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"Cisplatin-Induced Behavioural and Micro-Anatomical Alterations in Rat Brain Were Ameliorated by Seed Extract of *Raffia hookeri*"

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ABSTRACT
Cisplatin (CP) is used in the management of tumours but has side effects mediated in part by oxidative damage which limits its use. The seed extract of *Raffia hookeri* (SERH) with known antioxidant property was investigated to determine its capability to ameliorate perturbations in rat brain exposed to CP. Forty-eight female rats (151 – 210 g) were randomly divided into four equal groups (n = 12) viz: Group 1 (control, distilled water), Group 2 (100 mg/kg of SERH), Group 3 (CP, 7.5 mg/kg i.p, as single dose) and Group 4 (100 mg/kg of SERH + CP). The administration of SERH was orally via gavage for 14 days while CP was administered on the eighth day of experiment as a single dose. Forelimb grip strength and open field tests were carried out on day 15 of the experiment after which rats were euthanized followed by tissue processing for histology. Cisplatin reduced the total body weight, forelimb grip strength, transitions, rearings and groomings significantly ($p<0.05$) when compared with the control. Cisplatin similarly induced histological alterations of cerebral cortex neurons (CCN), granule cells (GC) and pyramidal neurons of cornu ammonis3 (PN). Quantitatively, CP significantly increased the pyknotic indices of CCN, GC and PN while it reduced the width of cerebral pyramidal neurons. These CP-induced alterations were ameliorated in the CP+SERH treatment group when compared with CP group. The SERH demonstrated neuroprotection against CP-induced behavioural and microanatomical alterations of cerebral and hippocampal neurons.

Key words: Histology, *Raffia hookeri*, rat, cerebral cortex, dentate gyrus, cornu ammonis.

INTRODUCTION
Cisplatin (cis-diamminedichloroplatinum-II) is an effective chemotherapeutic agent in the treatment of different solid tumors including cancers of the colon, cervix, ovary, testis and lung [1,3]. According to Ko and his co-workers, the chemotherapeutic mechanism is such that when cisplatin (CP) enters cells, its chloride ligands become replaced by water, and the hydrated species then reacts with nucleophilic sites on cellular macromolecules. Reports have also established that DNA binding is the main biological event that triggers the tumoricidal effects of cisplatin [1, 4], while the presence of CP adducts in DNA sequences is thought to trigger cell cycle arrest and apoptosis[5,6]**.** However, the clinical usefulness of CP has been hindered by undesirable side effects and toxicities such as ototoxicity, myelotoxicity, nephrotoxicity and neurotoxicity[7]. Studies on CP toxicity have revealed that it induces the generation of free radicals and increases production of reactive oxygen species (ROS) ultimately resulting in oxidative stress[8,9]. The use of CP can be enhanced by minimizing its side effect and thus preserve its chemotherapeutic efficacy by co-administering it with an effective antioxidant agent that may inhibit free radical generation. For example, D-methionine, a potent antioxidant, has been shown to ameliorate the cisplatin induced nephrotoxicity and emesis, even in chronic exposure[10].

We have previously reported on the ameliorative capacity of natural plants products with antioxidantive properties which mitigated cisplatin-induced histological alterations of rat brain namely, tomato pomace powder[11], *Launae taraxacifolia* leaves[12] and *Raffia hookeri* pulp extract which ameliorated the cisplatin-induced brain damage in Wistar rats[13].

Raffia palm (*Raphia hookeri)* belongs to the family Palmaceae and it is a monocotyledon plant, commonly found in tropical rainforest[14] and especially in West Africa where they are abundant in lowlands and swampy areas in Southwest Nigeria[15]. The plant has been found useful as edible palm oil (used for cooking and making margarine) and palm wine, while the fermented sap can be distilled into alcohol or local gin[16]. Traditional medicinal uses included treatment of stomach pain in infants and as antidiabetic[15]. Experimentally, the seed extract of *Raphia hookeri* was effective in attenuating hyperglycemia and ameliorated dislipidemia by minimizing the susceptibility of oxygen free radicals release[14]. Ogbuagu[17] had earlier demonstrated the presence of flavonoids and phenols in the seed extract of *Raphia hookeri* which may act as free radical scavengers, in addition to other compounds

like vitamin A, thiamine, riboflavin, nitrates and nitrites.

Important neurological functions such as cognitive and motor control are coordinated in mammals by the cerebral cortex, while memory coding and storage, emotions and behavior are associated with the hippocampus[18,19]. The effect of CP toxicity on the cortex and hippocampus might affect their microanatomy and physiology. We had earlier reported the ameliorative effect of some plant products with antioxidant properties on the neurotoxicity of on these important brain components[11,12,13]. Our literature search showed scanty information on the effect of the seed extract of *Raffia hookeri* (SERH) on Cisplatin-treated rat brain, hence this new work.

In view of this and in the context of continuous search for neuroprotection from CP side effects, we hypothesized that the reported antioxidant activity of SERH should be able to reduce oxidative damage that accompanies cisplatin-toxicity in brain tissue thus minimizing its neurotoxic effect as a neuroprotectant from possible CP-induced injuries.

The present study aimed to investigate the potential of SERH to exert preventive effects on CP behavioural changes and cell alterations in rat brain and so answer the research question of whether SERH might ameliorate the effect of CP in the brain of Wistar rat model.

MATERIALS AND METHODS

Plant materials and extraction process: *Raphia hookeri fruits were obtained from* the swamps of Oke Odan, Apete, Ibadan*, Nigeria in December, 2016.* Botanical identification and authentication of the fruits were done at the herbarium of Forestry Research Institute of Nigeria*, Ibadan, Nigeria* where a voucher number, *FHI 110540* was given and a specimen deposited. A modified method of Ogbuagu[17] was used for the cold extraction. Briefly, the exocarp of the fruits were removed, the mesocarp (pulp) was scraped from seeds and the *seeds dried* and grounded into powdery form for phytochemical screening and extraction. About 1 kg of the powder was used to obtain 98.1 g of the seed extract of *Raffia hookeri* (SERH*)* thus giving a 9.8% yield which was then stored in an air tight bottle and kept in a refrigerator till used.

Phytochemical screening: Phytochemical screening was performed on the seed using standard procedures (20*) for the following: f***lavonoids, alkaloids, saponins, tannins and anthraquinones.**

Experimental animals: Forty eight adult female Wistar rats weighing between 151 - 210 g were obtained from the Animal House of the College of Medicine, University of Ibadan, Nigeria and were acclimatized at the Department of Anatomy, University of Ibadan, for two weeks before being randomly assigned to experimental and control groups. They were housed in clean transparent plastic cages (39 x 29 x 27 cm) with wood shavings as bedding and were fed with rat chow and water *ad libitum*. Animals were humanely handled according to the acceptable guidelines on the ethical use of animals in research (21).

Chemicals and drugs: *Both Cisplatin* (manufactured by Korea United Pharm. Inc. Naojang, Chungnam, Korea)
and Ketamine hydrochloride (manufactured by Rotex Medica, Trittau, Germany) were purchased from Kunle-Ara Pharmacy, Ibadan, Nigeria.

Research Design: The forty eight adult female rats were randomized into four groups of twelve animals each as in Table 1

All administration were oral via gavage for 14 days while CP was intraperitoneal. The dosage and route of administration of cisplatin were based on the method of Ko *et al*[3], while that of SERH was according to Mbaka *et al*[14].

Behavioral tests: Behavioral tests were performed on 6 rats in each of the groups of animals on day 15 after weighing each rat.

Open field test: Rats were placed in the center of the open field and allowed to explore the apparatus for 5 minutes, after which, rats were returned to their cages and the floor of the box was cleaned with 70% ethyl alcohol and permitted to dry between tests to eliminate olfactory bias. This test was used to assess number of lines crossed, rearing, grooming and center square duration[22].

Forelimb Grip Strength Test: It involves the forepaws of the rats being placed on a horizontally suspended metal wire of 2 mm in diameter and 1 m in length, placed one meter above a landing area filled with soft bedding. Given a maximum time of 2 minutes, the length of time each rat was able to stay suspended before falling off the wire was recorded. This test reflects forelimb muscular strength in the animals[23].

Sacrifice and Sample collection: After the behavioral and forelimb grip strength tests, rats were euthanized by initial Ketamine 100 mg/kg followed by brain perfusion with 10% neutral buffered formalin. Thereafter rat brain were dissected out, removed, weighed and six brains preserved in the same fixative for histological analysis, while the remaining were used for the Golgi staining.

Histology and Histomorphometry: The tissues were processed at the Histological Laboratory, Department of Anatomy University of Ibadan, Nigeria. Rats' brain specimens were processed through the stages of fixation, dehydration, clearing, infiltration, embedding

and thereafter sectioned at 6 µm thickness with a Rotary Microtome (Leica RM2125 RTS, Germany). The ribbons were stained with haematoxylin and eosin according to the method of Bancroft and Gamble[24] to demonstrate general histology of the brain and possible microscopic alterations. After 24 hours, the perfused brains separated for Golgi staining were immersed in potassium dichromate solution for 5 days (5 changes every 24 hours) and then silver nitrate for 3 days (3 changes every 24 hours). Thereafter tissues were infiltrated for 30 minutes in molten wax, embedded in paraffin wax and cooled overnight at 4°C. The paraffin blocks were trimmed and sectioned at 60 µm, transferred into graded series of alcohol (80%, 90%, and two changes of 100%) for 2 minutes and cleared in xylene for 10 minutes. Tissues were thereafter mounted on glass slides using DPX as mountant. Thereafter, slides were viewed using Leica DM 500 digital light microscope (Germany) and images captured with Leica ICC50 E digital camera (Germany). Histomorphometric analyses were done using computerized image analyzers (Image J/Micro-Manager 1.4 and Digimizer Image Analysis Version 4.6.1). Using an objective lens (x 40) and an ocular lens (x 10), the viable and pyknotic neurons of the frontal cerebral cortex, cornu ammonis3 (CA3) and dentate gyrus of the brain were observed and counted with the software. The pyknotic index (PI) according to the method described[25], was calculated for in ten different areas of the slides of each of the interest area by 2 observers working independently. Photomicrograph calibrations were done using Image J/Micro-Manager 1.4[26].

Statistical Analysis: Values were expressed as mean ± SEM and were analyzed One-way Analysis of Variance (ANOVA) followed by post-hoc treatment using Dunnett's test (Graph Pad prism 5.04, San Diego CA, 2010). Differences were considered statistically significant at $P<0.05$.

RESULTS

Phytochemical screening: The phytochemical screening of SERH showed the presence of *f*lavonoids, alkaloids, saponins, tannins but absence of anthraquinones.

Effects of SERH on body weight and relative brain weight of rats treated with CP: Rats that received cisplatin (CP) passed copious water stool soiling their beddings in the first two days following CP administration. They also slowed down in physical activity but they became active from the third day. While there were weight increases in the CTL and SERH groups, those of CP and CP+SERH groups were reduced, whereas there was no significant differences in the relative brain weight across the groups (Table 2).

Table 1: Research Design

Groups	Treatment
CTL (n=12)	0.3 mL distilled water daily, served as control.
SERH (n=12)	100 mg /kg body weight of SERH
CP (n=12)	Cisplatin (7.5 mg/ kg body weight, i.p.) as single dose on day 8.
CP+SERH (n=12)	100 mg /kg body weight of SERH + Cisplatin (7.5 mg/kg body weight, i.p.) as single dose on day 8.

CTL, Control; SERH, seed extract of *Raphia hookeri*; CP, cisplatin.

Table 2: Effect of SERH on the body and brain weight changes in rats treated with CP

Group	CTL	SERH	CP	CP±SERH
Initial Wgt.	165±6.1	154±5.5	200.5±10.5	185±9.1
Final Wgt.	190±9.1	163±6.1	170±7.5	164.5±5.2
Wgt. changes	25±1.4	8.5±0.5	-30.5±1.7	-20.5±1.3
% Wgt. changes	15.2±1.03	5.5±0.6*	-15.2±1.02*	-11.1±0.8*
Brain Wgt.	1.7±0.01	1.67±0.01	1.73±0.03	1.69±0.02
R.B.W	0.89±0.01	1.02±0.02	1.02±0.03	1.02±0.03

Values are presented as mean ± S.E.M for six rats per group. CTL, Control; SERH, seed extract of *Raphia hookeri*; CP, cisplatin, CP+ SERH, cisplatin + seed extract of *Raphia hookeri*, Wgt- Weight (g), R.B.W.-Relative brain weight. * $P< 0.05$ versus Control group.

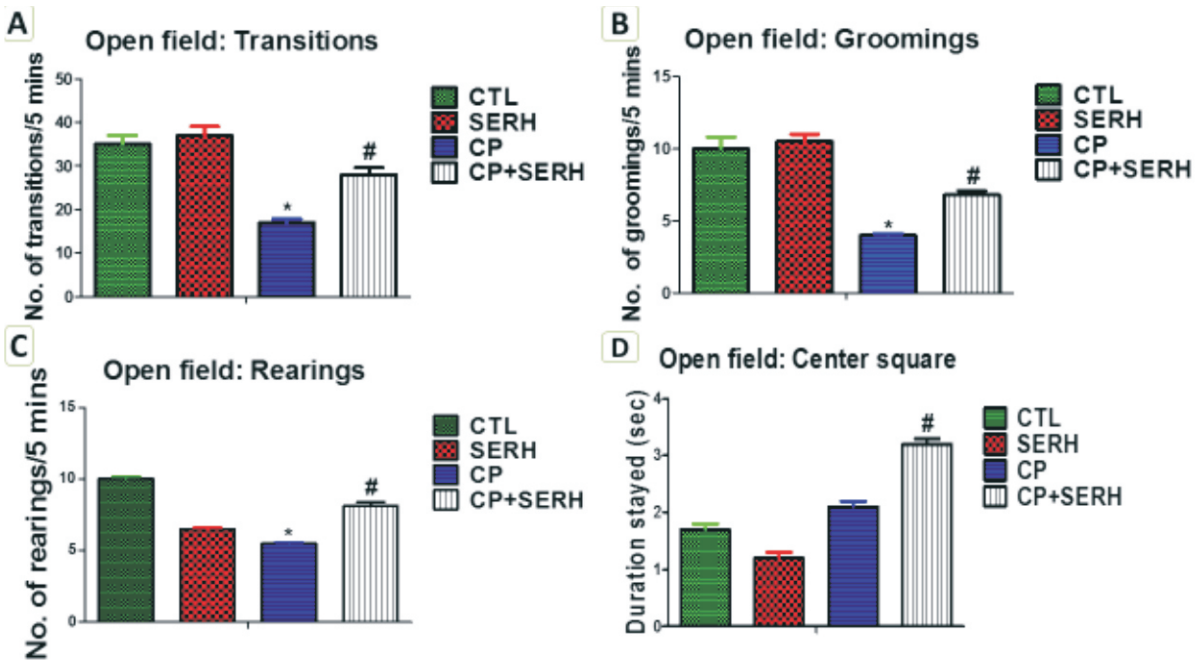

Figure 1: Histogram showing the effect of SERH on the behavioural parameters in rats treated with CP. Values are presented as mean ± S.E.M for six rats per group. CTL, Control; SERH, seed extract of *Raphia hookeri*; CP, cisplatin, CP+ SERH, cisplatin + seed extract of *Raphia hookeri*. * P< 0.05 versus Control group, # P< 0.05 versus CP group.

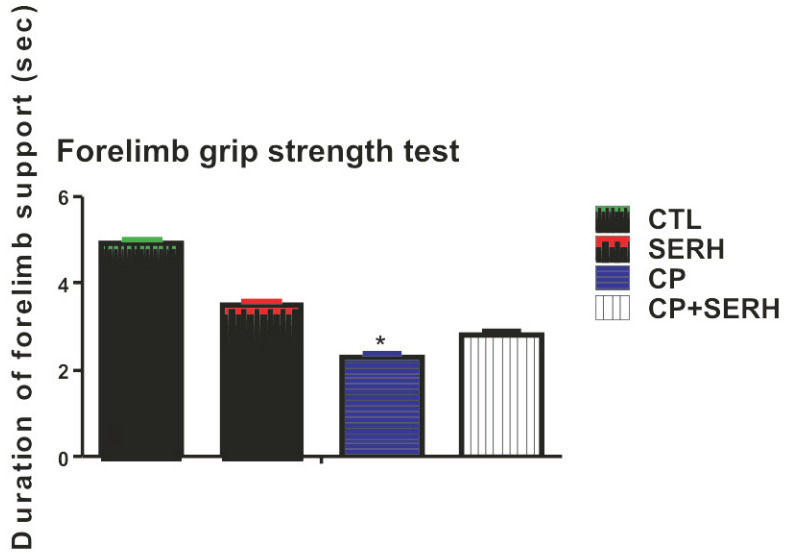

Figure 2: Histogram showing the effect of SERH on the forelimb grip test in rats treated with CP. Values are presented as mean ± S.E.M for six rats per group. CTL, Control; SERH, seed extract of *Raphia hookeri*; CP, cisplatin, CP+ SERH, cisplatin + seed extract of *Raphia hookeri*. * P< 0.05 versus Control group.

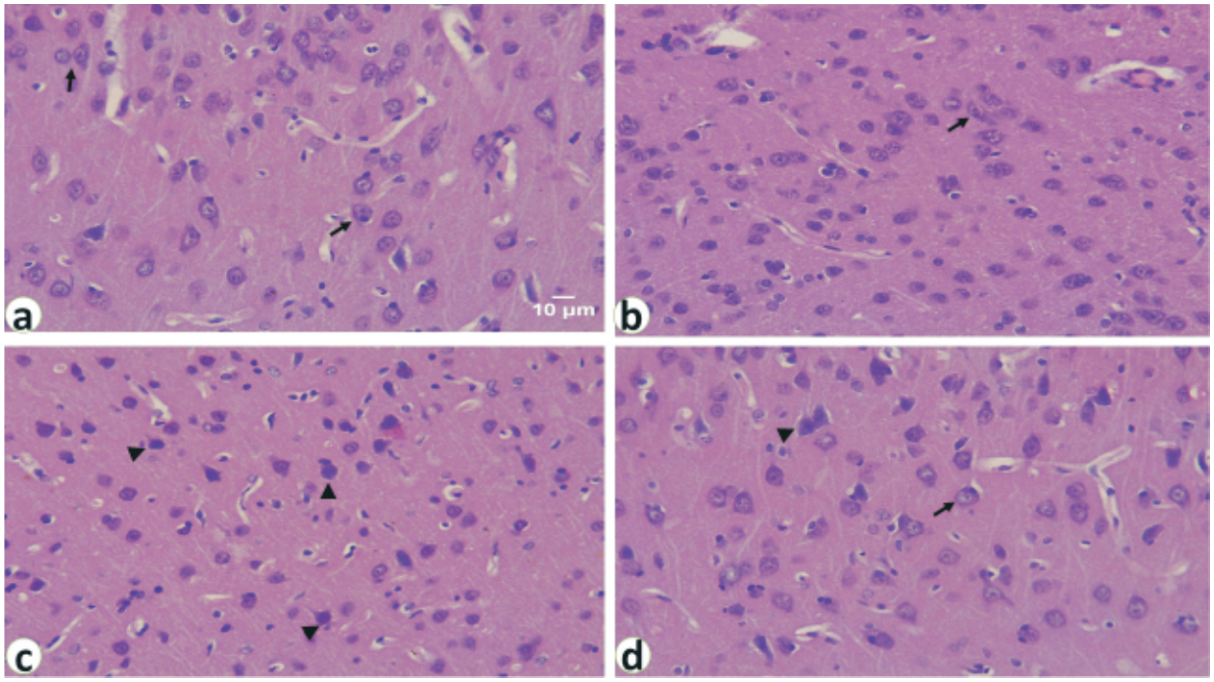

Figure 3: Representative stained sections of frontal **cerebral cortex** of rats: (a) Control group (b) SERH-treated (c) CP-treated (d) CP+SERH-treated. Normal cortical neurons in all groups are indicated by arrows. Dark (pyknotic) cortical neurons (arrowheads) are noted in Fig. 1c and scantily in Fig. 1d. which suggest neuronal degeneration. SERH; Seed Extract of *Raphia hookeri*; CP, cisplatin; CP+RHPE, Cisplatin + Seed Extract of *Raphia hookeri*. H&E. Scale bar for all figures = 10 µm.

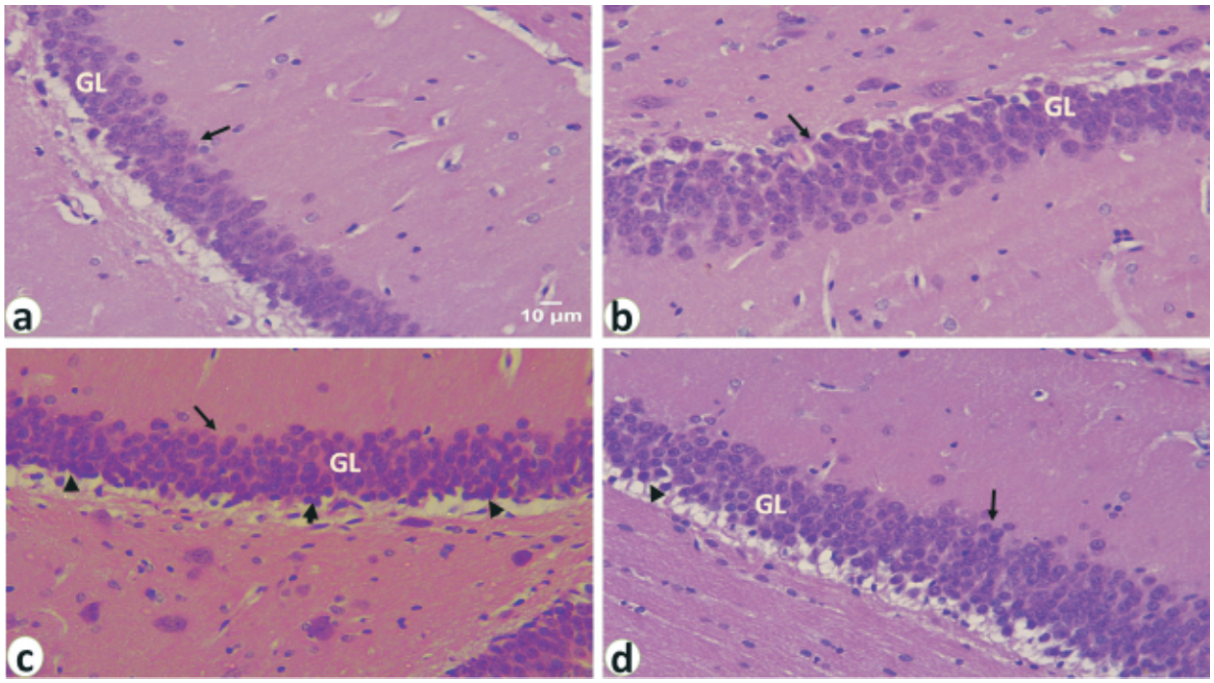

Figure 4: Representative stained sections of Dentate gyrus rat hippocampus: (a) Control group (b) SERH-treated (c) CP-treated (d) CP+SERH-treated. Normal granule neurons in the Granular layer (GL) are shown by arrows. Dark (pyknotic) granule neurons (arrowheads) are noted at basal cells of the GL in Fig. 2c and scantily in Fig. 2d. which suggest neuronal degeneration. SERH; Seed Extract of *Raphia hookeri*; CP, cisplatin; CP+SERH, Cisplatin + Seed Extract of *Raphia hookeri*. H&E. Scale bar for all figures = 10 µm.

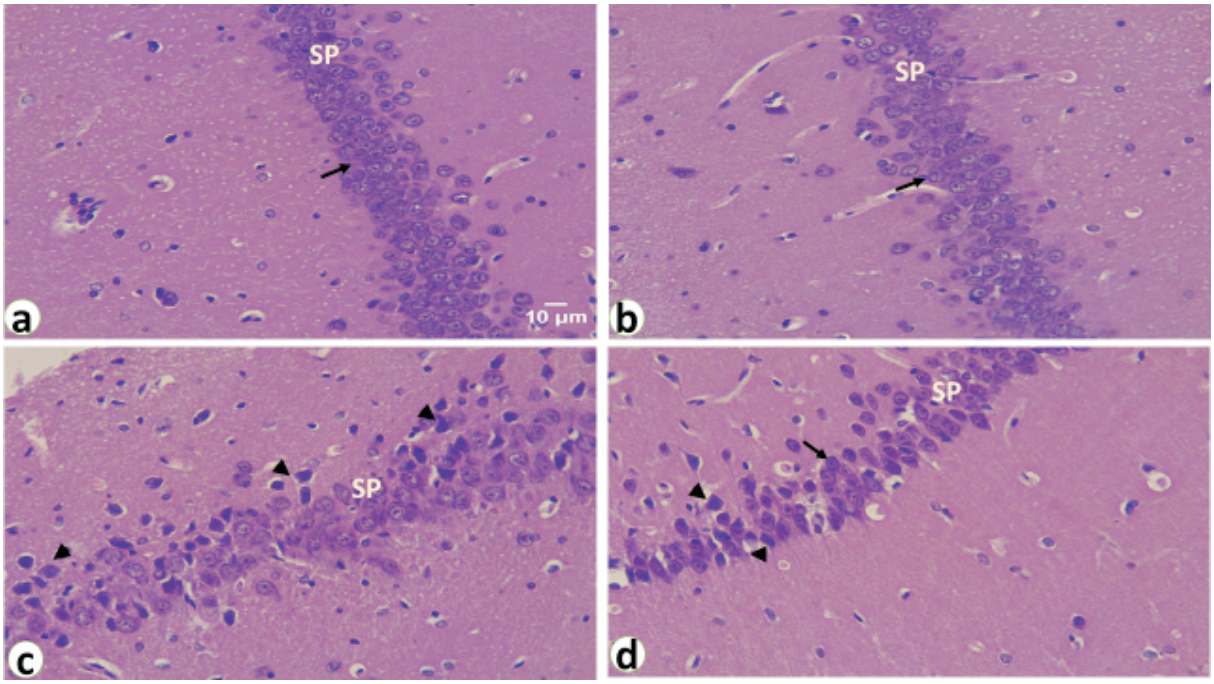

Figure 5: Representative stained sections of Cornu Ammonis3 of rat hippocampus: (a) Control group (b) SERH-treated (c) CP-treated (d) CP+SERH-treated. Normal pyramidal neurons in the Stratum pyramidalis (SP) indicated by arrows while the dark (pyknotic) granule neurons (arrowheads) are noted scattered among normal ones in Fig. 3c and scantily in Fig. 3d. indicating neuronal degeneration. SERH; Seed Extract of *Raphia hookeri*; CP, cisplatin; CP+RHPE, Cisplatin + Seed Extract of *Raphia hookeri*. H&E. Scale bar for all figures = 10 µm.

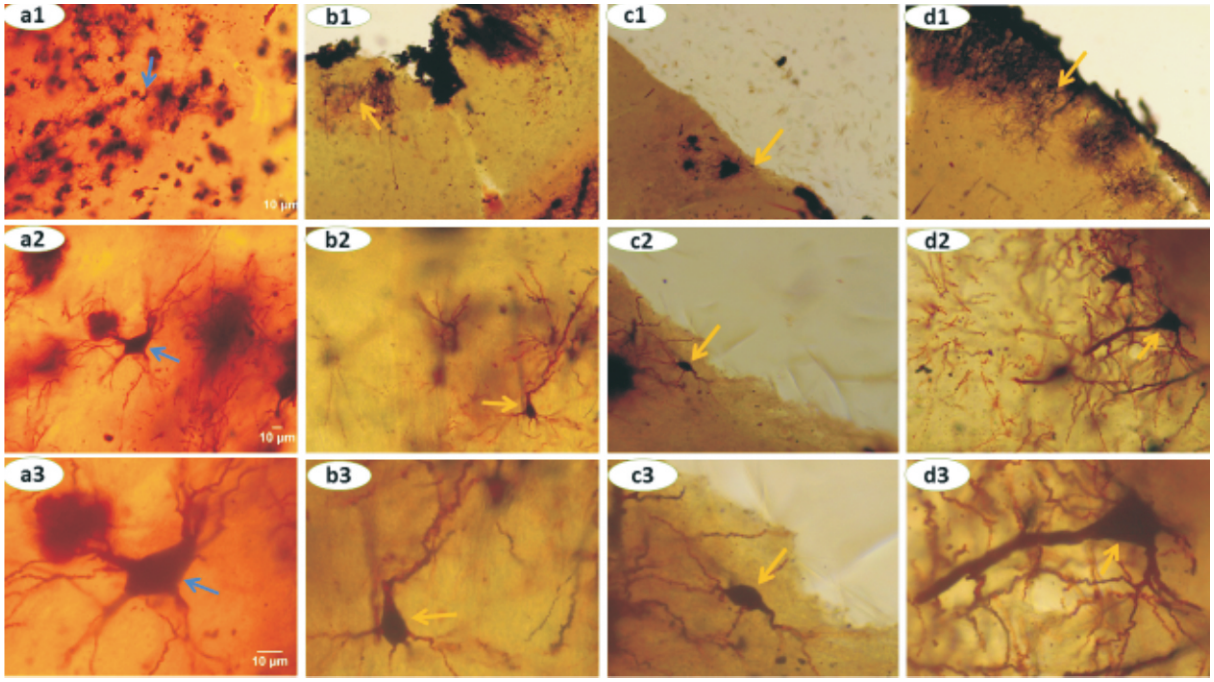

Figure 6: Representative Golgi stained sections of frontal cerebral cortex of rats: (a1-a3) Control group; (b1-b3) SERH-treated group; (c1-c3) CP-treated group; (d1-d3) CP+SERH-treated group. SERH, Seed Extract of *Raphia hookeri*; CP, cisplatin; CP+SERH, Cisplatin + Seed Extract of *Raphia hookeri*. Upper panel, a1-d1 represents figures at ×100 magnification; middle panel, a2-d2, is at ×400 magnification while the bottom panel, a3-d3, is at ×1000 magnification. The soma and dendrites of pyramidal neurons are well demonstrated in all groups. The dendritic arborization of the CP group are noted to be scanty compared with other groups. Scale bar for all figures = 10 µm.

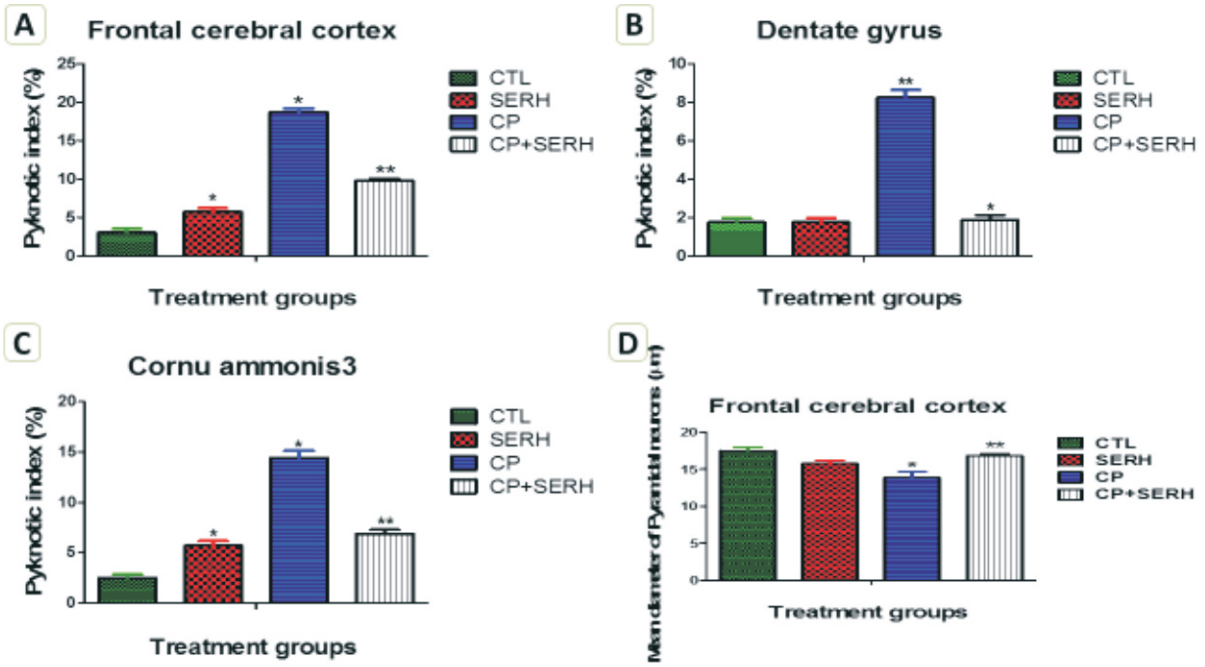

Figure 7: Histogram showing the effects of SERH on the histomorphometry of the neurons of the frontal cerebral cortex, dentate gyrus and cornu ammonis3 in rats treated with CP. Values are presented as mean ± S.E.M. of six rats. CTL, Control; SERH; Seed Extract of *Raphia hookeri*; CP, cisplatin; CP+SERH, Cisplatin + Seed Extract of *Raphia hookeri*. *P< 0.05 versus CTRL group, **P< 0.05 versus CIS group.

Effects of SERH on behavioral and forelimb grip strength test in rats treated with CP: Cisplatin significantly reduced the number of transitions, rearings, groomings and the duration of the forelimb grip strength ($p<0.05$) when compared with the control group as shown in Figures 1 and 2. Pretreatment with SERH significantly ameliorated these changes as observed in the CP+SERH rats relative to CP group. The duration of time spent in the center square was significantly increased by CP also (Figure 1).

Effects of SERH on the histology of the frontal cerebral cortex, dentate gyrus (DG) and cornu ammonis3 (CA3) in rats treated with CP: The histology of the frontal cerebral cortex show large round or oval neurons whose nuclei exhibit open chromatin pattern and visible nucleolus indicated by arrows in all the groups (Figure 3). Neurons of CP group are noted to show scattered dark (degenerated neurons) indicated by arrow heads. The representative photomicrographs of DG and CA3 show normal neurons with the exception of CP-treated groups which showed pyknotic neurons when compared with the control as depicted in Figures 4 and 5. Uniquely, only granule neurons in the basal layer of GL of DG exhibited dark neurons. The altered histologic features in the CP groups in the three brain parts were returned to near control-like features in CP+SERH groups with their neurons being large, rounded or oval with the nuclei showing open chromatin pattern and some showing nucleoli. Figure 6 showed that the dendritic arborization of pyramidal neurons of frontal appeared to be reduced in the CP treatment group.

Effects of SERH on the histomorphometry of the neurons of the frontal cerebral cortex, dentate gyrus and cornu ammonis3 in rats treated with CP: Figures 7a, 7b and 7c showed that CP significantly ($p<0.05$) increased the pyknotic indices of the neurons of frontal cerebral, DG and CA3, but reduced the mean diameters of the pyramidal neurons (Figure 7d) relative to the control. However, all these parameters were significantly ameliorated in the CP+SERH when compared with CP treatment.

DISCUSSION

We investigated the effect of the seed extract of *Raphia hookeri* (SERH) on cisplatin-induced behavioral changes as well as micro-anatomical alterations in rat brain. The numerical and histological results indicated that cisplatin (CP) elicited body weight reduction, behavioral changes and micro-anatomical alterations in the frontal cortex (FC), dentate gyrus (DG) and cornu ammonis3 (CA3) of adult Wistar rats.

The loss of body weight in the CP groups was probably due to water loss from diarrhea which resulted from CP-induced gastrointestinal toxicity[27], leading to CP's damage of the mucosal epithelial cells of rat's colon whose principal function is the recovery of water and salt from feces leading to formation of increasingly solid feces[28]. Studies have also attributed the weight loss in Cisplatin treated animals to reduced gastric motility, gastric distention and emesis[29,30]. The report of

weight loss in all rats treated with CP agreed with published findings[9,31]. The ability of the pretreatment of SERH with CP to maintain a steady weight gain compared with the CP group suggested a protective effect of the SERH.

The reduction of the transitions and rearings of the rats by CP indicated a reduction in the horizontal and vertical locomotive activities of the rats. When combined with reduction in groomings and forelimb grip strength in addition to increased center square duration of the rats by CP, this suggested possible reduction of the cognitive function and muscular activities and increased anxiety in the rats which agreed with published reports[11,32,33]. Assessment of rodent self-grooming is known to be useful for understanding of the neural circuits involved in complex sequential patterns of action[34]. The ability of SERH to ameliorate the transitions, rearings and groomings demonstrated in the CP+SERH groups suggested a protective factor in SERH which might protect these rats from becoming sluggish.

The presence of dark neurons in the FC, DG and CA3 were indications of altered micro-anatomy of these neurons[35] resulting from CP treatment which was consistent with previous reports [11,12,36]. The degeneration of FC neurons, increased pyknotic index and reduction of dendritic arborization of the pyramidal neurons as well as the reduction of the mean diameter of the pyramidal neurons of the frontal cortex in CP-treated rats all support the previous reports of cisplatin injury in rat cortical neurons[7,11,12,36]. The degeneration of cortical neurons induced by CP might explain the reduction in transition, rearing and forelimb grip test observed since the final control of fragmented distal digital movements in mammals is dependent on pyramidal tracts which are projection fibers from the cerebral cortex. Other cerebral cortical functions including limbic, decision making and cognition might also be affected[19].

The histology of the DG and CA3 neurons showed distortion as evidenced by the pyknotic neurons and increased pyknotic indices. The ability of CP to induce neurotoxicity with histological alteration was reported by Al-Moundhri *et al*[37], Gulec *et al*[38] and Owoeye *et al*[12] contrary to older reports that CP was unable to penetrate the blood-brain barrier (39). The possible consequence of the death of granule neurons of DG and pyramidal neurons of CA3 might be a distortion of the normal memory coding processing in the brain. From our findings, pyknotic neurons in the DG were limited to the subgranular layer which might limit the generation of new neurons and also affect the interneuronal relationship between the neurons and other cells of the dentate gyrus namely dentate pyramidal basket cells and mossy cells[40]. The afferent perforant pathway projections from layer II of the entorhinal cortex to the DG might also be affected resulting in faulty mossy fibre projection from DG to CA3. Because of scattered pyknotic pyramidal neurons of CA3 and the increased pyknotic index, the quality of projections of Schaffer's collateral from CA3 to CA1 and its ultimate projection to subiculum and entorhinal cortex might be compromised. The resultant effect might be impaired memory coding aside from other hippocampal functions like cognition and behavior in rats[33].

The neural damage by CP demonstrated by histological damage has been associated with free radical generation resulting in oxidative damage[7,12,36]. The presence of large amounts of long chain polyunsaturated fatty acids (PUFAs), high aerobic metabolism and low levels of antioxidants in brain tissue make the brain very susceptible to oxidative stress[41,42]. Oxidative stress is known to alter cell structure and function and might also reduce the antioxidant mechanisms resulting in DNA damage in biological systems which might explain the nuclei damage in the neurons of FC, DG and CA[9]. However, flavonoid-containing substances possess antioxidant activity which are capable of mitigating the effect of CP as is reportedly present in SERH[17,43]. The increased number of transitions, rearings, groomings, forelimb grip and mean diameter of frontal cortex pyramidal neurons on one hand and the improvement of the histology of the FC, DG and CA3 in the CP+SERH groups attest to the ameliorative effect of SERH with a possible mechanism via the antioxidant pathway. The overall effect of this amelioration by SERH treatment in both cerebral cortex and hippocampus would be an improvement in cognitive and memory coding processes [19,44,45].

Our limitations in this study includes the inability to isolate and characterize the active component in SERH which was responsible for the observed effects, using more elaborate techniques, such as electron microscopy and performing immunohistochemical investigations of the neurons to determine if neuronal death was due to apoptosis or necrosis.

CONCLUSION

Taken together, SERH ameliorated the behavioural changes and demonstrated neuroprotection against CP-induced micro-anatomical alterations of FC, DG and CA3 possibly through its antioxidant property. Since the development of n are beneficial in the identification of chemo-preventive agents and biologically active molecules, SERH could be further investigated for possible identification of promising therapeutic agents against CP toxicity.

Author contributions

Conceived and designed the experiments: OO.
Performed the experiments: EOA, FOA.
Analyzed the data: OO, EOA, FOA.
Wrote the manuscript: OO, EOA, FOA .
Edited the manuscript which a*ll authors read and approved*: OO, OSO, MOA .

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Conflicts of interest
None.

REFERENCES

1. Rousseau J, Barth R.F., Fernandez M., Adam J.F., Balosso J, Esteve F. et al. Efficacy of intracerebral delivery of cisplatin in combination with photon irradiation for treatment of brain tumors. J Neurooncol., 2010; 98(3):287-95.
2. Mokhtari M.J., Akbarzadeh A, Hashemi M, Javadi G, Reza R, Mehrabi M.R. et al.,. Cisplatin Induces Down Regulation of BCL2 in T47D Breast Cancer Cell Line. Adv Stud Biol., 2012; 4(1): 19 – 25.
3. Ko J-W., Lee I-C., Park S-H., Moon C., Kang S-S., Kim S.H. et al,. Protective effects of pine bark extract against cisplatin-induced hepatotoxicity and oxidative stress in rats. Lab Anim Res., 2014; 30(4): 174-180.
4. Jung Y, Lippard S.J. Direct cellular responses to platinum induced DNA damage. Chem Rev., 2007; 107(5):1387–1407.
5. Podratz J.L., Staff N.P., Froemel D., Wallner A., Wabnig F., Bieber A.J., et al,. Drosophila melanogaster: A new model to study cisplatin-induced neurotoxicity. *Neurobiol Dis., 2011;* **43(2)**: 330–337.
6. Hashem R.M., Safwat G.M., Rashed L.A., Bakry S. Biochemical findings on cisplatin-induced oxidative neurotoxicity in rats. Int J Adv Res., 2015; 3(10): 1222 – 1231.
7. Karavelioglu E., Boyaci M.G., Simsek N., Sonmez M.A., Koc R., Karademir M. et al.,. Selenium protects cerebral cells by cisplatin induced neurotoxicity. *Acta Cirúrgica Brasileira. 2015; 30(6): 394-400.*
8. Lu Y., Cederbaum AI. Cisplatin-induced hepatotoxicity is enhanced by elevated expression of cytochrome P450 2E1. Toxicol Sci., 2006; 89(2): 515-523.
9. Almutairi M.A., Alanazi W.A., Alshammari M.A., Alotaibi M.R., Alhoshani AR, Al-Rejaie S.S. et al,. Neuro-protective effect of rutin against Cisplatin-induced neurotoxic rat model. BMC Compl Alt Med., 2017; 17(1):472.
10. Ming-Tai L., Jiunn-Liang K., Te-Chung L. , Pei-Tsen C. , and Chu-Chyn O. Protective Effect of D-Methionine on Body Weight Loss, Anorexia, and Nephrotoxicity in Cisplatin-Induced Chronic Toxicity in Rats. Integrative Cancer Therapies. 2018; 17(3) 813–824
11. Owoeye O., Onwuka S.K. Tomato pomace powder ameliorated cisplatin-induced microanatomical alterations in brain of Wistar rats. Int J Biol Chem Sci., 2015; 9(1):1-11.
12. Owoeye O., Femi-Akinlosotu O.M., Adejuwon S.A. Launae taraxacifolia aqueous extract attenuates Cisplatin-induced neurotoxicity, by decreasing oxidative stress and neuronal cell death in rats. Arch Basic Appl Med., 2015; 3:71-78.
13. Owoeye O.,_Awoyemi F.O., Ajiboye E.O. Ameliorative effects of Raffia hookeri pulp extract on cisplatin-induced brain damage and consequent neurobehavioral changes in Wistar rats. *Niger. J. Physiol. Sci., 2018; 33(1): 075-082.*
14. Mbaka G.O., Ogbonnia S.O., Oyeniran K.J., Awopetu P.I. Effect of *Raphia hookeri* Seed Extract on Blood Glucose, Glycosylated Hemoglobin and Lipid Profile of Alloxan Induced Diabetic Rats. *Br. J Med Med Res., 2012; 2(4): 621-635.*
15. Dada F.A., Oyeleye S.I., Ogunsuyi O.B., Olasehinde T.O,, Adefegha SA, Oboh G, et al. Phenolic constituents and modulatory effects of Raffia palm leaf (Raphia hookeri) extract on carbohydrate hydrolyzing enzymes linked to type-2 diabetes. J Trad Complem Med., 2017; 7(4):494-500.
16. Afolayan A.O., Borokini T.I., Afolayan G.O. Sublethal Effects of Methanolic Extract of Raphia hookeri on the Reproductive Capacity of Clarias gariepinus. Advances in Zoology. 2014
17. Ogbuagu M.N.Vitamins, phytochemicals and toxic elements in the pulp and seed of raphia palm fruit (*Raphia hookeri*). Cirad/EDP Sciences. *Fruits. 2008;* 63: 297–302.
18. Afifi A.K., Bergman R.A. Functional neuroanatomy: Text and Atlas, 2nd edition, McGraw–Hill: New York. 2005; Pp 201–222.
19. Ellis H. Clinical Anatomy: Applied Anatomy for students and junior doctors. Blackwell, Oxford: UK., 2006;349-352.
20. Trease G.E., Evans W.C. A textbook of Pharmacognosy, 13th Edition. Balliere Tindall Ltd.:London. 1989; 176-180.
21. Public Health Service (PHS). Public health service policy on humane care and use of laboratory animals. *US Department of Health and Human Services*: Washington, DC., 1996; 99-158.
22. Mohammad S., Shahrnaz P., Masoud N., Moazamehosadat R., Nasser Z., Khadije E., et al. Walnut consumption protects rats against cisplatin - induced neurotoxicity. Neurotoxicol., 2010; 33(5):1314-21.
23. Tamashiro K.L.K., Wakayama T., Blanchard R.J., Blanchard C., Yanagimachi R. Postnatal growth and behavioral development of mice cloned from adult cumulus cells. Biol Reprod., 2000; 63(1):328–334.
24. Bancroft J.D, Gamble M. *Theory and Practice of Histology Techniques,* 6th edition. Churchill Livingstone Elsevier, Philadelphia. 2008; 83 - 134.
25. Taveira K.V.M, Kleber T.C., Carlos H.C., Luiza D.S.L. Morphological and Morphometric Analysis of the Hippocampus in Wistar Rats with Experimental Hydrocephalus. Pediatr Neurosurg. 2013; 48(3):163-7.
26. Edelstein A.D., Tsuchida M.A., Amodaj N., Pinkard H., Vale R.D., Stuurman N. Advanced

methods of microscope control using μManager software. J Biol Meth., 2014; 1(2) :10
27. Shahid, F., Farooqui, Z., Khan, F. Cisplatin-induced gastrointestinal toxicity: An update on possible mechanisms and on available gastroprotective strategies. European journal of Pharmacology. 2018; 827(15): 49-57.
28. Young B, Lowe S, Stevens A, Heath JW. Wheater's Functional Histology. A Text and Colour Atlas. Churchill Livingstone/Elsevier; 2006.
29. Malik N.M., Moore G.B., Smith G., Liu Y.L., Sanger G.J., Andrews P.L. Behavioral and hypothalamic molecular effects of the anti-cancer agent cisplatin in the rat: a model of chemotherapy related malaise? Pharmacol Biochem Behav., 2006; 83(1):9-20.
30. Gong Y, Liu Y, Liu F, et al. Ghrelin fibers from lateral hypothalamus project to nucleus tractus solitaries and are involved in gastric motility regulation in cisplatin-treated rats. Brain Res., 2017; 1659(1):29-40.
31. Adaramoye O.A., Azeez A.F., Ola-Davies O.E. Ameliorative effects of chloroform fraction of *Cocos nucifera* L. husk fiber against Cisplatin-induced toxicity in rats. Phcog Res., 2016; 8(2):89-96.
32. Ali B.H., Ramkumar A., Madanagopal T.T., Waly M.I., Tageldin M., Al-Abri S. et al. Motor and behavioral changes in mice with Cisplatin-induced acute renal failure. *Physiol Res., 2014; 63(1): 35-45.*
33. Elbatsh M.M., Shehata M.A. The neuroprotective effect of resveratrol on cisplatin-induced cognitive dysfunction. *Int J Biopharm. 2015;* 6(2): 107-114.
34. Kalueff, A. V., Stewart, A. M., Song, C., Berridge, K. C., Graybiel, A. M., Fentress, J. C. Neurobiology of rodent self-grooming and its value for translational neuroscience. Nature Reviews Neuroscience. 2016; 17(1): 45.
35. Stevens A, Lowe J. Pathology. 2nd Edition. Mosby,:Edinburgh. 2000; 23-33.
36. Kaya K., Ciftci O., Cetin A., Tecellioğlu M., Başak N. Beneficial effects of β-glucan against cisplatin side effects on the nervous system in rats. *Acta Cirúrgica Brasileira. 2016; 31(3): 198-205.*
37. Al Moundhri M.S., Al-Salam S., Al Mahrouqee A., Beegam S., Ali B.H. The Effect of Curcumin on Oxaliplatin and Cisplatin Neurotoxicity in Rats: Some Behavioral, Biochemical, and Histopathological Studies. J Med Toxicol. 2013; 29(1): 25–33.
38. Gulec M., Oral E., Dursun O.B., Yucel A., Hacimuftuoglu A., Akcay F. et al. Mirtazapine protects against cisplatin-induced oxidative stress and DNA damage in the rat brain. *Psych Clin Neurosci. 2013;* 67(1): 50–58
39. Gregg R.W., Molepo J.M., Monpetit V.J., Mikael N.Z., Redmond D., Gadia M. et al. Cisplatin neurotoxicity: the relationship between dosage, time, and platinum concentration in neurologic tissues, and morphologic evidence of toxicity. *J Clin Oncol, 1992;* 10(5): 795-803.
40. Amaral D.G., Scharfman H.E., Lavenex P. The dentate gyrus: fundamental neuroanatomical organization (dentate gyrus for dummies). *Prog Brain Res*. 2007; 163(1): 3–22.
41. Ebokaiwe A.P., Adedara I.A., Owoeye O., Farombi E.O. Neurotoxicity of Nigerian bonny light crude oil in rats. *Drug Chem Toxicol, 2013;* 36 (2): 187-195.
42. Chaudhary M., Joshi D.K., Tripathi S., Kulshrestha S., Mahdi A.A. Docosahexaenoic acid ameliorates aluminum induced biochemical and morphological alteration in rat cerebellum. Ann Neurosci, 2014; 21(1): 5-9.
43. Akpan E.J., Usoh I.F. Phytochemical screening and effect of aqueous root extract of Raphia hookeri (raffia palm) on metabolic clearance rate of ethanol in rabbits. Nig Soc Exp Biol, 2014; 16(1): 37 – 42.
44. Stepan J., Dine J., Eder M. Functional optical probing of the hippocampal trisynaptic circuit *in vitro*: network dynamics, filter properties, and polysynaptic induction of CA1 LTP. Front Neurosci., 2015; 9: 160.
45. Folarin O.R., Snyde, A.M., Peters D.G., Olopade F., Connor J.R, Olopade J.O. Brain Metal Distribution and Neuro-Inflammatory Profiles after Chronic Vanadium Administration and Withdrawal in Mice. Front. Neuroanat, 2017; 11(1):58.