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Mimosa pudica Ameliorated Dichlorvos Induced Neuro-oxidation

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Abstract

There has been a drastic widespread use and abuse of dichlorvos (DDVP) with increasing accidental and intentional exposure. Hence, the need for a local therapeutic remedy before medical intervention. This study aimed to show the ameliorative properties of *Mimosa pudica* (MP) on DDVP induced neurotoxicity. 50 adult BALB/c mice were randomly divided into 5 groups of 10 mice each. Mice were given normal feed or poisoned feed (2.5% of dichlorvos in feed) with or without MP extract treatment. The exposure lasted for 28 days and all administration was done orally. At the end of exposure, mice were euthanized using chloroform and perfused transcardially using 1X PBS followed by 10% formal saline for animals designated for histochemical evaluations. Exposure to DDVP reduces granular cell layer thickness, altered Nissl substance distribution, elevates expression of GFAP and CD68 compared to control, co-administration with MP extract did not considerably reversed these effects unlike DDVP+MP that showed similar observation to control mice. GR was significantly high in all groups than the control except DDVP+MP and the level of SOD was significantly reduced in DDVP mice compared to other groups. DDVP induced hippocampal alterations accompanied by oxidative stress reversed by subsequent treatment with MP extract.

Keywords: *Mimosa pudica*, Oxidative Stress, Hippocampus, DDVP

1. Introduction

Report from the World Health Organization showed that about 193,460 people died in 2012 due to unintentional poisoning where low- and middle-income countries account for 84% of these deaths. It is further stated that the use of pesticides accounts for the highest number of deaths by suicide every year which can be reduced by limiting access to poisonous pesticides.

Dichlorvos (DDVP) a type of organophosphate (OP) compound widely used in pest control for farm produce[1]. OP compounds use has been reported in recent times to be used widely in the control of household pests and preservation of agricultural produce[2,3]. OPs also include nerve gases[4] due to the use of OPs by terrorists, and some high profile assassinations[5,6] it has become a major chemical threat agent. OPs exposure inhibits the activity of anticholinesterase in the body system, this inhibition leads to the accumulation of acetylcholine at the synapse[7–10]. This accumulation leads to what is referred to as cholinergic crisis[5]. Symptoms of a cholinergic crisis include muscle fasciculation with subsequent flaccid

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paralysis, parasympathomimetic signs, respiratory failure and also centrally mediated seizures that can degenerate to status epilepticus due to its ability to cross the blood-brain barrier[6].

Apart from the canonical cholinergic effect of OPs, another mechanism of inducing neural damage by OPs has been identified. DDVP is mutagenic1, induce oxidative stress[10–13] and neuroinflammation[5]. Although major treatment for OP poisoning is the administration of cholinergic receptor blocker usually atropine. This has come with its deficiency as well[14]. Most people don't usually survive the poisoning before getting medical attention, and in the case of large-scale poisoning getting medical help to all victims may not be possible. Hence need for alternative treatment for OP poisoning.

Plants have been a good source of medicine since ancient times. It is a veritable source of medicine in low and middle-income countries[15]. *Mimosa pudica* (MP) is typically a short sensitive plant with twigs growing close to the ground and as high as about 0.5m and spreads about 0.3m. *Mimosa* has an erect stem, prickly, well-branched and slender structure. Its leaves are bipinnate, pale green in colour, fern-like with the propensity of closing when touched. They are quadri-pinnate, leaflets of between 15 to 25 pairs, usually reddish, bristly, acute, usually 1.5mm wide and 9 to 12 mm long. Extract of the plant is reported to have antimicrobial and antiprotozoal properties[15], wound healing[16] and aphrodisiac potentials[17]. Polyphenols from the plant are reported to be cytotoxic to skin cancer cells and also possess anti-dermatoheliotic properties together with anti-tyrosinase activity[18]. The plant extract is also documented to be rich in flavonoids, this which is suggested confer antioxidant properties on the plant[19]. This study is designed to assess the antidotal effect of MP on the DDVP induced neurotoxicity.

2. Material and Methods

2.1 Extract Preparation

The fresh leaves of MP were harvested during the raining season, they were identified and authenticated by botanist in Ekiti State University (EKSU) Ado-Ekiti Ekiti State Nigeria with herbarium number UHAE 2018017.

The leaves were rinsed first with distilled water and subsequently, normal saline to remove dirt and mycotoxins. The leaves were air-dried for five days under a shade and then pulverized into a fine powder using an electric blender (Solitaire electric blender (Mahariimpex model, Mumbai-India). 700 g of powdered MPwas added into 2149 ml of methanol and left for two (2) days to infiltrate. The solution was then filtered using Whatman filter paper. The filtrate was then concentrated at 35^0C in an oven to form a paste which was later air-dried to solid (MP extract). The extract was then reconstituted in water (Vehicle) for subsequent administration to the animals.

2.2 Feed Preparation

Dichlorvos (Sniper, DDVP) was purchased locally from Saro Agrosciences Limited Nigeria. 7 mL of DDVP was diluted in 300 ml of water to achieve 2.5% concentration of dichlorvos which was then mixed with 25 kg bag of animal feeds. This was labelled "**poisonedfeed**" .25% DDVP was used for poisoned feed based on its recommended exposure limit[20].

2.3 Animal Care

50 young healthy male BALB/c mice weighing between 20–30g were purchased from the animal holdings of Anatomy Department of Delta State University Abraka (DELSU), Delta State, Nigeria. They were housed in a standard mouse cage of 5 mice/cage under standard laboratory conditions of 12 hours alternating light and dark cycle.The mice were given feed and water *ad libitum*.

2.4 Animal Grouping

Mice were randomly divided into five (5) groups of ten (10) mice each. Group A (Control) have access to normal feed and water for 28 days with experimental handling alone, group B have access to normal feed and water together with MP extractorally for 28 days, group C (Toxicity Group) have access to the poisoned feed and water for 28 days, group D (Prophylactic Group) received poisoned feed with water and were administered MP extract concurrently and group E (Therapeutic Group) have access to poisoned feed with water for initial 14 days and subsequently given normal feed and water with MP extract for the remaining 14 days. MP extract was given at 400mg/kg body weight[21]. The summary of animals grouping is shown in Table 1.

2.5 Animal Sacrifice

The mice were sacrificed a day after the last day of exposure. Mice were deeply anaesthetized with chloroform in a desiccator. They were then perfused transcardially with normal saline to flush out blood. Five mice per group designated for biochemical assay have their brains dissected out and placed in a cold 10mM phosphate-buffered solution (1X PBS pH 7.4) and stored at -20^0C till further processing. Remaining 5 mice per group for histological and immunohistochemical studies were further perfused with 10% formal saline after normal saline to fix the animal. The whole brain was collected, and placed in a labelled specimen bottle containing 10% formal saline solution stored at 4^0C till further processing.

Table 1. Showing the experimental design

Groups	Dosage	Duration
A (Ctrl)	40 grammes of normal feed + water	28 days
B (MP)	400 mg/kg bw of *Mimosa pudica* in 0.1 ml orally	28 days
C (DDVP)	40 grammes of 2.5% DDVP conc. in feed (Poisoned feed PF) + water	28 days
D (DDVP/MP)	40 grammes of 2.5% DDVP conc. in feed (Poisoned feed PF) + 400 mg/kg bw of *Mimosa pudica* in 0.1ml orally	28 days
E (DDVP+MP)	40 grammes of 2.5% DDVP conc. in feed (Poisoned feed PF)	14 days
	400 mg/kg bw of *Mimosa pudica* orally	14 days

2.6 Histological and Immunohistochemical Procedure

Brain sample was processed for routine histological processing for haematoxylin and eosin (H&E) and Cresyl Fast Violet (CFV) stains following the departmental protocol[22]. Brain sections for immunohistochemistry were stained for astrocyte (rabbit anti-GFAP 1:1000 PA1239 BosterBio USA) and microglia (rabbit anti-CD68 1:200 M00144-1 BosterBio USA) as reported by Ishola *et al.*[23]. The primary was later labelled with goat anti-rabbit secondary antibody (FNSA-0041 1:1000 Wuhan Fine Biotech).

2.7 Microscopy

The histological and immune histochemical slides were viewed under a light microscope with an in-built camera (Opto-Edu China). Same areas were studied in all the brain slides and captured at x40 objective lens.

2.8 Biochemical Assay

Brain samples designated for biochemical assays were homogenized in 1X PBS centrifuged at 10,000 g for 10 minutes. The supernatants were used to evaluate the level of glutathione reductase (GR), superoxide dismutase (SOD), catalase (Cat) and acetylcholinesterase (AChE).

2.9 Statistical Analysis

Continuous data were expressed as means ± SEM.Values were analyzed using GraphPad prism version 5.0. P values was set at *0.05, 0.01 and 0.001.*

3. Results

3.1 MP Extract Restores Hippocampal Histomorphological Alterations Induced by DDVP

H&E stained slides (Figure 1) showed no histological alterations in the hippocampus of control and mice exposed to MP extract (group A and B) where as cellular distortions with reduced Granular Cell Layer (GCL) thickness was observed in the hippocampus of mice exposed to DDVP through feed (group C). Simultaneous administration of MP extract with DDVP still showed pockets of histological alterations in the hippocampus which is reduced than group C mice, while mice treated with MP extract after DDVP exposure showed no mark histological alterations in the hippocampus.

Hippocampal slides stained with CFV to show Nissl substances (Figure 2) was done. Group A and B mice showed the distribution of Nissl substance round the nucleus and the axonal hillock of the neurons. Mice exposed to DDVP feed showed reduced Nissl substance appearance with pockets of cellular degeneration. Co-administration of DDVP and MP extract did not fully ameliorate DDVP damage has there was the presence of mild distortions in Nissl substance distribution while administration of MP extract after DDVP exposure ameliorate DDVP damage by restoring Nissl substance distribution around the nucleus and axonal hillock.

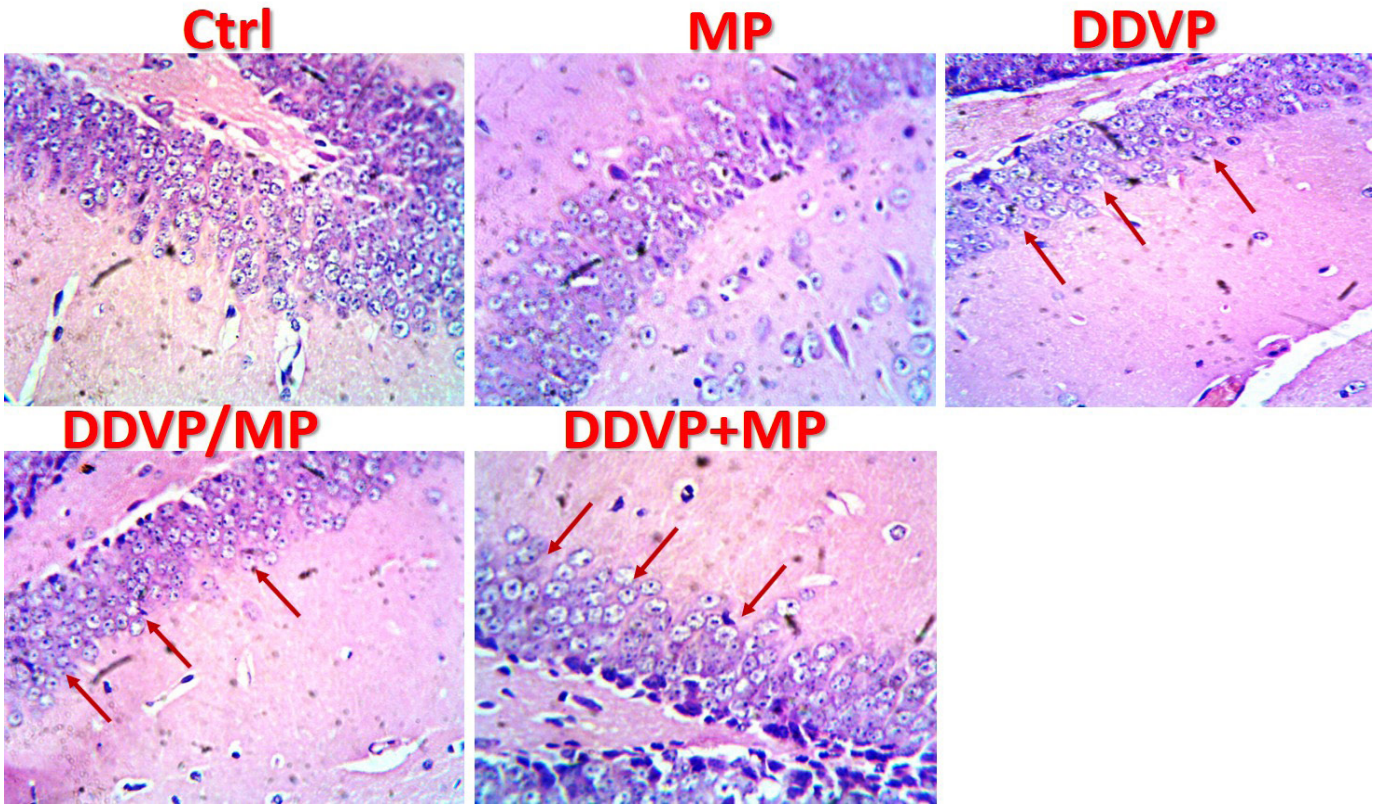


Figure 1. Representative histological slide of the hippocampus of experimental animals stained with H&E at x40 objective. Exposure to DDVP altered hippocampal histology by reducing the thickness of GCL of the hippocampus which was ameliorated by subsequent MP extract treatment but not simultaneous MP extract treatment (arrow heads).

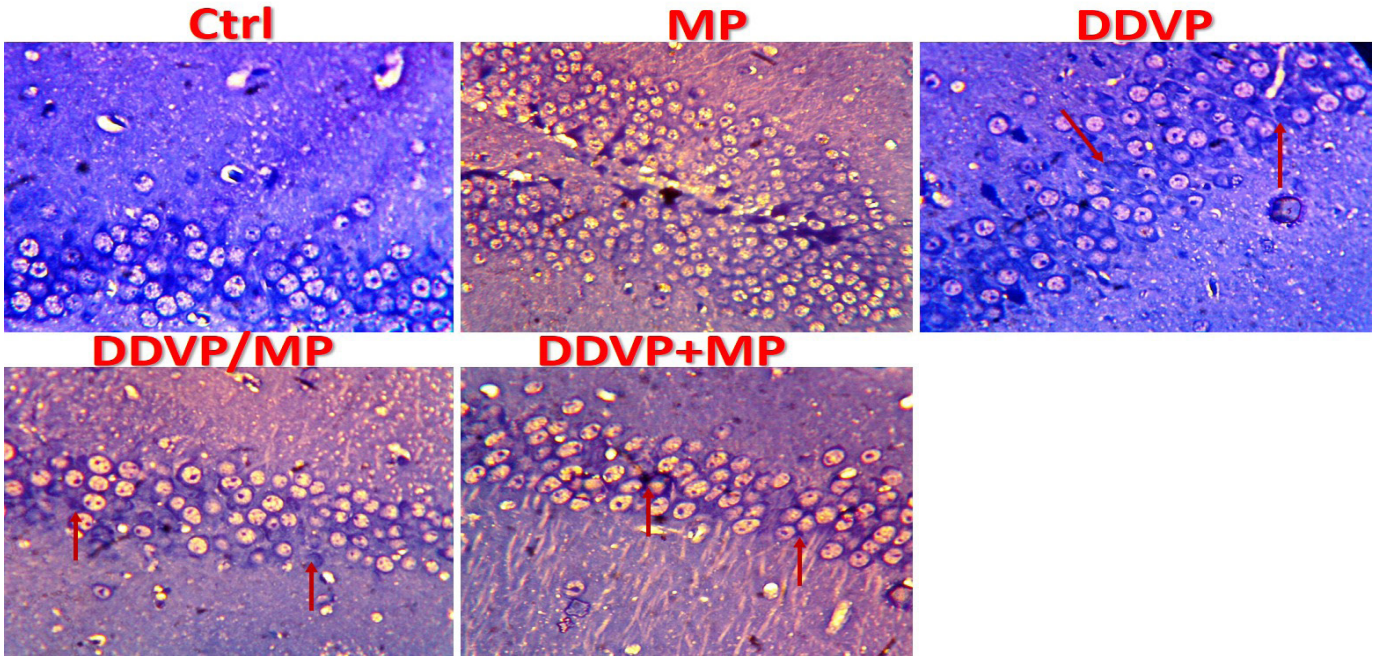


Figure 2. Representative histological slide of the hippocampus of experimental animals stained with CFV at x40 objective. Exposure to DDVP altered hippocampal Nissl substance distribution ameliorated by subsequent MP extract treatment but not simultaneous MP extract treatment (arrow heads).

3.2 MP Extract Treatment Ameliorates DDVP Induced Hippocampal Neuroinflammation

Neuroinflammation was assessed by checking for astrocytes and microglial expression using rabbit anti-GFAP and CD68 respectively. Expression of CD68 (microglia activity) (Figure 3) was similar between the control mice and MP extract-treated only mice. Mice exposed to DDVP feed showed high expression of CD68 than control, concomitant MP extract treatment with DDVP showed high CD68 expression than control though still reduced than DDVP mice while mice treated with MP extract after DDVP exposure showed similar CD68 expression to the control and MP treated only mice but reduced expression than DDVP mice. Observation from GFAP (astrocyte) expression (Figure 4) followed similar pattern as CD68 expression.

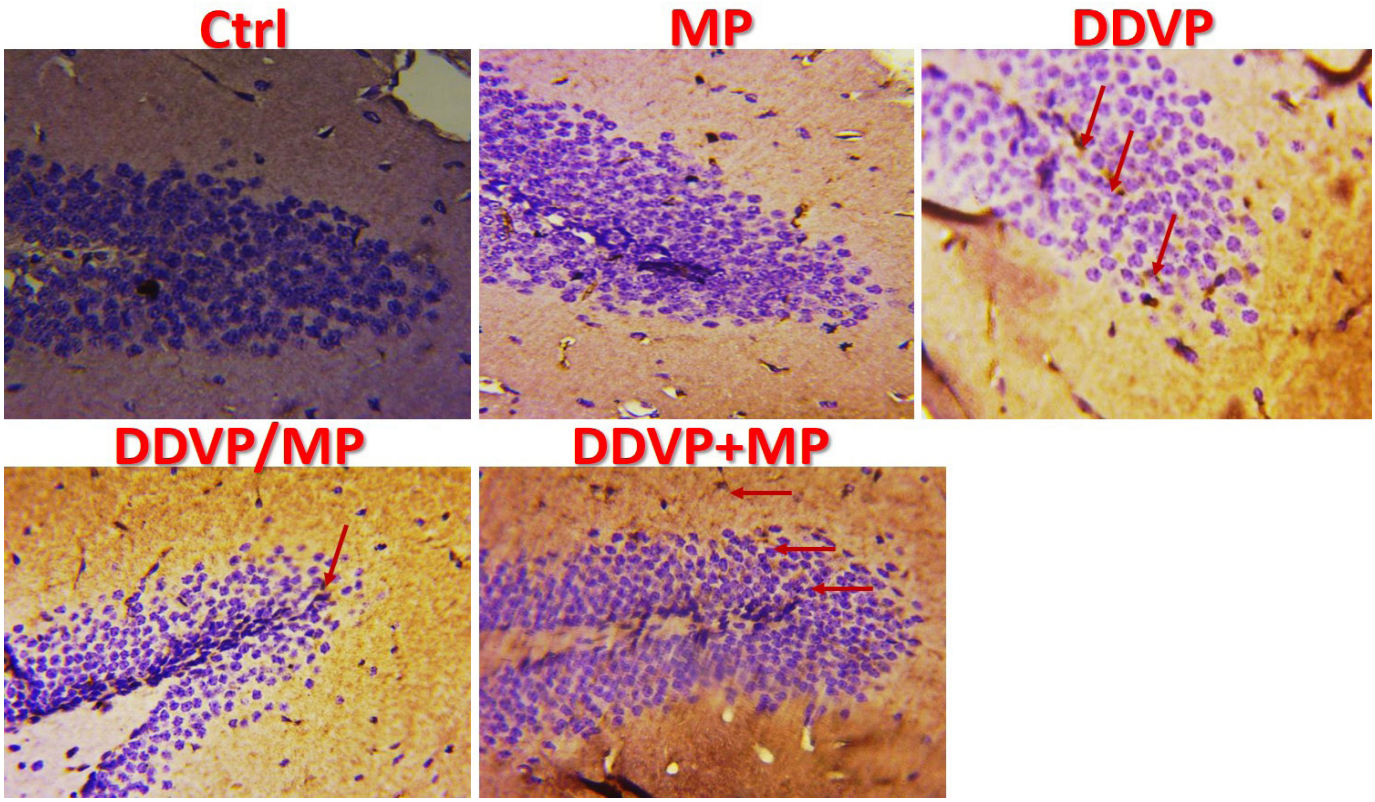


Figure 3. Representative immunohistochemical slide of the hippocampus of experimental animals showing CD68 (microglia) expression at x40 objective. Exposure to DDVP enhances CD68 expression than control while subsequent MP extract treatment after DDVP exposure reversed this effect.

3.3 MP Extract Reverses Oxidative Stress Induced by DDVP Exposure

Some oxidative stress parameters were evaluated to see their changes in DDVP exposure and the role of MP extract treatment. Glutathione Reductase (GR) an enzyme that plays crucial role in rummaging free radicals. The level of GR in the brain (Figure 5) was significantly *(**p<0.01, ***p<0.001)* upregulated in mice exposed to MP extract only, DDVP only and simultaneous DDVP and MP extract treatment. Subsequent MP extract treatment after DDVP exposure downregulate GR level similar to the level of control.

Superoxide Dismutase (SOD) another enzyme implicated in oxidative stress was also assessed. Brain level of SOD in the experimental mice (Figure 6) showed that MP extract only treated mice showed steady rise in the level of SOD compared to other groups. No significant difference was observed in the level of SOD across all groups.

Catalase which is responsible for converting hydrogen peroxide to water was also evaluated. DDVP exposure significantly (***p<0.01*) down regulates the level of catalase compared to control and other groups. No significant difference was observed across the rest of the group (Figure 7).

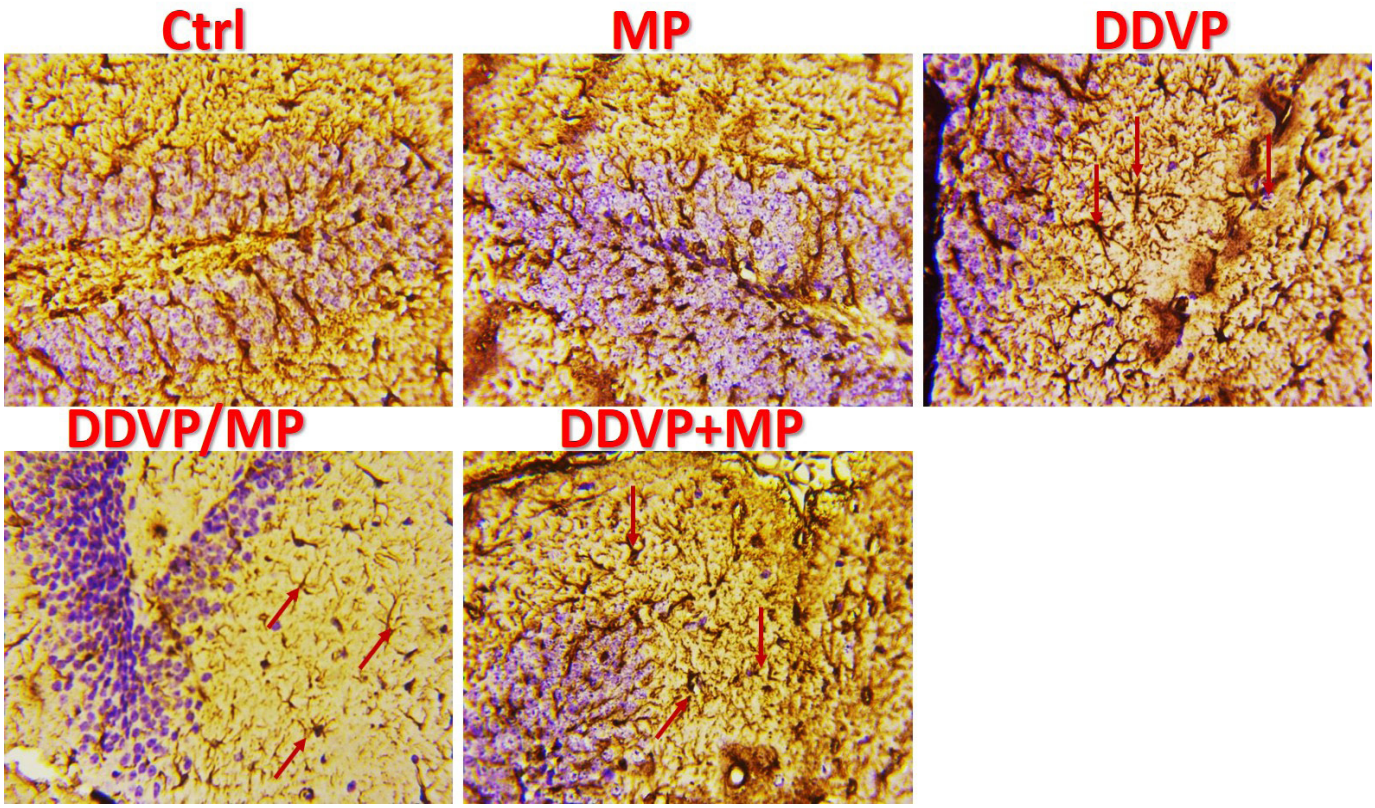


Figure 4. Representative immunohistochemical slide of the hippocampus of experimental animals showing GFAP (astrocyte) expression at x40 objective. Exposure to DDVP elevates GFAP expression than control while subsequent MP extract treatment after DDVP exposure ameliorate this effect.

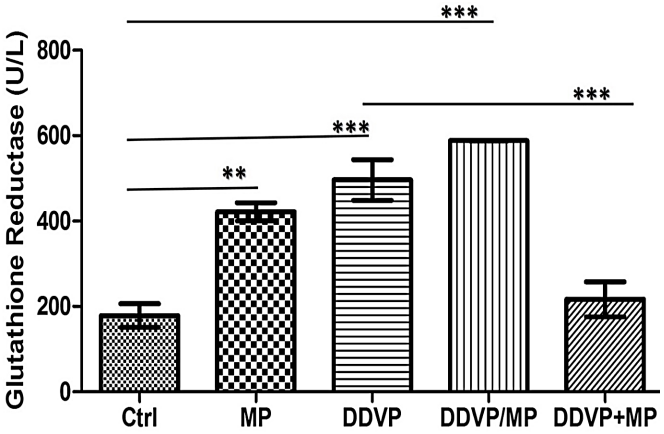


Figure 5. Graphical representation of glutathione reductase level in the brain of experimental animals. The level of brain glutathione reductase was significantly (***p<0.01, ***p<0.001*) upregulated in MP, DDVP and DDVP/MP compared to control but not DDVP+MP which is similar to control mice.

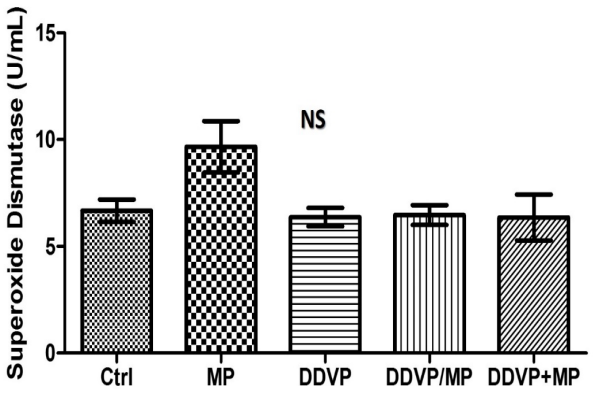


Figure 6. Graphical representation of the SOD level in the brain of experimental animals. No significant difference was observed in the level of SOD.

3.4 MP Treatment did not Alter the Level of Acetylcholinesterase (AChE) in the Brain

AChE an enzyme responsible for regulating acetylcholine transmission by degrading excess acetylcholine at the synapse was also checked in the brain of the experimental mice. Mice exposed to DDVP showed the highest level of AChE followed by DDVP/MP mice. MP and DDVP+MP mice showed a similar level of AChE with that of control mice. Statistical analysis showed no significant difference in the level AChE across all groups (Figure 8).

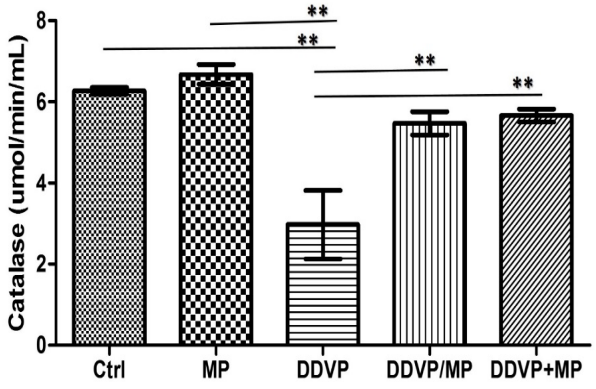


Figure 7. Graphical representation of the catalase level in the brain of experimental animals. DDVP mice showed significant (***p<0.01*) reduction compared to control and all other groups

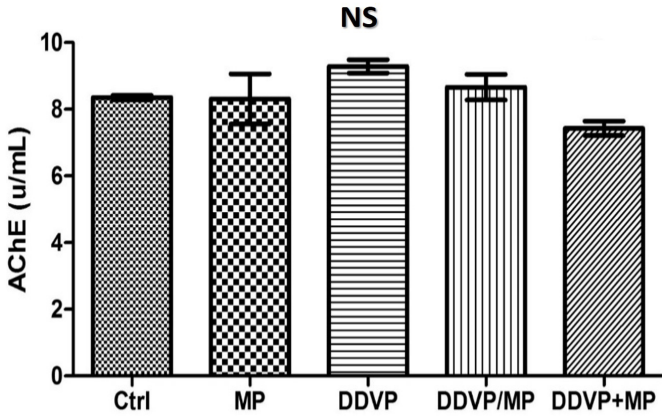


Figure 8. Graph showing AChE level in the brain of experimental animals. No significant difference was observed in the brain level of AChE in treated animals compared to control.

4. Discussion

Use of DDVP for pest control is wide spread among farmers and is getting popular in households as well for pest control. Although despite international regulations on the use of OPs, middle-and low-income countries which are investing in agriculture still uses OPs indiscriminately. Due to its household use accidental and intentional ingestion cannot be overlooked. In recent times many suicide reports have showed that DDVP is used as agent for committing suicide.

Widely treatment used in OP poisoning is administration of cholinergic blocker (e.g., atropine) which requires a medical expert for administration. Most people that suffered OP poisoning don't usually make it to the health facility to receive medical intervention. This made it imperative for a need for local intervention that can ameliorate OP poisoning before medical intervention.

We showed that exposure to DDVP through feed altered hippocampal histology with reduced granular cells in the hippocampus. This showed that DDVP is neurotoxic and can induce cell damage[13]. This observation is similar to earlier report that showed neural cell death in the hippocampus of DDVP exposed animals[2,3,24]. Similar observation was earlier reported on liver[25–27] where DDVP altered hepatocyte organisation. Lung damage has also been reported as a result of DDVP exposure[26]. Mice exposed to MP showed similar hippocampal histology with that of the control which indicates that MP extract is not neurotoxic. MP has been reported to be rich in antioxidant compounds[18,19,28]. Combined treatment with of MP extract with DDVP still showed some histological alterations though not much as that of DDVP only exposed mice. Whereas mice treated with MP extract after DDVP exposure indicates restoration of hippocampal alterations observed in DDVP only exposed mice. This observation is similar to our earlier report on liver where we showed that exposure to MP extract ameliorates liver damage induced by DDVP[25]. Previous result has showed that MP extract has healing properties[29] and it is reported to be a good source of antidote for brain poisoning[30].

Injury to the brain is usually accompanied by the activation of the glia cells[31,32]. The expression of microglia and astrocytes was more in mice exposed to DDVP feed than the control and MP extract-treated mice. This indicates that exposure to DDVP through feed induces neural insult in the mice which confirms earlier reports that DDVP is neurotoxic[3,24,33]. Co-administered mice with MP extract also showed high expression of glia cells but still lower than that of DDVP only exposed mice. Whereas, mice treated

with MP extract after DDVP exposure showed similar expression with that of the control mice. This observation indicates that although MP extract was able to reduce the effect of DDVP in the brain more ameliorative effect was seen after DDVP withdrawal. MP has been regarded as a potent antioxidant due to its rich source of phenols and flavonoids[18]. Reduced effect of cellular neuroinflammation observed and the restoration of hippocampal morphology may be attributed to the antioxidant properties of the plant. Similar observations have been recorded in vivo which showed that mimosa reduced inflammatory mediators[34].

In order to understand the mechanism of damage observed from the histochemical study, oxidative stress markers were evaluated to see the changes of these parameters. Glutathione Reductase (GR) which is an enzyme responsible for the reduction of glutathione disulfide to glutathione[35]. Glutathione which serve as important molecule to resist oxidative stress in cell[3]. The level of GR was significantly high in all treated group except DDVP+MP mice than the control mice. This means that there is a level of oxidative stress going on in the brain of the mice. Mice treated with MP extract alone still showing significant increase in the level of GR than the control which may means that MP extract induces slight oxidative stress as well, but the activity of glutathione in the cell has been shown not to be limited to oxidative stress alone but also with xenobiotics clearing[36,37]. This may explain why GR was high in MP extract-treated mice. Similar observation of increased GR with antioxidant treatment has also been recorded for quercetin (unpublished data). Subsequent treatment of MP after DDVP exposure brought the level of GR to control level indicating that MP confer its antioxidant properties on the oxidative stress induced by DDVP.

Superoxide dismutase (SOD) another enzyme necessary for breaking down superoxide into H_2O_2 and O_2 into[38]. Mice exposed to MP extract showed the highest level of SOD, though no significant difference was observed across the groups. Superoxide is generated by the mitochondria which serve as the powerhouse of the cell. No significant changes in the level of SOD indicates that the oxidative stress induced by DDVP is not through the mitochondria chain. This observation is contrary to other reports which showed that DDVP exposure alters energy metabolism of cells[11]. DDVP exposure has also been reported to increase the level of SOD[3,10,12] contrary to our observation. This disparity may be due to the mode of exposure of DDVP to the animals. While the authors administer doses of DDVP directly to the animals which is different to our own case which we added the DDVP to the feed. This addition to the feed may have reduce the severity of the DDVP effects compared to administering it directly.

Catalase an enzyme responsible for decomposing H_2O_2 into H_2O and O_2 was also evaluated. Mice exposed to DDVP showed significant reduction in the level of catalase compared to the control and other groups while no significant changes were observed between the control and other groups. This shows that DDVP reduced the activity of catalase hereby accumulating H_2O_2 in the cell. This observation is consistent with previous report that showed that DDVP exposure altered the level of catalase[3,10,13] although other reports showed that DDVP exposure doesn't change the level of catalase[12].

DDVP as with other OPs blocks the activity of acetylcholinesterase (AChE) in the body. The level of AChE was found to be high in mice exposed to DDVP which was reduced in mice co-administered with MP extract and with subsequent MP extract treatment. This is contrary to earlier reports of reduced AChE due to DDVP exposure[1,9,24]. This disparity may be due to the mode of exposure as animals will be exposed to low dose in our study as against direct dose exposure in the other study. The brain being a plastic organ which changes to suppress injury to it. Transiently blocking a receptor usually increases the distribution of the receptor in the brain[23]. This may account for the increase level of AChE observed in the DDVP exposed mice since the level of exposure is minimal compared to other reports that directly administered DDVP to the animals.

5. Conclusions

DDVP exposure through feed altered hippocampal morphology accompanied by oxidative stress which was reversed by subsequent treatment with MP extract.

6. Conflicts of Interest

Authors declare no conflict of interest regarding this work

7. Ethics Approval

Ethical approval was obtained from Delta State University ethical committee with approval number REC/FBMS/DELSU/18/35.

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