World Journal of Pharmaceutical Science and Research
www.wjpsronline.com
Research Article
ISSN: 2583-6579
SJIF Impact Factor: 5.111
Year - 2024
Volume: 3; Issue: 5
Page: 27-43

DANIELLIA OLIVERI LEAF METHANOL EXTRACT MITIGATES REDOX IMBALANCE AND NEURONAL DEGENERATION IN BRAIN OF WISTAR RATS EXPOSED TO DIMETHYLAMINE

***Ayobami T. E. and Adeleke G. E.**

Department of Biochemistry, Faculty of Pure and Applied Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.

Article Received: 31 July 2024 | | ***Article Revised: 22 August 2024*** | | ***Article Accepted: 13 September 2024***

***Corresponding Author: Ayobami T. E.**
Department of Biochemistry, Faculty of Pure and Applied Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.
DOI: https://doi.org/10.5281/zenodo.13868967

How to cite this Article: Ayobami T. E. and Adeleke G. E. (2024). *DANIELLIA OLIVERI* LEAF METHANOL EXTRACT MITIGATES REDOX IMBALANCE AND NEURONAL DEGENERATION IN BRAIN OF WISTAR RATS EXPOSED TO DIMETHYLAMINE. World Journal of Pharmaceutical Science and Research, 3(5), 27-43. https://doi.org/10.5281/zenodo.13868967

ABSTRACT

The present study examines the effects of *Daniellia oliveri* leaf methanol extract on DMA toxicity in blood and brain of male Wistar rats. Sohxlet extraction method was used to obtain *Daniellia oliveri* leaf methanol extract (DOLME). Seven groups (A – G) of rats (average weight of 154g) were used with five rats per group. Group A served as Control and group B was given intraperitoneal injection of DMA alone (10 mg/kg). Groups C to G were treated with DOLME (50, 100, 150, 200, 250, mg/kg) by oral gavage and DMA (10mg/kg) by intraperitoneal injection. The DOLME was administered every other day, while DMA was administered once per week. After 28days, the rats were sacrificed by cervical dislocation, while blood was collected for hematology. One portion brain tissue was processed for biochemical assays (superoxide dismutase, catalase, glutathione-s-transferase, reduced glutathione and glutathione peroxidase), while the hippocampus of the brain was fixed for histology and Immunohistochemical assays of glial fibriliary acidic protein (GFAP) and synaptophysin. The DMA significantly elevated RBC, hematocrit, WBC, monocytes and platelets relative to control group, which DOLME was able to reverse. The brain SOD and catalase activities were reduced, while GST was elevated relative to controls. The DOLME reversed the changes. The DMA caused hippocampal neuronal degeneration, which DOLME reversed. Strong expression of GFAP was noticed on degenerated astrocytes, while moderately high expression of neuronal synaptophysin was noticed in the brain hippocampus of rats treated with DMA. The DOLME caused mild to moderate expression of GFAP and mild expression of synaptophysin. The findings from this study reveal that *Daniellia oliveri* leaf methanol extract could potentially prevent Dimethylamine-induced hematological disorder and brain neuronal degeneration by improving antioxidant status in rat model.

KEYWORDS: Brain, *Daniellia oliveri*, glial fibriliary acidic protein, oxidative stress, synaptophysin.

1.0 INTRODUCTION

Medicinal plants have long been a reservoir of bioactive compounds, offering a myriad of biological activities (Souza and Passos, 2021). Among these plants, *Daniellia Oliveri* commonly known as "Black Ironwood" or "Ogea," has garnered attention for its phytochemical richness and diverse therapeutic potentials (Okunade and Olafadehan, 2019). Originating from the verdant landscapes of West Africa including Nigeria, Cameroon, Ghana, and Ivory Coast, this species is renowned for its ecological, economic and traditional medicinal significance (Agha *et al*., 2022). Phytochemical studies have demonstrated that *Daniellia Oliveri* is rich in alkaloids, flavonoids, tannins, and essential oils which are known for their antioxidant, anti-inflammatory, antimicrobial, and neuroprotective properties (Yaya *et al*., 2022). Alkaloids from this plant exhibit significant pharmacological effects, including antibacterial, analgesic, and anticancer activities (Badalyan and Rapior, 2021). Flavonoids such as quercetin, kaempferol, and myricetin have been shown to possess strong antioxidant and anti-inflammatory properties, contributing to the plant's potential in mitigating oxidative stress and inflammation in neural tissues (Sabri et al., 2021).

The traditional use of *Daniellia Oliveri* in West African medicine for treating respiratory disorders, gastrointestinal ailments, and inflammatory conditions underscores its broad therapeutic potential (Harris *et al*., 2023). Recent scientific investigations have confirmed the presence of bioactive compounds that contribute to its medicinal properties (Coker *et al*., 2021).The medicinal properties of this plant also offer potential for developing herbal remedies and nutraceuticals, resonating with the growing demand for natural health products (Sgroi, 2021). Traore *et al*. (2021) reported that methanol extract of *Daniellia Oliveri* inhibited the activity of 15-Lipooxygenase (LOX) enzyme in experimental animal model. The phytochemical composition of the plant has been responsible for its various pharmacological properties, which include antioxidant, antiproliferative, and tyrosinase inhibitory actions (Atolani and Olatunji, 2014, 2016; Naculima *et al*., 2021).

Dimethylamine is a secondary amine compound with significant industrial and biochemical relevance (Bugata *et al*., 2019). It is often used in the synthesis of pharmaceuticals, pesticides, and rubber chemicals (Igbal *et al*., 2021). *In vivo* metabolism of amines have been exemplified by arylamines (aromatic amines), which are metabolized via hepatic CYP 450-dependent *N*-hydroxylation (Turkesky *et al*., 1992), followed by phase II esterification of the *N*-hydroxylamine to reactive ester derivatives that covalently bind DNA (Herman and Synderwine, 1999). One of our previous studies indicated that Dimethylamine caused periportal cell infiltration in liver of rats, while Immunohistochemical staining revealed strong expression of CD 34, which is a diagnosis for tendency towards malignancy tumor incidence (Adeleke *et al*., 2017). The present study was designed to investigate the impacts of *Daniellia Oliveri* leaf methanol extract on hematological indices, redox imbalance and cytological alterations in brain tissue of Wistar rats exposed to dimethylamine.

2.0 MATERIALS AND METHODS

2.1Collection and extraction of *Daniellia oliveri* leaves

Daniellia oliveri leaves were collected at Obamoro village, via Iwo, Osun State in July, 2023. After air-drying for about three weeks, the leaves were pulverized, followed Sohxlet extraction using methanol and rotary evaporation to obtain *Daniellia oliveri* leaf methanol extract (DOLME).

2.2 Experimental design

Thirty-five male Wistar rats were purchased and divided into Seven groups (A – G) (average weight of 154 g) with five rats per group. Group A served as Control and group B was given intraperitoneal injection of DMA alone (10 mg/kg). Groups C to G were treated with DOLME (50, 100, 150, 200, 250, mg/kg) by oral gavage and DMA (10mg/kg) by intraperitoneal injection. The DOLME was administered every other day, while DMA was administered once per week for four weeks.

2.3 Sacrifice and Collection of blood and brain

After four weeks, the rats were fasted overnight and sacrificed by cervical dislocation. Blood was collected for both full blood count and differential, while brain was collected and divided into two portions. One portion of brain tissue was homogenized in pre-chilled Phosphate buffer (0.01M, pH 7.4) using Teflon homogenizer and then centrifuged at 10,000 x g for 15 minutes to obtain homogenate used for antioxidant assays, while the hippocampal portion of the brain was fixed for histology and Immunohistochemical assays (glial fibriliary acidic protein (GFAP) and synaptophysin).

2.4 Determinations of Protein and antioxidant parameters

The levels of protein in the brain homogenates were measured spectrophotometrically using a method described by Lowry *et al*., 1951. The levels of superoxide dismutase (Misra and Fridovich, 1971), catalase (Aebi, 1984), glutathione-S-transferase (Habig *et al*., 1974), reduced glutathione (Mitchell *et* al., 1973) and glutathione peroxidase (Paglia and Valentine, 1967) in the brain tissues were measured spectrophotometrically.

2.5 Histopathological examination

Ultra-thin sections (5 µm) of the formalin-fixed brain hippocampus were obtained using a microtome knife. The sections were stained with hematoxylin and Eosin (H&E) solutions, and then observed under microscope, taken the photomicrographs of the stained slides.

2.6 Immunohistochemical assays

Immunochemical staining of glial fibriliary acidic protein (GFAP) and synaptophysin in the hippocampus of brain tissue was carried out in paraffin-embedded tissue. The deparaffinised brain sections were subjected to the peroxidase labeled streptavidin-biotin technique, using themonoclonal antibody against the respective antigens. Peroxidase blocking was carried out on the sections by covering the sections with 3% hydrogen peroxide for 15 minutes. The sections were washed with phosphate buffered saline (PBS) and protein blocking was done using avidin for 15 minutes. The sections were washed with PBS and endogenous biotin in the liver was blocked for 15 minutes, followed by incubation with 5µg/ml each of anti- GFAP and anti- synaptophysin antibodies (diluted in 1:100) for 60 minutes. PBS was used to wash excess antibodies followed by application of secondary antibodies (LINK) on the sections for 15 minutes. The sections were washed and Horseradish peroxidase (HRP) label was applied on the sections for 15 minutes, and then washed with PBS for 5 minutes to remove unbound HRP. Visualization of the reaction products was done by immersing the section in Karnovsky solution (0.01% diaminobenzidine (DAB) in Tris buffer containing 0.05% hydrogen peroxide and 0.01% sodium azide). Excess DAB solution and precipitate were washed off with distilled water. The tissue sections were counterstained with Haematoxylin solution for 2 minutes. The slides were dehydrated in alcohol, cleaned in xylene, mounted in DPX mountant and then observed under microscope. Cells with specific brown color in the cytoplasm, cell membrane or nuclei, depending on the antigenic sites, were considered positive.

2.7 Statistical Analysis

Values were expressed as mean ± SD. Differences in the mean values were estimated statistically by one-way analysis of variance (ANOVA) by using the Statistical Package for Social Sciences (SPSS) software for Windows version 10.0 (USA). Significantly different values were taken at $P < 0.05$.

3.0 RESULTS

The result in table 1 shows that Dimethylamine (DMA) significantly ($p < 0.05$) elevated the levels of RBC and hematocrit relative to control group. The levels were however lowered by *Daniellia oliveri* leaf methanol extract (DOLME) against the DMA intoxication. The level of hemoglobin was not significantly affected by treatments with DMA and DOLME (Table 1). Treatment with DMA significantly ($p < 0.05$) increased the levels of WBC, monocytes and platelets relative to the controls, whereas DOLME lowered the levels against DMA treatment. The treatments were not able to cause significant change in the monocytes level (Table 2). As shown in table 3, the level of protein in the brain tissue of rats was not significantly affected. Dimethylamine was noticed to significantly reduce the activities of both superoxide dismutase and catalase enzymes in the brain tissue of the rats compared to control rats. However, while DOLME reduced the SOD, catalase activity was found to be increased at high doses against DMA treatment (Table 3). The result in figure 1 indicates that DMA increased the GST activity in the brain tissue compared to control, whereas DOLME reversed the effect at high doses. The treatments produced no significant ($p > 0.05$) change on the level of reduced glutathione in the tissue (Figure 2). The activity of glutathione peroxidase enzyme in the brain of the experimental rats showed no significant change between DMA and controls. Strikingly, the activity was lowered by DOLME at doses 50 and 150 mg/kg against DMA treatment (Figure 3).

Plates 1 A to G reveal the effects of the treatments with DMA and DOLME on the histology of the brain tissue of the rats. The tissue showed normal neuronal cells (Blue arrow), normal capillaries(Red arrow) and normal stroma (Slender arrow) in the control group (Plate 1 A), while DMA caused normal neuronal cells (Blue arrow) and degenerated neurons with pale appearance as indicated with lender arrows (Plate 1B).The results of brain histology of rats treated with DMA and DOLME (Plates 1 C, D, E, F and G) reveal presence of normal neuronal cells (indicated with blue arrows), while the cortex shows normal capillaries and stroma (Slender arrow). These features are comparable to that of the control rats (Plate 1A). The results in Plates 2A – E show the effects of treatments on the Immunohistochemical expression of glial fibrillary acidic protein (GFAP) in the brain tissue of the experimental rats.

Plates 2A,C. D and E show mild expression of fibrillary acidic protein (GFAP)on the normal astrocytes in the brain on treatment with DMA and DOLME, while treatment with DMA alone caused mild expression of GFAPon the astrocytes, which are degenerated, leaving few normal astrocytes. The Immunohistochemical study of the brain indicated the treatment with DMA and DOLME caused mild expression of Synaptophysin on the neurons (Plates 3 B, C D and E), against the control group that showed no expression of synaptophysinon the neuronal cells (Plate 3 A).

Table 1: Effects of *Daniellia Oliveri* leaf methanol extract and Dimethylamine on red blood cells, hemoglobin and hematocrit inthe experimental rats.

TREATMENT GROUPS	Red blood cells(10^{12}/L)	Hemoglobin (g/dL)	Hematocrit (%)
CONTROL	6.01 ± 0.24	13.78 ±0.21	31.23 ± 0.23
DMA (10mg/kg)	9.39 ± 0.90[a]	13.58± 1.77	36.52 ±5.50[a]
50mg/kgDOLME +DMA	5.58± 0.78[b]	11.83 ± 1.28	30.58 ± 3.20[b]
100mg/kgDOLME +DMA	5.76 ± 1.09[b]	12.18± 1.92	31,04 ± 5.80[b]
150mg/kgDOLME +DMA	5.19 ± 0.65[b]	11.38 ± 1.31	29.18 ± 2.87[b]
200mg/kgDOLME +DMA	5.26 ±1.37[b]	11.53 ± 2.94	28.13 ± 6.55[b]
250mg/kgDOLME +DMA	4.52 ±1.47[b]	9.98± 2.49	25.12± 7.26[b]

Values expressed as mean ± standard deviation, n = 5

a- significantly higher than control (P < 0.05); b - significantly lower than DMA group (P<0.05)

Table 2: Effects of *Daniellia Oliveri* leaf methanol extract and Dimethylamine on WBC, lymphocytes, monocytes and platelets in the experimental rats.

TREATMENT GROUPS	White blood cells (10^9/L)	Lymphocyte (10^9/L)	Monocytes (10^9/L)	Platelets (10^9/L)
CONTROL	5.97 ± 1.57	4.57 ± 1.13	0.57 ± 0.12	174.67 ± 75.48
DMA (10 mg/kg)	15.08± 3.04[a]	6.38± 2.50	0.91 ±0.37[a]	285.49 ± 23.56[a]
50mg/kgDOLME +DMA	7.13 ± 5.47[b]	6.625± 2.40	0.68 ± 1.03[b]	105.01 ± 27.43[b]
100mg/kgDOLME +DMA	6.76± 3.06[b]	5.22± 2.28	0.88± 0.52[b]	200.49 ± 46.68[b]
150mg/kgDOLME +DMA	5.68 ± 1.07[b]	4.53 ± 0.78	0.58 ±0.0b	168.56 ± 29.98[b]
200mg/kgDOLME +DMA	7.48 ± 4.22[b]	6.03 ± 3.25	0.75± 0.70[b]	123.93 ± 75.77[b]
250mg/kgDOLME +DMA	8.54 ± 1.74[b]	6.44 ± 1.25	0.69± 0.38[b]	185.32± 52.89[b]

Values expressed as mean ± standard deviation, n = 5

a- significantly higher than control (P < 0.05); b - significantly lower than DMA group (P<0.05)

Table 3: Effects of *Daniellia Oliveri* leaf methanol extract on Protein, Superoxide dismutase and Catalase levels in Brain of rats administered with dimethylamine.

TREATMENT GROUPS	Protein (µ/mg protein)	SOD $X10^{-5}$ (µ/mg protein)	Catalase (µ/mg protein)
CONTROL	2.72 ±0.72	15.98 ±9.74	92.92±65.21
DMA (10mg/kg)	3.06 ± 0.88	7.73±11.6[d]	69.64±24.44[a]
50 mg/kg DOLME + DMA	3.04 ± 0.73	2.11±1.78[c]	67.21±13.85
100mg/kg DOLME + DMA	2.54 ± 0.62	1.40 ±4.46[c]	82.30±25.25[b]
150mg/kg DOLME + DMA	2.82 ± 0.57	0.59±2.16[c]	91.30±15.73[b]
200mg/kg DOLME + DMA	2.20 ±0.62[b]	0.99±0.72[c]	96.48±30.44[b]
250mg/kg DOLME + DMA	2.38 ± 0.20[b]	0.27±1.68[c]	83.38± 7.39[b]

Values expressed as mean ± standard deviation, n = 5

a- significantly lower than control (P < 0.05); b - significantly higher than DMA group (P < 0.05); c – significantly lower than DMA group (p < 0.05); d- significantly lower than control (p < 0.05)

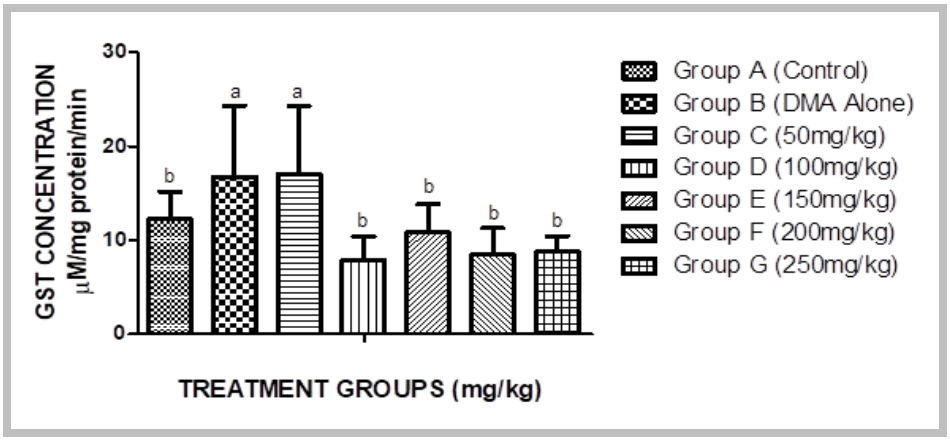

Figure 1: Effect of *Daniellia Oliveri* leaf methanol extract on Glutathione –S-transferase activity in brain of rats treated with DMA.

Values expressed as mean ± standard deviation, n = 5
a-significantly higher than control; b-significantly lower than DMA ($p<0.05$)

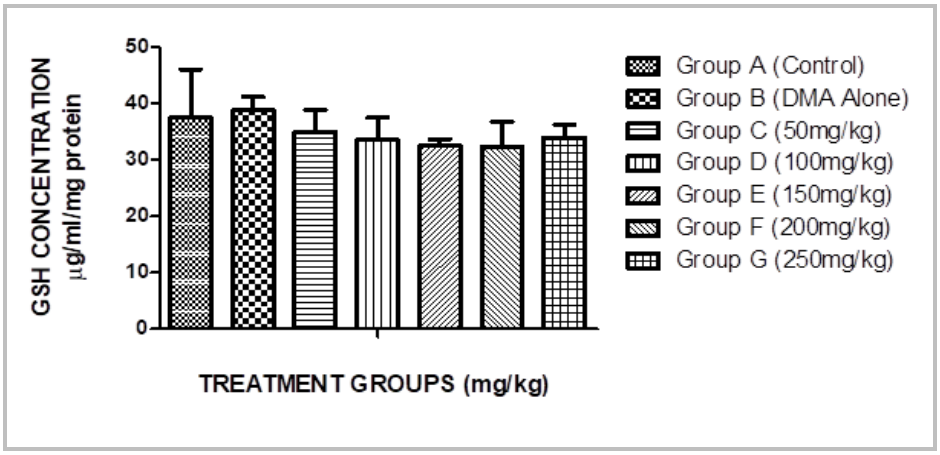

Figure 2: Effect of *Daniellia Oliveri* leaf methanol extract on reduced Glutathione concentration in brain of rats treated with dimethylamine.

Values expressed as mean ± standard deviation, n = 5

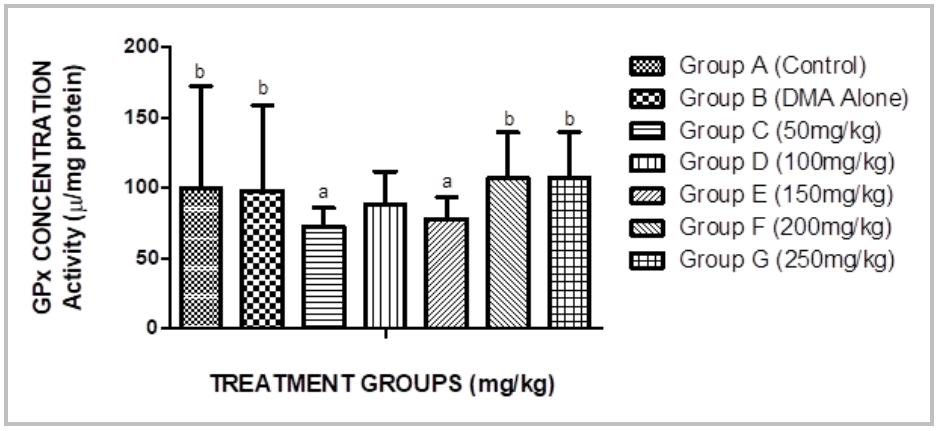

Figure 3: Effect of *Daniellia Oliveri* leaf methanol extract on Glutathione peroxidase activity in brain of rats treated with DMA.

Values expressed as mean ± standard deviation, n = 5
a- significantly lower relative to DMA group; b – comparable to control and DMA groups

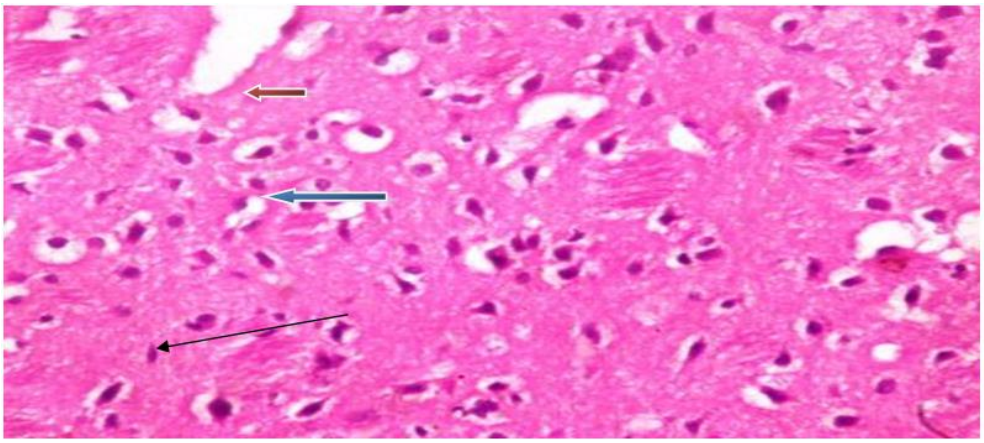

PLATE 1A: Brain (Control) - Normal neuronal cells (Blue arrow), normal capillaries(Red arrow) and normal stroma (Slender arrow) (x 400).

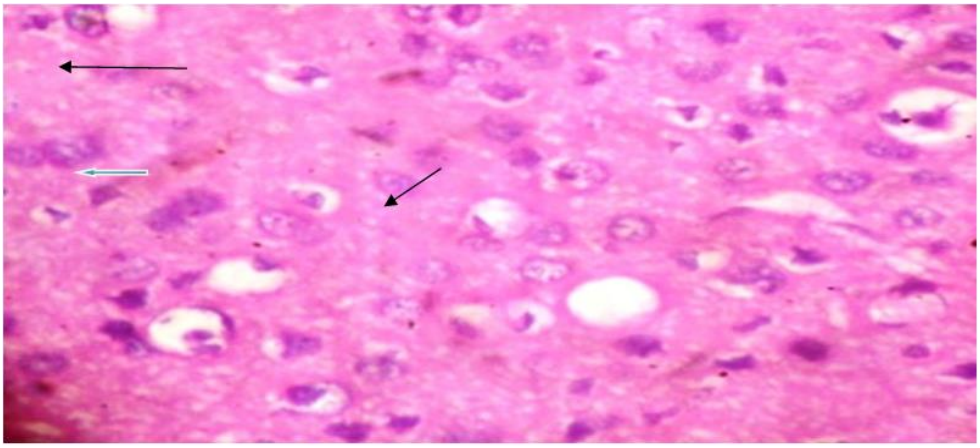

PLATE 1B: Brain (10 mg/kg DMA) - Cortex shows normal neuronal cells (Blue arrow) with some pale and degenerated neurons (Slender arrows) (x 400).

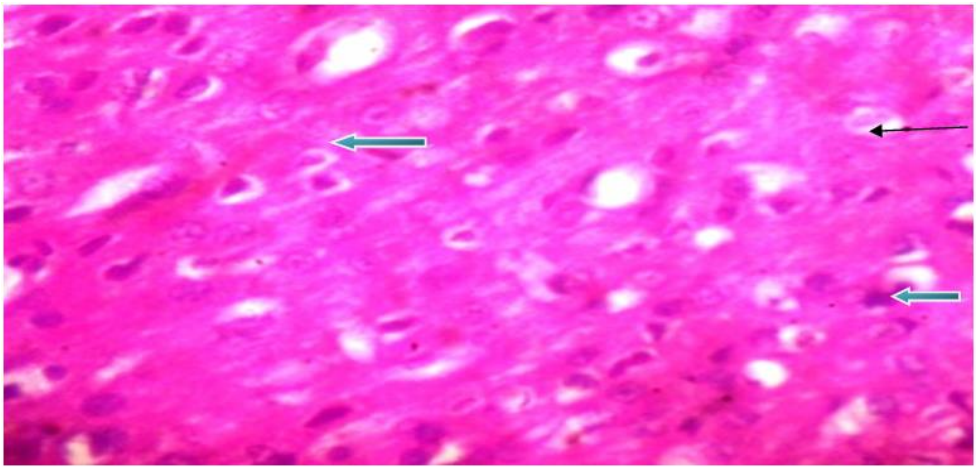

PLATE 1C: Brain (50mg/kg DOLME+10mg/kg DMA) - Normal neuronal cells (Blue arrows), the cortex shows neuronal cells, normal capillaries and stroma (Slender arrow) (x 400).

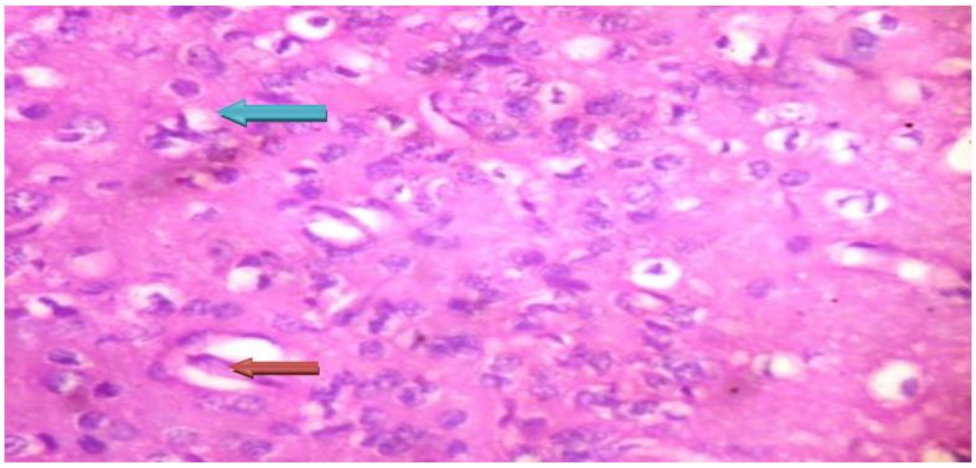

PLATE1D: Brain (100mg/kg DOLME+10mg/kg DMA) -Cortex shows neuronal cells (Blue arrow), normal capillaries and stroma (Red arrow) (x 400).

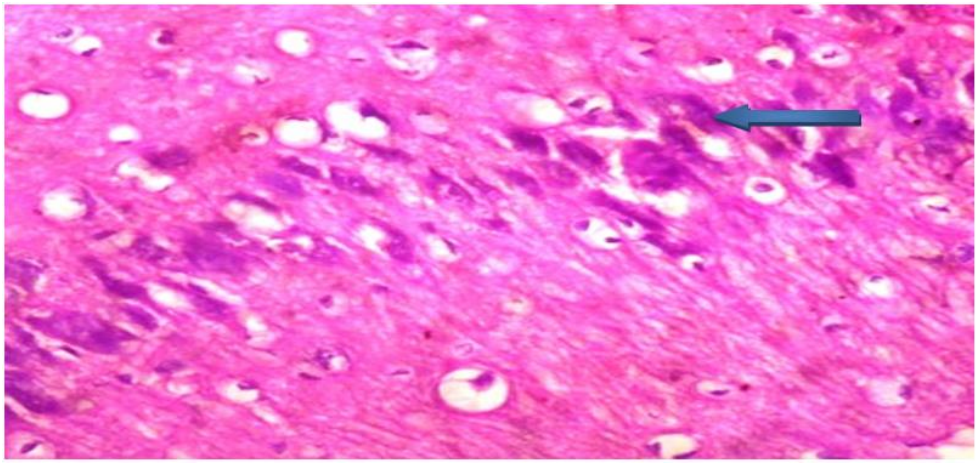

PLATE 1E: Brain (150mg/kg DOLME+10mg/kg DMA) - Cortex shows neuronal cells (Blue arrow), with normal capillaries and stroma (x 400).

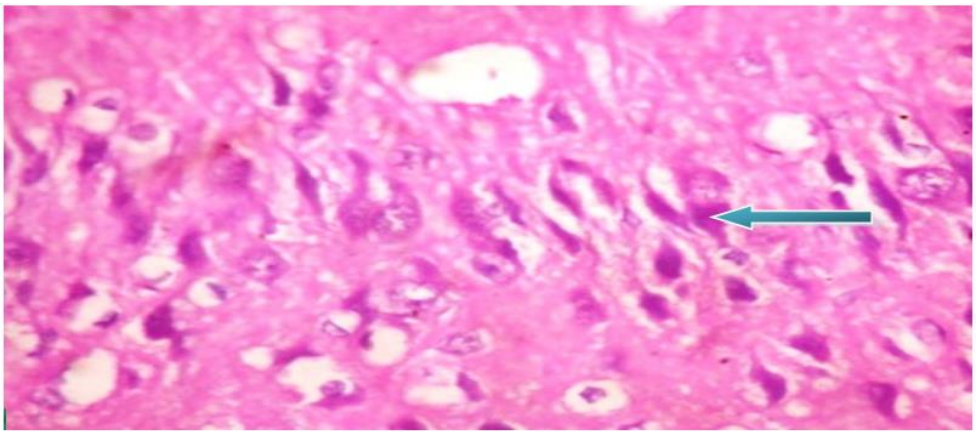

PLATE 1F: Brain (200mg/kg DOLME+10mg/kg DMA) - Cortex shows normal neuronal cells (Blue arrow) (x 400).

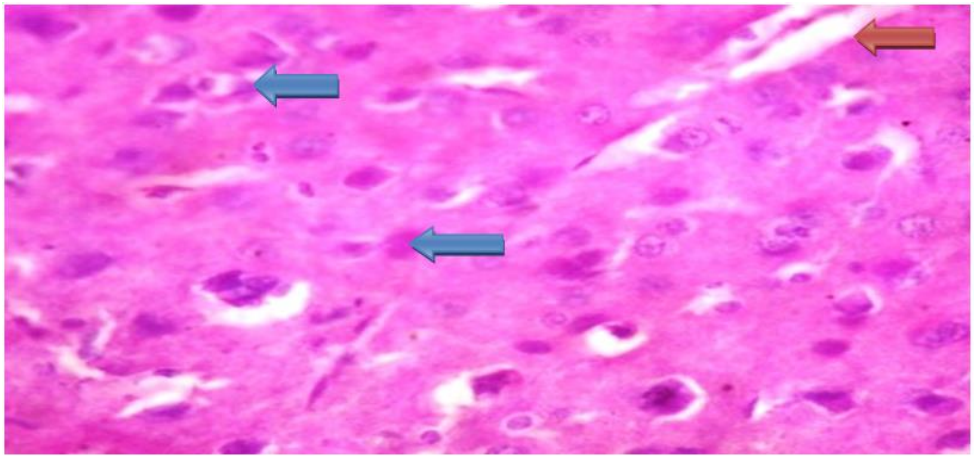

PLATE 1G: Brain (250mg/kg DOLME+10mg/kg DMA) -Cortex with normal neuronal cells (Blue arrows), and capillaries and stroma appear normal (Red arrows) (x 400).

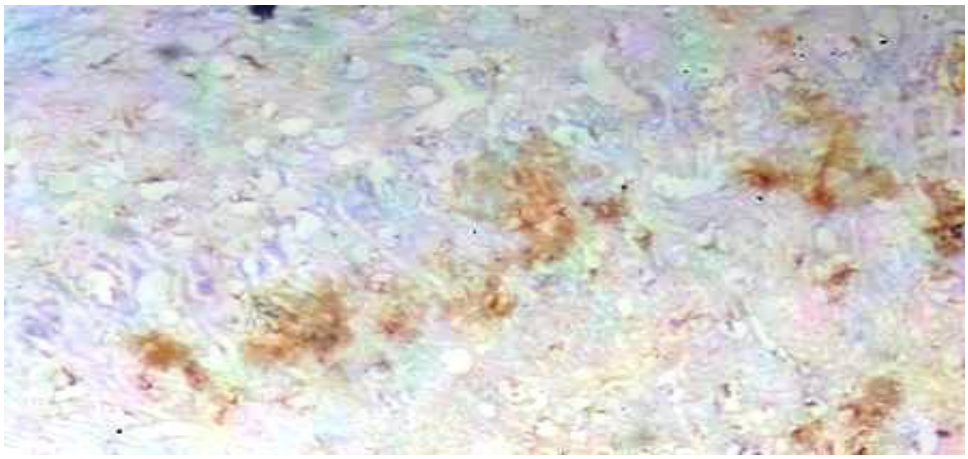

PLATE 2A: Hippocampal Brain (Control) – Mild expression of glial fibrillary acidic protein (GFAP) on the astrocytes, (x 400).

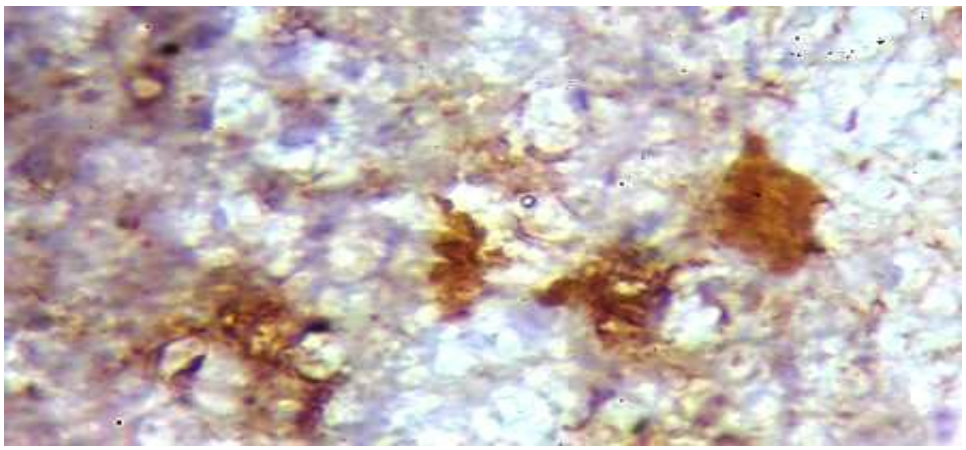

PLATE 2B: Hippocamal Brain (DMA 10 mg/kg) –Strong expression of glial fibrillary acidic protein (GFAP) on the few astrocytes; most astrocytes are degenerated (x 400).

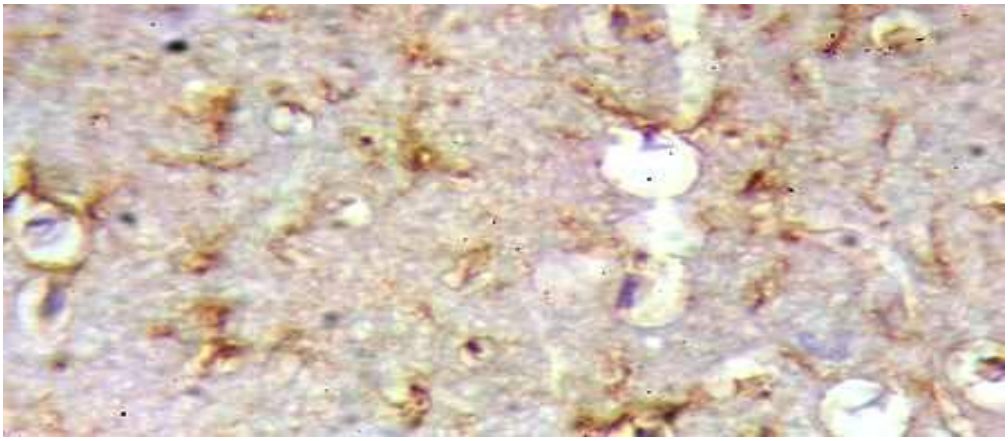

PLATE 2C: Hippocampal Brain (50mg/kg DOLME+10mg/kg DMA) - Moderate expression of glial fibrillary acidic protein (GFAP) on the astrocytes.

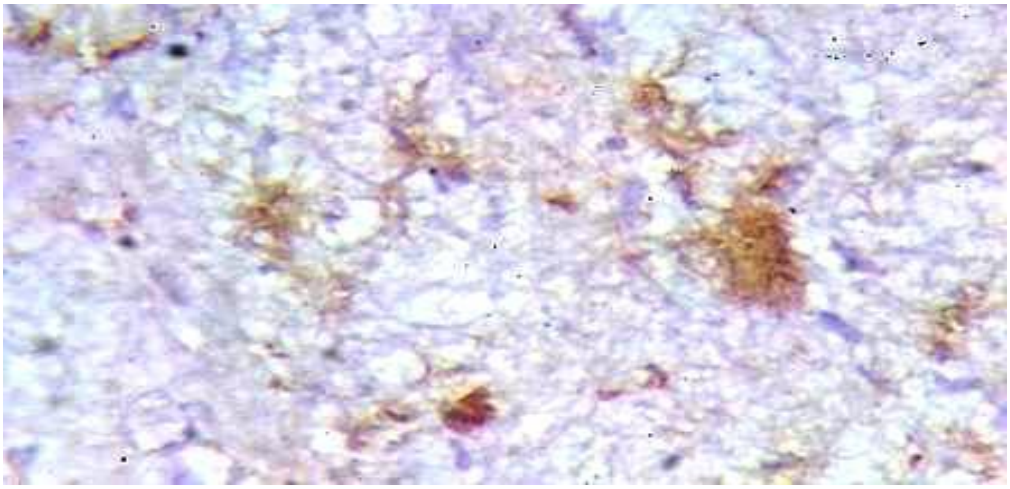

PLATE 2D: Hippocampal Brain (150mg/kg DOLME+10mg/kg DMA) - mild expression of glial fibrillary acidic protein (GFAP) on the astrocytes 9x 400).

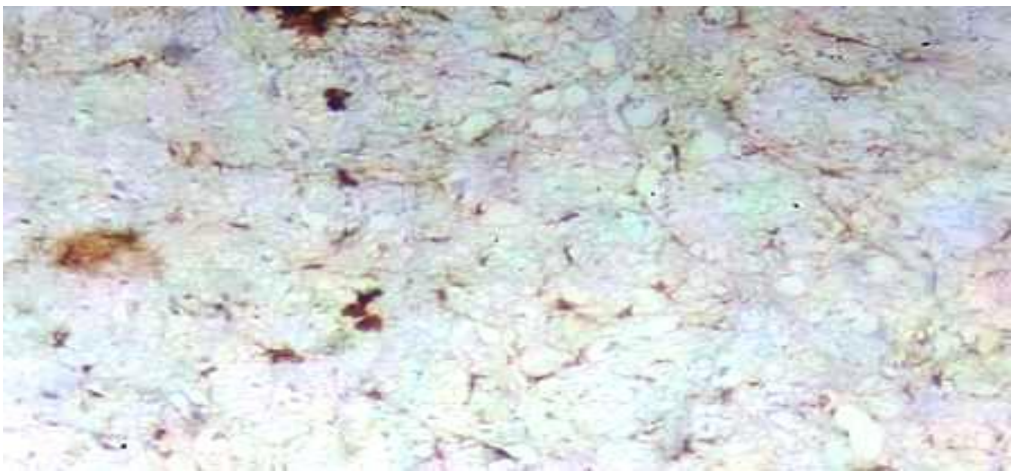

PLATE 2E: Hippocampal Brain (250mg/kg DOLME+10mg/kg DMA) - Mild expression of glial fibrillary acidic protein (GFAP) on the astrocytes (x 400).

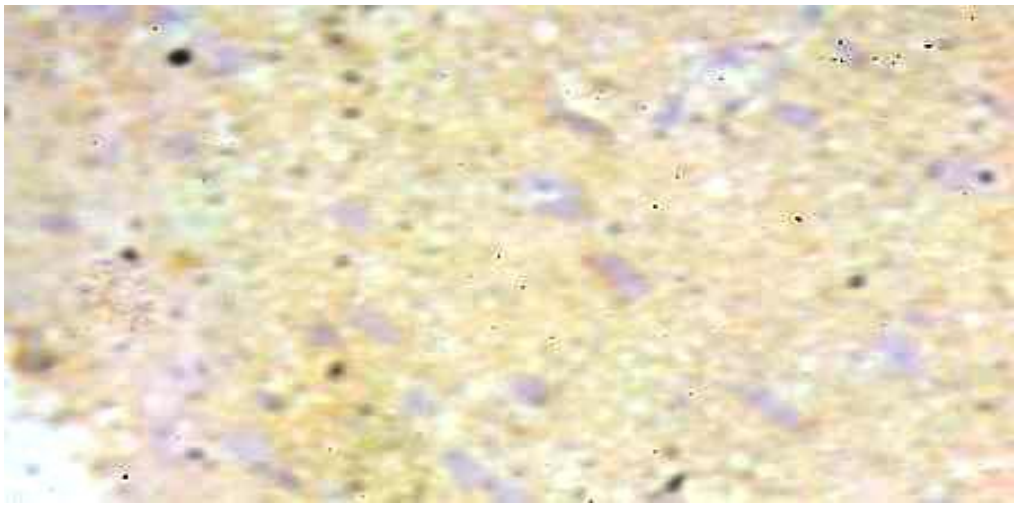

PLATE 3A: Hippocampal brain (Control) –Very mild expression of synaptophysinon the neurons (x 400).

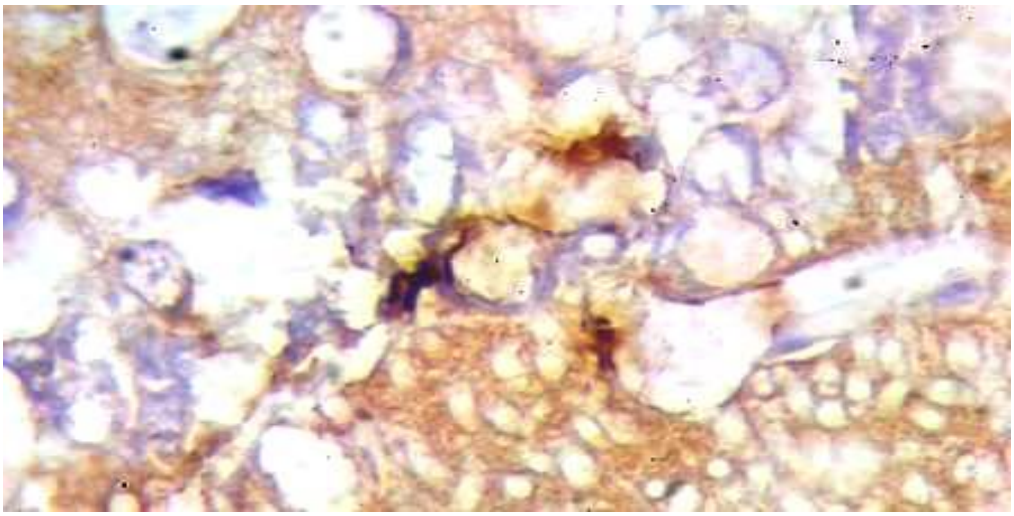

PLATE 3B: Hippocampal brain (10 mg/kg DMA)–Moderately high expression of Synaptophysin on the neurons (x 400).

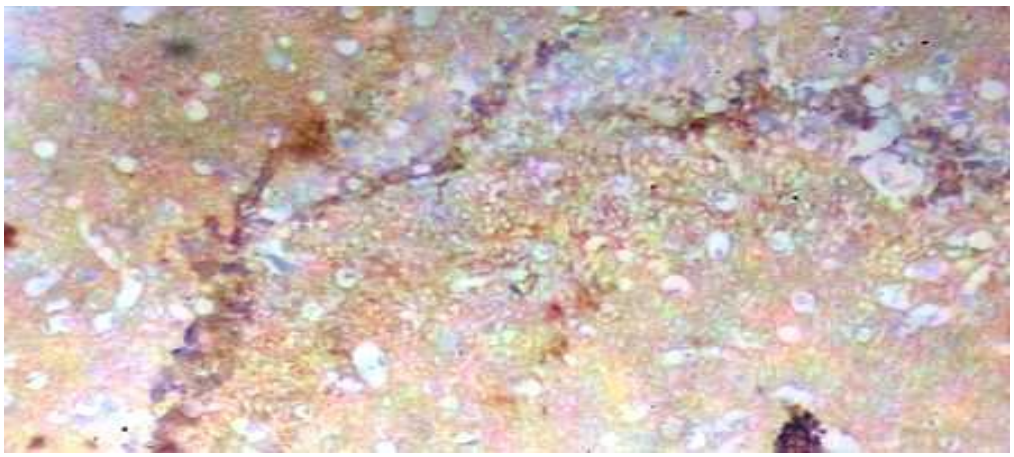

PLATE 3C: Hippocampal brain (50mg/kg DOLME+10mg/kg DMA) - Mild expression of synaptophysinon the neurons (x 400).

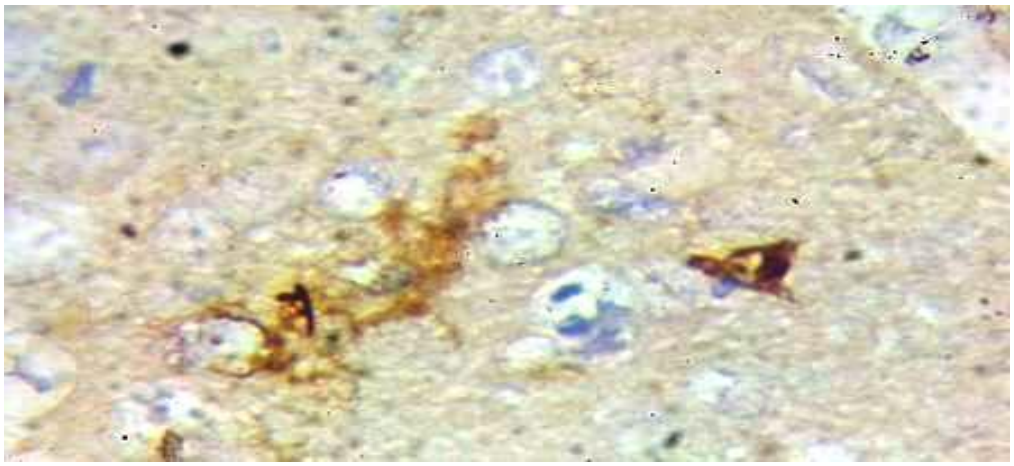

PLATE 3D: Hippocampal brain (150mg/kg DOLME+10mg/kg DMA) - Mild expression of synaptophysinon the neurons (x 400).

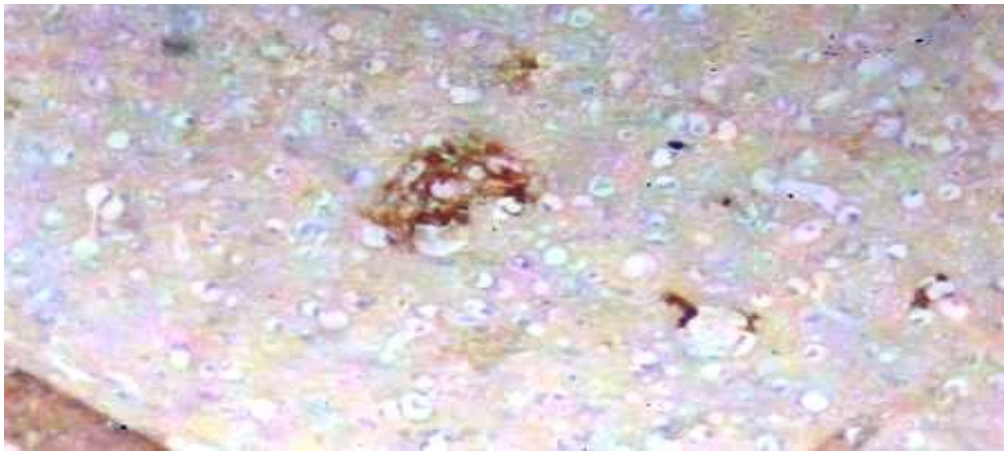

PLATE 3E: Hippocampal brain (250mg/kg DOLME+10mg/kg DMA) -Mild expression of synaptophysinonneurons (x 400).

4.0 DISCUSSION

This study investigated the potential of *Daniellia oliveri* leaf methanol extract (DOLME) on the toxic effect of Dimethylamine, a well-reported environmental toxicant, in the brain of experimental rat models. We examined the effects of the treatments on hematology, antioxidant status, histology and expressions of glial fibrillary acidic protein (GFAP) and synaptophysin in the rats brains. Dimethylamine elevated the levels of RBC and hematocrit, which were otherwise reduced by DOLME. The hemoglobin level was not appreciably altered by treatments with DMA and DOLME. The WBC, monocytes and platelets levels were increased by DMA, whereas DOLME reversed the effects of DMA treatment. Gao *et al*. (2021) reported that DMA could induce platelet activation. Ahmad *et al*. (2011) reported that *N*'- Nitrosodimethylamine (NDMA), a nitroso metabolite of DMA, increased the levels of total leukocytes (TLC), neutrophils, hemoglobin and hemolyzed RBC, and reduced lymphocytes in brain of albino rats.

Superoxide dismutase, catalase, total protein, glutathione-S-transferase, reduced glutathioneand glutathione peroxidase are key components involved in detoxifying reactive oxygen species (ROS) and maintaining cellular redox balance (Jena *et al*., 2023).The levels of protein in the brain tissue of rats were not observed to be remarkably affected in all the rats groups. However, Dimethylamine significantly reduced the activities of both superoxide dismutase and catalase

enzymes in the brain tissue comparable to control rats. The DOLME lowered the SOD, while catalase activity was elevated at high doses, mitigating DMA exposure. A study by Sulaimon *et al*. (2020) showed the potential of *D. oliveri* stem bark to increase the hepatic levels of SOD, catalase, GSH and GPx in Wistar rats treated with Carbon tetrachloride (CCl-4). Another recent study carried out by Sofidiya *et al*. (2023) revealed that D. oliveri stem bark elevated the levels of SOD, catalase, tumor necrosis factor- alpha and interleukin- 6 in mice exposed to xylene and carrageean. In this study, the activity of GST in brain was increased by DMA, but DOLME was able to reverse the effect at high doses of the extract. This study has indicated that DMA could antioxidant imbalance in the rat brain, which DOMLE upon could exert reversal. The levels of GSH and GPx were no significantly changed by DMA. *D. Oliveri* has been reported to be rich in phytochemicals like, flavonoids, alkaloids, tannins, and essential oils that possess antioxidant, anti-inflammatory, neuroprotective and antimicrobial potentials (Yaya *et al*., 2022). Olaleye *et al*. (2020) investigated the presence of high contents of phenolics, proanthocyanidins and flavonoids in *D. oliveri*, and concluded that these compounds could possibly be the underlying factors for the antioxidant potentials of the plant.

Histology of the hippocampal brain of the experimental rats showed normal neurons, capillaries and stroma in the control rats, whereas in the DMA group, there were degenerated neurons with pale appearance. The DOLME group was found with normal neuronal cells, capillaries and stroma, comparable to controls. Adeleke et al. (2017) documented that DMA treatment resulted in periportal cell infiltration in hepatocytes of a rat model.

Glial fibrillary acidic protein(GFAP) is a key intermediate filament (IF) protein associated with the maintenance of the cytoskeleton structure of glial cells, and supports the neighboring neurons and blood brain barrier (BBB) (Eng *et al*., 2000). This protein is encoded by a single gene located on chromosome 17q21 in human, and is uniquely present in the astrocytes of Central Nervous System (CNS), Schwann cells of Peripheral Nervous System (PNS) and glial cells of the enteric tissue (Yang and Wang, 2015). Four different isoforms of GFAP have been identified as Alpha (GFAP-α), Beta (GFAP-β), Delta (GFAP-δ/ε) and Kappa (GFAP-κ) isoforms. Mutations of GFAP have been linked to development of astrocytic inclusions, known as Rosenthal fibers, associated with Alexander Disease in brain (Hegemann *et al*., 2006). Messing *et al*. (2012) documented that Rosenthal fiber formation could be due to excessive accumulation of GFAP aggregates which are toxic to the astrocytes, causing degeneration of astroglia (astrocytes) and white matter in Alexander Disease. Through Immunohistochemical study, we observed that few astrocytes were present with strong expression of GFAP, and most of the astrocytes were degenerated in the hippocampus of brain of rats exposed to DMA alone. However, the brains of rats exposed to both DMA and DOLME showed normal astrocytes with mild to moderate expressions of GFAP. The strong expression of GFAP and astrocytic degeneration observed in the DMA group is an indication of neurodegenerative effect of this compound. This observed pathologic effect of DMA in the brain of the experimental rats could be attenuated by DOLME treatment. Choi *et al*. (2009) reported an enhanced expression of GFAP-δ/ε in human astrocytic tumor. An exposure to dimethylamine can lead to oxidative stress (Noushad *et al*., 2019), neurodegenerative and cognitive problems (Yang *et al*., 2020). Another study by Yuan *et al*. (2021) has also demonstrated the potential of DMA and metabolites to cause mutagenesis, genotoxicity and carcinogenesis.

We also examined the Immunohistochemical staining of hippocampal brains of the rats and detected that DMA alone caused moderately high expression of synaptophysin on the neurons, while the brains of controls, and those a co-treated with DMA and DOLME showed mild expression of the protein. Synaptophysin is an integral membrane glycoprotein occurring in the presynaptic vesicles of neurons for trafficking and targeting of Synaptobrevin II (sybII) to the synaptic

vesicles (SVs) during endocytosis. Synaptobrevin II (sybII) is a vesicular soluble NSF attachment protein receptor (SNARE) required for release of neurotransmitter. Gordon *et al*. (2011, 2016) documented that in synaptophysin knockout (null) neurons, endogenous sybII was diffusely distributed from nerve ends along the axon, indicating that synaptophysin is crucial to sybII targeting in neuronal vesicles. Alterations in expression or function of this protein could therefore adversely affect neuronal transmission and synaptic activity. Synaptophysin has been reported to be expressed independently of other neuronal differentiation markers; hence it could serve as a differentiation marker in neoplasms and neuroendocrine tumor (Wiedenmann *et al*., 1986). Furthermore, Quinn (1998) reported presence of synaptophysin–positive neurons and irregular white-matter synaptophysin in neocortical human brain, whereas synaptophysin-positive neurons were distributed broadly in normal brain. The moderately high expression of synaptophysin caused by DMA on the neuronal cells in hippocampus is an indication of neurotoxic effect of the compound at higher doses. Yu *et al*. (2022) reported a case study of a previously healthy operator at a Pharmaceutical industry, who accidentally splash his trousers with Dimethylamine borane, and developed blurred vision, nausea, dizziness, slow movement and general body weakness, three hours after the accident. Interestingly, the methanol extract of *Daniellia oliveri* leaves has shown potential to attenuate the DMA- induced neurotoxicity.

CONCLUSION

This study has demonstrated that Dimethylamine could exert some hematological effects, and induce brain damage via oxidative stress and neuronal degeneration, which *Daniellia oliveri* leaf methanol extract potentially reversed in the experimental rat model.

ACKNOWLEDGEMENT

We wish to acknowledge Mr Okedere Babajide Adedapo (Immunohistochemistry Laboratory, Genetic and bioethics Research Unit, College of Medicine, University of Ibadan, Oyo State, Nigeria) for the histological and Immunohistochemical studies.

Conflict of Interest: There was no conflict of interest between the authors.

Funding statement: The research was fully funded by the authors.

REFERENCES

1. Souza S. and Passos, M., Ethnobotanical use of Moringa Citrifolia: a Literature Review. Research Society and Development, 2021; 15(10): e352101523280.
2. Okunade S.A. and Olafadehan O. A., Rolfe (DanielliaOliveri) Seed meal as a protein source in locally produced Concentrates for lambs fed low quality basal Diet. Journal of the Saudi Society of Agricultural Sciences, 2019; 1(18): 83-88.
3. Agha H.M., Sidik N.J., Jawad A.H., Mohammed A.A., and Alkamil, A.A., Overview of ThaumatococcusDaniellii Plant, History, Uses, Benefits, and Characterization. Journal of Asian Scientific Research, 2022; 12(2): 80-90.
4. Yaya S.T., Claude M.J., Sirabana C. and Flavien T., Antipyretic Activity of Aqueous Extract of Danielliaoliveri Leaves (Rolfe, Hutch EtDalz) (Fabaceae). Scholars academic journal of pharmacy, 2022; 11(11): 193-199.
5. Badalyan S. and Rapior S., Perspectives of Biomedical Application of Macrofungi. Current Trends in Biomedical Engineering & Biosciences, 2021; 4(2): 403-408.

6. Sabri S., Takruri H., Ismail K., Nutrient Composition, antioxidant activities and anti-inflammatory Effect Of Jujube Fruit. Journal of Pharmacy and Nutrition Sciences, 2021; 11: 164-174.
7. Harris, N., Murphy, A., Green, A., Gámez, S., Nunez-Mir, G., Socio-ecological Gap Analysis To Forecast Species Range Contractions For Conservation. Proceedings of the National Academy of Sciences, 2023; 7(120): e2201942119.
8. Coker, M.E., Fasola, O.S., & Oaikhena, A.O., Antimicrobial Evaluation of Extracts and Fractions of Daniellaoliveri Leaf on Selected Clinical Enteropathogens. Nigerian Journal of Pharmaceutical Research, 2021; S(1): 61-71.
9. Sgroi L C., Lovino M A., Berbery E H., and Muller G V., Chartacteristics of drought in Argentina core crop region, Hydrology and Earth system science, 2021; 25(5): 2475-2490.
10. Traore M., Coulibaly A C., Yaro A., Kabre EWLM, Phenolic content, antioxidant and 15-lypoxygenase inhibitory effects Daniellia oliveri (Rolfe) Hutch and Dalziel (fabaceae). Internal journal of biochemistry research and review, 2021; 30(6): 10-18.
11. Atolani O., and Olatunji G A., Isolation and evaluation of antiglycation potential of polyalthric acid (Furanoterpene) from Daniellia oliveri. Journal of pharmacentic analyst, 2014; 4: 407-411.
12. Atolani O., Olatunji G A., Chemical composition, antioxidant and cytotoxicity potential of Daniellia oliveri (Rolfe) Hutch and Dalziel, Turkish J. of pharmacentical sciences, 2016; 13: 41-46.
13. Nacoulmna A., Campaore M., Meda N., Pottier L., Megalizzi V., Some 1 and Kiendrebeogo M., Antimelanogenesis effect of Daniellic acid isolated from Daniellia oliveri (Rolfe) Hutch and Dalziel (leguminosae) olecresin of Bartina. Open journal of medicinal chemistr, 2021; 11: 41-57.
14. Bugata L S P., Pitta V P., Gundu A R., Mohammed F R., Reddy U A., Kumar J M., and Mahboob M., Acute and subacute oral toxicity of copper oxide nanoparticles in female albino wistar rats. Journal of applied toxicology, 2019; 39(5): 702-716.
15. Igbal M. R., Bovine mastitis in fiji. Economic implications and management. Journal of Agricultural sci., 2021; 13(10): 162.
16. Turkesky RJ, Rossi SC, Welti DH, Lay JO Jnr., Kadlubar FF. Characterization of DNA-adducts formed in vivo by reaction *N*hydroxyl- 2- amino– 3 – methylimidazo (4,5, - f) quinoline and *N* – hydroxyl – 2 – amino – 3,8 – dimethylimidazo (4,5 – f) quinoxaline at the C8 and *N*2 atoms of guanine. Chem. Res. Toxicol, 1992; 5: 479–490.
17. Herman A.J. and Synderwine E.G., DNA adducts of heterocyclic amine food mutagens: Implications for mutagenesis and carcinogenesis. Carcinogenesis, 1999; 20(3): 353 – 368.
18. Adeleke G. E., Adedosu O.T., Afolabi O. K., Arinde O.O and Oyedokun T.M., Methanolic Leaf Extract of *Diospyros chloroxylon* Modulates Hepatic Redox Profile and Cell proliferation in Dimethylamine-treated rats. British Journal of Medicine & Medical Research, 2017; 21(12): 1-12.
19. Lowry OH, Rosbrough NJ, Farr AL, et al. Protein measurement with the Folin- phenol reagent. *J. Biol. Chem*., 1951; 193: 265-275.
20. Mistra HP, Fridovich I. The generation of superoxide radical during the autoxidation of ferredoxin. *J. Biol. Chem*., 1979; 246: 6886-6890.
21. Aebi H., Catalase in-vitro. In: Packer L. Editor. Methods in Enzymology. Academic Press, 1984; 121-126.
22. Habig W., Pabst M. and Jakoby W., Glutathione S-transferase. The first enzymatic step in mercapturic acid formation. J Biol Chem, 1974; 249: 7130–7139.

23. Mitchell J.R., Jollow D.J., Potter W.Z., Davis D.C., Gillette J.R. and Brodi B.B., Acetaminophen-induced hepatic necrosis. I. Role of drug metabolism. Jour. Pharmacol Exer. Therap, 1973; 187: 185-194.
24. Paglia, D.E. & Valentine, W.N., Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab Clin. Med*., 1967; 70: 158-169.
25. Gao Y, Zhang J., Chen H, Wang Z., Hou J., Wang L., Dimethylamine enhances platelet hyperactivity in chronic kidney disease model. LJ. Bioenerg. Biomembr, 2021; 53(5): 585- 595.
26. Ahmad A., Fatima R., Maheshwari V., Ahmad R., Effect of NDMA on red blood cell reheology and proteomic profiles of brain in male albino rats. Interdiscip. Toxicolol, 2011; 4(3): 125-131.
27. Jena K., Ananta S., and Akthar J., Environmental technology and innovation, 2023; 31(4617): 103200.
28. Jena A.B., Samal R., Bhol N.K. and Duttaroy A.K., Cellular Red-Ox system in health and disease: The latest update. Biomedicine and pharmacotherapy, 2023; 162: 114606.
29. Sulaimon L A., Obuotor E M., Rabiu L A., Sheu A M., Aliyu m., Shiro M Q., Antioxidant and hepatoprotective potentials of ethanol stem bark extract of Daniellia oliveri (rolfe) Hutch and Dalz (Caesalpiniaceae). Synergy, 2020; 11: 100067.
30. Sofidiya M O., Ikechukwu J U., Nnah V E., Olaleye O O., Basheeru K., Sowemimo A A., Ajayi A M., Anti-inflammatory and antinociceptive activities of Daniellia oliveri (Fabaceae) stem bark extract. Journal of ethnopharmacology, 2023; 309: 116337
31. Olaleye O.O., Sofidiya O. M., Ogba O. J., Lasore W., Antioxidant activities of Daniellia oliveri (Rolfe) Hutch. and Dalziel and Daniellia ogea (Harms) Rolfe ex Holland (Caesalpiniaceae): Tropical Journal of Natural Product Research (TJNPR), 2020; 4(5): 210 – 215.
32. Eng L F et al., Glial fibrillari acidic protein, GFAP-thirty one years (1969-2000) Neurochem. Res., 2000; 25: 1439-1451.
33. Yang Z. and Wang K.K.W., Glial fibrillari acidic protein, from intermediate filament assembly and gliosis to neurobiomarker trends. Neuroscience, 2015; 38(6): 364-374.
34. Hagemann T L et al., Alexander disease-associated glial fibrillari acidic protein mutations in mice induce resenthal fiber formation and a white matter stress response. Journal of nanosci, 2006; 2b: 11162-11173.
35. Messing A et al., Alexander diseases. Journal of neuroscience, 2012; 32: 2017-5023.
36. Choi K C et al., Enhanced glial fibrillary acidic protein-delta expression in human astrocytic tumor. Neuroscience letters, 2009; 463: 182-187.
37. Noushad S., Sajid U., Ahmed S., Saleem Y., Oxidative stress mediated neurodegeneration. Int. J. health sci. res., 2019; 2310-3841.
38. Yang Y., Wang L., Li X., Yu S., Wu Y., and Zhang Y., Neurotoxic effects of dimethylamine on zebrafish (Danio rerio) behavior, neurotransmitter and oxidative stress. Ecotoxicology and Environmental safety, 2020; 192: 110318.
39. Yuan C., Huang Y., Shu M., and Dai M., Genotoxicity of dimethylamine and its role in human health risk assessment. Environmental toxicology and pharmacology, 2021; 84: 103595.
40. Gordon S.L, Leube R.E., Cousin M.A., Synaptophysin is required for synaptobrecin in retrieval during synaptic vesicle endocytosis. J. Neurosci, 2011; 31: 14032 -14036.
41. Gordon S.L., Harper C.B, Smillie K.J, Cousin M.A., A fine balance of synaptophysin levels underlies efficient retrieval of Synaptobrevin II to synaptic vesicles. PLoS ONE, 2016; 11(2): e0149457.

42. Wiedenmann B., Frank W.W., Kuhn C., Moll R., Gould V E., Synaptophysin, a marker protein for neuroendocrine cells and neoplasms proc Natl Acad sci. USA, 1986; 83(10): 3500-3504.
43. Quinn B., Synaptophysin staining in normal brain: importance for diagnosis of ganglioglima. Am. J. Surg. Pathol, 1998; 22(5): 550 -556.
44. Yu G., Li Y., Jian T., Kan B., Cui S., Han P., Jian X., Neurotoxicity due todimethylamine borane poisoning via skin absorption. A case study, world J. emerg. Med, 2022; 13(6): 500-503.