state as compared to the fasted state because ADH levels are higher, and the ability of substrate shuttle mechanisms to transport reducing equivalents into the mitochondria is elevated. Food may also increase liver blood flow. The sugar fructose increases alcohol metabolism by providing substrates, which help to convert NADH to NAD+, and by enhancing mitochondrial oxygen uptake. The increase in the alcohol elimination rate by food was similar for meals of different compositions as there was no difference between carbohydrate, fat and protein on alcohol metabolic rate (Passanati *et al.*, 1990 andWissel, 1987).

v. **Biological Rhythms**: The rate of alcohol elimination varies with the time of day, being maximal at the end of thedaily dark period. This may be related to a body temperature cycle (Arthur, 2012).

vi. **Exercise**: unclear literature, most studies report a small increase in alcohol elimination rate, perhaps due to increased body temperature or catecholamine release.

vii. **Alcoholism:** Heavy drinking increases alcohol metabolic rate. Advanced liver disease will decrease the rate of ethanol metabolism (Martin, 1999).

viii. **Drugs:** Agents which inhibit ADH (pyrazoles, isobutyramide) or compete with ethanol for ADH(methanol, ethylene glycol) or which inhibit the mitochondrial

respiratory chain willdecrease the alcohol elimination rate. Antabuse (disulfiram) by inhibiting the elimination ofacetaldehyde slows alcohol metabolism (Arthur, 2012).

2.5 Anatomy of the Cerebrum

The **cerebrum** is a large part of the brain containing the cerebral cortex (of the two cerebral hemispheres), as well as several subcortical structures, including the hippocampus, basal ganglia, and olfactory bulb. In the human brain, the cerebrum is the uppermost region of the central nervous system. The telencephalon is the embryonic structure from which the cerebrum develops prenatally. In mammals, the dorsal telencephalon, or pallium, develops into the cerebral cortex, and the ventral telencephalon, or subpallium, becomes the basal ganglia.

The cerebrum is divided into approximately symmetric left and right cerebral hemispheres (Arnould-Taylor, 1998).The cerebrum is the largest part of the brain. Depending upon the position of the animal it lies either in front or on top of the brainstem. In humans, the cerebrum is the largest and best developed of the five major divisions of the brain. The cerebrum is the newest structure in the phylogenetic sense, and in mammals, it is the largest and most developed, out of all known species and tend to have thicker cortices (Nieuwenhuys *et al*. 1998).

The cerebrum is made up of the two cerebral hemispheres and their cortices, (the outer layers of grey matter), and the underlying regions of white matter (Arnould-Taylor, 1998). Its subcortical structures include the hippocampus, basal ganglia and olfactory bulb. The cerebrum consists of two cerebral hemispheres, separated from each other by a deep fissure called the longitudinal fissure.

The cerebral cortex, the outer layer of gray matter of the cerebrum, is found only in mammals. In larger mammals, including humans, the surface of the cerebral cortex folds to create gyri (ridges) and sulci (furrows) which increase the surface area (Angevine and Cotman, 1981).

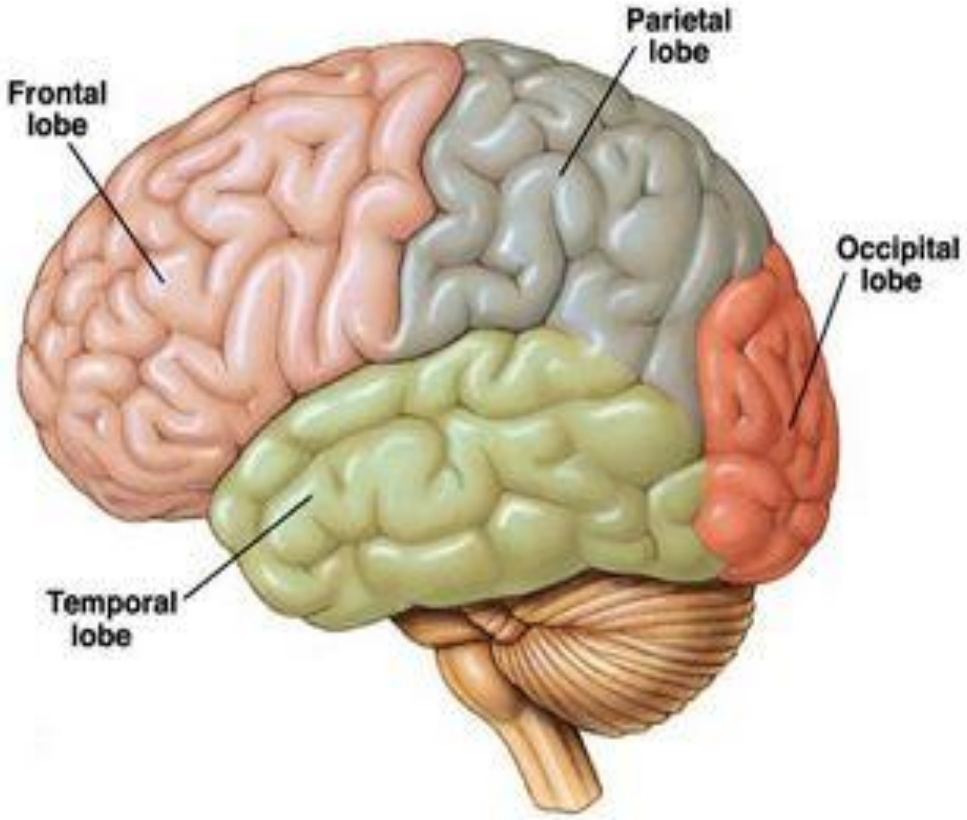


Figure 2.1: The lobes of the cerebral cortex (Falk *et al*., 2013).

The cerebral cortex is generally classified into four lobes: the frontal, parietal, temporal, and occipital lobes. The lobes are classify based on their overlying neurocranial bones (Rosdahl and Kowalski, 2008).

2.5.1Anatomy Frontal Lobe

The frontal lobe extends from the frontal pole to the central sulcus**.** On its lateral aspect, the frontal lobe extends from the frontal pole to the central sulcus, constituting the anterior one-third of the cerebral cortex. Its posteriormost gyrus, the precentral gyrus, consists of the primary motor area and is bordered anteriorly by the precentral sulcus and posteriorly by the central sulcus. The region of the frontal lobe located anterior to the precentral sulcus is subdivided into the superior, middle, and inferior frontal gyri. This subdivision is due to the presence, though inconsistent, of two longitudinally disposed sulci, the superior and inferior frontal sulci. The inferior frontal gyrus is demarcated by extensions of the lateral fissure into three subregions: the pars triangularis, pars

opercularis, and pars orbitalis. In the dominant hemisphere, a region of the inferior frontal gyrus is known as Broca's area, which functions in the production of speech.

On its inferior aspect, the frontal lobe presents the longitudinally disposed olfactory sulcus. Medial to this sulcus is the gyrus rectus (also known as the straight gyrus), and lateral to it are the orbital gyri. The olfactory sulcus is partly occupied by the olfactory bulb and olfactory tract. At its posterior extent, the olfactory tract bifurcates to form the lateral and medial olfactory striae. The intervening area between the two striae is triangular and is known as the olfactory trigone and it abuts the anterior perforated substance. On its medial aspect, the frontal lobe is bordered by the arched cingulate sulcus, which forms the boundary of the superior aspect of the

cingulate gyrus. The quadrangular-shaped cortical tissue anterior to the central sulcus is a continuation of the precentral gyrus and is known as the anterior paracentral lobule (Maria and Leslie, 2006).

2.5.1.1 Functional Regions of Frontal Lobe

2.5.1.1.1 Primary Motor Cortex (M1, Brodmann area 4)

The primary motor cortex is located on the precentral gyrus just rostral to the central sulcus. It is the source of cortical neurons that will project to the brainstem and spinal cord to activate neurons involved in the control of voluntary movements. It receives input from the neighboring primarysomatosensory area (S1, on the postcentral gyrus) and premotor cortex, as well as from the ventrallateral nucleus of the thalamus (a relay nucleus with projections from the cerebellum). These inputs modulate the output of M1 by providing information about the positioning, timing, and coordination of voluntary movements. The output of M1 goes by way of the internalcapsule to synapse in the brainstem (the projection referred to as the corticobulbar tract) or the spinal cord (the corticospinal tract). Damage to M1 will cause contralatereral motor deficits, initially a flaccid hemiplegia/hemiparesis and later a spastic hemiplegia/hemiparesis. Depending on the extent of cortical damage, these deficits may be localized to a specific region of the body or can be more widespread (*Alexandruet al., 2012)*.

2.5.1.1.2 Premotor Cortex (BA6)

The premotor cortex is located immediately rostral to M1. Its primary function is to assist in integration of sensory and motor information for the performance of an action (praxis). Thus it receives input from secondary somatosensory area (immediately caudal to S1 in the parietal

cortex) and the ventral anterior thalamic nucleus (a relay nucleus with projections from the basal ganglia, which themselves are a group of subcortical nuclei that modulate motor activity). The output of premotor cortex is to M1 and contralateral premotor area (by way of the corpus callosum). Damage to premotor cortex may result in apraxia; an acquired inability to carry out skilled actions that could previously be performed (but without paralysis), deficits in contralateral fine motor control, such as the performance of complex serial movements, and difficulty in using sensory feedback for the control and performance of movements (*Alexandruet al., 2012)*.

2.5.1.1.3 Frontal eye fields (BA8)

The frontal eye fields are located rostral to premotor cortex. Their primary function is associated with control of voluntary eye movements in the contralateral visual field for processes such as active visual search. Their connections with the rest of the brain are complex and beyond the scope of this discussion. Damage to the frontal eye fields will cause deficits in voluntary eye movement to the contralateral visual field (leading to active visual search deficits), but preserved passive eye movement (as in the following of a moving object) (*Alexandruet al., 2012)*.

2.5.1.1 4 Dorsolateral Prefrontal Cortices (BAs 45-49)

The dorsolateral prefrontal cortex makes up the largest proportion of frontal cortex, located rostral to the frontal eye fields and superior to orbitofrontal cortex. The functions of this region of the brain fall under the heading of "executive" processes, which in a general sense involves the ability to utilize sensory input from multiple modalities (ie. visual, auditory) in generation of appropriate responses. Its connections with the rest of the brain are extensive, but one circuit of

considerable importance involves input from the thalamus (primarily ventral anterior and mediodorsal nuclei) and output to the caudate nucleus of the basal ganglia. The function of the dorsolateral cortex is probably best reflected in the tasks used to assess dysfunction of this region (*Alexandruet al., 2012)*.

2.5.1.1.5 Cingulate Cortex/Supplementary Motor Area (BAs 24, 32)

The cingulate cortex is located in the medial portion of the cortex just superior to the corpus callosum. The supplementary motor area is located medial to the premotor cortex just anterior to M1. These regions of the brain have functions that are involved with drive and motivation along with environmental exploration. Their connections are with deep limbic structures of the brain (ie. basal forebrain structures such as the nucleus accumbens). Dysfunction in the cingulate/SMA are associated with several uniquely bizarre characteristics, including apathy and akinetic mutism (reflecting a loss of drive and motivation) along with complex attentional deficits and delayed habituation to external stimuli. The alien hand syndrome may also be present, whereby patients report experiencing a loss of conscious control over the movements and actions of their hand, which proceeds to "explore" the surrounding environment by, for instance, unbuttoning clothes (*Alexandruet al., 2012)*.

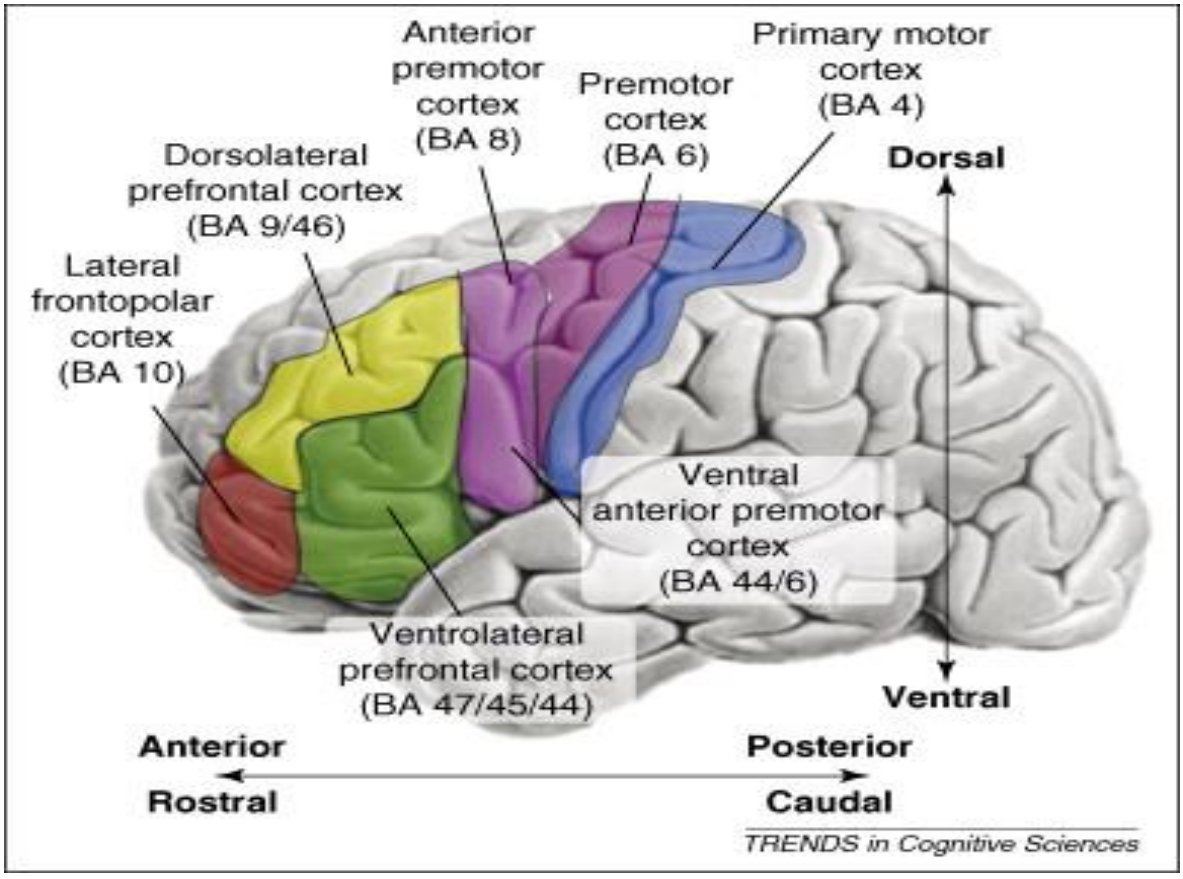


Figure 2.2: Functional region of frontal lobe.

2.5.2Histology of the Cerebral Cortex

The cerebral cortex is the layer of gray matter capping the white matter core of the cerebral hemispheres. Its thickness varies from 1.5 to 4.5 mm, with an average thickness of 2.5 mm. The cerebral cortex is thickest in the primary motor area (4.5 mm thick) and thinnest in the primary visual cortex (1.5 mm thick). The cortex is irregularly convoluted, forming gyri separated by sulci or fissures. The outer layer of the human cerebral cortex is around 0.2 m^2, but only one-third of this area is exposed to the surface, the rest being buried in sulci or incorporated in the insula. The number of neurons in the cerebral cortex is estimated at between 10 and 20 billion. Morphometric studies of the cerebral cortex in males and females demonstrate no gender-based differences in cortical thickness. On the other hand, they show neuronal density to be higher in the male with a reciprocal increase in neuropil and neuronal processes in the female. A relatively small area of the cerebral cortex in humans is specialized for receiving sensory input from the eyes, ears, and skin and for projecting motor output down the pyramidal tract to bring about movement. More than 80 percent of the cortex in humans serves an association function specially related to integrative and cognitive activities such as language, calculation, planning, and abstract reasoning (Afifi and Bergman, 2005).

The neurons of the cerebral cortex are of two functional categories; principal (projection) neurons and interneurons. The principal neurons provide corticocortical and corticosubcortical outputs. Interneurons are concerned with local information processing. Recent evidence suggests that the two neuronal types are generated in distinct proliferative zones. Principal neurons are derived from neuroepithelium in the ventricular zone. Interneurons, in contrast, arise from the

ganglionic eminence of the ventral telencephalon, which gives rise also to the basal ganglia. The cerebral cortex has its full complement of neurons (10 to 20 billion) by the 18th week of intrauterine life(Afifi and Bergman, 2005).

2.5.2.1 Principal (Projection) Neurons

Two types of cortical neurons belong to the principal category. They are the pyramidal neurons and the fusiform neurons. The excitatory neurotransmitter in both is glutamate or aspartate. Principal neurons constitute more than half of all cortical neurons(Afifi and Bergman, 2005).

2.5.2.1.1 Pyramidal Neurons

These neurons derive their name from their shape. The apex of the pyramid is directed toward the cortical surface. Each pyramidal neuron has an apical dendrite directed toward the surface of the cortex and several horizontally oriented basal dendrites that arise from the base of the pyramid. Branches of all dendrites contain numerous spines that increase the size of the synaptic area. A slender axon leaves the base of the pyramidal neuron and projects on other neurons in the same or contralateral hemisphere or else leaves the cortex to project on subcortical regions. The axon gives rise within the cortex to two types of axon collaterals. These are the recurrent axon collaterals (RACs), which project back on neurons in more superficial layers, and the horizontal axon collaterals (HACs), which extend horizontally to synapse on neurons in the vicinity. Pyramidal neurons are found in all cortical layers except layer I. They vary in size; most are between 10 and 50 µm in height. The largest are the giant pyramidal cells of Betz, which measure about 100 µm in height and are found in layer V of the motor cortex(Afifi and Bergman, 2005).

2.5.2.1.2 Fusiform, Spindle Neurons

These are small neurons with elongated perikarya in which the long axis is oriented perpendicular to the cortical surface. A short dendrite arises from the lower pole of the perikaryon and arborizes in the vicinity. A longer dendrite arises from the upper pole of the perikaryon and extends to more superficial layers. The axon enters the deep white matter. Fusiform neurons are found in the deepest cortical laminae(Afifi and Bergman, 2005).

2.5.2.2 Interneurons

Several types of cortical interneurons are recognized on the basis of dendritic architecture. They include the stellate neurons, the horizontal cells of Cajal, and the cells of Martinotti(Afifi and Bergman, 2005).

2.5.2.2.1 Stellate or Granule Neurons

These are small (4 to 8 μm) star-shaped neurons with short, extensively branched, spiny dendrites and short axons. They are most numerous in lamina IV.

Stellate cells are the only type of excitatory interneurons in the cortex. The neurotransmitter is glutamate. All other interneurons exert inhibitory influence by gamma-aminobutyric acid (GABA) (Afifi and Bergman, 2005).

2.5.2.2.2 Horizontal Cells of Cajal

These are small fusiform neurons with their long axes directed parallel to the cortical surface. A branching dendrite arises from each pole of the perikaryon, and an axon arises from one pole. The dendrites and axon are oriented parallel to the cortical surface. The horizontal cells of Cajal

are found only in lamina I and disappear or are rare after the neonatal period(Afifi and Bergman, 2005).

2.5.2.2.3 Cells of Martinotti

Giovanni Martinotti in 1890 first described cells whose axons ascend toward the surface of the cortex. Martinotti neurons are multipolar with short branching dendrites and an axon that projects to more superficial layers, giving out horizontal axon collaterals en route. The Martinotti neurons are found in deeper cortical laminae(Afifi and Bergman, 2005).

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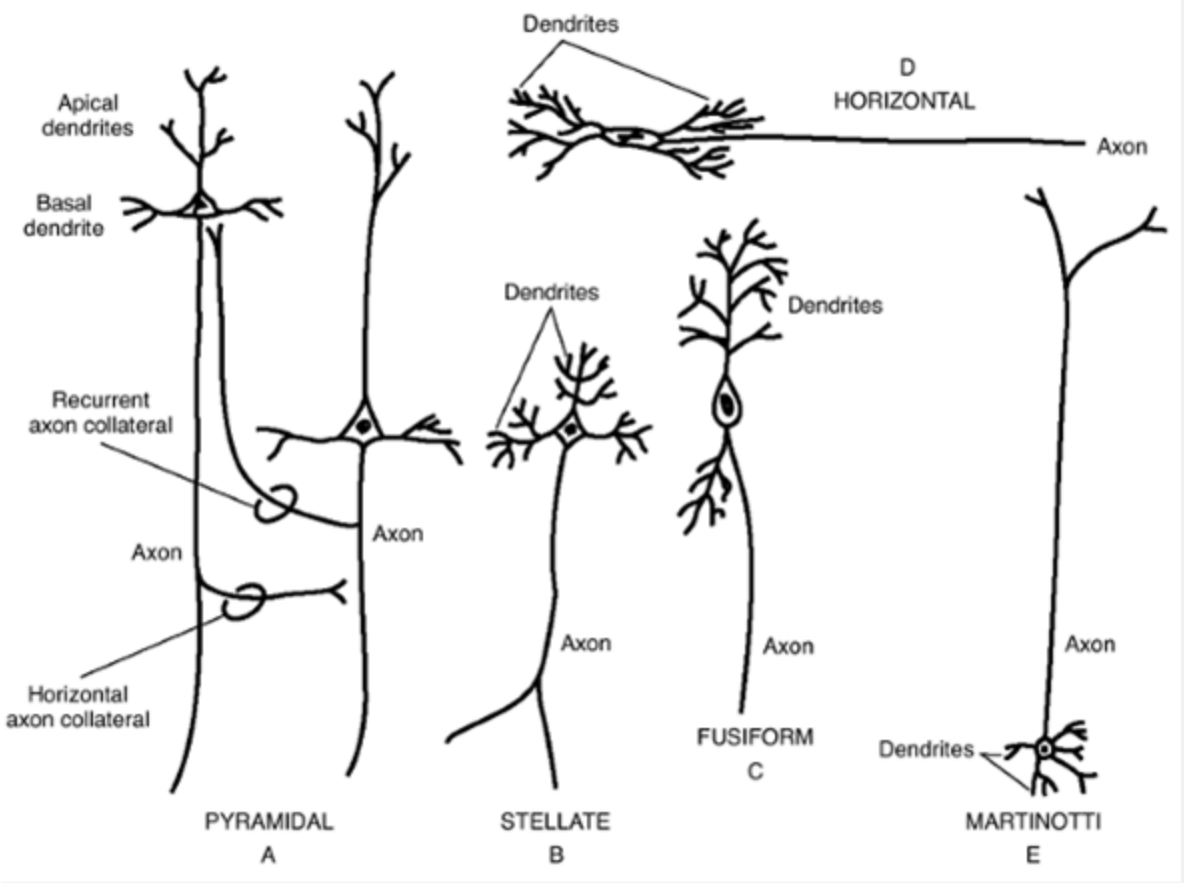


Figure 2.3: Schematic diagram of the various types of cortical neurons (Afifi and Bergmen, 2005).

2.5.3 Layers

The six layers of the neocortex are recognizable by about the seventh month of intrauterine life. The neurons in the six cortical layers develop in waves from the periventricular germinal matrix. Successive waves of migrating neuroblasts become situated progressively farther away from the germinal matrix (inside-out gradient of cortical histogenesis). Interruption of the normal process of migration or its arrest is associated with cortical gyral malformations such as agyria, pachygyria,micropolygyria, and heterotopia. Many of these are associated with mental retardation, seizures, and other neurologic deficits(Afifi and Bergman, 2005).

2.5.3.1 Layer I (Molecular, Plexiform)

Layer I consists primarily of a dense network of nerve cell processes among which are scattered sparse interneurons (horizontal cells of Cajal) and neuroglia. The nerve cell processes in this layer comprise projection axons from extracortical sites as well as axons and dendrites of neurons in other cortical areas. This layer of the cortex is primarily a synaptic area(Afifi and Bergman, 2005).

2.5.3.2 Layer II (External Granular)

Layer II consists of a dense packing of small and medium-sized pyramidal neurons and interneurons intermingled with axons from other cortical layers of the same and opposite hemispheres (association and commissural fibers), as well as axons and dendrites passing through this layer from deeper layers. The dendrites of pyramidal neurons in this layer project to layer I, while their axons project to deeper layers. This layer of the cortex contributes to the complexity of intracortical circuitry(Afifi and Bergman, 2005).

2.5.3.3 Layer III (External Pyramidal)

Layer III consists of pyramidal neurons that increase in size in deeper parts of the layer. The dendrites of neurons in this layer extend to layer I, while the axons project to other layers within the same and contralateral hemisphere (association and commissural fibers) or leave the hemisphere as projection fibers to more distant extracortical sites. This layer receives primarily axons of neurons in other cortical areas (association and commissural fibers), as well as axons of neurons in extracortical regions such as the thalamus. This layer contains the distinctive stripes of Kaes-Bekhterev(Afifi and Bergman, 2005).

2.5.3.4 Layer IV (Internal Granular)

Layer IV consists of pyramidal cells and densely packed small stellate cells with processes that terminate within the same layer, either on axons of other stellate cells or on axons of cortical or subcortical origin passing through this layer. The cell packing density in layer IV is the greatest of all cortical layers. Few of the larger stellate cells in this layer project their axons to deeper cortical layers. Layer IV is especially well developed in primary sensory cortical areas. The internal granular layer is the major recipient of thalamocortical fibers from modality-specific sensory relay nuclei (visual radiation, auditory radiation, and primary sensory radiation)(Afifi and Bergman, 2005).

2.5.3.5 Layer V (Internal Pyramidal)

This layer consists of large and medium-sized pyramidal cells, stellate cells, and cells of Martinotti. The cell packing density in this layer is the lowest of all cortical layers. The largest

pyramidal cells in the cerebral cortex (cells of Betz) are found in this layer (hence the name ganglionic layer). Dendrites of neurons in this layer project to the more superficial layers. Axons project on neurons in other cortical areas but mainly to subcortical sites (projection fibers) except the thalamus, which receives fibers from layer VI. This layer receives axons and dendrites arising in other cortical sites or in subcortical sites. It is also traversed by a dense band of horizontally oriented fibers; this is the internal band of Baillarger. Fibers originating in thalamic sensory nuclei contribute heavily to the formation of the lines of Baillarger, especially the outer one in lamina IV. The lines of Baillarger are thus prominent in primary cortical sensory areas(Afifi and Bergman, 2005).

2.5.3.6 Layer VI (Multiform)

Layer VI consists of cells of varying shapes and sizes, including fusiform cells and the cells of Martinotti, which are prominent in this layer. Dendrites of smaller cells arborize locally or in adjacent layers, while those of large neurons reach the molecular layer. Axons of neurons in this layer project to other cortical laminae or to subcortical regions.

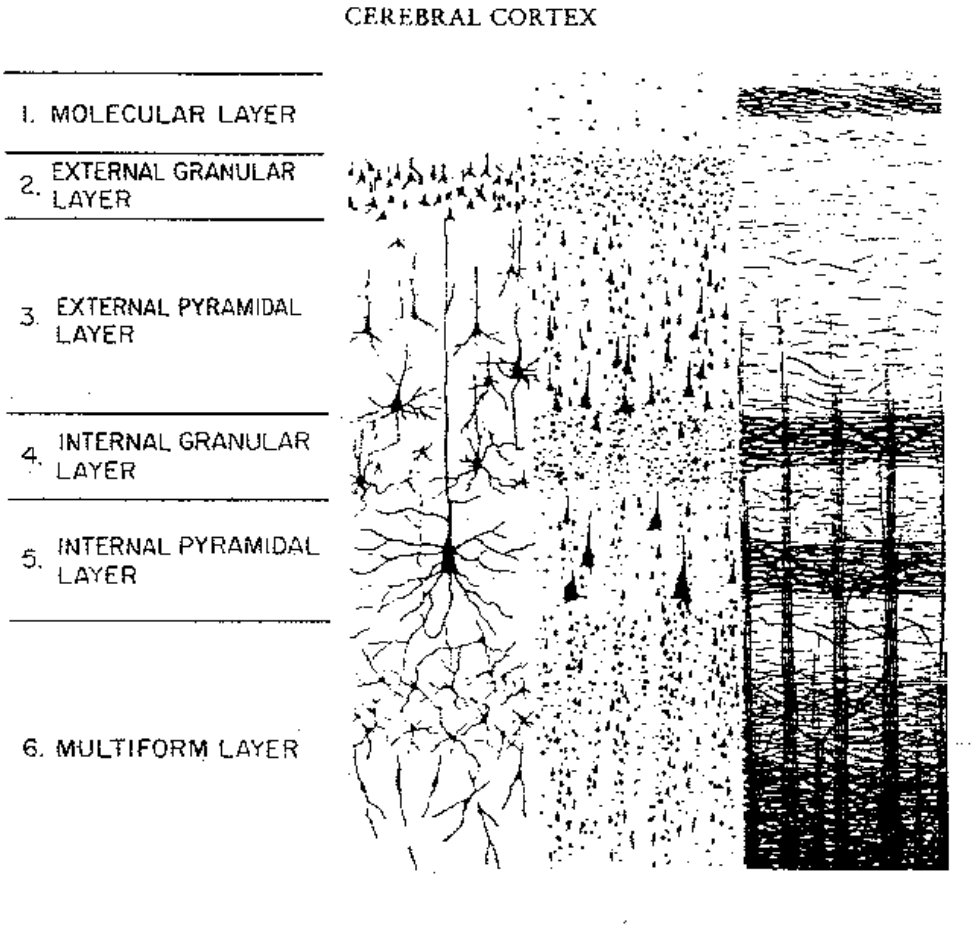


Figure 2.4: Layers of the cerebrum cortices (Ben, 1990).

2.6 Effect on Alcohol on Body and Organ Weight

In terms of caloric content, ethanol is a unique drug whose oxidation by alcohol dehydrogenase (ADH) produces 7.1 kcal per gram of ethanol oxidized. However, it is not known to what extent these calories are converted into body weight (Aguiar*et al.*, 2004).Pirola and Lieber (1976) reported that activation of the microsomal ethanol oxidizing system (MEOS) in hepatocytes of alcoholics might consume one third of the caloric content of ethanol and impair ATP synthesis. Lands and Zakhari (1991) suggested the existence of a futile cycle in ethanol metabolism, which would account for a loss of six ATPs per gram of ethanol oxidized. They proposed that ethanol may be oxidize to acetaldehyde, consuming three ATPs, and acetaldehyde may be again reduce to acetate, consuming three extra ATPs (Lands and Zakhari, 991).

However, there is some controversy about what happens to ethanol calories when consumption is moderate and not related to the metabolic changes of the liver that characterize chronic ethanol intake. It has been suggested that calories derived from ethanol may function as do calories derived from carbohydrates, producing a thermogenesis value of about 10% (Murgatroyd *et al.*, 1996 and Sonko *et al.*, 1994). This thermogenic value may be higher than that of fatty food (3%) and lower than that of proteins (about 20%) (Equier, 1999).

The contribution of calories provided by ethanol to body weight gain has also been studied but there seems to be no consensus about the data from animal and human studies.

Moderate ethanol consumption seems to favor an increase in fat storage and could result in weight gain, especially in overweight individuals (Lands, 1993). In an epidemiological study involving 89,538 women and 48,493 men, Colditz *et al.* (1991) reported a strong negative association between alcohol intake and body weight index for women and the absence of this association for men. Animal studies are consistent in reporting a decrease in the body weight of

rats receiving ethanol solutions as the only source of liquids. Concentrations of ethanol as low as 5% (v/v), which are similar to the ethanol content of a Brazilian beer, or as high as 40% (v/v), solution similar to spirit drinks, are related to decreased body weight gain (Macieira*et al.*, 1997). Similar results have been reported for 20% (v/v) ethanol solution (Laure-achagiotis *et al.*, 1990). Different results have been obtained for malnourished animals. Da-Silva et al. (1982), studying rats which had been treated with ethanol for 90 days, reported a significant weight gain by malnourished rats (50% food restriction) drinking a 20% (v/v) ethanol solution when compared to malnourished rats drinking water. He also reported improvement in somatic and motor development and a decrease in the mortality rate of the offspring of malnourished rats drinking low doses of ethanol (5%, v/v). These data suggest that malnourished rats can benefit from ethanol calories.Aguiar *et al.* (2004) suggest that well-nourished rats cannot utilize ethanol-derived calories and will lose weight when receiving a 10% ethanol concentration. Although the amount of calories ingested by well-nourished rats taking water and well-nourished rats taking ethanol was the same, they were not utilized to the same extent, in agreement with most literature reports on the effects of ethanol. This may be related to the toxicity of ethanol resulting in substrate maldigestion or nutrient malabsorption due to pancreatic, liver and small intestine complications (Bode and Bode, 1997; Organ, 1982). Taken together, these effects would result in malnutrition. Additionally, ethanol only produces energy when oxidized by ADH. Literature data suggest that there is a limit for the action of ADH, corresponding to 1.1gram ethanol per day in rats (Laure-Achagiotis *et al.*, 1990). Higher ethanol levels would activate the MEOS system, which would involve energy expenditure instead of energy production. Another explanation for energy consumption is the dehydration produced by the exclusive consumption of ethanol. It is therefore reasonable that rats drinking ethanol will lose weight (Aguiar *et al.*, 2004).

It is difficult to understand what could be occurring to malnourished rats. Ethanol-derived calories can be used for body weight up to the concentration of 20% (v/v). Would the expected toxicity of ethanol previously described be observed only at the highest concentration (40%)? Was ADH not saturated and was the MEOS not activated as expected at the 1.0 g/day concentration? Perhaps, the answers to these questions could be found in the important and complex physiological changes that occur when the food restriction model of malnutrition is imposed on rats. Since the restricted amount of food offered is consumed quickly, long periods of fasting would occur. Therefore, we should consider the changes that may occur in metabolism, especially those aimed at preserving the brain under fasting conditions. Since glucose is the only substrate for the brain, when it is not available from the diet, adaptations will be expected to occur in order to provide for it. Gluconeogenesis will occur at a high rate, using glycerol and amino acids as substrates. Only later will the brain adapt to using ketone bodies as substrates and to preserving proteins to some extent. It is reasonable to assume that ethanol calories will delay the proteolytic process by offering energy in the form of acetate and acetyl CoA to systemic functions, thus preserving glucose for brain utilization. Therefore, body weight loss would be retarded, resulting in a decreased weight loss among malnourished rats drinking ethanol. In fact, ADH activity per gram of liver seems to be increased among malnourished rats (Da-Silva and McLean, 1988). However, the process of taking calories to preserve body weight would be limited at higher ethanol doses due to the toxicity of the drug and to the well-known limits of the metabolic capacity of ADH (Aguiar *et al*., 2004).

2.7 Working Memory

From the published results in a vast neuropsychological, physiological, and imaging literature (Fuster, 2001), we now know that the mediation of cross-temporal contingencies, and therefore temporal integration, rely on two time-bridging functions of the lateral prefrontal cortex: working memory and set.

Working memory (Baddeley, 1986) is the temporary retention of an item of information; e.g., a sensor cue: for the solution of a problem or for a mental operation. Working memory is memory for the short term,rather than short-term memory. It is attention focused on an internal representation. I argued that working memory essentially consists of the temporary activation of a widely distributed cortical network of long-term memory. My argument is basedon the evidence that, during the short-term retention of sensory information for a prospective act, neurons inwidespread areas of the cortex exhibit sustained activation. Further, the working memory of a given stimuluscan elicit sustained neuronal activation in several areas of the cortex at the same time. The neuronal correlatesof working memory were first discovered, and havebeen repeatedly confirmed, in the prefrontal cortex of monkeys performing delay tasks (Fuster and Alexander,1971; Fuster *et al.*, 1982; Funahashi *et al.*, 1989; Miller *et al.*, 1996).

Working memory (WM) is a central concept in cognitive sciences. The prefrontal cortex constitutes the highest stage in the cortical hierarchy of executive memories (Funahashi, 2006; Constantinidis and Procyk, 2004; Fuster, 2001; Miler, 2000 and Goldman-Rackic, 1995), and it seems to be essential for integrating sensory information of different modalities with subsequent action in goal-directed behavior (Fuster, 2004; Wallis *et al.*, 2001, Tanji and Hoshi, 2001; and Levy and Goldman-Rakic, 2000).

Cells involved in WM ("memory cells") were first recorded in the dorsolateral prefrontal cortex (DLPFC) of monkeys performingdelayed-response tasks (Fuster, 1973; Kubota and Niki, 1971; and Fuster and Alexander, 1971) and have also been reported by other subsequent primate studies (Romo *et al.,* 1999, Rao et al., 1997; Miler *et al.,* 1996; Funahashi *et al*., 1990; Funahashi *et al.*, 1989; and Niki, 1974). Thepersistent delay activity recorded in those studies reflects themaintenance of a working-memory representation and therefore underlies the representation of retrospective, current, and prospectiveinformation (Cuttis and Lee, 2010). From the results of those studies, itseems that, in addition to persistent delay activity that is sustained throughout the whole delay period in WM tasks, task/setcells, eye movement-related responses, and phasic sensory responses, etc. (Funahashi and Takeda, 2002; Rao *et al*., 1997, Miler *et al.,* 1996; Funahashi *et al*., 1990, Funahashi *et al.,* 1989), two other general types of prefrontal neurons have also been studied (Verduzco-Flores *et al*., 2009 and Fuster, 1995). One is the so-called sensory-coupled cue cell, the discharge of which tends to diminish during the delay period of WM tasks. The other is the preparatory-set cell; its discharge tends to increase as the time for an expected behavioral response of a WM task approaches. These two types of cells may participate in two complementary processes: Sensory-coupled cells hold information of stimuli, and preparatory-set cells prepare for action in response to that information. These findings imply that the DLPFC plays a critical role in temporal organization of behavior by integrating action with recent sensory information across time (Fuster, 1997).

However, the mechanisms of temporal integration of sensory and action processing in cross-modal working memory remain unclear. Specifically, it is still unclear how the sensory component and the action component of cross-modal working memory networks, as well as the

component that mediates cross-temporal contingencies throughout the whole delay, are timely and selectively activated in the task (Wang *et al*., 2014).

2.7.1 Alcohol and Working Memory

There are bidirectional associations between executive function,a higher-order cognitive construct involved in the self-regulationof goal-directed behavior and alcohol use as reported by Lezak *et al*.,2012. Specifically, alcohol use appears to acutely affect executivefunction (Fillmore and Vogel-Sprott 2006; Duka and Twonshend,2004) in a dose-dependent manner (Guillot,2010), and lower baseline executive function appears to contribute to problematic alcohol use (Finn and Hall,2004; Nigg *et al*.,2006). Many studies examining executive function in relation to alcohol focus specifically on working memory (Peeters *et al*.,2014; Houben *et al.*2011; Day *et al*.,2015a), which has been conceptualized as a sub-component of executive functioning associated with updating information (Suchy,2009). Working memory is particularly relevant for alcohol-related behaviors; an individual who initiates a drinking episode with the intention of stopping after a set number of drinks or foregoing risks associated with uncontrolled drinking must rely, in part, on working memory to meet these behavioral goals. This potential for impaired control over drinking (Leeman *et al*.,2012) may in turn contribute to more alcohol-related problems.

Despite evidence demonstrating that working memory is acutely affected by alcohol consumption, studies examining the relationship between these variables have almost exclusively assessed working memory at a point prior to alcohol consumption. This is problematic, as it is possible that working memory may be differentially affected following alcohol consumption, independent of an individual's baseline working memory. Individual differences in alcohol-

induced changes of other executive functions such as impulse inhibition (Weafer and Fillmore, 2008) have been linked to subsequent drinking behaviors. However, little to no information exists in the literature on individual differences in working memory following alcohol use and how this might affect subsequent behaviors. Indeed, it has been noted that there is a dearth of research examining the ways in which alcohol-induced executive function impairments might influence drinking as well as other risky behaviors, such as cigarette smoking, illicit drug use, driving after drinking, overeating, and risky sexual behavior (Day *et al*., 2015). Therefore, gaining a better understanding of how individuals may differ in terms of alcohol-induced working memory decline, and how this may relate to alcohol use behavior, is critical.

The variability across studies in the effects of acute alcohol intoxication on working memory appears to be contingent upon one or more individual difference or task factors. It seems likely that certain kinds or components of working memory might be differentially sensitive to alcohol. Alcohol's effects could depend on working memory tasks' encoding or response demands. Varied results also could be related to experimental procedures that assessed performance at different rising or falling blood alcohol concentrations (BACs). Differences among sample populations might further contribute to inconsistent results across studies. For example, sample groups with different baseline working memory capacities might be differentially impaired by alcohol, based on the findings of Finn *et al.* (1999). Other participant characteristics, like drinking experience, tolerance, age, and health, also might differ across studies and influence their outcomes (Saults *et al*., 2007).

2.8 Alcohol and Reactive Oxygen Species (ROS)

Free radicals are atoms or molecules that have unpaired electrons, usually unstable and highly reactive (Finkel and Holbrook, 2000). Different radicals have very different chemical reactivities. It is usual to denote free radicals by the use of a superscripted dot (Hydroxyl radical OH˙Superoxide radical (O_2•-), Nitric oxide radical (NO˙), Lipid peroxy radical (LOO˙).

ROS are produced by mitochondria during oxidative metabolism through the one-electron reduction of molecular oxygen (O_2), forming superoxide anion (O_2•−). Superoxide is the proximal ROS produced by mitochondria and is converted to hydrogen peroxide (H_2O_2) through the action of superoxide dismutases (SODs) both within the mitochondria and in the cytosol. Complexes I, II, and III of the electron transport chain contain sites wherein electrons can prematurely reduce oxygen, resulting in the formation of superoxide (Turrens, 2003 and Murphy, 2009). As an inevitable by-product of oxidative phosphorylation (OxPhos), mitochondria generate over 90% of all intracellular reactive oxygen species (ROS), with conversion of 0.15 – 5 % of total oxygen consumed by resting cells (Halliwell and Gutteridge, 2007, Chance *et al.*, 1979, Boveris and Chance 1973, St. Pierre *et al.*, 2002).

In biology system, oxygen based radicals and nitrogen based radicals are two types of free radicals. Oxygen free radicals, such as superoxide, hydroxyl radicals, and peroxyl radicals, with the addition of non-radicals, such as hydrogen peroxide, hypochlorous acid and ozone, are known as reactive oxygen species (ROS), which are generated during the metabolism process of oxygen. Reactive nitrogen species (RNS), including nitrogen based radicals and non-radicals, such as nitrogen dioxide, nitric oxide radicals and peroxynitrite, are derived from nitric oxide and superoxide via inducible nitric oxide synthase (iNOS) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, respectively (Apel and Hirt, 2004; McCord, 2000). Due to their

special chemical characteristics, ROS/RNS can initiate lipid peroxidation, cause DNA strand breaks, and indiscriminately oxidize virtually all molecules in biological membranes and tissues, resulting in injury. However, since the body is able to remove ROS/RNS to a certain degree, these reactive species are not necessarily a threat to the body under physiological conditions (McCord, 2000; Mittler, 2002). As a matter of fact, ROS are required at certain level in the body to perform its important physiological functions. The generation of ROS is a natural part of aerobic life, which is responsible for the manifestation of cellular functions including signal transduction pathways, defense against invading microorganisms and gene expression to the promotion of growth or death (Finkel and Holbrook, 2000). Oxidative/nitrosative stress represents the bodies' imbalance in the production and the elimination of reactive oxygen and nitrogen species as well as decreased production of antioxidants (Sha*et al*., 2015).

In terms of oxidative stress, in specific physiological conditions, it is actually useful. For example, it could strengthen biological defense mechanisms during appropriate physical exercise and ischemia, and induce apoptosis to prepare the birth canal for delivery (Apel and Hirt, 2004; McCord, 2000). However, this is confined to particular situations, and in most other cases, large levels of ROS and oxidative stress will induce cell death through necrotic and/or apoptotic mechanisms, leading to cellular and tissue injury (Sha*et al.*, 2015).

Under normal physiological conditions 2-5% of oxygen is converted to reactive oxygen species, which are scavenged by the antioxidant system. These reactive oxygen species (ROS) can cause cellular damage by lipid peroxidation if not removed by antioxidant defenses. The antioxidant defense system includes antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and an ancillary enzyme glutathione reductase (GR), as well as antioxidant substances, such as reduced glutathione (GSH) and vitamins C and E. The brain

consumes 20% of the oxygen taken in by the body, and contains low levels of antioxidants and high amounts of peroxidizahle lipids. It may actually be more vulnerable to oxidative stress than other organs of the body (Somani, 1996; Somani, 1994). Therefore, it seems logical that stressors such as ethanol consumption may affect the antioxidant system and the amount of lipid peroxidation occurring in the brain. In brain mitochondria, the activities of glutathione peroxidase or glutathione reductase are relatively low (Satrustegui and Richter, 1984), but they do play a significant role in the elimination of H_2O_2 in brain tissues (Dringen *et al*., 1999 and Desagher *et al*., 1996).

There is evidence that ethanol produces alterations in the mitochondrial structure and function of several organs, both in laboratory animals and humans (Pachinger *et al.*, 1975). The mitochondria play an important role in the alcohol metabolism via the enzyme ALDH; this enzyme catalyzes the conversion of acetaldehyde into acetate. When this enzyme reaches a saturation point, the acetaldehyde escapes into the blood stream and leads damage to biomolecules such lipids, proteins and nucleic acids which results of the toxic side effects that are associated with alcohol consumption (Salvador and Alfredo, 2010). These changes affect the mitochondrial function decreasing respiratory rates (Albano, 2002) and ATP levels, and might result in increased production of reactive oxygen species (ROS) (Robin *et al*., 2005). Several enzymatic systems, including the cytochrome (CYP2E1), the mitochondrial respiratory chain and the cytosolic enzymes xanthine oxidase and the aldehyde oxidases have been implicated as sources of superoxide anion ($O_2^{\cdot -}$**)** and H_2O_2 in parenchyma cells during ethanol intoxication (Cederbaum, 1991). Numerous studies show that mitochondrial levels of ROS may be increased by chronic alcohol consumption as a consequence of increased mitochondrial CYP2E1 levels (Adam-Vizi, 2005 and Ronis *et al.*, 1996) as well as a by-product of the matrix enzyme α-

ketoglutarate dehydrogenase (Cederbaum, 1991). The CYP2E1 activity increases in alcohol-treated rodents (Liangpunsakul *et al*., 2005) and humans not only in alcohol abusers, but also in moderate alcohol consumers (Albano, 2006). It has a high rate of NADPH oxidase activity, which leads to the production of large quantities of O_2^- and H_2O_2 (Bailey*et al*., 1999).

It is postulated that the first metabolite of ethanol, acetaldehyde mediates many effects of ethanol in a brain, including behavioral, neurochemical and neurotoxic actions, and plays a crucial role in the development of alcoholism (Quertemont *et al*., 2005; Deitrich, 2004; Smith *et al*., 1997 and Hunt, 1996). This requires the presence of ethanol derivedacetaldehyde inside the brain. But the penetration from blood of acetaldehyde derived from peripheral ethanol metabolism is low due to the high aldehyde dehydrogenase (ALDH) activity in the liver and powerful blood–brain barrier for aldehydes represented by the same enzyme (Zimatkin, 1991). Another possibility of acetaldehyde appearance inside the brain is the production of acetaldehyde during the ethanol oxidation in situ (Zimatkin *et al*., 2006).

Acetaldehyde derived from the peripheral metabolism of ethanol penetrates from blood to brain with difficulty, becauseof the metabolic barrier represented by aldehyde dehydrogenase in capillary endothelium and surrounding astrocytes(Zimatkin, 1991; Westcott *et al*., 1980 and Sippel, 1974). Therefore,for its central action, acetaldehyde should be produced inside the brain. It seems possible because all of the main ethanol metabolizing enzymes (alcohol dehydrogenase, cytochrome 2E1 and catalase) are available in the brain tissue (Zimatkin and Deitrich, 1997). The slow, but measurable oxidation ofethanol, and the formation of acetaldehyde in the naive adult rat brain homogenates was demonstrated, and the crucial role of catalase in this process was established (Aragon *et al*., 1992; Gill *et al*., 1992; Hamby–Mason *et al*., 1997; Zimatkin *et al*., 1998). The significant oxidation of ethanol to acetaldehyde was found in fetal and neonatal

brain homogenates (Hamby–Mason *et al*., 1997 and Person *et al*., 2000), as well as in the primaryculture of astrocytes (Eysseric *et al*., 1997). In addition to catalase, the important role of CY 2E1 in ethanoloxidation in the rodent brains has been demonstrated using the specific enzyme inhibitors, mice with genetic deficiency in ethanol-metabolizing enzymes and brain subcellular fractionsknown to have differential activity of ethanol-metabolizing enzymes.

The brain is especially susceptible to the assaults perpetrated by ROS. This is because the brain as an organ is a major metabolizer of oxygen (20% of the body consumption) and yet has relatively Zimatkin *et al*. (2006) feeble protective antioxidant mechanisms. Thus it is especially vulnerable to oxidative stress. The brain contains a large amount of polyunsaturated peroxidizable fatty acids along with high levels of iron that act as a pro-oxidant and sometimes induce autophagic cell death in hemorrhagic stroke. Synaptic transmission involving dopamine and glutamate oxidation may also occur in the brain (Chen *et al*., 2013; Mehta *et al*., 2012 and Badjatia *et al*., 2012). In addition, lipid peroxidation leads to the production of toxic compounds such aldehydes or dienals (e.g., 4-hydroxynonenal) which may cause in turn neuronal apoptosis (McCracken *et al*., 2000).

At the DNA level, oxidative modifications may cause rapid depletion of intracellular energy by activating DNA repair enzymes. Energy scarcity after stroke will cause in turn endonuclease mediated DNA fragmentation, the key mechanism that leads to DNA-damage (Cui *et al*., 2000).

3.0 MATERIALS AND METHOD

3.1 Experimental Animals

For the purpose of this study, thirty-two (32)apparently healthy Wistar rats of both sexes were obtained from the Department of Human Anatomy Bello University, Zaria. The animals were housed in the animals House of the Department and were acclimatized for one weeks before the commencement of the experiment.

All animals were maintained on standard animal diet feeds and water. The animals were categorized into control and treatment groups. The animals were weighed before the commencement of the experiment and animals weighed between 80 and 120g.

3.1.1 Animal Feed

Pelletized growers feed manufactured by The Grand Cereals and Oil Mills Limited (GCOML) was obtained and used to feed the animals for the experiment.

Composition of feed include Crude protein (13%), Crude fibre (8%), Fat (15%), Calcium (0.80%),and phosphorus (0.40%).Other constituents include: cereals, essential amino acids/proteins, vegetables, animal protein, minerals, antibiotics, salt, antioxidant and Vitamins.

3.2 Drug Procurement

Sealed bottle of absolute ethanol manufacture by Guangdong Gannghua Sci- Tech Co., LtD, Shantoua, Guangdong, China was purchased and used for this research.

3.4 Set Up of Neurobehavioral Studies

For the purpose of this study; three neurobehavioral setups were used for the neurobehavioral test. These were Morris Water Maze (MWM) for testing learning and memory, elevated Plus Maze (EPM) for testing anxiety and Resident intruder test apparatus for test aggression and violence.

3.4 Other Materials

Other materials used during this research work included; Ketamine Injection, Digital microscope (celestron), Dissecting set, Fixative – Bouin`s fluid, heparinize bottle, oral carnula, digital weighing balance, dissecting glass slides, tissues, coverslips, saw dust for bedding, water bottles, bottles for fixing tissues fixatives and stains.

3.5 Experimental Design

Thirty-twoWistar (32)rats obtained for the purpose of this study was selectively categorized into four groups (Group A, Group B, Group C and Group D) with eight animals of both sexes in each group.

Group A was the control group and was administered with 1ml of distilled water while Group B, Group C and Group D were administered with 0.12g/kgbw of ethanol, 0.16g/kgbw of ethanoland 0.24g/kgbw of ethanol respectively. The ethanol was administered orally through oral cannula once daily for the period of 75days.

3.5.1 Body weight assessments

All experimental animals' body weights were taken at the onset of treatment, weekly throughout the study and at the end of the study before sacrifice using digit weighing scale.

3.5.2 Neurobehavioral studies

All experimental procedures were done in the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. The following neurobehavioral tests were conducted

3.5.2.1 Morris Water Maze

The Morris water maze is widely used to study spatial memory and learning. Animals are placed in a pool of water that is colored opaque with non-toxic tempera paint, where they must swim to a hidden escape platform. Because they are in opaque water, the animals cannot see the platform, and cannot rely on scent to find the escape route. Instead, animals must rely on external/extra-maze cues. As the animals become more familiar with the task, they are able to find the platform more quickly. Developed by Richard Morris in 1981, this paradigm has become one of the "gold standards" of behavioral neuroscience (Numez, 2008).

3.5.2.1.1 Setting up the Morris Water Maze

The main component of the water maze set up was a round pool, about 6 feet in diameter and about 3 feet deep.The water maze filled up with tap water in room temperature, escape platform is placed in the center of the pool. During training, it exposed, one inch above the water. This teaches the rat thatthere is a platform, and that it is the way to get out of the water. Later, after the animal is trained and ready for testing, the escape platform was just below the surface of the water, and was not visible because the water was made opaque with non-toxic paint.



Plate I: Setup for Morris Water Maze Paradigm

3.5.2.1.2 Training and testing for the water maze

For the water maze training, the platform was in the center of the pool and exposed one inch above the surface, so the animal knows that it's there, each animal was made to undergo three consecutive trials. The water maze has 4 starting positions: north, south, east, or west. The animal was taken to one of these positions and lower into the water by supporting it with hand and bringing it down gently into the water tail-end first, the animalswere allowed to swim and search for the platform for a maximum of 60 seconds.Once the rodent reaches the platform, the timer was stopped, and the time was recorded. If it doesn't find the platform in 60 seconds, then recorded timefor this trial is one minute and the same procedure was Repeat for two more trials, starting at a different direction for each trial.

3.5.2.2 Elevated Plus Maze

The elevated plus maze test is one of the most widely used tests for measuring anxiety-like behavior. The test is based on the natural aversion of mice for open and elevated areas, as well as on their natural spontaneous exploratory behavior in novel environments (Komada *et al*., 2008).

3.5.2.2.3 Setting up and testing for the Elevated plus maze

The apparatus consists of open arms and closed arms, crossed in the middle perpendicularly to each other, and a center area.The rats were housed with a 12-h light/dark cycle (lights on at 7:00 AM), Behavioral testingis performed between 9:00 AM and 6:00 PM. All the experimental rats are transferred to the behavior testing room 30 min prior to beginningthe first trial to habituate to

the condition of the behavior testing room.The rats were allowed to move freely about the maze for 5 min. The number of entries (an entry is defined as the center of mass of the mouse enters the arm) into each arm and the time spent in the open arms are recorded and these measurements serve as an index of anxiety-like behavior.The number of entries into the open arms and the time spent in the open arms are used as indices of open space-induced anxiety in mice.Ethanol (75%) was used to clean the apparatus after each successful explore by the rat to prevent odor cues from previous trials.

3.5.2.3 Resident intruder test

It is a Standardized Test for Aggression, Violence and Social Stress (Koolhaas *et al.*, 2013). The intruders in the resident-intruder paradigm will show defensive behavior in response to the offensive attacks by the resident. The paradigm therefore also allows one to study defensive behavior and social stress by using the intruder as the experimental animal.

3.5.2.3.1 Set up and testing for resident intruder test

For each resident was housed in a cage with a floor space of about half a square meter.Each resident was with a female for at least one week before the start of the experiments, which will facilitate the development of territoriality. At the same time this will prevent social isolation, which is known to be stressful for social animals and may lead to reduced welfare and aberrant forms of social behavior.The companion female was removed from the residential cage before the test and unfamiliar male (smaller in body size and weight) intruder was introduced into the home cage of the resident at the start of the test.Defensive aggressive behavior of the intruder rat was observed and record for duration of 5minutes.



Plate II:The setup for Resident intruder paradigm

3.6 Animal sacrifice

After day 75 of the administration, the Wistar rats were humanely sacrificed using ketamine injection (75mg/kgbw Intraperitoneal IP manufactured by SWISS parenteral PVT. LTD. 809, Kerala G.I.D.C., Nr. Bawla Dist., Ahmedabad-3822220, Gujarat, India) anesthesia and brain tissues from the animals were collected by opening through the mid-sagittal suture of the skull. One of the hemisphere frontal lobe was dissected out and homogenized in pH 7.4 phosphate buffer solution (PBS) for the estimation of Glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione and malondaldehyde (MDA). The remaining hemisphere frontal lobe was quickly fixed in Bouin's fluid for histological studies. All these procedures was carried out in the Department of Human Anatomy.

3.7 Biochemical assay

The homogenized tissue was centrifuge at 4000 rpm for 5 min and the supernatant was separated into a clean sample bottle and taken to the department of Biochemistry, Faculty of Life Sciences, Ahmadu Bello University, Zaria for the estimations of antioxidant marker.

3.7.1 Malondialdehyde assay

MDA levels in brain samples were measured by the method of Uchiyama and Mihara (1978). This method depends on the formation of MDA as an indicator of lipid peroxidation, which reacts with thiobarbituric acid producing thiobarbituric acid reactive substances (TBARS), measured spectrophotometrically at 532 nm.

3.7.2 Superoxide dismutase activity assay

Activity of SOD enzyme was evaluated by the method of Sun *et al. (*1988). The analysis of SOD was based upon the principle in which xanthine reacts with xanthine oxidase to produce superoxide radicals. The SOD activity was measured by the level of suppression of this reaction. Results were expressed as U/ml protein.

3.7.3 Catalase activity assay

Catalase enzyme activity was evaluated using a spectrophotometric test based on the yellow complex with molybdate and hydrogen peroxide, which was described in detail by Goth (1991) was expressed (U/mg).

3.7.4 Glutathione activity assay

Glutathione enzyme activity was evaluated using a spectrophotometric test based on the yellow complex ammonium tetracholoropalladate, which described in detail by Raggi *et al*., (1991) was expressed (ug/ml).

3.8 Histological Studies

The fixed brain tissue (Frontal lobe of the cerebrum) was processed in the department of Human Anatomy, Ahmadu Bello University, Zaria for routine histological process, stained with haematoxylin and eosin (H and E) for the demonstration of cytoplasm and nucleus, Golgi staining techniques for the demonstration of the neural processes.

3.9 Data Analysis

Data collected was presented as Mean±SEM and Mean Rank value was statistically analyses using Statistical Package for Social Science (SPSS), Version 20 (IBM, Incorp, NY). A *p*-value < 0.05 will be considered significant.

3.9.1 Body weight analysis

A Shapiro-Wilk's test (Shapiro and Wilk, 1965; Razali and Wah, 2011) and a visual inspection of their histogram, normal Q-Q plot and Box plot was conducted to test for the normality of the body weight (g) taken weekly before, during and after the experiment in four categories of male and female rats respectively. The four categories were Group A (Distilled Water), Group B (0.12g/kg of ethanol), Group C (0.16g/kg of ethanol) and Group D (0.24g/kg of ethanol).

The test was not statistically difference (p> 0.05). Therefore, the assumption of normality is valid (Cramer, 1998; Cramer and Howitt, 2004; Danne and Seward, 2011).

A one-way analysis of variance was conducted to assess if there were differences in body weight changes of categories in male and female wistar rats; the four categories were Group A (administered with distilled water), Group B (administered with 0.12g/kg of ethanol), Group C (administered with 0.16/kg of ethanol) and Group D (administered with 0.24g/kg of ethanol). The dependent continuous variable was the body weight taken weekly before, during and after the experiment.

3.9.2 Morris Water Maze Test Score Analysis

A Shapiro-Wilk's test (Shapiro and Wilk, 1965; Razali and Wah, 2011) and a visual inspection of their histogram, normal Q-Q plot and Box plot was conducted to test for the normality of the

escape time latency taken weekly before, at fifth week and tenth week of administration in the experiment in four categories of male and female rats respectively. The four categories were Group A (Distilled Water), Group B (0.12g/kg of ethanol), Group C (0.16g/kg of ethanol) and Group D (0.24 g/kg of ethanol).

The test was not statistically difference ($p > 0.05$). Therefore, the assumption of normality is valid (Cramer, 1998; Cramer and Howitt, 2004; Danne and Seward, 2011).

A repeated measure of analysis of variance was conducted to assess if there were differences in escape time latency (sec) of four groups of Wistar rats under three conditions; Before administration, fifth week of administration and tenth week of administration. The four groups were Group A (administered with distilled water), Group B (administered with 0.12g/kg of ethanol), Group C (administered with 0.16g/kg of ethanol) and Group D (administered with 0.24g/kg of ethanol). The dependent continuous variable was escape time latency. Post hoc test using Turkey's LSD was conducted to assess the significant difference between the groups.

3.9.3 Elevated Plus Maze Test Score Analysis

A Shapiro-Wilk's test (Shapiro and Wilk, 1965; Razali and Wah, 2011) and a visual inspection of their histogram, normal Q-Q plot and Box plot was conducted to test for the normality of the scored Elevated plus maze parameter (Percentage of open and close arm entries, and percentage of total time spent in open and close arm) in four categories of male and female Wistar rats under three conditions; Before administration, fifth week of administration and tenth week of administration. The four categories were Group A (Distilled Water), Group B (0.12g/kg of ethanol), Group C (0.16g/kg of ethanol) and Group D (0.24g/kg of ethanol).

The test was statistically difference ($p< 0.05$). Therefore, the assumption of normality is not valid (Cramer, 1998; Cramer and Howitt, 2004; Danne and Seward, 2011).

Friedman test was conducted to assess if there were differences in Percentage of open and close arm entries, and percentage of total time spent in open and close arm of four groups of Wistar rats under three conditions; Before administration, fifth week of administration and tenth week of administration. The four groups were Group A (administered with distilled water), Group B (administered with 0.12g/kg of ethanol), Group C (administered with 0.16g/kg of ethanol) and Group D (administered with 0.24g/kg of ethanol). The dependent continuous variable was Percentage of open and close arm entries, and percentage of total time spent in open and close arm. Post hoc test using bonferrioni adjustments was conducted to assess the significant difference with three conditions.

3.9.4 Resident intruder Test analysis

A Shapiro-Wilk's test (Shapiro and Wilk, 1965; Razali and Wah, 2011) and a visual inspection of their histogram, normal Q-Q plot and Box plot was conducted to test for the normality of the scored resident intruder test parameter (latency period of upright defense posture, submission, freeze, flight and percentage of defense scores) in four categories of male and female Wistar rats under three conditions; Before administration, fifth week of administration and tenth week of administration. The four categories were Group A (Distilled Water), Group B (0.12g/kg of ethanol), Group C (0.16g/kg of ethanol) and Group D (0.24g/kg of ethanol).

The test was statistically difference ($p< 0.05$). Therefore, the assumption of normality is not valid (Cramer, 1998; Cramer and Howitt, 2004; Danne and Seward, 2011).

Friedman test was conducted to assess if there were differences in latency period of upright defense posture, submission, freeze, flight and percentage of defense scores of four groups of Wistar rats under three conditions in male and female Wistar rats; Before administration, fifth week of administration and tenth week of administration. The four groups were Group A (administered with distilled water), Group B (administered with 0.12g/kg of ethanol), Group C (administered with 0.16g/kg of ethanol) and Group D (administered with 0.24g/kg of ethanol). The dependent continuous variables were latency period of upright defense posture, submission, freeze, flight and percentage of defense scores.

3.9.5 Biochemical assay analysis

A Shapiro-Wilk's test (Shapiro and Wilk, 1965; Razali and Wah, 2011) and a visual inspection of their histogram, normal Q-Q plot and Box plot was conducted to test for the normality of the antioxidant enzymes (Superoxide dismutase (SOD), Catalase (CAT), Glutathione (GSH) and Malonaldehyde (MDA)) activities in four categories of male and female rats respectively. The four categories were Group A (Distilled Water), Group B (0.12g/kg. of ethanol), Group C (0.16g/kg of ethanol) and Group D (0.24g/kg of ethanol).

The test was not statistically difference ($p > 0.05$). Therefore, the assumption of normality is valid (Cramer, 1998; Cramer and Howitt, 2004; Danne and Seward, 2011).

A one-way analysis of variance was conducted to assess if there were differences in antioxidant enzymes activities level of categories in male and female Wistar rats; the four categories were Group A (administered with distilled water), Group B (administered with 0.12g/kg. of alcohol), Group C (administered with 0.16 g/kg b. of alcohol) and Group D (administered with 0.24g/kg of

alcohol). The dependent continuous variable was antioxidant enzymes activities (SOD, CAT, GSH, and MDA).

4.0 RESULTS

4.1 Physical observation

During the course of the administration, we observed that there is stimulation of appetite up to the third week of administration follow by reduction in the intake of food until the end of the administration in all the groups administered with alcohol.

4.2 Body weight assessments

Result indicates the body weight(g) in first week of administration of Group 1(Control) (69.25±11.52) was significant lower compared to Group 2 (administered with 0.12g/kg body weight of ethanol per day)(105.75±7.28), Group 3 (administered with 0.16g/Kg body weight of ethanol per day) (99.00±5.94) and Group 4(administered with 0.24g/kg body weight of ethanol per day) (92.25±7.78) in male experimental rats. However, there was significant difference in weight from the second week to the end of administration (Figure 4.1).Also, in first week of administration of Group 4 (124.25±2.81) was significant higher compared to Group 3 (92.25±7.31), Group 2 (97.75±3.59) and Group 1(Control) (92.25±7.78), while in second week of administration, the same pattern was also observed but there was significant difference noted from the third week to the end of administration in female experimental rats (Figure 4.2).

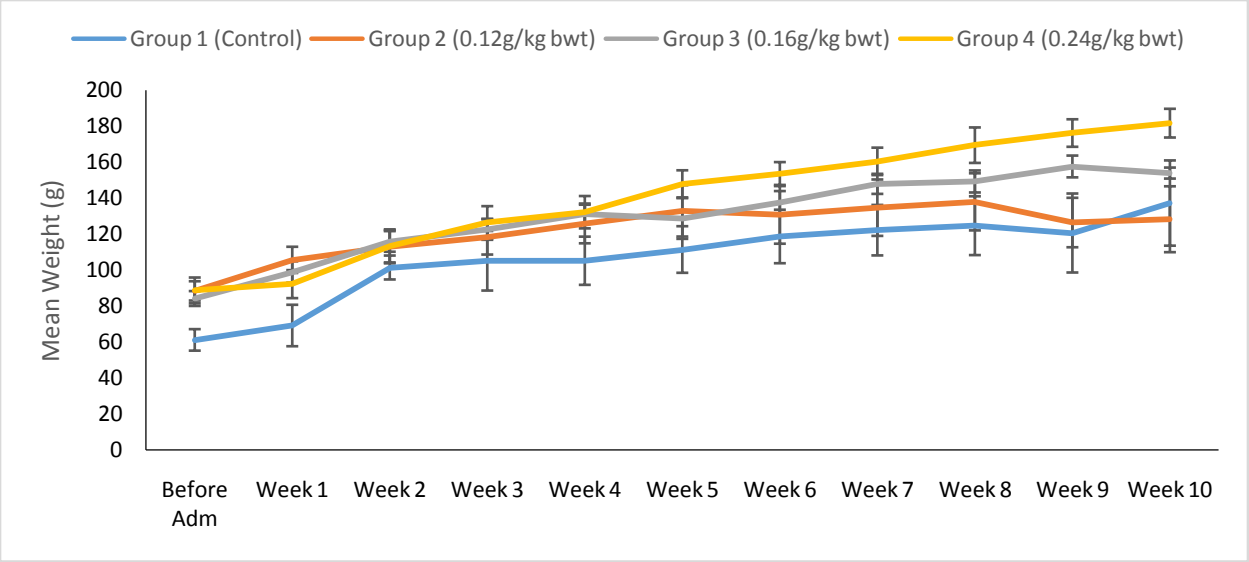


Figure 4.1. Mean weight (g) before, during and after administration of ethanol in male Wistar rats.

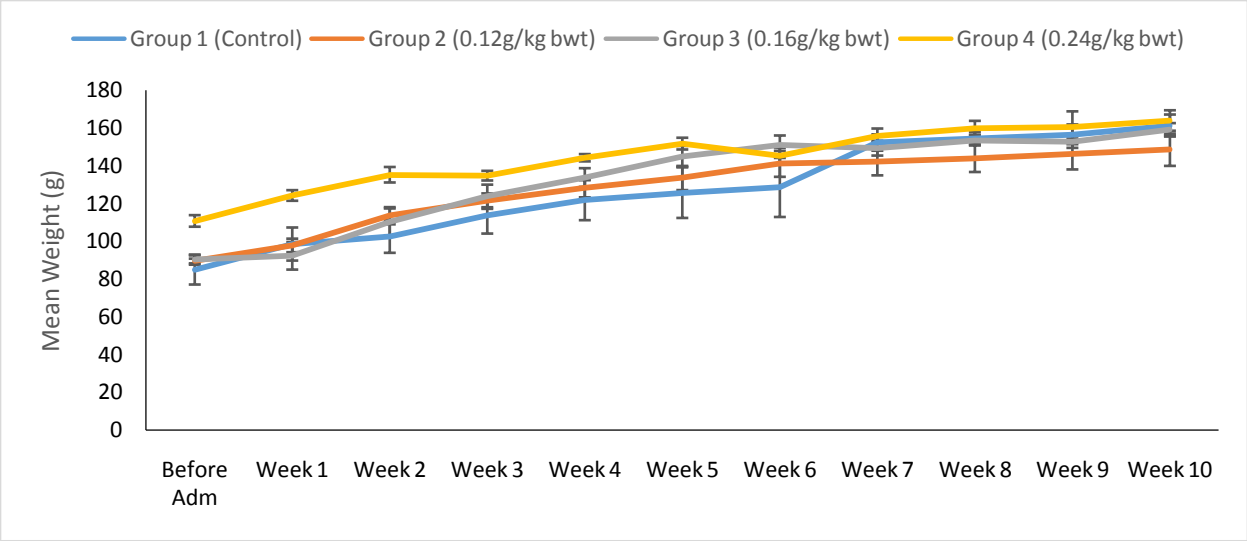


Figure 4.2.Mean weight (g) before, during and after administration of ethanol in female Wistar rats.

4.1.3Weight gain and brain body weight ratio

The result reveal that there is no significant difference in the weight gain and brain body weight ratio in male (Table 4.1) and female (Table 4.2) experimental rats.

Table 4.1: Weight gain (g) and brain body weight ratio as expressed as mean ± SEM of male experimental rats

	Group 1 (Control)	Group 2 (0.12g/kg Et)	Group 3 (0.16g/kg Et)	Group 4 (0.24g/kg Et)	*F*	*P*
Weight gain	79.67 ± 30.43	42.00 ± 23.35	68.00 ± 6.11	93.00 ± 11.21	1.33	0.324
% OWR	1.13 ± 0.20	1.22 ± 0.20	0.99 ± 0.02	0.85 ± 0.08	1.62	0.253

% OWR = Percentage Brain Body weight ratio, Weight gain = (Final weight (g) – Initial weight (g))

Table 4.2: Weight gain (g) and brain body weight ratio as expressed as mean ± SEM of female experimental rats

	Group 1 (Control)	Group 2 (0.12g/kg Et)	Group 3 (0.16g/kg Et)	Group 4 (0.24g/kg Et)	*F*	*P*
Weight gain	78.67 ± 16.18	59.00 ± 8.52	67.33 ± 6.12	53.25 ± 8.26	1.18	0.365
% OWR	1.07 ± 0.03	1.03 ± 0.03	1.13 ± 0.15	1.00 ± 0.13	0.33	0.801

% OWR = Percentage Brain Body weight ratio, Weight gain = (Final weight (g) – Initial weight (g))

4.4 Morris Water Maze test scores

In Male Wistar rats, the result indicates the escape time latency in group A (control) was not significantly different ($p > 0.05$) between before administration, at fifth week of administration and at tenth week of administration. while in group B (0.12g/kgbwt of ethanol), the escape latency at before administration (37.61±4.24) was significantly higher compare to at fifth week of administration (15.26±3.22) also in group C (0.16g/kgbwt of ethanol), the escape latency (Sec) at before administration (28.7±5.52) was significantly higher compare to at fifth week of administration (12.20±4.31) and at tenth week of administration (7.87±2.51) and in Group D (0.24g/kg of ethanol), the escape latency (Sec) at tenth week (8.63±2.88) of administration was significantly lower compare to fifth week (20.03±12.23) of administration and before administration (14.09±10.89). Table 4.3

Table 4.3: Escape time latency (sec) in Morris Water Maze test in male Wistar rats.

	Before Administration	Fifth Week of Administration	Tenth week of Administration	*F*	*p*
Group A (1ml of Distilled Water)	27.70 ± 15.13	17.14 ± 4.99	6.04 ± 2.23	4.71	0.089
Group B (0.12g/kg bwt Ethanol)	37.61 ± 4.24[a]	15.26 ± 3.22[a]	12.74 ± 3.44	12.16	0.020
Group C (0.16g/kg bwt Ethanol)	28.72 ± 5.52[ab]	12.20 ± 4.31[a]	7.87 ± 2.51[b]	83.05	0.001
Group D (0.24g/kg bwt Ethanol)	26.64 ± 9.64[b]	23.57 ± 1.84[a]	8.96 ± 3.05[sb]	14.28	0.005

Row sharing the same superscript are significantly different

In female Wistar rats, the result indicates the escape time latency in group A (control) and Group D (0.24g/kg) was not significantly different (p> 0.05) between before administration, at fifth week of administration and at tenth week of administration. while in group B (0.12g/kg of ethanol), the escape time latency at before administration (17.47±3.06) was significantly higher compare to at fifth week of administration (7.55±3.70) and at tenth week of administration (5.61±2.63), also in group C (0.16g/kg of ethanol), the escape time latency (Sec) at before administration (31.30±6.83) was significantly higher compare to at fifth week of administration (10.77±4.31). Table 4.4.

Table 4.4: Escape time latency (sec) in Morris water maze test female Wistar rats.

	Before Administration	Fifth Week of Administration	Tenth week of Administration	*F*	*p*
Group A (1ml of Distilled Water)	31.41 ± 7.84	20.76 ± 11.32	9.52 ± 4.23	5.78	0.066
Group B (0.12g/kg bwt Ethanol)	17.47 ± 3.06[ab]	7.55 ± 3.70[a]	5.61 ± 2.63[b]	14.77	0.005
Group C (0.16g/kg bwt Ethanol)	31.30 ±6.83[a]	10.77 ± 4.31[a]	8.77 ± 2.07	15.41	0.013
Group D (0.24g/kg bwt Ethanol)	14.63 ± 2.47	18.08 ± 7.96	18.41 ± 16.16	0.26	0.781

Row sharing the same superscript are significantly different

4.5Elevated plus maze test scores

In male rats, it was observed that there no significant (p> 0.05) difference in Percentage of open and close arm entries, and percentage of total time spent in open and close arm (Table 4.5), while the result indicates the mean rank of percentage of total time spent in open armbefore (3.00) was significantly (p< 0.05) higher compared to fifth week (1.75) and tenth week (1.25) of administration and mean rank of percentage of total time spent in close armbefore (1.00) was significantly (p< 0.05) lower compared to fifth week (2.25) and tenth week (2.75) of administration in female rats (Table 4.6).

Table 4.5: Percentage of open and close arm entries, and percentage of total time spent in open and close arm before, at fifth and at tenth week of administration in male Wistar rats.

		Mean Rank				
		Before	5th Week	10th Week	X^2	*P*
Group 1 (Control)	% open AE	1.33	2.50	2.17	2.60	0.273
	% close AE	2.50	1.67	1.83	1.27	0.529
	% time in OA	1.83	1.67	2.50	1.27	0.529
	% time in CA	2.17	2.33	2.50	1.27	0.529
Group 2 (0.12g/kg of ethanol)	% open AE	2.33	2.17	1.50	1.40	0.497
	% close AE	1.67	1.83	2.50	1.40	0.497
	% time in OA	2.67	2.00	1.33	2.67	0.264
	% time in CA	1.33	2.00	2.67	2.67	0.264
Group 3 (0.16g/kg of ethanol)	% open AE	2.00	2.50	1.50	3.00	0.223
	% close AE	2.00	1.50	2.50	3.00	0.223
	% time in OA	2.50	2.00	1.50	1.64	0.441
	% time in CA	1.50	2.00	2.50	1.64	0.441
Group 4 (0.24g/kg of ethanol)	% open AE	1.75	2.25	2.00	0.80	0.670
	% close AE	2.25	1.75	2.00	0.80	0.670
	% time in OA	2.50	1.50	2.00	2.00	0.368
	% time in CA	1.50	2.50	2.00	2.00	0.368

% open AE = percentage of open arm entries, % close AE = percentage of close arm entries, % time in OA = percentage of total time spent in open arm, % time in CA = percentage of total time spent in close arm.

Table 4.6: Percentage of open and close arm entries, and percentage of total time spent in open and close arm before, at fifth and at tenth week of administration in female Wistar rats.

		Mean Rank				
		Before	5th Week	10th Week	X^2	*P*
Group 1 (Control)	% open AE	2.17	1.17	2.67	2.82	0.148
	% close AE	1.83	2.83	1.33	3.82	0.148
	% time in OA	2.17	1.17	2.67	3.82	0.148
	% time in CA	1.83	2.83	1.33	3.82	0.448
Group 2 (0.12g/kg of ethanol)	% open AE	1.75	1.88	2.38	0.93	0.627
	% close AE	2.25	2.13	1.63	0.93	0.627
	% time in OA	3.00^{ab}	1.75^{a}	1.25^{b}	6.50	0.039
	% time in CA	1.00^{ab}	2.25^{a}	6.50^{b}	6.50	0.039
Group 3 (0.16g/kg of ethanol)	% open AE	2.83	1.17	2.00	5.00	0.082
	% close AE	1.17	2.83	2.00	5.00	0.082
	% time in OA	2.67	1.33	2.00	2.67	0.264
	% time in CA	1.33	2.67	2.67	0.264	0.264
Group 4 (0.24g/kg of ethanol)	% open AE	1.88	2.38	1.75	1.08	0.526
	% close AE	2.00	1.63	2.38	1.29	0.526
	% time in OA	1.50	2.00	2.50	2.00	0.368
	% time in CA	2.50	2.00	1.50	2.00	0.368

% open AE = percentage of open arm entries, % close AE = percentage of close arm entries, % time in OA = percentage of total time spent in open arm, % time in CA = percentage of total time spent in close arm. Rows sharing the same superscript are significantly different

4.6 Resident intruder test scores

The result indicates that the latency period of upright defense posture, submission, freeze, flight and percentage of defense scores were not significantly ($p > 0.05$) different from the three conditions (before, fifth week and tenth week of administration) in male (Table 4.7) and female (table 4.8) Wistar rats.

Table 4.7: Latency period of upright defense posture, submission, freeze, flight and percentage of defense scores before, at fifth and at tenth week of administration in male Wistar rats.

		Mean Rank				
		Before	5th Week	10th Week	X^2	*P*
Group 1 (Control)	LPDP	1.17	1.83	3.00	5.64	0.060
	LPS	1.50	2.17	2.33	2.00	0.368
	LPF	1.67	2.33	2.00	0.67	0.717
	LF	2.00	2.00	2.00	-	-
	%DS	1.67	2.00	2.33	0.67	0.717
Group 2 (0.12g/kg of ethanol)	LPDP	1.75	1.88	2.38	1.27	0.529
	LPS	1.50	1.88	2.63	3.00	0.223
	LPF	2.75	1.50	1.75	3.50	0.174
	LF	2.13	1.88	2.00	0.29	0.867
	%DS	2.50	1.25	2.25	3.50	0.171
Group 3 (0.16g/kg of ethanol)	LPDP	1.50	2.50	2.00	2.00	0.368
	LPS	1.83	1.83	2.33	2.00	0.368
	LPF	1.67	1.83	2.50	1.40	0.497
	LF	1.83	1.83	2.00	2.00	0.368
	%DS	1.33	2.00	2.67	2.67	0.264
Group 4 (0.24g/kg of ethanol)	LPDP	1.88	1.38	2.75	4.77	0.092
	LPS	1.75	2.50	1.75	4.00	0.135
	LPF	1.88	1.75	2.38	1.00	0.607
	LF	1.88	2.25	1.88	2.00	0.368
	%DS	1.75	1.75	2.50	1.50	0.472

LPDP = Latency of upright defense posture, LPS = Latency of submission, LPF = Latency of freeze, LF = Latency of flight, %DS = Percentage of defense score.

Table 4.8: Latency period of upright defense posture, submission, freeze, flight and percentage of defense scores before, at fifth and at tenth week of administration in female Wistar rats.

		Mean Rank				
		Before	5th Week	10th Week	X^2	*P*
Group 1 (Control)	LPDP	2.00	1.50	2.50	2.00	0.368
	LPS	1.67	2.17	2.17	0.545	0.761
	LPF	2.17	1.33	2.50	2.60	0.273
	LF	2.00	2.00	2.00	-	-
	%DS	16.7	1.33	3.00	4.67	0.097
Group 2 (0.12g/kg of ethanol)	LPDP	1.63	2.75	1.63	3.60	0.165
	LPS	2.38	2.50	1.13	4.93	0.085
	LPF	2.00	1.25	2.75	5.14	0.076
	LF	1.88	1.88	2.25	2.00	0.368
	%DS	2.00	1.50	2.50	2.00	0.368
Group 3 (0.16g/kg of ethanol)	LPDP	2.33	2.00	1.67	0.67	0.717
	LPS	2.67	2.00	1.33	3.20	0.202
	LPF	3.00	1.33	1.67	5.60	0.061
	LF	2.67	1.67	1.67	4.00	0.135
	%DS	3.00	2.00	1.00	6.00	0.050
Group 4 (0.24g/kg of ethanol)	LPDP	1.50	1.75	2.75	5.60	0.061
	LPS	1.50	1.75	2.75	5.60	0.061
	LPF	2.25	2.00	1.75	0.62	0.735
	LF	1.75	2.00	2.25	2.00	0.368
	%DS	2.13	1.88	2.00	0.13	0.936

LPDP = Latency of upright defense posture, LPS = Latency of submission, LPF = Latency of freeze, LF = Latency of flight, % DS = Percentage of defense score.

4.7 Biochemical studies

There was no significant difference found between the control and the groups administered with alcohol in all the antioxidant markers in males (table 4.9) and females (table 4.10) Wistar rats.

Table 4.9: Antioxidant enzymes activities in male Wistar rats as mean ± SEM

	Group A (distilled water)	Group B (0.12 g/kg ethanol)	Group C 0.16 g/kg ethanol	Group D 0.24g/kg ethanol	*F*	*p*
SOD (U/ml)	9.47 ± 2.12	9.48 ± 1.58	10.41 ±0.85	10.99 ±0.62	0.84	0.84
CAT (U/mg)	65.04 ±10.37	49.14±9.69	59.52±9.21	66.67±12.14	0.57	0.65
GSH (ug/ml)	7.75 ± 0.52	8.13 ±0.90	8.62 ± 0.34	8.35 ± 0.63	0.34	0.80
MDA (nMols/mg)	6898.53±675.19	6522.07±1669.05	4375.33±1235.88	3148.23±896.62	2.28	0.16

SOD = Superoxide dismutase, CAT = Catalase, GSH = Glutathione, MDA = Malonaldehyde

Table 4.10: Antioxidant enzymes activities female Wistar rats as mean ± SEM

	Group A (distilled water)	Group B (0.12g//kg ethanol)	Group C (0.16g/kg ethanol)	Group D (0.24g/kg ethanol)	*F*	*p*
SOD (U/ml)	10.53 ± 0.41	10.53 ± 0.00	11.23 ± 0.20	10.76 ±0.62	0.74	0.56
CAT (U/mg)	53.84 ± 14.20	63.51 ± 19.47	70.11 ± 13.57	69.35 ± 0.31	0.30	0.83
GSH (ug/ml)	8.94 ± 0.19	8.57 ± 0.60	9.16 ±0.33	9.65 ± 0.24	1.46	0.30
MDA (nMols/mg)	17698.57±6742.86	14624.53±3845.03	13799.53±3794.28	13430.60±4849.10	0.15	0.93

SOD = Superoxide dismutase, CAT = Catalase, GSH = Glutathione, MDA = Malonaldehyde

4.7 Histological studies

The result from the histological study reveal no histo-architecture distortion in the frontal lobe (internal Pyramidal cell layer) of the administered group compared to control in male Wistar rats while distortion in the histo-architecture in the group 4(0.24g/kg body weight of ethanol) compared to the control group in female Wistar rats.

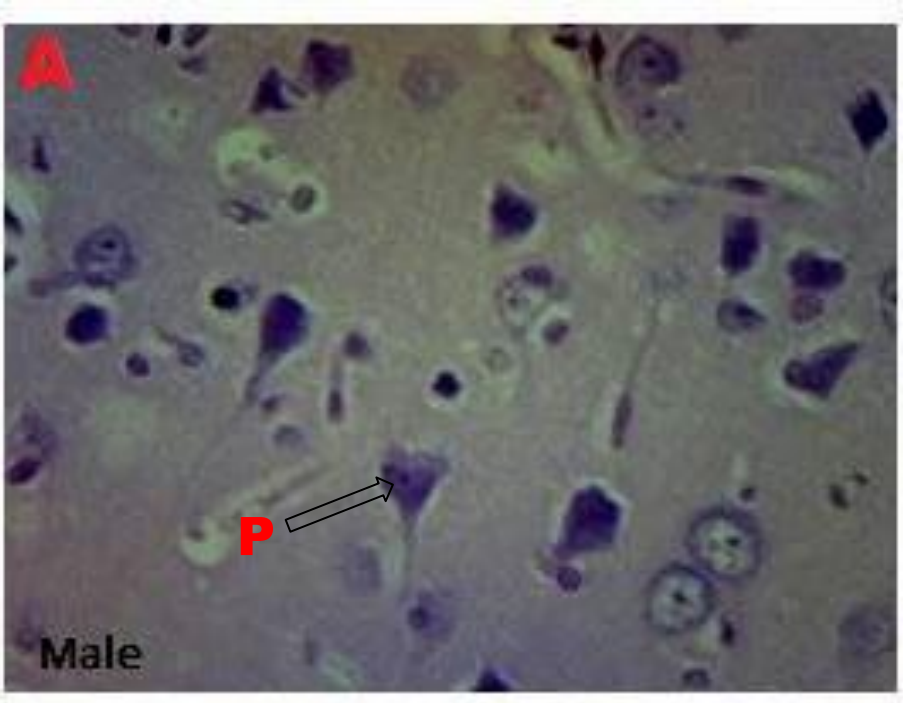


Plate III: Section of cerebrum (Frontal lobe: internal pyramidal layer) of the Control group (Administered with distilled water)of Male Wistar rat showing normal histology of the cerebrum. Pyramidal Neuronal cells (P). H and E (Mag. X 250)

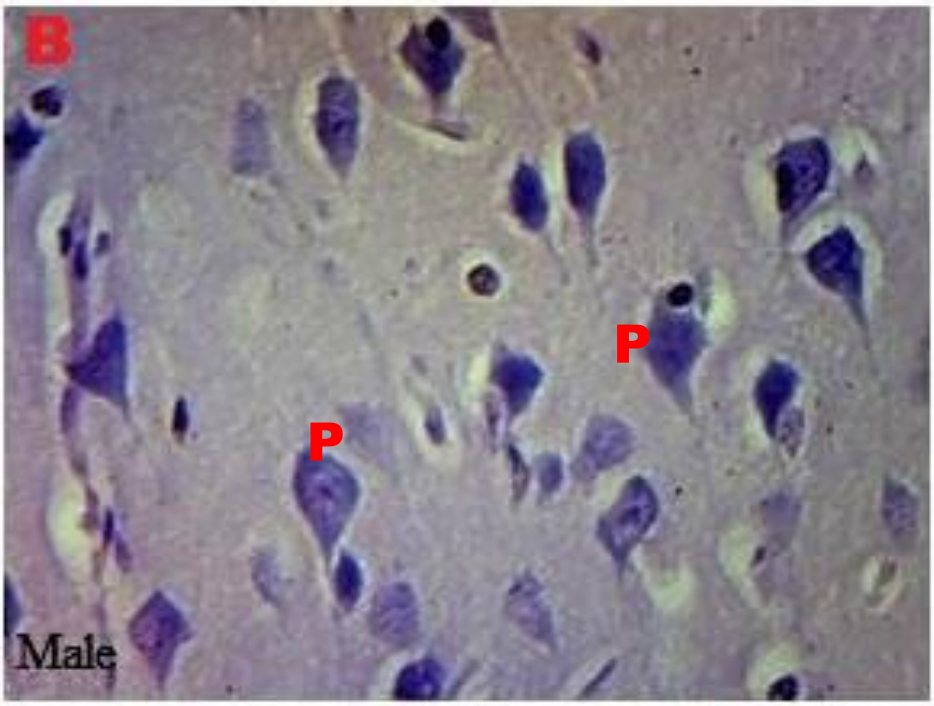


Plate IV: Section of cerebrum (Frontal lobe: internal pyramidal layer) of the Group 2 (Administered with 0.12g/Kg/body weight of ethanol) of Male Wistar rat showing histology of the cerebrum. Pyramidal Neuronal cells (P). H and E (Mag. X 250)

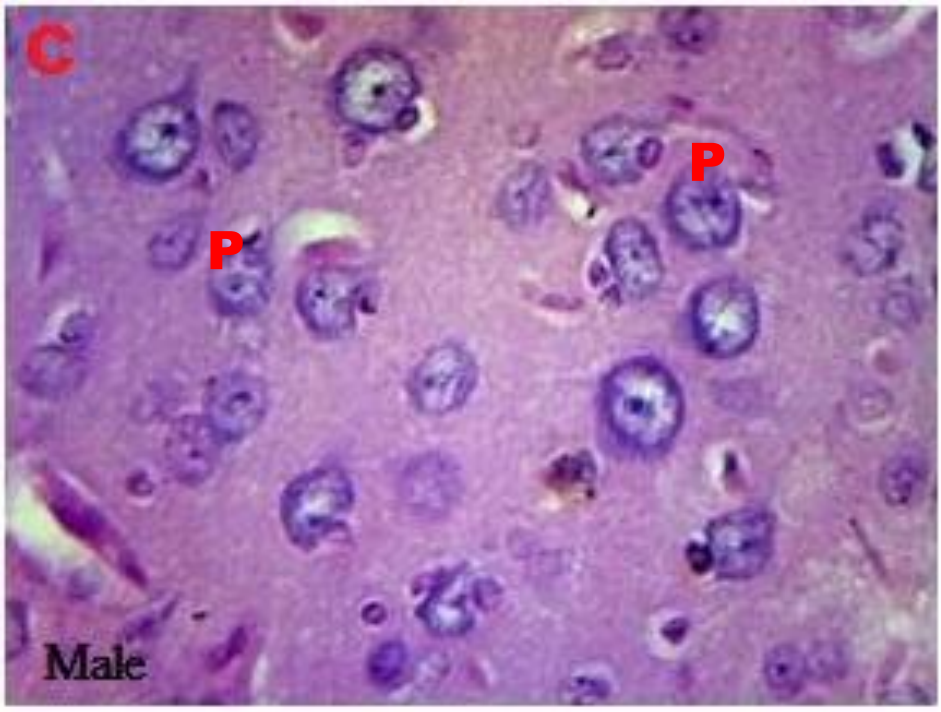


Plate V: Section of cerebrum (Frontal lobe: internal pyramidal layer) of the Group 3 (Administered with 0.16g/Kg/body weight of ethanol) of Male Wistar rat showing histology of the cerebrum. Pyramidal Neuronal cells (P). H and E (Mag. X 250)

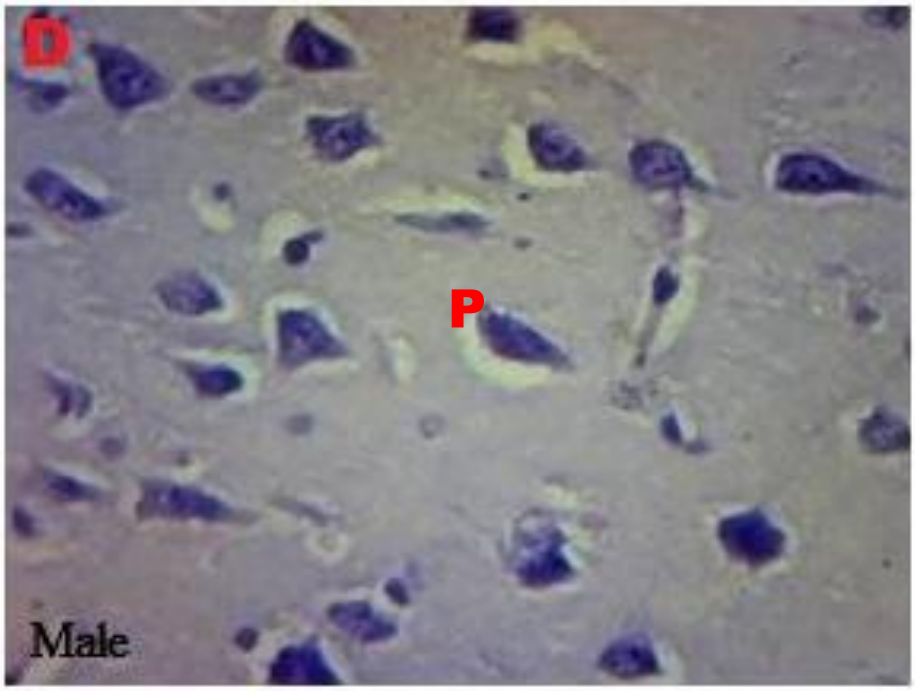


Plate VI: Sectionof cerebrum (Frontal lobe: internal pyramidal layer) of the Group 4 (Administered with 0.24g/Kg body weight of ethanol) of Male Wistar rat showing histology of the cerebrum. Pyramidal Neuronal cells (P). H and E (Mag. X 250)

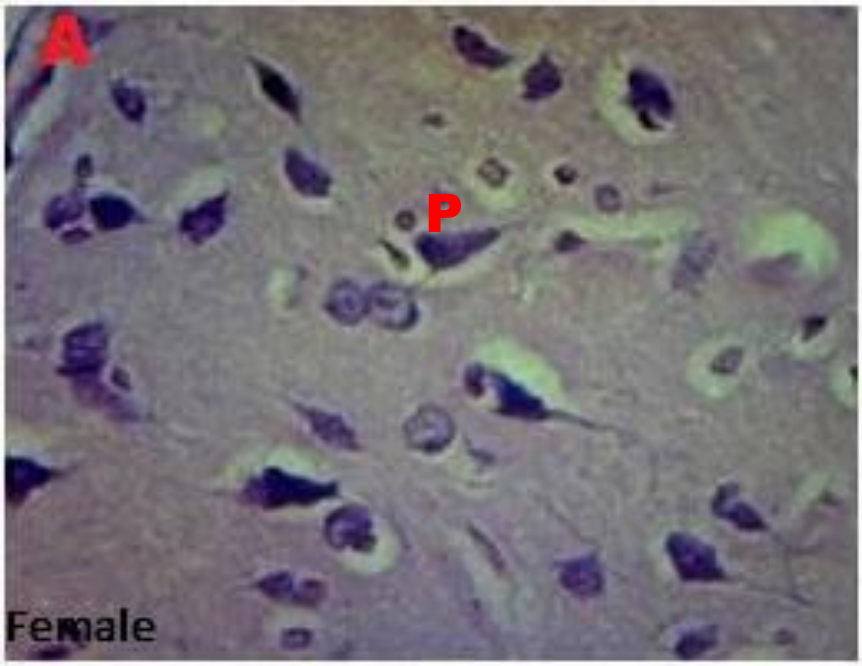


Plate VII: Section of cerebrum (Frontal lobe: internal pyramidal layer) of the Control (administered with distilled water) group of Female Wistar rat showing normal histology of the cerebrum. Pyramidal Neuronal cells (P). H and E (Mag. X 250)

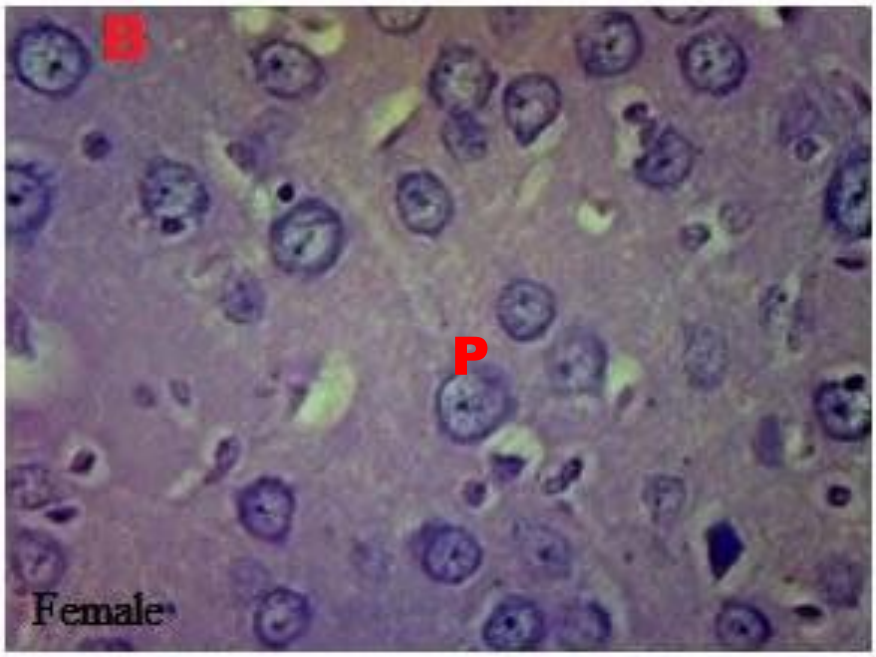


Plate VIII: Section of cerebrum (Frontal lobe: internal pyramidal layer) of the Group 2 (Administered with 0.12g/Kg/body weight of ethanol) of female Wistar rat showing histology of the cerebrum. Pyramidal Neuronal cells (P). H and E (Mag. X 250)

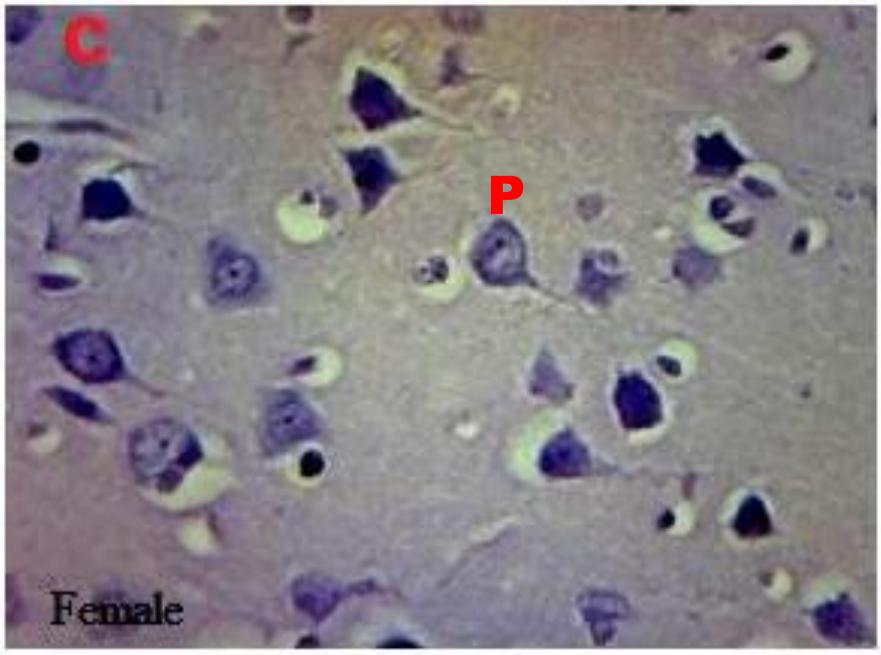


Plate IX: Section of cerebrum (Frontal lobe: internal pyramidal layer) of the Group 3 (Administered with 0.16g/Kg/body weight of ethanol) of female Wistar rat showing histology of the cerebrum. Pyramidal Neuronal cells (P). H and E (Mag. X 250)

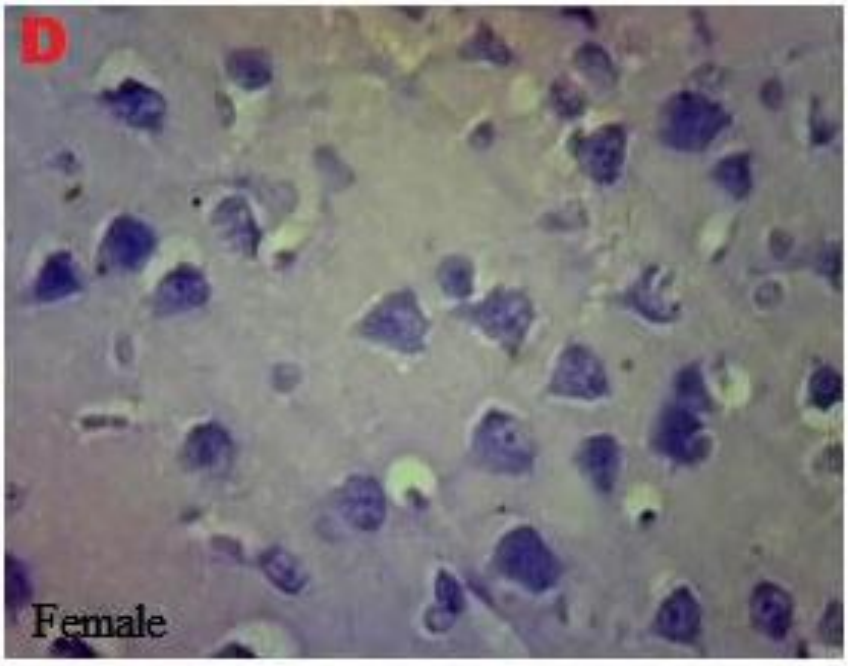


Plate X: Section of cerebrum (Frontal lobe: internal pyramidal layer) of the Group 4 (Administered with 0.12g/Kg/body weight of ethanol) of Male Wistar rat showing histology of the cerebrum. Pyramidal Neuronal cells (P). H and E (Mag. X 250).

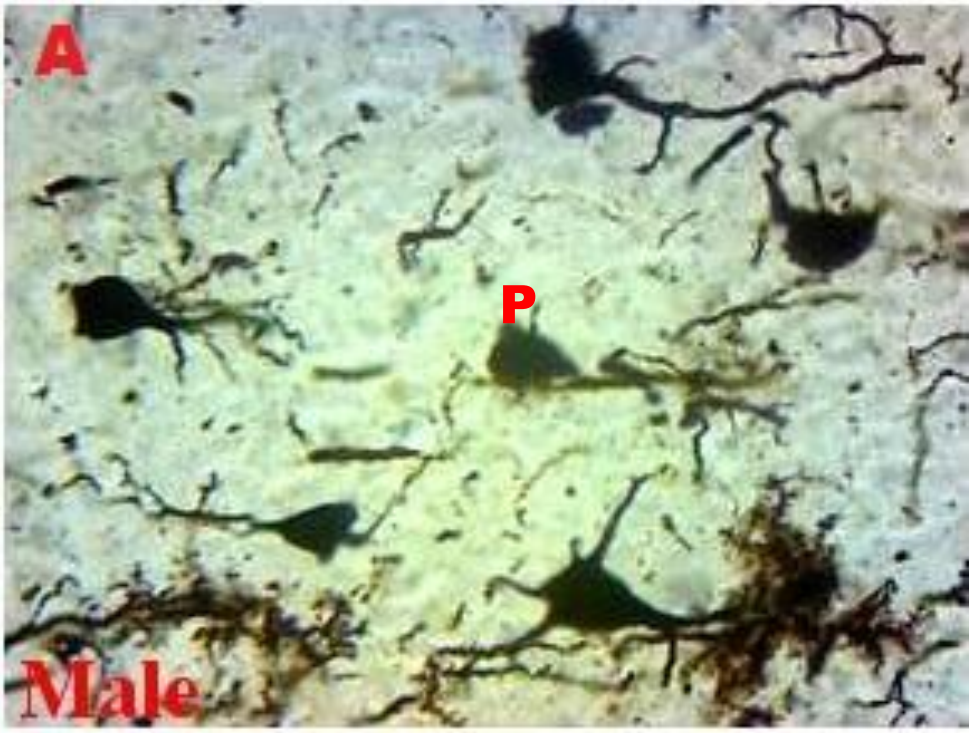


Plate XI: Section of cerebrum (Frontal lobe: internal pyramidal layer) of the Control (administered with distilled water) of Male Wistar rat showing histology of the cerebrum. Pyramidal Neuronal cells (P). Golgi stain (Mag. X 250).

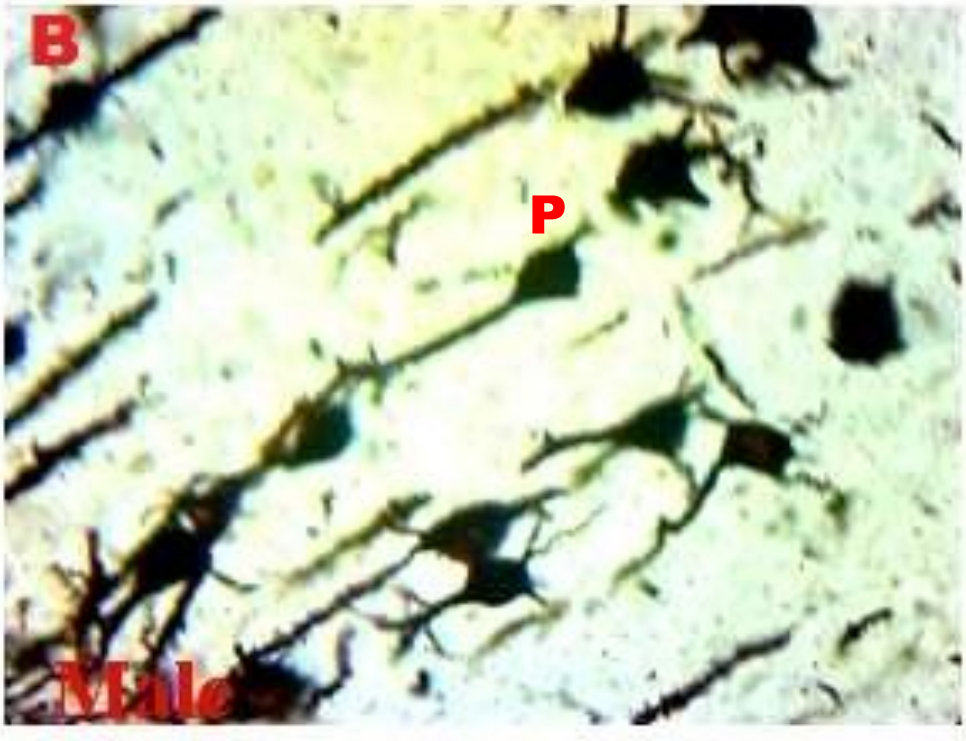


Plate XII: Section of cerebrum (Frontal lobe: internal pyramidal layer) of the Group 2 (Administered with 0.12g/Kg/body weight of ethanol) of female Wistar rat showing histology of the cerebrum. Pyramidal Neuronal cells (P). Golgi stain (Mag. X 250).

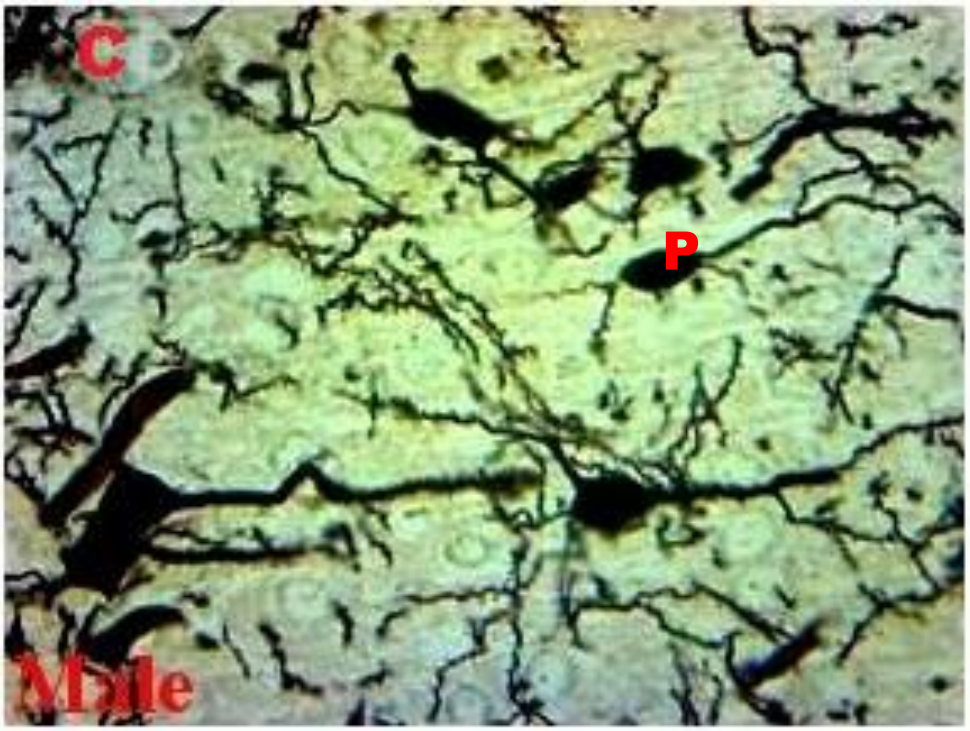


Plate XIII: Section of cerebrum (Frontal lobe: internal pyramidal layer) of the Group 3 (Administered with 0.16g/Kg/body weight of ethanol) of male Wistar rat showing histology of the cerebrum. Pyramidal Neuronal cells (P). Golgi stain (Mag. X 250).

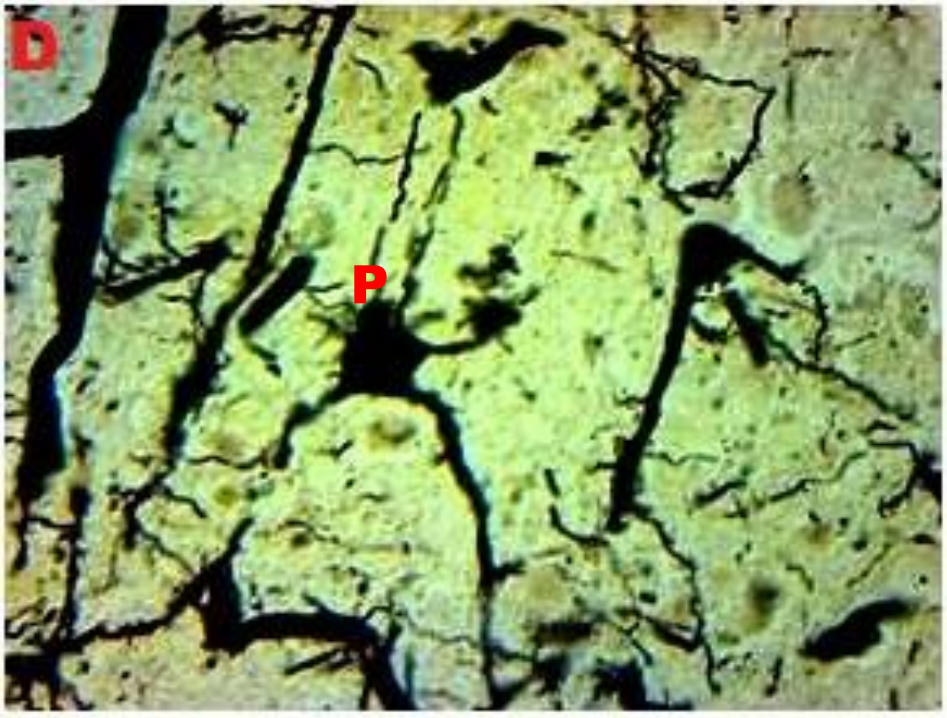


Plate XIV: Section of cerebrum (Frontal lobe: internal pyramidal layer) of the Group 4 (Administered with 0.24g/Kg/body weight of ethanol) of male Wistar rat showing histology of the cerebrum. Pyramidal Neuronal cells (P). Golgi stain (Mag. X 250).

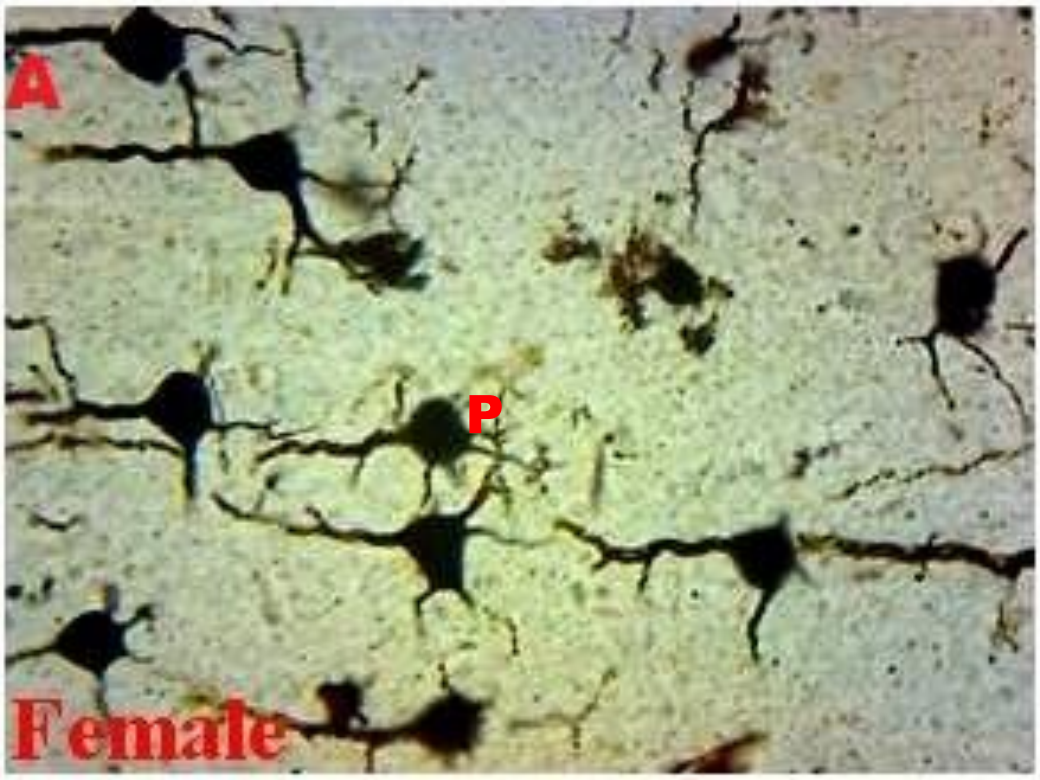


Plate XV: Sectionof cerebrum (Frontal lobe) of the control (Administered with distilled water) groupof female Wistar rat showing histology of the cerebrum. Pyramidal Neuronal cells (P). Golgi stain (Mag. X 250).

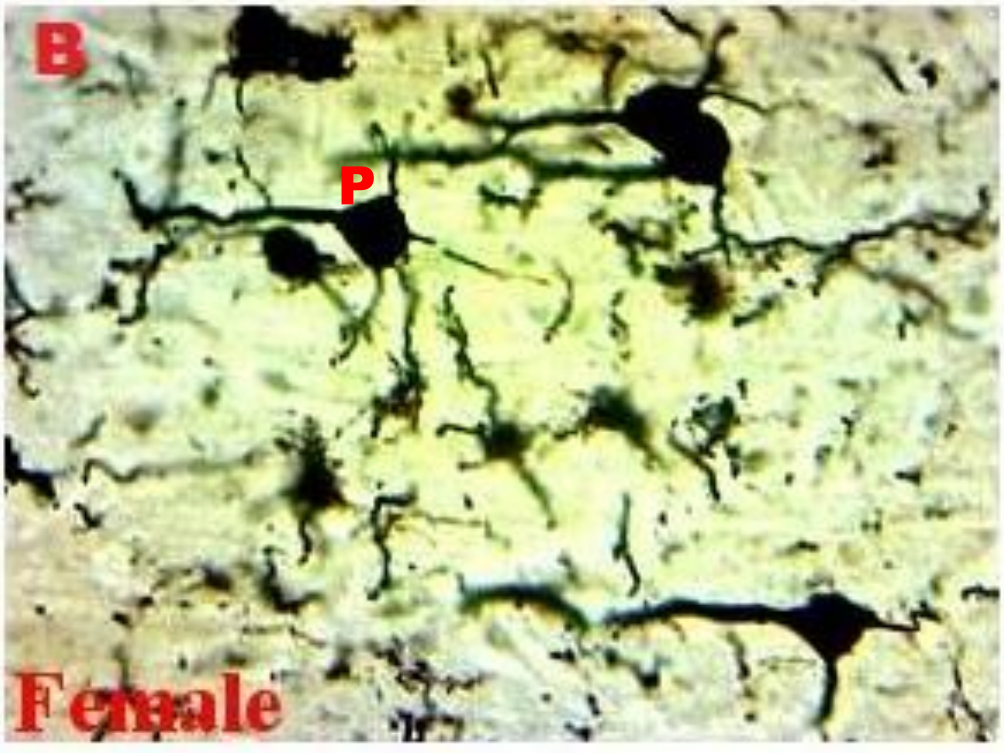


Plate XVI: Section of cerebrum (Frontal lobe: internal pyramidal layer) of the Group 2 (Administered with 0.12g/Kg/body weight of ethanol) of female Wistar rat showing histology of the cerebrum. Pyramidal Neuronal cells (P). Golgi stain (Mag. X 250).



Plate XVII: Section of cerebrum (Frontal lobe: internal pyramidal layer) of the Group 3 (Administered with 0.16g/Kg/body weight of ethanol) of female Wistar rat showing histology of the cerebrum. Pyramidal Neuronal cells (P). Golgi stain (Mag. X 250).

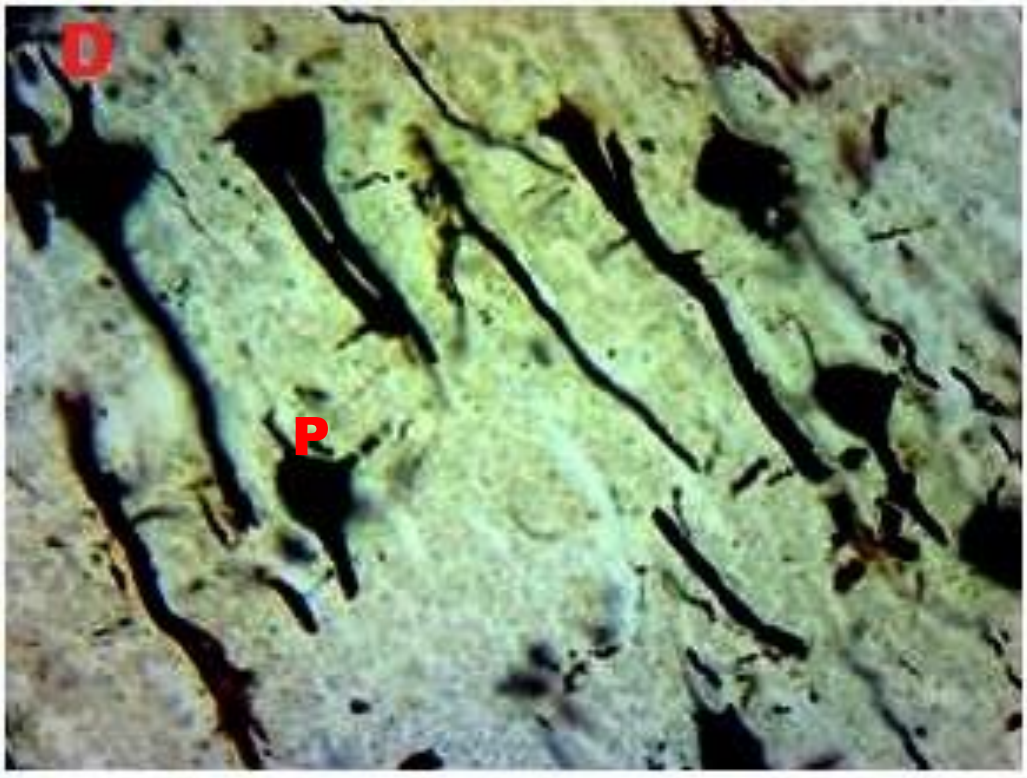


Plate XVIII: Section of cerebrum (Frontal lobe: internal pyramidal layer) of the Group 4 (Administered with 0.24g/Kg/body weight of ethanol) of female Wistar rat showing histology of the cerebrum. Pyramidal Neuronal cells (P). Golgi stain (Mag. X 250).

5.0 DISCUSSION

In the present study, we evaluate the effect of chronic oral consumption of low dose of alcohol on the cerebrum (frontal lobe) by using neurobehavioral, biochemical, histological and morphological method.

5.1 Physical observation

During the course of the administration, it was observed that there is stimulation of appetite up to the third week of administration follow by reduction in the intake of fooduntil the end of the administration in all the groups administered with alcohol. Alcohol is the second highest source of energy, on a per gram basis, of all the macronutrients, providing 29 kJ/g (7 kcal/g) (Foster and Marriott, 2006).

Unlike other macronutrients, there is minimal evidence for any reduction in food intake to compensate for the potential energy in alcohol. In contrast, moderate alcohol consumption prior to a test meal leads to a short-term increase in food intake. This stimulatory effect of alcohol is not apparent beyond acute administration, but the inability to reduce voluntary energy intake in response to energy from alcohol metabolism is evident over extended periods. Alcohol suppresses fatty acid oxidation, increases short-term thermogenesis and stimulates a number of neurochemical and peripheral systems implicated in appetite control, including inhibitory effects on leptin, glucagon-like peptide-1, and serotonin, and enhancement of gamma-aminobutyric acid, endogenous opioids and neuropeptide Y. All of these effects could lead to overeating, and mechanisms underlying appetite stimulation through alcohol require further substantiation (Yeomans *et. al.*, 2003).

Forsander, (1998) reported an experiments made in vitro in isolated tissue preparations and in perfused organs suggest that ethanol should be an excellent nutrient. The ethanol molecule contains much energy in which the body can utilize for its energy supply at least as efficiently as it uses the calories of ordinary food. Ethanol is a neutral water-soluble substance thatrequires no digestion before it is absorbed in to the blood by simple diffusion. It is transfer in to every cell of the body withoutthe participation of an active transport system. Althoughthesein vitroconditiondo notcorrespond to the situation in the intactbody,the conclusionthat ethanolis a goodnutrientis consistent with earlieralcoholconsumption studies(Richter,1953).Theenergyfromalcohol appearsto contribute to thetotalcaloricintake in thesameway asthenormalnutrientsdo,andit replacestheintake of othernutrientsin the sameway thatthe intakeof differentfoodcomponentsreplacesoneanother.Richter's find outthatvoluntaryalcoholintakedepresses foodconsumption in proportionto itsenergycontent hasbeen frequentlyreplicatedinlaboratorystudies (Eriksson, 1969). The results from human studies, however, have been lessconsistent. Some have shownthat alcohol depressed eatingalmostin relationto the caloriesobtainedfrom alcohol (Barboriac*et al*., 1978;Hillers andMassey,1985)while several otherexperimentsshowed thatethanol hadlittleor no effect on food intake and that the alcohol calories wereadded onto the calories from the food (Bebb *et al*., 1971; De-Castro and Orozco, 1990; Herbeth *et al*., 1988; Rissanen *et al.*,1987).

Ethanolinteractsnot only with the intakeof caloriesbutalsowith thechoiceof the food components. Althoughthe intakeof fat and protein may alsobe changed, thelargest effect is the suppression of carbohydrate consumption. In one experimentwith ratswith a particularlyhighcarbohydrate intake, it was shown that the carbohydrate consumptionwasreplaced by alcohol intake exactly in relation to the caloriccontent of the alcohol, while fat and protein consumption were not

influenced (Forsander, 1988). Also in several humanstudies, it hasbeen observed that alcoholdepresses the carbohydrate intake morethanthe othercomponent (Eddy *et al.,* 1971; Hurt *et al*., 1981; Neville *et al*., 1968; Windham*et al.*, 1983).As might beexpected, theintake isincreaseagainto their previouslevelswhenno more alcoholis consumed(Hurt *et al*., 1981).

The consumptionof food is regulatedby highly sophisticatedmechanismsinvolving interactions between peripheral metabolism and the central nervous system. For example, whenthe oxidationof glucoseis depressedandfat metabolismtakes over, as is the case in diabetes and related diseases (Randle*et al*., 1963), the consumptionof carbohydrates is depressed andthe fat intakeisincreased (Richter,1942). Itseemslikely that the intake of alcoholis controlledby thesesamemechanism(Forsander, 1994), with ethanol affecting the intake of other nutrients, and the nutrients, in turn, influencing the intake of alcohol.

5.2Body weight assessments

In the present study, it was observed that there is significant difference in the weight of the male Wistar rats that was administered with ethanol and control in first week of administration but no significant difference was recorded again in the experiment after first week while in female Wistar rats, the significant difference was observed up to the second week of administration.

The relation between alcohol consumption and body weight remains an enigma for nutritionists. This is an important problem because the average alcohol intake in adults is <10% of the total daily energy intake in several developed countries (Jéquier, 1999). Epidemiologic evidence on role of alcohol energy in body weight controldoes not show a clear relation between daily alcohol energy intake and body weight. However, most studies report that peopledo not compensate for the alcohol energy by decreasing non-alcohol food energy intake. Except in

aloholics, alcohol energy is usually added to total food energy intake. Therefore, moderatealcohol drinkers tend to consume more energy than non-drinkers do.

The paradox of increased alcohol-induced energy intake with no clear correlation between alcohol intake and body weight has led to the curious concept that alcohol energy has a low biological value (Lands and Zakhari, 1991; Liebar, 1991; and Pirola and Liebar, 1976). This hypothesis has not been confirmed byrecent metabolic investigations on the effect of alcohol intake onenergy expenditure and substrates oxidation in humans. Several studies carried out by using whole-body indirect calorimeters(Suter *et al.*, 1992; Suter *et al.*, 1994; and Murgatroyd, 1996) clearly showed that the body uses ethanol energy efficiently and that alcohol energy does count! Ethanol-induced thermogenesis has been studied by several groups of investigators;a mean value of <15% for ethanol-induced thermogenesishas been obtained (Suter *et al*., 1994; Murgatroyd, 1996; and Sonko *et al.*, 1994). After ethanol ingestion, the stimulation of energy expenditure induced by ethanol metabolism represents15% of the ethanol energy; thus, 85% of ethanol energyis available as metabolizable energy for other metabolic processes.

Ethanol-induced thermogenesis is smaller than protein-induced thermogenesis (<25%) and larger than carbohydrate-inducedthermogenesis (<8%) and lipid-induced thermogenesis (<3%).There is another way by which alcohol intake may alter bodyweight regulation. Ethanol is not stored in the body, but it is oxidizedin preference over other fuels. The addition of ethanol to a diet reduces lipid oxidation measured over 24 hours whereas oxidationof carbohydrate and protein are much less inhibited (Suter *et al*., 1992).Other studies confirm that alcohol ingestion reduces fat oxidation and favors a positive fat balance (Murgatroyd, 1996 and Sonko *et al.*, 1994). In summary, metabolicstudies show that ethanol energy is use with an efficiencycomparable with

that of a carbohydrate plusprotein meal and that it reduces fat oxidation. There is no reason to claim that ethanolenergy does not play a role in energy balance regulation.

5.3 Cognitive studies

A widely used method for assessing these cognitive processes in rodents is the Morris water maze, a classic test for examining spatial learning and memory (Barnhart *et al*., 2015).Morris Water Maze (MWM) was first established by neuroscientist Richard G. Morris in 1981 in order to test hippocampal-dependentlearning, including acquisition of spatial memory and long-term spatial memory (Morris, 1981). The main advantage being the differentiation between the spatial (hidden-platform) and non-spatial (visible platform) conditions (Eichenbaum*et al*., 1990; O'Keefe, 1979; and Scoville and Milner, 1957). Additional, the MWM testing environment reduces odor trail interference (Block, 1999). This has led the task to be usedextensively in the study of the neurobiology and neuropharmacology of spatial learning and memory. The MWM plays an important role in the validation of rodent models for neurocognitive disorders (Bromley-Brits *et al*., 2001). Many methodological variations of the MWM task have been and are being used by research groups in many different applications. However, researchers have become increasingly aware that MWM performance is influenced by factors such as apparatus or training procedure as well as by the characteristics of the experimental animals (sex, species or strain, age, nutritional state, exposure to stress or infection). Lesions in distinctbrain regions like hippocampus, striatum, basal forebrain, cerebellum and cerebral cortex were shown to impair MWM performance, butdisconnecting rather than destroying brain regions relevant for spatial learning may impair MWM performance as well. Spatial learning ingeneral and MWM performance in particular appear to depend upon the coordinated action of different brain regions

and neurotransmittersystems constituting a functionally integrated neural network. The MWM task has often been used in the validation of rodentmodels for neurocognitive disorders and the evaluation of possible neurocognitive treatments. Through its many applications, MWMtesting gained a position at the very core of contemporary neuroscience research (D'Hooge, and De Deyn, 2001).In the MWM, rats are placed in a large circular pool of water from which they can escape onto a hidden platform, which is located in the middle of one of the four imaginary quadrants of the pool. The platform is rendered invisible, both by ensuring that its top surface is just beneath the water surface and by making the water opaque (Morris, 1981). Thus. The platform offers no local cues to guide escape behavior. Normal rats very quickly learn to swim directly towards the platform from any starting position at the circumference of the pool. The accurate directionality of the escape behavior provides evidence that the rats escape by learning the spatial position of the platform relative to distal cues. Several measures are used to estimate the efficiency of the rat's performance: directionality, latency of finding the platform on each trial, length of the swim path as well as the

distribution of swimming time and swim-path in the four quadrants of the pool during transfer trials, in which the platform is removed from the pool, or during reversal trials in which the platform is moved to another quadrant of the pool.

From the present research, it was observed that there is consistence decrease in escape latency over time in dose dependent manner across the administered group in male Wistar rats while in female the consistency was noticed in group that was administered with highest dose of ethanol (0.24g/kg body weight per day). According to Morris (1981), the result reveal that is improved memory with decrease escape latency compare to control Wistar rats.

The review of Pohorecky (1977) provides strong psychological, behavioral and biochemical evidence for alcohol (ethanol) affecting the experimental behavior of animals and humans in a biphasic dose–dependent manner, with low-doses being excitory and high-doses depressant. For non-human mammalian biological end points (including, amongst others, the liver, kidney, heart, spleen, endocrine, embryonic development and birth weight), Calabrese and Baldwin's (2003) assessment of the toxicological and pharmacological literature demonstrated ethanol-induced biphasic dose responses over a wide range. In most cases, the low-dose hormetic responses were judged statistically significant and reliable. They observed striking similarities across studies in both hermetic dose-ranges and hormetic dose–response magnitudes, withthe maximum hormetic response approaching two-fold butwas usually 25–40% greater than the control value. Furthermore,the maximum hormetic response was generally within a factor of 2–4 of the estimated NOAEL (No Observed Adverse Effect Level) (Hayes, 2007). They argued against acommon explanatory mechanism, and for a diverse plethoraof underlying mechanisms. They also stated that regardless ofactual response mechanisms, the similarity of dose–responsecharacteristics likely reflected an important overall regulatorystrategy built into the framework of a homeostatic controlsystem. Calabrese and Baldwin (2003) further noted thatthese collective findings closely resembled the biphasic hormetic responses to a wide range of toxic chemical agents that they had previously reported (Calabrese and Baldwin, 1997).

While there are a considerable number of published investigations suggesting that chronic alcoholics have an increased susceptibility to infectious diseases (in particular pneumonia), Hallengren and Forsgren (1978) have presented evidence that at low to moderate blood alcohol concentrations there is an increased adherence, phagocytosis and chemotaxis of human polypmorphonuclear leucocytes. The magnitude of the detected response is consistent with that

seen for hormetic responses, with the hormetic concentration range being about four-fold. It would appear that these results have important ramifications for biomedical researchers and clinicians. Human health benefits of regular light-to-moderate alcohol consumption Vis-a`-Vis abstinence include decreased risks of dementia, diabetes, osteoporosis, and cardiovascular disease and death (Standridge *et al.,* 2004). Both case control and cohort epidemiological studies have consistently shown that light-to-moderate drinkers are at lower risk of cardiovascular disease and death than non-drinkers are, although heavier alcohol consumption can negatively affect the neurologic, gastrointestinal, hematologic, immune, psychiatric and musculoskeletal organ systems.

5.4 Anxiety Related Behavior Studies

Elevated plus maze described by File and co-workers (Pellow *et al*., 1985) is a widely used behavioral assay for rodents and it has been validated to assess the anti-anxiety effects of pharmacological agents and steroid hormones, and to define brain regions and mechanisms underlying anxiety-related behavior. Briefly, rats or mice are placed at the junction of the four arms of the maze, facing an open arm, and entries or duration in each arm are recorded by observer simultaneously for five minutes. Other ethological parameters (rears, head dips and stretched-attend postures) can also be observed. An increasein open arm activity (duration and/or entries) reflects anti-anxiety behavior. In our laboratory, ratsor mice are exposed to the plus maze on one occasion; thus, results can be obtained in five minutes per rodents. Unlike other behavioral assays used to assess anxiety responses that rely upon the presentation of noxious stimuli (i.e., electric shock, food/water deprivation, loud noises, exposure to predator odor, etc.) that typically produce a conditioned response, the elevated plus maze relies upon rodents'

proclivity toward dark, enclosed spaces (approach) and an unconditioned fear of heights/open spaces (avoidance) (Baenett. 1975).

Result obtained in the present study indicate that there was no significant difference in the open arm entries and duration across the experimental period in male Wistar rats, the shows that all the dosage of ethanol have not significant effect on the anxiety related behavior in male Wistar rats. While there was significant reduction in the percentage of open arm entries in female Wistar across the experimental period. Women absorb and metabolize alcohol differently than men. In general, women have less body water than men of similar body weight do, so that women achieve higher concentrations of alcohol in the blood after drinking equivalent amounts of alcohol (Frezza *et al*., 1990 and Taylor *et al*., 1996). In addition, women appear to eliminate alcohol from the blood faster than men. This finding may be explained by women's higher liver volume per unit lean body mass (Li *et al*., 1998 and Kwo *et al*., 1998), because alcohol is metabolized almost entirely in the liver (Levitt *et al*., 1997).

5.5Aggressive Related BehavioralStudies

Standardized method to measure offensive aggression and defensive behavior in a semi natural setting is resident intruder test as reported by Koolhaas *et al*. (2013).Aggressive behavior belongs to the natural behavioral repertoire of virtually all animal species. From a biological point of view, aggressive behavior can be considered as a highly functional form of social communication aimed at active control of the social environment (Koolhaas *et al*., 2013). This so-called resident-intruder paradigm allows the spontaneous and natural expression of both offensive aggression and defensive behavior in laboratory rodents in a semi natural laboratory setting. Defensive aggression is the form of aggressive behavior performed in response to an attack by another individual. It is distinctly different from offense in terms of its behavioral

expression and inhibitory controls (Blanchard *et al*., 2003). Note that extreme forms of defensive behavior can have violent characteristics. By recording the frequencies, durations, latencies and temporal and sequential patterns of all the observed behavioral acts and postures in the combatants during these confrontations, a detailed quantitative picture (ethogram) of offensive (resident) and defensive (intruder) aggression is obtained. The intruders in the resident-intruder paradigm will show defensive behavior in response to the offensive attacks by the resident. The paradigm therefore also allows one to study defensive behavior and social stress by using the intruder as the experimental animal.

In the present study, defensive aggression was studies and the result revealed that there is no significant difference in the control and experimental Wistar rats in male and female, this finding is agreement with what has been reported in various studies that Ethanol has long been known to affect a wide range of animal model and human behaviors in a biphasic dose-dependent manner with the low dose being stimulatory and the high dose being inhibitory (Pohorecky, 1977). Such bi-phasic responses have been recognized in animal studies for motor activity (Read *et al*., 1960; Buckalew and Cartwright, 1968; Carlsson *et al.*, 1972; Randall *et al*., 1975; Erickson and Matchett, 1975;Matchett and Erickson, 1977), open field activity tests (Sanders, 1976), performance in Y mazes (Leonard and Wiseman, 1970), positive food motivated operant behavior (Holloway and Vardiman, 1971; Holloway and Wansley, 1973; Glowa and Barrett, 1976; Leander *et al*., 1976), aversion responding-shock avoidance (Reynolds and VanSommers, 1960), electrical self-stimulation via bar pressing (Vrtunski *et al*., 1973; Carlson and Lydic, 1976), and aggression (Weitz, 1974; Peeke *et al*., 1975; Miezek and Barry, 1977). The mechanisms of such biphasic dose responses have long implicated the dopamine-norepinephrine system as a result of the use of early metabolic inhibitors, such as α-methyl-p-tyrosine, which

block catecholamine synthesis, thereby preventing the low dose stimulatory response (Calabrese, 2003).

5.6Biochemical Studies

Superoxide dismutase (SOD) activity, Catalase (CAT) activity, Glutathione and Malondialdehyde, which is a make for lipid peroxidation were estimated and it was found that even though there was slight increase in the level of these antioxidant mark, the increase was not significant. The increase in the antioxidant mark indicate that metabolism of alcohol lead to more production of reactive oxygen species as reported in the literature. There is evidence that ethanol produces alterations in the mitochondrial structure and function of several organs, both in laboratory animals and humans (Pachinger *et al.,* 1975). The mitochondria play an important role in the alcohol metabolism via the enzyme ALDH; this enzyme catalyzes the conversion of acetaldehyde into acetate. When this enzyme reaches a saturation point, the acetaldehyde escapes into the blood stream and leads damage to biomolecules such lipids, proteins and nucleic acids which results of the toxic side effects that are associated with alcohol consumption (Salvador and Alfredo, 2010). These changes affect the mitochondrial function decreasing respiratory rates (Albano, 2002) and ATP levels, and might result in increased production of reactive oxygen species (ROS) (Robin *et al*., 2005). Numerous studies show that mitochondrial levels of ROS may be increased by chronic alcohol consumption as a consequence of increased mitochondrial CYP2E1 levels (Adam-Vizi, 2005 and Ronis *et al.,* 1996) as well as a by-product of the matrix enzyme α-ketoglutarate dehydrogenase (Cederbaum, 1991).

The ROS generated from the metabolism is not enough to cause oxidative damage and therefore could be further use to benefit the cells, which is line with the concept mitohormetics as report, by Ristow and Schmeisser (2014).

5.7 Histological Studies

In the present study, light microscopic examination of routinely Haematoxylin and Eosin (H&E) and silver impregnation (Golgi) stained cerebral (frontal lobe) sections of Wistar rats reveal that no histo-architecture distortion in male Wistar rats while in female Wistar rats, vacuolation and loss of neuronal dendrite in the group administered with 0.24g/kg of ethanol compared to his male counterpart and female control.

The result obtained indicated that there are gender differences in alcohol pharmacokinetics, in agreement with Mumenthaler *et. al*. (2005) that reported that Women appear to become more impaired than men do after drinking equivalent amounts of alcohol, achieving higher blood alcohol concentrations even when doses are adjusted for body weight. This finding may be attributable in part to gender differences in total body water content. Significant gender differences in alcohol pharmacokinetics appear to include increased bioavailability and faster disappearance rates in women. Women have proportionally more body fat and less water than do men of the same body weights. Because alcohol is dispersed in body water, women reach higher peak BACs than men do after consuming equivalent doses of alcohol, even when doses are adjusted for body weight (Frezza *et al*. 1990; Taylor *et al*. 1996). Higher peak BACs in women may also reflect lower rates of FPM by gastric (Frezza *et. al.*, 1990; Vogel-Sprott, 1992) or hepatic ADH (Thomasson, 1995). The result of the male rats was also in agreement with the studies on Moderate ethanol consumption and increases hippocampal cell proliferation and neurogenesis in the adult mouse by Aberg *et. al*. (2005) and reported that Moderate ethanol consumption increased the proliferation of cells, which survived and developed a neural phenotype and ethanol consumption did not induce apoptosis, neither did it change differentiation nor the distribution patterns of the newly formed cells. The cell proliferation rate

in the dentate gyrus returned to basal levels 3 d after ethanol withdrawal. Moreover, concluded that voluntary ethanol intake by mice can change the rate of cell proliferation in the dentate gyrus. Since there was no increase in apoptosis concomitant with the ethanol-induced increase in neurogenesis, it is possible that the new cells in the dentate gyrus will contribute to the long-lasting changes of brain function after ethanol consumption.

6.0 CONCLUSION

In the present study, Histo-morphological, Biochemical and Neurobehavioral Evaluation of the frontal lobe following chronic alcohol consumption in Wistar rats. The results that chronic consumption of low dose of alcohol in Wistar rats can cause reduction in food intake, have no significant changes on body weight, improve cognitive activities in both male and female in dose dependent manner, has no effects on anxiety related behavior and violence and causes no significant changes in the production of reactive oxygen species.With respect to the findings of present result, chronic daily oral administration of lose dose of alcohol:

i. improve learning and memory in male and female Wistar rats
ii. does not significantly increase the production of reactive oxygen species which can cause oxidative damage in male and female Wistar rats.
iii. does not have a significant effect on anxiety and aggression in male and female Wistar rats.

6.1 Recommendation

i. Further studies are need to evaluate the effect of chronic administration of low dose of alcohol on the number of Neurons and Volume of the cerebrum using stereological techniques.

ii. In addition, further studies are required to investigate the effects of chronic consumption of alcohol on the mitochondrial gene expression, which is responsible for the mitohormetics effect of low dose of alcohol.

6.2 Contribution to knowledge

i. Chronic oral administration of alcohol at dose of 0.16g/kg and 0.24g/kg weight per day significantly (p <0.05) improvement in memory over time in male Wistar rats and at a dose of 0.12g/kg body weight per day also causes a significant improvement in memory in female Wistar rats.

ii. Chronic oral administration of alcohol at dose of 0.12g/kg, 0.16g/kg and 0.24g/kg body weight per day have no significant (p> 0.05) effect the production of reactive oxygen species in both male and female Wistar rats.

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