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Moringa oleifera Ameliorates Chlorpyrifos Induced Oxidative Stress in Male Wistar Rats

Onimisi B.O.[1], Zagga A.D.[2], Okolo R.U.[3], Abubakar M.S.[4], Usman Z.[1], Akor J.[5], Idris S.B[6]

[1]Lecturer, Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Usmanu Danfodiyo University, Sokoto, [2] Professor, Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Usmanu Danfodiyo University, Sokoto, [3]Associate Professor, Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Abuja, [4]Professor, Department of Veterinary Pathology Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, [5]Federal Medical Centre, BirninKebbi, Kebbi state, [6]Lecturer, Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto.

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Abstract

Background: Chlorpyrifos is a widely used organophosphate pesticide. It exerts its toxic effect by inhibiting of cholinesterases and induction of oxidative stress.

Methods: In the present study,Soya oil, Chlopyrifos,*Moringa oleifera*, Chlopyrifos + *Moringa oleifera*, were given to male rats through gavage for 2 weeks.The influence of chlorpyrifos on brain cholinesterase activity and indicators of oxidative stress malondialdehydecatalase, glutathione, superoxide dismutaseand also histopathological changes in the brain of Wistar rats was investigated compared to control group.

Results: There was significant increase in the brain malondialdehyde of the chlopyrifos group compared to the control and chlopyrifos+*Moringa oleifera* group. Also there were significant decreases in the superoxide dismutase, catalase, glutathione and acetylcholinesterase concentrations in the chlopyrifosgroup compared to the control and chlopyrifos + *Moringa oleifera* group. The rats showed significant changes in body, liver, kidneys and heart weights No significant haematological changes were seen following treatment. While some histopathological changes were detected in brain tissues in the chlopyrifos treated group, less histopathological changes were observed in chlopyrifos +*Moringa oleifera* treated groups at the end of the 2 weeks' study.

Conclusion: Overall, these results support the hypothesis that chlopyrifos causes oxidative stress and *Moringa oleifera*extract can help reduce the changes induced by chlopyrifos.

Keywords: chlopyrifos, oxidative stress, *Moringa oleifera*, relative organ weight.

Corresponding Author: Idris S.B, Lecturer,Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto.

E-mail: bankidris67@gmail.com

Tel: +2348136103222

Introduction

The use of pesticides in developing countries to improve and preserve farm yields exposes farmers and consumers alike to direct and/or indirect pesticide intoxication[1]. Although government regulatory agencies try to limit the market growth of pesticides, its use it still widespread. The most common pesticide used in developing countries ischlopyrifos (CPF)[2]. Chlorpyrifos (O, O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate) is a broad spectrumcrystalline organophosphate insecticide with agricultural and household applications[3]. Exposure to CPF could either be via respiratory, oral and dermal routes [3]. Exposure to CPF has been associated with developmental, neurological and reproductive effects [4,5,6]. CPF produces toxic effects mainly via the inhibition of acetylcholinesterase and other non-acetylcholinesterase dependent mechanisms such as induction of apoptosis, mitochondrial dysfunctionand freeradical generation in cells[7]. However, the latter is independent on the former. Increased free radical generation overwhelms the natural antioxidant defence of the body resulting in cell damage [8]. Boosting the antioxidant status via administration of extracts of plants rich in antioxidants may prevent cell damage. *Moringa oleifera* also known as drum stick or horse radish tree belongs to the plant family Moringaceae[9,10]. Mature *Moringa oleifera* leaves contain high amounts of antioxidants. [9]. Therefore, the present study explores the hematotoxic, brain histological and biochemical toxicity induced by CPF and the possible protective effects of *M. oleifera* extract.

Materials and Methods

Pesticides

Commercial grade CPF (20% EC, Termicot®, Sabero Organics, Gujarat limited, India), was bought from Main market, Sokoto. It was prepared by reconstituting in soya oil (Grand Cereals and Oil Mills Ltd., Jos, Nigeria). The pesticide mixture solutions were prepared as follows. The CPF + soya oil solution used in this work protected from light and stored at room temperature.

Plant Material, authentication and Preparation of Extract

Fresh *Moringa oleifera* leaves was bought in bulk from local Market; KofanGawo, Sokoto state Nigeria. The leaves were authenticated,and identification number obtained. Then, a specimen was kept in the herbarium of Department of Botany, Faculty of Biological Sciences, UsmanuDanfodiyo University,Sokoto where a voucher number UDUH/ANS/0225 was deposited.

The leaves were air dried in the laboratory at room temperature and grounded into powder with the help of an electrical grinder. The powder was subjected to sohxlet extraction with dehydrated alcohol. The extract obtained was filtered through Whatman filter paper and vacuum dried at 40-50°C to get a blackish green semisolid mass. From the yield obtained, 1 gram of the extract was dissolved in 10 ml of normal saline and mixed finely to make a solution. From this solution 200 mg/kg dose was calculated prior to daily use.

Experimental animals

A total of 24 adultmale adult Wistar rats were divided into 4 groups of 6 animals each: Group A: Soya oil, Group B: chlorpyrifos-treated (chlorpyrifos only), Group C: *Moringa oleifera*-treated and Group D *Moringa oleifera* + chlorpyrifos-treated Control group which received soya oil at a dose of 2 ml/kg was administered *via* gavage once per day for 2 weeks. Chlorpyrifos-treated group received chlorpyrifos dissolved in soya oil at a dose of 52.6 mg/kg once daily*via* gavage. *Moringa oleifera*-treated group was given *Moringa oleifera* dissolved in normal saline at a dose of 200 mg/kg once per day *via* gavage. *Moringa oleifera* + Chlorpyrifos-treated group received *Moringa oleifera* dissolved in normal saline (200 mg/kg) *via* gavage, then 30 minutes later, chlorpyrifos 52.6mg/kg was administered, also *via* gavage. The treatments were administered in the morning (between 07:00 and 9:00 h). On the 14th day of treatment, the rats in each group were sacrificed and dissected. The rats were euthanized with ketamine and xylazine combinations. Blood samples were collected from the heart, placed into sterile tubes, and centrifuged at 3500 rpm for 20 min to separate the serum. Blood samples were taken for the haematological tests. Brain

samples were taken to assess histological changes *via* light microscope examination. For the antioxidant enzymes and malondialdehyde concentrations, a known weight of the brain sample from each animal was homogenized in a known volume of ice-cold phosphate buffer to obtain a 10% homogenate. This was centrifuged at 3000 × g for 10 min to obtain the supernatant. The supernatant was then used to determine levels of MDA, SOD, GSH, CAT and AChE in the brain sample by measuring the absorbance of the samples in a UV spectrophotometer (Model 721A, Jeiferson Ltd, USA)

Determination of brain Malondialdehyde (MDA) and antioxidant enzyme activities

Malondialdehyde in brain tissue supernatant was separated and determined as conjugate with TBA as described by[11]. MDA reacts with TBA to form MDA -TBA complex. The absorbance was measured at 534 nm to determine the MDA concentration.

SOD activity was determined according to the method described by [12]by assaying the autooxidation and illumination of pyrogallol at 440 nm for 3 min. The SOD activity was expressed was U/mg of protein. Acetylcholinesterase activity was assayed by using the kinetic colorimetric method [13] and enzyme activity was expressed as nmol/mg protein.

CAT activity was measured according to the method described by [14]. This was measured by assaying the hydrolysis of H_2O_2 and the resulting decrease in absorbance at 240 nm over a 3 min period at 25 °C. Results were expressed as mmol/mg protein.

GPx activity was measured using H_2O_2 as substrate according to the method described by [12].

Haematological parameters

Blood samples with anti-coagulant EDTA were analyzed for hematological parameters [red blood cell (RBC) counts, hemoglobin, hematocrit, white blood cell (WBC) counts, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and platelets (PLT)] using autohematology analyser.

Histopathology

The brain harvested from the rats were fixed in Bouin Fluid and the areas of the cerebral cortex and cerebellum were dissected out. 7-10 mm semi-serial cuts slice tissues were dehydrated through ascending grades of ethanol (70%, 90% and 95% v/v), cleaned in xylene, and embedded in paraffin wax. The slides were stained with haematoxylin and eosin for light microscopic examination.

Statistics

The data were analyzedusing graphpad prism version 8. The significance of differences was calculated using one-way analysis of variance (ANOVA) followed by Tukey's procedure for multiple comparisons. $P<0.05$ was considered statistically significant

Results

The brain MDA concentration in the CPF treated group was significantly higher ($P < 0.001$) compared to those obtained in the soya oil, MOL, and CPF + MOL groups, respectively. The MDA concentration in CPF + MOL group was significantly higher compared to those from recorded in the S/oil group and MOL group ($P < 0.038$ and $P < 0.001$, respectively) (Table 1).

Table 1: Effect of exposure to soya oil (S/oil), chlorpyrifos (CPF), M. oleifera and chlopyrifos + M.oleifera on rat brain malonaldehyde, superoxide dismuthase, catalase, gluthathione and acetylcholinesterase concentration.

Oxidative Stress Markers	S/OIL	CPF	MOL	CPF + MOL
MDA conc. (nMol/mg protein)	274.7 ± 9.57	345.0 ± 13.1**	177.8 ± 14.6***	294.5 ± 9.3*
SOD conc. (U/mg protein)	32.30 ± 1.87	26.80 ± 0.95***	34.5 ± 1.29*	29.3 ± 2.5**
CAT conc. (mmol/mg protein)	171.5 ± 2.5	126.5 ± 13.3***	245.6 ± 9.67**	169.8 ± 6.5
GSH conc. (µmol/mg protein)	29.0 ± 0.8	21 ± 1.2***	33.8 ± 4.53**	25.0 ± 2.30*
AChe conc. (IU/mg protein)	4463.3 ± 190.9	4457.4 ± 106.7	4287.3 ± 22.5	4205.2 ± 70.1*

* Significant differences from the control group at $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$.

The brain SOD concentration in the CPF treated group was significantly lower (P < 0.001) compared to those obtained in the soya oil, MOL, and CPF + MOL groups, respectively. The SOD concentration in CPF + MOL group was significantly lower compared to those from recorded in the S/oil group and MOL group (P < 0.01 and P < 0.085, respectively) (Table 1).

The brain catalase concentration in the CPF treated group was significantly lower (P < 0.001) compared to those obtained in the soya oil, MOL, and CPF + MOL groups, respectively. There was no significant difference between the catalase concentration in CPF + MOL and S/oil group. However, the catalase concentration in the MOL group was significantly higher compared to those recorded in the S/oil groupand CPF + MOL groups (P < 0.01, respectively) (Table 1).

The brain gluthathione concentration in the CPF treated group was significantly lower (P < 0.001) compared to those obtained in the soya oil, MOL, and CPF + MOL groups, respectively. The GSH concentration in MOL group was significantly higher compared to those from recorded in the S/oil group (P < 0.01, respectively) (Table 1).

The brain acetylcholinesterase concentration in the CPF + MOL treated group was significantly lower (P < 0.05) compared to those obtained in the soya oil, MOL, and CPF, respectively. There was no significant difference in theAChe concentration in S/oil, MOL and CPF treated groups (Table 1).

Week one after the administration of chlorpyrifos, body weights of rats in the CPF group decreased significantly compared to those in the s/oil group(p < 0.05). While those in the MOL and MOL + CPF increased compared to their weights at week 0. By week 2, the rats in the CPF group differed significantly (p < 0.05) compared to those in the S/oil group. There was no significance between the weights of rats in S/oil, MOL and MOL + CPF groups (Table 2)

Table 2: Effects of Chlopyrifos and treatment with M. oleifera on body weights of rats administered CPFand treated with M. oleifera (Mean ± S.E., n = 6/group).

Weeks	S/OIL	CPF	MOL	CPF + MOL
0	230.9 ± 12.54	191.1 ± 6.59	164.0 ± 7.80	207.9 ± 4.53
1	256.0 ± 25.71	170.3 ± 83.71*	178.6 ± 15.32*	220.0 ± 6.91
2	258.7 ± 27.41	178.1 ± 34.18*	186.0 ± 35.70	231.0 ± 14.89

* Significant differences from the control group at p < 0.05.

Changes in haematological parameters

There were no statistically significant changes in the haematological parameters when control group was compared to CPF, MOL and CPF + MOL groupsat the end of the 14th day dosing period (Table 3).

Table 3: Haematological profiles of rats exposed to chlopyrifos and treated with *M. oleifera* (Mean ± S.E., n = 6/group)

Parameters	S/Oil	CPF	MOL	CPF + MOL
RBC (x 10^6 /µl)	8.52±1.09	7.30±0.30	8.42±0.61	7.702±0.41
Hemoglobin (g/dl)	18.34±1.06	15.40±0.61	15.67±0.35	16.98±1.09
Hematocrit (%)	47.06±6.06	38.18±1.38	45.77±3.74	42.28±2.28
MCV (fl)	55.20±0.65	52.30±1.15	54.23±1.11	54.85±0.42
MCHC (g/dl)	43.94±10.29	40.28±1.19	35.40±3.05	40.02±0.79
MCH (pg)	24.40±5.95	21.08±0.48	19.08±1.49	21.93±0.33
WBC (x 10^3 /µl)	12.98±2.33	19.28±2.37	24.77±4.28	13.53±3.01
Granulocytes (%)	31.36±9.87	24.14±1.77	26.03±2.81	29.43±8.53
Band Neutrophils (x 10^3 /µl)	3.50±0.32	4.66±0.67	6.77±1.59	3.483±0.70

Parameters	S/Oil	CPF	MOL	CPF + MOL
Lymphocytes (%)	60.62±9.87	67.86±1.84	66.53±3.12	62.53±8.59
Monocytes (%)	8.02±0.29	7.20±1.29	7.43±0.34	8.033±1.07
Platelets (x 10^3 /µl)	454.80±83.22	514.80±58.64	618.00±120.00	409.00±65.35

Relative organ weight

The relativeorgan weights (organ/body weight ratios) are shown in Figure 1. The relative liver, heart and kidney weight of rats treated with CPF increase significantly ($p < 0.01$,0.001 and 0.018, respectively) compared to those in the S/OIL, MOL and CPF + MOL groups.The relative weights of spleen were significantly decreased ($P < 0.05$) in the CPF groupcompared to those in the S/oil, MOL and CPF + MOL groups. However, there was no significant difference in the relative weight of seminal vesicle in all groups, although that of CPF treated group was lower compared to the other groups.

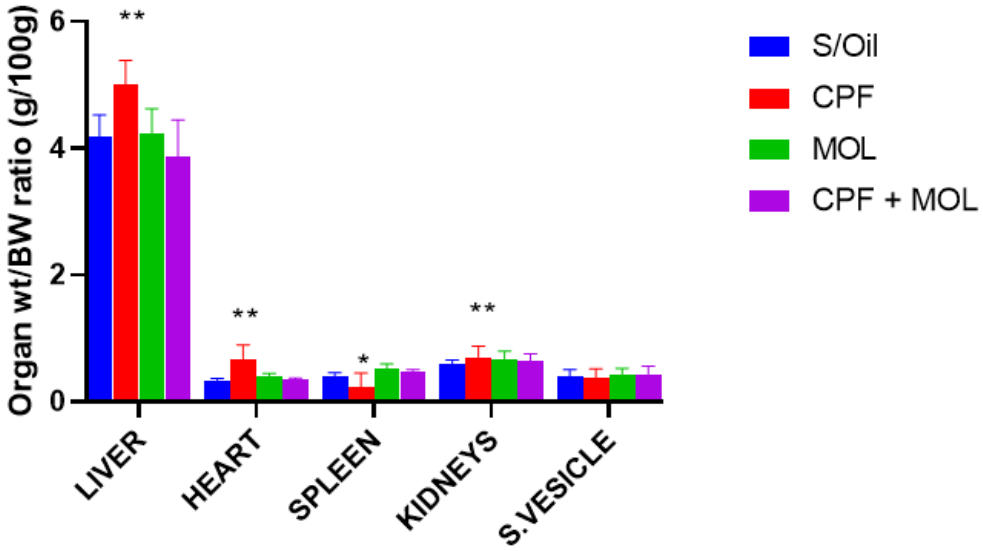

Figure 1: Relative organ weights of rats following treatment with S/oil, CPF, MOL and CPF + MOL. The results represent Means ± S.E. *Significant differences from the control group at *p < 0.05, **p < 0.01).

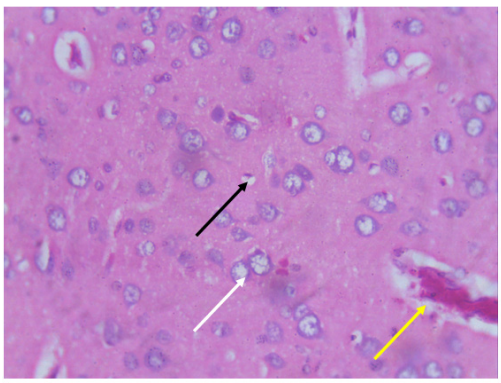

Figure 2: Photomicrograph of cerebral cortex of rat in the CPF group (H & E X 400). Showing Severe vacoulations(black arrow), Marginal clumping of Chromatin of Nuclei of Most Neurons (white arrow), Congested Blood Vessel surrounded by perivascular edema (yellow arrow)

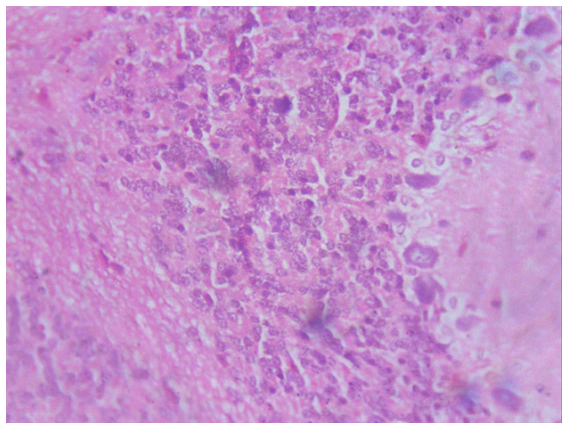

Figure 3: Cerebral cortex of MOL treated rats showing normal tissue (H & E x 400)

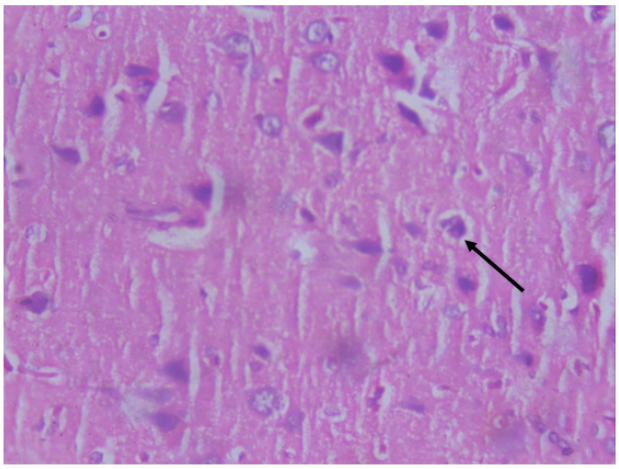

Figure 4: Cerebral Cortex of Group F (CPF + MOL), Showing Nearly Normal Morphological Appearance of Nerve Cells with Residual Fine Vacuolations (Black Arrow) of Pyramidal Cells. H&E X400.

Discussion

Experiments by previous researchers have reported various toxicological effects of CPF this is because CPF is lipophilic and easily cross the cell membrane to the cytoplasm[2]. This study investigated acute CPF induced toxicity and the ameliorative effect of MOL in male Wistar rats. CPF-treated animals also exhibited significantly higherMDA and decreased SOD, CAT and GSH activitiesthan the control group. Thesemay be as a result of peroxidation of membrane lipids and injury to cellular components as earlier reported by [5,7,15]. Oxidative stress occurs when the intracellular antioxidant defence system is overwhelmed by the generation of ROS, leading to increases in toxic molecules[5]. However, the amelioration of the lipoperoxidation in the group treated with CPF + MOL may be due to reduction in lipoperoxidative damage to the brain by MOL as demonstrated by its low MDA concentration in the present study. MOL extract is a rich source of antioxidants containing major bioactive compounds like quercetin, kaempferol, vitamin A and ascorbic acid, which are responsible for its antioxidant activities [9,10]. Irreversible inhibition of acetylcholinesterase in the peripheral and central nervous systems contributes to the cholinergic syndrome induced by an acute exposure to organophosphate insecticides[16]. In the present study, CPF caused a significant reduction in brain acetylcholinesterase concentration which was ameliorated with MOL treatment.

Body and relative organ weights are pointers of somegeneral toxicity in animals after exposure to toxicants[1]. In this study, statistically significant decreases were seen in rats treated with CPF at week 1 and 2, respectively. However, there was increased body weight seen in rats in the CPF + MOL group. The increase in body weight in the CPF + MOL group may be because of the vitamins and minerals present in MOL which restores appetite and It was observed that the relative weights of the liver, kidneys and adrenals were significantly increased in CPF group as compared to those rats in the SO, MOL and CPF + MOL groups the controls. Also, there was a significant decrease inthe spleen weight of rats in the CPF group. The decrease may be as a result of damage to internal organs caused by pesticides[17].

Histopathology is a widely used biomarker for the investigation of pesticide toxicity [18,19]. The result of the brain antioxidant markers tests also corroborated the histopathological lesions of severe vacuolations in the cerebral cortex, marginal clumping of chromatin of nuclei of neurons and congested blood vessel surrounded by perivascular oedema seen in the CPF treated group in this study (Figure 2). CPF is known to induce various histopathological changes in the organs of rats as those seen in the present study [20]. Therefore, the results of this study provides insights that *Moringa oleifera* supplementation may mitigate oxidative stress in individuals who are at risk of acute CPF exposure.

Conclusion

Overall, it could be concluded that chlopyrifos induces oxidative stress in male Wistar rats. The analysis revealed higher MDA and decreased SOD, CAT and GSH activities in the CPF treated group than the control group. The oxidative stress may be attributed to peroxidation of membrane lipids and injury to cellular components. *Moringa oleifera* proved effective against oxidative damage. These results are encouraging enough to further pursue the molecular mechanisms involved in the antioxidant protection provided by the plant.

Ethical Clearance: Sought from UDUS Ethical committee

Conflict of interest: The authors have no conflict of interest to disclose

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