

Research Article

Cisplatin-induced neurotoxicity in rat brain was attenuated by *Sida acuta* ethanol extract

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Abstract

Neurotoxicity is associated with Cisplatin (CP) chemotherapy, and oxidative stress has been associated with the pathogenesis. *Sida acuta* ethanol extract (SAEE) may prevent neurotoxicity through its known antioxidant properties. The study aimed to examine the potential protective effects of SAEE against cisplatin-induced microanatomical alterations in rat brain. Twenty-eight rats were randomized equally into four groups: control (1 mL distilled water); SAEE (200 mg/kg p.o.); CP (7 mg/kg i.p.); CP+SAEE (7 mg/kg; 200 mg/kg, respectively i.p and p.o.). All treatments lasted 14 days. On the 15th day, behavioural studies were done after which all the rats were euthanized. Histology and histomorphometry of brain tissue were carried out. CP significantly ($p < 0.05$) reduced the frequency of locomotion, rearing, grooming and the forelimb strength of rats. Similarly, the densities of viable neurons of the cerebral cortex, dentate granule cell layer of dentate gyrus, cornu ammonis3 field of hippocampus propria and Purkinje cells (PCs) of cerebellum were significantly ($p < 0.05$) reduced by CP. Histologically, neuronal loss and neuro-degeneration was evident in all the different parts of the brain examined, being most marked on the PCs. However, pre- and concomitant treatment of rats with SAEE with CP reduced the damaging effect of CP in all of brain tissues. SAEE demonstrated neuroprotective effects against CP-induced neuro-degeneration, behavioural alteration and reduction in densities of viable brain neurons. These effects may be attributed to the antioxidant capabilities of SAEE.

Key Words: Cisplatin, *Sida acuta*, neurotoxicity, Purkinje cells, neuroprotective.

INTRODUCTION

Cisplatin [cis-Diammine Dichloro Platinum (II)] (CP) is a commonly used chemotherapeutic agent in the treatment of solid tumors in various organs including testes, ovary, breast, bladder, lung, colon, rectum, uterine cervix and head and neck cancers (Ozyurt et al., 2006; Sanchez-Gonzalez et al., 2011; Gulec et al., 2013). While CP is reported to be clinically effective, the major side effects viz: renal toxicity, neurotoxicity, myelotoxicity, ototoxicity, haematological toxicity and hepatotoxicity are dose limiting and occur acutely or after repeated doses (Ozyurt et al., 2006; McWhinney et al., 2009). Following nephrotoxicity, neurotoxicity is the next common side effect of CP-based chemotherapeutics and these main adverse effects limit the drug utilization in cancer treatment (Ozyurt et al., 2006).

The mechanism of CP toxicity has been reported to include apoptosis, DNA damage, induction of oxidative stress and inflammation (Ali et al., 2014). Some reports showed that neurotoxicity and hepatotoxicity are due to damage to the mitochondria causing increases in mitochondrial lipid peroxidation and protein carbonyl contents in brain tissues (Altun et al., 2010; Bansode et al., 2014) and that CP-induced formation of oxygen free-radicals elicit a fall in plasma antioxidant levels because of oxidative stress (Ozyurt et al., 2006; Owoeye et al., 2015) and that antioxidants effectively protect neurons from CP-induced neurotoxicity (Hamers et al., 1993; Owoeye and Onwuka, 2015). Brain tissue is highly

sensitive to damage by free radicals because of its high use of oxygen, its high concentration of polyunsaturated fatty acids and its low concentration of cytosolic antioxidants (Ebokaiwe et al., 2013).

Different parts of the rat brain has specific functions: the cerebral cortex is involved in various functions ranging from cognition to vision, auditory, visual etc., the hippocampus is involved in memory formation and storage as well as some limbic functions, while the cerebellum is involved in co-ordination of motor function and balance (Snell, 2012). The histology and functions of the brain may be altered by its susceptibility to damage by neurotoxic drugs like CP.

We have previously tested antioxidant-rich plants (*Launaea taraxacifolia* and *Lycopersicon esculentum*) and reported their ability to ameliorate the neurotoxicity and tissue damage exhibited by CP in rat brain (Owoeye et al., 2015; Owoeye and Onwuka, 2015). The flavonoids content and antioxidant activity of *Sida acuta* leaf had previously been reported (Benzouzi et al., 2004; Ekor et al., 2010; Bahar et al., 2013). However, literature is scanty on its effect on CP's toxicity in the rat brain, hence this current study to evaluate its ability to modulate the effect of CP on rat brain histology.

Therefore, the aim of our current study was to investigate the possible protective effects of *Sida acuta* ethanol extract against Cisplatin-induced neurotoxicity and thus be able to answer the research question: "Can *Sida acuta* ethanol extract modulate Cisplatin-induced alterations in rat brain?"

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Healthy fresh leaves of *Sida acuta* were harvested from bushes behind the Department of Fisheries and Wildlife, University of Ibadan, Nigeria in April 2014. The leaves were identified at the Botany Department, University of Ibadan, Nigeria and then authenticated at the Forestry Research Institute of Nigeria (FRIN) Ibadan, Nigeria, where a voucher sample with the Forest Herbarium Identification Number FHI 110161 was deposited.

Animal Protocol

Experiments were performed on 28 adult male Wistar rats whose average weight was 197±10.8 g, obtained from the College of Medicine Animal House, University of Ibadan, Nigeria, and housed in well-ventilated plastic cages, provided with rat pellets and water *ad libitum*. The animals were maintained under standard conditions of temperature and humidity with alternating 12 h light/dark cycles. The experimental protocols were carried out according to the guidelines set by the University of Ibadan Ethical Committee, ensuring its conformation to the acceptable guidelines on the ethical use of animals in research (Public Health Service, 1996).

Phytochemical Screening

Phytochemical analyses of the leaves of *Sida acuta* was carried out using simple standard chemical tests based on the protocol described by Harborne (1973) at the Department of Pharmacognosy, University of Ibadan, Nigeria.

Extraction procedures of *Sida acuta* ethanol extract (SAEE).

The leaves were washed under running tap water to remove impurities and then air-dried at room temperature for seven days. The dried leaves were blended and 550 g of pulverized leaves was obtained. Extraction of plant constituent was by cold maceration in 99.9% ethanol (3 x 2.5 L) at room temperature, stirred daily over a period of 96 hours. The solvent-extract mixture was filtered with Whatmann filter paper and the extract solution obtained concentrated in a rotary vacuum evaporator until a solid residue was formed which weighed 25.5 g, giving a percentage yield of 4.6%. The dry (solid) extract was termed *Sida acuta* ethanol extract (SAEE).

Preparation, dosage and administration of SAEE

From a stock solution of 1 g SAEE per 10 mL of distilled water, the dissolved extract (SAEE) was administered orally to the animals at a dose of 200 mg/kg based on the published method of Ekor *et al.* (2010).

Chemicals and drugs

Cisplatin was manufactured by Korea United Pharm. Inc. (Naojang, Chungnam, Korea) and purchased from Kunle-Ara Pharmacy, Ibadan, Nigeria. Ketamine hydrochloride was manufactured by Rotex Medica, Trittau, Germany. All other reagents were of analytical grade and were obtained from the British Drug Houses (Poole, Dorset, UK).

Experimental induction of neuropathy by Cisplatin

Cisplatin was administered intraperitoneally twice during the course of the experiment on days 7 and 14 at a dose of 7 mg/kg body weight and was administered 1 hour after oral

administration of SAEE. The dosage of Cisplatin was based on the method of Ateşşahin *et al.* (2006) and Ozyurt *et al.* (2006).

SAEE (*Sida acuta* ethanol extract), CP (Cisplatin). Cisplatin was administered intraperitoneally (i.p.) one hour after SAEE administration. Other preparations were administered orally using a clean intra-gastric gavage. Rats were euthanized on the 15th day of experiment.

Experimental Design

The details of the experimental design are as shown in Table 1:

Table 1:

Animal grouping and Research Design

Group	Treatment
I	Control, (1 mL distilled water) daily for 14 days.
II	SAEE (200 mg/kg body weight) daily for 14 days.
III	CP (7 mg/kg body weight) i.p on days 7 and 14 of experiment.
IV	SAEE (200 mg/kg body weight) daily for 14 days + CP (7 mg/kg body weight) i.p. on days 7 and 14 of experiment.

Behavioural Tests

On experimental day 15, rats in each group were weighed and then subjected to behavioural studies to evaluate motor function, exploration, locomotion and anxiety parameters. Motor function test was evaluated by the forelimb grip strength test, while exploration, rearing, locomotion and anxiety tests were conducted in the open field box.

Forelimb Grip Strength Test: This test involved the forepaws of the rats being placed on a horizontally suspended metal wire (measuring 2mm in diameter and 1m in length). Placed one meter above a landing area filled with soft bedding. The length of time each rat was able to stay suspended before falling off the wire was recorded. A maximum time of 2 minutes is given to each rat after which it will be removed. The test reflects muscular strength in the animals (Olopade *et al.*, 2012).

Open Field Test. This test was carried out on day 15 of experiment. The animals were placed for 5 min in an open-field arena. The apparatus, made of wood covered with impermeable formica, had a white floor of 100 × 100 cm (divided by black lines into 25 squares of 20 × 20 cm) and 40-cm high white walls. Each rat was placed at the center of the open field and was left free to explore the unfamiliar arena; the total number of squares crossed and rearing was measured (Cai *et al.*, 2010). The quadrant was considered crossed when the animal have four paws in the adjacent square. The floor and walls of the box were wiped with 70% ethanol and allowed to dry after completing the test for each rat to exclude olfactory bias (Ibironke and Fasanmade, 2015).

Histological process

After completing the behavioural tests, all rats were euthanized with Ketamine 100 mg/kg body weight i.p. followed by cervical dislocation. From each rat the whole brain was dissected and brain weights recorded. The cerebral cortex, hippocampus and cerebellum were separated and fixed in 10% neutral buffered formalin (10% NBF), dehydrated through graded ethanol series, cleared in xylene, embedded in

paraffin wax and then sectioned at 5 – 6 µm thickness with a Rotary Microtome (Leica RM2125 RTS). Sections were stained with haematoxylin and eosin (Bancroft and Gamble, 2008) and evaluated under light microscope (Olympus CH, Japan). Images were captured with a Sony DSC-W 30 digital camera (Japan) while photomicrograph calibration was done with Micro-Manager (Edelstein *et al.*, 2014).

Histomorphometric Studies

Histomorphometric analysis were done using computerized image analyzer (TSView CX image software file version 6.2.4.3 and Image motic 2000 (China). On the tissue slides of cerebral cortex, granule cell layer of dentate gyrus and stratum pyramidalis of CA3, the number of viable cerebral cortical neurons, granule cells and pyramidal cells respectively were counted. Viability of neurons was based on presence of dispersed chromatin, distinct nucleoli and absence of features of cell death like pyknosis and karyolysis or karyorrhexis at high power.

Statistical Analysis

Data were presented as Mean ± SEM and analysed using one way ANOVA (Analysis of Variance) test, followed by LSD post hoc using the statistical software package IBM SPSS statistics 20. The statistical significance was set at p < 0.05, for the null hypothesis being true by chance and the confidence interval at 95% level.

RESULTS

General Observations

Two rats in the cisplatin group died before the completion of the experiments while rats in other groups remained active throughout.

Phytochemical Analysis

The phytochemical evaluation of the leaves of *Sida acuta* showed the presence of flavonoids tannins, alkaloids, cardenolides, anthraquinones, steroids and terpenoids.

Open field test.

As displayed in Table 2, CP administration significantly (p<0.05) reduced the frequencies of locomotion, rearing, grooming and the forelimb grip strength compared with control values while concomitant treatment of CP plus SAEE elevated these values significantly (p<0.05) relative to CP group.

Table 2:

Behavioural changes in rats treated with Cisplatin and *Sida acuta* ethanol extract.

Treatment	Line Crossing	Rearing	Grooming	Forelimb gri strength (s)
Control	42.10 ±5.00	11.12 ±3.0	16.12 ±3.60	5.75 ±1.34
SAEE	38.00 ±4.04	11.00 ±3.0	15.23 ±5.10	4.60 ±1.00
CP	24.12 ±4.00*	7.41 ±1.50*	9.00 ±2.30*	1.84 ±0.20*
CP+SAEE	43.75 ±5.34**	9.25 ±3.13**	15.16 ±4.28**	4.8 ±1.15**

Data are expressed as mean ±SEM of five rats per group. SAEE, 200 mg/kg *Sida acuta* ethanol extract; CP, Cisplatin, 7 mg/kg. *P<0.05 versus Control; **P<0.05 versus CP

Histological parameters

Cerebral cortex: Cerebral cortical neurons show viable normal histological appearance. The margins of the neurons were distinct (VN) with nucleoli distinctively observed (Plates 1A, 1B and 1D) whereas those of the Cisplatin group (Plate 1C) show overall reduced basophilic staining, degenerated neurons and vacuolated areas shaped around the neurons.

Dentate gyrus of hippocampus: Cisplatin treated group (Plate 2C) shows denuded granule neurons most of which are pyknotic in contrast to other groups where the neurons are healthy and layered in several rows (Plates 2A, 2B and 2C). Cisplatin treatment caused loss of granule cells of the dentate granule cells relative to other groups.

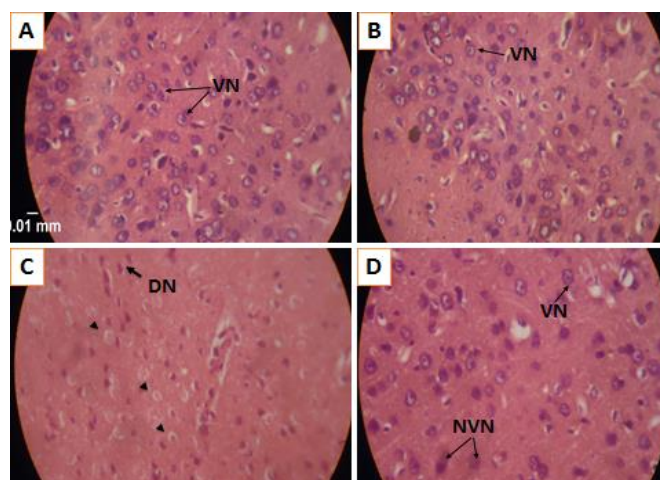


Plate 1: Representative stained sections of cerebral cortex of rats: (A) Control (B) SAEE-treated (C) Cisplatin-treated (D) Cisplatin+SAEE-treated. SAEE, *Sida acuta* ethanolic extract, 200 mg/kg; Cisplatin, 7 mg/kg; VN, viable cortical neurons; NVN, non-viable cortical neuron; DN, degenerated neurons. Arrowheads, vacuolated neuron spaces. H&E. Calibration bar = 0.01mm (10 µm).

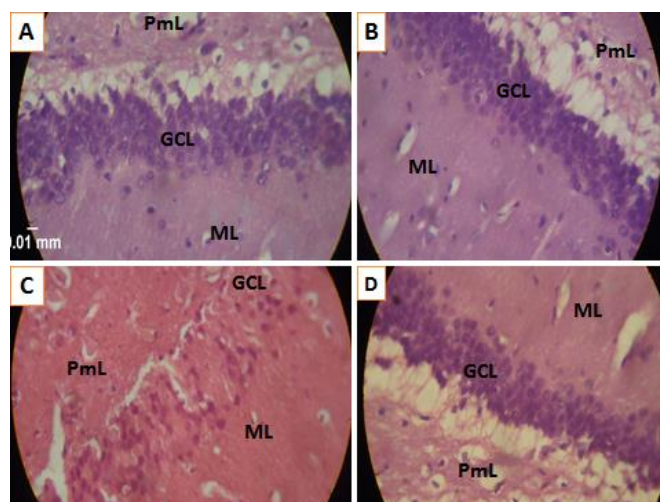


Plate 2: Representative stained sections of dentate gyrus of rats: (A) Control (B) SAEE-treated (C) Cisplatin-treated group (D) Cisplatin+SAEE-treated. SAEE, *Sida acuta* ethanolic extract 200 mg/kg; Cisplatin, 7 mg/kg; ML, molecular layer; GCL, granular cell layer; PmL, polymorphic layer. H&E. Calibration bar = 0.01mm (10 µm) for all figures.

The blades of granule cell layer of dentate gyrus of rats: Plate 3A show CP-treated group (*low power*) compared with CP-treated group (*high power*) Plate 3B. Arrows point to portions of neuron loss in the crest (C) in the proximal part of the suprapyramidal blade (SPB) relative to the infrapyramidal blade (IPB) of granular cell layer.

Cornu ammonis3 (CA3) of hippocampus propria: Plates 4A and 4B show CA3 with normal pyramidal neurons with visible and distinct nucleoli. Cisplatin treated group (Plate 4C) shows disordered arrangement of pyramidal neurons and degenerated neurons (arrowheads). Plate 4D exhibits a good measure of recovery of the neurons as an effect of co-treatment of CP with SAEE.

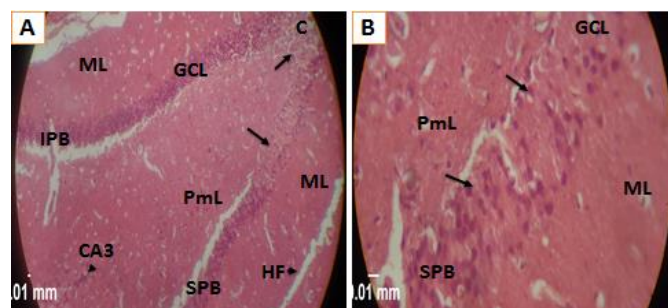


Plate 3: Representative stained sections of the blades of granule cell layer of dentate gyrus of rats: (A) Cisplatin-treated group (*low power*) (B) Cisplatin-treated group (*high power*). SAEE, *Sida acuta* ethanolic extract 200 mg/kg; Cisplatin, 7 mg/kg; ML, molecular layer; GCL, granular cell layer; PmL, polymorphic layer; CA4, cornu ammonis4; HF, hippocampal fissure. Arrows point to portions of neuron loss in the crest (C) and proximal part of suprapyramidal blade (SPB) of granular cell layer relative to the infrapyramidal blade (IPB). H&E. Calibration bar = 0.01mm (10 μ m) for both figures.

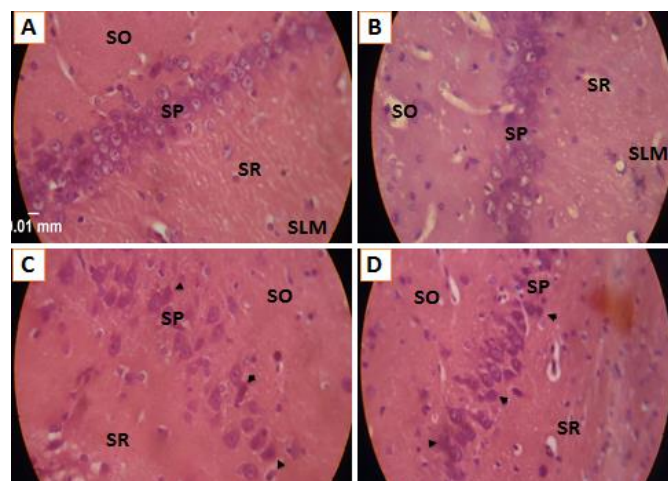


Plate 4: Representative stained sections of Cornu Ammonis3 of rats: (A) Control rats (B) SAEE-treated (C) Cisplatin-treated (D) Cisplatin+SAEE. *Sida acuta* ethanolic extract 200 mg/kg; Cisplatin, 7 g/kg mercuric chloride; SO, stratum oriens; SP, stratum pyramidalis; SR, stratum radiatum, SLM, stratum lacunosum-moleculare. Arrowheads indicate degenerated neurons. H&E. Scale bar, 0.01mm (10 μ m) for all figures.

Cerebellar cortex: Plates 5A, 5B and 5C show normal histological features of the cortex of adult cerebellum with large soma of Purkinje cells (arrowheads) exhibiting basophilia. The Purkinje cells are arranged typically in single

row at the junction of the molecular layer with the granular layer. The Purkinje neurons of cisplatin-treated groups show complete dissolution of the nucleus with eosinophilic cytoplasm (Plate 5C) which is evidence of karyolysis.

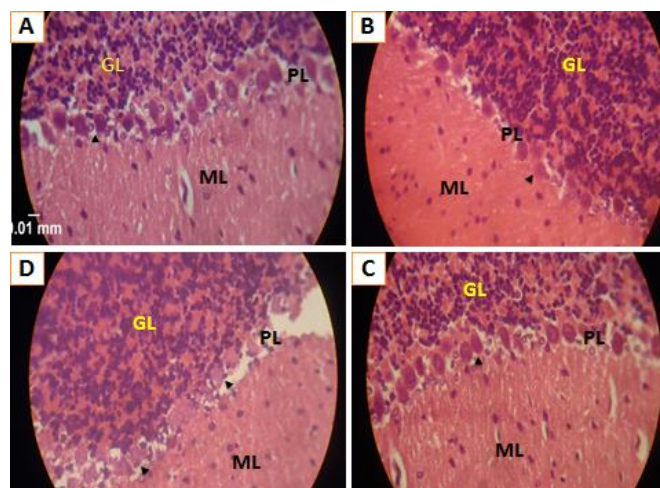


Plate 5: Representative stained sections of Cerebellar cortex of rats: (A) Control rats (B) SAEE-treated (C) Cisplatin-treated (D) Cisplatin+SAEE. *Sida acuta* ethanolic extract 200 mg/kg; Cisplatin, 7 g/kg mercuric chloride; ML, molecular layer; GL, granular layer; PL, Purkinje layer. Arrowheads indicate Purkinje neurons. H&E. Scale bar, 0.01mm (10 μ m) for all figures.

Histomorphometry

Table 3 shows that Cisplatin treatment elicited a significant ($p < 0.05$) reduction in the densities of the viable neurons of the cerebral cortex, pyramidal neurons of CA3 and the granule cells of the dentate gyrus relative to control. Concomitant treatment of CP with SAEE ameliorated the effect by elevating the densities of these neurons significantly ($p < 0.05$) when compared with CP group.

Table 3:

Effects of Cisplatin and SAEE treatment on the densities of viable neurons in cerebral cortex and hippocampal region

Groups	CCtx (no/nm ²)	CA 3 (no/nm ²)	DG (no/nm ²)
Control	3.35±0.62	7.26±0.81	18.86±1.69
SAEE	3.57±0.53	6.36±0.84	14.17±0.78
CP	0.84±0.32*	3.24±0.41*	10.23±0.93*
CP+SAEE	2.58±0.24**	5.25±0.42**	17.86±1.97**

Data presented as Mean \pm SEM of five rats per group. CCtx, cerebral cortex; DG, dentate gyrus; CA3, cornu ammonis3; EESA, *Sida acuta* ethanol extract 200 mg/kg; CP, Cisplatin, 7 mg/kg. * $P < 0.05$ versus Control, ** $P < 0.05$ versus CP

DISCUSSION

The present study showed that Cisplatin (CP) induced significant micro-anatomical alterations in the cerebral cortex (CC), dentate gyrus (DG), cornu ammonis3 (CA3) and cerebellar cortex of adult albino rats. *Sida acuta* ethanol extract given as pretreatment and concomitant treatment ameliorated the CP-induced changes as well as the behavioral changes.

It was observed that CP-induced alterations in rat brain ranged from loss of cerebral cortical neurons, loss of dentate granule cells in the dentate gyrus which was more marked in the crest and proximal part of the suprapyramidal blade of the granular cell layer, death and distortion of the layering of the

pyramidal neurons of the CA3 and karyolysis of Purkinje cells of the cerebellum. The report of CP's inability to penetrate the blood-brain barrier (Gregg et al., 1992) contradicted our findings that showed it actually caused brain damage. However, our observations CP caused brain toxicity agreed with earlier reports (Gulec et al., 2013; Owoeye et al., 2015). The association of CP-induced neurotoxicity with histological damage was reported by Al Moundhri et al. (2012) which this experiment has amplified. The report of induction of cell death in vivo and in vitro cell by CP in both the cerebellum and hippocampus (Bottone et al., 2012) agreed with cell death in different parts of the brain as presented. Neuronal death in the forebrain of CP-treated rats might affect decision making, coordination of cortical functions, as well as cognition. The widespread neuronal loss with vacuolated spaces and degenerated neurons we observed is in agreement with and similar to the report of Karavelioglu et al. (2015) and this might affect the tracts that originates like the corticospinal tract and their functions e.g poor control of fragmented distal digital movements.

In the CP-treated rats, the loss of granular neurons in the proximal portion of the suprapyramidal blade (inner blade) of the DG was more than the infrapyramidal blade (outer blade) and so also affected was the crest of dentate granule cell layer (DGCL) (Amaral et al., 2007; Hussein and George, 2009). The reason for the preference of the inner blade by CP was not quite obvious. Most of the surviving cells were pyknotic when compared with cells from other groups and this neuron degeneration agreed with previous report of CP damage of granule cells (Owoeye et al., 2015). The effect of extensive death of granule neurons will be the effect on other cells of the dentate gyrus e.g dentate pyramidal basket cell located along the interface between DGCL and the polymorphic layer (PoL), and the mossy cells domiciled in the PoL (Amaral et al., 2007). Since mossy fibres from granule cells project to CA3, mossy cells and the molecular layer, the effect of cell death will reduce the perforant pathway projection from layer II of entorhinal cortex. Potentially, the subgranular zone cells responsible for production during adult life of new granule cells might also be affected (Amaral et al., 2007). The overall effect of the massive granule cell death might be reduction of their role in hippocampal functions of declarative memory formation and storage (Stepan et al., 2015).

The observation of cellular death and distortion occasioned by CP treatment of the regular layering of pyramidal neurons of CA3, the dark pyramidal neurons, widening of intercellular spaces and scantiness of pyramidal neurons with dispersed chromatin are all evidences of the toxicity of cisplatin which are in agreement with our earlier findings (Owoeye et al., 2015; Owoeye and Onwuka, 2015). The report of Bottone et al. (2012) that CP induces cell death in the hippocampus in in agreement with our findings of cell death in both DG and CA3. Damage to CA3 pyramidal neurons might reduce the efficacy of the projection of mossy fibres from DGCL and the projections of CA3's Schaffer's collaterals to CA1. The death of pyramidal neurons might also reduce the incoming and outgoing information from them thus reducing the efficiency of formation and storage of episodic memory for which the hippocampus is associated (Amaral et al., 2007; Stepan et al., 2015; Alberini and Kandel, 2016).

Cisplatin toxicity of Purkinje cells (PCs) was the most severe as there was complete loss of the characteristic basophilia of their soma, which was replaced by eosinophilia, and karyolysis, an observation in agreement with earlier

reports of Owoeye and Onwuka (2015) and Bottone et al. (2012) who reported that CP induced cell death in the cerebellum. According to Ito (2002), PCs are crucial elements in the cerebellum that control motor functions, such as movement, motor learning, and body balance; in addition they are the sole output neurons in the cerebellar cortex, sending inhibitory signals to target neurons of the cerebellar nuclei. It will imply therefore, that the degeneration of PCs observed may result in spinocerebellar ataxias and other movement and posture impairments (Pisu et al., 2004; Kim et al., 2009). This will be due to the cutting off of excitatory projections from climbing, mossy and parallel fibres from the PCs. So also rendered ineffective from the PCs will be the inhibitory impulses from Golgi, stellate and basket cells (Ito, 2002). Therefore, PCs projection to cerebellar nuclei will be compromised and ineffective. The sum effect of the lesion observed in brain of CP-treated rats might therefore affect cerebellar modulated activities.

These histological observations agreed with earlier reports that CP-induced neurotoxicity is associated with histological damage (Arrieta et al., 2011; Owoeye et al., 2015). The damage might also explain the significant reduction of behavioural parameters and densities of viable neurons of cerebral cortex, dentate gyrus and cornu ammonis3. It is conceivable that reduction in neuronal density would affect the efficiency of these neurons. However, pretreatment and concomitant treatment of CP with SAEE provided neuroprotection from the micro-anatomical alterations observed in the cerebral cortex (CC), dentate gyrus (DG), cornu ammonis3 (CA3) and cerebellar cortex of the rat as demonstrated in the histology, behavioural studies report and the histomorphometry results. In effect, SAEE treatment will reduce and ameliorate the possible effect the CP treatment would have caused. The neuroprotective effect demonstrated by *Sida acuta* leaf extract in this experiment was probably due to its flavonoids content and antioxidant property as already demonstrated (Ekor et al., 2010; Bahar et al., 2013).

Of all the neurons studied in our experiment, the most severe effect of CP was on the PCs, hence the plan to further study these neurons by immunohistochemical analysis using antibodies to calbindin D28k, a specific PC marker in the cerebellum (Kim et al., 2009) which might provide more understanding of the pathophysiology of PCs disorder in this situation.

In conclusion, Cisplatin treatment induced significant degeneration and micro-anatomical alterations in the cerebral cortical neurons, granule cells of dentate gyrus, pyramidal neurons of cornu ammonis3 and Purkinje neurons of cerebellum of the adult male Wistar rats. However, *Sida acuta* ethanol extract has shown the capacity to protect these neurons from the cisplatin toxicity and may be further investigated for consideration as a potential neuroprotectant against cisplatin neuropathy.

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REFERENCES

Alberini, C.M., and E.R. Kandel. 2015. The Regulation of Transcription in Memory Consolidation. *Cold Spring Harb Perspect Biol* 2015; 7:a021741. Cold Spring Harbor Laboratory

- Press. Retrieved from <http://cshperspectives.cshlp.org/> on 14/01/2016.
- Ali, B.H., Ramkumar, A., Madanagopal, T.T., Waly, M.I., Tageldin, M., Al-abri, S., Fahim, M., Yasin, J. and A. Nemmar. 2014. Motor and behavioral changes in mice with Cisplatin-induced acute renal failure *Physiol. Res.* 63: 35-45.
- 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.
- Altun, Z.S., Gunes, D., Aktas, S., Erbayraktar, Z, and N. Olgun. 2010. Protective effects of acetyl-L-carnitine on cisplatin cytotoxicity and oxidative stress in neuroblastoma. *Neurochem. Res.* 35: 437-443.
- Amaral, D.G., Scharfman, H.E. and P. Lavenex. 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 J. Sotelo. 2011. Retinoic acid reduces chemotherapy induced neuropathy in an animal model and patients with lung cancer. *Neurology*, 77: 987-995.
- Ateşşahin, A, Karahan, I, Türk, G, Gür, S, Yılmaz, S, Çeribaş, AO. 2006. Protective role of lycopene on cisplatin-induced changes in sperm characteristics, testicular damage and oxidative stress in rats. *Reproductive Toxicol.* 21(1): 42-47.
- Bahar, E., Ara J., Alam M., Nath B., Bhowmik U., Runi N. 2013. In-vitro antioxidant and thrombolytic activity of methanol extract of *Sida acuta*. *Journal of Pharmacognosy and Phytochemistry.* 2(2): 125-133.
- Bancroft, J.D. and Gamble M. 2008. *Theory and Practice of Histology Techniques*, 6th edition. Churchill Livingstone Elsevier, Philadelphia; 83 - 134.
- Bansode, VJ, Vyawahare NS, Munjal NJ, PN, Amrutkar PS, Sontakke SR. 2014. Neuroprotective effect of ethyl pyruvate in vincristine and cisplatin induced neuropathic pain. *International Journal of Nutrition, Pharmacology, Neurological Diseases.* 4(4): 214-223.
- 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.
- Kim, B.J., Lee SY, Kim HW, Park E-J, Kim SJ, So I, Jeon J-H. 2009. Optimized Immunohistochemical Analysis of Cerebellar Purkinje Cells Using a Specific Biomarker, Calbindin D28k. *Korean J Physiol Pharmacol.* 13: 373-378.
- Cai, L., Yan, X.B., Chen, X.N., Meng, Q.Y. and Zhou, J.N. 2010. Chronic all-trans retinoic acid administration induced hyperactivity of HPA axis and behavioral changes in young rats. *Eur. Neuropsychopharmacol.* 20, 839-847.
- 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 AD, Tsuchida MA, Amodaj N, Pinkard H, Vale RD, and Stuurman N. 2014. Advanced methods of microscope control using µManager software. *Journal of Biological Methods*, 2014 1(2):e11 doi:10.14440/jbm.2014.36
- Ekor M., Odewabi A.O., Bakre A.G., Oritogun K.S., Ajayi T.E., Sanwo O.V. 2010. Comparative evaluation of the protective effect of the ethanolic and methanolic leaf extracts of *Sida acuta* against hyperglycaemia and alterations of biochemical and haematological indices in alloxan diabetic rats. *J. Pharma and Toxicol.* 5(1): 1-12.
- Gregg R.W., Molepo J.M., Monpetit V.J., Mikael N.Z., Redmond D., Gadia M, and Stewart D.J. 1992. Cisplatin neurotoxicity: the relationship between dosage, time, and platinum concentration in neurologic tissues, and morphologic evidence of toxicity. *Journal of Clinical Oncology*, 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. *Psychiatry and Clinical Neurosciences*, 67: 50-58
- Harmers F.P.T., Brakkee J.H., Cavalletti E., Tedeschi M., Marmonti L., Pezzoni G., Neijt J.P, and Gispen W.H. 1993. Reduced glutathione protects against cisplatin-induced neurotoxicity in rats, *Cancer Res*, 53: 544-549.
- Harborne J.B. 1973. *Phytochemical Methods: A guide to modern techniques of plant analysis*. Fakenham Press Ltd. Great Britain. 182-201.
- Hussein A.A.W. and George N.T. 2009. Histological and Morphological Analysis of the Hippocampal Subfields in the Adult Rat. *J Fac Med Baghdad.* 51(3): 323-327.
- Ibironke G.F and Fasanmade A.A. 2015. Analgesic and Central Nervous System Depressant Activities of Kolaviron (a Garcinia kola biflavonoid complex). *Afr. J. Biomed. Res.* 18: 217- 223.
- Ito M. 2002. Historical review of the significance of the Cerebellum and the role of Purkinje cells in motor learning. *Ann. N.Y. Acad. Sci.* 978: 273-288.
- Karavelioglu E, Boyaci MG, Simsek N, Sonmez MA, Koc R, Karademir M, Guven M, Eser E. 2015. Selenium protects cerebral cells by cisplatin induced neurotoxicity. *Acta Cirúrgica Brasileira.* 30(6): 394-400.
- McWhinney S.R., Goldberg R.M, and McLeod H.L. 2009. Platinum neurotoxicity pharmacogenetics. *Mol. Cancer Ther*, 8: 10-16.
- Olopade, F.E., Shokunbi, M.T. and Siren, A. 2012. The relationship between the ventricular dilatation, neuropathological and neurobehavioural changes in hydrocephalic rats. *Fluids and Barriers of the CNS*, 9:19
- 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. *Archives of Basic and Applied Medicine* 3:71-78.
- Özyurt B, Güleç M, Özyurt H, Ekic F, Atış O, Akbas A. 2006. The effect of antioxidant caffeic acid phenethyl ester (CAPE) on some enzyme activities in cisplatin-induced neurotoxicity in rats. *Eur J Gen Med*, 3(4):167-172
- Pisu MB, Roda E, Avella D, Bernocchi G. 2004. Developmental plasticity of rat cerebellar cortex after cisplatin injury: inhibitory synapses and differentiating Purkinje neurons. *Neuroscience*, 129: 655-664.
- 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 PD, Lopez-Hernandez FJ, Lopez-Novoa JM, Morales AI. 2011. An integrative view of the pathophysiological events leading to cisplatin nephrotoxicity. *Crit Rev Toxicol* 41: 803-821.
- Snell R.S. 2006. *Clinical Neuroanatomy 6th Edition*. Lippincott Williams and Wilkins. Pp. 219-364.
- Stepan J., Dine J., 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