

Ameliorative Effects of *Raffia hookeri* Pulp Extract on Cisplatin-induced Brain Damage and Consequent Neurobehavioural Changes in Wistar Rats

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Summary: Cisplatin (CIS), a known anticancer drug, has side effects initiated by oxidative damage which hinders its use. *Raffia hookeri* pulp extract (RHPE), reported to possess antioxidant activity should mitigate cisplatin toxicity. The present study examined the potential of RHPE to reduce brain damage in rats exposed to cisplatin. Forty eight female rats (150 g – 220 g) were randomized into four groups (n = 12) viz: Group 1 served as control received distilled water daily, Group 2 received 100 mg/kg body weight of RHPE, Group 3 received CIS (7.5 mg/kg body weight, intraperitoneally) as single dose, Group 4 received 100 mg/kg body weight of CIS+RHPE. The RHPE was given orally via gavage for 14 days while the single dose of cisplatin was administered on the eighth day of experiment. Behavioral tests namely: transitions, rearings, groomings and forelimb grip strength were carried out on 15th day of the experiment after which rats were euthanized followed by histology and histomorphometry. Cisplatin significantly (p<0.05) reduced the percentage body weight changes, transitions, rearings, groomings and forelimb grip strength compared with the control group, whereas treatment with CIS+RHPE significantly (p<0.05) increased these parameters compared with Cisplatin treatment. Cisplatin also caused histological alterations of Purkinje neurons, pyramidal neurons of Cornu ammonis3, granule cells and cerebral cortex neurons. It significantly (p<0.05) reduced the diameter of Purkinje (9.1±0.59 µm) compared with control (14.41±0.31 µm) and pyramidal neurons (11.32±0.05 µm) compared with control (17.03±0.54 µm). Rats in the CIS+RHPE had their histology considerably improved compared with those of cisplatin. In conclusion, RHPE reversed the behavioural changes and demonstrated neuroprotection against CIS-induced behavioural changes and microanatomical alterations of cerebellar, hippocampal and cerebral neurons.

Keywords: Cisplatin, *Raffia hookeri*, neuroprotection, neurons, behavioural tests.

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INTRODUCTION

Cisplatin (cis-DDP cis Diammine Dichloro Platinum (II)), a platinum-based drug is used as an antineoplastic agent with wide spectrum of use in various tumors including the lung, kidney, ovary, testis, bladder, head, neck, and brain (Mokhtari et al., 2012; Sanchez-Gonzalez *et al.* 2011). The antitumor activity of Cisplatin (CP) has been linked to its ability to form adducts with DNA, which could cause cross-linking of DNA strands (Dasari and Tchounwou, 2014; Hasheem et al., 2015). However, the use of CP is restricted due to its various side effects such as neurotoxicity, nephrotoxicity, ototoxicity, hepatotoxicity and testicular toxicity. These effects have been attributed majorly to the generation of reactive oxygen species (ROS) which could interfere with the antioxidant defense system (Tsimberidou et al., 2010).

Raffia palm leaf (*Raffia hookeri*), a monocot belonging to the family Arecaceae is usually found in lowland swamps throughout Western and Central Africa, Asia and South America (Oduah and

Ohimain, 2015). All parts of the plant are well utilised by locals for various things ranging from building materials as twine, rope; personalized items like baskets, placemats, hats, shoes to consumables like oil, wine and food (Akinola et al., 2010; Afolayan et al., 2014). Its fruit is large, cone-shaped with a single hard nut having an outer layer of overlapping reddish brown scales and in-between the outer layer of scales and the hard seed is a yellow, mealy, oil-bearing mesocarp or pulp (Mbaka *et al.*, 2012). The pulp extract of *Raphia hookeri* was shown to contain vitamins C and E, carotenes, niacin, alkaloid, saponins, flavonoids and phenols which explains its antioxidant activity (Edem et al., 1984; Akpan and Usoh, 2004; Dada et al., 2017). Flavonoids and tannins as phenolic compounds in plants are a major group of compounds that act as primary antioxidants by scavenging free radicals (Polterait, 1997).

The mammalian cerebral cortex is associated with cognitive and motor control. The hippocampus is involved in emotions, behaviour, memory coding and storage, while the cerebellum is the integrative center

for voluntary skeletal muscle control and equilibrium. The cerebellum also co-ordinates different muscle groups so that the muscle movements are fluent and precise (Fonnum and Lock, 2000; Afifi and Bergman, 2005; Ellis, 2006). The involvement of these important brain components in cisplatin toxicity may alter their microscopic anatomy and physiology as previously demonstrated (Owoeye and Onwuka, 2015; Owoeye et al., 2015). There is scanty information on the effect of the pulp extract on neural tissue. We hypothesized that the antioxidant activity of *Raphia hookeri* pulp extract (Edem et al., 1984; Akpan and Usoh, 2004; Dada et al., 2017) should be able to reduce oxidative damage that accompanies cisplatin-toxicity in brain tissue thus minimizing its effect.

The present study was carried out to assess the possible ameliorative effect of *Raphia hookeri* pulp extract (RHPE) in cisplatin-induced neurotoxicity and so answer the research question of whether RHPE may mitigate the neurotoxic effect of cisplatin in a Wistar rat model.

MATERIALS AND METHODS

Extraction Procedure

Ripe fruits of *Raphia hookeri* (RH) were obtained from the swamps of Oke Odan, Apete, Ibadan, Nigeria in December, 2016 and authenticated at Forestry Research Institute of Nigeria, Ibadan, Nigeria with FHI number 110540. The hard, tough and scaly exocarp of the fruits were removed and discarded and the soft, mealy mesocarps (pulp) scraped from the seeds. Extraction of the pulp was carried out applying a modified method of Afolayan et al. (2014). Briefly, 1 kg of the dried pulp was grounded into powdery form was soaked in 2.5 L of pure ethanol and stirred at 2 hours interval. After the first collection was filtered the pulp powder was soaked again in 1.5 L of ethanol and stirred at 2 hourly intervals for 72 hours to allow complete extraction. The ethanol solvent containing the extract was collected using muslin bag after which the effluent was further filtered using Whatman filter paper 1 and the filtrate then concentrated using rotary evaporator set at 40°C after which it was stored in an air tight bottle and kept in a refrigerator at 4°C till used. The final yield termed *Raphia hookeri* pulp extract (RHPE) was 130.8 g gave a percent yield of 13%.

Phytochemical screening

Phytochemical screening was performed using standard procedures (Sofowora, 1993). The pulp was screened for flavonoids, alkaloids, saponins, tannins, terpenoids, anthraquinones and cardiac glycosides.

Experimental animals

Forty-eight adult female Wistar rats weighing between 150 - 220 g were obtained from the Animal House of the College of Medicine, University of

Ibadan, Nigeria. They were acclimatized at the Department of Anatomy, University of Ibadan, for two weeks before being assigned randomly to experimental and control groups using random numbers. 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 (Public Health Service, 1996). In chemotherapy, the use of drugs including cisplatin for cancer treatment might involve both male and female patients. It is needful therefore to mimic this true life exposure of females to cisplatin, hence we designed this present study using female rats as has been reported (Akman et al., 2015; Kumar et al., 2017).

Chemicals and drugs

Cisplatin manufactured by Korea United Pharm. Inc. (Naojang, Chungnam, Korea) and Ketamine hydrochloride was 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 six groups of twelve animals each as follows:

Group 1 (n=12): CTRL, 0.3 mL distilled water daily, served as control.

Group 2 (n=12): RHPE, 100mg /kg body weight of *Raphia hookeri* pulp extract

Group 3 (n=12): CIS, Cisplatin at single dose 7.5 mg/kg body weight, i.p.

Group 4 (n=12): CIS+RHPE, 100mg /kg body weight *Raphia hookeri* pulp extract + Cisplatin 7.5 mg/kg body weight, i.p. as single dose.

The RHPE administration was oral via gavage for 14 days while the single dose of cisplatin at 7.5 mg/kg body weight, i.p. was administered on the eighth day of experiment, 1 hour after administration of RHPE extract. The dosage and route of administration of cisplatin were based on the method of Ko *et al.*, (2014), whereas those of RHPE was according to Mbaka *et al.* (2013).

Behavioural tests

Behavioural 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 open field box 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 assessed horizontal locomotion (number of squares crossed), and vertical locomotion (number of rearings) and grooming (number of times the rat cleaned its body)

(Mohammad et al., 2010). Each animal was given two trials at 30 minutes interval and the average taken.

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. Each animal was given two trials at 30 minutes interval and the average taken. This test reflects forelimb muscular strength in the animals (Tamashiro et al., 2000).

Sacrifice and Sample collection

After the behavioural tests, rats weighed and were thereafter euthanized using Ketamine 100 mg/kg intraperitoneally and euthanized by transcardiac perfusion with 10% neutral buffer formalin after an initial wash off with 200 mL of normal saline. The perfused rat was then laid prone on the dissecting board, cranium was opened and the whole brain carefully excised, rinsed in normal saline and weighed. The cerebellum was dissected out and the cerebral hemisphere divided into two sagittally and then fixed in 10% formalin for histological analysis.

Relative brain weight was calculated by the equation: weight of whole brain (g) / final body weight of rat (g) x 100.

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 μ m 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 (2008) 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 μ m, 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 cerebral cortex, cornu ammonis3 (CA3) and frontal cerebral cortex of the brain were observed and counted. The pyknotic index (PI) according to the method described (Taveira et al., 2013), 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 (Edelstein et al., 2014).

Statistical Analysis

Data was expressed as Means \pm Standard Error of Mean (SEM). Significant differences between groups were calculated using Student's t-test and $p < 0.05$ was considered statistically significant.

RESULTS

Phytochemical screening

The phytochemical screening of RHPE conducted showed the presence of flavonoids, terpenoids, saponins, alkaloids, steroids and tannins but tested negative for the presence of cardiac glycosides and anthraquinones.

Effects of RHPE on body weight and relative brain weight in rats treated with cisplatin

The immediate 48 hours following cisplatin administration, the rats had diarrhoea and weakness, but thereafter picked up gradually. There was significant percentage weight reduction in the CIS group compared with CTRL ($p < 0.05$) whereas there was a significant increase in the RHPE compared with CIS group (Table 1). However, the relative brain weight alterations were not significant (Table 1).

Table 1: Effect of Cisplatin and RHPE on the body and brain weight changes in rats

Group	CTRL	RHPE	CIS	CIS+RHPE
Initial body Wt.	162.5 \pm 8.4	162 \pm 7.5	167 \pm 7.5	200.5 \pm 14.4
Final body Wt.	189 \pm 11.3	187 \pm 10.2	148 \pm 7.5	170 \pm 8.5
Body Wt. changes	26.5 \pm 1.5	25 \pm 1.3	19 \pm 1.1	30.5 \pm 1.7
% Wt. changes	16.3	15.4	11.4*	15.2#
Brain Wt.	1.69 \pm 0.02	1.63 \pm 0.02	1.63 \pm 0.02	1.71 \pm 0.03
R.B.W	0.89	0.87	1.10	1.01

Values are presented as Mean \pm Standard error of mean for six rats per group. CTRL, Control, RHPE; *Raphia hookeri* pulp extract; CIS, cisplatin only, CIS+RHPE, cisplatin + *Raphia hookeri* pulp extract, Wt- Weight (g), R.B.W.-Relative brain weight. * $P < 0.05$ versus Control group, # $P < 0.05$ versus CIS group.

Effects of RHPE on behavioural and forelimb grip strength test in rats treated with cisplatin

Cisplatin significantly ($p < 0.05$) reduced the transitions, rearings, grooming when compared with the control group as shown in Figure 1. Control values of number of (transitions 35 ± 2.0 ; rearing 10.5 ± 0.2 and grooming 10 ± 0.5) were reduced by CIS (17 ± 0.8 , 5.5 ± 0.4 and 4.0 ± 0.16 respectively). Similarly CIS significantly ($p < 0.05$) reduced the duration of the forelimb grip strength to 2.2 ± 0.3 seconds when compared with control of 4.9 ± 0.58 seconds. However, pretreatment with RHPE significantly ($p < 0.05$) ameliorated these changes as observed in the CIS+RHPE rats relative to CIS group findings for all the parameters with the exception of duration of the forelimb grip strength as depicted in the figure.

Effects of RHPE on the histology of cerebellar cortex, cornu ammonis3 (CA3), dentate gyrus (DG) and cerebral cortex in rats treated with cisplatin

The histology of the cerebellar cortex in the Control group (Figure 2) exhibited normal cytoarchitecture with three normal layers namely molecular, Purkinje and granular and the Purkinje cells (Pc) exhibiting basophilic nuclear and normal shapes. The Pc of the CIS group exhibited features of degenerative alterations ranging from deeply eosinophilic cell bodies, shrinkage of the neuronal bodies with loss of their regular outlines as in Figures 2C relative to those of the control. The representative photomicrographs of CA3, DG and cerebral cortex were similarly normal in all groups except the CIS groups which showed neuronal alterations when compared with the control as depicted in Figures 3, 4 and 5. It is observed that

histologic features were returned to near control in CIS+RHPE groups with their neurons large, rounded or oval with open chromatin pattern and some with nucleoli. Plate 5 showed that the dendritic arborization of pyramidal neurons of frontal were reduced in CIS group.

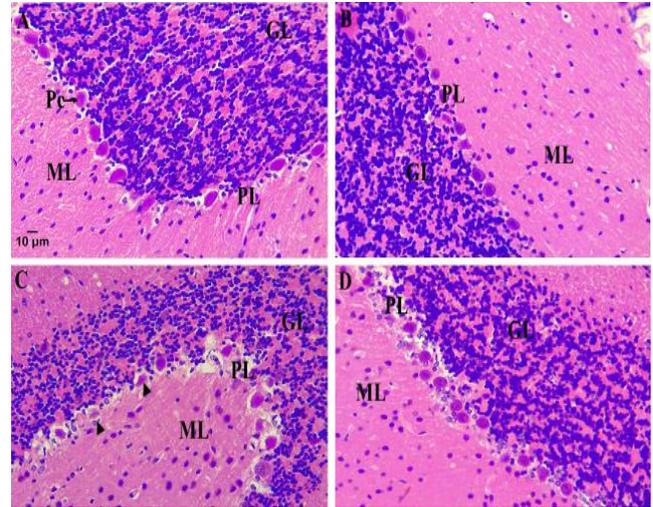


Figure 2: Representative stained sections of cerebellum of rats: (A) CTRL group (B) RHPE-treated (C) CIS-treated (D) CIS+RHPE treated. CTRL, Control; RHPE, *Raphia hookeri* pulp extract; CIS, cisplatin only; CIS+RHPE, cisplatin + *Raphia hookeri* pulp extract. ML, molecular layer; PCL, Purkinje cell layer; GL, granular layer. CIS-treated shows varying degree of degenerated Purkinje neurons (arrowheads). H&E. Scale bar = 10 µm for all figures.

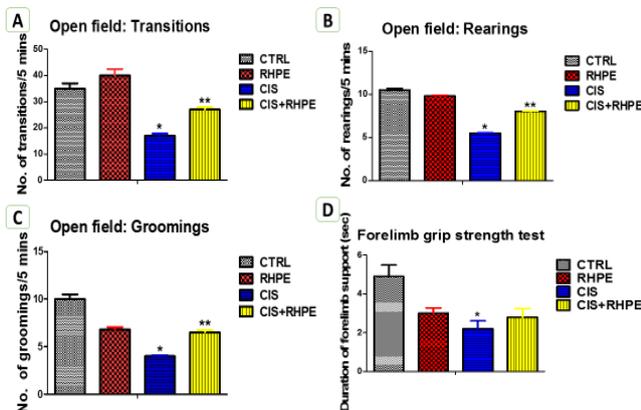


Figure 1: Histogram showing the effect of RHPE on behavioural parameters and forelimb grip strength test in rats treated with CIS. Values are presented as Mean ± S.E.M. of six rats. CTRL, Control; RHPE, *Raphia hookeri* pulp extract; CIS, cisplatin only; CIS+RHPE, cisplatin + *Raphia hookeri* pulp extract. * $p < 0.05$ versus CTRL group, ** $p < 0.05$ versus CIS group.

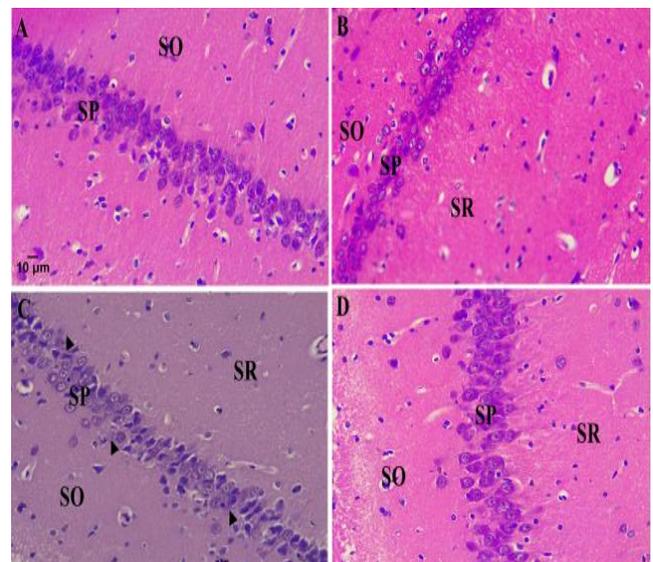


Figure 3: Representative stained sections of Cornu Ammonis 3 of rat hippocampus: (A) CTRL group (B) RHPE-treated (C) CIS-treated (D) CIS+RHPE treated. CTRL, Control; RHPE, *Raphia hookeri* pulp extract; CIS, cisplatin only; CIS+RHPE, cisplatin + *Raphia hookeri* pulp extract. SO, stratum oriens; SP, stratum pyramidalis; SR, stratum radiatum. Dark pyramidal neurons (arrowheads) are noted in CIS-treated brains. H&E. Scale bar = 10 µm for all figures.

Effects of RHPE on the histomorphometry of Purkinje cells, Pyramidal neurons of frontal cerebral cortex and Pyknotic indices of frontal cerebral cortex and cornu ammonis3 in rats treated with cisplatin.

Figure 6 demonstrated that CIS significantly ($p < 0.05$) reduced the diameters of Purkinje neurons of the cerebellum ($9.10 \pm 0.59 \mu\text{m}$) compared with the control ($14.44 \pm 0.31 \mu\text{m}$) and that of pyramidal neurons of frontal cortex ($11.32 \pm 0.05 \mu\text{m}$) when compared with the control ($17.03 \pm 0.54 \mu\text{m}$).

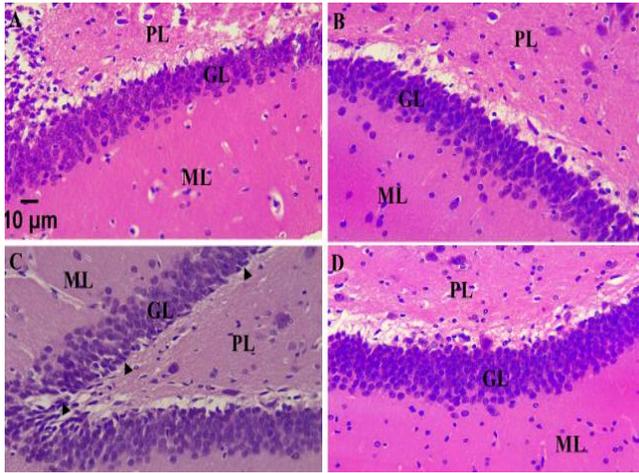


Figure 4: Representative stained sections of Dentate gyrus of rats: (A) CTRL group (B) RHPE-treated (C) CIS-treated (D) CIS+RHPE treated. CTRL, Control; RHPE, *Raphia hookeri* pulp extract; CIS, cisplatin only; CIS+RHPE, cisplatin + *Raphia hookeri* pulp extract. ML, molecular layer; PL, Polymorphic layer; GL, granular layer. Dark granule neurons (arrowheads) in CIS-treated brains are restricted to the innermost layer of cells. H&E. Scale bar = 10 μm for all figures.

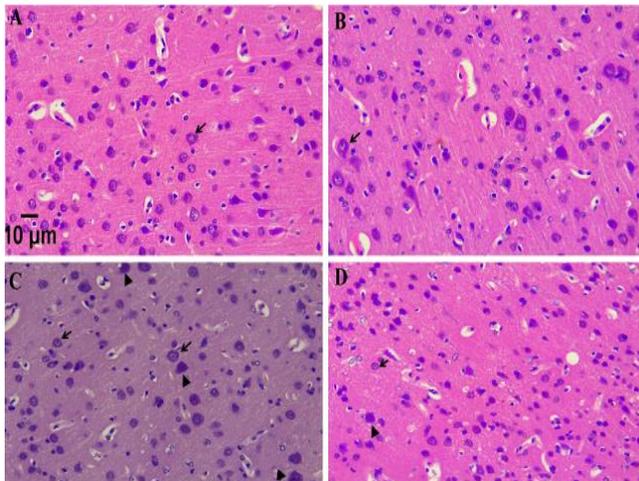


Figure 5: Representative stained sections of cerebral cortex of rats: (A) CTRL group (B) RHPE-treated (C) CIS-treated (D) CIS+RHPE treated. CTRL, Control; RHPE, *Raphia hookeri* pulp extract; CIS, cisplatin only; CIS+RHPE, cisplatin + *Raphia hookeri* pulp extract. Some of the CIS-treated cortical neurons exhibit pyknosis (arrowheads). H&E. Scale bar = 10 μm for all figures.

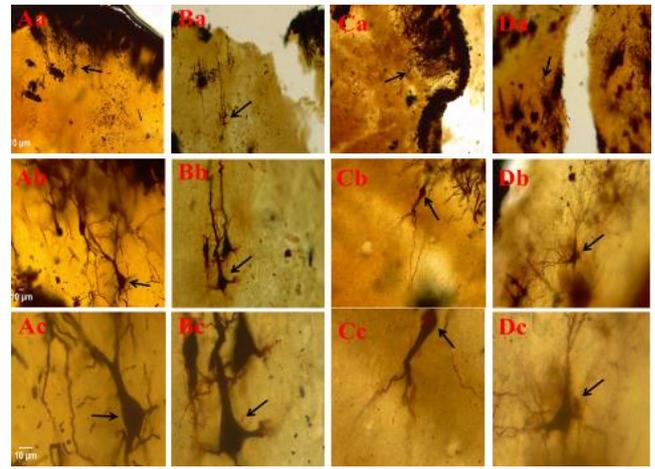


Figure 6: Representative Golgi stained sections of cerebral cortex of rats: (Aa-Ac) CTRL group; (Ba-Bc) RHPE-treated; (Ca-Cc) CIS-treated; (Da-Dc) CIS+RHPE treated. CTRL, Control; RHPE, *Raphia hookeri* pulp extract; CIS, cisplatin; CIS+RHPE, cisplatin + *Raphia hookeri* pulp extract. Upper panel, Aa-Da represents all the groups at $\times 100$ magnification; middle panel, Ab-Db is at $\times 400$ magnification while the lowermost panel, Ac-Dc is at $\times 1000$ magnification. Dendritic arborization appears reduced in group C as shown in Cb and Cc. Scale bar = 10 μm for all figures.

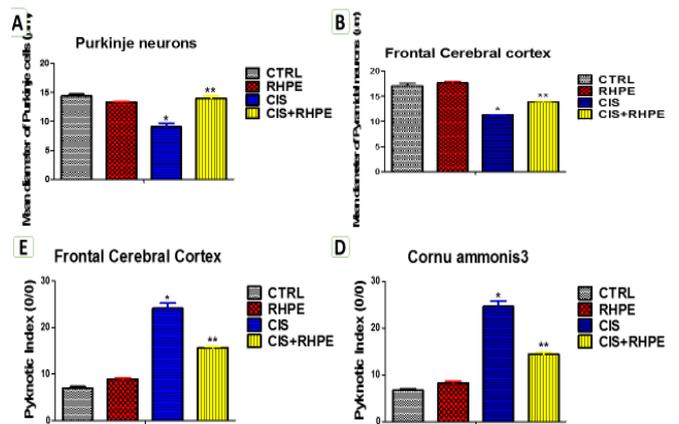


Figure 7: Histogram showing the effects of RHPE on the histomorphometry of Purkinje neurons, Pyramidal neurons of frontal cerebral cortex and Pyknotic indices of frontal cerebral cortex and cornu ammonis3 in rats treated with cisplatin. Values are presented as Mean \pm S.E.M. of six rats. CTRL, Control, RHPE; *Raphia hookeri* pulp extract; CIS, cisplatin only; CIS+RHPE, cisplatin + *Raphia hookeri* pulp extract. * $p < 0.05$ versus CTRL group, ** $p < 0.05$ versus CIS group.

Similarly, CIS significantly ($p < 0.05$) increased the pyknotic indices of both the pyramidal neurons of CA3 (24.62 ± 1.12) compared with the control (6.7 ± 0.33) and that of the frontal cortical neurons (24.14 ± 1.09) relative to the control (6.93 ± 0.38). However, all these parameters were significantly reversed to near control levels by pretreatment with RHPE when compared with CIS treatment.

DISCUSSION

The results showed that cisplatin (CIS) induced significant behavioural as well as micro-anatomical alterations in the cerebellum, cornu ammonis3 (CA3), dentate gyrus (DG), and frontal cerebral cortex (FCC) of adult albino rats.

The weight loss in the CIS group was possibly due to diarrhoea the rats had following CIS administration. This might be due to damage to water-absorptive mucosa epithelium of the rat colon from CIS injury. The water and possible salt loss might have caused the overall weight loss in agreement with previous studies (Ko et al., 2014; Ali et al., 2014; Adaramoye et al., 2016). The brain weight change in the groups were not significantly altered possibly because of the short duration of the diarrhoea and early recovery of the rats.

The reduction by CIS of both horizontal locomotion (number of lines crossed) and vertical locomotion (number of rearings) as well as forelimb muscle strength (duration of hanging on to the metal wire) by rats suggested possible reduction of the nervous and muscular activities of the rats which agreed with published reports (Ali et al., 2014; Owoeye and Onwuka, 2015). It was however, noted that co-treatment of CIS with RHPE reduced the effect of CIS alone thus ameliorating the reduction observed. The overall effect of these changes was to make the rats sluggish.

Damage to the Purkinje cells of the cerebellum as observed histologically agreed with previous reports of CIS on cerebellum (Bottone et al., 2012; Owoeye and Onwuka, 2015; Owoeye et al., 2015). This damage posed a danger to the smooth and effective role the cerebellum performs as a motor stabilizing control system since it receives continual feedback information about intended movement and actual movements (Chaudhary et al., 2014). Degeneration of Purkinje cells, the principal efferent pathway of the cerebellum may lead to different forms of ataxia and an unstable gait (Kim et al., 2009). Our findings of neural death in the cerebellum agrees with the report of induction of cell death *in vivo* and *in vitro* by CIS in both the cerebellum (Bottone et al., 2012). The alteration of Purkinje neurons as shown in this study may be responsible for the associated behavioural and muscular strength changes observed.

The observations of CIS-induced alterations in rat CA3 and DG neurons showed distortion of the architecture of these hippocampal parts. Earlier reports indicated the inability of CIS to penetrate the blood-brain barrier (Gregg et al., 1992). Further studies had shown that it could penetrate this barrier to induce neurotoxicity with histological alteration (Al Moundhri et al., 2012; Gulec et al., 2013; Owoeye and Onwuka, 2015; Owoeye et al., 2015).

The effect of death of pyramidal neurons of CA3 and granule neurons of DG will be on the memory coding process in the brain as both will affect incoming perforant pathway projections from layer II of the entorhinal cortex. The death of granule neurons in the subgranular layer might affect the generation of new neurons as well as affect other cells of the dentate gyrus namely dentate pyramidal basket and mossy cells (Amaral et al., 2007). The damage to mossy fibres from granule cells projecting to CA3 might also affect the quality of projections of Schaffer's collateral from CA3 to CA1 and its ultimate projection to subiculum and entorhinal cortex. We propose that the overall effect of these alterations, that is the elevated pyknotic index of CA3 pyramidal neurons and hippocampal components in general might lead to impairment of declarative memory formation, memory storage and behaviour of the rats (Ellis, 2006; Stepan et al., 2015; Folarin et al., 2017).

The observed death of FCC neurons, increased pyknotic index and reduction of dendritic arborization of the pyramidal neurons of the cortex in CIS-treated rats are in agreement with previous report of cisplatin injury in rat cortex (Karavelioglu et al., 2015; Owoeye and Onwuka, 2015; Owoeye et al., 2015). Since the final control of fragmented distal digital movements in mammals are controlled by the corticospinal tracts originating from the cortex, the death of cortical neurons induced by CIS might explain the reduced strength of the grip of the rats as observed in the forelimb grip test.

Cisplatin-induced neurotoxicity has been associated with histological damage (Al Moundhri et al., 2012; Arrieta et al., 2011) shown to be mediated via oxidative damage (Turan et al., 2013). Brain tissue contains large amounts of long chain polyunsaturated fatty acids (PUFAs), low levels of antioxidants and high aerobic metabolism which make it very susceptible to oxidative stress (Ebokaiwe et al., 2013; Chaudhry et al., 2014). Our observation of reduction of the CIS-induced behavioural and micro-anatomic alterations in rats co-treated with RHPE suggested neuroprotection of Purkinje neurons, pyramidal neurons of CA3, granule neurons of DG of the hippocampal formation and neurons of the FCC. This supports the hypothesis of ameliorative potential of RHPE as shown in the CIS+RHPE group. *Raffia hookeri* pulp has been shown to possess antioxidant property (Edem et al., 1984; Akpan and Usuh, 2004; Dada et al., 2017) due to its flavonoid contents, but we did not study anti-oxidative property in this study.

Taken together, RHPE reversed the behavioural changes and demonstrated neuroprotection against cisplatin-induced and micro-anatomical alterations of cerebellar, hippocampal and cerebral cortex neurons possibly through its antioxidant property. Further studies are warranted to isolate and characterize the

active component in RHPE responsible for these observed effects.

REFERENCES

- Adaramoye, O.A., Azeez, A.F. and Ola-Davies, O.E. (2016). Ameliorative effects of chloroform fraction of *Cocos nucifera* L. husk fiber against Cisplatin-induced toxicity in rats. *Phcog Res.* 8:89-96.
- Afifi, A.K. and Bergman, R.A. (2005). *Functional neuroanatomy: Text and Atlas*, 2nd edition, McGraw-Hill, New York: 201-222.
- Afolayan, A.O., Borokini, T.I. and Afolayan, G.O. (2014). Sublethal Effects of Methanolic Extract of *Raphia hookeri* on the Reproductive Capacity of *Clarias gariepinus*. *Advances in Zoology*. Article ID 615908. Available at: <http://dx.doi.org/10.1155/2014/615908>. Accessed August 27, 2017.
- Akinola, F.F., Oguntibeju, O.O., Adisa, A.W. and Owojuyigbe, O.S. (2010). Physico-chemical properties of palm oil from different palm local factories in Nigeria. *J. Food Agric. Environ.* 8: 264-269.
- Akman, T., Akman, L., Erbas, O., Terek, M.C., Taskiran, D. and Ozsaran, A. (2015). The preventive effect of oxytocin to Cisplatin-induced neurotoxicity: an experimental rat model. *Biomed Res Int.* 2015:167235. DOI: 10.1155/2015/167235
- Akpan E.J. and Usoh, I.F. (2004). Phytochemical screening and effect of aqueous root extract of *Raphia hookeri* (raffia palm) on metabolic clearance rate of ethanol in rabbits. *Nigerian Society for Experimental Biology*, 16(1): 37 - 42.
- Al Moundhri, M.S., Al-Salam, S., Al Mahrouqee, A., Beegam, S. and Ali, B.H. (2012). The effect of curcumin on oxaliplatin and cisplatin neurotoxicity in rats: Some behavioral, biochemical, and histopathological studies. *J. Med. Toxicol*, doi: 10.1007/s13181-012-0239-x.
- Ali, B.H., Ramkumar, A., Madanagopal, T.T., Waly, M.I., Tageldin, M., Al-abri, S., Fahim, M., Yasin, J. and Nemmar, A. (2014). Motor and behavioral changes in mice with Cisplatin-induced acute renal failure *Physiol. Res.* 63: 35-45.
- Amaral, D.G., Scharfman, H.E. and Lavenex, P. (2007). The dentate gyrus: fundamental neuroanatomical organization (dentate gyrus for dummies). *Prog Brain Res.* 163: 3-22.
- Arrieta, O., Hernandez-Pedro, N., Fernandez-Gonzalez-Aragon, M.C., Saavedra-Perez, D., Campos-Parra, A.D., Rios-Trejo, M.A., Ceron-Lizarraga, T., Martinez-Barrera, L., Pinedan, B., Ordonez, G., Ortiz-Plata, A., Granados-Soto, V. and Sotelo, J. (2011). Retinoic acid reduces chemotherapy induced neuropathy in an animal model and patients with lung cancer. *Neurology*, 77: 987-995.
- Bancroft, J.D. and Gamble M. (2008). *Theory and Practice of Histology Techniques*, 6th edition. Churchill Livingstone Elsevier, Philadelphia: 83 - 134.
- Bottone, M.G., Santin G., Piccolin V.M., Bo V.D. and Bernocchi G. (2012). Cisplatin neurotoxicity induces cell death *in vitro* and *in vivo*. In: Kojima T, Morita Y (Eds). *Cisplatin: Pharmacology, clinical uses and adverse effects*. NOVA Science Publishers, Inc.: 123-140.
- Chaudhary, M., Joshi, D.K., Tripathi, S., Kulshrestha, S. and Mahdi, A.A. (2014). Docosahexaenoic acid ameliorates aluminum induced biochemical and morphological alteration in rat cerebellum. *Ann Neurosci.* 21(1): 5-9.
- Dada, F.A., Oyeleye, S.I., Ogunsuyi, O.B., Olasehinde, T.O., Adefegha, S.A., Oboh, G. and Boligon AA. (2017). 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). Available at: <http://dx.doi.org/10.1016/j.jtcme.2017.01.003>. Accessed August 03, 2017
- Dasari, S. and Tchounwou, P.B. (2014). Cisplatin in cancer therapy: Molecular mechanisms of action. *Euro J Pharmacol.* Volume 740, 5 October 2014, Pages 364-378. <https://doi.org/10.1016/j.ejphar.2014.07.025>
- Ebokaiwe, A.P., Adedara I.A., Owoeye O, and Farombi E.O. (2013). Neurotoxicity of Nigerian bonny light crude oil in rats. *Drug Chem Toxicol*, 36 (2): 187-195.
- Edelstein, A.D., Tsuchida, M.A., Amodaj, N., Pinkard, H., Vale, R.D. and Stuurman, N. (2014). Advanced methods of microscope control using μ Manager software. *J Biol Meth.* 1(2):e11 doi:10.14440/jbm.2014.36
- Edem, D.O., Eka, O.U. and Ifon, E.T. (1984). Chemical evaluation of the nutritive value of the raffia palm fruit (*Raphia hookeri*) *Food Chemistry*. 15(1): 9-17
- Ellis, H. (2006). *Clinical Anatomy: Applied Anatomy for students and junior doctors*. Blackwell, Oxford, UK: 349-352.
- Folarin, O.R., Snyder, A.M. Peters, D.G., Olopade, F., Connor, J.R. and Olopade, J.O. (2017). Brain Metal Distribution and Neuro-Inflammatory Profiles after Chronic Vanadium Administration and Withdrawal in Mice. *Front. Neuroanat.* 11:58. doi: 10.3389/fnana.2017.00058
- Fonnum, F. and Lock, E.A. (2000). Cerebellum as a target for toxic substances. *Toxicol Lett.* 15: 112-113: 9-16.
- Gregg, R.W., Molepo, J.M., Monpetit, V.J., Mikael, N.Z., Redmond, D., Gadia, M, and Srewart, D.J. (1992). Cisplatin neurotoxicity: the relationship between dosage, time, and platinum concentration

- in neurologic tissues, and morphologic evidence of toxicity. *J. Clin. Oncol.* 10(5): 795-803.
- Gulec, M., Oral, E., Dursun, O.B., Yucel, A., Hacimuftuoglu, A., Akcay, F. and Suleyman H. (2013). Mirtazapine protects against cisplatin-induced oxidative stress and DNA damage in the rat brain. *Psych. Clin. Neurosci.* 67: 50–58
- Hashem, R.M., Safwat, G.M., Rashed, Laila, A. and Bakry, S. (2015). Biochemical findings on cisplatin-induced oxidative neurotoxicity in rats. *Int. J. Adv. Res.* 3(10): 1222 – 1231.
- Karavelioglu, E., Boyaci, M.G., Simsek, N., Sonmez, M.A., Koc, R., Karademir, M., Guven, M. and Eser, E. (2015). Selenium protects cerebral cells by cisplatin induced neurotoxicity. *Acta Cirúrgica Brasileira.* 30(6): 394-400.
- Kim, B.J., Lee, S.Y., Kim, H.W., Park, E-J., Kim, J., Kim, S.J., So, I. and Jeon, J-H. (2009). Optimized Immunohistochemical Analysis of Cerebellar Purkinje Cells Using a Specific Biomarker, Calbindin D28k. *Korean J Physiol Pharmacol.* 13: 373 - 378.
- Ko, J-W., Lee, I-C., Park, S-H., Moon, C., Kang, S-S., Kim, SH. and Kim, J-C. (2014). Protective effects of pine bark extract against cisplatin-induced hepatotoxicity and oxidative stress in rats. *Lab Anim Res* 2014: 30(4), 174-180.
- Kumar, P., Sulakhiya, K., Barua, C.C. and Mundhe, N. (2017). TNF- α , IL-6 and IL-10 expressions, responsible for disparity in action of curcumin against cisplatin-induced nephrotoxicity in rats. *Mol Cell Biochem.* 431:113-122.
- Mbaka, G.O., Ogbonnia, S.O., Oyeniran, K.J. and Awopetu, P.I. (2012). Effect of *Raphia hookeri* Seed Extract on Blood Glucose, Glycosylated Haemoglobin and Lipid Profile of Alloxan Induced Diabetic Rats, *Br. J. Med. Med. Res.*, 2(4): 621-635.
- Mohammad, S., Shahrnaz, P., Masoud, N., Moazamehosadat, R., Nasser, Z., Khadije, E. and Fatemeh, A. (2010). Walnut consumption protects rats against cisplatin - induced neurotoxicity. *Neurotoxicol.* oi:10.1016/j.neuro.2012.08.004. Accessed 02/11/2014.
- Mokhtari, M.J., Akbarzadeh, A., Hashemi, M., Javadi, G., Reza, R., Mehrabi, M.R., Farhangi, A. and Mohammadi, H. (2012). Cisplatin Induces Down Regulation of BCL2 in T47D Breast Cancer Cell Line. *Adv. Stud. Biol.*, 4(1): 19 – 25.
- Oduah, A.A. and Ohimain, E.I. (2015). Ethnobotany of raffia palm (*Raphia hookeri*), productivity assessment and characterization of raffia palm oil from the Niger Delta, Nigeria *Res. J. Phytomed.* 01[01] 2015. Available at: www.asdpub.com/index.php/rjpp (Online). Accessed August 02, 2017
- Owoeye, O. and Onwuka, S.K. (2015). Tomato pomace powder ameliorated cisplatin-induced microanatomical alterations in brain of Wistar rats. *Int. J. Biol. Chem. Sci.* 9(1):1-11.
- Owoeye, O., Femi-Akinlosotu, O.M. and Adejuwon S.A. (2015). *Launae taraxacifolia* aqueous extract attenuates Cisplatin-induced neurotoxicity, by decreasing oxidative stress and neuronal cell death in rats. *Arch. Basic Appl. Med.* 3:71-78.
- Polterait, O. (1997). Antioxidants and free-radical scavengers of Natural Origin. *Current. Org. Chem.* 1: 415-440.
- Public Health Service (PHS) 1996. Public health service policy on humane care and use of laboratory animals. US Department of Health and Human Services, Washington, DC, PL. 99-158.
- Sanchez-Gonzalez, P.D., Lopez-Hernandez, F.J., Lopez-Novoa, J.M. and Morales, A.I. (2011). An integrative view of the pathophysiological events leading to cisplatin nephrotoxicity. *Crit Rev Toxicol* 41: 803-821.
- Stepan, J., Dine, J. and Eder, M. (2015). Functional optical probing of the hippocampal trisynaptic circuit in vitro: network dynamics, filter properties, and polysynaptic induction of CA1 LTP. *Front Neurosci.* 9: 160.
- Tamashiro, K.L.K., Wakayama, T., Blanchard, R.J., Blanchard, C. and Yanagimachi, R. (2000). Postnatal growth and behavioral development of mice cloned from adult cumulus cells. *Biol Reprod.* 63:328–334.
- Turan, M.I., Turan, I.S., Mammadov, R., Altinkaynak, K. and Kisaoglu, A. (2013). The Effect of Thiamine and Thiamine Pyrophosphate on Oxidative Liver Damage Induced in Rats with Cisplatin. *BioMed Research International*, Article ID 783809: 1-6.