Original Article

Microscopic Assessments of the Effect of *Phoenix dactylifera* L. in a Rat Model of Mercury-Triggered Cerebral M1 Changes

Abel Nosereme Agbon, Helen Ochuko Kwanashie[1], Wilson Oliver Hamman[2], Austin Oseloka Ibegbu[3], Hamisu Sule[2], Murtala Hamza Yahaya[4], Rachael Henry[2], Andrew Ekpenyong Ivang[5]

Department of Human Anatomy, Neuroanatomy and Neurosciences Research Unit, Faculty of Basic Medical Sciences, College of Medical Sciences, Ahmadu Bello University, [1]Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Science, Ahmadu Bello University, [2]Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, Ahmadu Bello University, Zaria, [3]Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, Alex Ekwueme Federal University Ndufu Alike, Ikwo, [4]Department of Human Anatomy, Faculty of Basic Medical Sciences, Yusuf Maitama Sule University, Kano, Nigeria, [5]Department of Clinical Biology, Clinical Anatomy Unit, College of Medicine and Pharmacy, University of Rwanda, Rwanda

Abstract

Context: Mercury is a widespread environmental and industrial pollutant that exerts toxic effects on vital organs. The cerebrum, composed of cortical areas such as the primary motor cortex (M1), is a vulnerable target of mercury toxicity within the central nervous system. *Phoenix dactylifera* is used in folk medicine to treat diverse disorders, such as loss of consciousness, memory disturbances, and nervous disorders. **Aim:** This study microscopically evaluated the neuroprotective effect of aqueous fruit pulp extract of *P. dactylifera* (AFPD) on mercury-triggered M1 changes in Wistar rats. **Materials and Methods:** Twenty-four Wistar rats were divided into six groups (I–VI; $n = 4$). Group I was administered distilled water (2 ml/kg); Group II administered mercuric chloride (MCL, 5 mg/kg); Group III administered Vitamin C (100 mg/kg) + MCL (5 mg/kg); Groups IV, V, and VI were administered AFPD (250 mg/kg, 500 mg/kg, and 1000 mg/kg, respectively) followed by MCL (5 mg/kg). Neuroprotective property was evaluated by microscopic assessment of M1 region applying histological techniques and analysis of histometric features of M1 neurons. **Statistical Analysis Used:** One-way ANOVA and paired sample *t*-test were used. **Results:** Microscopic examination of MCL-treated cerebral sections revealed M1 histoarchitectural distortion and neurodegenerative changes such as pyknosis, neuronal shrinkage, chromatolysis, loss of pyramidal neurites, and altered Nissl substance reactivity, relative to the control. Administration of AFPD remarkably ameliorated MCL-triggered M1 changes, especially at dose 500 mg/kg with neuroprotective property comparable to the reference drug, Vitamin C. **Conclusion:** AFPD is potentially efficacious in ameliorating mercury-triggered microscopic alterations in M1 region of Wistar rats. The neuroprotective property of AFPD could be attributed to antioxidant properties of constituent phytochemicals.

Keywords: Histochemistry, histology, histometry, neurodegeneration, neuroprotection, primary motor cortex

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Introduction

Mercury is a heavy metal that exists in three predominant forms: elemental mercury (Hg^0), organic mercury (such as methyl mercury), and inorganic mercury (mercuric chloride [MCL] – $HgCl_2$).[1,2] Mercury is a widespread environmental and industrial pollutant that exerts toxic effect on a variety of vital organs by triggering severe structural and physiological alterations in the tissues of animals and humans.[3,4] Mercury exposure can result from inhalation, ingestion, or absorption through the skin and may be highly toxic once absorbed into bloodstream.[4] The major sources of mercury load in humans are food contamination, drug and vaccine preservatives, dental amalgams, or occupational exposure.[5] Acute and chronic exposure to mercury has been established to cause a variety of neurological or psychiatric disorders.[6-8] Elevated production of reactive oxygen species (ROS) which elicit oxidative stress is a well-known pathophysiology of mercury-triggered neuronal injury.[9-11]

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Address for correspondence: Dr. Abel Nosereme Agbon, Neuroanatomy and Neurosciences Research Unit, Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, Ahmadu Bello University, Nigeria.
E-mail: bellulu09@gmail.com

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The central nervous system (CNS) is one of the most vulnerable organs affected by mercury toxicity. Within the CNS, two of the most often affected areas are the cerebral cortex and the cerebellum.[12,13] The cerebral cortex is composed of relevant cortical areas which play a critical role in the programming and execution of voluntary movements.[14-16] The largest of these cortical areas is the primary motor cortex (M1) anatomically characterized by large neurons, the Betz cells.[17,18]

In low-income countries, about 80% of the people rely on phytomedicine for primary healthcare.[19] In traditional practices of medicine, numerous plants have been used in the management and treatment of neurological disorders, including neurodegenerative diseases.[20] *Phoenix dactylifera* (date palm) is a member of the monocot family *Arecacea* called "Nakhla."[21] *P. dactylifera* fruit is a good source of energy, vitamins, essential minerals, and several medicinal values.[22-24] Folklorically, *P. dactylifera* is widely used for the treatment of various ailments, such as fever, memory disturbances, inflammation, paralysis, loss of consciousness, and nervous disorders.[25-27] Several pharmacological studies have demonstrated beneficial properties of *P. dactylifera* against different environmental toxins.[28-30] Recently, more attention has been paid to the role of natural agents, especially plants, with antioxidant properties that may have more antioxidant activity than Vitamins C and E,[31,32] including their neuroprotective actions.[33-35]

This study evaluated the neuroprotective effect of AFPD on mercury-triggered M1 cerebral changes in Wistar rats using microscopic assessments.

Materials and Methods

Plant material

Dried *P. dactylifera* (date palm) fruits were obtained from a local market in Zaria, Kaduna state, Nigeria, authenticated in the Herbarium Unit of the Department of Biological Sciences, Faculty of Life Sciences, Ahmadu Bello University (ABU), Zaria, and the voucher specimen number 7130 was provided.

Extract preparation and phytochemical screening

P. dactylifera fruit pulp extraction and phytochemical screening were conducted in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, ABU, Zaria. The method of maceration as reported by Agbon *et al.*[29] for the preparation of aqueous fruit extract of *P. dactylifera* was adopted. The method described by Trease and Evans[36] for qualitative phytochemical screening of secondary metabolites was adopted.

Experimental animals

Wistar rats weighing 125 ± 15 g were obtained from Animal House of the Department of Human Anatomy, Faculty of Basic Medical Sciences, ABU, Zaria, and housed in new wired cages in the same facility where rats acclimatized for a week before the commencement of the experiments. The rats were housed under standard laboratory condition, light and dark cycles of 12 h, and were provided with food (rat chow) and water *ad libitum*. The rats were categorized into control and treatment groups. The treatment groups were administered, in addition to feed and water, AFPD/mercury/Vitamin C for 2 weeks.

Drug

MCL was obtained and used as a neurotoxicant for the experiment. The product is manufactured by British Drug Houses Chemicals, Poole, England.

Vitamin C (ascorbic acid) was obtained and used for the experiment as standard antioxidant. The product is manufactured by Emzor Limited, Lagos, Nigeria.

Experimental procedure

Twenty-four Wistar rats were divided into six groups (I–VI) of four rats each. Group I served as control and was administered distilled water (2 ml/kg). Cortical cerebral damage was induced in rats by the administration of MCL as reported by Sheikh *et al.*[37] Group II was administered MCL (5 mg/kg; that is, 12.5% of LD_{50})[37] only. Group III was administered Vitamin C (100 mg/kg)[38] followed by MCL (5 mg/kg); Groups IV, V, and VI were administered AFPD (250 mg/kg, 500 mg/kg, and 1000 mg/kg, respectively, that is, 5%, 10%, and 20% of LD_{50}, respectively),[39] concomitantly followed by MCL (5 mg/kg). All administrations were via oral route and lasted for 2 weeks [Figure 1].

At the end of the experiment, rats were euthanized using chloroform anesthesia, and brains were harvested and fixed in Bouin's fluid.

Physical observation

During the period of experiment, the rats were observed for any change in physical activity and behavioral pattern, such as playing and eating. Absolute body weights (BWs) before (initial weight [IW]) and after the experiments (final weight [FW]) were weighed (using digital weighing scale, Kerro BL 20001, 0.1 g); weight change (FW − IW) was computed and compared. Harvested whole brains were equally weighed (using digital weighing scale, Acculab VICON; VIC-303, USA, 0.001 g) and organosomatic index was computed (BW/final BW × 100).[40,41] Recorded weight values were statistically compared.

Histological and histochemical studies

Fixed brains were processed using histological techniques by making a coronal section at the cerebrum 7.4 mm posterior to the junction between the olfactory bulb and cerebrum to target the M1 region as indicated by the rat brain atlas.[42] The layer V of M1 region was primarily examined to assess for treatment-associated changes in the large pyramidal neurons (Betz cells) critically involved in motor-related functions [Figure 2a and b].). Paraffin processed histological brain sections were stained with Haematoxylin and Eosin (H&E) stains to demonstrate general histoarchitectural features and, histochemical stains (Cresly Violet and Golgi stains to

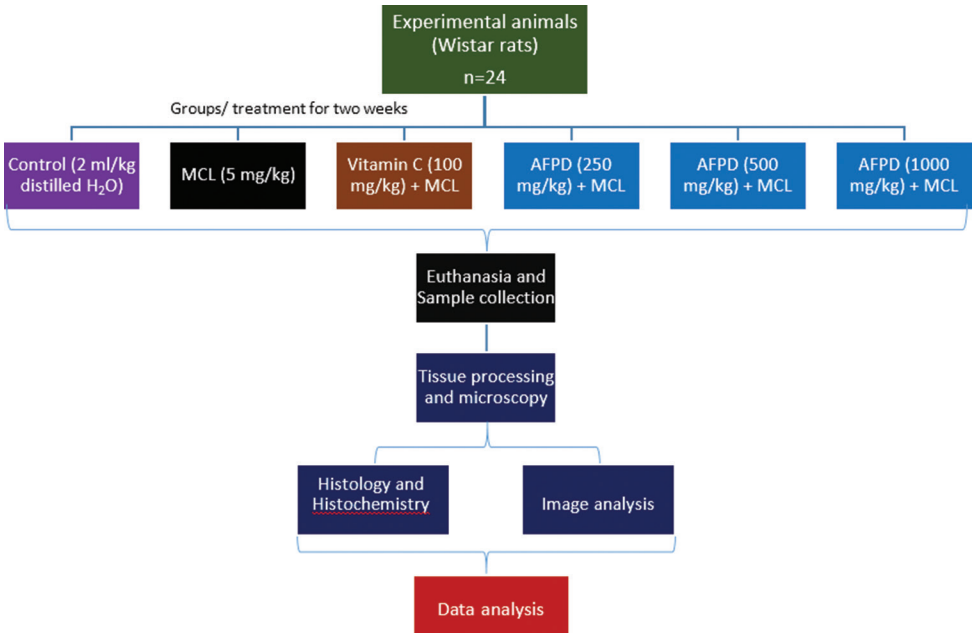

Figure 1: Experimental protocol. $n = 4$; AFPD: Aqueous fruit extract of *Phoenix dactylifera*, MCL: Mercuric chloride

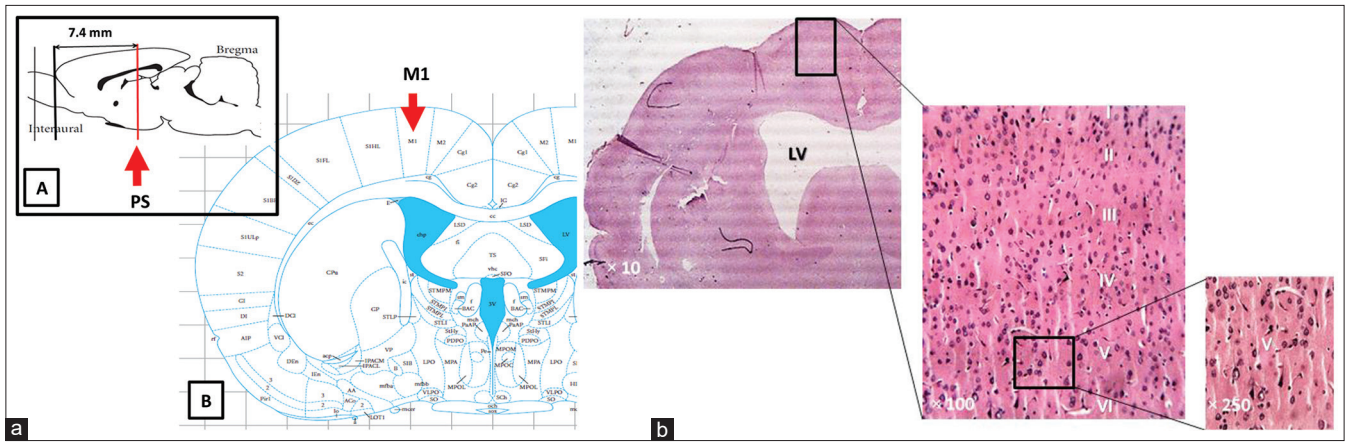

Figure 2: (a) Snipped image indicating region of the brain section (A) and the M1 region of the cerebral cortex (B). M1: Primary motor area, PS: Plane of brain section.[42] (b) Coronal section of the cerebrum of Wistar rat with layers (I–VI) of M1 region. H and E stain. M1: Primary motor area, H and E: Hematoxylin and eosin, LV: Lateral ventricle

demonstrate Nissl substances and neuritis, respectively). Tissues were processed in the Histology Unit of the Department of Human Anatomy, ABU, Zaria, and light microscopy and micrography were conducted in the Microscopy and Stereology Research Laboratory of the same facility.

Image analysis

Microscopic image analysis involved characterization of histometric features and quantification of NS reactivity of layer V neurons in the M1 region. CV stain, an excellent neuronal (cell body-specific) stain and useful for the demonstration of NS in neurons,[43,44] was employed for this study.

Histometric analysis involved measuring the soma area and perimeter of the pyramidal neurons from CV-stained micrographs (digital microscopic images) using a light microscope (HM-LUX, Leitz Wetzlar, Germany) with a 40/0.65 × objective (×400), micrometer slide (1 mm graduated in 0.01 mm units; that is divided ×10 into 100 µm units), and computer running imaging software (AmScope MT version 3.0.0.5, USA), according to the manufacturer's instruction.[45] Three different micrographic fields were randomly captured[46,47] in the M1 region, and 5–10 neurons that met the criteria for selection (that is, layer V pyramidal neurons, with well-outlined nucleus in the cell profiles) were randomly selected; using the AmScope imaging software polygon tool, soma area and perimeter were measured and analyzed [Figure 3a].

Quantification of NS reactivity involved measuring the staining intensity of NS using CV stained micrographs and a computer running image analysis software (ImageJ, U.S.

National Institute of Health, Bethesda, Maryland, USA) according to the manufacturer's instruction. The ImageJ region of interest (ROI) manager tool for analysis of specific areas of the micrographs was employed to limit bias values, resulting from nonidentical image quality (image acquisition setting and exposure times).[48,49] The modal gray values for three ROIs were obtained; means were computed and analyzed [Figure 3b].

Data analysis

Data obtained were expressed as mean ± standard error of the mean, and the presence of significant difference among means of the groups was determined using one-way ANOVA with least significant difference *post hoc test* for significance. Paired sample *t*-test was employed for the comparison of means as appropriate. Values were considered statistically significant when $P < 0.05$. Data were analyzed using the statistical software, Statistical Package for the Social Sciences (IBM SPSS v 21.0 Armonk, NY: IBM Corp) and Microsoft Office Excel 2013 for charts.

Results

Phytochemical analysis

Phytochemical screening of AFPD produced positive reaction for metabolites such as flavonoids, saponins, and tannins, while anthraquinone, a negative reaction [Table 1].

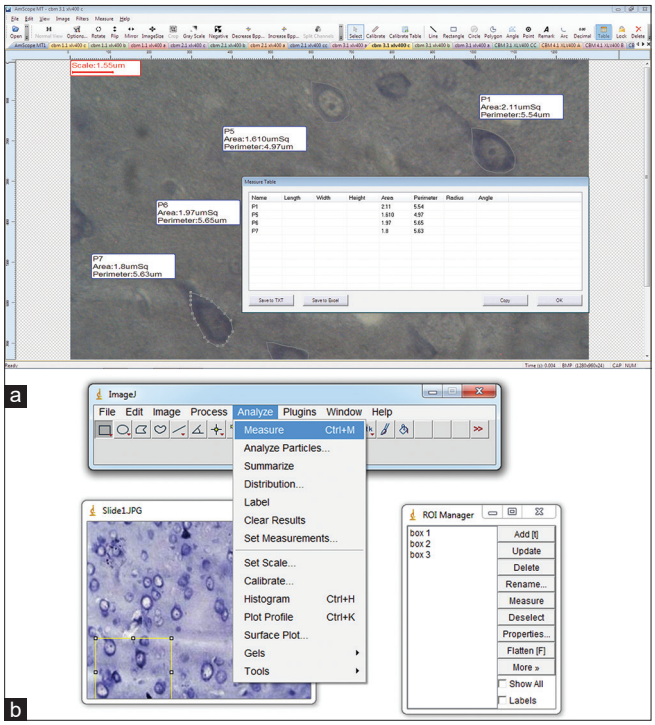

Figure 3: (a) Histometry using a Cresly violet stained micrographs with a computer running imaging software (AmScope MT version 3.0.0.5, USA). (b) Quantification of Nissl substance reactivity using Cresly violet stained micrographs with a computer running image analysis software (ImageJ, NIH, USA)

Physical observation

During the period of administration, physical activities of the rats were observed. Rats in the control group were observed to exhibit normal physical activities, such as movement and playfulness, whereas rats in the treatment groups exhibited decreased activity, especially in MCL-treated group.

The weights of the rats, in all groups, were observed to have increased when IW and FW were compared. Remarkable ($P < 0.05$) increases in weights were observed with the control and AFPD (250 mg/kg and 500 mg/kg) + MCL-treated groups. However, there was no remarkable difference ($P > 0.05$) in weight changes (that is, difference in IW and FW) when treated groups were compared with the control [Figure 4a]. Relative to the control, organosomatic index (relative organ weight) revealed increase in all treated groups, especially ($P < 0.05$) with Vitamin C + MCL-treated group [Figures 4b].

Histological and histochemical examination

Microscopic examination of histological sections of the rats' cerebral cortex, particularly layer V of M1 region,

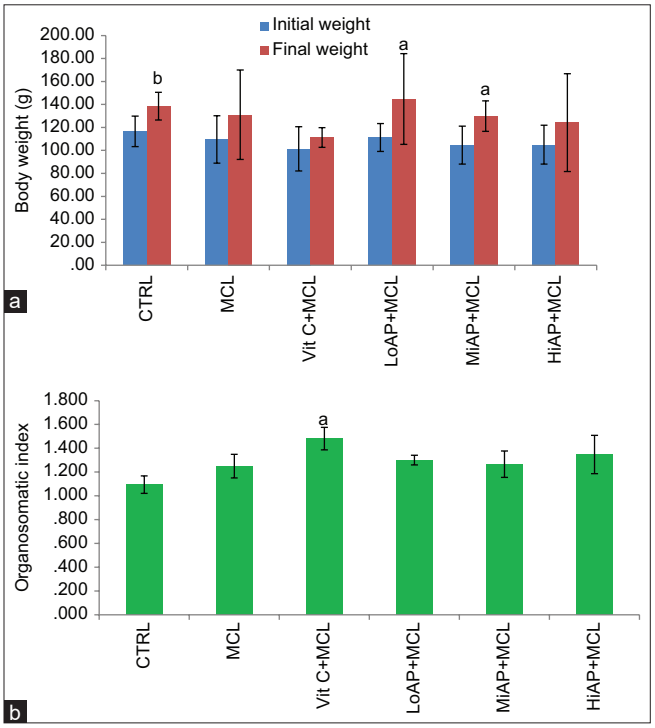

Figure 4: (a) Absolute body weight comparison of Wistar rats. $n = 4$; mean ± SEM; paired sample *t*- test, $^{a}P < 0.05$; $^{b}P < 0.001$ when IW and FWs were compared. One-way ANOVA least significant difference *post hoc* test, $P > 0.05$ when weight changes (FW – IW) were compared with the control. CTRL: Control (distilled H_2O 2 ml/kg), LoAP, MiAP, and HiAP: Aqueous fruit extract of *Phoenix dactylifera* (250, 500, and 1000 mg/kg, respectively), MCL: $HgCl_2$ (5 mg/kg), Vit C: Vitamin C (100 mg/kg), FW: Final weight, IW: Initial weight. (b) Comparison of organosomatic index of Wistar rats. $n = 4$; mean ± SEM; one-way ANOVA least significant difference *post hoc* test, $^{a}P < 0.05$, when compared with the control. CTRL: Control (distilled H_2O 2 ml/kg), LoAP, MiAP, and HiAP: Aqueous fruit extract of *Phoenix dactylifera* (250, 500, and 1000 mg/kg, respectively), MCL: $HgCl_2$ (5 mg/kg), Vit C: Vitamin C (100 mg/kg)

Table 1: Phytochemical constituents of aqueous fruit pulp extract of *Phoenix dactylifera*

Constituents	Alkaloid	Anthraquinones	Cardiac glycoside	Carbohydrates	Flavonoids	Saponin	Tannin
Inference	+	–	–	+	+	+	+

+: Positive (present), –: Negative (absent)

stained with H and E, CV, and Golgi stains revealed the following:

Histological sections of the control group showed normal histoarchitecture; the characteristic appearance of neurons arranged into six layers, the layers differing in neuron morphology, size, and population density. Particularly, the ganglionic layer (layer V) revealed large pyramidal (ganglion/Betz) cells of the motor cortex and stellate cells. CV staining for NS revealed normal appearance of distinctly stained large pyramidal neurons, and Golgi staining for neurites revealed normal appearance of large pyramidal neuron axonal and dendritic processes [Figures 5a, 6a, and 7a]. Histological sections of MCL-treated group revealed distortion in histoarchitecture and neuronal degenerative changes, such as neuronal shrinkage and perineuronal vacuolations, pyknotic nuclei (pyknotic necrosis), and chromatolysis. Golgi staining revealed loss of pyramidal neuron processes, while CV staining revealed pyknotic nuclei and chromatolysis [Figures 5b, 6b, and 7b]. Histological sections of Vitamin C + MCL-treated and AFPD + MCL-treated groups revealed mild distortion of the histoarchitecture of the cerebral cortex. The histological features of the Vitamin C-treated group were comparable with the control [Figures 5c, 6c, and 7c]. Histological features of the AFPD-treated groups showed mild neurodegenerative changes, such as chromatolysis, pkynotic necrosis, and satellitosis/gliosis. Histoarchitectural preservation conferred by the administration of AFPD was dose dependent, except for 1000 mg/kg AFPD + MCL-treated group when compared to the control [Figures 5d-f, 6d-f, and 7d-f].

Histometric analysis

Histometric features (soma area and perimeter) of pyramidal neurons in the layer V of M1 region revealed remarkable ($P < 0.05$) decrease in soma area of all the treated groups, except AFPD (250 and 1000 mg/kg)-treated groups when compared to the control. Besides, remarkable ($P < 0.05$) difference was observed when AFPD (250 and 1000 mg/kg)-treated groups were compared to the MCL-treated group [Figure 8a]. Relative to control, soma perimeter revealed remarkable ($P < 0.05$) decrease with MCL-treated group. Remarkable ($P < 0.05$) difference was observed when Vitamin C + MCL-treated group and AFPD (250 and 500 mg/kg) + MCL-treated groups were compared to the MCL-treated group [Figure 8b].

Nissl substance reactivity

Analysis of the staining intensity of NS to quantify NS distribution in the layer V neurons of the M1 region revealed remarkable difference in all the treated groups, except AFPD (250 and 500 mg/kg)-treated groups when compared to the control [Figure 9].

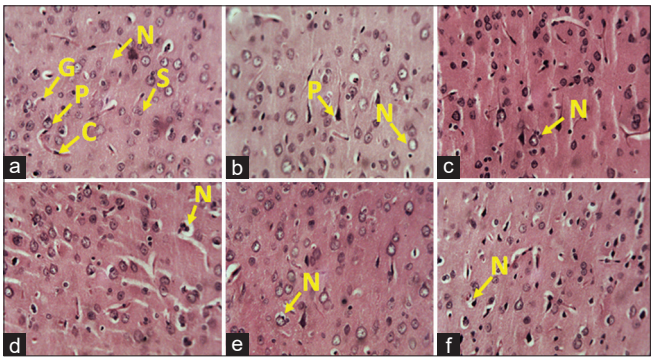

Figure 5: Micrograph of M1 cerebral region (layer V) of Wistar rat (H and E stain, × 250). (a) Control (2 ml/kg distilled water) group with normal histoarchitecture. C: Capillary – Blood vessel, G: Glial cell, N: Neuropil, P: Pyramidal cell, S: Stellate cell. (b) Group administered $HgCl_2$ (5 mg/kg) with distortion in the histoarchitecture. P: Pyramidal cell, N: Neuronal degeneration – Necrosis and chromatolysis. (c) Group administered Vitamin C (100 mg/kg) and $HgCl_2$ (5 mg/kg) with mild distortion in the histoarchitecture. N: Neuronal degeneration – Satellitosis. (d) Group administered AFPD (250 mg/kg) and $HgCl_2$ (5 mg/kg) with mild distortion in the histoarchitecture. N: Neuronal degeneration –gliosis/satellitosis. (e) Group administered AFPD (500 mg/kg) and $HgCl_2$ (5 mg/kg) with mild distortion in the histoarchitecture. N: Neuronal degeneration – gliosis. (f) Group administered AFPD (1000 mg/kg) and $HgCl_2$ (5 mg/kg) with mild distortion in the histoarchitecture. P: Pyramidal cell, N: Neuronal degeneration – Perineuronal vacuolation and gliosis, AFPD: Aqueous fruit pulp extract of *Phoenix dactylifera*

Discussion

In this study, phytochemical analysis of AFPD was conducted, and the neuroprotective effect of AFPD against mercury-triggered cerebral changes was evaluated by the assessment of Wistar rats' physical activity, weight changes, and M1 region microscopic features.

Phytochemical studies

Qualitative phytochemical analysis of AFPD revealed the presence of metabolites such as flavonoids, saponins, and tannins, which have been reported to possess antioxidant activities and exert neuroprotective actions in models of neurological disorders.[50,51] Findings agree with reported phytochemicals present in fruit extract of *P. dactylifera*.[27,39,52]

Physical observation

Decreased physical activity exhibited by MCL-treated rats reflects treatment-related toxicity. Finding is in consistence with reports on drug-related toxicity; altered physical activity manifesting as sluggishness and loss of appetite indicates drug-related toxicity.[53-55] Body weight serves as a sensitive indicator of the general health status of an animal and useful as a pointer of adverse effect of drugs and chemicals.[54,56] In this study, the trend of BW across the treatment period revealed

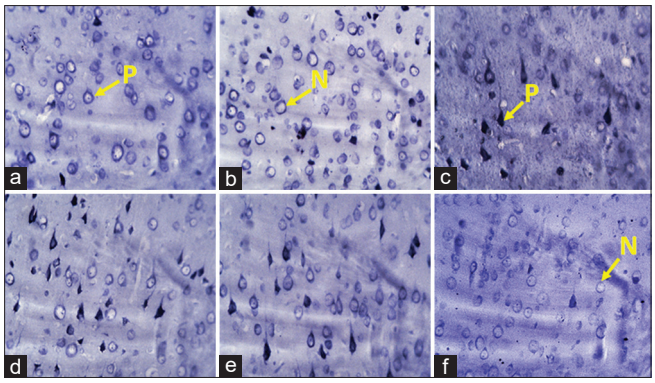

Figure 6: Micrograph of M1 cerebral region (layer V) of Wistar rat (CV stain, ×250). (a) Control (2 ml/kg distilled water) group with normal histoarchitecture. P: Pyramidal cell. (b) Group administered $HgCl_2$ (5 mg/kg) with distortion in the histoarchitecture. N: Neuronal degeneration –Chromatolysis. (c) Group administered Vitamin C (100 mg/kg) and $HgCl_2$ (5 mg/kg) with normal histoarchitecture. P: Pyramidal cell. (d) Group administered AFPD (250 mg/kg) and $HgCl_2$ (5 mg/kg) with relatively normal histoarchitecture. (e) Group administered AFPD (500 mg/kg) and $HgCl_2$ (5 mg/kg) with relatively normal histoarchitecture. (f) Group administered AFPD (1000 mg/kg) and $HgCl_2$ (5 mg/kg) with mild distortion in the histoarchitecture. N: Neuronal degeneration – Chromatolysis, AFPD: Aqueous fruit pulp extract of *Phoenix dactylifera*

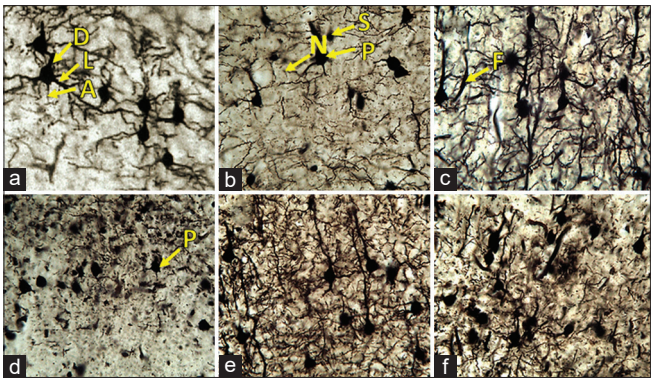

Figure 7: Micrograph of M1 cerebral region (layer V) of Wistar rat (Golgi stain, ×250) (a) Control (2 ml/kg distilled water) group with normal histoarchitecture. A: Axon, D: Dendrite, L: Lateral dendrite. (b) Group administered $HgCl_2$ (5 mg/kg) with distortion in the histoarchitecture – Neuronal process loss. S: Stellate cell, P: Pyramidal cell. (c) Group administered Vitamin C (100 mg/kg) and $HgCl_2$ (5 mg/kg) with relatively normal histoarchitecture. F: Fusiform cell. (d) Group administered AFPD (250 mg/kg) and $HgCl_2$ (5 mg/kg) with distortion in the histoarchitecture – Neuronal process loss. P: Pyramidal cell. (e) Group administered AFPD (500 mg/kg) and $HgCl_2$ (5 mg/kg) with relatively normal histoarchitecture. (f) Group administered AFPD (1000 mg/kg) and $HgCl_2$ (5 mg/kg) with mild distortion in the histoarchitecture – Neuronal process loss. AFPD: Aqueous fruit pulp extract of *Phoenix dactylifera*

that MCL-treated group lagged behind with no remarkable difference between IW and FW. This could be attributed to treatment-related altered growth pattern. Altered normal growth pattern has been associated with deleterious exogenous substances as a result of disruption in the normal physiological and biochemical processes of biological systems.[4] However, remarkably increased BW observed with AFPD-treated group could be attributed to the high caloric content in *P. dactylifera*, which has been reported as a good source of energy and rich in nutrients.[24,57] Observed difference in relative organ weight compared to the control could be attributed to variations in absolute BW at the termination of the study, rather than manifestation of toxicity. Thus, AFPD has the potentials to maintain and/or improve appetite and normal growth pattern during experimental exposure to adverse exogenous substances when compared to the reference drug, Vitamin C.

Histological and histochemical studies

Exposure to mercury has been reported to trigger neuropathological conditions associated with physiological and structural alterations in the CNS.[58,59] Histoarchitectural distortion or degenerative changes of neural tissue are indicative of neurotoxicity.[60-62] In this study, observed degenerative changes such as necrosis, chromatolysis, and loss of pyramidal neurites in the M1 region of MCL-treated rats are suggestive of treatment-related toxicity. Necrosis, a pathologic type of cell death resultant from extrinsic insults such as toxins, has been associated with neuronal degeneration.[63,64] Chromatolysis is a histopathological manifestation in response to axonal injury, whereby the nucleus and chromatin are pushed to the perikaryon periphery.[43,63,65] Findings are in concordance with reports on the neurotoxic properties of heavy metals, such as mercury, lead, cadmium, and arsenic with the capacity to induce nervous tissues damage.[58,66] Ibegbu *et al.*[67] reported that administration of MCL at different doses produced clumping and necrosis of cortical cerebral cells in rats. Mercury has been reported to interact with a wide range of cellular targets and affect multiple cellular functions, such as inhibition of neuronal ion channels,[68] alteration of mitochondrial function,[10,69] disruption of presynaptic and postsynaptic functions,[70] and damage to neuronal cytoskeleton components and DNA structures.[71,72]

Ascorbic acid is a well-established antioxidant compound.[73,74] In this study, histological features of the Vitamin C + MCL-treated group were comparable with that of the control, which indicates histoarchitectural preservation and neuroprotective property. This finding is in consistence with the reports of Ibegbu *et al.*[67] and Kumar *et al.*;[75] ascorbic acid which participates in several beneficial cellular functions such as antioxidant protection ameliorated mercury-induced cortical cerebral damage in rats. Antioxidants play a significant role in the reversion of mercury-induced injury by forming inert complexes and inhibiting toxic effects on neurons.[76] AFPD + MCL-treated groups revealed histoarchitectural preservation that manifested as mild neurodegenerative changes of the M1 region. This implies that AFPD has neuroprotective properties against mercury-induced neurotoxicity. Findings are in agreement with reports on the neuroprotective activity of *P. dactylifera*.[26,77,78] Kalantaripour *et al.*[61] reported that the extract of *P. dactylifera* protected cortical neurons against ischemia reperfusion-induced insults in rats. Thus, AFPD neuroprotective property is comparable to that of the reference drug, Vitamin C.

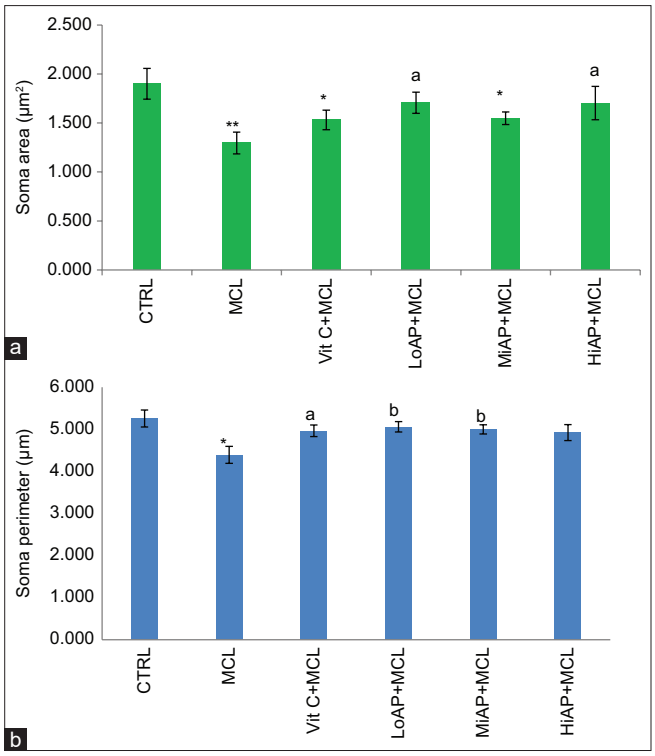

Figure 8: (a): Effect of *Phoenix dactylifera* on histometric characteristics (soma area) of pyramidal neurons in layer V of M1 cerebral region of Wistar rats. n = 5–10 cells/3 fields; mean ± SEM; one-way ANOVA least significant difference *post hoc* test, *$P < 0.05$, **$P < 0.01$ when compared with the control; $^{a}P < 0.05$ when compared with MCL. CTRL: Control (distilled H_2O 2 ml/kg); LoAP, MiAP, and HiAP: Aqueous fruit extract of *Phoenix dactylifera* (250, 500, and 1000 mg/kg respectively), MCL: $HgCl_2$ (5 mg/kg), Vit C: Vitamin C (100 mg/kg). (b) Effect of *Phoenix dactylifera* on histometric characteristics (soma perimeter) of pyramidal neurons in layer V of M1 cerebral region of Wistar rats. n = 5–10 cells/3 fields; mean ± SEM; one-way ANOVA least significant difference *post hoc* test, *$P < 0.05$ when compared with the control; $^{a}P < 0.05$, $^{b}P < 0.01$ when compared with MCL. CTRL: Control (distilled H_2O 2 ml/kg); LoAP, MiAP, and HiAP: Aqueous fruit extract of *Phoenix dactylifera* (250, 500, and 1000 mg/kg, respectively), MCL: $HgCl_2$ (5 mg/kg), Vit C: Vitamin C (100 mg/kg)

Histometric studies

Histometric quantification provides an objective basis for comparison of histological observation. It increases precision compared with direct visual appraisal and improves assessment of certain histological change, providing for accurate statistical grading.[55,79,80] Observed remarkable difference in histometric characteristics (soma area and perimeter) of pyramidal neurons in the layer V of the M1 region is indicative of treatment-related cytoarchitectural changes. Histometric parameters are directly applied and related to tissue function.[80,81] Decreased perikaryon size, a pointer to neuronal cytoplasmic shrinkage, has been attributed to stress-induced morphological changes at the light microscopic level.[82,83] Histometric findings corroborate with histologic observations in this study. Thus, AFPD has potentials to preserve neuronal cytoarchitectural features following mercury-triggered changes in M1 region.

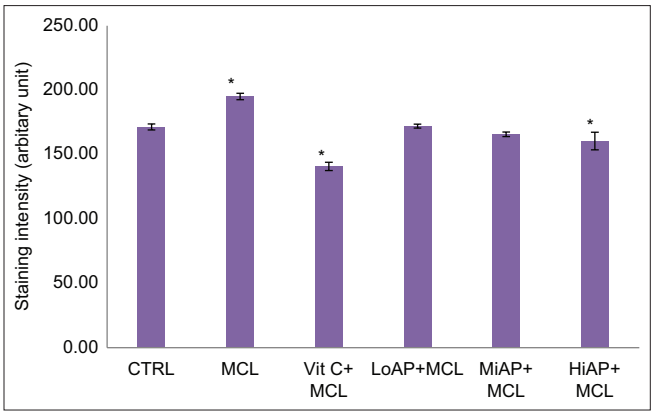

Figure 9: Effect of *P. dactylifera* on Nissl substance reactivity in the neurons of layer V of M1 cerebral region of Wistar rats. Mean ± SEM; One way ANOVA LSD post hoc test, *=$P<0.05$ when compared with the control; CTRL= Control (distilled H_2O 2 ml/kg); LoAP, MiAP and HiAP= Aqueous fruit extract of *P. dactylifera* (250, 500 and 1,000 mg/kg respectively); MCL= $HgCl_2$ (5 mg/kg); Vit C= Vitamin C (100 mg/kg)

Nissl substance reactivity

CV is a qualitative histopathological stain for Nissl bodies (essentially the rough endoplasmic reticulum [RER]) in neuronal cell bodies.[44] Quantification of NS reactivity by measuring tissue staining intensity to a Nissl stain serves as an important tool for objectively grading microscopic images, which could be associated to cellular activity and integrity.[43,84,85] Remarkable difference in staining intensity of M1 layer V neurons is suggestive of treatment-related cytoarchitectural changes. Observed increase in staining intensity with MCL-treated group relative to the control could be associated to increased protein synthesis to accommodate for axonal sprouting in response axonal injury, which is a characteristic of cytoarchitectural pathological change, chromatolysis.[65,86] This finding correlates with histologic observations in this study. Reduced staining intensity relative to the control observed in Vitamin C + MCL-treated and AFPD (1000 mg/kg) + MCL-treated groups is indicative of neurotoxicity. Degenerating neurons stain very poorly, rather than darker, with CV as a result of disassociation of ribosomes from the RER, which occurs in the early stages of cell degeneration.[87] Similar staining intensity relative to the control observed in AFPD (250 and 500 mg/kg)-treated groups could imply cytoarchitectural preservation. The M1 region of the brain is involved in motor processes, and proper regulation of its cellular and structural proteins is critical for its functionality.[88] Thus, AFPD has potentials to preserve neuronal cytoarchitectural features following mercury-triggered histochemical alterations in the M1 region compared to the reference drug, Vitamin C.

Neuroprotective properties of AFPD could be attributed to the potent antioxidant activities of constituent phytochemicals. Antioxidant agents play a critical role in the protection from deleterious exogenous substances in biological systems.[51,89,90] Saponins act as neuroprotective agents via pathways such as anti-inflammatory, antioxidant, and antiapoptotic pathways.

Modulation of neurotransmitters, tau phosphorylation inhibition, and neural networks regeneration has been reported as possible mechanisms.[50,91] Polyphenolics, including flavonoids, found in plants have been reported to be strong ROS scavengers, antioxidants, and protectors of neurons from lethal damage and are able to chelate metal ions.[92-94] Flavonoids exert diversity of neuroprotective actions within the brain which includes potential to protect neurons against injury triggered by neurotoxins and ability to suppress neuroinflammation.[95,96] Thus, AFPD could benefit the brain through its neuroprotective property, protecting neurons from the actions of ROS by utilizing its antioxidant property,[97-99] and could be a potential candidate for application in the management and treatment of ROS-induced neurodegenerative diseases.

Conclusion

AFPD, especially at dose 500 mg/kg, is potentially efficacious in ameliorating mercury-triggered microscopic alterations in the M1 cerebral region of Wistar rats. The neuroprotective property of AFPD is comparable to the reference drug and could be attributed to antioxidant properties of constituent phytochemicals.

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Conflicts of interest

There are no conflicts of interest.

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