The Journal of Anatomical Sciences

Email: anatomicaljournal@gmail.com

J. Anat Sci 14(1)

Moringa oleifera Ameliorates Chlorpyrifos -Induced Hippocampal Region –Histoarchitectural Changes in Wistar rat

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ABSTRACT

Intensive use of Chlorpyrifos (CPF) especially in developing countries for crop protection and improve yield has been reported to cause neurodegenerative diseases in animal models and humans. Therefore, there is a need to assess the phytotherapeutic potentials of *Moringa oleifera* in CPF-induced neurotoxicity. This study was conducted to evaluate the ameliorative efficacy of ethanol leaf extract of *Moringa oleifera* (EEM) on CPF -induced hippocampal and dentate gyrus neurotoxicity using histological methods. Thirty Wistar rats were categorized into five (5) groups with six rats in each group. The control group received Soya oil (2 ml/kg), another group received EEM (200 mg/kg), another group administered CPF (52.6 mg/kg), and two other groups were administered CPF (52.6 mg/kg) and subsequently treated with EEM (200 mg/kg) and Vitamin C (100 mg/kg), respectively. Treatments were via the oral route and lasted for fourteen days, while CPF administration was once on the first day of experimentation. On day 15, rat brains were harvested and fixed in Bouin's fluid, and histological assessment was conducted using Hematoxylin and Eosin (H&E) stains for routine histological analysis. Histopathological examination revealed normal histological architecture of neurons in the control and EEM groups, while CPF (52.6 mg/kg) group revealed distorted histoarchitecture of the hippocampal and dentate gyrus regions. While CPF (52.6 mg/kg) + EEM (200 mg/kg) group showed improved histoarchitecture. In conclusion, ethanol leaf extract of *Moringa oleifera* possesses potential neuroprotective properties against chlorpyrifos-triggered neurodegenerative changes in the hippocampal and dentate gyrus regions of Wistar rats.

Keywords: Oxidative stress, Histoarchitecture, Pyknosis and Chromatolysis.

INTRODUCTION

Exposure to environmental pollution and substances including chemicals, heavy metals, herbicides and pesticides have been reported to have adverse effects on the health status and normal brain functioning of humans and animals[1,2,3]. Pesticides are a diverse group of chemicals designed for the prevention and elimination of destructive plants, insects, rodents and fungi[4,5]. Intensive use of pesticides has been reported to have serious environmental problems due to their slow biodegradability[5,6]. Chlorpyrifos (CPF), a broad-spectrum organophosphate insecticide, is one of the most used organophosphate insecticides in domestic and industrial applications all over the world[7].

There are reports associating CPF with endocrine disruption, cardiovascular diseases and neurological disease conditions[8,9,10]. Exposure to CPF is mainly through inhalation, dermal absorption or ingestion of residues in the diet[11]. The neurotoxic pathophysiology of CPF is via acetylcholinesterase (AChE) inhibition at cholinergic synapses and neuromuscular junctions[12] resulting in cholinergic toxicity with nicotinic and muscarinic effects[13]. Additionally, CPF has been reported to induce oxidative stress in rodents[14,15]. An epidemiology study reveals performance impairment in a neurobehavioral study among fifty-seven CPF applicators for the cotton crop[16]. Neurological studies in rodents have identified the hippocampal region as a target for the neurotoxic effects of CPF exposure[17,18,19].

Oxidative stress is a mechanism of CPF neurotoxicity and its main target is the hippocampus which is one of the most vulnerable brain regions to oxidative damage[20,21,22]. The hippocampus is a complex brain structure embedded deep in the temporal lobe of the cerebral cortex, it has a major role in learning and memory, spatial navigation, emotional behaviour and the regulation of hypothalamic functions[23,24]. *Cornu ammoni* (CA: CA1, CA2, CA3) and dentate gyrus are the two major divisions of the hippocampal formation. These parts are separated by the hippocampal sulcus and curve into each other[25]. Alteration in the basic structure of the hippocampus as a result of exposure to potential neurotoxicants such as CPF had been linked to neurological and psychiatric disorders including Alzheimer's disease (AD), epilepsy and, Parkinson's disease (PD)[25,26].

Moringa oleifera is one of the most commonly researched medicinal plants because of its pharmacological properties beneficial to human health. Moringa is reported to provide seven times more vitamin C than oranges, ten times more vitamin A than carrots, seventeen times more calcium than milk, nine times more protein than yoghurt, fifteen times more potassium than bananas and twenty-five times more than spinach[27]. The potential neuroprotective properties of *M. oleifera* had been connected to its antioxidant activities[28,29]. The neurotoxic effects of CPF exposure are moderately established in animal models. There is a need to assess the phytotherapeutic potentials of this plant, *M. oleifera*, in CPF-induced neurotoxicity. This study, therefore, assessed the neurotherapeutic properties of *M. oleifera* on CPF-induced hippocampal and dentate gyrus histoarchitectural distortion in rats.

MATERIALS AND METHODS

Plant Materials: *Moringa oleifera* leaves were collected from a Local Market in Sokoto, Sokoto State, Nigeria, and provided with a Voucher Specimen Number: UDUH/ANS/0225 after identification in the Herbarium Unity, Botany Department, Faculty of Biological Sciences, Usman Danfodiyo University Sokoto (UDUS), Sokoto, Nigeria. The maceration method described by Sidhuraju and Becker,[30] for the extraction of ethanol leaves extract of *M. oleifera* (EEM) was adopted.

Experimental Animal: Thirty (30) apparently healthy male Wistar rats (180 to 200 g) were procured from the Animal House, Department of Biochemistry, Faculty of Sciences, UDUS, Sokoto. The rats were housed in wired cages, before the commencement of the study, and the rats were allowed to acclimatize for two weeks. The housing was according to standard laboratory conditions, light and dark cycles of 12 hrs provided and fed, rat chow and water *ad libitum*.

CHEMICALS AND DRUGS

Chlorpyrifos (CPF): Commercial grade CPF (liquid, 20% EC, Termicot®, Sabero Organics, Gujarat limited, India) was obtained from a certified agrochemical store in Sokoto, and used as a neurotoxin in this study. It was prepared by reconstituting in soya oil (Grand Cereals and Oil Mills Ltd., Jos, Nigeria).

Vitamin C: Ascorbic acid (Vitamin C) tablets were obtained and used as a reference drug to evaluate the therapeutic property of EEM. The product is manufactured by Emzor Pharmaceutical Industries Ltd, Lagos, Nigeria.

Ketamine: Ketamine (Ketamine Hydrochloride injection USP, 50 mg/ml) was obtained and used for anaesthesia. The product is manufactured by Swiss Parenteral PVT Ltd, Gujarat, India.

Experimental Design: Thirty Wistar rats were categorized into five (5) groups with six rats in each group. The control group was administered Soya oil (2 ml/kg), another group was administered EEM (200 mg/kg), another group administered CPF (52.6 mg/kg), and two other groups were administered CPF (52.6 mg/kg) and subsequently treated with EEM (200 mg/kg) and Vitamin C (100 mg/kg), respectively. Treatments were via the oral route and lasted for fourteen days, while CPF administration was once on the first day of experimentation.

At the end of the experiment, the rats were euthanised using Ketamine (75 kg/mg i.p[31]) anaesthesia, and the rats were decapitated and skulls dissected to remove the brain. Harvested brains were fixed in Bouin's fluid for histological processing.

Histological Studies: Fixed brain samples were processed using histological techniques by making sagittal sections to target the hippocampus. The hippocampal regions: dentate gyrus, CA3, CA2, and CA1 were primarily examined under a light microscope (Optical Microscope; HM-LUX, Leitz Wentzler, Germany). Histological paraffin sections were stained with Haematoxylin and Eosin (H&E) stains for demonstration of histoarchitecture in the Histopathology Laboratory, Usman Danfodiyo University Teaching Hospital,

Sokoto, Nigeria. Microscopy and micrography (using a digital microscopic camera, MA 500 AmScope®, USA) were conducted in the Microscopy and Stereology Research Laboratory, Department of Human Anatomy, Ahmadu Bello University, Zaria, Nigeria.

RESULTS

In this study, histology examination of the hippocampal regions (dentate gyrus, CA3, CA2, CA1) of Wistar rats were examined and the following features were observed:

The dentate gyrus of the control showed normal histoarchitectural features with well-defined pyramidal neurons and granule cell layer. Relative to the control, the section of the EEM (200 mg/kg)-treated group revealed relatively normal histoarchitectural features of the dentate gyrus, while the CPF (52.6 mg/kg)-treated group showed neurodegenerative changes as histoarchitectural distortion: pyknotic neuron, chromatolysis. CPF (52.6 mg/kg)- and EEM (200 mg/kg)- treated group showed mild distortion of the histoarchitectural as vacuolation when compared to the control, also the CPF (52.6 mg/kg)- and Vitamin C (100 mg/kg)-treated group showed distortion of the histoarchitectural features of the Dentate gyrus as vacuolation and chromatolysis.

Section of the hippocampal CA3 region of the control group showed normal histoarchitectural features with well-preserved pyramidal neurons. The CA3 region of the EEM (200 mg/kg)-treated group also revealed relatively normal histoarchitectural features when compared with the control while CPF (52.6 mg/kg)-treated group revealed severe distortion of the histoarchitectural features as pyknotic neurons and perinuclear vacuolation. The CPF (52.6 mg/kg)- and EEM (200 mg/kg), and CPF (52.6 mg/kg)- and Vitamin C (100 mg/kg)-treated groups showed slight histoarchitectural distortion as perinuclear vacuolation when compared with the control**.**

CA2 region of the hippocampus in the control group revealed normal histoarchitectural features with well-preserved pyramidal neurons, also, relative to the control there were relatively normal histoarchitectural features in the EEM (200 mg/kg)-treated group. CPF (52.6 mg/kg)-treated group revealed severe histoarchitectural distortion as pyknotic neurons, chromatolysis and perinuclear vacuolation. CPF (52.6 mg/kg)- and EEM (200 mg/kg)-treated group showed relatively normal histoarchitectural features of the CA2 region while CPF (52.6 mg/kg)- and Vitamin C (100 mg/kg)-treated group showed slight histoarchitectural distortion as chromatolysis

CA1 region of the hippocampus in the control group revealed normal histoarchitectural features with well-preserved pyramidal neurons, EEM (200 mg/kg)-treated group also revealed relatively normal histoarchitectural features with well-preserved pyramidal neurons. CPF (52.6 mg/kg)-treated group showed severe histoarchitectural distortion as pyknotic neurons, chromatolysis, and perinuclear vacuolation. The CPF (52.6 mg/kg)- and EEM (200 mg/kg), and CPF (52.6 mg/kg)- and Vitamin C (100 mg/kg)-treated groups showed relatively normal histoarchitectural features of the CA1.

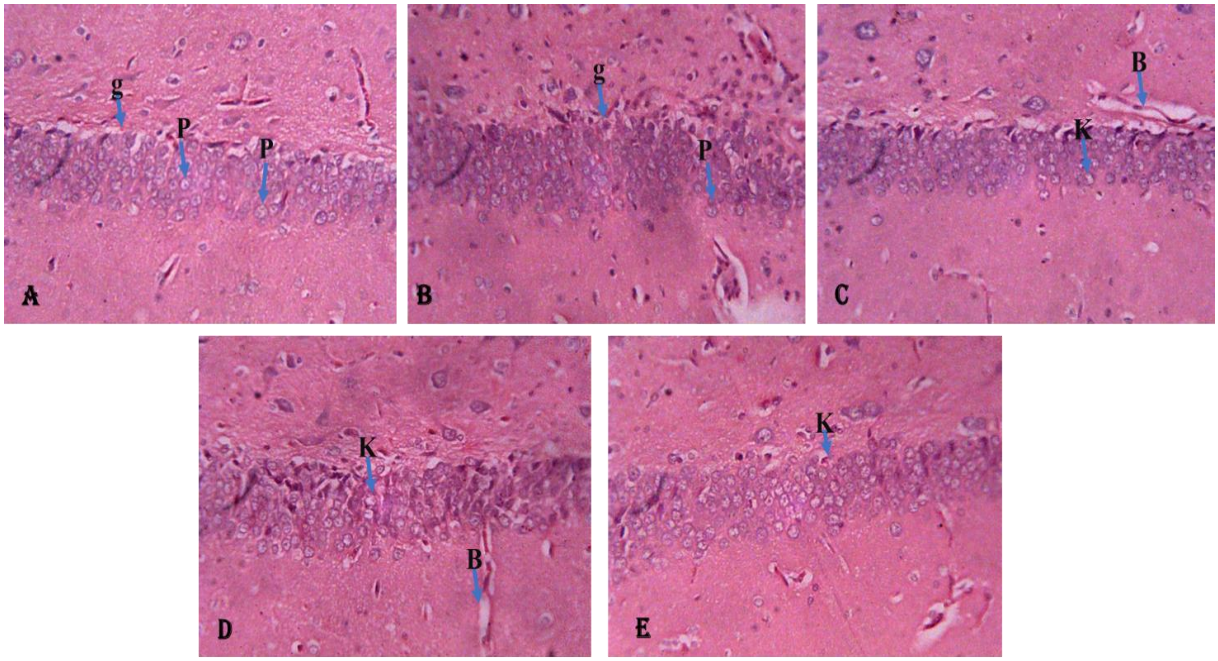

Figure 1: Micrograph of dentate gyrus of Wistar rats.

A. Control group (soya oil (2 ml/kg): P: Pyramidal neurons; g: granule cell layer. **B. EEM (200 mg/kg)**: P: Pyramidal neurons; g: granule cell layer. **C. CPF (52.6 mg/kg)**: K: Pyknotic neuron and perinuclear vacuolation; B: Blood vessel. **D. CPF (52.6 mg/kg) + EEM (200 mg/kg)**: K: Pyknotic neuron and perinuclear vacuolation; B: Blood vessel. **E. CPF (52.6 mg/kg) + Vitamin C (100 mg/kg)**: K: Pyknotic neuron and perinuclear vacuolation (x250)

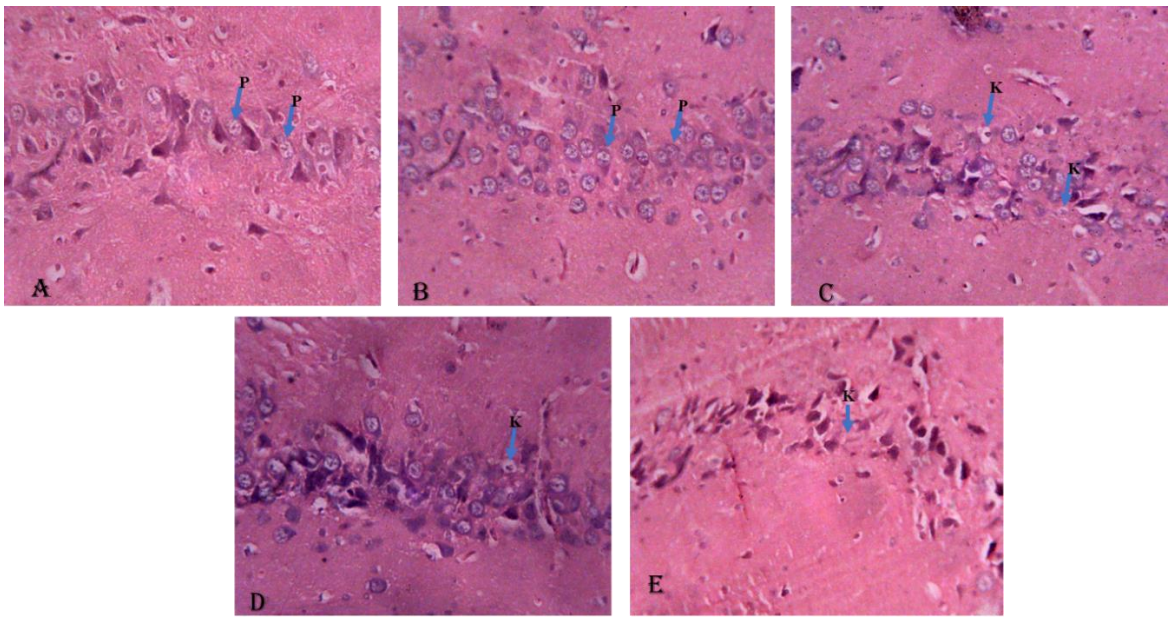

Figure 2: Micrograph of CA3 of Wistar rat

A. Control group (soya oil 2 ml/kg): P: Pyramidal neurons. **B. EEM (200 mg/kg)**: P: Pyramidal neurons. **C. CPF (52.6 mg/kg)**: K: Pyknotic neuron and perineuronal vacuolation; **D. CPF (52.6**

mg/kg) + EEM (200 mg/kg): K: Pyknotic neuron and perineuronal vacuolation. **E. CPF (52.6 mg/kg) + Vitamin C (100 mg/kg)**: K: Pyknotic neuron and perineuronal vacuolation (x250)

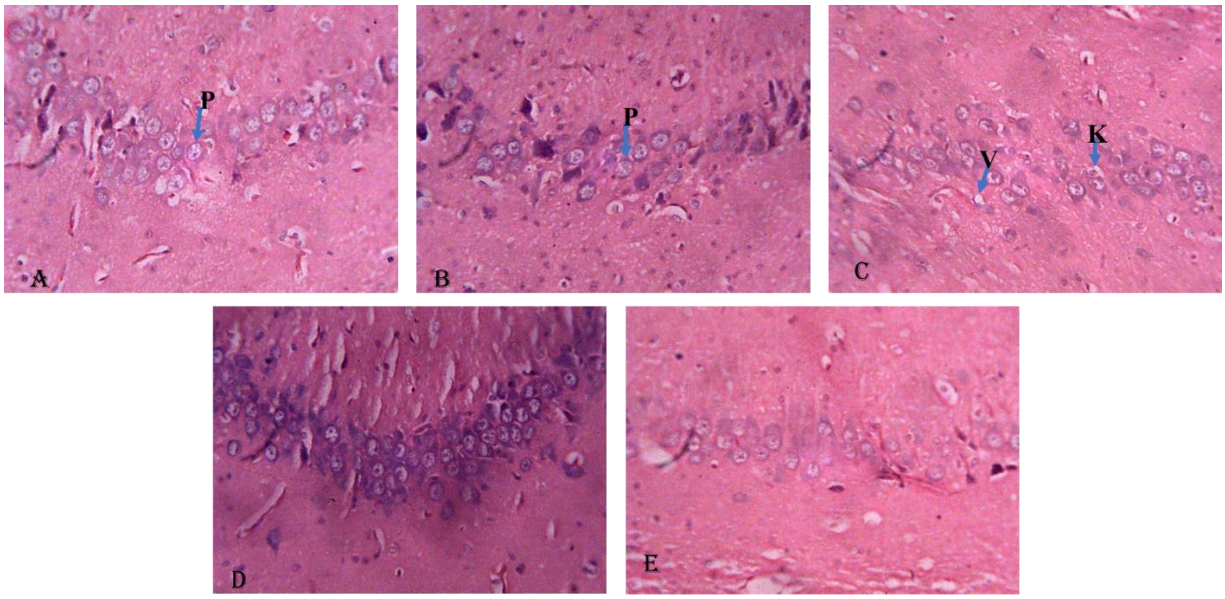

Figure 1: Micrograph of CA2 of Wistar rat.

A. Control group (soya oil 2 ml/kg): P: Pyramidal neurons. **B. EEM (200 mg/kg)** P: Pyramidal neurons. **C. CPF (52.6 mg/kg):** C: Pyknotic neuron; V: Pyknotic neuron and cytoplasmic Vacuolation. **D. CPF (52.6 mg/kg) + EEM (200 mg/kg)**: Relatively normal histoarchitectural features. **E. CPF (52.6 mg/kg) + Vitamin C (100 mg/kg)**: Relatively normal histoarchitectural features

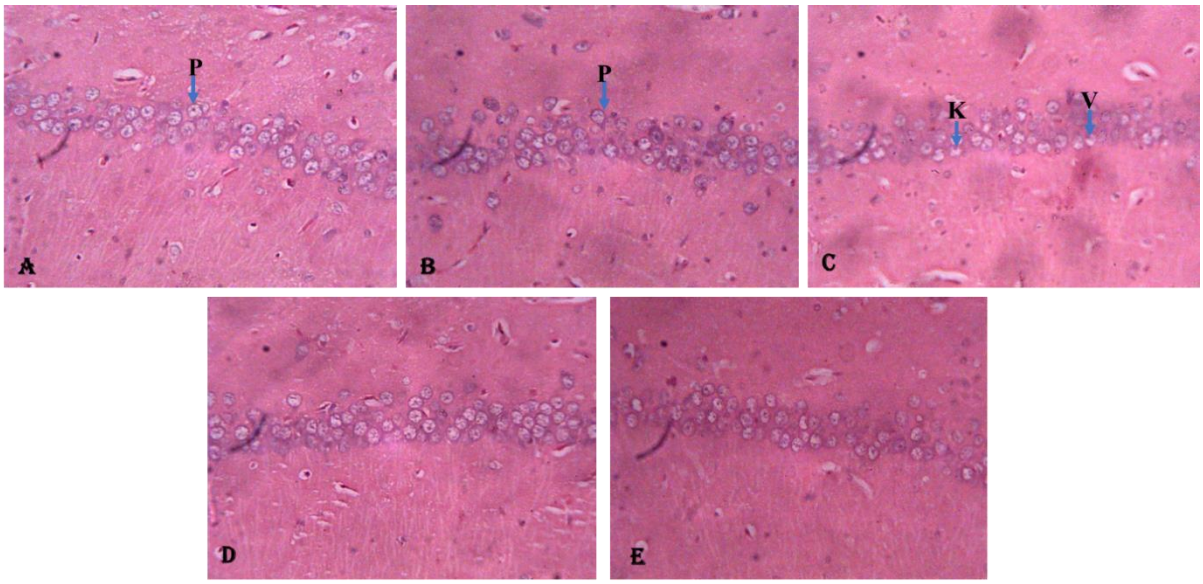

Figure 1: Micrograph of CA1 of Wistar rats

A. Control group (soya oil 2 ml/kg) P: Pyramidal neurons. **B. EEM (200 mg/kg)** P: Pyramidal neurons. **C. CPF (52.6 mg/kg)**: K: Pyknotic neuron; V: Perinuclear vacuolation.

D. CPF (52.6 mg/kg) + EEM (200 mg/kg): Relatively normal histoarchitectural features. **E. Group 5 CPF (52.6 mg/kg) + Vitamin C (100 mg/kg)**: Relatively normal histoarchitectural features.

DISCUSSION

This study assessed the ameliorative properties of ethanol leaf extract of *Moringa oleifera* on the hippocampal regions of Wistar rats exposed to chlorpyrifos-induced neurotoxicity using histological assessment.

Pathological changes have been associated with neurodegeneration in different regions of the brain in animal models resulting from exposure to environmental neurotoxicants[32,33]. Neurotoxins are common risk factors for chronic neurodegenerative changes, although molecular mechanisms engaged in the pathogenesis of diseases are still indistinct, oxidative stress, excitotoxicity, inflammation and apoptosis have been associated with neurodegenerative diseases including AD, epilepsy and PD in which clinical hallmarks comprise cognitive and motor impairments[34].

Exposure to organophosphorus insecticides like CPF can induce both acute toxicity and long-term neurological deficits[35,36,37]. In this study, neurodegenerative features observed presented as histoarchitectural distortion including chromatolysis, perineuronal vacuolation, pyknotic nuclei and pyknotic necrosis in the hippocampus following CPF treatment are suggestive of CPF-induced neurotoxicity. The findings are in agreement with previous studies.

Kaur *et al.*[38] and Mahmoud *et al.*[39] reported neurodegeneration in different regions of the brain including a decrease in cell density, pyknotic, degenerated neuron and, perineuronal vacuolation thereby reflecting chlorpyrifos-induced neurotoxicity. Pyknotic neurons of the pyramidal cells of the hippocampal region (dentate gyrus CA3, CA2 and CA1) of the hippocampus were histoarchitectural distortion observed in the CPF-treated group[40]. Exposure to CPF can induce both acute toxicity and long-term neurological deficits[35,36,37]. Several mechanisms by which exposure to CPF induces neurotoxicity include inhibition of acetylcholinesterase enzyme (AChE) in synaptic junctions of the nervous system[41] and through the generation of oxidative stress[15,42]. As a result of acetylcholinesterase enzyme inhibition, acetylcholine accumulated in the synapse causes repeated and uncontrolled stimulation of the post-synaptic axon leading to the death of the animal[43]. Chlorpyrifos induces oxidative damage through the generation of free radicals that result in oxidative damage, alteration of mitochondrial complex I in neurons and, lipid peroxidation resulting in cytotoxicity and cytoarchitectural distortion[42]. Neurological tissue has a high rate of oxidative metabolism, consuming about 20% of the cardiac output. Since CPF is polyunsaturated fatty acid (PUFAs), the brain is especially vulnerable to oxidative stress[44,45].

This study observed mild distortion to relatively normal histoarchitectural features of the hippocampus and dentate gyrus regions treated with CPF and EEM relative to the control is suggestive of ameliorative properties against CPF-induced neurodegenerative changes. The findings in

this study are in line with the previous report on the neuroprotective properties of *M. oleifera* following neuropathological changes associated with environmentally-induced neurotoxicity. Mahmoud *et al.*[46] reported ethanol leave extract of *M. oleifera* attenuated neuroinflammation, oxidative stress and apoptosis in the hippocampus and cerebral cortex of the hepatic encephalopathy experimental model. In an earlier study, *M. oleifera* pre-treatment mitigated sub-chronically exposed CPF-induced oxidative damages in rats. *Moringa oleifera* alleviated cypermethrin-induced neurotoxicity by reducing mitochondrial dysfunction, apoptotic markers, and increased acetylcholinesterase activity[47].

Several natural agents, especially of plant origin, have been known to be advantageous in the improvement of xenobiotic-induced neurotoxicity. *Moringa oleifera* leaves extracts have been reported to have a neuroprotective impact by progressing neuronal survival and neurite outgrowth[48]. The leaves extract had a defensive impact against Alzheimer's disease by adjusting the neuronal monoamine levels and electrical impulses[49]. Furthermore, it was reported that another potential neuroprotective activity is through its, saponins, and flavonoids phytochemicals in the leaves[50]. Giacoppo *et al*.,[51] reported protection against neurodegeneration due to the glucosinolates, which offer protection to neurons from cytotoxicity and oxidative stress through the prevention of reactive oxygen species, DNA crumbling and, membrane rupture.

In conclusion, ethanol leaves extract of *Moringa oleifera* possesses potential neuroprotective properties against chlorpyrifos-triggered neurodegenerative distortion in the hippocampus and dentate gyrus of Wistar rats. This study focused on histoarchitecture following treatments in rats. Thus, the need for further investigation on the efficacy and potentiality of *M. oleifera* leaves using other methodologies of assessment, like neurobehaviour, immunohistochemistry, stereology and molecular studies.

Financial support and sponsorship: Nil.

Conflicts of interest: There are no conflicts of interest.

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