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Full Length Research Article

Comparative Microscopic Assessments of the Effect of Aqueous and Ethanol Extracts of *Phoenix dactylifera L.* in a Rat Model of Mercury-Triggered Hippocampal Changes

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Summary: Mercury is an environmental neurotoxicant that triggers structural and physiological alterations in different brain parts. The hippocampus is associated with learning and memory, and injury to this brain part may lead to behavioural and cognitive changes. *Phoenix dactylifera* (date palm) has been demonstrated to possess a variety of medical benefits. This study comparatively assessed the neuroprotective property of aqueous and ethanol fruit pulp extracts of *P. dactylifera* in a rat model of mercury-triggered hippocampal changes using microscopic examinations. Twenty-eight Wistar rats were divided into seven groups (I–VII, n=4). Group I (control) was administered distilled water (2ml/kg); group II was administered mercuric chloride, $HgCl_2$ (5mg/kg); group III was administered vitamin C (100mg/kg) as reference drug +$HgCl_2$; groups IV and V were administered aqueous extract (250mg/kg and 500mg/kg, respectively) +$HgCl_2$, while groups VI and VII were administered ethanol extract (250mg/kg and 500mg/kg, respectively) +$HgCl_2$. Extracts' neuroprotective property were evaluated using histological and histometric assessments of CA1 and CA3 hippocampal sub-regions. Results revealed cytoarchitectural changes including karyopyknosis, basophilic necrosis and remarkably decreased histometric features of hippocampal pyramidal neurons in $HgCl_2$-treated group relative to control. Administration of the extracts remarkably ameliorated mercury-induced degenerative changes by preservation of cytoarchitectural features comparable to reference drug. Comparatively, neuroprotective efficacies of the extracts are relatively similar, especially at doses of 500mg/kg and could be attributed to antioxidant activities of constituent phytochemicals. Results suggest that aqueous and ethanol fruit pulp extracts of *P. dactylifera* may prove efficacious in ameliorating mercury-triggered microscopic alterations in the hippocampus of Wistar rats.

Keywords: *Neurodegeneration; Neuroprotection; Histology; Histometric; Oxidative stress*

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INTRODUCTION

Severe illness and sudden death in humans has been implicated to environmental pollution. Heavy metals including cadmium, lead and mercury have been classified among major causes of environmental pollution (Chen and Chen, 2001; Galadima et al., 2011; Branco et al., 2017). Mercury is a potent contaminant present in various environmental media and food across the globe at levels that adversely affect biological systems, exerting toxic effect on a variety of vital organs in the human body (UNEP, 2002; Jha et al., 2019). Mercury is an established neurotoxicant causing structural and physiological alterations in different brain parts by eliciting oxidative stress which results to neurological deficits (Xu et al., 2012; Phukan et al., 2019). Subcortical limbic structures like the hippocampus is associated with learning and memory, and injury to this brain part may lead to behavioural and cognitive changes (Snell, 2010; Owoeye and Farombi, 2015; Fogwe et al., 2020).

Several plants in the human diet, containing numerous natural compounds with vast medicinal benefits, have been used in folk medicine to treat different types of ailments like cognitive disorders, including neurodegenerative diseases (Kumar and Khanum, 2012). *Phoenix dactilyfera L.* (date palm) is a plant that belongs to the family Arecaceae (Ahmed et al., 2008); its fruits reported as a good source of energy and essential vitamins and elements (Usama et al.,

2009; Farooqui et al., 2019), and different parts of the plant claimed to have medicinal benefits used for the treatment of diversity of ailments including paralysis, memory disturbances, loss of consciousness and nervous disorders (Nadkarni, 1976; Saha et al., 2017). *P. dactilyfera* has been scientifically demonstrated to possess a variety of pharmacological activities including amelioration of the deleterious effects toxins, antioxidant and neuroprotective activities (Allaith and Abdul, 2005; Vyawahare et al., 2009; El-Far et al., 2016). There is need to empirically demonstrate the neuroprotective properties of certain solvent extract forms of *P. dactilyfera* against different environmental toxins in different brain regions.

This study comparatively assessed the neuroprotective property of aqueous and ethanol fruit pulp extracts of *P. dactylifera* in a rat model of mercury-triggered hippocampal changes using microscopic examinations.

MATERIALS AND METHODS

Plant Material: Dried *P. dactylifera* (date palm) fruits were obtained from Samaru Market in Zaria, Kaduna State, Nigeria and authenticated in the Herbarium Unit of the Department of Biological Sciences, Faculty of Life Sciences, Ahmadu Bello University (ABU), Zaria with the Voucher Specimen Number 7130.

Extraction and phytochemical screening of *P. dactylifera* fruit was carried out in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, ABU, Zaria. The method of maceration as reported by Agbon *et al.* (2013) and Abdul-Wahab *et al.* (2010) for the preparation of aqueous fruit pulp extract of *P. dactylifera* (AFPD) and ethanol fruit pulp extract of *P. dactylifera* (EFPD), respectively, were adopted. The method described by Trease and Evans (2002) for qualitative phytochemical screening of secondary metabolites was adopted.

Experimental Animals: The experimental animals (Wistar rats) weighing 130 ± 20 g were obtained from Animal House of the Department of Human Anatomy, Faculty of Basic Medical Sciences, ABU, Zaria and housed in new cages in the same facility were rats acclimatized for a week before experimentation. The rats were housed under standard laboratory condition, light and dark cycles of 12 hours, and were provided with food (rat chow) and water *ad libitum*.

Drug: Mercury in the form of mercuric chloride ($HgCl_2$) was obtained and used as neurotoxicant for this study. The product is manufactured by British Drug Houses Chemicals, Poole, England.
Vitamin C (ascorbic acid) was obtained and used for this study as reference antioxidant drug. The product is manufactured by Emzor Limited, Lagos, Nigeria.

Experimental Protocol: Twenty-eight (28) Wistar rats were divided into seven (7) groups (I – VII) of four rats each. Hippocampal neurotoxicity was induced in rats by the administration of $HgCl_2$ as reported by Sheikh *et al.* (2013) Group I (control) was administered distilled water (2 ml/kg); group II was administered $HgCl_2$ (5 mg/kg); group III was administered vitamin C (100 mg/kg; Raghu-Jetti et al., 2014) as reference drug followed by $HgCl_2$ (5 mg/kg); groups IV and V were administered AFPD (250 mg/kg and 500 mg/kg, respectively), while groups VI and VII were administered EFPD (250 mg/kg and 500 mg/kg, respectively). Treatment period lasted fourteen (14) days. All administrations were via oral route (*See* Figure 1).

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

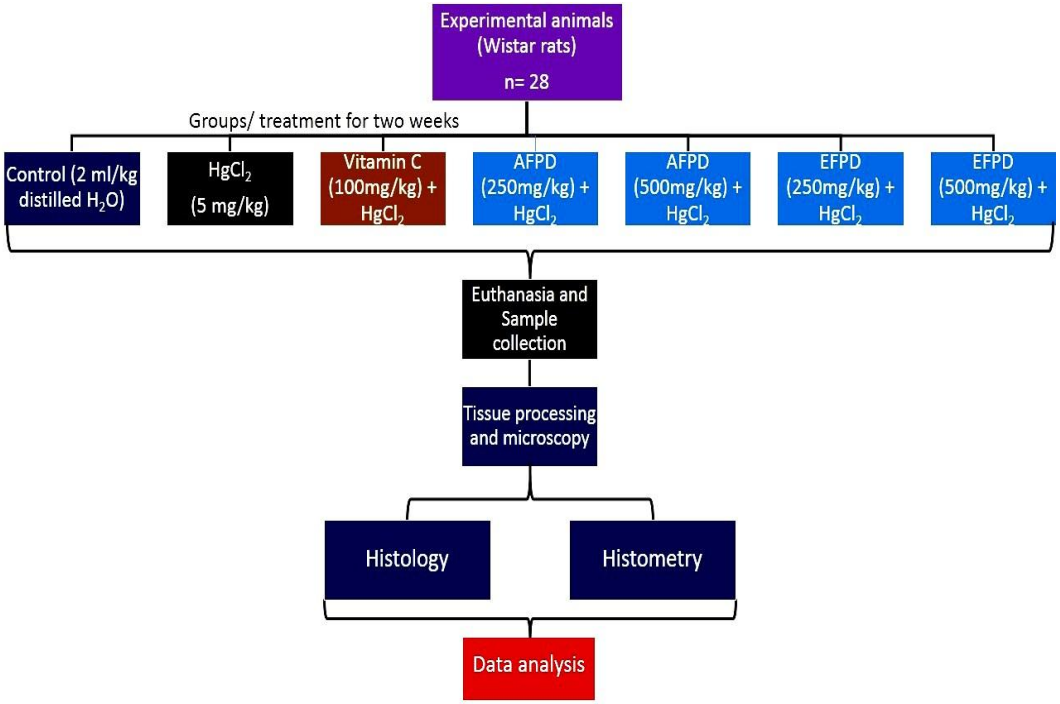

Figure 1:
Experimental protocol
n = 4; AFPD= Aqueous fruit pulp extract of Phoenix dactylifera; EFPD= Ethanol fruit pulp extract of Phoenix dactylifera; $HgCl_2$= Mercuric chloride

Physical Observation
During the period of experiment, the rats were observed for changes in physical activity and behavioral pattern, like playing and eating. Absolute body weight before (initial weight, IW) and after the experiment (final weight, FW) were weighed (using digital weighing scale, Kerro BL 20001, 0.1 g) and values were statistically compared.

Histological Studies
Fixed brains were processed using histological techniques by making a coronal section at the caudal region of cerebrum to target the hippocampus. Histological brain sections were stained for light microscopy with Haematoxylin and Eosin (H & E) and Cresly Violet (CV) stains to demonstrate cytoarchitectural features of *cornu ammonis* (CA; CA1 and CA3 regions) of the hippocampus. Tissues were processed in the Histology Unit, Department of Human Anatomy, ABU, Zaria and, light microscopy and micrography conducted in the Microscopy and Stereology Research Laboratory of the same facility.

Histometric studies: Histometric analysis was used as an objective basis for comparison of histological observation (Asuquo et al., 2007; Huda and Zaid et al., 2007). Briefly, histometry involved measuring the soma area and perimeter of pyramidal neurons of hippocampal CA1 and CA3 regions from CV (*CV is an excellent neuronal, cell body-specific stain (Suvarna et al., 2019))* stained micrographs (digital microscopic images). Histometric analysis was conducted using a light microscope (HM-LUX, Leitz Wetzlar, Germany) with a 40/ 0.65 x objective (x 400 magnification), micrometer slide (1 mm graduated in 0.01 mm units; that is divided 10x into 100 μm units) and computer running imaging software (AmScope MT *version* 3.0.0.5, USA) according to the manufacturer's instruction (Using the AmScope Microscope Cameras, 2012). Three different micrographic fields were randomly captured (Jelsing, 2006; Oliveira et al., 2015) in the CA1 and CA3 hippocampal regions and 5 - 10 neurons that met the criteria for selection (*that is, pyramidal neurons, with well-outlined nucleus in the cell profiles*) were randomly selected; using the AmScope imaging software polygon tool, soma area and perimeter were measured and analyzed.

Data Analysis
Data obtained were expressed as mean ± S.E.M; paired sample *t*–test was employed for the comparison of means and one way ANOVA with least significant difference (LSD) *post hoc test* for presence of significant difference among means of the groups. Values were considered significant when $p< 0.05$. Data were analyzed using the statistical software, Statistical Package for the Social Sciences (IBM SPSS *v 21.0* SPSS Inc., Chicago, USA) and Microsoft Office Excel 2013 for charts.

Physical observation: During the period of treatment, rats in the control group were observed to exhibit normal behavioural pattern of eating and physical activities, like movement and playfulness, while rats in other groups exhibited reduced activity, especially $HgCl_2$- treated group. The absolute body weights of rats in all groups were observed to have increased remarkably ($p<0.05$) when IW and FW were compared, except for $HgCl_2$-, Vitamin C+ $HgCl_2$ and EFPD (250 mg/kg)+ $HgCl_2$-treated groups (Table 2).

RESULTS

Phytochemical Analysis: Phytochemical screening of AFPD and EFPD produced positive reaction for secondary metabolites like, flavonoids, saponins and tannins (Table 1).

Physical observation: During the period of treatment, rats in the control group were observed to exhibit normal behavioural pattern of eating and physical activities, like movement and playfulness, while rats in other groups exhibited reduced activity, especially $HgCl_2$- treated group. The absolute body weights of rats in all groups were observed to have increased remarkably ($p<0.05$) when IW and FW were compared, except for $HgCl_2$-, Vitamin C+ $HgCl_2$ and EFPD (250 mg/kg)+ $HgCl_2$-treated groups (Table 2).

Table 1:
Phytochemical constituents of fruit pulp extracts of *P. dactylifera*

Constituents	Alkaloid	Anthraquinones	Cardiac glycoside	Carbohydrates	Flavonoids	Saponin	Tannin
Inference (AFPD)	+	–	–	+	+	+	+
Inference (EFPD)	+	–	–	+	+	+	+

+ = Positive (Present); **–** = Negative (Absent); **AFPD=** Aqueous fruit extract of *Phoenix dactylifera*; **EFPD=** Ethanol fruit extract of *Phoenix dactylifera*;

Table 2:
Absolute body weight comparison of Wistar rats

Group	Treatment	IW (g)	FW (g)	*t*	*p*-value
I	Control (H_2O 2 ml/kg)	116.63±13.28	138.50±12.01	8.919	0.000
II	$HgCl_2$ (5mg/kg)	109.50±20.67	131.00±38.92	2.261	0.073
III	Vit C (100mg/kg) + $HgCl_2$ (5mg/kg)	101.40±19.27	111.20±8.55	0.735	0.503
IV	AFPD (250mg/kg) + $HgCl_2$ (5mg/kg)	111.25±12.09	144.75±39.51	3.310	0.045
V	AFPD (500mg/kg) + $HgCl_2$ (5mg/kg)	104.57±16.55	129.86±13.23	2.881	0.028
VII	EFPD (250mg/kg) + $HgCl_2$ (5mg/kg)	144.25±36.37	159.25±59.36	1.730	0.182
VIII	EFPD (500mg/kg) + $HgCl_2$ (5mg/kg)	117.50±21.42	138.00±21.61	4.889	0.016

n= 4; mean ± SEM; Paired sample *t*- test; **AFPD=** Aqueous fruit extract of *Phoenix dactylifera*; **EFPD=** Ethanol fruit extract of *Phoenix dactylifera*; **Vit C**= Vitamin C; **$HgCl_2$**= Mercuric chloride; **FW**=Final weight; **IW**= Initial weight.

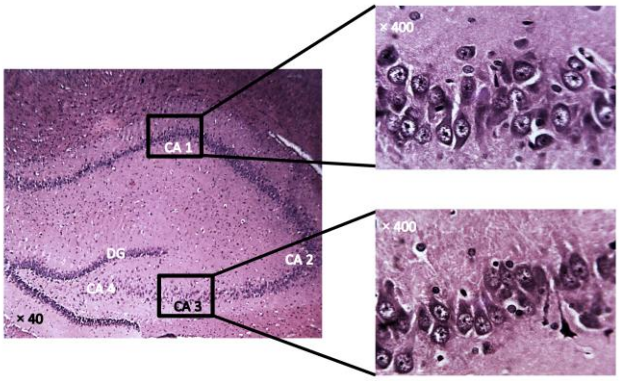

Plate 1:
Coronal section of the hippocampus of Wistar rat with subregions (CA1 - CA4). H and E stain. *Cornu ammonis* (CA); Dendate gyrus (DG)

Histological Examination: Histological examination of sections of the hippocampus (CA 1 and CA 3 regions) of rats, stained with H & E and CV stains revealed the following: The rat hippocampus (*hippocampus proprius*) is subdivided into four regions (CA1 - CA4) according to density, size and branching of axons and dentrites of the pyramidal cells. Each of these regions consists of three layers: *stratum molecular* (molecular layer), *stratum pyramidale* (pyramidal layer) which contains bodies of the pyramidal cells *and stratum multiforme* (multiform layer). The continuation of CA3 in the concavity of dendate gyrus (*fascia dentata*) is the CA4. CA1 is characterized by densely packed medium sized cells; CA3 is the region with large less densely packed cells, while CA2 is a transition field between CA3 and CA1 (Plate 1). The control group showed normal histoarchitecture of CA1 and CA3 regions: the characteristic pattern of an ordered sheet of neurons (pyramidal and granule cells) whose cell bodies are all packed together (Plates 2a and 3a). The large neurons are the giant pyramids of CA3 and other interneurons such as stellate, fusiform and basket cells of Cajal, which differ from the pyramidal and granule cells, observed most clearly in CA3 (Plates 2a and 3a).

Histological sections of $HgCl_2$-treated group revealed histoarchitectural distortion of CA1 and CA3 regions; neurodegenerative changes like, irregular arrangement of CA1 hippocampal neurons, satellitosis, perineuronal vacuolation, pyknotic and basophilic necrosis (Figures 2b, 3b, 4b and 5b). However, hippocampi of vitamin C + $HgCl_2$-, AFPD+ $HgCl_2$- and EFPD+ $HgCl_2$- treated groups, revealed mild histoarchitectural distortions of CA1 and CA3 regions relative to the control. The histological features of the vitamin C-treated group showed chromatolysis and karyorrhexis (Plates 2c, 3c, 4c and 5c); AFPD-treated groups showed histoarchitectural changes like karyopyknosis and satellitosis (Figures 2d - e, 3d - e, 4d - e and 5d – e) and EFPD-treated groups showed changes such as, karyorrhexis, eosinophilic necrosis, perineuronal vacuolations and pyknotic necrosis (Plates 2f - g, 3f - g, 4f - g and 5f - g). AFPD and EFPD administration conferred preservation of the histoarchitecture in a dose dependent manner compared to the control

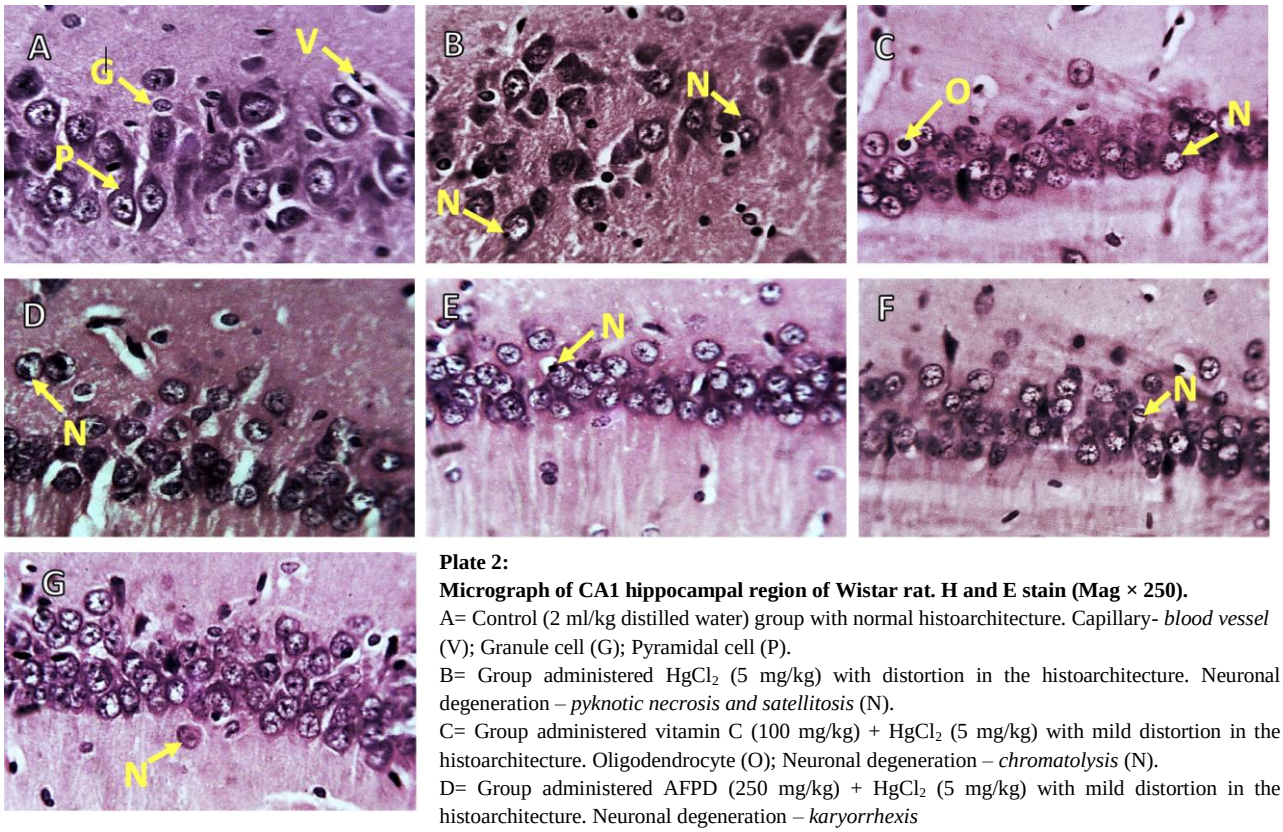

Plate 2:
Micrograph of CA1 hippocampal region of Wistar rat. H and E stain (Mag × 250).
A= Control (2 ml/kg distilled water) group with normal histoarchitecture. Capillary- *blood vessel* (V); Granule cell (G); Pyramidal cell (P).
B= Group administered $HgCl_2$ (5 mg/kg) with distortion in the histoarchitecture. Neuronal degeneration – *pyknotic necrosis and satellitosis* (N).
C= Group administered vitamin C (100 mg/kg) + $HgCl_2$ (5 mg/kg) with mild distortion in the histoarchitecture. Oligodendrocyte (O); Neuronal degeneration – *chromatolysis* (N).
D= Group administered AFPD (250 mg/kg) + $HgCl_2$ (5 mg/kg) with mild distortion in the histoarchitecture. Neuronal degeneration – *karyorrhexis*
E= Group administered AFPD (500 mg/kg) + $HgCl_2$ (5 mg/kg) with mild distortion in the histoarchitecture. Neuronal degeneration – *pyknotic necrosis and perineuronal vacuolation* (N); F= Group administered EFPD (250 mg/kg) + $HgCl_2$ (5 mg/kg) with mild distortion in the histoarchitecture. Pyramidal cell (P); Neuronal degeneration – *pyknotic necrosis and perineuronal vacuolation* (N); G= Group administered EFPD (500 mg/kg) + $HgCl_2$ (5 mg/kg) with mild distortion in the histoarchitecture. Pyramidal cell (P); Neuronal degeneration – *esinophilic necrosis* (N).

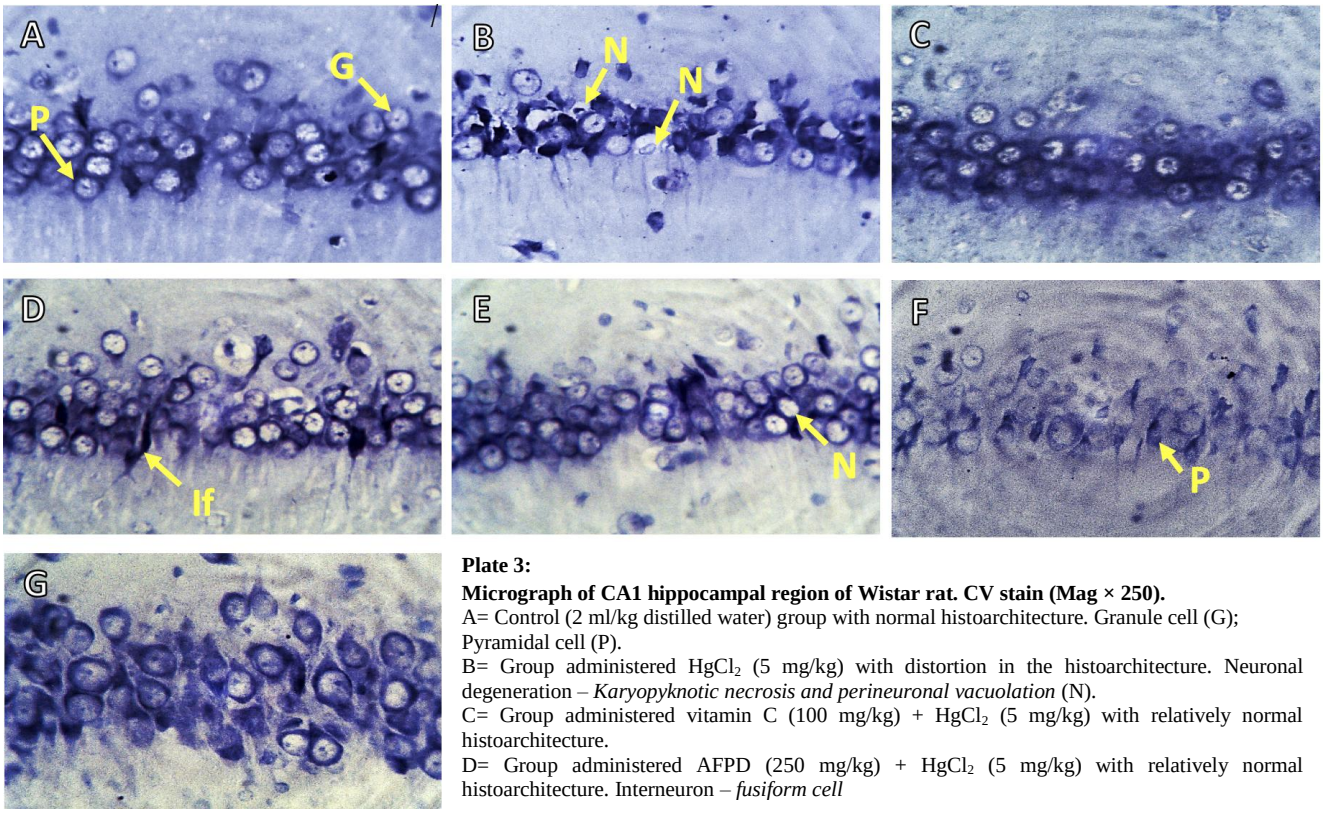

Plate 3:
Micrograph of CA1 hippocampal region of Wistar rat. CV stain (Mag × 250).
A= Control (2 ml/kg distilled water) group with normal histoarchitecture. Granule cell (G); Pyramidal cell (P).
B= Group administered $HgCl_2$ (5 mg/kg) with distortion in the histoarchitecture. Neuronal degeneration – *Karyopyknotic necrosis and perineuronal vacuolation* (N).
C= Group administered vitamin C (100 mg/kg) + $HgCl_2$ (5 mg/kg) with relatively normal histoarchitecture.
D= Group administered AFPD (250 mg/kg) + $HgCl_2$ (5 mg/kg) with relatively normal histoarchitecture. Interneuron – *fusiform cell*
E= Group administered AFPD (500 mg/kg) + $HgCl_2$ (5 mg/kg) with mild distortion in the histoarchitecture. Neuronal degeneration – *chromatolysis* (N).
F= Group administered EFPD (250 mg/kg) + $HgCl_2$ (5 mg/kg) with relatively normal histoarchitecture. Pyramidal cell (P).
G= Group administered EFPD (500 mg/kg) + $HgCl_2$ (5 mg/kg) with relatively normal histoarchitecture.

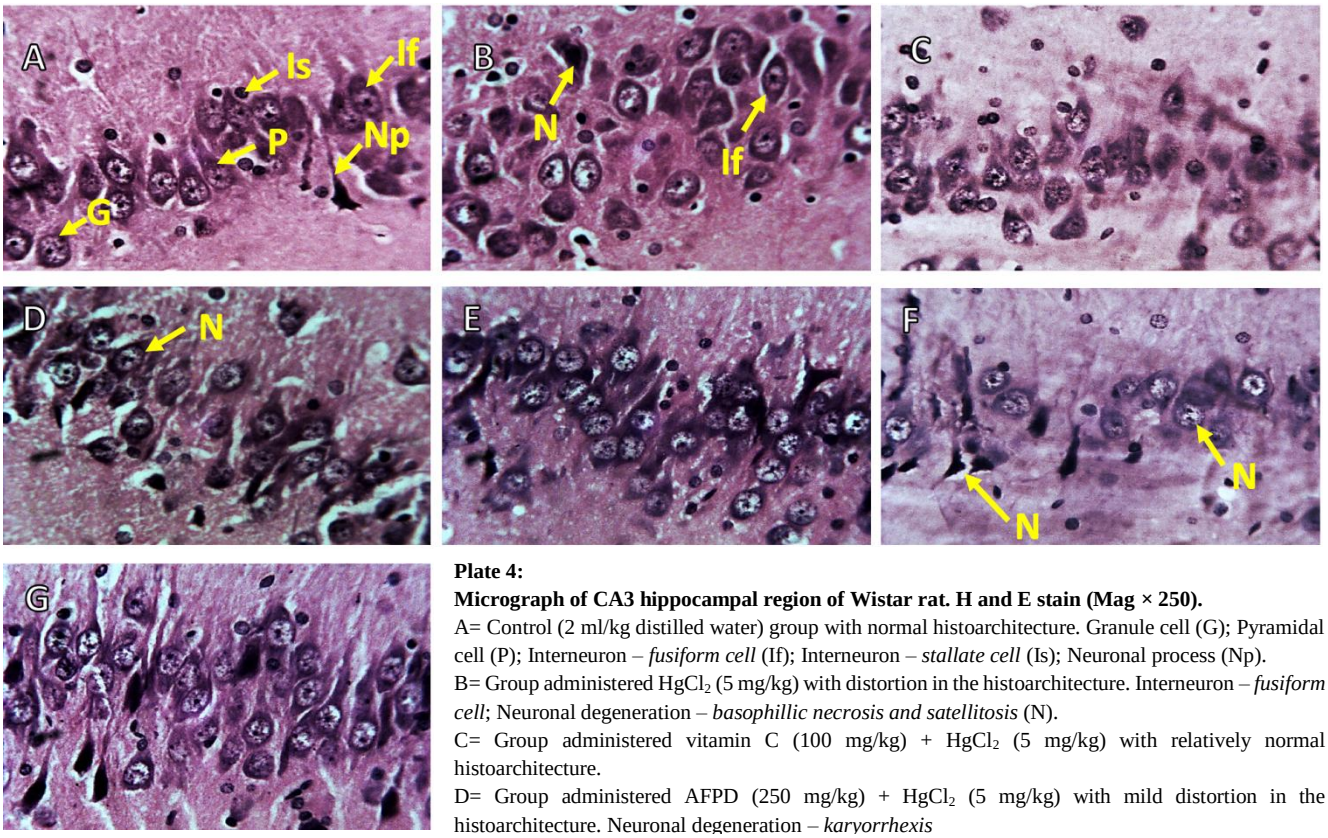

Plate 4:
Micrograph of CA3 hippocampal region of Wistar rat. H and E stain (Mag × 250).
A= Control (2 ml/kg distilled water) group with normal histoarchitecture. Granule cell (G); Pyramidal cell (P); Interneuron – *fusiform cell* (If); Interneuron – *stallate cell* (Is); Neuronal process (Np).
B= Group administered $HgCl_2$ (5 mg/kg) with distortion in the histoarchitecture. Interneuron – *fusiform cell*; Neuronal degeneration – *basophillic necrosis and satellitosis* (N).
C= Group administered vitamin C (100 mg/kg) + $HgCl_2$ (5 mg/kg) with relatively normal histoarchitecture.
D= Group administered AFPD (250 mg/kg) + $HgCl_2$ (5 mg/kg) with mild distortion in the histoarchitecture. Neuronal degeneration – *karyorrhexis*
E= Group administered AFPD (500 mg/kg) + $HgCl_2$ (5 mg/kg) with relatively normal histoarchitecture.
F= Group administered EFPD (250 mg/kg) + $HgCl_2$ (5 mg/kg) with mild distortion in the histoarchitecture. Pyramidal cell (P); Neuronal degeneration – *karyorrhexis and perineuronal vacuolation* (N).
G= Group administered EFPD (500 mg/kg) + $HgCl_2$ (5 mg/kg) with relatively normal histoarchitecture.

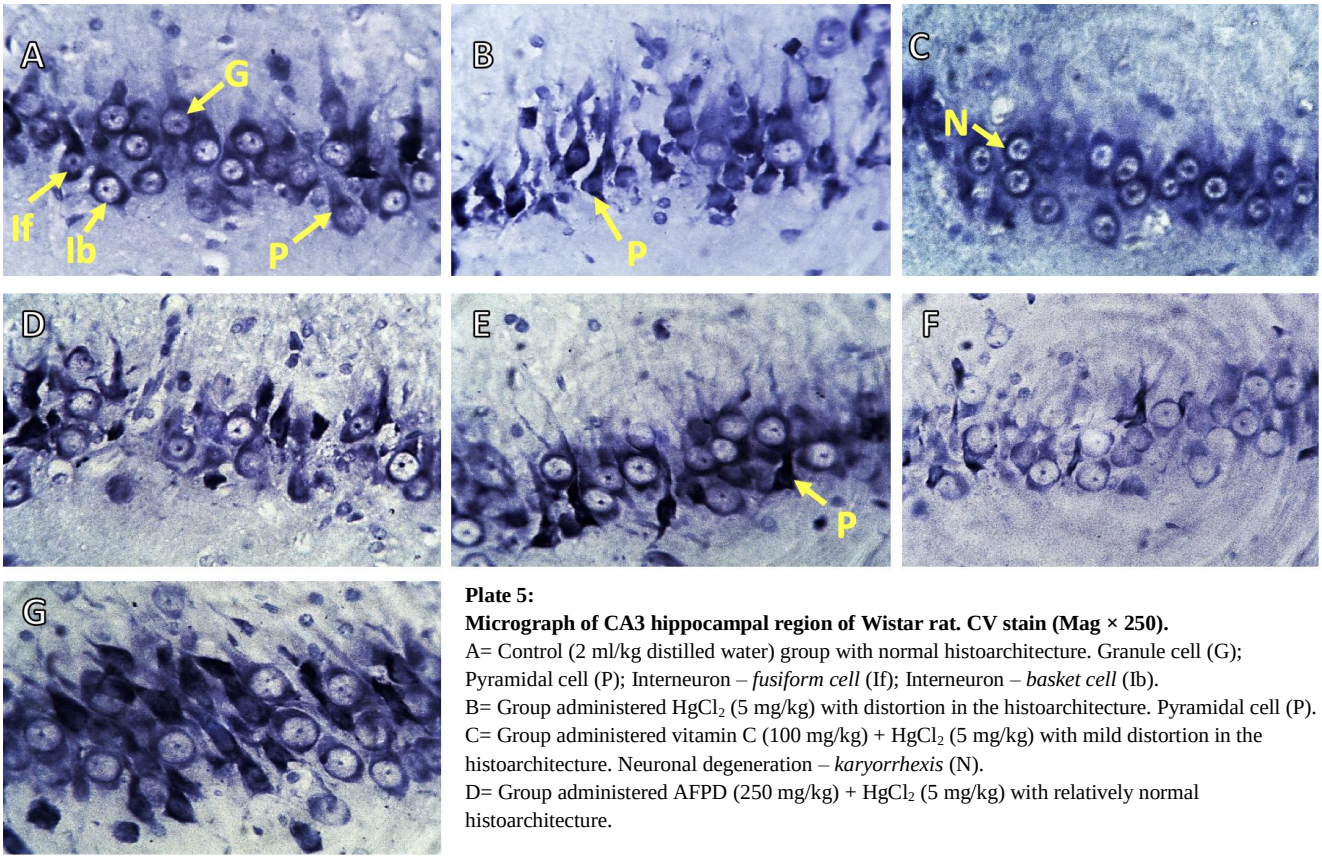

Plate 5:
Micrograph of CA3 hippocampal region of Wistar rat. CV stain (Mag × 250).
A= Control (2 ml/kg distilled water) group with normal histoarchitecture. Granule cell (G); Pyramidal cell (P); Interneuron – *fusiform cell* (If); Interneuron – *basket cell* (Ib).
B= Group administered $HgCl_2$ (5 mg/kg) with distortion in the histoarchitecture. Pyramidal cell (P).
C= Group administered vitamin C (100 mg/kg) + $HgCl_2$ (5 mg/kg) with mild distortion in the histoarchitecture. Neuronal degeneration – *karyorrhexis* (N).
D= Group administered AFPD (250 mg/kg) + $HgCl_2$ (5 mg/kg) with relatively normal histoarchitecture.
E= Group administered AFPD (500 mg/kg) + $HgCl_2$ (5 mg/kg) with relatively normal histoarchitecture. Pyramidal cell (P).
F= Group administered EFPD (250 mg/kg) + $HgCl_2$ (5 mg/kg) with relatively normal histoarchitecture.
G= Group administered EFPD (500 mg/kg) + $HgCl_2$ (5 mg/kg) with relatively normal histoarchitecture.

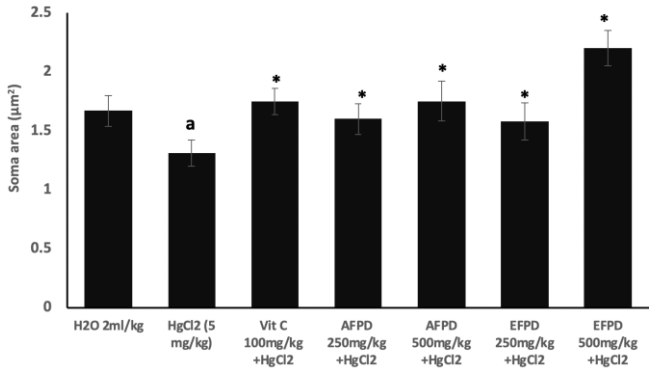

Figure 2a
Effect of *P. dactylifera* on histometric characteristic (soma area) of pyramidal neuron in the CA1 hippocampal region of Wistar rats.
n = 20 ± 5 (*5-10 cells/ 3 fields*); mean ± SEM; One way ANOVA *LSD post hoc test*, **a** =p<0.05 when compared with the control (2 ml/kg distilled H_2O), ***** =p<0.05 when compared with $HgCl_2$. **AFPD=** Aqueous fruit extract of *Phoenix dactylifera*; **EFPD=** Ethanol fruit extract of *Phoenix dactylifera*; **Vit C**= Vitamin C; **CA**= *Cornu Amonis.*

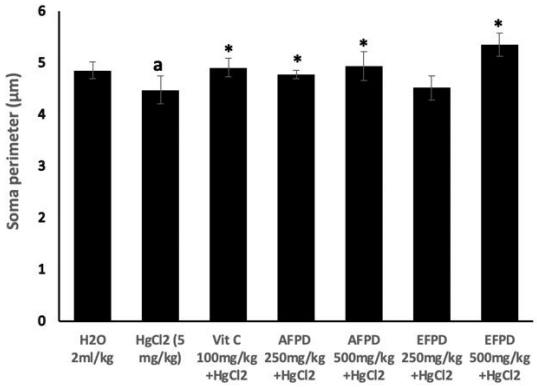

Figure 2b
Effect of *P. dactylifera* on histometric characteristic (soma perimeter) of pyramidal neuron in the CA1 hippocampal region of Wistar rats.
n = 20 ± 5 (*5-10 cells/ 3 fields*); mean ± SEM; One way ANOVA *LSD post hoc test*, **a** =p<0.05 when compared with the control (2 ml/kg distilled H_2O), ***** =p<0.05 when compared with $HgCl_2$. **AFPD=** Aqueous fruit extract of *Phoenix dactylifera*; **EFPD=** Ethanol fruit extract of *Phoenix dactylifera*; **Vit C**= Vitamin C; **CA**= *Cornu Amonis*

Histometric Analysis: Histometric features of CA1 pyramidal neuron revealed remarkable ($p<0.05$) decrease in soma area and perimeter of $HgCl_2$- treated group compared to the control. Relative to the $HgCl_2$-treated group, striking ($p<0.05$) difference in histometric features were observed with the vitamin C + $HgCl_2$-, AFPD+ $HgCl_2$- and EFPD+ $HgCl_2$ -treated groups (Figures 2a and b).

Histometric features of CA3 pyramidal neuron revealed remarkable ($p<0.05$) decrease in soma area of $HgCl_2$-treated group relative to the control. Moreover, relative to the $HgCl_2$-treated group, striking ($p<0.05$) difference in neuronal soma area was observed with vitamin C + $HgCl_2$-, AFPD+ $HgCl_2$- and EFPD+ $HgCl_2$ -treated groups (Figure 3a). Neuronal soma perimeter revealed significant decrease with $HgCl_2$- and AFPD (250 mg/kg) + $HgCl_2$-treated groups when compared to the control. Comparing neuronal soma perimeter of $HgCl_2$-treated group with AFPD+ $HgCl_2$- and EFPD+ $HgCl_2$ -treated groups revealed remarkable difference (Figure 3b).

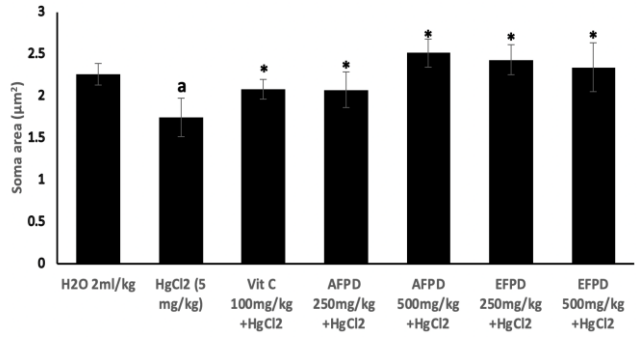

Figure 3a
Effect of *P. dactylifera* on histometric characteristic (soma area) of pyramidal neuron in the CA3 hippocampal region of Wistar rats.
n = 20 ± 5 (*5-10 cells/ 3 fields*); mean ± SEM; One way ANOVA *LSD post hoc test*, **a** =p<0.05 when compared with the control (2 ml/kg distilled H_2O), ***** =p<0.05 when compared with $HgCl_2$. **AFPD=** Aqueous fruit extract of *Phoenix dactylifera*; **EFPD=** Ethanol fruit extract of *Phoenix dactylifera*; **Vit C**= Vitamin C; **CA**= *Cornu Amonis*

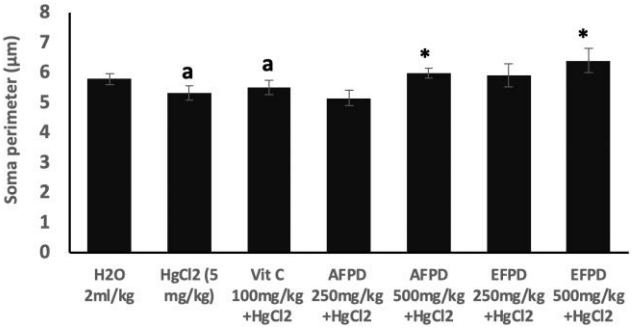

Figure 3b
Effect of *P. dactylifera* on histometric characteristic (soma perimeter) of pyramidal neuron in the CA3 hippocampal region of Wistar rats.
n = 20 ± 5 (*5-10 cells/ 3 fields*); mean ± SEM; One way ANOVA *LSD post hoc test*, **a** =p<0.05 when compared with the control (2 ml/kg distilled H_2O), ***** =p<0.05 when compared with $HgCl_2$. **AFPD=** Aqueous fruit extract of *Phoenix dactylifera*; **EFPD=** Ethanol fruit extract of *Phoenix dactylifera*; **Vit C**= Vitamin C; **CA**= *Cornu Amonis*

DISCUSSION

In this study, phytochemical analysis of AFPD and EFPD were conducted and, the neuroprotective effect of AFPD and EFPD against mercury-triggered hippocampal changes was comparatively evaluated by the assessment of Wistar rats' physical activity, absolute body weight changes and microscopic features of CA1 and CA3 hippocampal regions.

Phytochemical analysis of AFPD and EFPD revealed the presence of similar metabolites like flavonoids, saponins, and tannins which have been reported to possess antioxidant and neuroprotective properties in models of neurological disorders (Chen et al., 2015; Sarian et al., 2017). Findings agree with reported phytochemicals present in fruit extract of *P. dactylifera* (Raghu-Jetti et al., 2014; Bouhlali et al., 2017).

Altered physical activity exhibited by $HgCl_2$-treated group is suggestive of treatment-related toxicity. Loss of appetite and sluggishness has been associated with drug-related toxicity (Salawu et al., 2009; Agbon et al., 2014). Body weight change is a sensitive pointer to the general health status of an animals and serves as indicator of the deleterious effects of drugs and chemicals (Mukinda and Syce, 2007; Salawu et al., 2009). Observed striking increase in the trend of absolute body weight with AFPD and EFPD-treated groups, when IW and FWs were compared, could be attributed to the high caloric content in *P. dactylifera* which has been reported as a good source of energy and rich in nutrients (Ghnimi et al., 2017; Al-Mssallem et al., 2019; Hussain et al., 2020).

The hippocampus has several functions, playing critical role in short- and long-term memory and spatial navigation (Eichenbaum and Cohen, 2014; Ekstrom and Ranganath, 2018). The relevance of hippocampal CA1 and CA3 subregions in hippocampal functions have been reported by several researchers (Kesner et al., 2005; Kesner, 2013; Dimsdale-Zucker et al., 2018).

Observed histoarchitectural distortion of the hippocampal regions, like irregular arrangement of CA1 hippocampal neurons, pyknosis and basophilic necrosis are indicative of $HgCl_2$ treatment-triggered neurodegenerative changes. Findings are in accordance with the reports of Falluel-Morel et al. (2007) and Ranjan et al. (2015) on the vulnerability of hippocampus to mercury showing detrimental changes; cytoarchitectural distortion of hippocampal neurons in response to mercury exposure.

The integrity of CA1 architecture is important because of the particular vulnerability of the neurons in the pyramidal layer to toxic events (Seidman, 2011). Typically, the neurons are packed together, arranged in one or two very dense rows (Slomianka, 2011). Depolarization or irregular arrangement of CA1 neurons observed in this study is suggestive of treatment-related degenerative changes. Finding is in line with the reports on preferential degeneration of CA1 pyramidal cells following insult (Ruan et al., 2007; Seidman, 2011). Wu *et al.* (2016) observed disorganization and decrease in the number of hippocampal neurons in Sprague Dawley rats exposed to mercury. Observed basophilia of cytoplasm and pyknosis of neurons, which histologically characterizes neuronal atrophy associated with a wide variety of irreversible neuronal injuries resulting to progressive cell death in several degenerative disorders (Seilhean et al., 2004) is indicative of treatment related toxicity. Several lines of evidence have associated the main neurotoxic mechanism of mercury to induction of oxidative stress (Shanker et al., 2004; Farina et al., 2013; Abdel-Zaher et al., 2017).

In this study, administration of vitamin C ameliorated mercury-induced hippocampal changes. Findings are in agreement with reports on neuroprotective activity of ascorbic acid following heavy metal intoxication (El-Sokkary and Awadalla, 2011; Kumar et al., 2018). Raghu-Jetti *et al.* (2014) reported less severe neurodegenerative changes in hippocampal subregions of fluoride exposed animals treated with ascorbic acid. Treatment with AFPD and EFPD ameliorated mercury-induced hippocampal changes by conferring histoarchitectural preservation. Findings are in consistence with reports related to neuroprotective properties of plant products which had been shown to exert neuroprotective effects (Kim et al., 2008; Khazdair et al., 2019; Phukan et al., 2019) against experimentally-induced neuronal injury.

Mild neurodegenerative changes observed in hippocampal CA1 and CA3 subregions of vitamin C-, AFPD- and EFPD-treated groups is suggestive of histoarchitectural preservation and neuroprotection. Neuroprotection refers to the relative preservation of neuronal structure and/ or function (Casson et al., 2012; Sairazi and Sirajudeen, 2020). Relative preservation of neuronal integrity implies a reduction in the rate of neuronal

damage in the presence of a neurodegenerative insult such as mercury. Ascorbic acid participates in several beneficial cellular functions including antioxidant protection which plays critical roles in the reversion of mercury-induced injury by forming inert complexes and inhibiting toxic effects on neurons (Kumar et al., 2018; Teleanu et al., 2019). Neuroprotective properties of natural agents including plants have been attribute to antioxidant activities (Hwang et al., 2012; Kim et al., 2015; Sairazi and Sirajudeen, 2020). Plant phytochemical constituents including flavonoids and tannin have been reported to exhibit great antioxidant activities which provide protection against oxidative stress (Chang et al., 2012; Hwang et al., 2015). Thus, AFPD and EFPD neuroprotective property is comparable to that of the reference drug, vitamin C.

Histometric quantification provides for accurate statistical grading for comparison of histological observation, increasing precision and improving assessment of certain histological change compared with direct visual appraisal (Huda and Zaid et al., 2007; Agbon et al., 2016). In this study, remarkably decreased histometric features (soma area and perimeter) of CA1 and CA3 pyramidal neurons in $HgCl_2$-treated group are an indication of treatment-related cytoarchitectural changes. Decreased perikaryal size, an attribute of neuronal cytoplasmic shrinkage, has been associated to stress-induced cytoarchitectural changes (Insausti et al., 1997; Mohammad et al., 2012). Histometric parameters are directly applied and related to tissue function (Pearse and Mark, 1974; Huda and Zaid et al., 2007). Heavy metals, including mercury, have been reported to undergo redox cycling reactions and possess the ability to produce reactive radicals such as superoxide anion radical and nitric oxide in biological systems (Jomova and Valko, 2011; Farina et al., 2013; Cariccio et al., 2018). Neuronal shrinkage has been reported as direct toxic effect or role of mercuric chloride in neurodegenerative progression (Ramzi and Stanely, 1994; Ghusoon et al., 2012; Jha et al., 2019).

Relative to the $HgCl_2$-treated group, striking difference in histometric features observed in vitamin C-, AFPD- and EFPD-treated groups is suggestive of cytoarchitectural preservation and neuroprotection. Neuroprotective effects of various phytochemicals are associated with reduced levels of oxidative stress (Kumar and Khanum, 2012; Gombeau et al., 2019). Wan Ismail and Mohd Radzi (2013) reported increased levels of endogenous antioxidants in the brain and remarkable reduction in neuronal damage in form of neuronal shrinkage, atrophy and necrosis in rats treated with *P. dactylifera* fruit. Histometric findings corroborate with histologic observations in this study. Thus, AFPD and EFPD have potentials to preserve neuronal cytoarchitectural features exposed to $HgCl_2$-triggered degenerative changes. Comparatively, the neuroprotective efficacy of AFPD and EFPD are relatively similar, especially at doses of 500 mg/kg.

Antioxidant activities have been implicated for neuroprotective property of *P. dactylifera* (Kalantaripour et al., 2012; Agbon et al., 2017; Essa et al., 2019). Phytochemicals including flavonoids, saponins and tannins have been reported to be potent scavengers of reactive oxygen species, metal ions chelators (El Sohaimy et al., 2015; Komaki et al., 2015), protectors of neurons from lethal damage induced by neurotoxins (Pujari et al., 2011) and, exert multiplicity of neuroprotective actions in *in vivo* and *in vitro* models of neurological disorders (Lobo et al., 2010; Chang et al., 2012; Hussain et al., 2020). Relevance of the structural and physiological integrity of CA1 and CA3 hippocampal subregions in hippocampal functionality has been established by several researchers (Kesner et al., 2005; Drew and Huckleberry, 2017; Liang et al., 2020). Ross *et al.* (2009) and Farovik *et al.* (2010) have reported severe memory impairment after selective CA3 and CA1 damage. The potential ability of *P. dactylifera* phytochemicals to suppress neuroinflammation and neuronal injury indicates its potential to promote memory, learning and cognitive function (Komaki et al., 2015).

In conclusion, results suggest that aqueous and ethanol fruit pulp extracts of *P. dactylifera* may prove efficacious in ameliorating mercury-triggered microscopic alterations in the hippocampus of Wistar rats. Neuroprotective property was dose dependent and relatively similar efficacy for both extract forms, AFPD and EFPD. Neuroprotective property could be attributed to antioxidant activities of constituent phytochemicals. Thus, aqueous and ethanol fruit pulp extracts of *P. dactylifera* are potential candidates for application in the management and treatment of mercury-induced neurodegenerative changes and related disease conditions.

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REFERENCES

Abdel-Zaher, M., Taha, M.M.M., Ahmed, H.G. (2017). Neurodegenerative disorders associated with mercuric chloride toxicity in mice and the role of some antioxidant. Int. J. Sci. Res. 6(4):1253-1260.

Abdul-Wahab, A., Mabrouk, M.A., Joro, J.M., Oluwatobi, S.E., Bauchi, Z.M., John, A.A. (2010). Ethanol extract of *phoenix dactylifera l.* prevents lead induced hematotoxicity in rats. Cont. J. Biomed. Sci. 4:10-15.

Agbon, A.N., Abubakar, M.G., Enemali, F.U., Mahdi, O., Bobbo, K.A., Sule, H., Yahaya, M.H., Okoh C.C. (2017). Assessment of Ethanol Fruit Extract of *Phoenix dactylifera L*. (Date Palm) on Mercuric Chloride-induced Cerebral and Cerebellar Alterations in Wistar Rats. J. Anat. Sci. 8(1):188-201.

Agbon, A.N., Ingbian, S.D., Dahiru, A.U. (2014). 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. 1:204-209.

Agbon, A.N., Kwanashie, H.O., Hamman, W.O., Ibegbu, A.O., Akpulu, P.S., Ogirima, N.A. (2016). 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. J. Anat. Sci. 7(2):44-54.

Agbon, A.N., Kwaneshie, H.O., Hamman, W.O. (2013). Antidiarrheal activity of aqueous fruit extract of *Phoenix dactylifera* (date palm) in Wistar rats. Br. J. Pharmacol. Toxicol. 4(3):121-127.

Ahmed, M.B., Hasona, N.A., Selemain, H.A. (2008). Protective effects of extract from dates (*Phoenix dactylifera* l.) and ascorbic acid on thioacetamide-induced hepatotoxicity in rats. Iran J. Pharm. Res. 7(3):193-201.

Allaith, F., Abdul, A.A. (2005). *In vitro* evaluation of antioxidant activity of different extracts of *Phoenix dactylifera* fruits as

functional foods. Deutsche Lebensmittel Rundschau. 101:305-8.

Al-Mssallem, M.Q., Alqurashi, R.M., Al-Khayri, J.M. (2019). Bioactive compounds of date palm (*Phoenix dactylifera L.*). Springer Nature Switzerland AG. p. 2

Asuquo, O.R., Igiri, A.O., Olawoyin, O.O., Eyong, E.U. (2007). Correlation of histological and histometric changes in rats testes treated with chloroquine phosphate. Nig. J. Physiol. Sci. 22(1-2):135-139.

Biglari, F., Abbas, F.M.A., Azhar, M.E. (2008). Antioxidant activity and phenolic content of various date palm (*Phoenix dactylifera*) fruits from Iran. Food Chem. 107:1636-1641.

Bouhlali, E.T., Ramchoun, M., Alem, C., Ghafoor, K., Ennassir, J., Zegzouti, Y.F. (2017). Functional composition and antioxidant activities of eight Moroccan date fruit varieties (*Phoenix dactylifera L.*). J. Saudi Soci. Agri. Sci. 16:257–264.

Branco, V., Caito, S., Farina, M., Teixeira da Rocha, J., Aschner, M., Carvalho, C. (2017): Biomarkers of mercury toxicity: Past, present, and future trends, J. Toxicol. Environ. Health, Part B. 1-36.

Cariccio, V.L., Samà, A., Bramanti, P., Mazzon, E. (2018). Mercury Involvement in Neuronal Damage and in Neurodegenerative Diseases. Biol. Tr. Elem. Res. 187(2):341-356.

Casson, R.J., Chidlow, G., Ebneter, A., Wood, J.P., Crowston, J., Goldberg, I. (2012). Translational neuroprotection research in glaucoma: a review of definitions and principles. Clin. Exp. Ophthalmol. 40(4):350–357.

Chang, C.L., Lin, C.S., Lai, G.H. (2012). Phytochemical characteristics, free radical scavenging activities, and neuroprotection of five medicinal plant extracts. Evidence-Based Comp. Alt. Med. 1-8.

Chen, Y., Chen, M. (2001). Heavy metal concentrations in nine species of fishescaught in coastal waters off Ann-ping S.W. Taiwan. J. Food Drug Anal. 9(2):219.

Chen, Y., Sun, H., Huang, L., Li, J., Zhou, W., Chang, J. (2015). Neuroprotective Effect of Radix Trichosanthis Saponins on Subarachnoid Hemorrhage. Evidence-Based Compl. Alt. Med. 2015;1-10.

Dimsdale-Zucker, H.R., Ritchey, M., Ekstrom, A.D., Yonelinas, A.P., Ranganath, C. (2018). CA1 and CA3 differentially support spontaneous retrieval of episodic contexts within human hippocampal subfields. Nature Communications, 9(294): 1-8.

Eichenbaum, H., Cohen, N.J. (2014) Can we reconcile the declarative memory and spatial navigation views on hippocampal function? Neuron. 83:764–770.

Ekstrom, A.D., Ranganath, C. (2018) Space, time, and episodic memory: the hippocampus is all over the cognitive map. Hippocampus. 28:680–687.

El Sohaimy, S.A., Abdelwahab, A.E., Brennan, C.S., Aboul-enein, A.M. (2015). Phenolic content, antioxidant and antimicrobial activities of Egyptian date palm (*Phoenix dactylifera L.*) fruits. Australian J. Basic Appl. Sci. 9:141–147.

El-Far, A.H., Hazem, M.S., Mohamed, M.A., Soad, K.A., Shaker, A.M. (2016). Date palm (*Phoenix dactylifera*): Protection and remedy food. Curr. Tr. Nutr. 2016;1(2):1-10.

El-Sokkary, G.H., Awadalla, E.A. (2011). The protective role of vitamin c against cerebral and pulmonary damage induced by cadmium chloride in male adult albino rat. *The Open* Neuroendocrinology J. 4:1-8.

Essa, M.M., Singh, V., Guizani, N., Manivasagam, T., Thenmozhi, A.J., Bhat, A., Ray, B., Chidambaram, S.B. (2019). *Phoenix dactylifera L.* fruits date fruit ameliorate oxidative stress in 3-NP intoxicated PC12 cells. Int. J. Nutr. Pharm. Neurol. Dis. 9:41-7.

Falluel-Morel, A., Sokolowski, K., Sisti, H.M., Zhou, X., Shors, T.J., Dicicco-Bloom, E. (2007). Developmental mercury exposure elicits acute hippocampal cell death, reductions in neurogenesis, and severe learning deficits during puberty. J. Neurochem. 103:1968-81.

Farina, M., Avila, D.S., da Rocha, J.B., Aschner, M. (2013). Metals, oxidative stress and neurodegeneration: a focus on iron, manganese and mercury. Neurochem. Intern. 62(5):575-594.

Farooqui, S., Khan, W., Pandey, S., Hussain, Z. (2019). Study of sustenance properties of dates. J. Emer. Tech. Inn. Res. 6(1):1-5.

Farovik, A., Dupont, L.M., Eichenbaum, H. (2010). Distinct roles for dorsal CA3 and CA1 in memory for sequential nonspatial events. Learn Mem.17:12-17.

Fogwe, L.A., Reddy, V., Mesfin, F.B.(2020) Neuroanatomy, Hippocampus In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing.

Galadima, A., Garba, Z.N., Leke, L., Almustapha, M.N., Adam, I.K. (2011). Domestic water pollution among local communities in Nigeria - causes and consequences. Euro. J. Sci. Res. 52(4):592-603.

Ghnimi, S., Umer, S., Karim, A., & Kamal-Eldin, A. (2017). Date fruit (*Phoenix dactylifera L.*): An underutilized food seeking industrial valorization. NFS J., 6:1-10.

Ghusoon, A.K.A., Rajiha, A.A.N., Eman, H.Y.A. (2012). Assessment the therapeutic effects of aqueous extracts of *Cilantro* and *Garlic* in mercuric chloride poisoning in rats. Iraqi J. Vet. Med. 36(2):231-243.

Gombeau, K., Bezerra de Oliveira, R., Sarrazin, S.L.F., Veras Mourão, R.H., Bourdineaud, J.P. (2019). Protective effects of *Plathymenia reticulata* and *Connarus favosus* aqueous extracts against cadmium- and mercury-induced toxicities. Toxicol. Res. 35(1):25-35.

Huda, M.A., Zaid, A.A. (2007). Photo-histometry a modified computer assisted morphometric measurement program. Fac. Med. Baghdad. 49(1):1.

Hussain, M.I., Farooq, M., Syed, Q.A. (2020). Nutritional and biological characteristics of the date palm fruit (*Phoenix dactylifera L.*) – A review. Food Biosci. 34(100509):1-12.

Hwang, S., Shih, P., Yen,G. (2015). Citrus flavonoids and effects in dementia and age-related cognitive decline. Diet Nutr. Dem. Cogn. Decl. 80:869–878.

Hwang, S., Shih, P., Yen, G. (2012). Neuroprotective effects of citrus flavonoids. J. Agric. Food Chem. 60(4):877–885.

Insausti, A.M., Gaztelu, J.M., Gonzalo, L.M., Romero-Vives, M., Barrenechea, C., Felipo, V. (1997). Diet induced hyperammonemia decreases neuronal nuclear size in rat entorhinal cortex. Neurosci. Lett. 231:179–181.

Jelsing, J., Gundersen, H.J.G., Nielsen, R., Hemmingsen, R., Pakkenberg, B. (2006). The postnatal development of cerebellar Purkinje cells in the Göttingen minipig estimated with a new stereological sampling technique – the vertical bar fractionators. J. Anat. 209:321-331.

Jha, A., Saidullah, B., Bubber, P. (2019). A study on prooxidative and neurotoxic effects of mercury chloride in rats. EC Pharmacol. Toxicol. 7(2):112-124.

Jomova, K., Valko, M. (2011). Advances in metal-induced oxidative stress and human disease. Toxicol. 10,283(2-3):65-87.

Kalantaripour, T.P., Asadi-Shekaari, M., Basiri, M., Gholaamhosseinian, A.N. (2012). Cerebroprotective effect of date seed extract (*Phoenix dactylifera*) *on* focal ischemia in male rats. J. Biol. Sci. 12(3):180-185.

Kesner, R.P. (2013). A process analysis of the CA3 subregion of the hippocampus. Frontiers in Cell. Neurosci. 7.78:1-17.

Kesner, R.P., Hunsaker, M.R., Gilbert, P.E. (2005). The role of CA1 in the acquisition of an object-trace-odor paired associate task. Behav. Neurosci. 119:781-786.

Khazdair, M.R., Anaeigoudari, A., Hashemzehi, M., Mohebbati, R. (2019). Neuroprotective potency of some spice herbs, a literature review. J. Trad. Comp. Med. 9:98-105.

Kim, S., Nah, S.Y., Rhim, H. (2008). Neuroprotective effects of ginseng saponins against L-type Ca^{2+} channel-mediated cell death in rat cortical neurons. Biochem. Biophys. Res. Comm. 365(3):399–405.

Kim, W., Kim, D.W., Yoo, D.Y., Jung, H.Y., Kim, J.Y., Kim, D. *et al.* (2015). Antioxidant effects of Dendropanax morbifera Léveille extract in the hippocampus of mercury-exposed rats. BMC Comp. Alt. Med. 15:247.

Komaki, A., Hoseini, F., Shahidi, S., Baharlouei, N. (2015). Study of the effect of extract of Thymus vulgaris on anxiety in male rats. J. Trad. Com. Med.1-5.

Kumar, A., Saini, R.V., Saini, A.K. (2018). Neuroprotective role of ascorbic acid: antioxidant and non-antioxidant functions. Asian J. Pharm. Clin. Res. 11(10):30-33.

Kumar, G.P., Khanum, F. (2012). Neuroprotective potential of phytochemicals. Pharmacogn. Rev. 6(12)81-90.

Liang, X., Hsu, L.M., Lu, H., Ash, J. A., Rapp, P. R., Yang, Y. (2020). Functional connectivity of hippocampal CA3 predicts neurocognitive aging via CA1–frontal circuit. Cerebral Cortex. 30:4297-4305.

Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacog Rev* 2010;4:118-126.

Mohammad, H., Ebrahim, E., Ali, H. (2012). The effect of prenatal exposure to restraint stress on hippocampal granule neurons of adult rat offspring. Iran J. Basic Med. Sci. 15(5):1060-1067.

Mukinda, J.T., Syce, J.A. (2007). Acute and chronic toxicity of the aqueous extract of Artemisia afra in rodents. J. Ethnopharmacol. 112:138-144.

Nadkarni, K.M. (1976). Indian Materia Medica, Vol. 1, Popular Prakashan. Bombay. p. 1175–81.

Oliveira, A.C., Pereira, M.C., Santana, L.N. da S., Fernandes, R.M., Teixeira, F.B., Oliveira, G. B., et al. (2015). Chronic ethanol exposure during adolescence through early adulthood in female rats induces emotional and memory deficits associated with morphological and molecular alterations in hippocampus. J. Psychopharmacol. 29(6):712-724.

Owoeye, O., Farombi, E.O. (2015). Tomato pomace protects against mercuric chloride-induced neurodegeneration and motor abnormality in adult rat. Int. J. Biol. Chem. Sci. 9(3):1142-1153.

Pearse, A., Marks, R. (1974). Measurement of section thickness in quantitative microscopy with special reference to histometry. J. Clin. Path. 27:615.

Phukan, P., Namasudra, S., Bawari, M., Sengupta, M. (2019). Neuroprotective effects of aqueous extract of *Hydrocotyle javanica* in ameliorating neurobehavioral alteration induced by mercury . Asian J. Pharm. Clin. Res.12(1):374-379.

Pujari, R.R., Vyawahare, N.S., Kagathara, V.G. (2011). Evaluation of antioxidant and neuroprotective effect of date palm (*Phoenix dactylifera L*.) against bilateral common carotid artery occlusion in rats. Indian J. Exp. Biol. 49(8):627-633.

Raghu-Jetti, R., Raghuveer, C.V., Mallikarjuna-Rao, C., Somayaji, S.N., Prakash-Babu, B. (2014). Neuroprotective effect of Ascorbic acid and *Ginkgo biloba* against Fluoride caused Neurotoxicity. IOSR JESTFT. 8(1):30-36.

Ramzi, V.K., Stanely, I. (1994). Robbins pathological basis of diseases. 5th ed. Philadelphia, London, Toronto, Monterial. p.183-5.

Ranjan, B., Husain, S.M.D., Kumar, K., Maheshwari, T.P. (2015). Comparative study of histo-pathological effects of mercury on cerebrum, cerebellum and hippocampus of adult albino rats. Ann. Int. Med. & Den. Res. 1(1):21-4.

Ross, R.S., Brown, T.I., Stern, C.E. (2009). The retrieval of learned sequences engages the hippocampus: Evidence from fMRI. Hippocampus.19:790-799.

Ruan, Y.W., Zou, B., Fan, Y., Li, Y., Lin, N., Zhang, Y. *et al.* (2007). Morphological heterogeneity of CA1 pyramidal neurons in response to ischemia. J. Neurosci. Res. 85:193-204.

Saha, S., Barua, B., Sikdar, D. (2017). Phytochemical screening, phenolic content and antioxidant activity of wild date palm (*Phoenix sylvestris* Roxb.) fruit extracted with different solvents. Int. Food Res. J. 24(6):2534-2542.

Sairazi, N.S.M, Sirajudeen, K.N.S. (2020). Natural products and their bioactive compounds: neuroprotective potentials against neurodegenerative diseases. Evidence-Based Comp. Alt. Med. 2020:1-30

Salawu, O.A., Chindo, B.A., Tijani, A.Y., Obidike, I.K., Salawu, T.A., Akingbasote, A.J. (2009). Acute and sub-acute toxicological evaluation of the methanolic stem bark extract of *Crossopteryx febrifuga* in rats. Afr. J. Pharm. Pharmacol. 3(12):621-626.

Sarian, M.N., Ahmed, Q.U., So'ad, S.Z., Alhassan, A.M., Murugesu, S., Perumal, V., Syed- Mohamad, S.N., Khatib, A., Latip, J. (2017). Antioxidant and antidiabetic effects of flavonoids: A structure-activity relationship based study. Biomed. Res. Int. 2017:1-14.

Seidman, R.J.(2011). Normal gross brain and microscopy. Neuropathology, Department of Pathology Stony Brook University.

Seilhean, D., De Girolami, U., Gray, F. (2004). Basic pathology of the central nervous system. In textbook of Escourolle and Poirier Manual of Basic Neuropathology 4th ed. Butterworth Heinemann an imprint of Elsevier, Philadelphia. p.2-4.

Shanker, G., Aschner, J.L., Syversen, T., Aschner, M. (2004). Free radical formation in cerebral cortical astrocytes in culture induced by methylmercury. Brain Res. Mol. Brain Res. 128:48-57.

Sheikh, T.J., Patel, B.J., Joshi, D.V., Patel, R.B., Jegoda, M.D. (2013). Repeated dose oral toxicity of inorganic mercury in Wistar rats: biochemical and morphological alterations. *Vet. World.* 6(8):563-567.

Slomianka, L. (2011). Hippocampal pyramidal cells: the reemergence of cortical lamination. Brain Str. Func. 216(4):301-17.

Snell, R.S. (2010). Introduction and organization of the nervous system. Clinical Neuroanatomy, 7th ed. Philadelphia, Lippincott Williams & Wilkins. 1: p 2.

Suvarna, S.K., Layton, C., Bancroft, J.D. (2019). Stains for Nissl substance; Techniques for staining neurons in Bancroft's Theory and Practice of Histological Techniques. 8th Ed., Elsevier, China. p. 308.

Teleanu, R.L., Chircov, C., Grumezescu, A.M., Volceanov, A., Teleanu, D.M. (2019). Antioxidant therapies for neuroprotection-A Review. J. Clin. Med. 8(1659):1-23.

Trease, G.E., Evans, W.C. (2002). Phytochemicals in: Pharmacognosy. 15th ed. W.B. Sanders Publishers, London.

United Nations Environment Programme, UNEP: Global Mercury Assessment, UNEP Chemicals, Geneva, Switzerland, 2002.

Usama, B., El-Gazzar, A.H., El-Far Hussein, A.A. (2009). The ameliorative effect of *Phoenix dactylifera* extract on CCl_4 hepatotoxicity in New Zealand rabbits. J. Appl. Sci. Res. 5(9):1082-1087.

Using the AmScope Microscope Cameras (2012). Part 1, AmScope Microscope, USA. p. 1–12.

Vyawahare, N., Pujari, R., Khsirsagar, A., Ingawale, D., Patil, M., Kagathara, V. (2009). *Phoenix dactylifera*: An update of its Indegenous uses, phytochemistry and pharmacology. Internet J. Pharmacol. 7:1:1531-2976.

Wan Ismail, W.I., Mohd Radzi, M.N.F. (2013). Evaluation on the benefits of date palm (*Phoenix dactylifera*) to the Brain. Alt. Integ. Med. 2(4):1-3.

Wu, J., Cheng, G., Lu, Z., Wang, M., Tian, J., Bi, Y. (2016). Effects of methyl mercury chloride on rat hippocampus structure. Biol. Trace Elem. Res. 171:124-30.

Xu, F., Farkas, S., Kortbeek, S., Zhang, F., Chen, L., Zamponi, G.W. *et al.* (2012). Mercury-induced toxicity of rat cortical neurons is mediated through N-methyl-D-Aspartate receptors. Mol. Brain. 5:(30)1-14.