Journal of Anatomical Sciences

Email:anatomicaljournal@gmail.com

J Anat Sci 12 (1)

Neurobehavioral and Microscopic Assesements of Methanol Fruit Extract of *Phoenix Dactylifera* on Lead Acetate-Induced Cerebellar Changes in Wistar Rats

[1,6]Henry R, *[1,6]Agbon AN, [1]Yakubu U, [1]Simon D, [1]Shuaib YM, [1,6]Sule H, [2,7]Yahaya MH, [3]Usman IM, [4]Ivang AE, [1]Oladimeji O, [5,8]Bobbo KA and [5,9]Mahdi O

[1]Neuroanatomy and Neurosciences Research Unit, Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, Ahmadu Bello University, Zaria, Nigeria.
[2]Department of Human Anatomy, Faculty of Basic Medical Sciences, Yusuf Maitama Sule University, Kano, Nigeria
[3]Human Anatomy Department, Faculty of Biomedical Sciences, Kampala International University, Uganda.
[4]Clinical Anatomy Unit, Department of Clinical Biology, College of Medicine and Pharmacy, University of Rwanda.
[5]Department of Human Anatomy, College of Medical Sciences, Gombe State University, Gombe, Nigeria
[6]Microscopy and Stereology Research Unit, Department of Human Anatomy, Ahmadu Bello University, Zaria, Nigeria.
[7]Department of Anatomy and Neuroscience, Graduate School of Medicine, Osaka University, Japan.
[8]UPM-MAKNA Cancer Research Laboratory, Institute of Bioscience, Universiti Putra Malaysia, Selangor, Malaysia.
[9]Department of Human Anatomy, Neuroscience and Behavioural Laboratory, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Malaysia

Corresponding Author: Henry R
E-mail: henryrachel99@gmail.com; +234(0)7031663971

ABSTRACT

Lead is a highly toxic substance, exposure to which can produce a wide range of adverse health effects on humans including neurological conditions. *Phoenix dactylifera* (date palm) is of vast medicinal and nutritious values. This study assessed the neuroprotective effect of methanol fruit pulp extract of *Phoenix dactylifera* (MFPD) following lead acetate (PbA)-triggered cerebellar changes in Wistar rats. Twenty-four rats were divided into six groups (n=4): Control group administered distilled water (2ml/kg); PbA (120mg/kg) group; Vitamin C (100mg/kg)+PbA group; MFPD (250 mg/kg)+PbA group, MFPD (500mg/kg)+PbA group and MFPD (1000mg/kg)+PbA group. Treatments were via oral route for a period of 14 days. Neuroprotective property of MFPD was evaluated using neurobehavioral assessment (beam walking performance for motor coordination and balance) and microscopic examination of cerebellar cortex applying histological and histochemical staining techniques and quantification of Nissl substance distribution (NSD) using a computer running image analysis software (ImageJ, NIH, US). In PbA-treated group, results revealed neurodegenerative changes as remarkable ($p<0.05$) motor coordination impairment as altered beam walk performance and cortical cerebellar cytoarchitectural distortions including pyknotic nuclei, perineuronal vacoulation and satellitosis and remarkably reduced NSD. However, administration of *MFPD* remarkably ameliorated *PbA*–induced motor coordination impairment by reduced latency time to perform the beam walking task and ameliorated cerebellar changes by preserving cortical cerebellar cytotoarchitecture, especially with MFPD 500mg/kg-treatment. Findings suggest that MFPD possess neuroprotective activity which could be attributed to antioxidant properties of its constituent phytochemicals and, could be of potential benefit in the treatment and/ or management of heavy metal-triggered neurodegenerative-related disorders.

Keywords: Antioxidant, Beam walking, Cytoarchitecture, Neurodegeneration, Neuroprotection

INTRODUCTION

Heavy metals occur as natural constituents of the earth crust and are persistent environmental contaminants since they cannot be degraded or destroyed. Although these metallic elements are lacking in abundance, they are not lacking in significance[1, 2]. Some well-established toxic metallic elements are: arsenic, cadmium, mercury and lead [3, 4, 5]. Lead is a widely spread toxic metal found in the environment and of potential danger to human health due to its multifaceted action with a broad range of physiological and biochemical dysfunctions [6, 7, 8]. Exposure to lead is unavoidable as it occurs through many routes including contaminated air, water, soil, food, and consumer products. Compared to other organ-systems, the central nervous system appears to be the most sensitive and chief target for lead-triggered toxicity. [9, 10, 11]

The cerebellum is a region of the brain that plays an important role in motor control. [12, 13] Cerebellar-related injuries or pathologies clinically presents with neurodegenerative movement disorders in fine movement, equilibrium, posture, and motor learning. [14, 15] Cerebellar dysfunction may occur in association with exposure to a wide variety of environmental neurotoxins including heavy metals such as lead. [16, 17, 10]

The role of traditional medicine in the management and treatment of health related issues are invaluable on a global scale. [18, 19, 20] Recently, scientists have begun investigating the biological activities of natural agents, especially medicinal plants, including their benefits as neuroprotective agents. [21, 22, 23] Dates (*Phoenix dactylifera*) are one of the members of the palm family *Arecaceae*, or *Palmae* [24]. *P. dactylifera* fruits are important component of diet in the arid and semiarid regions of the world, [25] a good source of energy, vitamins and minerals such as, phosphorus, iron, potassium and calcium. [26, 27] Folkorically, *P. dactylifera* is used in the treatment of various ailments which include memory disturbances, fever, inflammation, paralysis, loss of consciousness and nervous disorders. [25, 28, 29]

Lately, several pharmacological investigations have been conducted on *P. dactylifera* and have demonstrated health beneficial properties in *in vitro* and *in vivo* models. [30, 31, 32, 33] Thus, this study evaluated the neuroprotective effect of methanol fruit pulp extract of *P. dactylifera* (MFPD) against lead acetate-triggered cerebellar changes in an experimental animal model.

MATERIALS AND METHODS

Plant Materials: Dried *Phoenix dactlylifera L.* (date palm) fruits were obtained from a local (Samaru) market in Zaria, Kaduna State, Nigeria. The plant was authenticated with a Specimen Voucher Number of 7130 in the Herbarium Unit of Department of Botany, Faculty of Life Sciences, Ahmadu Bello University (ABU), Zaria.

Extract Preparation and Phytochemical Screening: Preparation of methanol fruit pulp extract of *P. dactylifera* (MFPD) and phytochemical screening was carried out in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, ABU, Zaria. The method of maceration for extraction was adopted and briefly described as follows:

The pulp (mesocarp) of the dried *P. dactylifera* fruits were manually separated from the pits (seeds) and pulverized into powder using laboratory mortar and pestle. About 250 g of the powder was soaked in 2 liters of absolute methanol in a conical flask. After 24 hours, the solution (a mixture of *P. dactylifera* fruit powder and methanol) was filtered using a filter rag and funnel. The filtrate was allowed to settle for a while, followed by decantation of the supernatant. The supernatant was gently heated (steamed) to dryness in an evaporating dish (Royal Worcester; made in England) using H-H Thermometer Water Bath (Mc Donald Scientific International– 22050Hzl.0A International Number) at 60ºC. A yield of 18.8% of the extract was obtained.. Qualitative phytochemical screening of MFPD for secondary metabolites was carried out according to the method described by Trease and Evans. [34]

Experimental Animals: A total of twenty-four Wistar rats (weighing 120-130 g) were obtained from the Animal House of the Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, ABU Zaria and housed in new wired cages in same facility to acclimatize for a week before the commencement of the experiments under standard laboratory conditions, 12 hours light-dark cycle. The rats were all given rat chow and water *ad libitum.* The rats were categorized into treatment groups and were administered distilled water/ MFPD/ lead acetate/ Vitamin C/ in addition to water and food.

Drugs: Lead acetate (PbA) was obtained from Steve Moore Chemicals, Zaria and used as neurotoxicant for the experiment. The product is manufactured by British Drug Houses Limited (BDH) Laboratory Chemicals Division, Poole, England.

Ascorbic acid (Vitamin C), an established antioxidant, [35] was obtained from Al-Husna Pharmaceuticals, Zaria and used as reference drug to evaluate the activity of MFPD. The product is manufactured by Emzor Pharmaceutical Industries Limited, Lagos, Nigeria.

Experimental Protocol: Twenty-four rats were divided into six groups of four rats each. The control group was administered distilled water (2 ml/kg); another group was administered PbA (120 mg/kg); another group was treated with Vitamin C (100 mg/kg)+PbA (120 mg/kg); three other groups were treated with MFPD (250 mg/kg)+PbA (120 mg/kg), MFPD (500 mg/kg)+PbA (120 mg/kg) and MFPD (1000 mg/kg)+PbA (120 mg/kg). All administrations were via oral route for a period of two weeks. This study

was conducted according to the guidelines of the institutional research ethics committee, Ahmadu Bello University Committee on Animal Use and Care.

Physical Observation: During the experimental period, the rats were observed for any change in physical activity and behavioral pattern such as eating habit and agility. Absolute body weights before (initial weight, IW) and after (final weight, FW) the experiment were measured using a digital weighing scale and compared. Weight change difference was computed by subtracting initial from final body weight (FW – IW) and compared statistically.

Neurobehavioral Studies: The beam walking test was used to assess for motor coordination and balance of rats by allowing the rats to traverse a narrow beam to reach an enclosed safety box. A modification of the beam walking apparatus as reported by Carter *et al.* [36] was adopted. The beam walking apparatus consisted of an elevated platform connected to a 100 cm long wood beam with a width of 3 cm. The beam was placed horizontally, 50 cm above the floor surface with one end mounted to a narrow support connected to start platform 10 by 10 cm [37] and the other end attached to a goal box (20 by 20 by 20 cm). The start point was placed by a bright light source to motivate the rats to traverse the beam. The beam walking performance (BWP) was assessed by latency time to traverse the beam recorded in seconds (s) and the number of foot (hind limb or paw) slips.

Prior to the test (before treatment), rats were habituated to the beam walking apparatus daily for 3 days at the Neuroanatomy and Neurosciences Research Laboratory, Department of Human Anatomy, ABU, Zaria. Rats were tested after 14 days of treatment.

Histological and Histochemical Studies: At the end of the experiment, rats were euthanized using chloroform anaesthesia and whole brain harvested and fixed in Bouin's fluid for 72 hours. Fixed brains were processed for light microscopic examination using histological paraffin sections stained with Hematoxylin and Eosin (H&E) and Cresyl fast violet (CFV) stains for demonstrating the general histological features, neuronal cell bodies and cytoplasmic Nissl substances.

Histological tissue processing was conducted in the Histology Unit, Department of Human Anatomy, ABU, Zaria and light microscopy (*HM-LUX, Leitz Wetzlar, Germany*) and micrography (*using a digital microscopic camera, MA 500 AmScope®, USA*) conducted in the Microscopy and Stereology Research Laboratory of the same facility. Tissue sections of cortical cerebellar region (*See* Figure 1) were blindly examined for histopathalogical changes.

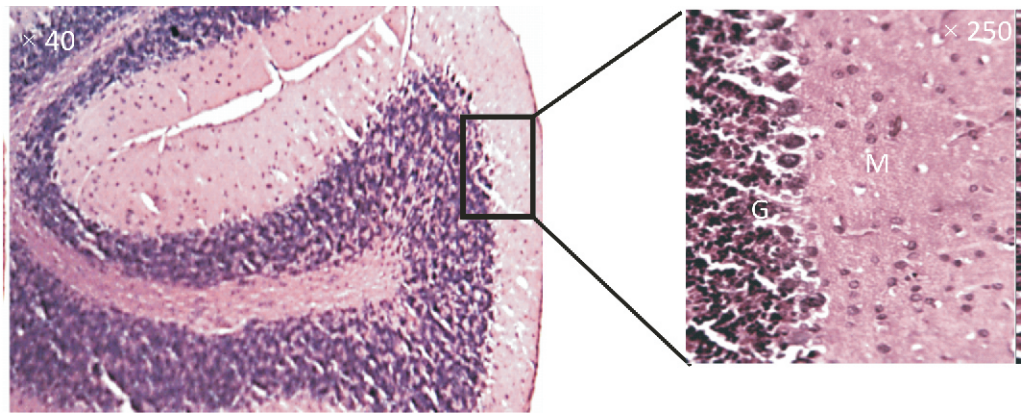

Figure 1: Section of the cerebellum of Wistar rat indicating cortical region. H and E stain. Molecular cell layer (M); and Granular cell layer (G).

Quantification of Nissl Substance Distribution: Nissl substance (NS) distribution in cortical cerebellar neurons was measured by the quantification of NS reactivity to CFV, an excellent neuronal (cell body-specific) stain which is useful for the demonstration of NS in neurons. [38,39]

Quantification of NS reactivity involved measuring the staining intensity from CFV stained micrographs (digital microscopic images) using a computer running image analysis software (ImageJ, NIH, US) according to the manufacturer's instruction [40]. The ImageJ region of interest (ROI) manager tool for analysis of specific areas of the micrographs was employed to limit bias values resulting from non-identical image quality (image acquisition setting and exposure times) . [41, 42] The modal gray values for three ROI were obtained, means computed and analyzed (*See* Figure 2).

Data Analysis: Results obtained were expressed as mean ± S.E.M and presence of significant differences among means of the groups were determined using one-

way ANOVA with least significant difference (LSD) *post hoc test* for significance. Paired *t*-test was employed for the comparisons of means as appropriate. Values were considered significant when $p < 0.05$. Data were analyzed using the statistical software, Statistical Package for the Social Sciences (SPSS *version* 18.0; PASW Statistics for Windows, SPSS Inc., Chicago, USA) and Microsoft Office Excel 2013 for charts.

RESULTS

Phytochemical Screening: Phytochemical analysis of MFPD revealed positive and negative reactions for some secondary metabolites as indicated in Table 1.

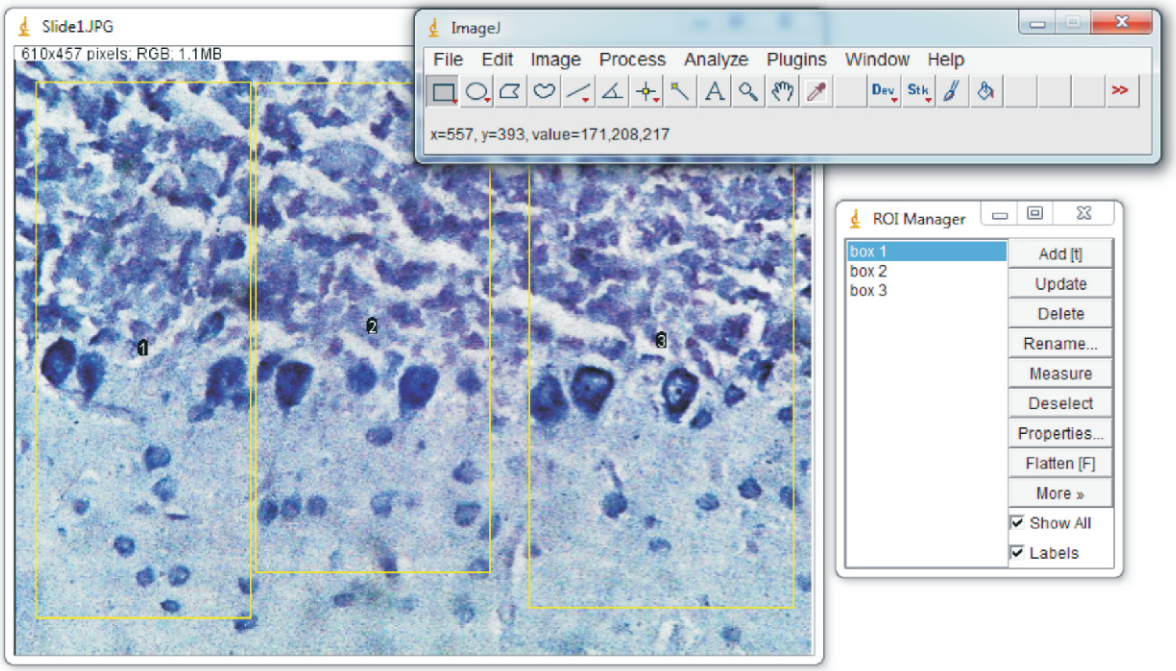

Figure 2: Quantification of Nissl substance reactivity using cresyl fast violet stained micrographs with a computer running image analysis software (ImageJ, NIH, USA)

Table 1: Phytochemical constituents of MFPD

Constituents	Inference
Alkaloid	-
Anthraquinone	–
Carbohydrate	+
Cardiac glycoside	+
Flavonoid	+
Saponin	+
Steroid and tripenone	+
Tannins	+

+ = Present, – = Absent; MFPD= Methanol fruit extract of *Phoenix dactylifera*

Physical observation: Physically observed changes in behavioural patterns revealed normal physical activities in the control group while, reduced agility as sluggishness and weakness were manifested by the PbA-treated groups.

Comparison of the IW and FW revealed increase in all the groups, especially ($p< 0.05$) in the control, MFPD (250 mg/kg) + PbA- and MFPD (500 mg/kg) + PbA-treated groups (Figure 3a). Relative to the control, comparison of weight change difference revealed remarkable ($p<0.05$) difference in all the groups, except in MFPD (250 mg/kg) + PbA-treated group (Figure 3b).

Neurobehavioral Studies: The BWP with respect to latency time to cross the beam, revealed remarkable ($p<0.05$) difference in Vit C + PbA-, MFPD (500 mg/kg) + PbA- and MFPD (1,000 mg/kg) + PbA-treated groups when latency time at pre-treatment and day-14 treatment were compared (Figure 4a). BWP at day-14 treatment showed decrease ($p<0.05$) latency time in Vit C + PbA-, MFPD (500 mg/kg) + PbA- and MFPD (1,000 mg/kg) + PbA-treated groups when compared to the control and PbA-treated group (Figure 4b).

Relative to number (frequency) of foot slips on the beam walk apparatus, increased ($p<0.05$) frequency was observed only in PbA-treated group. While, no foot slips - to - reduced frequency ($p<0.05$) was observed in the MFPD (250 mg/kg, 500 mg/kg and 1000 mg/kg) + PbA-treated groups. (Figure 4c). BWP at day-14 treatment showed increase ($p<0.05$) in foot slip frequency with PbA-treated group when compared to control. While, Vit C + PbA- and MFPD (250 mg/kg, 500 mg/kg and 1000 mg/kg) + PbA-treated groups revealed reduced frequency when compared to PbA-treated group (Figure 4d).

Figure 3a: Comparison of absolute body weight of Wistar rats

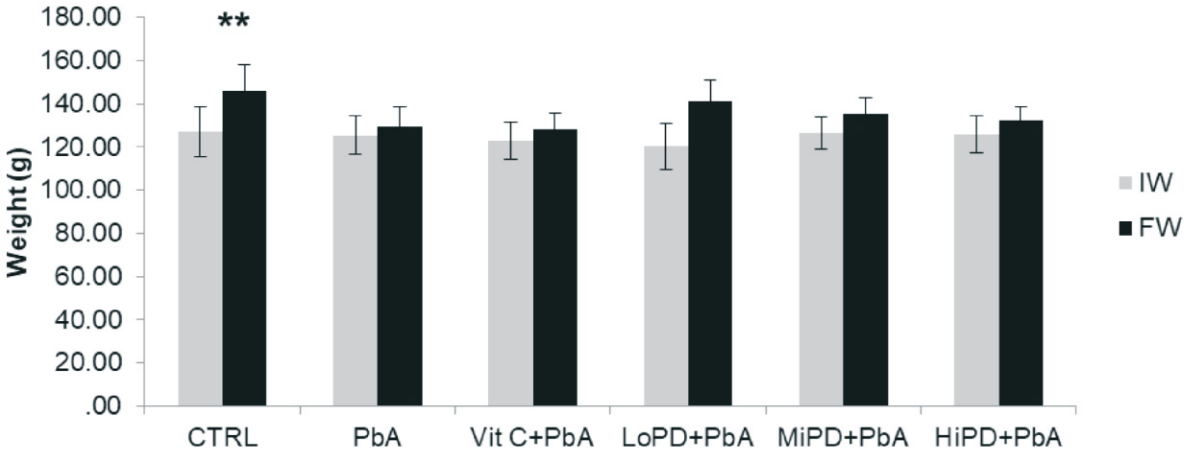

Figure 3a: Comparison of absolute body weight of Wistar rats

n=4; mean± SEM, paired *t*-test; *= $p<0.05$, **= $p<0.01$ significant difference when IW and FW was compared. CTRL= Control (distilled H_2O 2 ml/kg); PbA= Lead acetate (120 mg/kg); Vit C= Vitamin C (100 mg/kg); LoPD= 250 mg/kg MFPD; MiPD= 500 mg/kg MFPD; HiPD= 1000 mg/kg MFPD; MFPD= Methanol fruit extract of *Phoenix dactylifera*; IW= Initial weight; FW= Final weight.

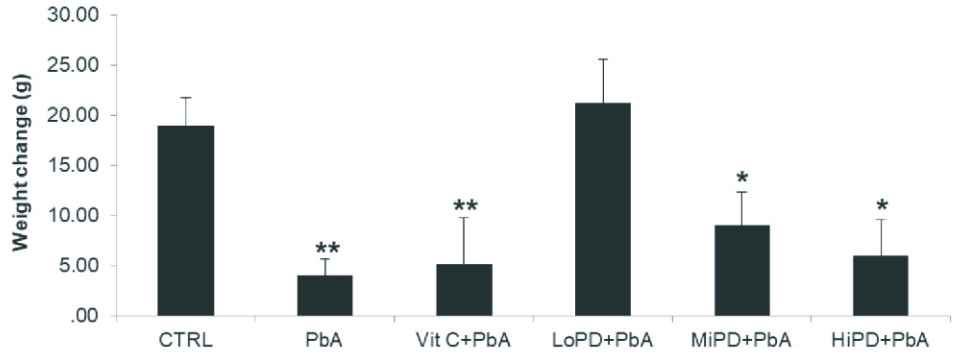

Figure 3b: Comparison of weight change difference of Wistar rats

n=4; mean± SEM, one-way ANOVA; *= p<0.05, **= p<0.001 significant difference when weight change difference was compared to control. CTRL= Control (distilled H_2O 2 ml/kg); PbA= Lead acetate (120 mg/kg); Vit C= Vitamin C (100 mg/kg); LoPD= 250 mg/kg MFPD; MiPD= 500 mg/kg MFPD; HiPD= 1000 mg/kg MFPD; MFPD= Methanol fruit extract of *Phoenix dactylifera*.

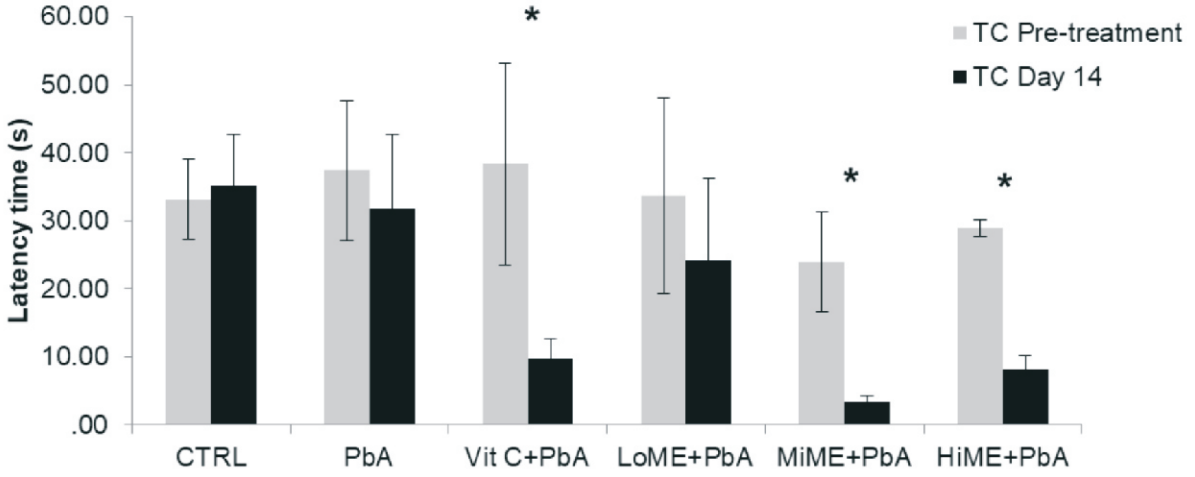

Figure 4a: Comparison of beam walk performance (latency time) in Wistar rats

n=4; mean ± SEM, paired *t*-test; *= p<0.01 significant difference when TC pre-treatment and tcday 14 treatment. CTRL= Control (distilled H_2O 2 ml/kg); PbA= Lead acetate (120 mg/kg); Vit C= Vitamin C (100 mg/kg); LoPD= 250 mg/kg MFPD; MiPD= 500 mg/kg MFPD; HiPD= 1000 mg/kg MFPD; MFPD= Methanol fruit extract of *Phoenix dactylifera*; TC= Time to cross the beam.

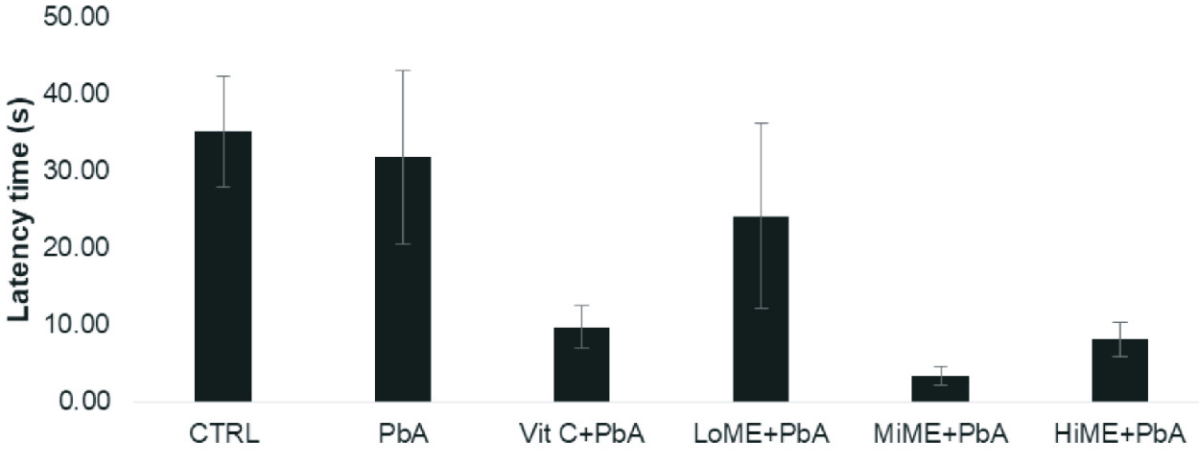

Figure 4b: Effect of MFPD on Wistar rats exposed to PbA in beam walk performance (latency time) at day-14 treatment

n=4; mean ± SEM, one-way ANOVA; *= p<0.01 significant difference when compared to the control; a= p<0.01, significant difference when compared to the PbA-treated group. CTRL= Control (distilled H_2O 2 ml/kg); PbA= Lead acetate (120 mg/kg); Vit C= Vitamin C (100 mg/kg); LoPD= 250 mg/kg MFPD; MiPD= 500 mg/kg MFPD; HiPD= 1000 mg/kg MFPD; MFPD= Methanol fruit extract of *Phoenix dactylifera*.

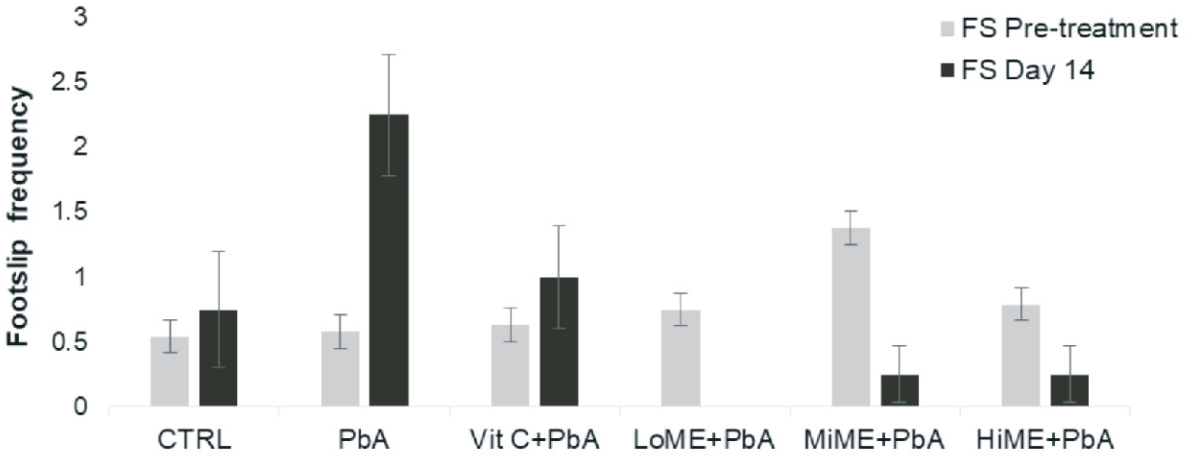

Figure 4c: Comparison of beam walk performance (foot slip frequency) in Wistar rats

n=4; mean ± SEM, paired *t*-test; *= p<0.05 significant difference when comparing pre-treatment and day 14 treatment. CTRL= Control; PbA= Lead acetate; Vit C= Vitamin C; LoME= 250 mg/kg MFPD; MiME= 500 mg/kg MFPD; HiME= 1000 mg/kg MFPD; MFPD= Methanol fruit extract of *Phoenix dactylifera*; FS= foot-slips.

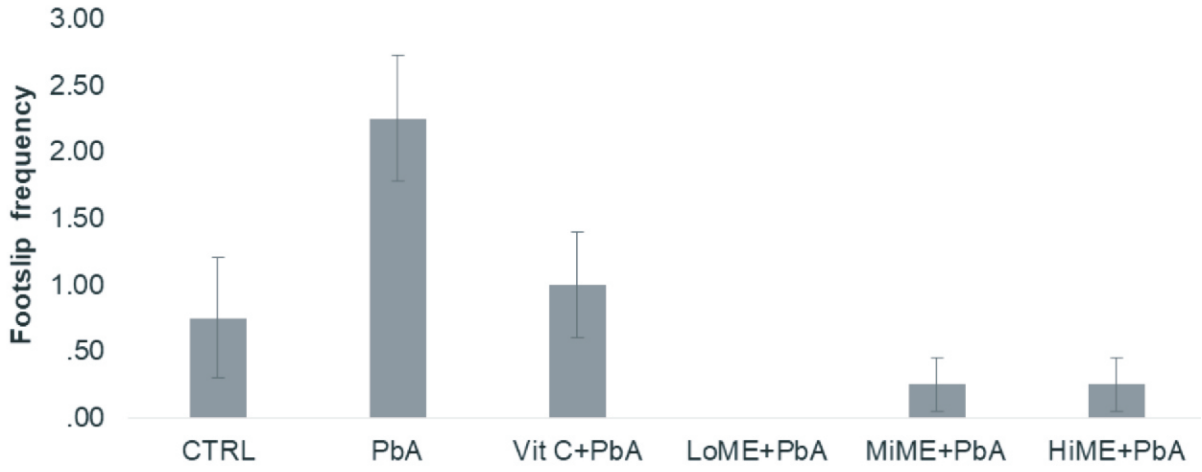

Figure 4d: Effect of MFPD on Wistar rats exposed to PbA in beam walk performance (foot slip frequency) at day-14 treatment

n=4; mean ± SEM, one-way ANOVA; *= p<0.01 significant difference when compared to the control; a= p<0.01, significant difference when compared to the PbA-treated group. CTRL= Control; PbA= Lead acetate; Vit C= Vitamin C; LoME= 250 mg/kg MFPD; MiME= 500 mg/kg MFPD; HiME= 1000 mg/kg MFPD; MFPD= Methanol fruit extract of *Phoenix dactylifera*.

Histological and Histochemical Examination: Histological and histochemical examinations of cerebellar sections using H&E stains for demonstrating the general histological features and CFV stain for neuronal cell bodies and cytoplasmic Nissl substance, respectively, revealed the following:

The cerebellar sections of the rats in the control group showed normal cytoarchitecture with the three distinctive cortical layers: an outer molecular layer with distinct neurons, an intermediate Purkinje cell layer and an inner granular layer. Histochemical (CFV) staining revealed normal features of neuronal cell bodies with optimal staining intensity for Nissl substances (Figure 5a and 6a).

The section of the cerebellar cortex of rats treated with PbA showed neurodegenerative changes as distortions in the cytoarchitecture including perineuronal vacuolation and satellitosis, cytoplasmic shrinkage and pyknotic nuclei/ necrosis of Purkinje cells. CFV staining revealed Purkinje cell shrinkage and reduced Nissl substance staining intensity, especially in the granular cell layer when compared to the control (Figure 5b and 6b).

Examination of the cerebellar cortex of Vitamin C- and MFPD-treated rats revealed cytoarchitectural preservation as mild distortions in the cytoarchitecture when compared to the pathological changes observed in the PbA-treated rats. The cytoarchitectural features of the Vitamin C + PbA-treated group was relatively normal compared to the control (Figure 5c and 6c). MFPD (250 mg/kg, 500 mg/kg and 1000 mg/kg) + PbA-treated groups showed mild distortions - to-relatively normal cytoarchitectural features of the cerebellar cortex compared to the control (Figures 5d - f and 6d - f).

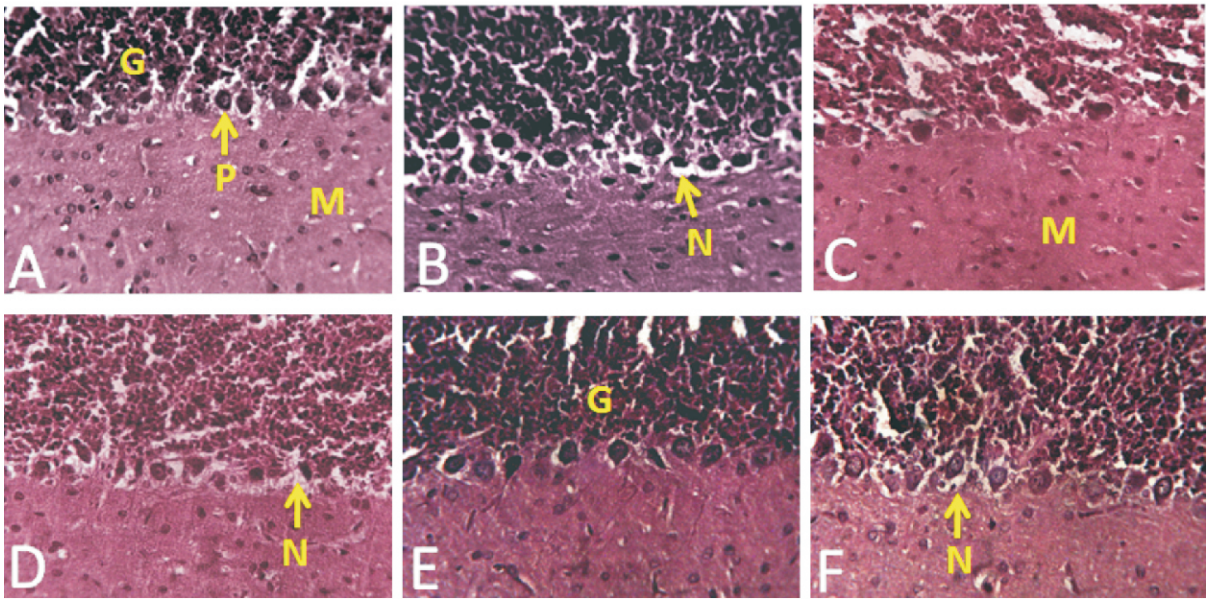

Figure 5: Micrograph of cerebellar cortex section of Wistar rats. H and E stain (Mag ×250)
A= Section of cerebellar cortex of the control (distilled H_2O 2 ml/kg) with normal histoarchitecture. G= Granular layer; P= Purkinje cell; M= Molecular layer
B= Section of cerebellar cortex of PbA (120 mg/kg)-treated group with distortion in the histoarchitecture. N= Neurodegeneration- shrinkage of Purkinje cells, *perineuronal vacuolation* and *satellitosis.*
C= Section of cerebellar cortex of Vitamin C (100 mg/kg) + PbA-treated group with relatively normal histoarchitecture. M= Molecular layer.
D= Section of cerebellar cortex of MFPD (250 mg/kg) + PbA-treated group with mild distortion in the histoarchitecture. N= Neuronal degeneration- *perineuronal vacuolation.*
E= Section of cerebellar cortex of MFPD (500 mg/kg) + PbA-treated group with normal histoarchitecture G= Granular layer.
F= Section of cerebellar cortex of MFPD (1000 mg/kg) + PbA-treated group with mild distortion in the histoarchitecture. N= Neuronal degeneration- *perineuronal vacuolation* and *satellitosis.*

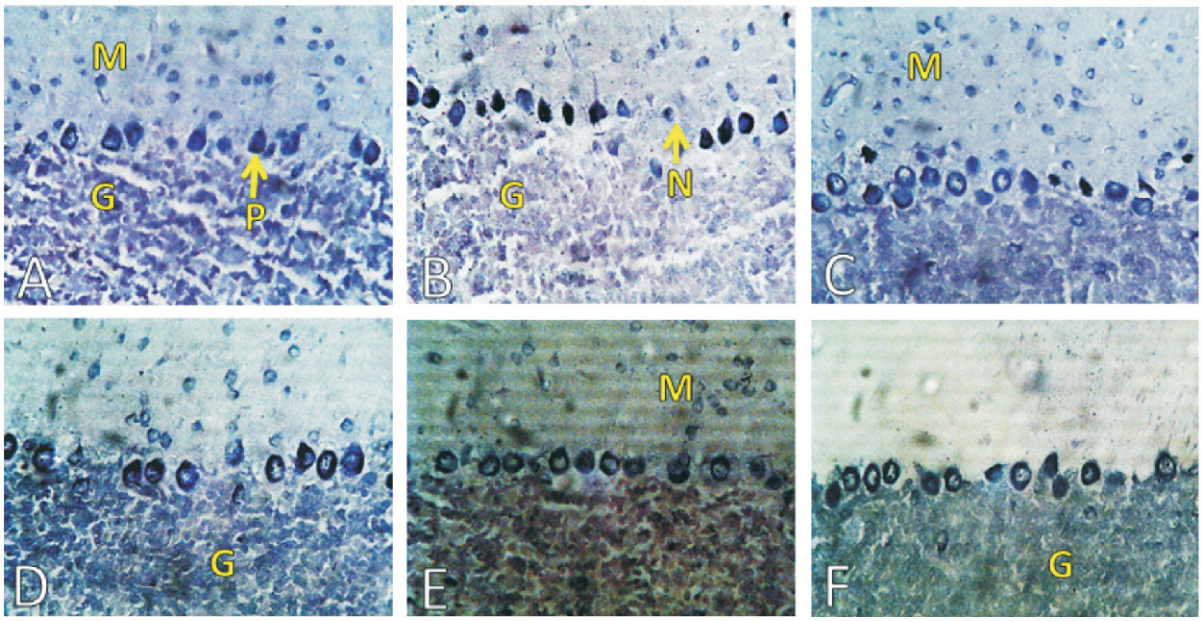

Figure 6: Micrograph of Cerebellar Cortex section of Wistar rats. CFV Stain (Mag ×250)
A= Section of cerebellar cortex of the control (distilled H_2O 2 ml/kg) with normal cytoarchitecture of neuronal cell bodies

expressing optimal staining intensity of cytoplasmic Nissl substances. G= Granular layer; P= Purkinje cell; M= Molecular layer.
B= Section of cerebellar cortex of PbA (120 mg/kg)-treated group with distorted cytoarchitecture expressing reduced staining intensity of Nissl substances. G= Granular layer; N= Neurodegeneration- *perineuronal vacoulation.*
C= Section of cerebellar cortex of Vitamin C (100 mg/kg) + PbA-treated group with relatively normal cytoarchitecture expressing optimal staining intensity of Nissl substances. M= Molecular layer.
D= Section of cerebellar cortex of the MFPD (250 mg/kg) + PbA-treated group with relatively normal cytoarchitecture expressing optimal staining intensity of Nissl substances. G= Granular layer
E= Section of cerebellar cortex of MFPD (500 mg/kg) + PbA-treated group with relatively normal cytoarchitecture expressing reduced staining intensity of Nissl substances. M= Molecular layer.
F= Section of cerebellar cortex of MFPD (1000 mg/kg) + PbA-treated group with relatively normal cytoarchitecture expressing optimal staining intensity of Nissl substances. G= Granular layer.

Nissl Substance Distribution: Analysis of the staining intensity of NS to quantify NS distribution in cortical cerebellar neurons revealed remarkable ($p<0.05$) difference in PbA- and MFPD (500 mg/kg and 1000 mg/kg) + PbA-treated groups when compared to the control (Figure 7).

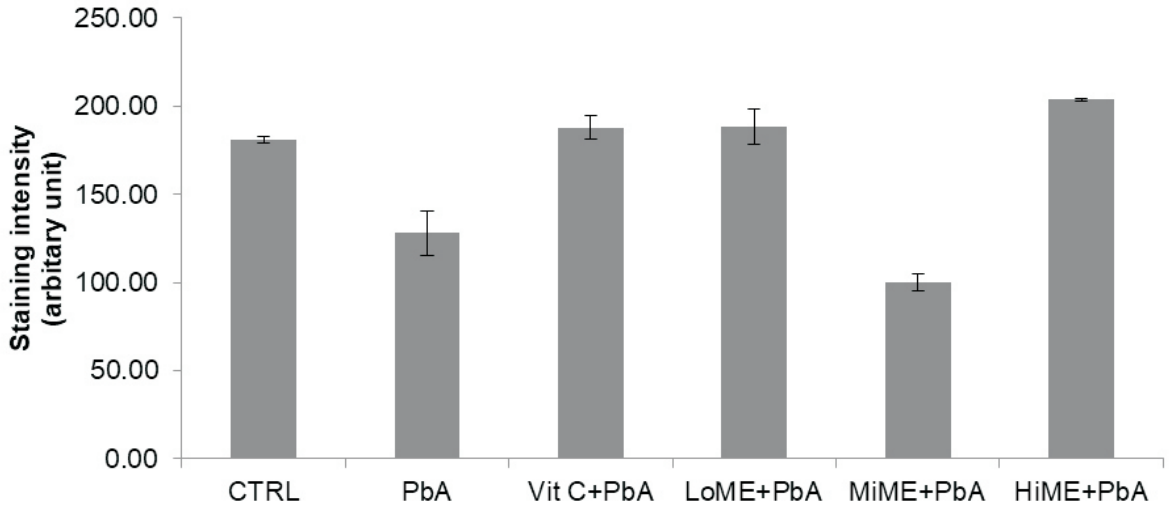

Figure 7: Effect of *P. dactylifera* on Nissl substance distribution in the cortical cerebellar **neurons of Wistar rats.** Mean ± SEM; One way ANOVA; ***=** $p<0.05$ significant difference *when compared to the control.* CTRL= Control (distilled H_2O 2 ml/kg); PbA= Lead acetate (120 mg/kg); Vit C= Vitamin C (100 mg/kg); LoPD= 250 mg/kg MFPD; MiPD= 500 mg/kg MFPD; HiPD= 1000 mg/kg MFPD; MFPD= Methanol fruit extract of *Phoenix dactylifera*.

DISCUSSION

In this study, phytochemical analysis of MFPD was conducted and neuroprotective effect of MFPD on cerebellar cortex following lead acetate exposure to rats was assessed applying physical, neurobehavioral and light microscopic approaches. Phytochemical screening of MFPD revealed the presence of flavonoids, tannins, saponins and steroid which have been reported to have neuroprotective activity in *in vivo* and *in vitro* models. [43, 44, 45]

Decreased physical activity in behavioral patterns exhibited by PbA-treated rats is suggestive of treatment-related toxicity. Finding is in line with reports on drug related toxicity in association with altered physical activity which could manifest as sluggishness and reduced agility or playfulness. [11, 42]

A responsive indicator of the general wellbeing of an experimental animal model is the status of its absolute body weight. [46] Remarkable increase in the trend of body weight (*from initial to final*) across the study period in MFPD-treated groups and difference in weight change could be associated to treatment related response. Several experimental studies have associated changes in absolute body weight as a useful indicator that reflects the deleterious effects of chemicals and drugs. [47, 48, 49] Exposure to noxious substances, including heavy metals like lead, in *in vivo* studies have been implicated in altered normal growth pattern consequent to disruptions in physiological and biochemical processes of biological systems. [50, 51] However, remarkably increased body weight observed with MFPD treatment could be attributed to essential nutrients with high caloric value, such as carbohydrates and lipids present in *Phoenix dactylifera.* [52, 53] *Phoenix dactylifera* fruit has been reported as a good source of energy and rich in nutrients. [54, 27]

The cerebellum plays a critical role in the coordination of voluntary movements with respect to timing, gait and balancing. [55, 56] In the study, motor coordination and

balance was assessed based on BWP in certain parameters (latency time to cross the beam and foot-slips frequency) across the period of study. Decreased observation in these parameters indicate improved BWP which could be associated to optimal motor coordination and balance functionality, while increased parameter values implied functional deficit. [31, 58, 59] Observed altered BWP as remarkable increase in foot slips frequency is suggestive of functional deficit in motor coordination and balance. Several studies have reported the involvement of heavy metals including lead in cerebellar toxicity and dysfunction which is usually accompanied by impaired motor coordination as different forms of ataxia and unstable gait. [60, 61, 62, 51] Finding is in consistence with Flora et al. [63] who reported impairment in motor coordination as a result of lead intoxication in rats. Established pathophysiology of lead intoxication on neuronal cells is by free radical generation that results in oxidative stress and excitotoxicity due to the presence of glutamatergic receptors. [60, 64, 65] Thus, ameliorating excitotoxicity and/ or oxidative stress is critical in neuroprotection. [66,67,68]

Strikingly improved BWP as decreased parameter values observed in the groups administered Vit C and MFPD is indicative of preservation of normal function and improvement in motor coordination and balance functionality. Ascorbic acid has been reported to participate in several beneficial functions including antioxidant protection and maintenance of motor coordination skills [69, 70, 35]. Polyphenolic contents of *P. dactylifera* including flavonoids have been reported to have antioxidant activity and are excellent scavengers of reactive oxygen species. [71,72,73] Improvement in motor coordination and balance observed with MFPD-treatment could be attributed to free radical scavenging activity and amelioration of excitotoxicity by acting as glutamate antagonists. This finding is consistent with the reports of Yusuf *et al.* [74] and Lazarus *et al.* [11] who reported extract of *Phoenix dactylifera* has neuroprotective properties against chemically-induced neurotoxicity.

Neurodegenerative changes are associated with neurotoxicity and neuropathological conditions in different parts of the brain. [75, 76] Neurodegenerative changes; cytoarchitectural distortions including Purkinje cell layer-related perineuronal vacoulations and karyopyknotic necrosis observed in cerebellar sections of the PbA-treated rats could be attributed to lead-triggered neurotoxicity. Findings are in line with the reports on heavy metal compounds demonstrated to induce nervous tissue damage with Purkinje cells most sensitive elements of the cerebellar cortex to these neurotoxins. [61, 74] Lead causes generation of reactive oxygen species (ROS) which results in critical damage to various biomolecules like DNA, enzymes, proteins and membrane based lipids, while simultaneously it impairs the antioxidant defense system. [77,78,65]

Neuroprotection is a term commonly attributed to pharmacological strategies that can prevent, slows down the progression of, or even reverse neurodegeneration. [67, 79, 80] In this study, administration of Vitamin C ameliorated lead-induced cerebellar damage by preservation of cortical cerebellar cytoarchitecture. Result is in accordance with report on the neuroprotective activity of ascorbic acid following heavy metal-induced neuropathological changes in *in vivo* models. [81] Kumar *et al.* [82] and Teleanu *et al.* [35] reported that ascorbic acid participates in several beneficial cellular functions including antioxidant protection by potentiation of endogenous antioxidant defense system and scavenging of free radicals.

Various naturally occurring antioxidants (nutrient antioxidants) like vitamins, flavonoids and herbal antioxidants have been reported for the prevention and treatment of lead-induced toxicity and oxidative stress in particular. These natural agents have the ability to scavenge ROS at molecular level and chelate metal ions, thereby reversing the toxic effects. [83, 19] Observed mild cortical cerebellar neurodegenerative changes in MFPD-treated rats is suggestive of the protective property of MFPD. This finding is in agreement with reports on the neuroprotective effect of *Phoenix dactylifera*. [84, 85, 86] Histological examination revealed optimal neuroprotective property of MFPD with dose 500 mg/kg comparable to the reference drug, Vitamin C.

In light microscopy, CFV staining has affinity for reactivity with basophilic sub-cellular components [39] including the rough endoplasmic reticulum (RER) and free ribosomes which appear as basophilic granular areas (Nissl bodies), thus a useful tool for the quantification of NS distribution in neurohistology. CFV reactivity with degenerating neurons is poor, appearing lighter than darker, as a result of disassociation of ribosomes from the RER associated with stages of neuronal degeneration. [87, 88] In this study, observed remarkable reduction in NS distribution in cortical cerebellar neurons of PbA-treated group could be attributed to the neurodegenerative activity of lead exposure. Finding is in line with the reports of Ajibade *et al.*, [89, 90] reporting loss of NS in cerebellar neurons and nuclear shrinkage following drug-induced toxicity. Observed cytoarchitectural preservation and optimal distribution of NS in Vit C and MFPD-treated groups relative to the control is suggestive of neuroprotective activity which may be involved in the potentiation and/ or modulation of neuronal biochemical processes. Findings thus corroborate histological outcomes in this study.

CONCLUSION

Results of this study suggest that methanol fruit pulp extract of *Phoenix dactylifera* is potentially neuroprotective by ameliorating lead acetate-induced alterations in the cerebellar cortex of Wistar rats. The

neuroprotective property of *P. dactylifera* was relatively similar to that of Vitamin C, and could be attributed to the antioxidant activities of its phytochemical constituents. Thus, *P. dactylifera* could be of potential benefit in the treatment and/ or management of heavy metal-triggered neurodegenerative-related disorders and disease conditions.

CONFLICT OF INTEREST

Authors hereby declare that there is no conflict of interest regarding the publication of this article.

ACKNOWLEDGEMENTS

Authors wish to acknowledge and appreciate the Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, Ahmadu Bello University, Zaria-Nigeria for providing the facilities to conduct this study.

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