Original Research Article

Effect of *Dacryodes edulis* (African pear) ethanolic leaf extract on the cytoachitecture of the prefrontal cortex and long term learning and memory in Wistar rats of Ketamine-induced neurotoxicity

ABSTRACT

Introduction: Ketamine is a therapeutic drug that is mostly used as an anaesthetic. It is a drug of abuse due to its pleasant psychotic effects, thereby leading to cases of neurotoxicity. Drug-induced neurological disorders usually affect the normal functioning of brain parts, including the prefrontal cortex and hippocampus. *Dacryodes edulis* (African pear) is a medicinal plant known for its medicinal qualities.

Aims: The aim of this study was to investigate the effect of *Dacryodes edulis* ethanolic leaf extraction the cytoarchitecture of the prefrontal cortex using hematoxylin and eosin staining technique. Another objective was to determine spatial learning and memory in the rats using Morris water maze method.

Place and duration of study: College of Medical Sciences animal house, Faculty of Basic Medical Sciences, University of Calabar (January 2023 to February 2023). Neurobehaviour laboratory, Physiology department (March 2023). Histopathology department, University of Calabar Teaching Hospital (March 2023).

Methods: Thirty (30) rats were divided into five groups of six rats each. The control group was designated as group A, while the ketamine control group (group B) was administered with 100mg/kg body weight "BW" of Ketamine only. Groups C, D and E were given Ketamine (100mg/kg BW) + Diazepam (0.3mg/kg BW), Ketamine (100mg/kg BW) + 1000mg/kg BW *Dacryodes edulis* ethanolic leaf extract and Ketamine (100mg/kg BW) + 500mg/kg BW *Dacryodes edulis* ethanolic leaf extract respectively for 28 days. The histology of the prefrontal cortex was determined using hematoxylin and eosin staining method, while spatial learning and memory was assessed using Morris water maze method.

Results: The histological result revealed marked atrophic cellular changes and significant reduction in neuronal cell count (hypoplasia) in the prefrontal cortex of groups B rats (treated with ketamine alone) and C (treated with ketamine and diazepam) as revealed by the cytometric study. Group D rats treated with treated with 1000mg/kg BW of *Dacryodes edulis* extract displayed slight neuronal cell atrophy but an increase (hyperplasia) in neuronal cell count when compared to groups B and C, while group E rats treated with treated with 500mg/kg BW of *Dacryodes edulis* extract showed marked atrophic cellular changes and a reduction in neuronal cell count.

The neurobehavioral study with the use of Morris water maze revealed significant decrease in escape latency in group D during the acquisition and reversal training period when compared to groups B, C and E. This is indicative of improved spatial learning and memory capability in the group D rats. During the probe trial day, group A exhibited the longest duration spent in the retention quadrant, while group D rats (1000mg/kg BW of *Dacryodes edulis* extract) spent a longer time in the quadrant when compared to groups B, C and E. The result therefore indicates ameliorative potential of the leaf extract but in a dose-dependent pattern.

Conclusion: The leaf extract of *Dacryodes edulis* can be considered as an alternative neuroprotective drug in cases of ketamine-induced neurodegeneration.

Keywords: *Dacryodes edulis*, ketamine, histology, prefrontal cortex, Morris water maze.

INTRODUCTION

The prefrontal cortex is a part of the brain that is situated around the frontal lobe of the cerebrum. It is a key brain region that is involved in planning complex cognitive behaviour, expressing personality traits, making decisions and regulating social behaviour (38). Therapeutic drugs used on the brain can become a source of worry when utilized inappropriately, leading to neurotoxicity. One of such drugs is ketamine which is a dissociative drug known for its use as a recreational drug due to its pleasant hallucinogenic and euphoric side effects. In surgery situations, it is sometimes used with benzodiazepines in order to help counteract the adverse psychological symptoms that may present thereafter (27). The neurotoxicity caused by its abuse is said to be associated with oxidative tissue damage to brain tissue, as well as neuro-cognitive deficits. The brain is really sensitive to damage by reactive-oxygen species because of its relatively small antioxidant capacity (30). Ketamine overdose can lead to numerous troubling symptoms that can impact mental and physical functioning (31,30,5). A percentage of adults (10-20%) go through adverse psychiatric effects after anesthesia with

ketamine, and these include dysphoria, hallucination and emergence delirium (24,40). Ketamine functions as an antagonist on the N-Methyl-D-Aspartate “NMDA” receptors, which are ubiquitously distributed within the central nervous system. Specifically, it exerts its non-competitive binding affinity to phencyclidine-binding site on the NMDA-receptor, thereby impeding the entry of Ca^{2+} into the cells. Consequently, this disruption of calcium-mediated signaling between cortico-cortical and cortico-subcortical regions culminates in multifaceted pharmacological effects, including analgesia, perturbation of neural plasticity and induction of dissociative anesthesia (32,20). Many studies have illustrated the therapeutic impact of medicinal plants, especially in the amelioration of tissue toxic effects caused by exposure to toxic substances (10,11,1,12,4,17,3). One of such plants is *Dacryodes edulis* (African pear) which has a history of uses medicinally. It is also known as African plum, African palm, native pear, bush butter tree, Eben or Ube (in Nigeria). The fruit is highly nutritious, comprising of lipids, proteins and provitamins. Many health-enhancing chemical constituents such as alkaloids, tanins, flavonoids and saponins are present in parts of the plant including its leaves. The bio-constituents obtained from its leaves are said to exhibit antioxidant, anti-sickle cell and antimicrobial activities (26). According to Sadhwani (33), antioxidants in plants have been utilized for the treatment and management of many illnesses, including neurological disorders. The rich antioxidant property of the leaf makes it relevant for this study which involves its effect on the prefrontal cortex and neuro-behaviour in rats of ketamine-induced neurotoxicity.

MATERIALS AND METHODS

Experimental design:

Thirty (30) adult male albino Wistar rats (140-200g) were procured from the College of Medical sciences animal house, University of Calabar. The rats were sheltered in optimal environmental conditions of humidity, temperature and daylight/dark cycle. They were fed with standard rodent feed and distilled water *ad libitum.* They were kept in this environment for a period of twenty-one days before the commencement of the experiment proper. Furthermore, the rats were divided into 5 groups and placed in properly ventilated plastic cages labelled A to E, with each cage containing six rats Group A represented the normal control and was administered with animal feed and distilled water only; group B (ketamine control group) received 100mg/kg BW of Ketamine intra-peritoneally “IP” only; group C received 100mg/kg BW Ketamine (IP) and 0.3mg/kg BW Diazepam (IP), group D received 100mg/kg BW (IP) and 1000mg/kg BW *Dacryodes edulis* ethanolic leaf extract; while group E received 100mg/kg BW Ketamine (IP) and 500mg/kg BW *Dacryodes edulis* ethanolic leaf extract.

The extract was given orally to the rats with the aid of an oro-gastric tube, while ketamine and diazepam were administered to the rats intra-peritoneally. The ketamine and the leaf extract was administered to the rats (in groups B, D and E) concurrently for four weeks, while it was administered concurrently with Diazepam (in group C rats) for the same period. Twenty-four hours after the last administration, the experimental animals were subjected to Morris water maze (MWM) test in order to assess spatial learning and memory using Morris method. The animals were then sacrificed and the brain was removed from the skull. The brain was then removed, with the prefrontal cortex dissected and processed for histological staining.

Drug:

Ketamine hydrochloride (PharmaTher, USA) and Diazepam (Roche, USA) injectibles were procured from Bez pharmacy in Calabar Municipality, Calabar, Cross River state, Nigeria.

Plant acquisition, identification and preparation of extract:

Fresh leaves of *Dacryodes edulis* were meticulously sourced from a residence in Ikot Omin, 8 miles, Calabar, Cross River state. The leaves were identified and authenticated at the department of Botany, Faculty of Science, University of Calabar. They were then given a voucher number Bot/Herb/UCC/0189.

Plant extract preparation: The fresh *Dacryodes edulis* leaves obtained were subsequently washed and air-dried in the laboratory in order to maintain their quality. The dried samples were blended into powder and 100g of the powdered leaves was immersed in 80% alcohol. The mixture was vigorously agitated using an electric blender. To obtain a purified filterate, the mixture underwent a double-filtration process, using chess cloth and Whatman No.1 filter paper. The filtrate was obtained and concentrated using a rotary evaporator with regulated temperatures of 40^{o}C to 50^{o}C, yielding a crude paste residue which was then preserved (in a sterile container) in a cool dry environment until time for use.

Acute toxicity test:

Sixteen rats were employed for the determination of the lethal dose "$LD"_{50}$ of *Averrhoa carambola* fruit extract using Lorke's method. The rats were separated into four groups (3 rats each) for the first phase and received 500mg, 1000mg, 1500mg and 2000mg/kg BW respectively. They were observed for twenty-four hours. The second phase involved two groups of 2 rats each which were given

4000mg and 6,000mg/BW of the extract respectively. There was no death recorded and this agrees with a study by Ononamadu which illustrated that the leaf extract is non-lethal in any concentration (26). The LD_{50} was therefore taken as >5,000mg/kg.

Morris water maze test: Twenty-four hours after the last administration, the rats were made to undergo Morris water maze "MWM" test so as to assess their spatial learning and memory. Extra maze visual cues were introduced to the animals to help them locate an escape platform hidden under the surface of non-transparent water. Their success in this activity depends largely on their capacity to memorize and master positions within the immediate environment. The concealed platform type of MWM presents a spatial memory test that is associated with hippocampal injury, while the detectable platform version of MWM is not associated with the hippocampus. The Morris water maze activity went on for a period of seven days. The rats were trained for 6 days; acquisition training with the concealed escape platform (North-West quadrant) was done for the first 3 days, while reversal training with the concealed escape platform (South-East quadrant) was done between day 4 and 6. Day 7 involved a probe trial which was conducted without an escape platform.[10]

Histological study with hematoxylin and eosin stain "H&E":

The paraffin slides containing prefrontal cortical tissue underwent a dewaxing process involving two rounds of exposure to xylene for 5 minutes each. Rehydration was done using decreasing concentrations of alcohol of alcohol and thereafter, rinsing under tap water. The sections were then subjected to staining with hematoxylin, then counter-staining with Eosin. After rinsing, the sections underwent dehydration (using ascending grades of alcohol) and clearing in xylene. Following this, the sections were allowed to air-dry, then few drops of dibutylphthalate polystyrene xylene "DPX" were applied to the slide surfaces before a coverslip was placed on top. The resultant slide was then viewed under a light microscope for examination[2].

A cytometry study of sections of the hippocampus was done using Image J (version 1.54):

It was further analysed and visualized with the use of Microsoft Excel (version 2023). This was done to show the average number of cells, as well as the average size of cells across the sections.

RESULTS AND DISCUSSION

According to a study by Shafri *et al.,*(35) neurotoxic tissue damage is possible in cases of ketamine overdose. There exist documented characteristics of degenerating neuronal tissue. These include hypertrophied neuronal cells, eosinophilic neurons, astrogliosis, reactive microglial cells, nuclear

pyknosis, finely vacuolated neuropil adjacent to degenerating neurons and vacuolation within the hippocampal molecular layer (14,18).

The resultant cytometric study showed marked atrophic cellular changes and a significant reduction in neuronal cell count (hypoplasia) in the photomicrograph of the prefrontal cortex (H & E) of groups B (Plate 2) and C (Plate 3) rats treated with 100mg/kg BW of ketamine (Figures 1 & 2). Group A (Plate 1) rats displayed a normal prefrontal cortical microstructure (Figures 1 & 2). Group D (Plate 4) rats treated with treated with 1000mg/kg BW of *Dacryodes edulis* extract displayed slight neuronal cell atrophy but increase (hyperplasia) in neuronal cell count when compared to groups B and C (Figures 1 & 2), while group E (group 5) rats treated with treated with 500mg/kg BW of *Dacryodes edulis* extract showed marked atrophic cellular changes and a reduction in neuronal cell count (Figures 1 & 2).

The neurobehavioral study with the use of Morris water maze revealed significant decrease in escape latency in group D (Figure 3) (throughout the acquisition period) when compared to groups A, B, C and E. This is indicative of improved spatial learning and memory capability in the group D rats. During reversal training period, a significant decrease in escape latency was observed in groups A and D (Figure 4) when compared to groups B, C and E, also indicative of learning enhancement in group D rats. During the probe trial day, group A (Figure 5) exhibited the longest duration spent in the retention quadrant (South/West), while groups B, C and E spent far less time in the same quadrant. Interestingly group D rats (Fig. 5) that were treated with the higher dose (1000mg/kg BW of *Dacryodes edulis* extract) of the extract spent more time in the retention quadrant compared to groups B, C and E. Several literatures exist on ketamine inducing dose-dependent memory impairment assessed using MWM (36,23,7). The probe trial results indicate that group D rats displayed unimpaired spatial working memory, implying that the administered extract has the potential to augment learning and memory in rodents. The mechanism of action by this plant in counteracting the effects of ketamine may be as a result of its antioxidant property. This is in line with Eru *et al.*(10), Lauriane *et al.*(22), Eru *et al.*(12), Folawiyo *et al.*(13) and Beppe *et al.*(3) who reported that *Talinum triangulare, Ocimum bailicum, Telfara occidentalis, Ocimum gratissimum* and *Albizia adianthifolia* were able to enhance learning and memory due to their high antioxidant capacity.

The histological changes observed in group B rats imply cellular degeneration and give evidence to the fact that ketamine overdose can cause neurodegeneration as reported by Giroux *et al.*(16), Shafri *et al.*(35) and Ding *et al.*(7). The precise underlying mechanism responsible for this degeneration

remains elusory, but based on postulations and theoretical considerations, it has been hypothesized that the process generation of free radicals may be consequential in the given context (39). Intriguingly, a research by Deveci and his team gave more illustration on this intricate process. They said that through continuous exposure to Ketamine, the NMDA receptor can undergo upregulation and create a molecular environment that is conducive for toxic accumulation of calcium within neuronal cells following cessation of ketamine administration. This dysregulation calcium homeostasis then serves as a catalyst for heightened production of reactive oxygen species “ROS”, which in turn triggers a cascade of events that lead to oxidative tissue damage (6,37). The neuronal cell degeneration as well as the cognitive impairment (MWM test) observed in group C may be due to the effect of ketamine being administered concurrently with Diazepam, even though diazepam stands as one of the drugs used medically to reduce the risk of neurotoxicity from NMDA receptors (27,25). According to Perez(28) and Kalsi *et al.*(20), ketamine used in combination with drugs such as diazepam can lead to dire and unpredictable outcomes such as decreased heart function, memory loss, coma and eventual death.

The results obtained also a dose-dependent pattern in the efficacy of *Dacryodes edulis* leaf extract in promoting neuronal cell repair and reproduction. Amongst the experimental groups, group D demonstrated the most significant ameliorative potential on neuronal cells in the prefrontal cortex. The findings suggest that the therapeutic effects of the extract can be attributed to its rich phenol (antioxidant) content. As suggested by Sadhwani (33) and Pharm-Huy *et al.*(29), phenols possess the ability to counteract excessive free radicals, thereby safeguarding cells against their toxic effects. Futhermore, phenols have been implicated in the therapy and management of many neurological disorders. The findings of this research align with previous studies conducted by Eru *et al*.(10), Eru *et al*.(11), Boussadia *et al.*(4), Anani *et al.*(1), Seddik *et al.*(34), and Kharoubi *et al.*(21), Ifiok *et al* (19). Eru *et al.*(8) and Eru *et al*(9). which have reported on ameliorative effects of plants rich in phenols on areas of the brain affected by toxic substances. Overall, the ameliorative effects (both in the histological study and MWM test) were better in group D than that of group E rats. This may be due to the decreased neuroprotective action considering the administration of a lower dose of the extract.

CONCLUSION

The present study illustrated ameliorative potentials of *Dacryodes edulis* ethanolic leaf extract on the prefrontal cortex of rats of ketamine-induced neurotoxicity, as well as neurobehaviour but in a dose-dependent pattern. It also showed that the administered dosage of ketamine for the given period of

time led to neuronal cell injury in the prefrontal cortex, as well as cognitive impairment in the rats, thereby confirming neurotoxicity.

ETHICAL CONSIDERATION

All authors hereby declare that “Principles of laboratory animal care” (NIH publication No. 85-23, revised 1985) were followed as well as specific national laws where applicable. All experiments have been examined and approved by Faculty Animal Research Ethics committee, Faculty of Basic Medical Sciences, University of Calabar, Cross River state, Nigeria and given a registration number 190ANA3023.

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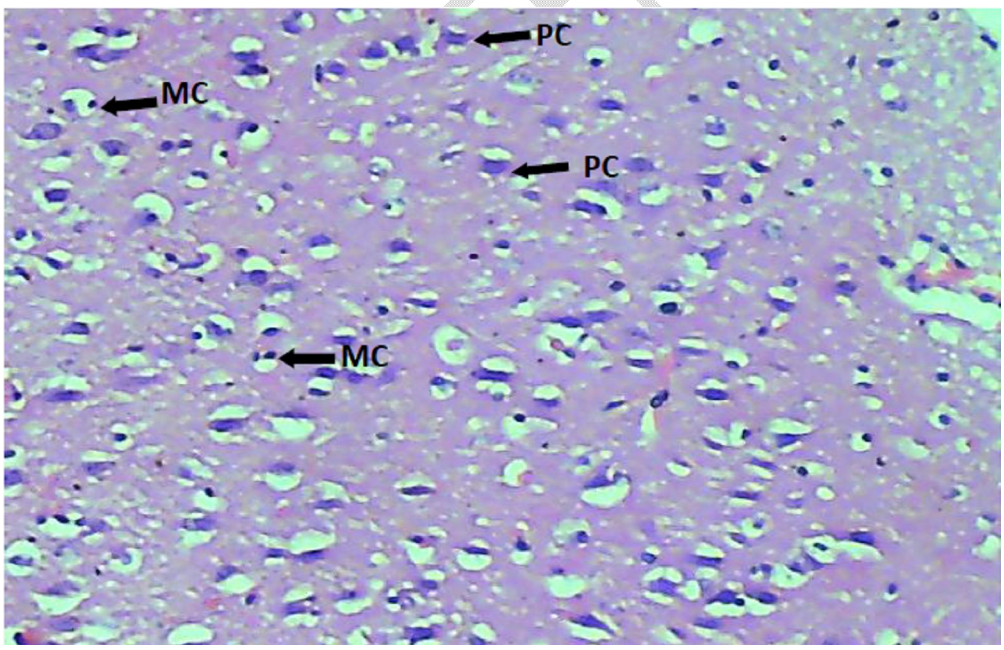

Plate 1: Photomicrograph (X400) of a unit of the prefrontal cortex (H&E-stained section) of group A rats (normal control group) displaying evenly distributed neuronal cell bodies of various sizes and shapes, with predominant pyramidal cells (PC) having triangular cell shapes and a thin rim of cytoplasm. There are also abundant neuropil and scattered microglia cells (MC).

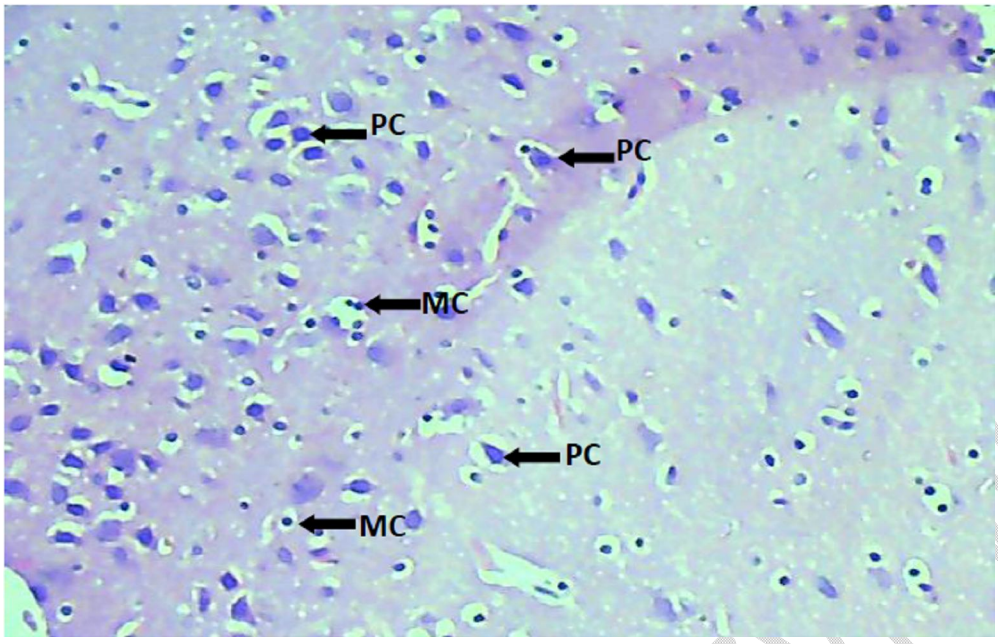

Plate 2: Photomicrograph (X400) of a unit of the prefrontal cortex (H&E-stained section) of group B rats treated with 100mg/kg BW ketamine showing evenly distributed neuronal cell bodies of various sizes and shapes, with predominant sparsely populated pyramidal cells (PC). The neuronal cell bodies display atrophic changes. There are also abundant neuropil and scattered microglia cells (MC) present with predominant pyramidal cells which have triangular cell shapes and a thin rim of cytoplasm. There are also abundant neuropil and scattered microglia cells present.

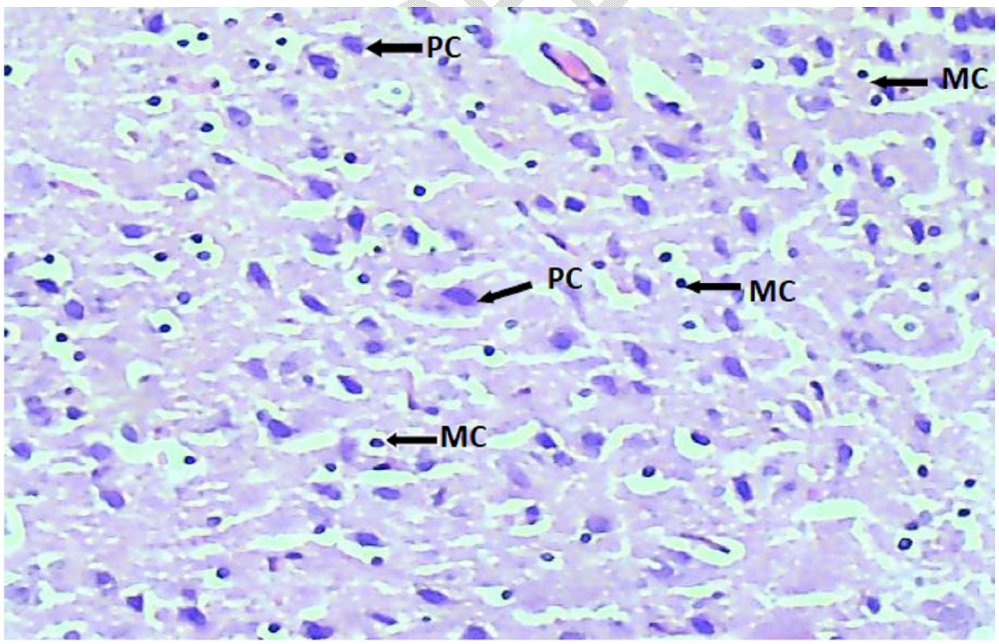

Plate 3: Photomicrograph (X400) of a unit of the prefrontal cortex (H&E-stained section) of group C rats (treated with ketamine and 0.3mg/kg BW diazepam) showing evenly distributed neuronal cell bodies of various sizes and shape with predominant sparsely pyramidal cells (PC) having a triangular cell shape and a thin rim of cytoplasm. The neuronal cell bodies display atrophic changes. There are abundant neuropil and scattered microglia cells (MC) present.

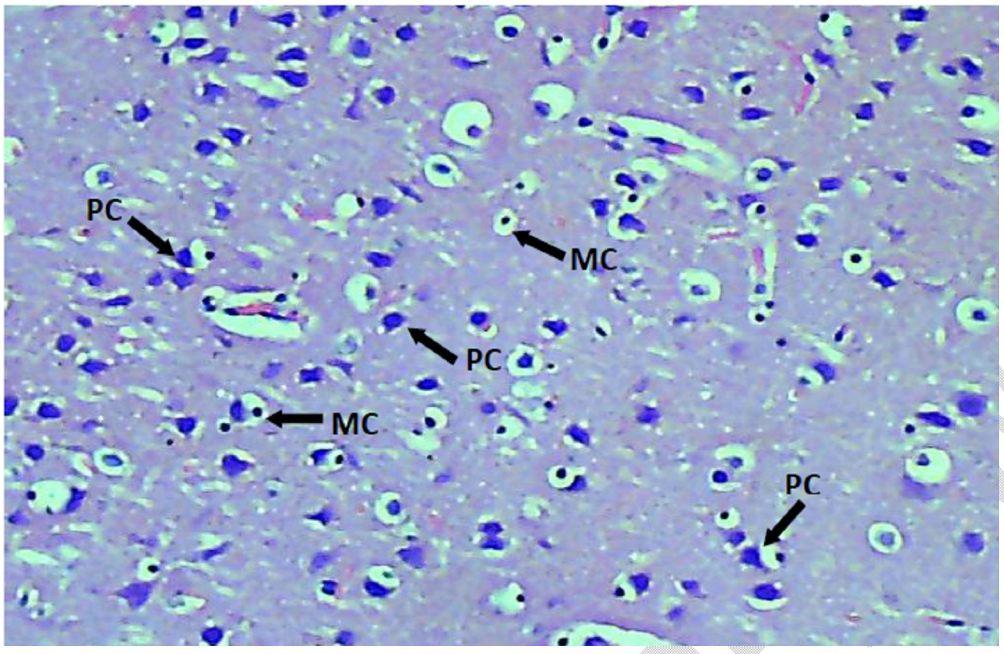

Plate 4: Photomicrograph (X400) of a unit of the prefrontal cortex (H&E-stained section) of group D rats (treated with ketamine + 1000mg/kg BW *Dacryodes edulis* extract) showing densely populated neuronal cell bodies of various sizes and shapes. There are proliferating microglia cells (MC) scattered among the neuronal cells.

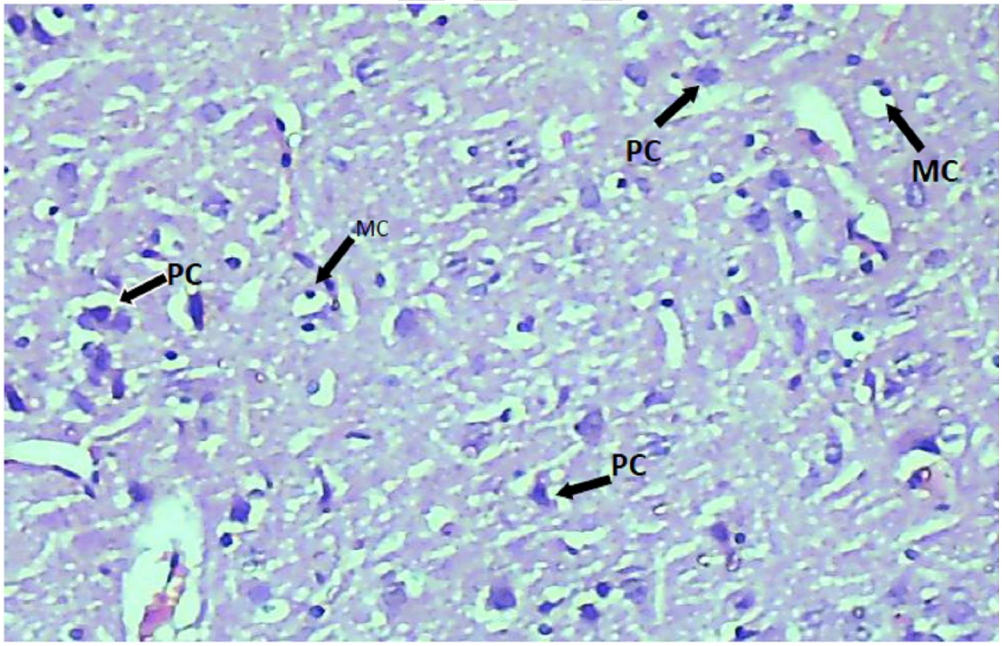

Plate 5: Photomicrograph (X400) of a unit of the hippocampus (H&E-stained section) of group E rats (treated with ketamine + 500mg/kg BW *Dacryodes edulis* extract) showing densely populated neuronal cell bodies of various sizes and shapes. There are proliferating microglia cells (MC) scattered among the neuronal cells.

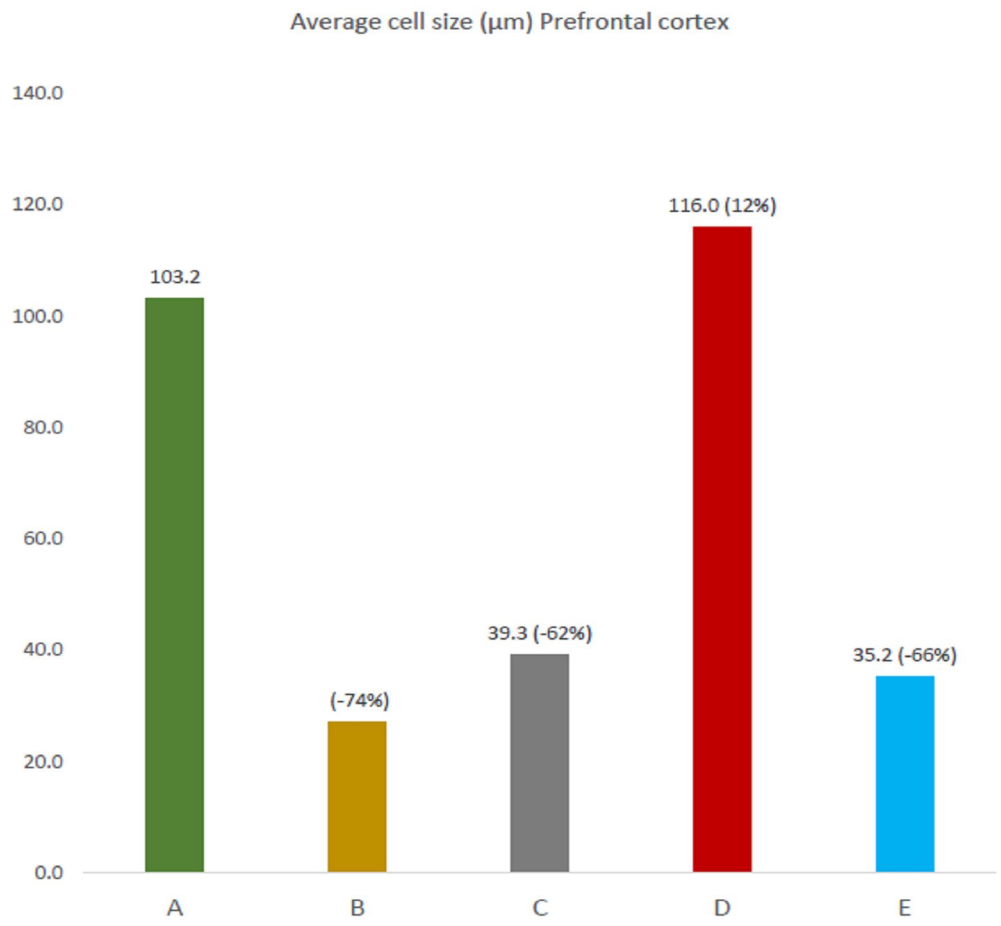

Figure 1: Effect of ketamine, diazepam and the different doses of *Dacryodes edulis* leaf extract (1000mg/kg BW and 500mg/kg BW) on prefrontal cortical neuronal cell size based on the H&E sections. Y-axis = average size (µm) of cells in section, X-axis = experimental groups (B to E) showing % increase or decrease in average cell size compared to group A.

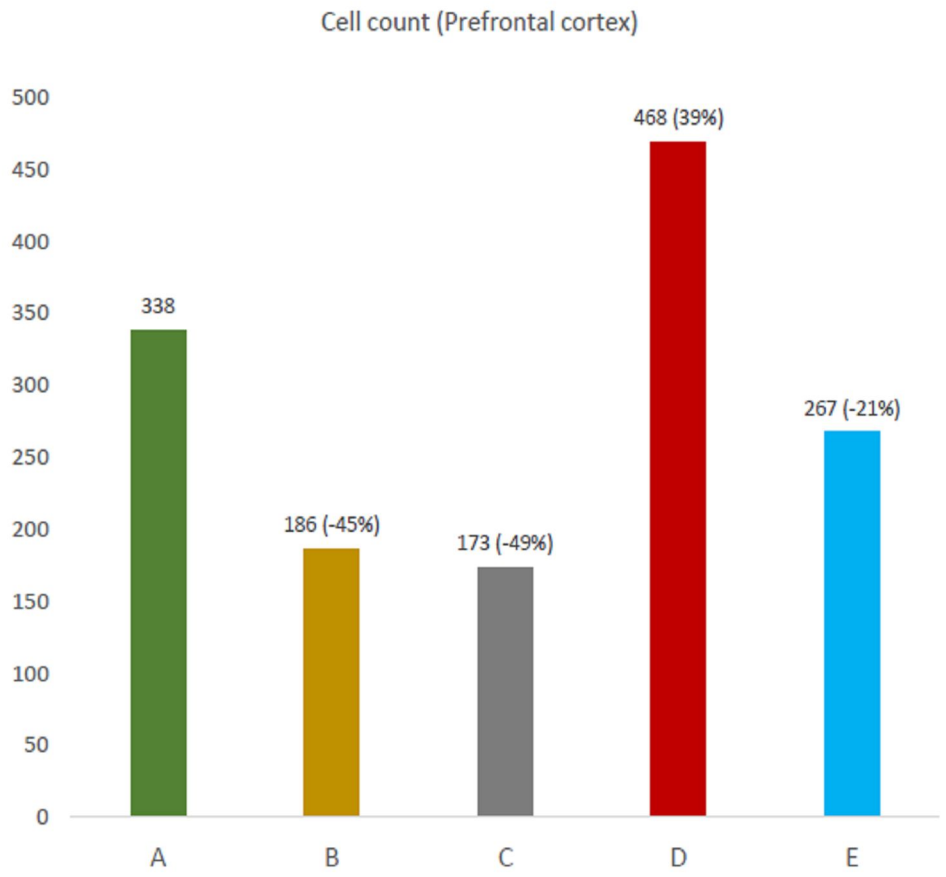

Figure 2: Effect of ketamine, diazepam and the different doses of *Dacryodes edulis* leaf extract (1000mg/kg BW and 500mg/kg BW) on prefrontal cortical neuronal cell count based on the H&E sections. Y-axis = average number of cells in section, X-axis = experimental groups (B to E) showing % increase or decrease in average cell count compared to group A.

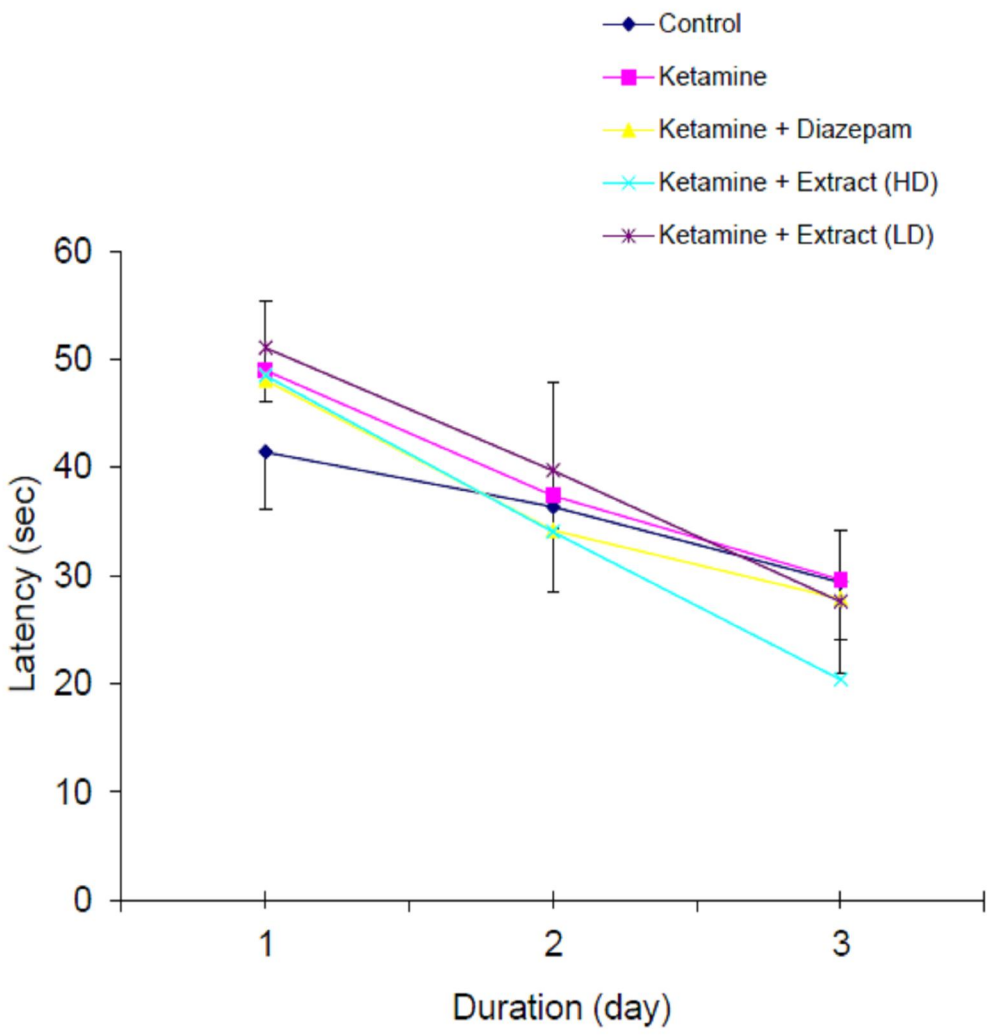

Figure 3: Comparison of swim latency during acquisation training on days 1, 2 and 3 of Morris water maze test.
Values are expressed as mean +SEM.

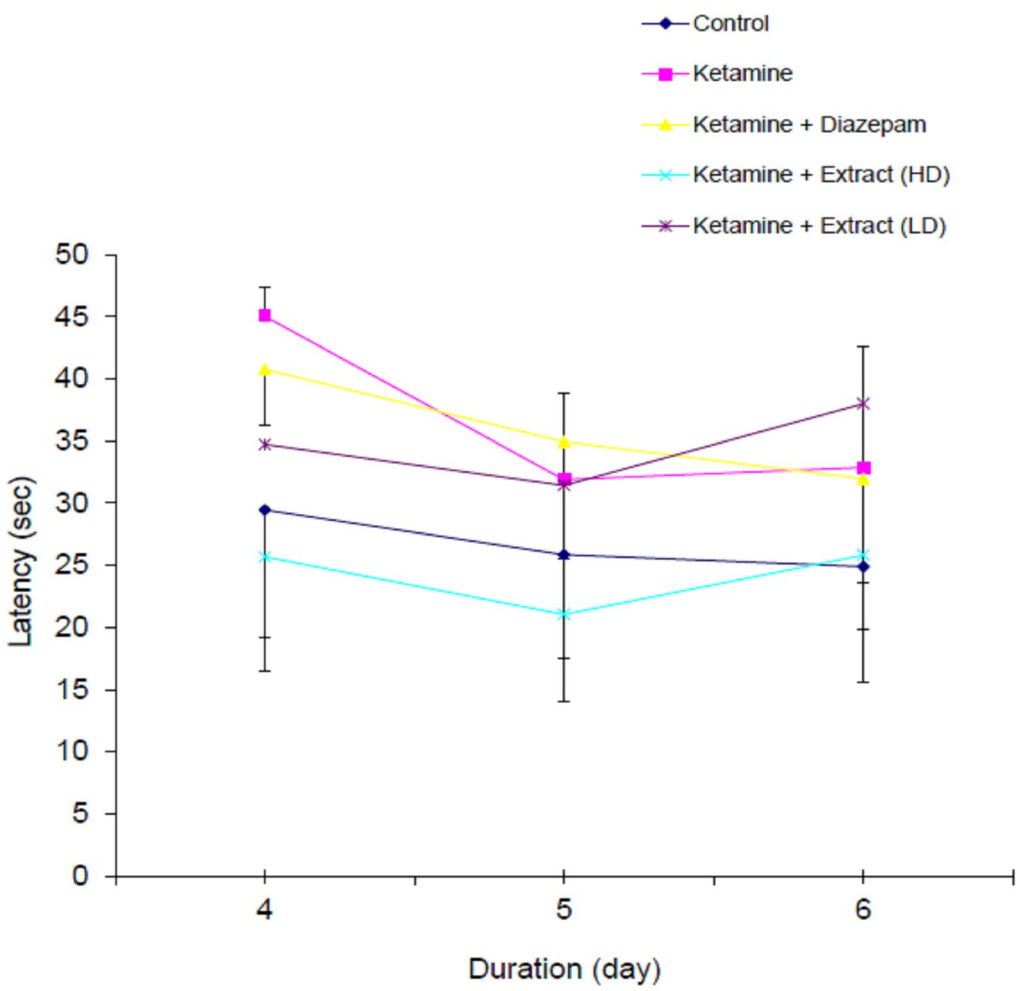

Figure 4: Comparison of swim latency during reversal training on days 4, 5 and 6 of Morris water maze test.
Values are expressed as mean +SEM.

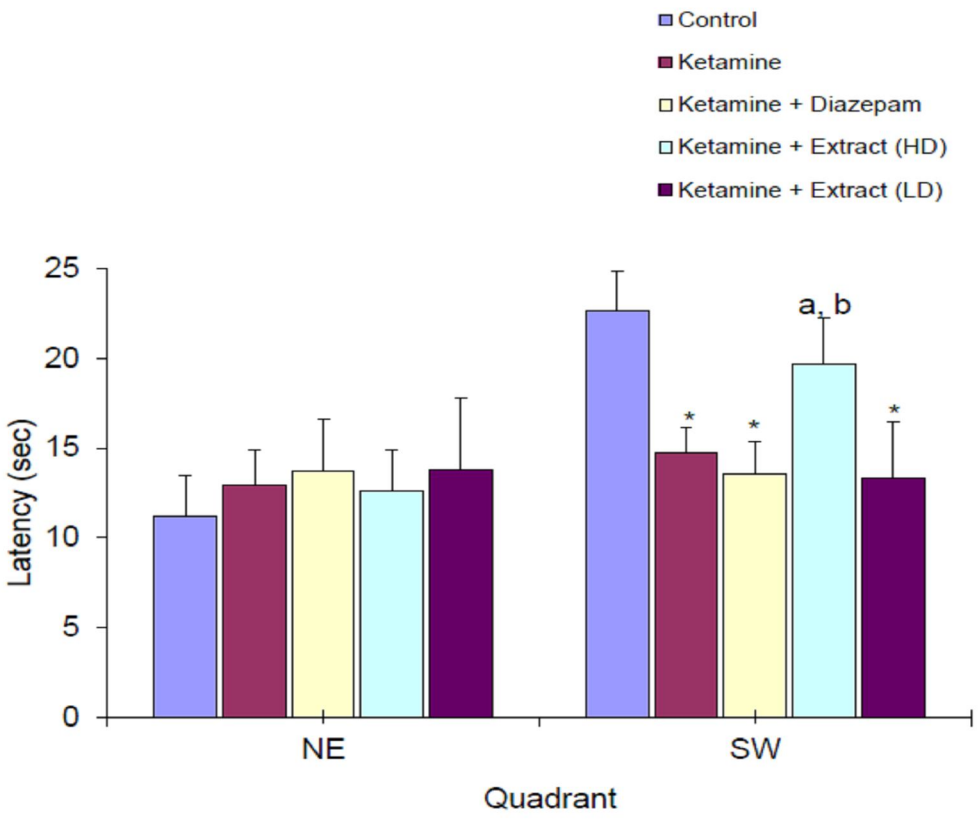

Figure 5: Swim latency in the north east and south west quadrants during probe trial of the Morris water maze test.
Values are expresed as mean +SEM.
* = significantly different from normal control at $p < 0.05$
a = significantly different from ketamine control at $p < 0.05$
b = significantly different from diazepam at $p < 0.05$