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ASSESSMENT OF THE NEUROTOXICITY OF ETHANOL LEAF EXTRACT OF *Ficus benjamina* (WEEPING FIG) ON BRAIN OF WISTAR RATS

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ABSTRACT

Ficus benjamina has been proven to have medicinal potential, especially in alleviating some brain pathologies but there are few works on its neurotoxic effect. This study investigated the acute toxicity and effect of ethanol crude leaf extract of *Ficus benjamina* on the histology of some parts of the brain. Twenty-nine (29) Wistar rats weighing 123g-190g were used for the study. LD_{50} was determined with nine (9) rats using Lorke's method. Twenty (20) rats were divided into four (4) groups of 5 animals each. The control group was administered water and feed. Low dose (500 mg/kg), medium dose (1000 mg/kg), and high dose (1500 mg/kg) of extract were administered for twenty-one (21) days. On the 21st day, all the animals were weighed and sacrificed and their brains were harvested and processed histologically by the formalin-fixed-paraffin wax embedding method. The tissue blocks were sectioned and stained using haematoxylin and eosin, Periodic acid Schiff, and GFAP (glial fibrillary acidic protein). The results showed that the LD_{50} was ≤5000 mg/kg. There was no statistical significant difference in the body weights of the animals before and after the experiment ($p=0.985$). Histopathological changes showed normal histology of cells in the choroid plexus, hippocampal CA3 area, and cerebral frontal cortex, with normal glycogen and astrocytes in the control. The low-dose group had mild reactive astrogliosis. There was mild atrophy, vacuolation, and mild reactive astrogliosis in the medium-dose group. The high-dose group had marked hypertrophy/hyperplasia, blood vessel dilation, vacuolation, necrosis, and mild reactive astrogliosis. The results suggest that prolonged consumption of the medium and high doses of the leaf extract of *Ficus benjamina* may cause marked neurotoxicity. The low dose is safer for administration.

KEYWORDS: *Ficus benjamina*, acute toxicity, Brain, GFAP, Choroid plexus. Cerebral cortex,

INTRODUCTION

Medicinal plants have been known to cause neurotoxicity and naturally occurring substances in these plants have been responsible for this neurotoxic effect (Mensah *et al*., 2019; NINIDS, 2022). Neurotoxicity occurs when the nervous system's normal functions are disrupted by natural or man-made hazardous substances called neurotoxicants.

Neurotoxicity affects the structure and functions of the brain. The brain performs diverse functions, key among which are in learning, memory, and production of cerebrospinal fluid (CSF) performed by the cerebral cortex, hippocampus, and choroid plexus respectively (Waxman, 2013). These functions might be disrupted by toxins.

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According to Robertson (2019), brain cells are highly vulnerable to toxicity due to their high metabolic rate and the effect of toxicity on the brain will depend on various factors such as the type of neurotoxin, the exposure dose, the ability to metabolize and excrete the toxin, the recovery ability of affected structures, and the vulnerability of the cellular target. These toxins from medicinal plants cross the blood-brain barrier (in the cerebral cortex and hippocampus) or blood-CSF barrier (in the choroid plexus) to cause injury (Waxman, 2013; Kauffman *et al*., 2012).

Medicinal plants have been greatly utilized since ancient times as medicine for various ailments. Some of the applications of these medicinal plants include antifungal, anti-diabetic, anticancer, antioxidant, antipyretic, hypotensive, and anti-dysentery properties as well as ameliorating male infertility (Ogba *et al*., 2023; Asuquo *et al*., 2023; Imran *et al*., 2014; Ofem *et al*., 2023).

One of the medicinal plants that have diverse medicinal potentials is *Ficus benjamina* (Imran *et al*. 2014). *Ficus benjamina* commonly known as the weeping fig is a member of the Moraceae (fig) family having *Ficus Linn* and *benjamina* as its genus and species respectively (ITIS, 2022). *Ficus benjamina* is a house plant that thrives outdoors in the tropics (PIER, 2014). In Nigeria, it is widely used as an ornamental plant and eaten by goats as fodder. Its extracts have been used to treat ailments such as skin disorders, inflammation, leprosy, malaria, and cancer. *Ficus benjamina* is rich in many phytochemicals such as flavonoids, alkaloids, benjaminide, and essential oil (Singh *et al*., 2019, Imran *et al*., 2014). These phytochemical compounds are responsible for their anticonvulsant, antimicrobial, antioxidant, antiplasmodial, and antipyretic properties (Imran *et al*., 2014, Mumtaz *et al*., 2018; Singh *et al*., 2019). FIG. 1 is the *Ficus benjamina* plant.

A recent study reported the anti-convulsant and neuro-protective properties of *Ficus benjamina* attributed to its rich serotonin content (Singh *et al*., 2023). However, there is paucity of data on its toxic effect on the brain. Hence, the rationale of this study is to determine the LD_{50} and investigate the effect of ethanol crude leaf extract of *Ficus benjamina* on the histology of cerebral cortex, hippocampus, and choroid plexus of the brain of Wistar rats.

FIG. 1: *Ficus benjamina* plant leaves (Source: Fieldwork, 2023)

MATERIALS AND METHODS

Plant collection and extract preparation

Ficus benjamina leaves were obtained from 50 Webber Street, Calabar, Cross River State. Authentication of the leaves was done by Dr. Effa B. Effa of the Department of Plant and Ecological Studies, University of Calabar, Calabar. The fresh leaves were rinsed with clean water, to remove dust particles and debris then allowed to drain completely. The leaves were air-dried at room temperature for 10 days and the dried leaves were pulverized (blended) mechanically into dry powder using a manual blender. Then, 650g of the powdered leaves was extracted using 2000 ml of 95% ethanol for 48 hours. The extract was double-filtered with chess cloth, then with Whatmann No.1 filter paper. The filtrate obtained was concentrated under reduced pressure at 45^{o}C with a rotary evaporator and then to complete dryness using a vacuum water bath to yield 56g of ethanol crude extract. The prepared extract was placed in an airtight container and stored in a refrigerator at 4^{o}C until use.

Experimental animals and ethical approval

Twenty-nine (29) Wistar rats weighing 123-190g were procured from the Animal House of the College of Medical Sciences, University of Calabar. The rats were housed in standard laboratory cages and were allowed to acclimatize for two weeks before the commencement of the study. They were provided with standard rat feed and water. Ethical approval was obtained from the Animal Research Ethical Committee of the Faculty of Basic Medical Science, University of Calabar, Calabar with Number: FAREC/PA/UC/049.

Oral LD50 determination

LD_{50} determination was carried out with nine Wistar rats using Lorke's method described by Udonkang *et al*. (2018). The nine (9) rats were divided into three (3) groups having three (3) rats each. Each group was administered orally different doses of the ethanol crude leaf using the orogastric tube. Group I was given 100 mg/kg, 500 mg/kg, and 1000 mg/kg to each rat. In group 2, the rats were administered doses of 1500 mg/kg, 2500 mg/kg, and 3500 mg/kg each, and in Group 3, 4500 mg/kg, 5500 mg/kg, and 6500 mg/kg were given to each rat. At the end, the rats are observed for death or physical changes after 24 hours.

Experimental design and procedure

Twenty (20) rats were divided into four groups of five animals each. The control group was administered water and feed. Low-dose, medium dose and high-dose groups were administered 500 mg/kg, 1000 mg/kg, and 1500 mg/kg body weight of the extract respectively by oral route using an orogastric tube for twenty-one (21) days.

After twenty-one (21) days, the rats were weighed and sacrificed using Ketamine (100 mg/kg body weight) intraperitoneal injection. The rats were dissected at the skull starting from the occipital bone, temporal bones, parietal bones, and lastly frontal bone. The brains were harvested and fixed in 10% Neutral buffered formalin for 72 hours before further histological techniques were carried out. The fixed brain samples were grossed, put in a plastic tissue cassette, and processed using the routine formalin-fixed-paraffin wax embedding method. After processing, the brain tissues were embedded in molten paraffin wax. Afterward, the tissue blocks were sectioned using a rotary microtome at 3 μm. The sections obtained were stained for general tissue structure with Haematoxylin & Eosin (Bancroft *et al*., 2019), Periodic acid Schiff (Bancroft *et al*., 2019), and GFAP (Bancroft *et al*., 2019) techniques. Slides were viewed with the x10 and x40 objectives of the OMAX (China) light microscope. Photomicrographs were taken using AmScope (USA) digital camera and software.

Statistical analysis

Statistical analysis of data obtained was done with SPSS (version 20). The body weights of the rats were analyzed using Pearson's Chi-square test for differences between categories (body weights before and after the experiment). Analysis of Variance (ANOVA) was used to analyze the GFAP staining intensities. All results were expressed as mean$\pm$standard deviation and were statistically significant at $p \leq 0.05$.

RESULTS

LD_{50} determination

The LD_{50} of the ethanol crude leaf extract of *Ficus benjamina* is shown in Table 1. There was no mortality after administration of the extract in Phases 1, 2, and 3. The LD_{50} value was ≤5000 mg/kg body weight.

Table 1: The LD50 of ethanol crude leaf extract of *Ficus benjamina*

Phase	Group	No. of rats	Dose (mg/kg)	Mortality after 24 hours	LD_{50} value (mg/kg)
	1	1	100	0/1	
1	2	1	500	0/1	
	3	1	1000	0/1	
	1	1	1500	0/1	≤5000
2	2	1	2500	0/1	
	3	1	3500	0/1	
	1	1	4500	0/1	
3	2	1	5500	0/1	
	3	1	6500	0/1	

Body weight measurement of the animals

The body weight measurements of the rats are shown in Table 2. There was no statistically significant decrease in body weight across the groups before and after the experiment (χ^2=0.152; p=0.985).

Table 2: Body weight measurements of the Wistar rats

Group	Initial weight (Mean±SD)	Final weight (Mean±SD)	Statistics
Control	167.60±29.21	156.15±27.64	
Low dose	154.75±15.07	144.30±12.42	χ^2=0.152
Medium dose	150.93±17.45	143.65±16.12	p = 0.985
High Dose	173.20±12.05	163.70±11.27	
Total	161.62±20.47	151.95±18.91	

Key- SD= standard deviation

Photomicrographs

The choroid plexuses of the rats in the experimental groups are shown in Fig. 2. The histology of the choroid plexus of rats in the control group revealed normal epithelial cells, normal connective tissue, and blood vessels in the control and low-dose groups. The medium dose group showed normal epithelial cells and mildly enlarged blood vessels but the high dose group showed marked hypertrophy, proliferation of epithelial cells, enlargement of connective tissue with vacuolation, and marked enlargement of blood vessels within the ventricle.

In FIG. 3 is the hippocampal CA3 area of the rats. In the control group, the CA3 area showed numerous glia cells in all layers, numerous pyramidal cells in the pyramidal cell layer and normal cells in the polymorphic layer. The CA3 area of rats in the low-dose group showed numerous glia cells in all layers, few pyramidal cells, and normal cells in the polymorphic layer. The CA3 area of rats in the medium dose group showed vacuolation around a few pyramidal cells, and glia cells in all layers. The CA3 area in the high dose group showed vacuolation, numerous glia cells in all layers, and scanty pyramidal cells in the pyramidal cell layer.

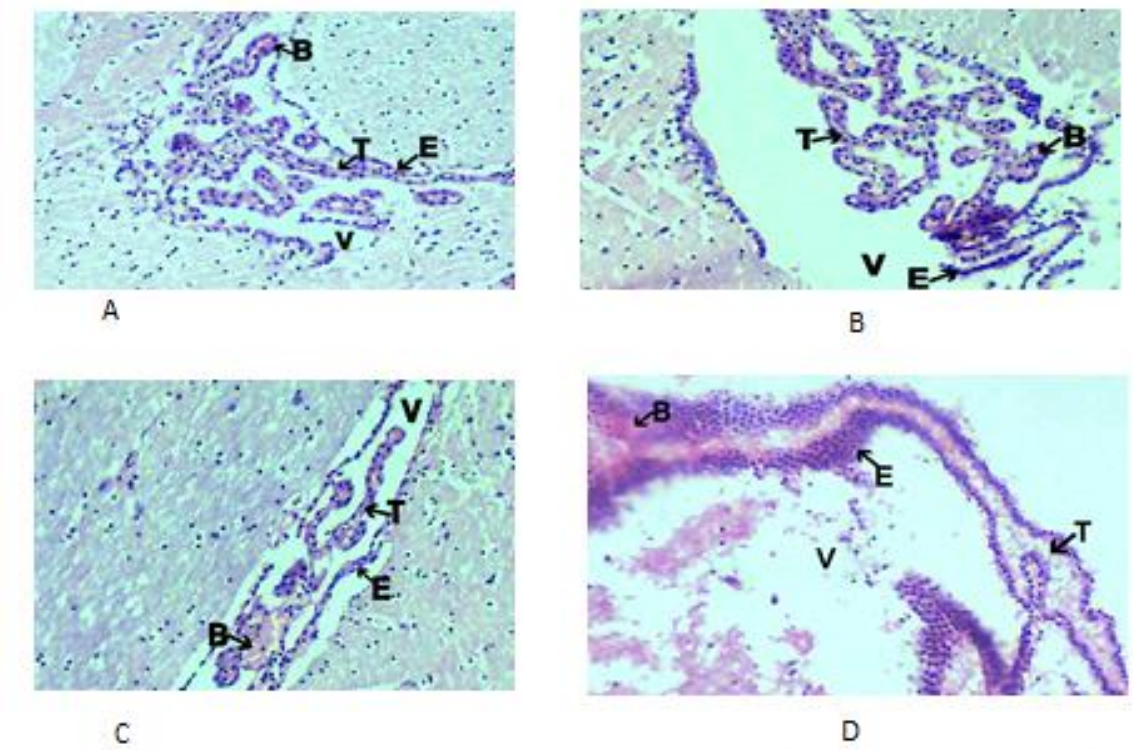

FIG. 2: Choroid plexuses of rats in (A) control (B) low dose (500 mg/kg) (C) medium dose (1000 mg/kg) (D) high dose (1500 mg/kg) groups. Keys: epithelial cells (E), connective tissue (T), blood vessel (B) and ventricle (V). Haematoxylin and eosin x100 .

The frontal cerebral cortex of rats stained with H&E is shown in FIG.4. The frontal cortex of the control group showed normal cells and blood capillary in the molecular layer, numerous granule cells in the external granular layer, and numerous pyramidal cells in the polymorphic layer. The frontal cortex of rats in the low-dose group showed normal cells in the molecular layer, few granule cells in the external granular layer, and proliferation of pyramidal cells and blood capillary in the polymorphic layer. The frontal cortex of rats in the medium dose group showed normal cells in the molecular layer, enlarged granule cells, and mild enlargement of blood capillary in the external granular layer with proliferation of pyramidal cells in the polymorphic layer. The frontal cortex of rats in the high dose group showed proliferation of cells in the molecular layer and few granule cells, and mild enlargement of blood capillary in the external granular layer. There was atrophy and proliferation of pyramidal cells in all layers. The glycogen staining of the frontal cortex of the rats showed normal staining of glycogen in the neuropil for all the experimental groups with the PAS staining.

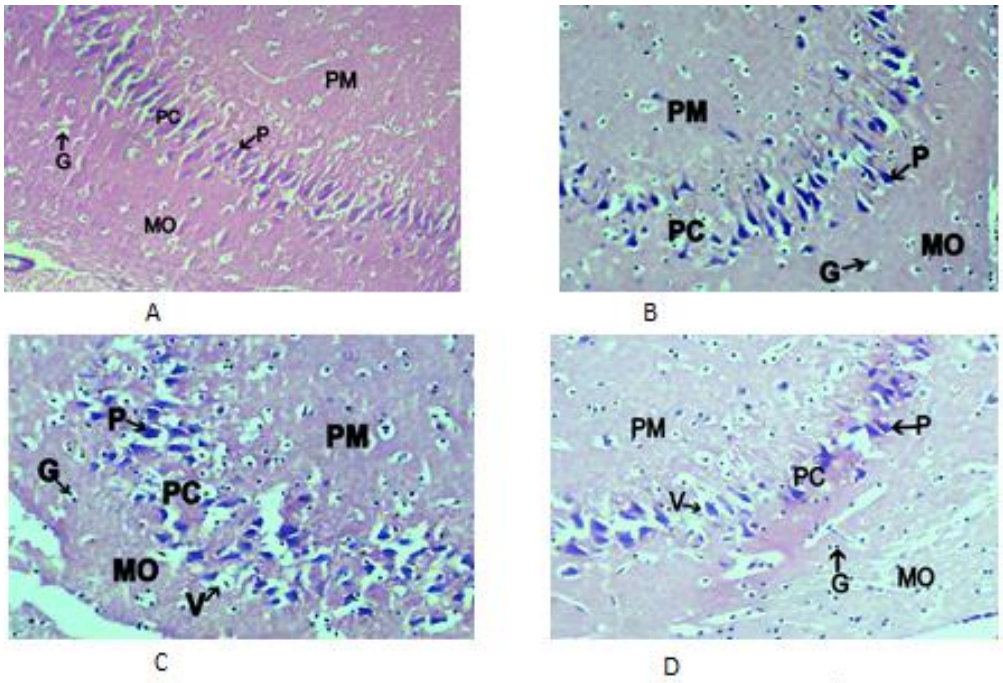

FIG. 3: Hippocampus CA3 areas of rats in (A) control (B) low dose (500 mg/kg) (C) medium dose (1000 mg/kg) (D) high dose (1500 mg/kg) groups. Keys: Pyramidal cells (P), glia cells (G), vacoulations (V), molecular layer (MO), pyramidal cell (PC) layer, and polymorphic layer (PM). Haematoxylin and eosin x100.

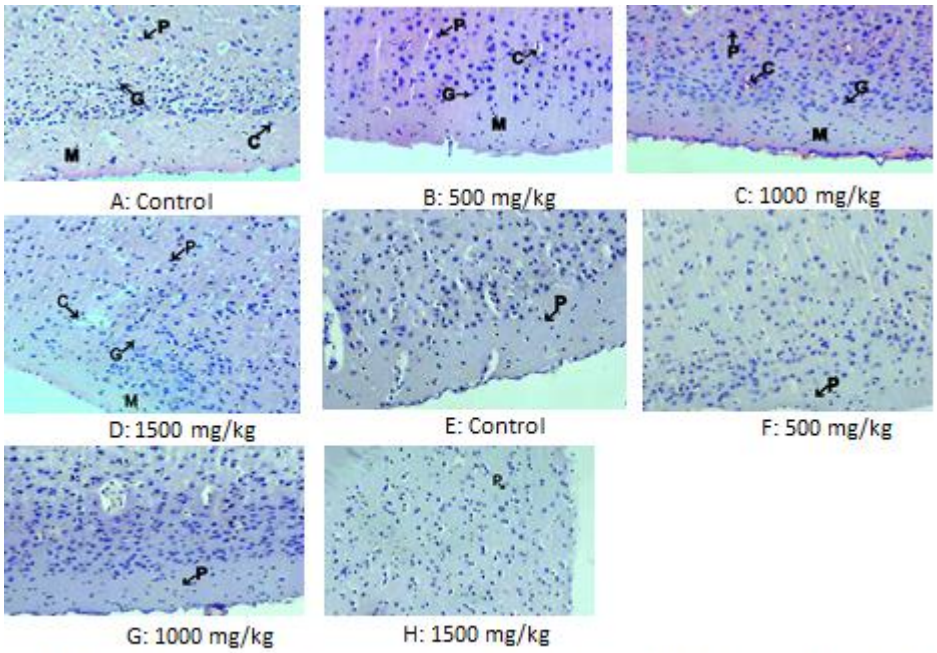

FIG. 4: Frontal cerebral cortex of rats stained with H&E (A, B, C, D) and PAS (E, F. G, H). Keys: Pyramidal cells (P), granule cells (G), Molecular layer (M), capillary (C) in H&E. P (glycogen) in PAS. Mag. X100.

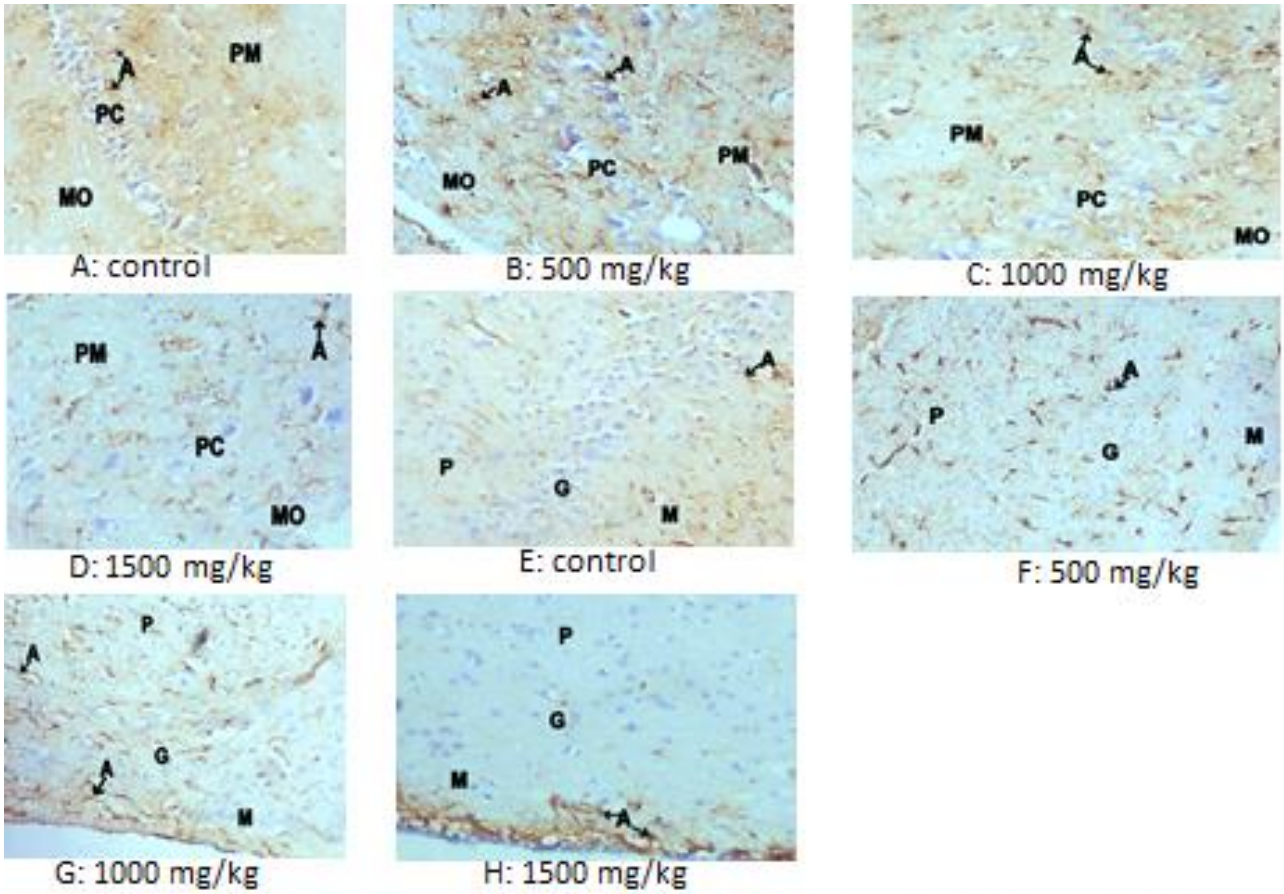

FIG. 5: Hippocampus (A, B, C, D) and frontal cortex (E, F. G, H) of rats stained with GFAP. Keys: Astrocyte (A), molecular layer (MO), pyramidal cell (PC) layer, polymorphic layer (PM), Pyramidal cells (P), granule cells (G), Molecular layer (M). GFAP X100.

FIG.5 is the hippocampus and frontal cortex of rats stained for GFAP. The CA3 area of rats in the control group showed normal star-shaped astrocytes. The CA3 areas of the rat in the low, medium, and high dose groups showed mild enlargement and proliferation of astrocytes. The frontal cortex in the control group showed normal numerous astrocytes in all layers. The frontal cortex of rats in the low-dose, medium-dose, and high-dose groups showed mild enlargement of astrocytes in all layers. The astrocytes (A), molecular layer (M), external granular layer (G), and polymorphic layer (P) are shown.

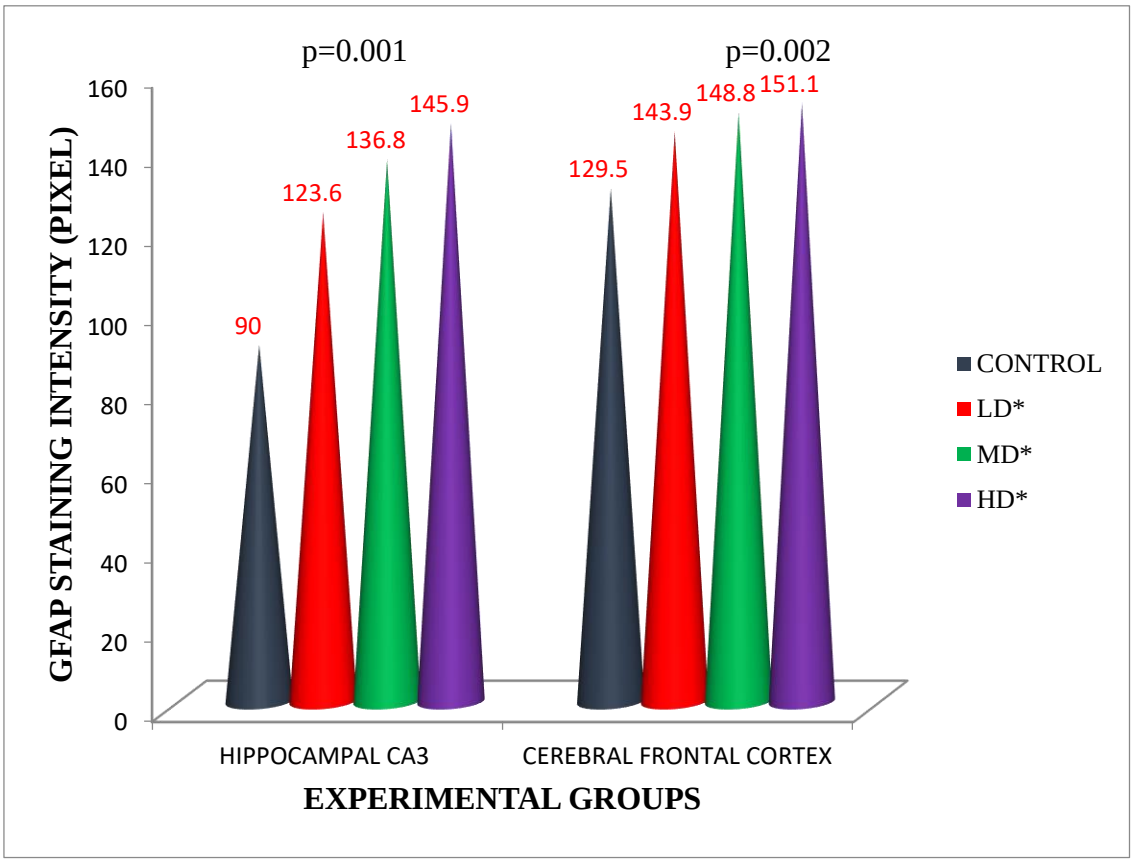

FIG. 6: GFAP staining intensity among the experimental groups

FIG. 6 is the GFAP staining intensity among the experimental groups. The CA3 had a dose-dependent increase in intensity of 90.0±23.11px, 123.6±20.87px, 136.8±17.04px, 145.9±23.54px in the control, low-dose, medium-dose, and high-dose groups respectively. This staining intensity was statistically significant between the control and the test groups (F=13.228, p=0.001). The staining intensity in the cerebral frontal cortex was 129.5±13.7px, 143.9±13.1px, 148.8±14.5px, 151.10±8.1px in the control, low-dose, medium-dose, and high-dose groups respectively. This dose-dependent increase in intensity of staining was statistically significant between the control and the test groups (F=5.924, p=0.002)

DISCUSSION

In this study, none of the rats used for the lethality study died and had LD_{50} ≤5000 mg/kg range of showing relatively low acute toxicity. This LD_{50} range is classified in Category 5 of acute toxicity hazard categories and shows that although the leaf extract is relatively safe, it might cause harm to vulnerable populations (ILO, 1956). This is similar to the result obtained by Ladaf *et al*. (2023) who showed that at LD_{50} of ≤5000 mg/kg body weight, no mortality was recorded following administration of aqueous leaf extract of *Ficus benjamina*. The body weights of the rats were not significantly reduced in this study which showed that the extract did not cause any weight change. This is similar to the result of no significant change in body weight reported by Larbie *et al*. (2016) using 500 mg/kg aqueous leaf extract of *Ficus pumila*.

In the histological findings, the prolonged consumption and higher doses of the extract resulted in epithelial hypertrophy, hyperplasia, vasodilation, and vacuolation in the choroid plexus. The choroid plexus is responsible for cognitive functions, such as spatial learning and memory in addition to the production of CSF. Choroid plexus epithelial hypertrophy and enlargement have been linked to inflammation, increase in volume, and cognition impairment (Lizano *et al*., 2019). Hyperplasia and hypertrophy of the epithelial cells of the choroid plexus can also cause an increase in ventricular pressure as a result of an increase in CSF production (Lizano *et al*., 2019). Thus, this finding has suggested that this enlargement might lead to an increase in ventricular pressure and impairment in cognition.

In the hippocampus of the rats, inflammation and necrosis in the CA3 areas were observed. The CA3 area is prominent in carrying out memory retention. These changes might affect the memory consolidation functions of the hippocampus, thereby affecting cognition. Similarly, the frontal cortex had necrosis of granule cells, pyramidal cells hyperplasia, and blood capillary vasodilation. Injury to the hippocampus and cerebral cortex has been linked to cognition impairment (Lizano *et al*., 2019).

However, there was no impairment in glycogen storage as the result showed normal staining intensity in all groups. This suggests that the extract does not interfere with glucose metabolism in the brain.

The hippocampal CA3 area and cerebral frontal cortex also showed mild reactive astrogliosis characterized by hypertrophy of astrocytes. The astrocytes had an increase in staining intensity in a dose dependent manner. Astrocytes are prominent during cellular injury. The astrocytes become hypertrophic because of the overexpression of the intermediate filament GFAP (Ben Haim *et al*., 2015). Astrogliosis is a neuro-protective response against assault on the brain. This is because astrocytes perform neuro-inflammatory functions and play a primary defense response against toxins entering the brain as they form the blood-brain barrier (Ferrer, 2017).

These neurotoxic effects of the plant extract could be attributed to the presence of alkaloids, particularly the pyrrolizidine alkaloids. *Ficus benjamina* has a rich content of pyrrolizidine alkaloids which are compounds that are secondary metabolites of plants (Singh and Sharma, 2023). These pyrrolizidine alkaloids have been reported to cause neurotoxicity in animals (Schramm *et al*., 2019). The neurotoxicity is believed to occur due to cellular adducts formed from interaction between pyrrole esters and cellular proteins. The pyrrole esters are end products of catabolism of pyrrolizidine alkaloids by cytochrome p450 enzymes in the liver. These adducts have been reported to cause cellular proliferation, membrane damage, and haemorrhage in the liver (Singh and Sharma, 2023, Lin and Tujios, 2023, Udonkang *et al*., 2024). The neurotoxic effects seen in the choroid plexus, hippocampus, and cerebral cortex indicate the assault on the blood-CSF and blood-brain barriers by the toxin. This is supported by Kaufmann *et al*. (2012) who stated that necrosis, inflammation, and astrogliosis are markers of neurotoxicity.

CONCLUSION

In this study, the ethanol crude leaf extract of *Ficus benjamina* has LD_{50} ≤5000 mg/kg and did not reduce the body weights of the rats. Histopathological changes observed were epithelial cells hyperplasia and hypertrophy, vasodilation, and vacuolation in the choroid plexus at high dose. Necrosis of glia and pyramidal cells and vacuolation were observed in the hippocampus at medium and high doses. Granule and pyramidal cells hyperplasia and vasodilation were seen in the cerebral frontal cortex at medium and high doses. The extract caused mild reactive astrogliosis in the hippocampus and frontal cortex in all test groups. The degree of the pathological changes was dose-dependent. *Ficus benjamina* extract is safer at 500 mg/kg. Hence, the extract should be administered at this low dose to avoid neurotoxic effects.

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