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Histological and Histochemical Studies on the Neuroprotective Effect of Aqueous *Fruit* Extract of *Phoenix Dactylifera L. (Date Palm) on Mercury–Induced Cerebellar* Damage *in Wistar Rats*

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ABSTRACT

The cerebellum is vulnerable to damage from a variety of sources such as degenerative diseases, infectious processes and toxins, like, mercury. In traditional medicine, various parts of *Phoenix dactylifera* (date palm) are used to treat disorders, such as, loss of consciousness, nervous disorders, memory disturbances, etc, in different parts of the world. The neuroprotective effect of aqueous fruit extract of *Phoenix dactylifera (AFPD) was assessed against mercury*-induced cerebellar damage in Wistar rats. Twenty-four Wistar rats were divided into six groups (A-F) of four rats each. Group A (control) was administered distilled water (0.5 ml/kg p.o) while, groups B-F were treatment groups. Cerebellar damage was induced in rats by the administration of mercury ($HgCl_2$). Group B was administered $HgCl_2$ (5mg/kg p.o) only. Group C was administered vitamin C (100mg/kg) as reference drug; groups D-F were administered AFPD *(250mg/kg, 500mg/kg and 1000mg/kg p.o, respectively)* followed, concurrently, by $HgCl_2$ (5mg/kg p.o) for a period of 14 days. Neuroprotective activity was studied by histologic examination of brain sections (cerebellar cortex) applying routine (H&E) and histochemical (cresly fast violet) staining techniques. Histologic examination of cerebellar sections of *mercury*–intoxicated rats revealed cortical degenerative changes, such as, perineuronal vacuolations and degeneration of Purkinje cells, and alteration in the general histoarchitecture of cerebellar cortex. The administration of *AFPD* remarkably ameliorated *mercury*–induced neuronal damage in rats, dose-dependently, comparable to the reference drug, vitamin C. Result suggests that *AFPD* is a potential candidate for application in the management and treatment of chemically-induced neurodegenerative diseases, and neuroprotective activity is probably due to its constituent antioxidant properties.

Keywords: Cerebellar cortex, Histology, Mercury, Neuroprotection, *Phoenix dactylifera*

INTRODUCTION

Severe illness and sudden death in human beings has been implicated on environmental pollution, centuries before now. Pollution can be air, water or land and can result from mining, automobile exhaust, agricultural and industrial activities among others. Classified among major causes of environmental pollution, are heavy metals. Heavy metals occur as natural constituents of the earth crust, and are persistent environmental contaminants since they cannot be degraded or destroyed. Although these elements are lacking in abundance they are not lacking in significance[1]. Some well-established toxic metallic elements are: arsenic, cadmium, lead and mercury.[2]

Environmental mercury levels have increased considerably since the on-set of the industrial age. Mercury is now present in various environmental media and food (especially fish) all over the globe at levels that adversely affect humans and wildlife. Widespread exposures are occurring due to human generated sources, and past practices have left a legacy of mercury in landfills, mine tailings, contaminated industrial sites, soils and sediments[3]. Consequently, mercury is a potent environmental contaminant that exerts toxic effect on a variety of vital organs in the human body. It exists in three predominant forms: elemental (Hg^0), organic (such as methylmercury - H_3CHg), and inorganic (mercuric chloride - $HgCl_2$) mercury[4, 5]. Each form has its own effects, routes of absorption and tissue specificity.

The cerebellum is part of the brain responsible for motor movements, coordination, balance, equilibrium and muscle tone[6]. The cerebellum is vulnerable to damage from a variety of sources such as developmental defects, degenerative diseases, infectious processes, chronic alcoholism, trauma and tumors[7]. Cerebellar

injuries have been reported to result from toxins, such as, mercury[8,9,10].

In traditional practices of medicine, numerous plants in the human diet containing a large number of natural compounds with wide range of medicinal actions, have been used to treat different types of diseases, such as cognitive disorders, including neurodegenerative diseases.[11] Recently, scientists have begun investigating the biological activities of medicinal plants, including their neuroprotective actions[12,13].

Phoenix dactilyfera L. (date palm) belongs to the family Arecaceae.[14] It is believed to be indigenous to the countries around the Persian Gulf.[15] *P. dactylifera* fruits are important component of diet in the arid and semiarid regions of the world [16] and are a good source of energy, vitamins (vitamin A, B_1, B_2, B_3 and C), and a group of elements like phosphorus, iron, potassium, and a significant amount of calcium.[17, 18, 19] In folk medicine, different parts of the plant have been claimed to be used for the treatment of diversity of ailments which includes: memory disturbances, fever, inflammation, paralysis, loss of consciousness and nervous disorders.[20] It has been scientifically reported to possess a variety of pharmacological activities, such as, antiulcer, hepatoprotective, antimutagenic, antidiarrhoeal, anti-inflammatory, antioxidant and neuroprotective activities.[21,22,23,24,25]

The aim of this study was to histologically and histochemically assess the neuroprotective effect of aqueous fruit extract of *Phoenix dactylifera* (AFPD) against mercury-induced cerebellar toxicity in Wistar rats.

MATERIALS AND METHODS

Plant Material

Dried *P. dactylifera (date palm)* fruits were obtained from a local market (Samaru) in Zaria, Kaduna, Nigeria and, authenticated and deposited in the Herbarium unit of the Department of Biological Sciences, Faculty of Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria with the Voucher Specimen Number of 7130.

Extract Preparation

Extraction of P. dactylifera fruit was carried out in the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Kaduna, Nigeria. The method of maceration as reported by Agbon *et al.* [25] for the preparation of aqueous fruit extract of *P. dactylifera* was adopted.

Experimental Animals

Experimental animals (Wistar rats) weighing 125±15 g were obtained from Animal House of the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria, Kaduna, Nigeria and housed in new wired cages in the same animal house were rats acclimatized for two weeks prior to the commencement of the experiments. The rats were housed under standard laboratory condition, light and dark cycles of 12 hours, and were provided with standard rodent pellet diet 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 a period of 14 days. The rats and their organs (brain) were weighed at the end of the study and organ (brain)/ body weight index computed.

Drug

Mercuric chloride was obtained and used as neurotoxicant for the experiment. The product is manufactured by British Drug Houses (*BDH) 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 (24) Wistar rats (male and female) were divided into six groups (A - F) of four rats each. Group A (control) was administered distilled water (0.5 ml/kg) while, groups B - F were treatment groups. Cerebellar neurotoxicity was induced in rats by the administration of mercury – *mercuric chloride* as reported by Sheikh *et al.* [26] Group B was administered *mercuric chloride* (5 mg/kg; *that is, 12.5% of* LD_{50}*; mercuric chloride* LD_{50} *oral in rats = 40 mg/kg*) [26] only. Group C was administered vitamin C (100 mg/kg) [27]; groups D, E and F were administered aqueous fruit extract of *P. dactylifera (250 mg/kg, 500 mg/kg and 1,000 mg/kg, respectively (that is, 5%, 10 % and 20% of* LD_{50} *respectively; aqueous fruit extract of P. dactylifera* LD_{50} *oral in rats = 5000 mg/kg)* [28] followed, concurrently, by *mercuric chloride* (5 mg/kg) for a period of 14 days. All administrations were via oral route.

Histological and Histochemical Studies

At the end of the experiment, rats were euthanized and brain organs harvested. Harvested brain organs were fixed in Bouin's fluid and tissues processed for routine histological examination, stained with Haematoxylin and Eosin (H&E) and histochemical (Cresly Fast Violet, CFV) stains and examined under the light microscope.

Histometric studies

Histometric studies involved measuring the cell area and perimeter of the Purkinje cells of cerebellar cortex using a light microscope (HM-LUX, Leitz Wetzlar, Germany) with a 40/ 0.65 x objective (x 400 magnification) and, a micrometer slide (1 mm graduated in 0.01 mm units; that is divided 10x into 100 μm units) and computer running imaging soft ware (AmScope MT *version* 3.0.0.5, USA) according to the

manufacturer's instruction.

CFV stained micrographs (digital microscopic images) were used for the histometry. Three micrographs were randomly captured [29, 30] in the brain region - cerebellar cortex [31, 32] and 5 - 10 cells were randomly selected, measured and averaged in the three micrographs.

Data Analysis
Results obtained were analysed using the statistical soft ware, Statistical Package for the Social Sciences (SPSS *version 18.0*) and results 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 sample *t*–test was employed for the comparison of means as appropriate. Values were considered significant when $p \leqslant 0.05$.

RESULTS

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 animals in the treatment groups exhibited decreased activity, especially in mercury ($HgCl_2$) - treated group.

The weights of the rats, in all groups were observed to have increased when initial and final weights were compared. Remarkable ($p<0.05$) increases in weights were observed with the control and extract- treated groups, especially, aqueous fruit extract of *P. dactylifera* (250 mg/kg and 500 mg/kg) doses. However, there were no significant differences ($p>0.05$) in weight changes (difference in initial and final weights) when treated groups were compared with the control (Table 1).

The relative organ weights (brain weight/ body index) were computed and compared with the control. Relative to the control, there was increase in relative organ weight, especially with vitamin C- treated group (Table 2).

Histological and Histochemical Examination
Histological examination of sections of cerebellar cortex of rats, stained with routine (H&E) histological and histochemical (CFV) stains revealed the following:

The cerebellar sections of rats in the control group revealed normal histoarchitecture of the cerebellar cortex; the distinctive appearance of the three cortical layers: outer molecular layer and inner granular layer, between these layers is a monolayer, Purkinje cell layer with flask-shaped Purkinje cells. Histochemical (CFV) staining for Nissl substances revealed normal appearance of distinct intensely stained cortical neurones (**Figures 1A and 2A**).
The cerebellar sections of mercury-treated rats revealed histoarchitectural distortion of the cerebellar cortex; neurodegenerative changes, such as and perineuronal vacuolations, neuronal cytoplasmic shrinkage and gliosis. CFV staining revealed Pukinje cell and internal granule cell necrosis, and indistinct neurones due to reduced staining intensity (Figures 1B and 2B).

Examination of the cerebellar sections of vitamin C- and aqueous fruit extract of *P. dactylifera* - treated rats, followed by mercury treatment, revealed mild cerebellar cortex histoarchitectural distortions compared to the severe distortions of the mercury-treated. The histological features of the vitamin C-treated group showed perineuronal vacuolations, satellitosis and internal granule cell necrosis (Figure 1C and 2C).

Histological features of the aqueous fruit extract of *P. dactylifera* (250 mg/kg, 500 mg/kg and 1,000 mg/kg) - treated groups revealed mild neurodegenerative changes, such as perineuronal vacuolations, satellitosis, outer molecular cell and internal granule cell necrosis. Aqueous fruit extract of *P. dactylifera* administration conferred preservation of the histoarchitecture dose-dependently compared to the control (Figure 1 D-F and 2 D-F).

Histometric Analysis
Histometry involved measuring the cell area and perimeter of neurones (Purkinje cells of the cerebellar cortex) of the rats.

Cerebellar cortex histometric characteristics of the Purkinje cells showed decrease in the cell area and perimeter of all the treated groups relative to the control. However, differences were only significant ($p<0.01$) in cell area when compared to the control. Besides, remarkable ($p<0.05$) difference in Purkinje cell area was observed in aqueous fruit extract of *P. dactylifera* (250 mg/kg)-treated group when compared to the mercury-treated group (Table 3).

DISCUSSION

Decreased physical activity exhibited by mercury-treated rats reflects treatment-related toxicity. This is in concordance with reports on drug-related toxicity; altered physical activity manifesting as sluggishness and loss of appetite indicates drug-related toxicity.[33, 34, 35] Body weight changes serve as a sensitive indication of the general health status of animal,[34] and used as an indicator of adverse effect of drugs and chemicals.[36] Changes in the body weight of Wistar rats treated with mercury lagged behind the extract treated. This is in consistence with the reports of Jadhav *et al.*[37] and Wadaan.[38]

Relative organ weight increase in treated groups relative to the control indicates treatment-related toxicity. Increased relative brain weight has been reported with heavy metal intoxication.[39] Ghusoon *et al.*[40] reported macroscopic brain lesions of rats treated

orally with mercuric chloride appeared, swollen and edematous, with severe congestion of meningeal blood capillaries.

Cells death has been reported to result from neuronal degeneration.[41] Cell death may result from necrosis, a pathologic type of cell death that occurs from extrinsic insults to the cells or after abnormal stresses, such as chemical injury or toxin, thermal, traumatic and mechanical factors.[42, 43, 44] The Purkinje cells and the granule cells are the most important targets in cerebellum for toxic substances.[45] Neurodegenerative changes, such as, perineuronal vacuolations, neuronal cytoplasmic shrinkage and gliosis, Pukinje cell and internal granule cell necrosis, and indistinct neurones due to reduced staining intensity observed in mercury-treated rats implies treatment related toxicity. Findings are in consonance to studies related to heavy metals induced cell injury on cerebellar structures.[46, 47] Heavy metals disrupt the mechanisms release of neurotransmitters regulated by calcium, but also induced damage to mechanisms of DNA repairs.[48] Oral administration of mercuric chloride has been reported to induce cerebellar degenerative changes.[40] Inorganic mercury, a more reactive form of mercury, can cross the blood–brain barrier and accumulate in the brain at higher concentrations.[49, 50] It interacts with many macromolecules and exhibits a long latency of neurotoxicity.[51, 52] Mercuric chloride disturbs neuronal network structure and induces cell apoptosis in cortical neurones. These actions involve a degradation of β-tubulin, an important component of the neuronal cytoskeleton, and these effects are evoked by NMDA receptor function.[5] Rough endoplasmic reticulum and free ribosomes appear as basophilic granular areas (Nissl bodies) with Cresy Fast Violet staining in light microscopy. Reduction in Nissl boches has been reported in relation to neuronal degeneration.[53] The observed neuronal degeneration: loss of Nissl bodies with consequent reduced staining intensity of the Nissl substances in the cerebellar section of the mercury-treated rats in this study is in concordance with reports by the earlier findings of Classen *et al.* [54] and Ajibade *et al.* [55, 56], reporting loss of Nissl substance in cerebellar neurones and shrinkage of the nucleus following chemically induced toxicity.

Mild histoarchitectural distortion, such as perineuronal vacuolations, satellitosis, outer molecular cell and internal granule cell necrosis compared to the control observed from cerebellar sections of rats treated with vitamin C and aqueous fruit extract of *P. dactylifera* followed by the administration of mercury suggests mercury induced neurotoxicity. The brain is made up of neurones and neuroglia, the neurones being responsible for sending and receiving nerve impulses or signals. The microglia and astrocytes are essential for ensuring proper functioning of neurones and, are quick to intervene when neurones become injured or stressed. As they are sentinels of neurone well-being, pathological impairment of microglia or astrocytes could have devastating consequences for brain function. It is assumed that neuroglial activation is largely determined by neuronal signals.[57] Satellitosis, a condition marked by an accumulation of neuroglia cells around damaged or necrosed neurones of the central nervous system, often as a prelude to neuronophagia,[58] as observed in this study is indicative of treatment related toxicity. Acute injury causes neurones to generate signals that inform neuroglia about the neuronal status. Depending on how severe a degree of neuronal injury, neuroglia will either nurse the injured neurones into regeneration or kill them if they are not viable. These types of neuroglial responses are considered to represent normal physiological and neuroprotective responses.[11]

Administration of aqueous fruit extract of *P. dactylifera* showed preserved histoarchitecture of the cerebellar cortex parenchyma and cytoarchitectural preservation of neuronal cell Nissl substance in Wistar rats. This implies that, the extract have a protective effect against mercury-induced neurotoxicity. Results from this study are in consistence with reports on the neuroprotective effect of *P. dactylifera* extract.[35] Pujari *et al.* [59] reported that, pretreatment with extract of *P. dactylifera* attenuated cortical neurones against bilateral common carotid artery occlusion induced oxidative stress neuronal damage in Wistar rats. Wan Ismail and Mohd Radzi[60] reported that, neuronal damage in the form of shrinkage, atrophy and necrosis of neurones is greatly reduced and there is an increase in the levels of endogenous antioxidants in the brain of rats treated with *P. dactylifera* (date palm) fruit extract.

Histometry provides a sound basis for comparison of histological observation. It offers objectivity, increases precision compared with direct visual appraisal and makes statistical analysis easier. It improves assessment of certain histological change, which though may be recognizable by the eye, are accurately graded and their progression better appreciated by histometric quantization.[61, 62, 63]

In this study, histometry of the cell area and perimeter of neurones (Purkinje cells of the cerebellar cortex) of the Wistar rats were conducted. The cerebellar Purkinje cells which are among the largest cells in the central nervous system, are of primary interest, and have been the subject of several investigations concerning number and size, as these cells constitutes the only output elements of the cerebellar cortex.[32, 64, 65] Remarkable difference in histometric characteristics (particularly, cell area) of the Purkinje cells of cerebellar cortex is indicative treatment-related toxicity. Histometric parameters are directly applied and correlated to tissue function.[63, 66] Decrease in the perikaryal size – neuronal cytoplasmic shrinkage, has been attributed to stress-induced structural changes at the light microscopic

level.[67, 68] Thus, histometric characteristics correlate with histologic observations.

Aqueous fruit extract of *P. dactylifera* administration conferred preservation of the histoarchitecture dose-dependently compared to the control. Antioxidant effects have been implicated for neuroprotective activity of *P. dactylifera*.[69, 70, 71, 72] Phytochemicals, such as flavonoids, saponins, tannins and alkaloids have been reported to exert neuroprotective actions in animal and cell culture models of neurological disorders.[73, 74, 75] The presence of flavonoids, saponins and tannins have been reported in phytochemical screening of aqueous fruit extract of *P. dactylifera*.[28]

The polyphenolics including flavonoids, found in many plant extracts, have been shown to be strong reactive oxygen species (ROS) scavengers, antioxidants and protectors of neurones from lethal damage [59, 76] and are able to chelate metal ions.[77] Flavonoids exert a multiplicity of neuroprotective actions within the brain, including a potential to protect neurones against injury induced by neurotoxins, an ability to suppress neuroinflammation, and the potential to promote memory, learning, and cognitive function.

Therefore, antioxidant activities of the extract could be implicated for neuroprotection; aqueous fruit extract of *P. dactylifera* benefits the brain through neuroprotective property, protecting the brain from the actions of ROS by utilizing its antioxidant.[60]

CONCLUSION

The result of the present study suggests that, the aqueous fruit extract of *Phoenix dactylifera* may prove efficacious in ameliorating mercury-induced histologic and histochemical alterations in the cerebellar cortex of Wistar rats. The neuroprotective property of extract, relative to the standard (vitamin C), is somewhat similar, and could be attributed to antioxidant properties of constituent phytochemicals, such as flavonoids. Thus, aqueous fruit extract of *Phoenix dactylifera* is a potential candidate for application in the management and treatment of ROS-induced neurodegenerative diseases, and its regular consumption may be helpful for health.

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Table 1: Weight change and weight comparison of Wistar rats at *initial* (*day 1*) and *final* (*day 14*) day of treatment

Group	Treatment	FW-IW (g)	IW (g)	FW (g)	*t*	p-value
A	Control (H_2O 0.5ml/kg)	21.14±2.70	116.63±13.28	138.50±12.01	8.919	0.000
B	$HgCl_2$ (5mg/kg)	29.00±10.71	109.50±20.67	131.00±38.92	2.261	0.073
C	Vit C (100mg/kg) + $HgCl_2$ (5mg/kg)	8.57±11.38	101.40±19.27	111.20±8.55	0.735	0.503
D	AFPD (250mg/kg) + $HgCl_2$ (5mg/kg)	24.00±3.02	111.25±12.09	144.75±39.51	3.310	0.045
E	AFPD (500mg/kg) + $HgCl_2$ (5mg/kg)	25.29±8.78	104.57±16.55	129.86±13.23	2.881	0.028
F	AFPD (1000mg/kg) + $HgCl_2$ (5mg/kg)	18.71±7.28	105.00±16.86	124.20±42.54	1.329	0.255

n = 4; mean ± SEM; Paired sample *t*- test; One way ANOVA *LSD post hoc test. p>0.05 when compared with the control.* AFPD= *Aqueous fruit extract of Phoenix dactylifera*; Vit C= Vitamin C; FW=Final weight; IW= Initial weight; FW-IW= Weight change.

Table 2: Relative organ-body weight of Wistar rats treated with aqueous fruit extract of *P. dactylifera* against *mercury*–induced neurotoxicity

Group	Treatment	Brain/body weight Index
A	Control (H_2O 0.5ml/kg)	1.094 ± 0.073
B	$HgCl_2$ (5mg/kg)	1.250 ± 0.100
C	Vit C (100mg/kg) + $HgCl_2$ (5mg/kg)	1.481 ± 0.095*
D	AFPD (250mg/kg) + $HgCl_2$ (5mg/kg)	1.301 ± 0.040
E	AFPD (500mg/kg) + $HgCl_2$ (5mg/kg)	1.266 ± 0.111
F	AFPD (1000mg/kg) + $HgCl_2$ (5mg/kg)	1.347 ± 0.161

n = 4; mean ± SEM; One way ANOVA *LSD post hoc test.* *= *p<0.05 when compared with the control.* AFPD= *Aqueous fruit extract of Phoenix dactylifera*; Vit C= Vitamin C.

Table 3: Effect of aqueous fruit extract of *P. dactylifera* on histometric characteristics of Purkinje cells in the cerebellar cortex of Wistar rats

Group	Treatment	Cerebellar cell area (*μm*)	Cerebellar cell perimeter (*μm*)
A	Control (distilled H_2O 0.5 ml/kg)	664.09 ± 25.04	24.78 ± 0.41
B	$HgCl_2$ (5 mg/kg)	492.64 ± 51.51 ^{c}	21.09 ± 1.08
C	Vit C (100 mg/kg) + $HgCl_2$ (5 mg/kg)	447.71 ± 21.97 ^{c}	22.88 ± 0.68
D	*AFPD* (250 mg/kg) + $HgCl_2$ (5 mg/kg)	375.65 ± 26.96 ^{c}*	19.52 ± 0.97
E	*AFPD* (500 mg/kg) + $HgCl_2$ (5 mg/kg)	506.40 ± 21.37 ^{b}	22.85 ± 0.55
F	*AFPD* (1000 mg/kg) + $HgCl_2$ (5 mg/kg)	437.44 ± 28.96 ^{c}	20.92 ± 0.75

n = 8; mean ± SEM; One way ANOVA *LSD post hoc test* a/*=*p<0.05;* b =*p<0.01;* c =*p<0.001 when compared with the control/ $HgCl_2$.* AFPD= *Aqueous fruit extract of Phoenix dactylifera;* Vit C= *Vitamin C.*

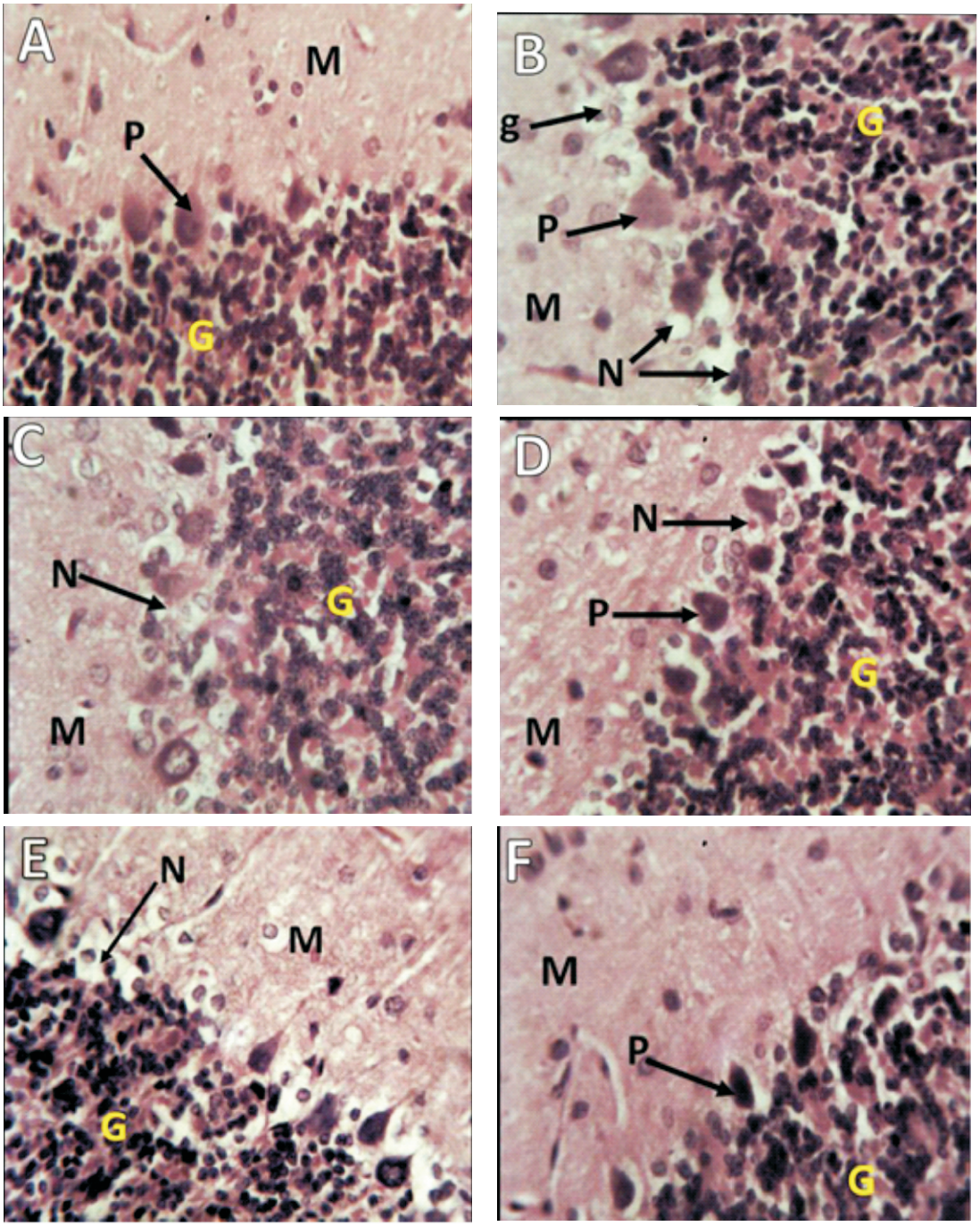

Figure 1: Micrograph of brain section (cerebellar cortex) of Wistar rat. H and E stain (Mag x 400).

A= Section of cerebellum of the control (untreated) group showing normal histology of the cerebellar cortex. Granular layer (G); Molecular layer (M); Purkinje cell (P).

B=: Section of cerebellum of the group administered mercuric chloride (5 mg/kg) showing distortion in the histology of the cerebellar cortex. Granular layer (G); Molecular layer (M); Purkinje cell (P); Neuronal degeneration – *perineuronal vacuolations and necrosis* (N); Gliosis (g)

C= Section of cerebellum of the group administered vitamin C (100 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebellar cortex. Granular layer (G); Molecular layer (M); Purkinje cell (P); Neuronal degeneration - *perineuronal vacuolations and satellitosis* (N).

D= Section of cerebellum of the group administered AFPD (250 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebellar cortex. Granular layer (G); Molecular layer (M); Purkinje cell (P); Neuronal degeneration – *perineuronal vacuolations and necrosis* (N).

E= Section of cerebellum of the group administered AFPD (500 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebellar cortex. Granular layer (G); Molecular layer (M); Purkinje cell (P); Neuronal degeneration - *perineuronal vacuolations and necrosis* (N).

F= Section of cerebellum of the group administered AFPD (1000 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebellar cortex. Granular layer (G); Molecular layer (M); Purkinje cell (P).

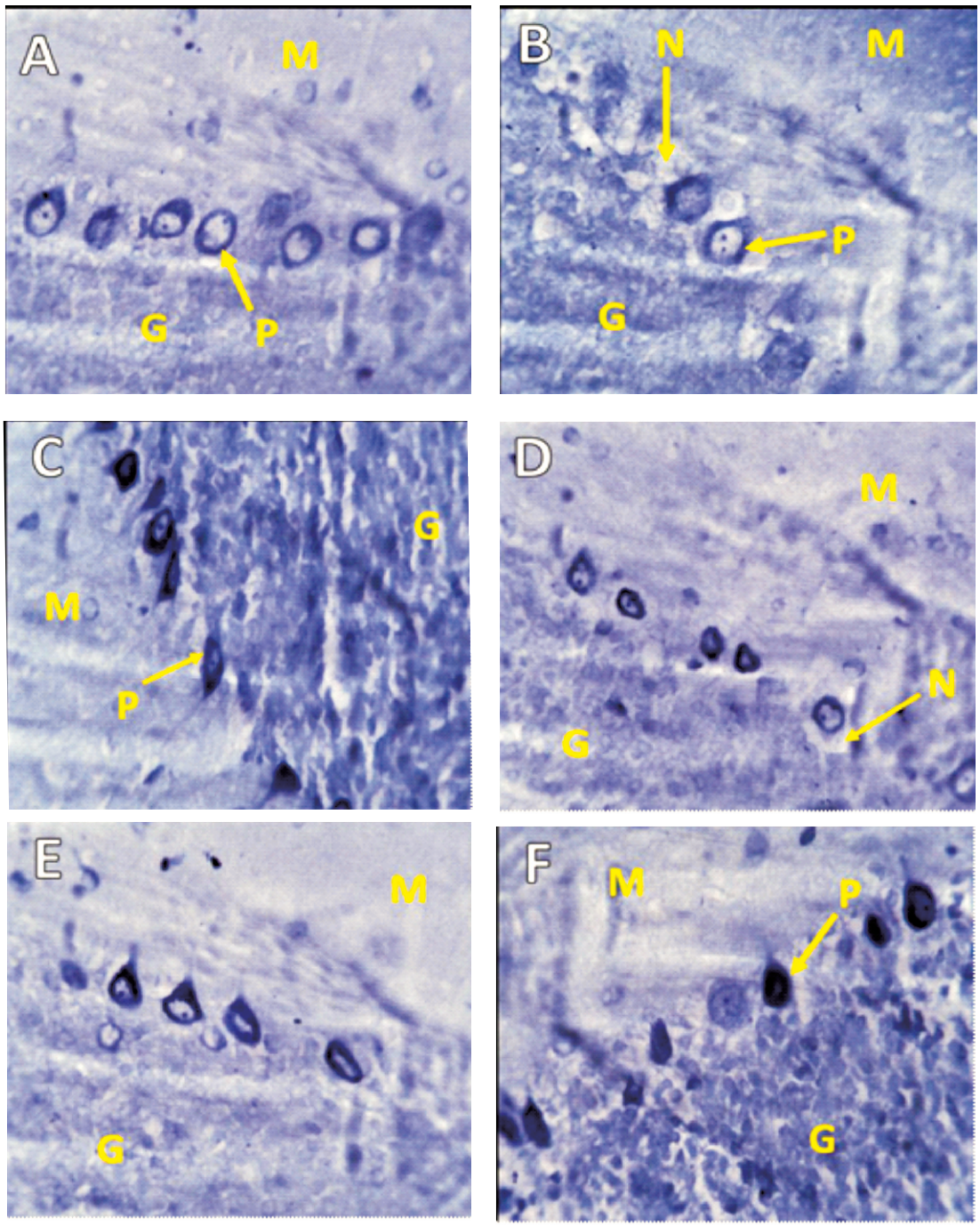

Figure 2: Micrograph of brain section (cerebellar cortex) of Wistar rat. CFV stain (Mag x 400).

A= Section of cerebellum of the control (untreated) group showing normal histology of the cerebellar cortex. Granular layer (G); Molecular layer (M); Purkinje cell (P).

B=: Section of cerebellum of the group administered mercuric chloride (5 mg/kg) showing distortion in the histology of the cerebellar cortex. Granular layer (G); Molecular layer (M); Purkinje cell (P); Neuronal degeneration – *perineuronal vacuolations and necrosis* (N).

C= Section of cerebellum of the group administered vitamin C (100 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebellar cortex. Granular layer (G); Molecular layer (M); Purkinje cell (P).

D= Section of cerebellum of the group administered AFPD (250 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebellar cortex. Granular layer (G); Molecular layer (M); Neuronal degeneration – *perineuronal vacuolations and necrosis* (N).

E= Section of cerebellum of the group administered AFPD (500 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebellar cortex. Granular layer (G); Molecular layer (M).

F= Section of cerebellum of the group administered AFPD (1000 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebellar cortex. Granular layer (G); Molecular layer (M); Purkinje cell (P).

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