

Histological Assessment of the Effect of *Phoenix Dactylifera* (Date Palm) on Cortico-ponto-cerebellar Structures in Paraquat Model of Neurotoxicity

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ABSTRACT

Background: Herbicides like paraquat (PRQ) are widely used as weed control, predisposing humans to a variety of altered neurological functions. The integrity of brain regions, including structures of the cortico-ponto-cerebellar (CPC) pathway, are involved in the homeostasis of motor activities and coordination. Pharmacological studies have demonstrated the health benefits of *Phoenix dactylifera*. This study evaluated the ameliorative effect of *P. dactylifera* fruits pulp extract against PRQ-triggered CPC changes in Wistar rats using histological assessments. **Materials and Methods:** Twenty-five rats were categorized into five groups (n=5); control was administered 2ml/kg distilled H₂O, another group received 11.35mg/kg PRQ, another received 11.35mg/kg PRQ+10mg/kg *L-dopa* as reference drug, while two other groups received 11.35mg/kg PRQ+500mg/kg aqueous fruit extract of *P. dactylifera* (AFPD) and 11.35mg/kg PRQ+1,000mg/kg AFPD, respectively for twenty-eight days. Histological examination was conducted using Haematoxylin and Eosin stains to assess the ameliorative effect of AFPD following PRQ-induced CPC changes. **Results:** Relative to the control, sections of the PRQ (11.35mg/kg)-treated group revealed neurodegenerative changes as cytoarchitectural distortions in CPC structures manifesting as pyknotic nuclei and neuronal necrosis, chromatolysis, perineuronal vacuolations and satellitosis, and shrunken Purkinje cells and gliosis. However, *L-dopa* (10mg/kg)- and AFPD (500 and 1,000mg/kg) treatments ameliorated PRQ-induced changes by preserving the CPC cytoarchitecture manifesting as mild distortions to relatively normal cytoarchitecture when compared to the control. AFPD revealed neuroprotection comparable to the reference drug, *L-dopa*. **Conclusion:** AFPD has potential ameliorative properties against PRQ-induced CPC neurodegenerative changes in Wistar rats. AFPD neuroprotective properties could be attributed to constituent bioactive compounds with potent antioxidant activities against oxidative stress-related PRQ neuropathological changes.

Keywords: Antioxidant, Histology, Neuroprotection, Oxidative stress.



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INTRODUCTION

Herbicides are widely used in agricultural settings, predisposing humans to continuous exposure resulting in a variety of adverse health conditions and altered neurological functions triggered by herbicides toxicity^[1]. Environmental neurotoxicants including paraquat (PRQ) are commonly used worldwide as weed control^[2]. It is a member of the bipyridylum family, a broad spectrum herbicides^[3]. Substantial studies from animal models braced by clinical-pathological reports have implicated PRQ as a potential neurotoxicant following exposure^[4,5]. Its mechanism of toxicity is via the redox cycle, generating oxidative stress in the mitochondria of cells that triggers disruption of significant biochemical and physiological processes, cell death, and multiple organ failure^[5]. Several studies have associated PRQ exposure to produce progressive, permanent, and cumulative neurotoxicity with risk of clinical conditions including movement disorders and neurodegenerative diseases like Parkinson's diseases^[6].

The brain plays critical role in the functionality and survival of a biological species. The integrity of different brain regions involved in the homeostasis of motor activities and coordination are critical in the optimal functionality of the biological system. Important elements of such homeostasis are the structures of the cortico-ponto-cerebellar pathway^[7,8]. In the central nervous system (CNS), the cortico-ponto-cerebellar (CPC) structures are integral elements of the corticofugal fibre system forming a great calibre of a group of white matter (fibres) in the basis pontis. The CPC fibres consist of two neuronal circuitries: the cortico-pontine fibres which project to nuclei in the pons and, ponto-cerebellar fibres which decussate at the midline projecting to the cerebellum via the middle cerebellar peduncle. From the cerebral cortex, CPC pathways are the major input towards the cerebellum linking several areas in the cerebral cortex with the cerebellum for movement

coordination and regulation^[8]. Alteration in the basic structures of CPC as a result of exposure to environmental neurotoxins such as PRQ could result in functional deficits with clinical implications.

Phoenix dactylifera (date palm) is a well-known plant, mostly in areas with an almost desert-like climate [9]. In traditional medicine, various parts of *P. dactylifera* are used in the management and treatment of diversities of ailments including neurological conditions. Several pharmacological studies have demonstrated the potential benefits of *P. dactylifera* fruits in *in vitro* and *in vivo* assessments^[10,11]. Recently, several researchers have demonstrated the benefit of *P. dactylifera* as a neuroprotective agent^[12,13]. There is a need to assess the phytotherapeutic potentials of this plant in environmentally triggered neurotoxicity.

This study histologically assessed the ameliorative effect of *P. dactylifera* extract against paraquat induced changes in cortico-ponto-cerebellar structures of Wistar rats.

MATERIALS AND METHODS

Plant Material

Dried date palm fruits ('the yellow medium sized' variety) were collected in Zaria, Kaduna State, Nigeria, and given a Voucher Specimen Number: 21104 after identification in the Herbarium Unit, Botany Department of Faculty of Life Sciences, Ahmadu Bello University (ABU), Zaria. The maceration method described by Agbon *et al.*^[14] for the preparation of aqueous fruit pulp extract of *Phoenix dactylifera* (AFPD) was adopted and, carried out in the Department of Pharmacognosy and Drug

Experimental Animals

Development, Faculty of Pharmaceutical Sciences, ABU, Zaria.

Twenty-five apparently healthy male Wistar rats (100 to 150 g) were procured from the Animal

House, Faculty of Pharmaceutical Sciences, ABU, Zaria. The rats were housed in wired cages in the Animal House, Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, ABU, Zaria, and were allowed to acclimatize for two weeks before the commencement of the study. The housing was according to standard laboratory conditions, dark and light cycles of 12hrs provided and fed, rat chow and water *ad libitum*.

Drugs

□ Paraquat (PRQ)

Paraquat (Paracot® Paraquat Dichloride) was obtained from Muazu and Sons Agrochemical Store in Zaria and used as a neurotoxin in this study. The product is manufactured by Hubei Xianlong Chemical Industry Co, China.

□ Levodopa (*L-dopa*)

Levodopa (Sinemet) tablet was obtained from Rukkayat Pharmaceutical Store in Zaria and used as the reference drug to evaluate the therapeutic property of AFPD. The product is manufactured by Mylan Pharmaceuticals, Inc., Morgantown, USA.

□ Ketamine

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

Experimental Design

Twenty-five rats were categorized into five groups with five rats in each group. The control group was administered distilled H₂O (2 ml/kg), another group was administered PRQ (11.35 mg/kg), another group administered PRQ (11.35 mg/kg)+*L-dopa* (10 mg/kg), while two other groups were administered PRQ (11.35 mg/kg)+AFPD (500 mg/kg) and PRQ (11.35

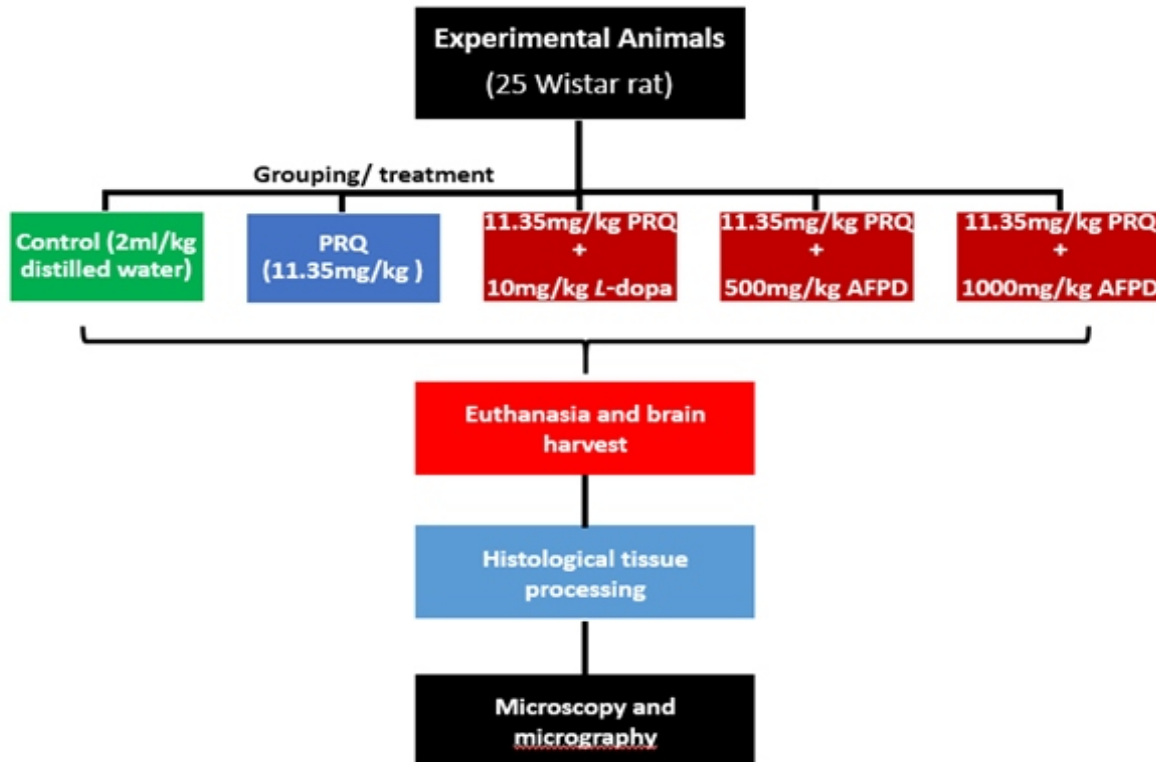


Figure 1a: Experimental design

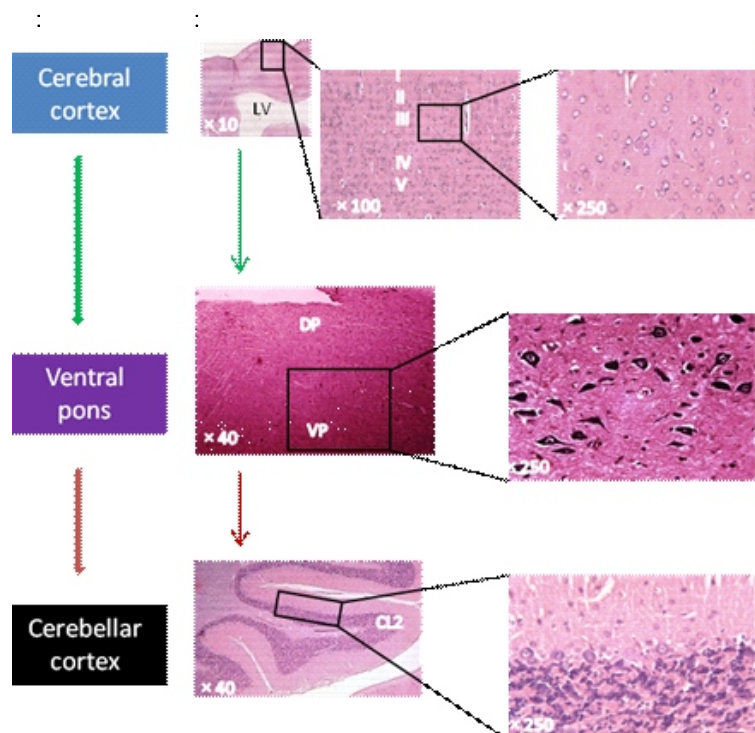


Figure 1b: Structures of the cortico-ponto-cerebellar pathway

LV: Lateral ventricle; I-V: Layers I-V of cerebral cortex; DP: Dorsal pons; VP: Ventral pons; CL2: Cerebellar cortex, 2nd lobule.

mg/kg)+AFPD (1,000mg/kg), respectively. All treatments were via the oral route and lasted for twenty-eight days (Table 1). This study was conducted according to the guidelines of the institutional research ethics committee, Ahmadu Bello University Committee on Animal Use and Care: ABUCAUC/2018/097.

At the end of the experiment, the rats were euthanised using Ketamine (75kg/mg i.p; Kurdi *et al.* [15]) anaesthesia, the rats were decapitated and skull dissected to remove the brain. Harvested brains were fixed in Bouin's fluid for histological processing (see Figure 1a).

Histological Studies

Fixed brain samples were processed using histological techniques by making coronal and sagittal sections to target the cerebrum, pons, and cerebellum. Layer III (external pyramidal layer) of the cerebral cortex, ventral pontine region of the pons, and the CL2 (second cerebellar lobule) of the

cerebellar cortex were primarily examined under the light microscope (Optical Microscope; HM-LUX, Leitz Wetzlar, Germany) (see Figure 1b). Histological paraffin sections were stained with Hematoxylin and Eosin (H&E) stains for demonstration of histoarchitecture in the Histology Unit, Department of Human Anatomy, ABU, Zaria. Microscopy and micrography (using Digital microscopic camera, MA 500 AmScope®, USA) were conducted in the Microscopy and Stereology Research Laboratory of the same facility.

RESULTS

In this study, histological examination of the cerebral cortex (layer III; external pyramidal layer), ventral pons, and cerebellar cortex (CL2; second cerebellar lobule) of Wistar rats were conducted and the following features were observed:

The cerebral cortex of the control showed normal histoarchitectural features with characteristic six (I - VI) layers of the cerebral cortex. In particular, layer III revealed stellate and pyramidal cells with well-outlined cytoplasm, prominent nuclei, and relatively dispersed neuroglia (See Figure 1; only layers I - V are demonstrated in the figure). Relative to the control, the section of the PRQ (11.35 mg/kg)-treated group showed neurodegenerative changes as cytoarchitectural distortions: pyknotic nucleus/ necrosis, chromatolysis, perineuronal vacuolation, and satellitosis. The *L-dopa* (10 mg/kg)- and AFPD (500 mg/kg)-treated groups revealed mild distortion of the cytoarchitecture as perineuronal vacuolation and satellitosis. AFPD (1,000 mg/kg) showed relatively normal cytoarchitecture when compared to the control (Figure 2).

Section of the pons revealed two distinct pontine regions; ventral and dorsal (See Figure 1b). The ventral pontine region of the control group revealed normal cytoarchitecture of stellate cells and characteristic large pontine neurons with distinguishable nuclei and well-preserved cytoplasm, radiating neuronal processes. The ventral pontine region of the PRQ (11.35 mg/kg)-treated group showed distortion in cytoarchitecture as perineuronal vacuolation and gliosis. The *L-dopa* (10 mg/kg)- and AFPD (500 mg/kg)-treated groups revealed relatively normal cytoarchitecture, while the AFPD (1,000 mg/kg) showed mild cytoarchitectural distortion as perinuclear vacuolation when compared to the control (Figure 3).

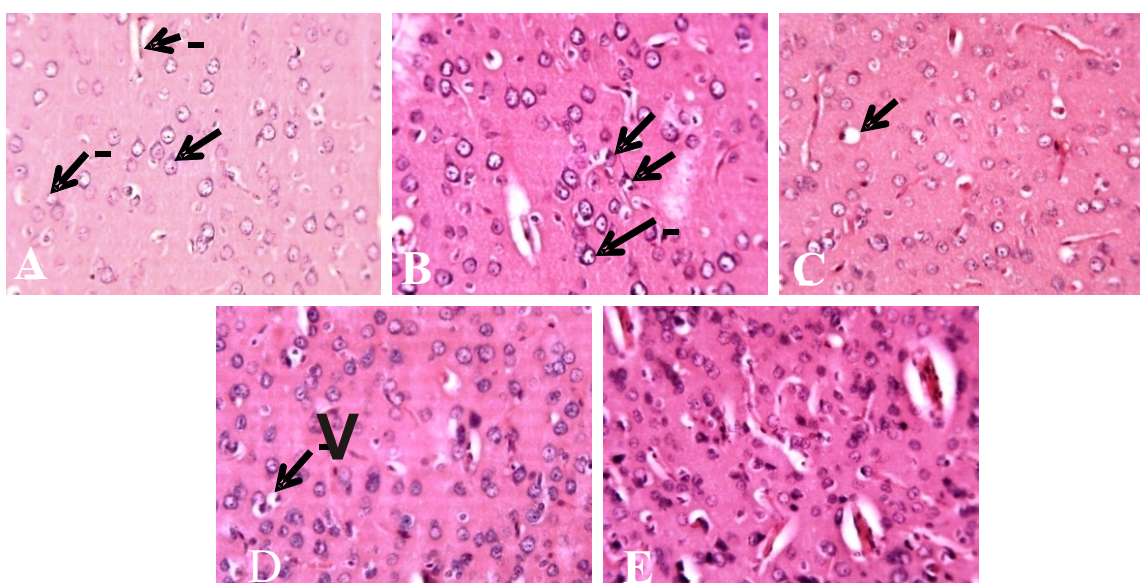


Figure 2: Micrograph of cerebral cortex (layer III) of Wistar rats (H&E x250).

A= Control (distilled water 2ml/kg) group with normal histoarchitecture. B: Blood Vessel; P: Pyramidal cell; S: Stellate cell.

B= Paraquat (11.35 mg/kg) treated group with histoarchitectural distortions. N: Pyknotic nucleus/ necrosis; C: Chromatolysis; V: Perineuronal vacuolation and satellitosis.

C= Paraquat (11.35 mg/kg) and L-dopa (10 mg/kg) treated group with mild histoarchitectural distortions. V: Perineuronal vacuolation.

D= Paraquat (11.35 mg/kg) and AFPD (500 mg/kg) treated group with mild histoarchitectural distortions. V: Perineuronal vacuolation and satellitosis.

E= Paraquat (11.35 mg/kg) and AFPD (1,000 mg/kg) group with relatively normal histoarchitecture

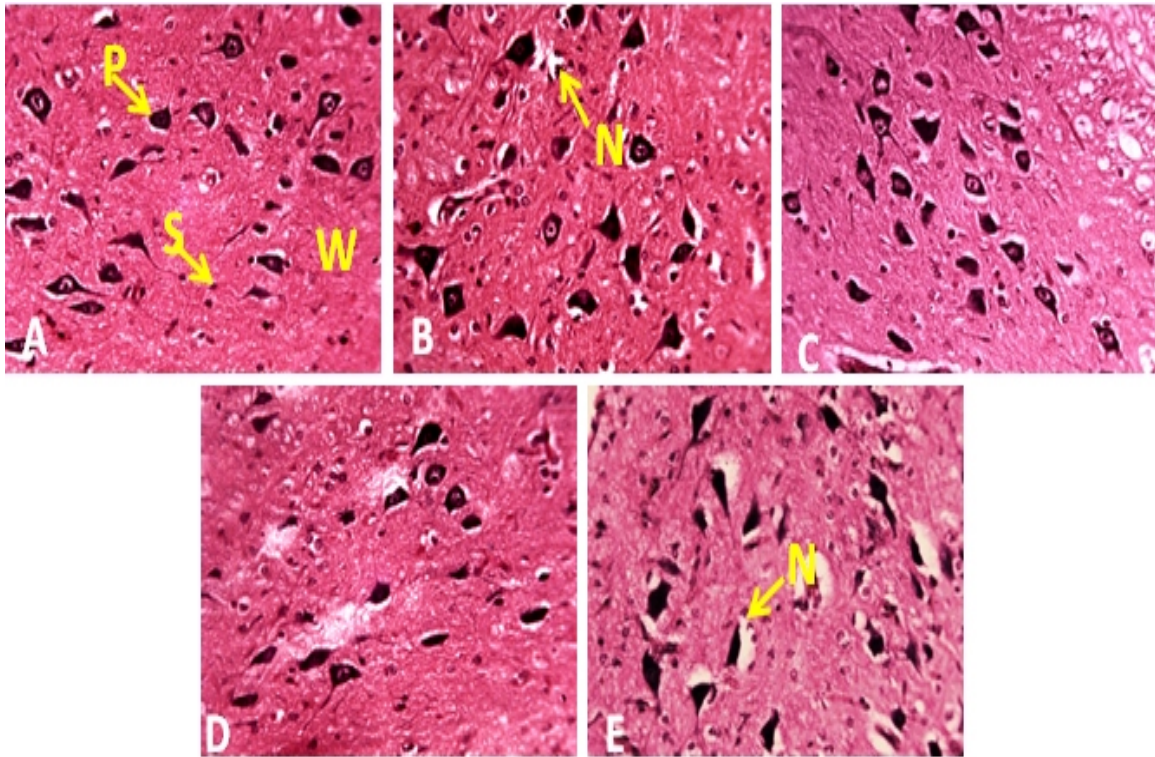


Figure 3: Micrograph of ventral pons of Wistar rats (H&E x250).

A= Control (distilled water 2ml/kg) group with normal histoarchitecture. P: Pontine neurons; S: Stellate cell; W: White matter.

B= Paraquat (11.35 mg/kg) treated group with histoarchitectural distortions. N: Perinuclearvacuolation and gliosis.

C= Paraquat (11.35 mg/kg) and L-dopa (10 mg/kg) treated group with relatively normal histoarchitecture.

D= Paraquat (11.35 mg/kg) and AFPD (500 mg/kg) treated group with relatively normal histoarchitecture. E= Paraquat (11.35 mg/kg) and AFPD (1,000 mg/kg) group with histoarchitectural distortions. N: Perinuclearvacuolation and gliosis.

The cerebellar cortex in the control group showed normal histoarchitecture with characteristic layers; molecular, Purkinje cell, and granular layers of the lobules of the cerebellar cortex (See Figure 1b). The CL2 of the control showed normal cytoarchitecture with stellate cells in the molecular layer, well-defined Purkinje cells in the Purkinje cell layer, and a variety of cells, including the granular cells, in the granular layer. Relative to the control, PRQ (11.35 mg/kg) treated group revealed

neurodegenerative changes as distortion in the cytoarchitecture of cells as pyknotic Purkinje neurons, perineuronal vacuolation, shrunken Purkinje cells, and gliosis.

The L- dopa (10 mg/kg) and AFPD (1,000 mg/kg) treated groups showed mild cytoarchitectural distortion as perineuronal vacuolation and gliosis, while the section of the cerebellar cortex of AFPD (500 mg/kg) treated group showed relatively normal cytoarchitecture when compared with the control group (Figure 4).

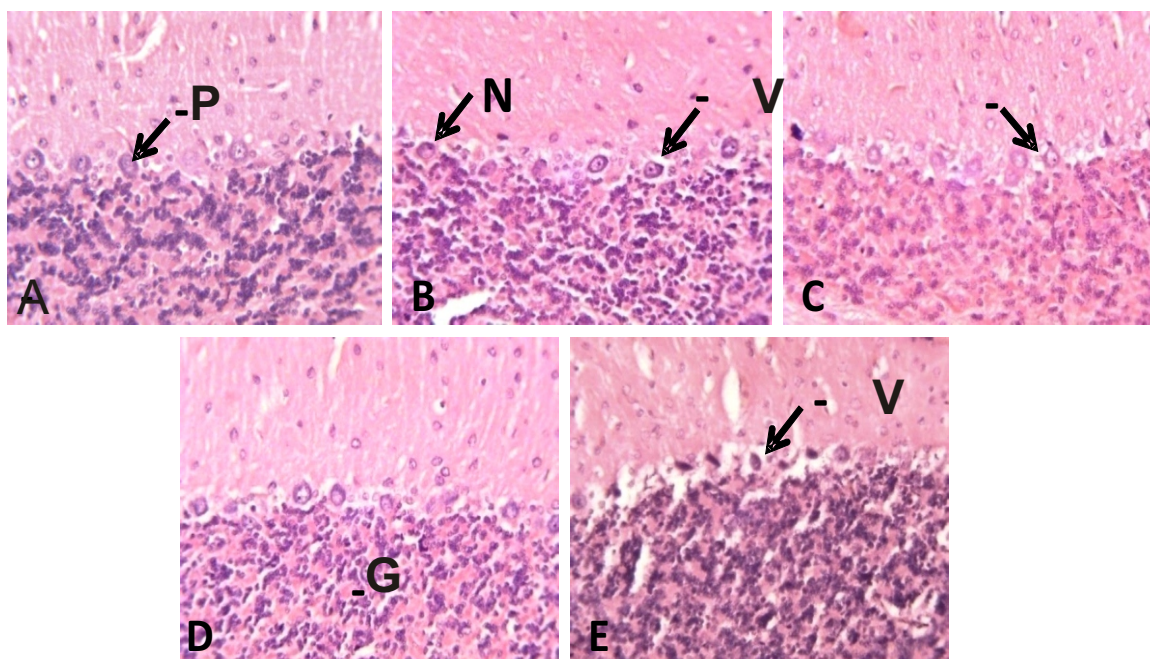


Figure 4: Micrograph of cerebellar cortex of Wistar rats (H&E x250).

A= Control (distilled water 2ml/kg) group with normal histoarchitecture. G: Granular layer; M: Molecular layer; P: Purkinje Cell.

B= Paraquat (11.35 mg/kg) treated group with histoarchitectural distortions. N: Pyknotic neurons; V: Perineuronalvacuolation, shrunken Purkinje cell and gliosis.

C= Paraquat (11.35 mg/kg) and L-dopa (10 mg/kg) treated group with mild histoarchitectural distortions. V: Perineuronalvacuolation.

D= Paraquat (11.35 mg/kg) and AFPD (500 mg/kg) treated group with relatively normal histoarchitecture.

E= Paraquat (11.35 mg/kg) and AFPD (1,000 mg/kg) group with histoarchitectural distortions. V: Perineuronalvacuolation and gliosis.

DISCUSSION

In this study, the ameliorative property of aqueous fruit extract of *Phoenix dactylifera* on cortico-ponto-cerebellar structures of Wistar rats exposed to paraquat neurotoxicity was studied 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^[16,17]. Observed neurodegenerative changes which presented as histoarchitectural distortions including pyknotic nuclei and pyknotic necrosis, chromatolysis,

perineuronal vacuolation and shrunken neuronal somata, perineuronal satellitosis, and gliosis in the brain regions of interest following PRQ treatment are suggestive of PRQ-induced neurotoxicity.

Findings are in agreement with the report of neurotoxic properties of the environmental toxin, PRQ. Rappold *et al.*^[18] reported cytoarchitectural distortion and cell deaths in rodents treated with PRQ. Different regions of the central nervous system, especially the brain, are vulnerable to PRQ toxicity resulting in neuronal damage and glial

cells reactivity^[19]. Niveditha *et al.*^[6] reported neurodegenerative changes which appear as cellular vacuolar lesions following acute and multiple-doses of PRQ and, Edobor *et al.*^[20] reported cytoarchitectural distortions such as pyknotic nuclei and perineuronal vacuolation following PRQ treatment in Wistar rats. Neurodegenerative changes have been associated with environmental stressors like PRQ^[21]. Several mechanisms by which PRQ treatment induces neurotoxicity including excitotoxicity, alteration of mitochondrial complex I activity, α -synuclein aggregation, autophagy, and alteration of dopamine metabolism have been reported. However, oxidative stress generation has been proposed as the central mechanism for the toxicological effects of PRQ^[21,22]. Paraquat causes oxidative damage through a free radical mechanism that result in oxidative damage and lipid peroxidation in the brain^[23]. Paraquat is a strong oxidative stress inducer that participates in the formation of reactive oxygen species (ROS), by alteration of mitochondrial complex I in neurons resulting in cytotoxicity and cytoarchitectural changes^[24]. Paraquat has been demonstrated to cross blood-brain barrier in experimental animal models which trigger neurodegenerative changes. Neurodegenerative changes including neuronal loss have been associated with neurodegenerative diseases including PD and Alzheimer's disease (AD) in which clinical hallmarks comprise motor and cognitive impairments^[18,25,26]. Thus, alteration in the structural integrity of CPC, some of the brain regions involved in the homeostasis of motor activities and coordination^[7,8], as a result of exposure to PRQ could be associated with neurological conditions such as neurodegenerative movement disorders.

In this study, observed mild distortion to relatively normal cytoarchitecture with *L-dopa* and AFPD treatments relative to the control is suggestive of ameliorative properties against PRQ induced neurodegenerative changes. Findings are in line with reports from studies related to the beneficial activities of *L-dopa* as a remarkable agent in the

management of certain pathological changes resulting from oxidative stress associated with neurodegenerative disease conditions like PD^[27,28]. *L-dopa* protects neuronal cells against ROS-induced toxicity by eliciting up-regulation of endogenous antioxidant molecules such as glutathione^[29].

Several natural agents, especially of plant origin, have been demonstrated to be beneficial in the amelioration of xenobiotic triggered neuropathology and hence their potential for therapy^[30,31,32]. *Phoenix dactylifera* has been reported to have high nutritional value and beneficial properties that identified it as a potential nutraceutical agent^[33,34]. Findings in this study are in line with reports on the neuroprotective properties of *Phoenix dactylifera* following neuropathological changes associated with environmentally induced oxidative stress in biological systems. Pujari^[35,36] reported the neuroprotective effect of *P. dactylifera* against oxidative stress and neuronal damage in an animal model. Additionally, Agbon *et al.*^[37] and Yusuf *et al.*^[38] reported fruit extracts of *P. dactylifera* demonstrated remarkable neuroprotection against cytoarchitectural changes triggered by environmental neurotoxicants in cortical cerebral and cerebellar neurons of rats. Edobor *et al.*^[20] demonstrated neuroprotective properties of *P. dactylifera* against PRQ-induced neuropathologies in cortico-nigral structures of rats. *Phoenix dactylifera* contains a variety of phytonutrients including polyphenolic compounds reported to have strong antioxidant activities associated with neuroprotective properties in *in vivo* studies^[33,39]. Polyphenols are bioactive compounds with strong antioxidant activities that scavenge free radicals or quench ROS necessary for neuroprotection^[40,41]. One of the major polyphenolics present in the fruit extract of *Phoenix dactylifera* is the secondary metabolite, flavonoids^[34,42]. Flavonoids possess strong antioxidant and anti-inflammatory activity in a biological system and, have been reported to possess neuroprotective properties in models of neurological disorders^[43,44,45]. Thus, AFPD,

especially at dose 500 mg/kg, possesses neuroprotective properties comparable to the reference drug, *L-dopa*.

Pathological neurodegenerative changes in different regions of the brain associated with neurological disease conditions such as movement disorders and, neurodegenerative diseases like PD and AD share similar features including oxidative stress^[46]. The brain is exposed to oxidative stress due to its high rate of oxygen consumption and low detoxification mechanisms^[47,48] which makes the brain susceptible to exogenously triggered oxidative stress. Neuroprotection is a pharmacological intervention that slows down the progression of neurodegeneration by working in synergy with endogenous defense systems to alleviate responses or reactions resulting from ROS formation^[48,49]. *L-dopa*, although established as a beneficial pharmacological agent for the management of oxidative stress-associated neurological changes in parts of the brain responsible for motor functionality and homeostasis, has shown manifestation of *L-dopa*-induced complication such as personality and behavioral changes and fluctuations, and reduced period of benefits have been reported in clinical trials^[27,50]. Recently, several investigations have emphasized the need for the optimization of therapies and identification of novel formulation for neurodegenerative pathologies and related clinical conditions^[51]. Neuroprotective antioxidants from plants like *P. dactylifera*, could help in the prevention, treatment, and management of ROS-triggered neurodegenerative conditions as they play a critical role as constituent bioactive components and are potent deactivators of free radicals in biological systems, thus its health benefits. Additionally, bioactive compounds such as flavonoids from AFPD could be beneficial as an adjunct treatment for the optimization of an orthodox medicament for ROS-induced neurodegeneration.

CONCLUSION

Aqueous fruit pulp extract of *Phoenix dactylifera* possesses potential ameliorative properties against paraquat-triggered neurodegenerative changes in cortico-ponto-cerebellar structures of Wistar rats. Neuroprotective properties could be attributed to bioactive compounds present in AFPD with potent antioxidant activities against ROS-associated PRQ neurodegenerative changes.

The emphasis in this study was on histological changes following treatments in rats. Thus, the need to further investigate the efficacy and potentiality of AFPD using other methodologies of assessment, like biochemistry, immunohistochemistry, and molecular biology. Additionally, other solvent extract forms of *Phoenix dactylifera* fruit pulp could be assessed as a potential medicament for oxidative stress-associated neuropathologies and neurodegeneration to enhance formulation and ultimately, its commercialization.

Conflict of Interest

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

REFERENCES

1. Jayasinghe SS, Seneviratne SA. Neurotoxic effects of paraquat. *Galle Med. J.* 2016;21(2), 21-24.
2. Rashidipour M, Maleki A, Kordi S, Birjandi M, Pajouhi N, Mohammadi E, Heydari R, Rezaee R, Rasoulilian B, Davari B. Pectin/chitosan/tripolyphosphate nanoparticles: efficient carriers for reducing soil sorption, cytotoxicity, and mutagenicity of paraquat and enhancing its herbicide activity. *J. Agric. Food Chem.* 2019 May 1;67(20):5736-45.
3. Huang Y, Zhan H, Bhatt P, Chen S. Paraquat degradation from contaminated environments: current achievements and perspectives. *Front.*

- Microbiol. 2019 Aug 2;10:1754.
4. Smeyne RJ, Breckenridge CB, Beck M, Jiao Y, Butt MT, Wolf JC, Zadory D, Minnema DJ, Sturgess NC, Travis KZ, Cook AR. Assessment of the effects of MPTP and paraquat on dopaminergic neurons and microglia in the substantia nigra pars compacta of C57BL/6 mice. *PLoS one*. 2016 Oct 27;11(10):e0164094.
 5. Hajji K, Mteyrek A, Sun J, Cassar M, Mezghani S, Leprince J, Vaudry D, Masmoudi-Kouki O, Birman S. Neuroprotective effects of PACAP against paraquat-induced oxidative stress in the *Drosophila* central nervous system. *Human Molecular Genetics*. 2019 Jun 1;28(11):1905-18.
 6. Niveditha S, Ramesh SR, Shivanandappa T. Paraquat-induced movement disorder in relation to oxidative stress-mediated neurodegeneration in the brain of *Drosophila melanogaster*. *Neurochem. Res*. 2017 Nov;42(11):3310-20.
 7. Palesi F, De Rinaldis A, Castellazzi G, Calamante F, Muhlert N, Chard D, Tournier JD, Magenes G, D'Angelo E, Gandini Wheeler-Kingshott CA. Contralateral cortico-ponto-cerebellar pathways reconstruction in humans *in vivo*: implications for reciprocal cerebro-cerebellar structural connectivity in motor and non-motor areas. *Sci. Rep*. 2017 Oct 9;7(1):1-3.
 8. Enokizono M, Sato N, Ota M, Shigemoto Y, Morimoto E, Oba M, Sone D, Kimura Y, Sugai K, Sasaki M, Ikegaya N. Disrupted cortico-ponto-cerebellar pathway in patients with hemimegalencephaly. *Brain Dev.* 2019 Jun 1;41(6):507-15.
 9. Aladele SE, Fajimi O, Adetunji OA, Gidado RM, Solomon BO, Okere AU, Odofin WT. Cultivation of *Phoenix dactylifera* L.(date palm) for combating desertification and enhanced livelihood: NACGRAB R and D Focus. In IV International Date Palm Conference 882 2010 Mar 15 (pp. 81-87).
 10. Vyawahare N, Pujari R, Khsirsagar A. *Phoenix dactylifera*: An update of its indigenous uses, phytochemistry and pharmacology. *Int. J. Pharmacol*. 2009;7(1)
 11. Agbon AN, Ingbian SD, Dahiru AU. Preliminary histological and histochemical studies on the neuroprotective effect of aqueous fruit extract of *Phoenix dactylifera* L.(Date Palm) on atesunate-induced cerebellar damage in wistar rats. *Sub-Saharan Afr J Med*. 2014 Oct 1;1(4):204.
 12. Al-Alawi RA, Al-Mashiqri JH, Al-Nadabi JS, Al-Shihi BI, Baqi Y. Date palm tree (*Phoenix dactylifera* L.): natural products and therapeutic options. *Front. Plant Sci*. 2017 May 23;8:845.
 13. Djaoudene O, López V, Cásedas G, Les F, Schisano C, Bey MB, Tenore GC. *Phoenix dactylifera* L. seeds: A by-product as a source of bioactive compounds with antioxidant and enzyme inhibitory properties. *Food Funct*. 2019;10(8):4953-65.
 14. Agbon AN, Kwaneshie HO, Hamman WO. Antidiarrheal activity of aqueous fruit extract of *Phoenix dactylifera* (DATE PALM) in Wistar rats. *Br. J. Pharmacol. & Tox*. 2013 Jun 25;4(3):121-7.
 15. Kurdi SM, Theerth KA, Derva RS. Ketamin: Current applications in anesthesia, pain and critical care. *Anesth Essays Res* 2014;8:283-90.
 16. Eze SM, Musa AS, Umana UE, Sadeeq AA, Agbon AN, Peter AS. Evaluation of the Effects of Water Melon Seed Oil (*Citrullus lanatus*) on Cadmium Induced Cerebral Toxicity in Male Wistar Rats. *J. Anat. Sci*. 2021;12(2), 59 68
 17. Henry R, Agbon AN, Yakubu U, Simon D, Shuaib YM, Sule H.,..., Mahdi O. Neurobehavioral and microscopic assessments of methanol fruit extract of *Phoenix dactylifera* on lead acetate-induced cerebellar changes in Wistar rats. *J. Anat. Sci*. 2021;12:95-108.
 18. Rappold PM, Cui M, Chesser AS, Tibbett J, Grima JC, Duan L, Sen N, Javitch JA, Tieu K. Paraquat neurotoxicity is mediated by the dopamine transporter and organic cation transporter-3. *PNAS*. 2011 Dec

- 20;108(51):20766-71.
19. Tieu K. A guide to neurotoxic animal models of Parkinson's disease. Cold Spring Harb. Perspect. Med. 2011 Sep 1;1(1):a009316.
 20. Edobor HD, Musa SA, Umana UE, Oderinde GP, Agbon AN. Neuroprotective effect of *Phoenix dactylifera* (date palm) on paraquat triggered cortico-nigral neurotoxicity. J. Neuro. Behav.. 2021 Sep 1;8(3):199.
 21. Franco R, Li S, Rodriguez-Rocha H, Burns M, Panayiotidis MI. Molecular mechanisms of pesticide-induced neurotoxicity: Relevance to Parkinson's disease. Chem. Biol. Interact. 2010 Nov 5;188(2):289-300.
 22. Zhang XF, Thompson M, Xu YH. Multifactorial theory applied to the neurotoxicity of paraquat and paraquat-induced mechanisms of developing Parkinson's disease. Lab. Investig. 2016 May;96(5):496-507.
 23. McCormack AL, Atienza JG, Johnston LC, Andersen JK, Vu S, Di Monte DA. Role of oxidative stress in paraquat-induced dopaminergic cell degeneration. J. Neurochem. 2005 May;93(4):1030-7.
 24. Cochemé HM, Murphy MP. Complex I is the major site of mitochondrial superoxide production by paraquat. Int. J. Biol. Chem. 2008 Jan 25;283(4):1786-98.
 25. Jankovic J. Parkinson's disease: clinical features and diagnosis. J. Neurol. Neurosurg. Psychiatry. 2008 Apr 1;79(4):368-76.
 26. Kalyn M, Hua K, Mohd Noor S, Wong CE, Ekker M. Comprehensive analysis of neurotoxin-induced ablation of dopaminergic neurons in zebrafish larvae. Biomedicines. 2019 Dec 28;8(1):1.
 27. Aquino CC, Fox SH. Clinical spectrum of levodopa-induced complications. Mov. Disord. 2015 Jan;30(1):80-9.
 28. LeWitt PA. New levodopa therapeutic strategies. Parkinsonism Relat. Dis. 2016 Jan 1;22:S37-40.
 29. Olanow CW. Levodopa: effect on cell death and the natural history of Parkinson's disease. Mov. Disord. 2015 Jan;30(1):37-44.
 30. Ullah R, Khan M, Shah SA, Saeed K, Kim MO. Natural antioxidant anthocyanins A hidden therapeutic candidate in metabolic disorders with major focus in neurodegeneration. Nutrients. 2019 Jun;11(6):1195.
 31. Agnihotri A, Aruoma OI. Alzheimer's disease and Parkinson's disease: a nutritional toxicology perspective of the impact of oxidative stress, mitochondrial dysfunction, nutrigenomics and environmental chemicals. J Am Coll Nutr. 2020 Jan 2;39(1):16-27.
 32. Rajput SA, Wang XQ, Yan HC. Morin hydrate: A comprehensive review on novel natural dietary bioactive compound with versatile biological and pharmacological potential. Biomed. Pharmacother. 2021 Jun 1;138:111511.
 33. Hussain MI, Farooq M, Syed QA. Nutritional and biological characteristics of the date palm fruit (*Phoenix dactylifera* L.) A review. Food Biosci.. 2020 Apr 1;34:100509.
 34. Younas A, Naqvi SA, Khan MR, Shabbir MA, Jatoi MA, Anwar F, Inam-Ur-Raheem M, Saari N, Aadil RM. Functional food and nutra-pharmaceutical perspectives of date (*Phoenix dactylifera* L.) fruit. J. Food Biochem. 2020 Sep;44(9):e13332.
 37. Agbon AN, Abubakar MG, Enemeli FU, Mahdi O, Bobb KA, Sule H, Yahaya MH, Okah CC. Assessment of Ethanol Fruit extract of *Phoenix dactylifera* L. (Date Palm) on mercury chloride induced cerebral and cerebellar alterations in Wistar rats. J. Anat. Sci. 2017;8(1):188-201.
 38. Yusuf AO, Buraimoh AA, Agbon AN, Raji KB, Akpulu PS. Preliminary histological studies on the effect of aqueous fruit extract of *Phoenix dactylifera* L. (date palm) on lead acetate-induced cerebellar damages in Wistar rats. Afr. J. Cell. Path. 2017 Jan 31;8(1):1-8.
 39. Pujari RR, Vyawahare NS, Thakurdesai PA. Neuroprotective and antioxidant role of *Phoenix dactylifera* in permanent bilateral common carotid occlusion in rats. J. Acute

- Dis. 2014 Jan 1;3(2):104-14.
40. El Sohaimy SA, Abdelwahab AE, Brennan CS, Aboul-Enein AM. Phenolic content, antioxidant and antimicrobial activities of Egyptian date palm (*Phoenix dactylifera* L.) fruits. *Australian J. Basic Appl. Sci.* 2015;9,141147.
 41. Echegaray N, Pateiro M, Gullon B, Amarowicz R, Misihairabgwi JM, Lorenzo JM. *Phoenix dactylifera* products in human health A review. *Trends Food Sci Technol.* 2020 Nov 1;105:238-50.
 42. Moss JW, Ramji DP. Nutraceutical therapies for atherosclerosis. *Nat. Rev. Cardiol.* 2016 Sep;13(9):513-32.
 43. Chen Y, Sun H, Huang L, Li J, Zhou W, Chang J. Neuroprotective effect of Radix *Trichosanthis* Saponins on subarachnoid hemorrhage. *Evid.-based Complement. Altern.* 2015 Jan 1;2015.
 44. Sarian MN, Ahmed QU, Mat So'ad SZ, Alhassan AM, Murugesu S, Perumal V, Syed Mohamad SN, Khatib A, Latip J. Antioxidant and antidiabetic effects of flavonoids: A structure-activity relationship based study. *Biomed Res. Int* 2017 Nov 28;2017.
 45. Agbon AN, Kwanashie, HO, Hamman WO, Ibegbu AO, Henry R, Sule H, ... & Oladimeji, OJ. Comparative Microscopic Assessments of the Effect of Aqueous and Ethanol Extracts of *Phoenix dactylifera* L. in a Rat Model of Mercury-Triggered Hippocampal Changes: *P. dactylifera* Neuroprotection against Mercury-triggered Neurotoxicity. *Nig. J. Phy. Sci.* 2021;36(1),91-100.
 46. He J, Zhu G, Wang G, Zhang F. Oxidative stress and neuroinflammation potentiate each other to promote progression of dopamine neurodegeneration. *Oxid. Med. Cell. Longev.* 2020 Oct;2020.
 47. Goodfellow MJ, Borcar A, Proctor JL, Greco T, Rosenthal RE, Fiskum G. Transcriptional activation of antioxidant gene expression by Nrf2 protects against mitochondrial dysfunction and neuronal death associated with acute and chronic neurodegeneration. *Exp. Neurobiol.* 2020 Jun 1;328:113247.
 48. Hrelia P, Sita G, Ziche M, Ristori E, Marino A, Cordaro M, Molteni R, Spero V, Malaguti M, Morroni F, Hrelia S. Common protective strategies in neurodegenerative disease: focusing on risk factors to target the cellular redox system. *Oxid. Med. Cell. Longev.* 2020 Aug 1;2020.
 49. Salamon A, Zádori D, Szpisjak L, Klivényi P, Vécsei L. Neuroprotection in Parkinson's disease: facts and hopes. *J. Neural Transm. Suppl.* 2020 May;127(5):821-9.
 50. Beaulieu-Boire I, Lang AE. Behavioral effects of levodopa. *Mov. Dis.* 2015 Jan;30(1):90-102.
 51. Tambasco N, Romoli M, Calabresi P. Levodopa in Parkinson's disease: current status and future developments. *Curr. Neuropharmacol.* 2018 Oct 1;16(8):1239-52.