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Observed Heamatological and Neurohistochemical Alterations in Hippocampal formation Of Adult Wistar Rats (Rattus norvegicus) following Sub-acute Monosodium Glutamate Exposure

Odubela Olukayode Olusola [1], Fakunle Ponle Bamidele [2,*], Ehiremen Samuel Ehimare [1], Alli Zainab Tobilola [1], Adeleye Ademola Stephen [1] and Dawodu Adekunle Jonathan [3]

[1] *Department of Anatomy, Faculty of Basic Medical Sciences, Obafemi Awolowo College of Health Sciences, Olabisi Onabanjo University, Sagamu, Ogun State, Nigeria.*
[2] *Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.*
[3] *Olabisi Onabanjo University Teaching Hospital, Obafemi Awolowo College of Health Sciences, Olabisi Onabanjo University, Sagamu, Ogun State, Nigeria.*

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Abstract

Introduction: Exposure to harmful substances either by biological, chemical or physical agents which can result to changes with memory cognition underlines neurotoxicity. Monosodium glutamate (MSG) is a combination of sodium and glutamic acid primarily used as a flavoring enhancer in food processing. The hippocampus is a part of the limbic system that helps in both spatial memory and the consolidation of information from short-term to long-term memory.

Aim: To assess the Heamatological and Neurohistochemical effects of Monosodium Glutamate on Hippocampus of adult wistar rats.

Methods: Thirty (30) adult wistar rats weighing 200±50g were randomly assigned into 3 groups (n=10). The control group received distilled water, T1 group received 2g/kg bd.wt of MSG and T2 group received 2g/kg bd.wt of MSG. All the rats were exposed for a duration of 4 weeks except T2 group that were allowed a 2-week withdrawal period. The animals were sacrificed by cervical dislocation followed by hippocampal specimen's excision which were processed for routine histological staining techniques, Heamatological studies and biochemical quantifications. All data obtained were statistically analyzed using GraphPad prism version 5.

Results: The Body weight (P value=0.4478), Brain weight(P value< 0.0001), Organ weight (P value < 0.0001), LDH (P value < 0.0001), SOD (P value < 0.0001), CAT (P value < 0.0001), PCV (P value=0.0080), Hb (P value=0.0537) and RBC (P value=0.0005) showed significant decrease at T1 groups and significant increase at T2 groups while the WBC (P value < 0.0001) shows a significant increase at T1 and a significant decrease at T2 when compared to the control groups. The histological findings showed the alterations in the hippocampal architecture of the T1 group and slight regeneration of hippocampal tissues in T2 group when compared to the control group.

Conclusion: These findings add to a thorough comprehension of the neurobiological impacts of MSG and highlight the need for dietary guidelines and legislative actions to protect brain function.

Keywords: Neurotoxicity; Monosodium Glutamate (MSG); Hippocampus; Antioxidant activity

* Corresponding author: Fakunle Ponle Bamidele

1. Introduction

Neurotoxicity refers to the changes in neurophysiology caused by exposure to harmful substances which can result to changes with memory, cognition, mood, or even lead to the start of psychiatric diseases (1). Multiple processes can lead to neurotoxicity, such as direct injury to neurons, inflammation, oxidative stress, and disruption of neurotransmitter systems (2). Depending on exposure type, neurotoxic exposure may result in central-nervous system damage, affective disturbances, and/or neurocognitive disruptions. It can result from exposure to various chemical agents, drugs, environmental toxins, or pathological conditions (3).

Heavy metals, pesticides, pharmaceuticals, and environmental contaminants are examples of compounds that can be neurotoxic (4). There are two types of neurotoxic exposures; chronic exposure is most frequently investigated in relation to neurotoxicity caused by heavy metals, such as lead, mercury, and arsenic. Chronic exposure can also result in a wide range of varied and long-lasting symptom manifestations, including fatigue, reduced fine and gross motor function, slowed processing speed, and generally worse cognitive performance (5). Acute exposure can result in rapid onset of nausea, headaches, altered cognition, and emotional disturbances (4). Prolonged exposure commonly results in neurodegeneration which are mental symptoms. Psychiatric symptoms could include heightened anxiety, anger, and depression. Numerous neurological conditions and symptoms, such as cognitive decline, mobility issues, and mental problems, can be brought on by neurotoxicity (6). The neurotoxic potential of compounds is evaluated using a variety of techniques, such as in vitro tests and animal investigations (6).

A neurotransmitter called glutamate has the potential to produce excitatory neurotoxicity at excessive extracellular concentrations. This can trigger the calcium balance and raise the generation of reactive oxygen species (ROS) (7). An aberrant accumulation in the extracellular space of the brain can results in neurodegenerative disorders such as Alzheimer's, Parkinson's and Huntington's disorders (8). Inhibiting glutamate toxicity by targeting the excessive breakdown process is seen as a viable technique for treating neurodegenerative diseases (9).

Therapy for symptoms and assistance comes after removing or minimizing exposure to the harmful drug. The prognosis is based on the severity of the neurological impairment, the duration and level of exposure. After treatment, a lot of people recover fully. While some people may live, their recovery may be incomplete. Certain situations can result in death when exposed to neurotoxicants (10).

1.1. Monosodium glutamate

Monosodium glutamate (MSG) is a combination of sodium and glutamic acid which is primarily used as a flavoring enhancer in food processing, giving food an umami taste that intensifies the meaty, savory flavor, much like naturally occurring glutamate does in stews and meat soups (11). The amino acid glutamic acid is present in food naturally. Monosodium glutamate is also known as sodium glutamate which is the sodium salt of L-glutamic acid, an amino acid (12). Its IUPAC name is sodium 2-aminopentanedioate, in both the International Numbering System (INS) and the European system for food additives. MSG is designated with code number 621 (13).

Worldwide, the fermentation process utilizing Corynebacterium glutamicum or closely related species produces about 1.9 million tons of MSG yearly (14).The two major active ingredients in taste enhancers are salt (NaCl) and MSG. Shops on the street, supermarkets, and outdoor markets carry a variety of brands of culinary spices (15). Certain brands, such as A-one, Vedan, Star Maggi, Ajinimoto, Knorr, Royco, Doyin, Jumbo (cubes), Aluba Shrimp Seasoning (powdered), Salsa, and Tasty are among them, according to literature (16). MSG has been used for more than a century to season food in which numerous studies have been conducted on its safety. It contains 61% less sodium than table salt and one study found that substituting part of the table salt with MSG could reduce the sodium content of a soup by 32% without compromising its flavor (17).

Several other studies have also used MSG to preserve flavor while lowering sodium levels in soups, stocks, seasonings, instant noodle products, meats, and snacks like chips and nuts and the best results were obtained when MSG was used in place of table salt (13). Additionally, there may be benefits for elderly individuals with appetite disorders. MSG is also used medically to treat hepatic coma and to improve the taste of tobacco (18). The essential ingredient of MSG, glutamate, is found in high concentrations in the body and is derived from meals containing free glutamate or dietary protein (19). In the small intestine, aspartate and glutamine are the other two amino acids that are broken down with glutamate. It is found in 20–40% of most proteins, which means it serves as a substrate for protein synthesis (20). These are the main glutamate and glutamine active transporters: excitatory amino acid carrier 1 (EAAC1) (intestine), glutamate/aspartate transporter 1 (GLAST1), and glutamate transport (GLT1) (stomach). Competitively blockable,

these transporters are dependent on sodium ions. In the intestines, glutamate has the ability to convert into free amino acids, which can then be further broken down (16).

Chronic MSG use has been linked to a number of health issues, including dyslipidemia, obesity, diabetes, cardiovascular disease, hypertension, respiratory disorders; sleep disorders, and neuroendocrine abnormalities, including anxiety and depression. Hepatotoxicity, reproductive toxicity, genotoxicity, and renal toxicity are only a few of the detrimental effects of MSG (21). Furthermore, pathological illnesses caused by the neurotoxic effects of MSG include depression, anxiety, stroke, Parkinson's disease, Alzheimer's disease, addiction, and epilepsy (22). Thus, it has been demonstrated that MSG induces oxidative stress by generating free radicals, which results in oxidative DNA damage, membrane biomolecule peroxidation, and cell death. Thus, it is not unexpected that consuming MSG could contribute to the onset and advancement of the majority of metabolic illnesses for which it has been linked because of its ability to cause oxidative stress in functioning tissues (16).

1.2. The brain

The human brain is made up of over 100 billion neurons, which are cells that process information. It is possibly the most complicated biological system (23). Nervous tissue makes up the brain, an organ that controls movement, senses, emotions, language, thought, memory, and task-evoked responses (24).The human brain consists of the cerebrum, cerebellum, and brainstem. From the ectodermic layer of the embryo, the human brain develops through a process called neurulation, which takes 20 to 25 years on average to reach maturity (25). The development of the neural tube during the third or fourth week of pregnancy marks the beginning of it all in a systematic and chronological order (26). Subsequently, the cerebral cortex folds to expand its size and surface area, resulting in a more intricate structure, as a result of cell migration and proliferation (24).

The central nervous system (CNS) is made up of the brain and spinal cord. The autonomic nervous system (ANS) and the somatic nervous system (SNS) are two subtypes of the peripheral nervous system (PNS) (27). Peripheral nerve fibers, which transmit information from the CNS to the skeletal muscle, and motor fibers, which transmit information from the CNS to the CNS, make up the SNS. The sympathetic nervous system (SNS), the parasympathetic nervous system (PaNS), and the enteric nervous system (ENS) make up the autonomic nervous system (ANS), which regulates the smooth muscle of the viscera and glands (28). Numerous head and body regions are connected to the brain by nerves, which results in a variety of voluntary and involuntary actions (22). Basic bodily processes including breathing, digesting, sweating, and shaking are managed by the autonomic nervous system (ANS) (29). They also help to regulate gut blood flow; motility, and absorption, neurotransmitters like norepinephrine, epinephrine, dopamine, and serotonin have lately attracted attention due to their involvement in gut physiology and CNS pathology (24).

In addition to making up 2% of the human body weight, the brain uses 20% of all oxygen in the body and 15% of cardiac output. The brain uses 20% of the body's energy when it is at rest (27). An extra 5% more energy is consumed by the brain when it is working on a task, indicating that intrinsic functions account for the majority of the brain's energy usage (25). Glucose serves as the brain's primary energy source. The brain uses ketone bodies as its main energy source when blood glucose levels are low. Lactate can be used by the brain as an energy source during exercising (30).

A neurodegenerative disease known as "reactive gliosis" is triggered by brain injury to multiply astrocytes, an immune response (26). Glial fibrillary acidic protein (GFAP) overexpression is a direct result of brain tissue damage, which also drives morphological and molecular alterations (31). In contrast, EGFR promotes axonal regeneration and speedy recovery when it is inhibited. EGFR permits the change from non-reactive to reactive astrocytes. This indicates that reactive astrocytes multiply and enlarge; resulting in the creation of glial scars (32). Aging-related neuronal degeneration can impact various brain regions, exacerbating mobility, memory, and cognitive issues. Deficits in motor function result from Parkinson disease, which is caused by the loss of dopamine-producing neurons (23). Alzheimer's disease is brought on by aberrantly folded proteins depositing in the brain, which causes neuronal degeneration (33). A genetic mutation that raises the production of the neurotransmitter glutamate is the cause of Huntington illness (28). Glutamate overdose results in the death of basal ganglia neurons, which impairs movement, cognition, and mental health. When blood flow is interrupted, neurons die, which leads to vascular dementia (24).

1.2.1. The hippocampus

The hippocampus is a part of the limbic system that helps in both spatial memory and the consolidation of information from short-term to long-term memory (34).In humans and other mammals, there are two brain regions called hippocampi that are located on opposing sides of the brain. The term "Ram's horn" comes from the C-shaped frontal region of the hippocampus, which has an outline that resembles a ram's horn. Ammon's Horn is another name for it, named for an Egyptian deity with a ram's head (35). Its anterior extremities are lengthy and have a few ridges and

grooves in between. Because of its resemblance to an animal's paw, it is known as pes hippocampi (pes: foot). Moving backward, the hippocampus gets smaller until it ends beneath the corpus callosum's splenium (36).

The hippocampus is found in the medial temporal lobe of the brain which has a distinctively curved form that makes it resemble a sea horse. Situated deep within the brain, the limbic system is referred to as a "primitive brain" (35). The frontal limbic lobe is the amygdala, while the posterior limbic lobe is the hippocampus. Adult humans have 3-3.5 cm3 of hippocampal volume on each side of the brain, whereas 320–420 cm3 of cortex volume is present in the brain (36). As a result, the volume of the hippocampal region is 100 times smaller than that of the cerebral cortex. The hippocampal formation (HF) and the parahippocampal region (PH) are the two primary components that make up the hippocampus area. The hippocampus forms a curved elevation along the inferior horn of the lateral ventricle's floor, approximately 5 cm in length, and is situated above the subiculum and medial parahippocampal gyrus (37). Its frontal end is larger, and the pes hippocampi, a paw-like structure, may have two or three shallow grooves. A scalloped strip of the cortex, the DG is connected inferiorly to the subiculum, laterally to the hippocampal proprius, and medially to the fornix fimbria. The hippocampal sulcus is located between DG and Cornu Ammonis. The four categories of Cornu Ammonis are CA1, CA2, CA3, and CA4. The subiculum and the hippocampus are connected via the entorhinal cortex (36).

Pyramidal cells in a single layer sandwiched between plexiform layers make up the hippocampus's trilaminar archicortex (38). These three fields are CA1, CA2, and CA3. Field CA3, on the one end, borders the dentate gyrus's hilus, and field CA2, on the other. In the hippocampal region, dentate granule cells on their proximal dendrites supply mossy fiber to the largest pyramidal cells, known as field CA3 pyramidal cells (39).The pyramidal cell layer is approximately ten cells thick throughout in this field. Sometimes it's difficult to tell where CA2 and CA3 cross. There is the highest density of packing in the pyramidal cell layer in the CA2 field. There are three different levels that comprise CA regions: The polymorphic layer is made up of basal dendrites of pyramidal cells, inhibitory basket cell bodies, horizontal trilaminar cells, and axons of pyramidal neurons. Primary excitatory neurons in the hippocampus are found in the pyramidal layer, which houses their cell bodies. The dentate gyrus granule cells' projections to the CA3 region, or mossy fibers, are represented by synapses in this layer (40). Apical dendrites of pyramidal cells, some interneurons, septal and commisural fibers, Schaffer collateral fibers (the projection from CA3 to CA1), and perforant route fibers (fibers from superficial layers of the entorhinal cortex shallow levels) are all found in the molecular layer (36)

The hippocampus is undoubtedly a highly significant component of the brain, serving a variety of purposes that distinguish humans from other animals. It is necessary for spatial navigation in addition to controlling memory and learning (41).

2. Materials and methods

2.1. Materials

2.1.1. Experimental Animals

Under standard laboratory conditions, 30 adult wistar rats (weighing 200±50 g) were obtained from the animal holdings of Anatomy Department of Olabisi Onabanjo University. They were housed in an extremely sanitary and favorable environment with a 12-hour light-dark cycle. The hygienic and nutritional conditions of Joyful Feed Nigeria Limited, Sabo, Sagamu, Ogun state, provided the animals with standard laboratory mouse chow.

2.1.2. Procurement of MSG

The MSG was purchased from a MEX Pharmacy, Sagamu, Nigeria while the Department of Pharmacology and Therapeutics of Olabisi Onabanjo University verified and authenticated it.

2.1.3. Ethical Approval

The Ethics Committee of Research at the Faculty of Basic Medical Sciences (FBMS) at Olabisi Onabanjo University provided approval, ensuring that ethical considerations were met in accordance with the guidelines and regulations governing the use and care of rats.

2.1.4. Animal care

The "Guide to the care and use of Laboratory Animals Resources" (NRC, 1985) and the guidelines established by the National Health Research Ethics Committee (NHREC) and Institutional Animal Care and Use Committees (IACUC) were followed in all experimental investigations.

2.1.5. Animal Grouping

A total number of 30 rats weighing 200±50 g were used for this study. The animals were randomly divided into three (3) groups, T1 group, T2 group and Control group C respectively.

2.1.6. Animal Treatment

The T1 group was given 2 g/kg of monosodium glutamate for duration of four (4) weeks. Monosodium glutamate (2g/kg) was administered to the T2 group for 4 weeks, followed by a 2 weeks withdrawal period. Control group were given distilled water for 4 weeks as previously describes by (42)

2.2. Determination of body weight

The body weight of the rats was closely observed using the digital weighing balance. The wellbeing of the rats was then examined using this record and the impact of monosodium glutamate treatment and distilled consumption on the body weight of the treatment group, withdrawal group and the control group was observed. Using the digital weighing balance, each rat in the group was weighed separately. As the total body weight of the rats in a group divided by the total number of rats in that group, the mean body weight value was determined.

2.3. Animal sacrifice and organ collection

The animals were sacrificed through cervical dislocation at the end of the administration process. Before the animals were sacrificed by cervical dislocation, a non-heparinized capillary tube was inserted into the eyes of the adult wistar rats in the control group, Treatment group (T1), and Withdrawal group (T2) to obtain blood samples for the EDTA bottles. Using medical scissors to separate the rat's head from its body and then remove the skin surrounding it, the adult wistar rats' brains were harvested likewise the hippocampus. Rats in the control, T1 and T2 groups were the subjects of this procedure.

2.4. Determination of brain weight and the organ weight

After the brain was extracted from the skull, weighing machine was used to assess its weight. In a similar manner, the weighing machine was used to determine the weight of each hippocampus that was collected from the control group, Treatment group (T1), and withdrawal group (T2). The aim of this approach was to evaluate and track the effects of oral Monosodium glutamate administration on the hippocampus of adult wistar rats.

The brain-to-animal weight ratio was used to determine each rat's proportional brain weight.

It was determined that the rat's relative brain weight was equal to its brain weight divided by its animal body weight.

$$\text{Weight of organs relative to body} = (\text{weight of organs/body}) \times 100$$

2.5. Histochemical analysis

Brain forceps were used to carefully remove the brains from the skull of each of the rats. The brains were then weighed, and one hemisphere of the brain which contained the hippocampus was stored in 10% formalin.

All brain tissues, including the hippocampal regions, were routinely treated for H&E stain (Carson *et al.,* 2015) and histochemical stains (Cresyl violet,Venero et at, 2000, Luxol fast blue, (Sheehan *et al.,* 1991) and Bielschowsky (Carson *et al.,* 2015)) after being fixed for 24 hours. The second hemisphere brain tissues from each rat were homogenized and utilized in a quantitative active enzyme assay for LDH and oxidative stressors like SOD Misra and Fridovich method (1972).and CAT Sinha method (1972).

2.6. Biochemical analysis

The second hemisphere brain tissues from each rat were homogenized and utilized in a quantitative active enzyme assay for LDH (Jennifer, 2023).and oxidative stressors like SOD Misra and Fridovich method (1972).and CAT Sinha method (1972).

2.7. Photomicrography

Using Leica DM-750 photomicroscope from central research laboratory of Obafemi Awolowo University Nigeria, all the slides obtained were viewed, examined and photographed. A bright light Java Application Software (image J Software) used for Image analysis.

2.8. Statistical analysis

For statistical analysis, data were analyzed by both one-way (for weight analysis) and one-way analysis of variance (ANOVA) using GraphPad Prism (version 9.5.1) software. The results were expressed as mean standard deviation.

3. Result

3.1. Morphometric analysis

3.1.1. Average body weight

The three groups (control, T1, and T2) had their average body weight measured every two days for four (4) weeks while the animals were under observation. This data revealed notable morphological alterations in the rats. Results indicated that from week one to week four, the control group's average body weight increased significantly. A decrease in average body weight was observed in T1 and T2 respectively and this shows that T1 value is non-significant when compared to control group. T2 value is non-significant when compared to T1.

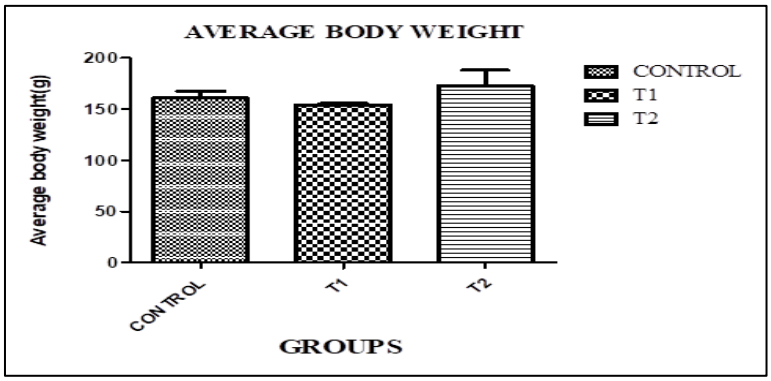


Figure 1 Statistical analysis for average body weight of rats across the three groups

All the values are expressed as mean ± standard error of mean (SEM). Analysis of data was done using GraphPad Prism version 5 for Windows. Differences between groups were analysed by one-way ANOVA. Differences were considered nonsignificant (F=0.8796; P value=0.4478)

3.1.2. Relative brain weight (RBW)

The brain weight data for the three groups - control, test 1, and test 2 - offers a glimpse into potential morphological variations associated with MSG exposure. Control Group (Brain Weight: 1.63g): The control group exhibits the highest average brain weight among the three groups. Test 1 Group (Brain Weight: 1.50g): In contrast, the test 1 group presents a slightly lower average brain weight compared to the control group. Test 2 Group (Brain Weight: 1.58g): The test 2 group demonstrates a slightly higher average brain weight compared to test 1 and is closer to the control group's weight.

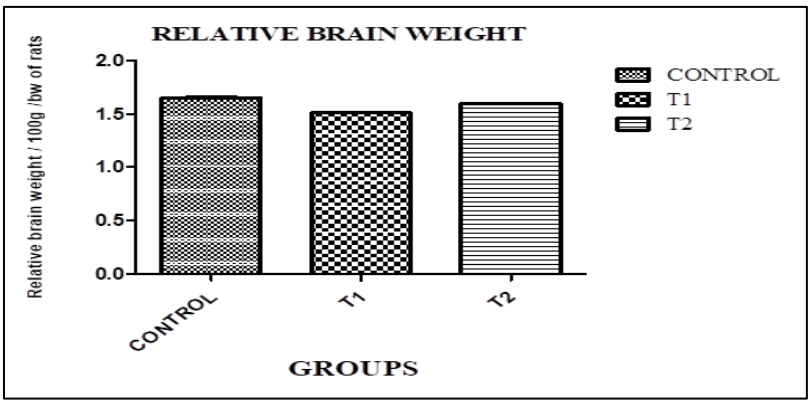


Figure 2 Statistical analysis of rats' relative brain weight

All the values are expressed as mean ± standard error of mean (SEM). Analysis of data was done using GraphPad Prism version 5 for Windows. Differences between groups were analysed by one-way ANOVA followed by Dunnet post-hoc test. Differences were considered significant (F=91.50; P value< 0.0001). Values were significant when compared to the control group

3.1.3. Average organ weight (HIPPOCAMPUS)

The animals were analyzed and average organ weight of rats for the three groups (control, T1 and T2) were recorded. The result shows that T1 value is significant when compared to the control group. T2 value is significant when compared to T1.

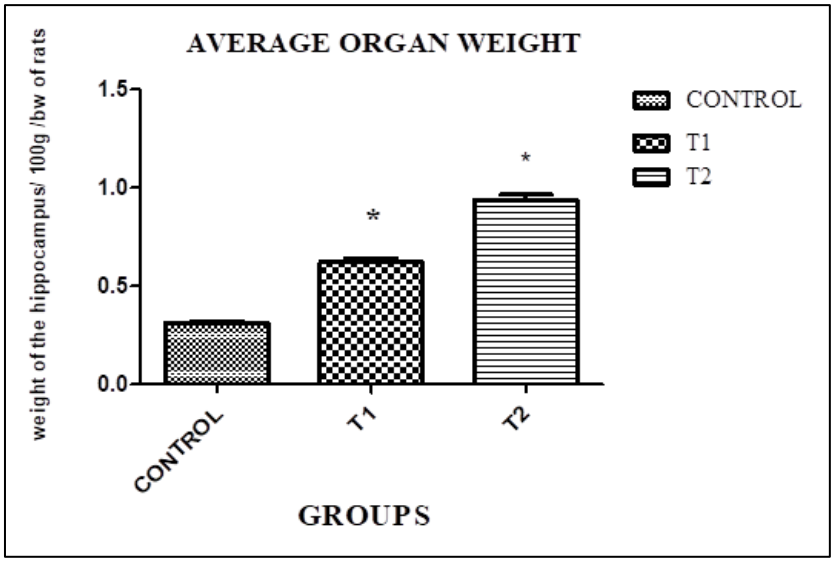


Figure 3 Statistical analysis of the rats' organ weight among the three groups

All the values are expressed as mean ± standard error of mean (SEM). Analysis of data was done using GraphPad Prism version 5 for Windows. Differences between groups were analysed by one-way ANOVA. Differences were considered significant (F=228.6; P value < 0.0001)

3.1.4. Lactate dehydrogenase (LDH)

The LDH activity of the hippocampus for the three groups (control, T1 and T2) were analyzed and recorded. The control group demonstrates the highest range among the three groups which serve as a baseline for lactate dehydrogenase analysis. The result of the T1 value is significant compared to control group. T2 value is significant when compared to T1.

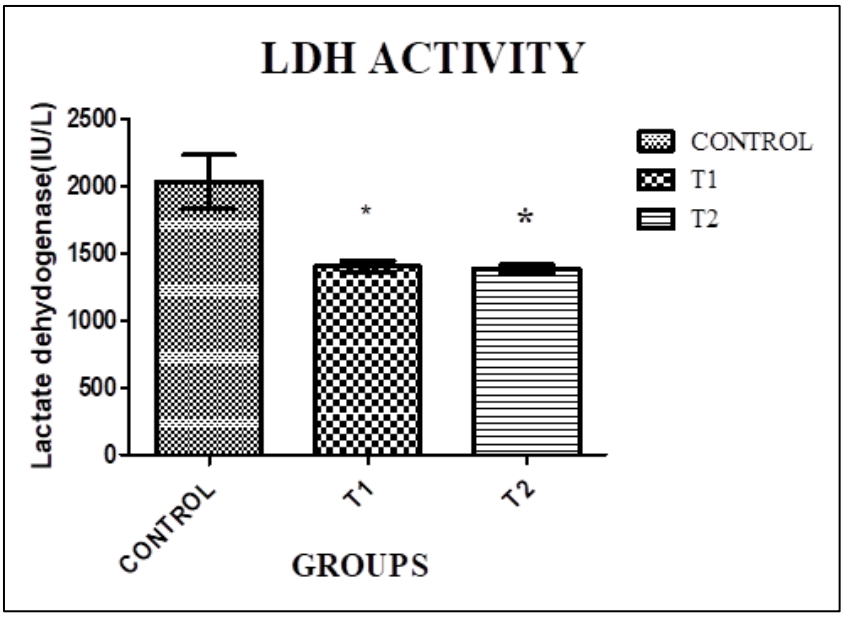


Figure 4 Statistical analysis of the LDH activity among the three groups

All the values are expressed as mean ± standard error of mean (SEM). Analysis of data was done using GraphPad Prism version 5 for Windows. Differences between groups were analysed by one-way ANOVA. Differences were considered significant (F=41.73; P value < 0.0001)

3.1.5. Superoxide dismutase (SOD) activity

The SOD activity of the hippocampus for the three groups (control, T1 and T2) were analyzed and recorded. The result of the T1 value is significant compared to control group. T2 value is significant when compared to T1.

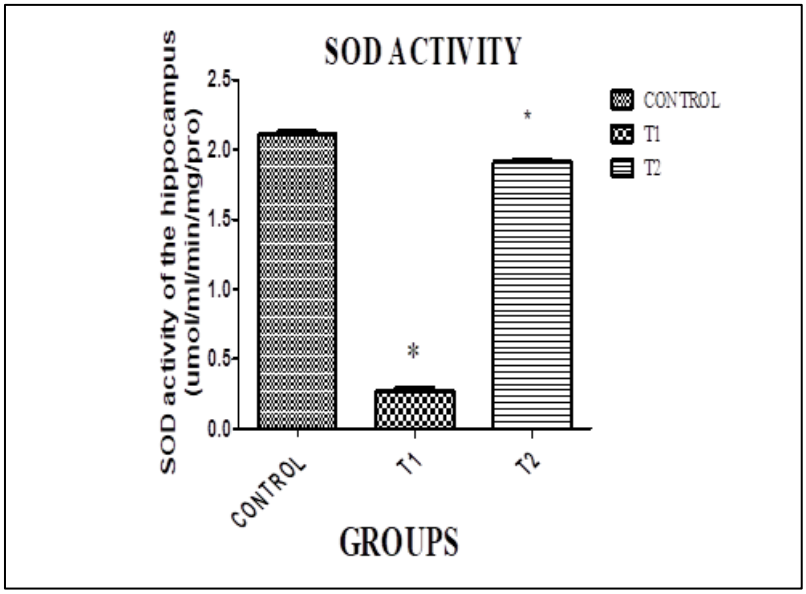
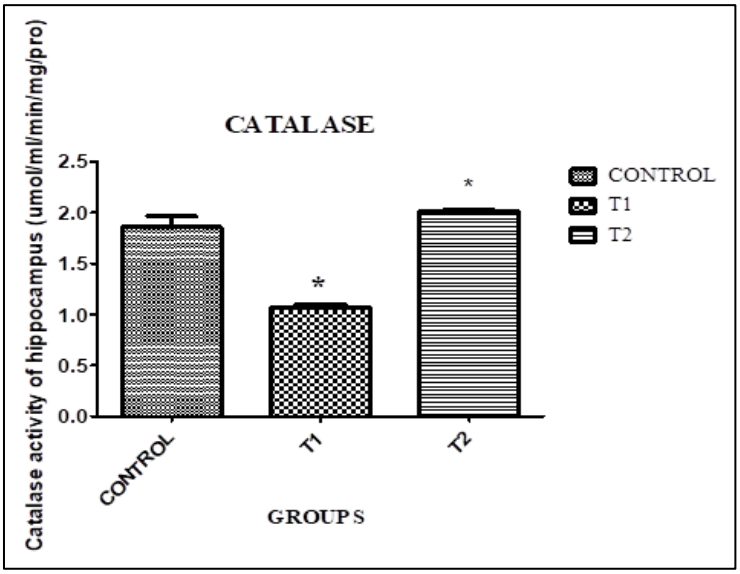


Figure 5 Statistical analysis of the SOD activity among the three groups

All the values are expressed as mean ± standard error of mean (SEM). Analysis of data was done using GraphPad Prism version 5 for Windows. Differences between groups were analysed by one-way ANOVA. . Differences were considered significant (F=1914; P value < 0.0001)

3.1.6. Catalase

The Catalase activity of the hippocampus for the three groups were analyzed and recorded. The result of the T1 value is significant compared to control group. T2 value is significant when compared to T1.



All the values are expressed as mean ± standard error of mean (SEM). Analysis of data was done using GraphPad prism version 5 for windows 9. Differences between the groups were analyzed by one way ANOVA. Differences were considered significant (F=63.03; P value < 0.0001)

Figure 6 Statistical analysis of Catalase activity among three groups

3.1.7. Park cell volume (PCV)

The animals park cell volume were analyzed and average PCV of rats for three groups (control, T1 and T2) were recorded. The result of the T1 value is significant compared to control group. T2 value is significant when compared to T1.

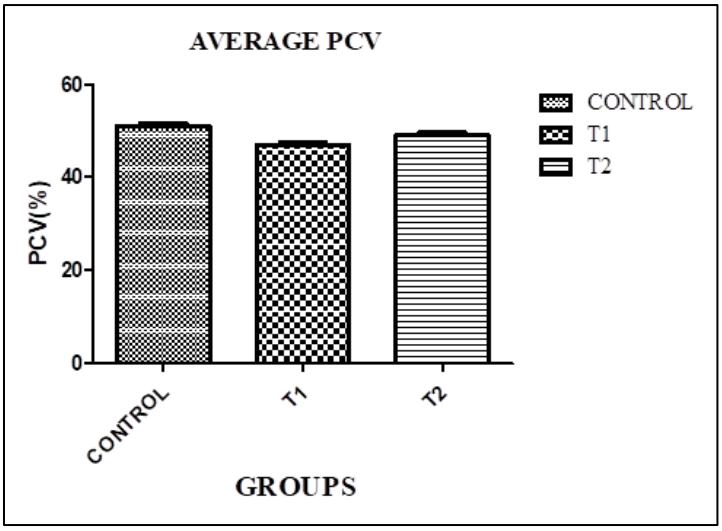


Figure 7 Statistical analysis of rats PCV among three groups

All the values are expressed as mean ± standard error of mean (SEM). Analysis of data was done using GraphPad prism version 5 for windows 9. Differences between the groups were analyzed by one way ANOVA. Differences were considered significant (F=12.00; P value =0.0080)

3.1.8. Hemoglobin (Hb)

The animals hemoglobin were analyzed and average Hb of the rats for the three groups (control, T1 and T2) were recorded. The results shows that T1 value is nonsignificant when compared to control group. T2 value is nonsignificant when compared to T1.

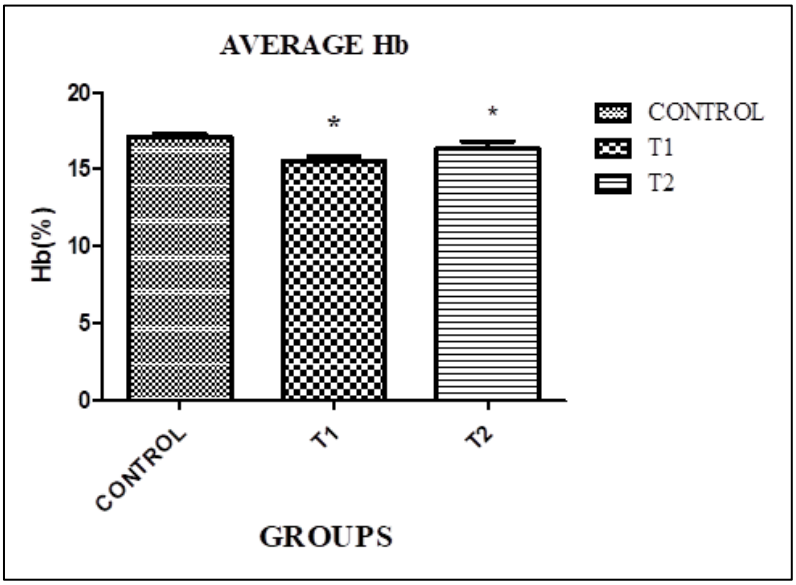


Figure 8 Statistical analysis of rats Hb among three groups

All the values are expressed as mean ± standard error of mean (SEM). Analysis of the data was done using GraphPad prism version 5 for window 9. Differences between the groups were analysed by one way ANOVA. Differences were considered nonsignificant (F=4.954; P value=0.0537)

3.1.9. WHITE BLOOD CELL (WBC)

The animals white blood cell were analyzed and average WBC of rats for three groups (control, T1 and T2) were recorded. The results shows that T1 value is significant when compared to control group. T2 value is significant when compared to T1.

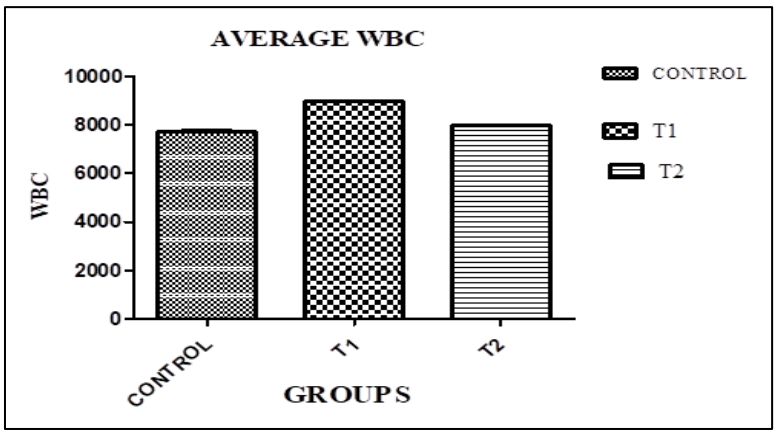


Figure 9 Statistical analysis of rats WBC among three groups

All the values are expressed as mean ± standard error of mean (SEM). Analysis of data was done using GraphPad prism version 5 for window 9. Differences between the groups were analysed by one way ANOVA. Differences were considered significant (F=282.5; P value < 0.0001).

3.1.10. Red blood cell (RBC)

The animals red blood cell were analyzed and average RBC of rats for three groups (control, T1 and T2) were recorded. The results shows that T1 value is significant when compared to control group. T2 value is significant when compared to T1.

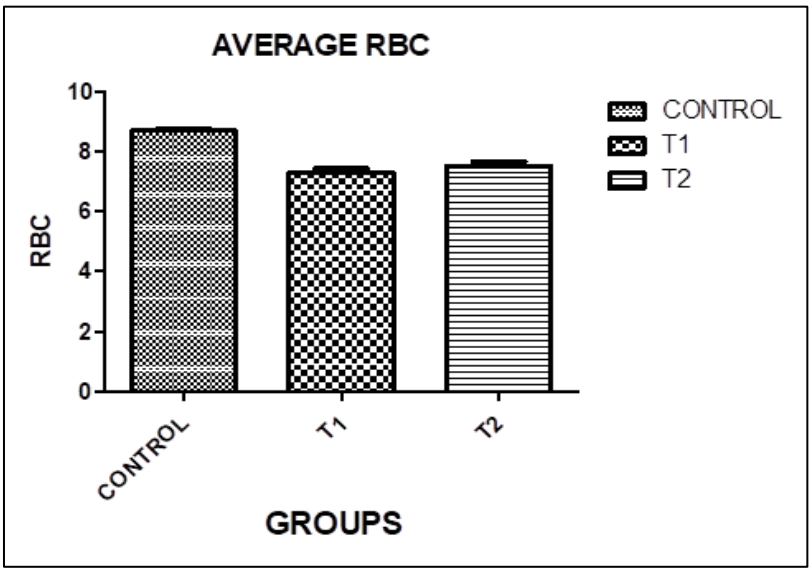


Figure 10 Statistical analysis of rats RBC among three groups

All the values are expressed as mean ± standard error of mean (SEM). Analysis of data was done using GraphPad prism version 5 for window 9. Differences between the groups were analysed by one way ANOVA. Differences were considered significant (F= 35.45; P value=0.0005)

3.2. Histological analysis

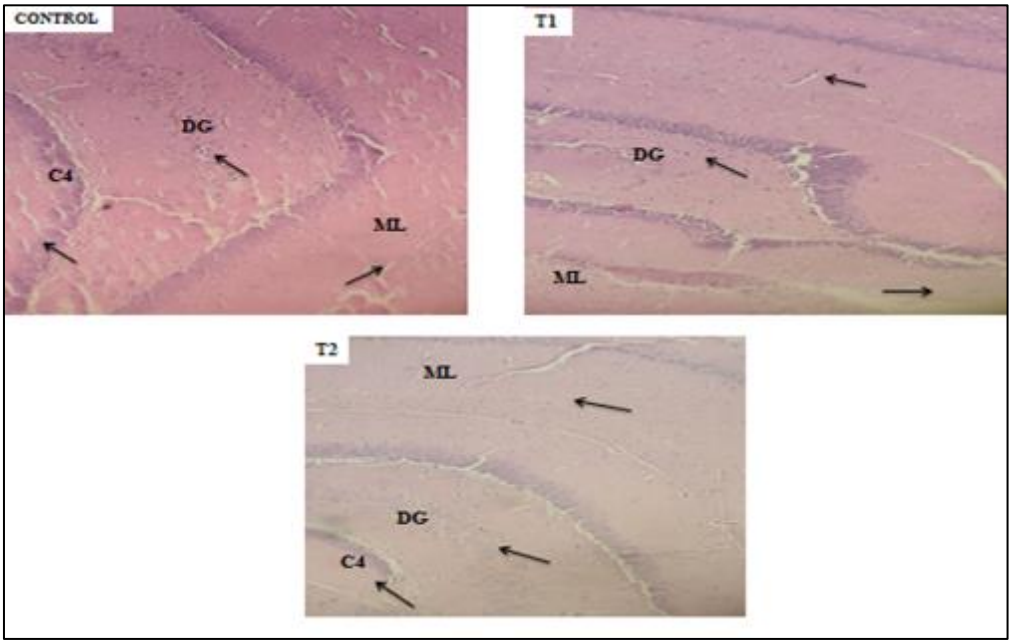


Figure 11 Photomicrograph of section of the hippocampus stained with H&E stain (MAG X100)

Control group: shows normal architecture of the histology of the hippocampus with well differentiated neuronal and glial cells showing oriented pyramidal cells of CA4 region and the Dentate Gyrus (DG) with a well-defined molecular layer (ML). **T1 Group** shows poorly stained layer of granular cells with poorly stained granular cells of the dentate gyrus (DG) which denotes degeneration of the neurons.**T2 Group** shows poorly stained pyramidal cells of CA4 region and the Dentate Gyrus (DG) present with poorly molecular layer (ML).

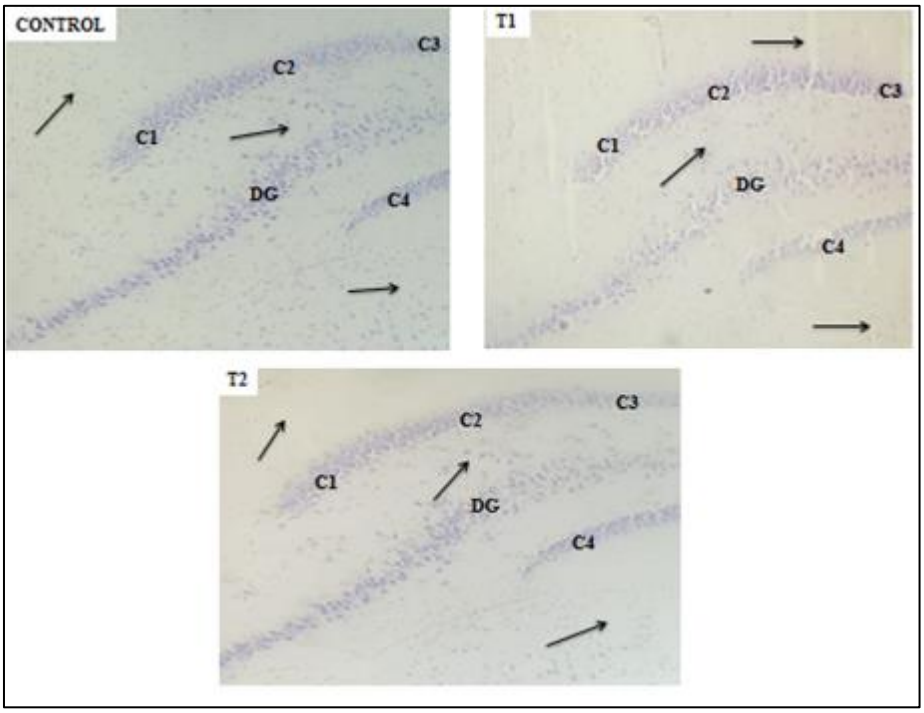


Figure 12 Photomicrographs of section of the hippocampus stained with Cresyl-Echt violet stain (MAG X100)

Control group: shows normal architecture of the histology of the hippocampus with well distributed Nissl substances in the neurons of the hippocampus. C1, C2, C3 and CA4 regions including the Dentate Gyrus and the Molecular layers are well presented. **T1 group** shows layers of compact granular cells within dentate gyrus (DG) with abnormal cellular degeneration of the cellular presentation with numerous sites of dissolution of the Nissl substances and the neurons were noticed to be disorganized and poorly oriented. **T2 Group** shows poorly stained pyramidal cells of the hippocampal regions including Dentate Gyrus (DG) and the molecular layer with reduced zone of dissolution of Nissl substances were noticed.

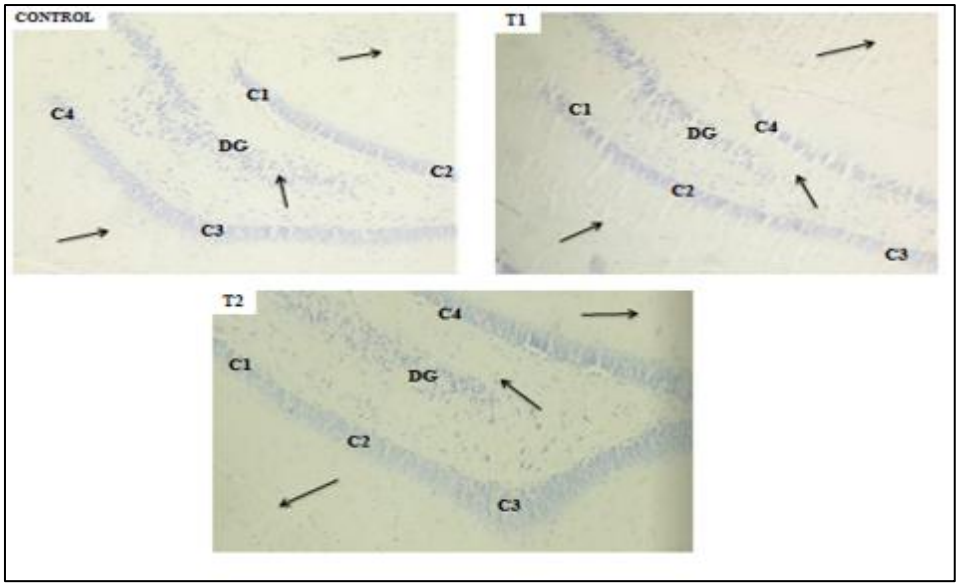


Figure 13 Photomicrographs of section of the hippocampus stained with Luxol fast blue stain (MAG X100)

Control Group: shows normal architecture of the histology of the hippocampus with well oriented pyramidal cells, myelin fibers, neutrophils and nerve cells well presented. **T1 Group** shows abnormal cellular degeneration of the cellular presentation and the myelin fibers and nerve cells were noticed to be disorganized and poorly oriented.**T2 Group** shows poorly stained pyramidal cells of hippocampal regions including the Dentate Gyrus (DG), Molecular layer, myelin fibers and the nerve cells were noticed to be disorganized and poorly oriented.

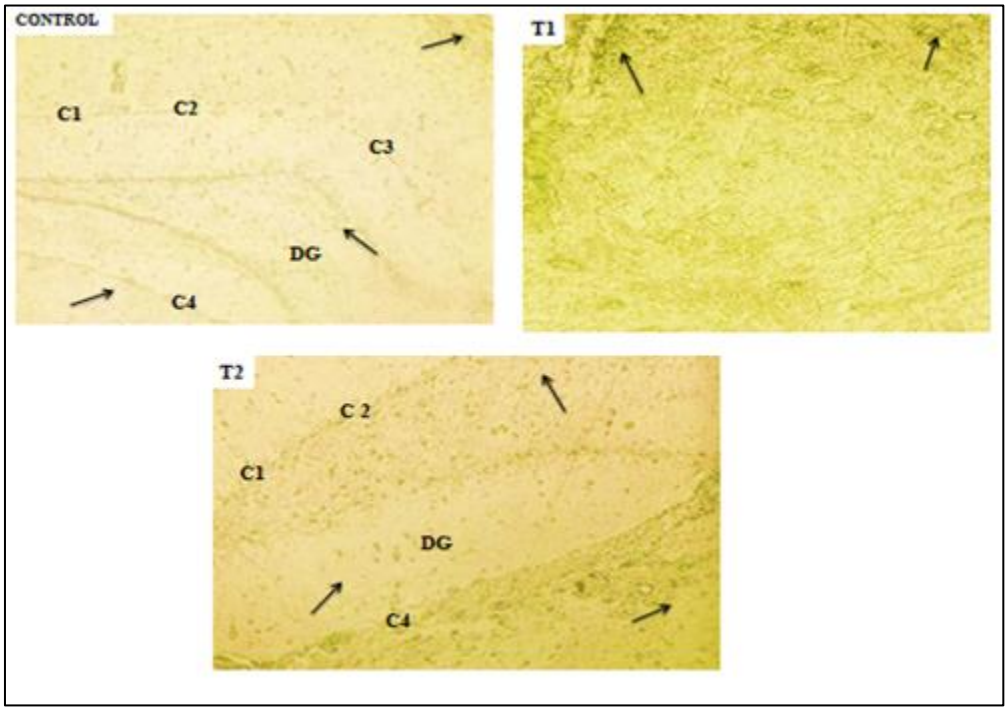


Figure 14 Photomicrographs of section of the hippocampus stained with Bielschowsky stain (MAG X100)

Control Group: shows normal tissue of the hippocampus and the neurons with no injury upon the yellow background. **T1 Group**: Due to the high dose in T1, the fibers are evenly distributed and the senile plaques, the axons and the neurofibrillary tangles were noticed upon the yellow background.**T2 Group**: The fibers are undergoing a recovery process with some forms of lesions. The neurons form as a consequence of plaque aggregation. Arrows indicates Neurofibers decomposition accumulated in the hippocampus upon the yellow background.

4. Discussion

Monosodium glutamate is the sodium form of L-glutamic acid which is commonly used as a flavor enhancer in food preparations with taste known as “umami” (sweet, sour, salty, and bitter). It has been found via several human and

animal clinical trials that MSG contributes to a number of health issues, including the development of diabetes, obesity, and reproductive system diseases, genotoxicity, neurotoxicity, liver and kidney failure with numerous metabolic disorders including "Chinese restaurant syndrome" which include migraine, diarrhea, weakness, vomiting, stomach discomfort, and pressure in the chest (43). Neurodegenerative disease is a diverse range of age-related illnesses which is characterized by the gradual degeneration or death of neurons in the central or peripheral nervous systems (44). However, other neurodegenerative disorders (NDDs) like Alzheimer's, Parkinson's, Huntington's, and multiple sclerosis have also been linked to neuronal over-activation. The most common dietary ingredient, monosodium glutamate (MSG), raises the brain's free glutamate content, which puts people at risk for epilepsy and non-dissociative disorders (NDDs) (45). This study thoroughly examined a number of criteria to gain a comprehensive understanding of the effects of exposure to monosodium glutamate (MSG) on hippocampus of adult wistar rats.

This study included measurements of the hippocampal histomorphology and histomorphometry, evaluations of total body weight, and measurements of particular biochemical markers. First, compared to the control group, the study found no significant difference in the body weight of the rats exposed to MSG (P value=0.4478; F=0.8796). According to (42) 2013, this result implies that the experimental individuals' total weight did not significantly alter as a result of MSG administration on a macroscopic level. Turning now to the assessment of brain weight, the study found that, although statistically significant, the brain weights of the treatment groups had decreased. According to (46), a thorough reaction to MSG exposure is implied by this slight drop in brain weight, underscoring the significance of taking into account both macroscopic and microscopic alterations in neural structures (P value< 0.0001; F=91.50). The hippocampal complex, an area crucial for memory and learning, was investigated (47). An important finding was that the hippocampal weights of treatment group (T1) and the withdrawal group (T2) increased significantly. According to the study done by(48)., 2018, this major finding raises important questions about the specific effects of MSG on the hippocampus, highlighting the need for more research into the hidden processes behind this change. In addition to morphological evaluations, the study expanded to include biochemical indicators such as catalase, lactate dehydrogenase (LDH), and superoxide dismutase (SOD). These markers function as cellular function and oxidative stress indicators (49). A remarkable difference was found between treatment group and withdrawal group when the hippocampal superoxide dismutase (SOD) activity was measured. SOD activity was lower in treatment group (T1) (50), which may indicate that this important antioxidant enzyme is down-regulated in response to exposure to monosodium glutamate (MSG). Conversely, SOD activity increased in the withdrawal group (T2), suggesting a different reaction. The hippocampus's oxidative stress dynamics are subtly examined in light of this disparity in SOD activity. SOD activity in T1 was found to be reduced, which may indicate a weakened antioxidant defense system that leaves hippocampus cells more vulnerable to oxidative injury. On the other hand, greater SOD activity in T2 suggests that there may have been a deliberate attempt to offset increased oxidative stress, which begs the question of what adaptive or compensatory mechanisms were activated by MSG exposure (P value < 0.0001; F=1914). Lactate dehydrogenase (LDH) is found as an enzyme which can be found in most tissues and it is used to check for any alteration in tissues which could result in damages, injuries, or infections which can either be chronic or acute (51). There is a reduction in the level of LDH activity in the treatment group (T1) and withdrawal (T2) when compared to the control group. T1 showed a low LDH level when compared to control group while T2 exhibit a lower LDH level when compared to T1. According to (52), the study correlates with this study to shows that there is a deficiency in the level of glucose (pyruvate) in the LDH activity following the administration of MSG (P value < 0.0001; F=41.73). Catalase (CAT) activity in the hippocampal regions showed a comparable pattern. Parallel to this, T1 showed lower CAT activity, whereas T2 showed higher CAT activity. This important brain region's regulation of oxidative stress is further complicated by the simultaneous modification of both SOD and CAT activity in response to MSG intake (53).

T1 group decreased CAT activity may indicate a weakened detoxification capacity for hydrogen peroxide, which would exacerbate oxidative stress (54). In contrast, the increased CAT activity in T2 group would suggest a better ability to reduce oxidative damage by effectively breaking down hydrogen peroxide into oxygen and water (11). The complicated equilibrium of antioxidant defense mechanisms in the hippocampus upon exposure to MSG is highlighted by these findings (P value < 0.0001; F=63.03). A thorough investigation into the underlying molecular pathways is important, as evidenced by the varying responses in SOD and CAT activity throughout treatment groups. To learn more about how hippocampus cells respond to oxidative stress generated by MSG, future research endeavors may explore the precise genetic and molecular elements governing these reactions. The hematological parameter consists of the park cell volume, hemoglobin and white blood cell. The park cell volume (PCV) showed that there is a slight reduction in treatment (T1) group compared to the control group. There is a slight increase in the withdrawal (T2) group when compared to T1 following MSG administration (P value=0.0080; F=12.00). The hemoglobin level reduced in the treatment (T1) group compared to control group while there is a slight increase of hemoglobin level in withdrawal (T2) group when compared to the treatment group and it is statistically nonsignificant (P value=0.0537; F=4.954). There is a significant increase in white blood cell (WBC) of the treatment (T1) group when compared to control group which has a lower range. Withdrawal (T2) group is significantly lower to treatment (T1) group (P value < 0.0001; F=282.5).

A distinct molecular layer, well-oriented pyramidal cells in the CA4 area, and a well-defined Dentate Gyrus (DG) with a well-defined Molecular Layer (ML) characterizes the hippocampal architecture of the control group. Healthy hippocampal histology is established by this baseline observation. In comparison, there is a significant difference in the T1 group. The Dentate Gyrus (DG) granular cells seem to be badly stained, as does the layer of granular cells. This implies that exposure to MSG may have disrupted the granular layer's cellular architecture, which could have an effect on histology. Likewise, aberrant histological characteristics are seen in the T2 group. The molecular layer exhibits poorly defined properties, and the pyramidal cells in the CA4 area, the Molecular Layer (ML), and the Dentate Gyrus (DG) are all weakly stained. This pattern highlights the structural alterations caused by MSG in this withdrawal group (T2) and points to a more extensive effect on the molecular layer as well as the pyramidal cells. The observed differences in staining patterns across the groups could indicate changes in the shape, density, or possibly viability of cells within the hippocampus regions caused by MSG using H&E staining. These histological alterations can be a sign of pathological processes affecting the hippocampal tissue, such as excitotoxicity. The Dentate Gyrus (DG) of the T1 group shows notable alterations in Cresyl Violet staining, which are typified by layers of compact granular cells. This tight arrangement indicates possible changes in cellular organization or density, indicating histological modifications brought on by exposure to MSG. The molecular layer also exhibits aberrant cellular degeneration, which suggests microscopic structural alterations. Interestingly, the Nissl substance's disarray and improper orientation emphasize the specific effects of MSG on these cells in more detail. Histological abnormalities are also seen in the T2 group, most prominently in the pyramidal cells of the CA4 region, the Molecular Layer (ML), and the Dentate Gyrus (DG). The irregularly arranged Nissl material and the badly stained pyramidal cells together suggest that MSG exposure has caused a change in the cellular architecture. These changes are seen in several hippocampus areas, highlighting the extensive effect on cellular integrity. Both the T1 and T2 groups' histological analyses showed disarray and poor orientation, which may have been caused by excitotoxic mechanisms activated by high glutamate levels. These findings suggest cellular stress or injury (26). The Dentate gyrus (DG) of the hippocampus is well oriented with prominent pyramidal cells, myelin fibers, neutrophils and nerve cells in the control group via Luxol fast blue stain. The T1 group demonstrates abnormal cellular degeneration and disorganized poorly oriented neurons, myelin fibers and nerve cells. The hippocampus of the T2 group showed poorly stained pyramidal cells and the dentate gyrus (DG), neurons, molecular layer, nerve cells and myelin fibers were noticed to be poorly stained and slightly disorganized (36). Bielschowsky stain is a histological stain used in the demonstration of senile plaques and neurofibrillary tangles via silver impregnation (55). There is a normal distribution of the neuronal decomposition in the control group showing normal histology of the hippocampus with no injury via Bielschowsky stain. The senile plaques, Neurofibers and the axons were prominent and evenly distributed in the T1 group. There is an undergoing recovery process with some forms of lesions in the T2 group.

5. Conclusion

The administration of MSG causes changes in brain weight, altered oxidative stress and histological architecture of hippocampus. The aggregate results of these findings add to a thorough comprehension of the neurobiological impacts of MSG and highlight the need for dietary guidelines and legislative actions to protect brain function.

Compliance with ethical standards

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Disclosure of conflict of interest

All Authors Declare that there’s no competing interest

Statement of ethical approval

Anatomical Sciences Research Ethics Committee of Olabisi Onabanjo University Sagamu Campus, Ogun State Nigeria

Author Contribution

All authors have equal contributions in completing the present research work. All authors read and approved the final manuscript

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