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Cimetidine ameliorated the effect of Cisplatin treatment on brain of Wistar rats

Owoeye O and Olaoye, HO
Department of Anatomy, College of Medicine, University of Ibadan, Ibadan, Nigeria.

Corresponding Author: Owoeye O
E-mail: o.owoeye@mail.ui.edu.ng; oowoeye2001@yahoo.com
+234-8033239973; Fax: 234-2-8103043

ABSTRACT
Cisplatin (CIS) is an anti-neoplastic drug used for chemotherapy of solid organ malignancies. Unfortunately, it activates processes that induce oxidative damage in the brain (neurotoxicity). Cimetidine (CTD) is a Histamine$_2$ receptor blocker reported to exhibit antioxidative function. This study investigated the effect of CTD on CIS neurotoxicity in rat. The study was carried out on 40 male Wistar rats randomized into four groups; A, B, C and D, (n=10) and treated as follows: Group-A rats (control) were injected intraperitoneally (IP) with 0.5 mL/kg saline once daily for 14 days, Group-B rats were IP administered 100 mg/kg CTD once daily for 14 days, Group-C rats received 7.5 mg/kg Cisplatin IP on day 7, Group-D rats were IP administered 100 mg/kg CTD for 14 days and CIS (7.5 mg/kg) on day 7. Behavioural tests were performed on the experimental rats from day 15 to 17 post-treatment after which they were sacrificed under anaesthesia (Ketamine hydrochloride 100 mg/kg). Brain sections were processed into paraffin blocks and H&E stained. Results showed significant weight loss in CIS only group while CTD treatment reduced weight loss significantly ($p<0.05$) in CTD+CIS group. Behavioural study showed that cimetidine treatment in animals of CTD+CIS group enhanced exploratory ability of the rats in the group, while CIS only group animals exhibited impaired exploratory ability with significantly lower discrimination index. Histological sections of the cerebellum and hippocampus of CIS group of animals showed multiple alterations in their cytoarchitecture, whereas sections from CTD+CIS treated animals appeared normal. In conclusion, these findings suggest that cimetidine exhibited ameliorative effect against cisplatin-induced behavioural deficits and cytoarchitectural distortions of the brain cells of rats.

Keywords: Cisplatin, Cimetidine, Neurotoxicity, Rats.

INTRODUCTION
Studies have reported that chemotherapy is the treatment of choice for most cases of cancer[1]. Some reports listed associated side effects of chemotherapy to include neurotoxicity[2,3,4,5,6]. Reported neurotoxic drugs include cisplatin, vincristine, vinblastine, vinorelbine, oxaliplatin, cytarabine, ifosfamide, 5-fluorouracil and methotrexate among others[7,8]. The major toxic effects associated with a high-dose cisplatin regimen[9] include peripheral neurotoxicity, myelosuppression, ototoxicity and nephrotoxicity, The toxicity of cisplatin [cis-diamminedichloroplatinum-II, molecular formula: $Pt(NH_3)2Cl_2$] has various pathophysiological mechanisms proposed to explain it, one of which is that cisplatin kills malignant cells and peripheral neurons by means of the mechanism of apoptosis[10]. Although the onset of toxicity is reported to be delayed until a cumulative dose higher than 300 mg/m^2 has been given[11], single doses of cisplatin in laboratory animals have been reported to cause neural damage affecting central nervous structures like the cerebellum, hippocampus and cerebral cortex via oxidative mechanism[12,13]. The cisplatin toxicity has also been linked to the induction of generation of free radicals and increased production of reactive oxygen species (ROS) which then results in oxidative stress and its consequences[14,15]. Others have reported that cisplatin and other platinum drugs undergo aquation (hydrolysis), which is important for the drug to form a complex with the target DNA. This leads to the formation of positively charged molecules (free radicals) that then cross link with DNA, forming the DNA/platinum adducts[16,3]. Although the cellular damage is believed to be due to the formation of these DNA adducts, another complex composed of platinum-DNA-protein crosslinks (DPCLs) has been proposed as a mechanism for the platinum antitumor activities, and studied specifically with cisplatin[17]. When DNA covalently links with protein, the resulting DPCLs complexes are able to disrupt nuclear metabolism and spatial organization of chromatin, and also inhibit DNA replication and repair[18]. Histamine is a biogenic amine that plays important roles in various physiological, biochemical and behavioral functions [19,20,21,22]. Histamine is a diamine that has four different receptors namely H_1, H_2, H_3 and H_4, all of which are G-protein coupled[23]. While H_1 receptors mediate its pro-inflammatory, allergic inflammation and vascular

activities, H_2 receptors mediate the release of gastric acid, H_3 receptors mediate effects on brain and nerve endings and feedback inhibition for histamine and other neurotransmitters, while the H_4 receptors are involved in immunomodulation involving leukocyte chemotaxis[24,23,25].

Cimetidine (as Cimetidine hydrochloride, molecular formular $C_{10}H_{17}ClN_6S$ with molecular weight 288.80) is a member of the class of guanidines. It is a known potent histamine H_2 receptor antagonist for inhibiting excessive histamine-induced gastric acid secretion and is used worldwide to treat peptic ulcers and gastric aspiration syndrome[26,24]. The antioxidative property of cimetidine was reported by Lambat et al[26] who in their study assessed the levels of free radicals and demonstrated that cimetidine reduced the generation of superoxide anion formed in a nitroblue-tetrazolium assay and also reduced the iron-induced rise in lipid peroxidation in rat brain homogenates.

Neurotoxicity continues to be a burdensome side effect of platinum-based chemotherapy which prevents administration of the full dosage and sometimes leads to treatment withdrawal[27,28,29]. The different functions of the brain include the processing of incoming sensory information, motor coordination and cognition by the cerebral cortex; memory formation and storage in the hippocampus; and maintenance of balance and equilibrium by the cerebellum. Any or all of these functions might be affected in cisplatin neurotoxicity.

However, information on the probable protective effect of cimetidine on cisplatin-induced neurotoxicity in rats is scanty. Since literature has demonstrated that antioxidative agents can reduce or inhibit cisplatin-induced toxicity[30,31,12], it is therefore plausible to hypothesize that based on the reported antioxidative potential of cimetidine, Lambat et al[26], it might protect rat brain from the toxic effects of cisplatin when co-administered with it. The present study aimed to investigate the potential of cimetidine to exert preventive effects on cisplatin-induced toxicity on rat brain and so answer the research question of whether cimetidine can ameliorate the toxic effect of cisplatin in the brain of rats when both are co-administered.

MATERIALS AND METHODS

A total of forty (40) adult male Wistar rats weighing between 150-200 g were obtained from the Central Animal House, University of Ibadan, Ibadan. The animals were housed in clean transparent plastic cages (39 x 29 x 27 cm) with wood shavings as bedding (five rats per cage), fed with rat feed (Ladokun Feeds, Ibadan, Nigeria) and water *ad libitum*. The rats were acclimatized for one week prior to the onset of the experiment and weighed prior to, during and at the end of the experiment. Animals were humanely handled according to the acceptable guidelines on the ethical use of animals in research[32].

Drugs: Cisplatin (Korea United Pharm. Inc. Naojang, Chungnam, Korea), Cimetidine (GlaxoSmithKlein, Cork, Ireland) and Ketamine hydrochloride (RotexMedica, Trittau, Germany).

Research Design: The 40 Wistar rats were randomized into four groups (N=10):
Group-A (control group) were given 0.5 mL/kg saline (i.p.) daily for 14 days.
Group-B (cimetidine group) administered 100 mg/kg cimetidine (i.p.) once daily for 14 days.
Group-C (cisplatin group) administered 7.5 mg/kg cisplatin (i.p.) on day 7.
Group-D (cimetidine and cisplatin group) administered 100 mg/kg cimetidine (i.p.) for 14 days while cisplatin (7.5 mg/kg i.p.) was given on day 7.
The dosage and route of administration of the drugs were based on published literature (Cisplatin: 7.5 mg/kg body weight[33,34]; Cimetidine: 100 mg/kg body weight[35,36]).

Morphological study: Percentage change in rat body weight and brain to body weight index were calculated using the standard formulae.

Neurobehavioural study: The rats were made to undergo the open field behavioural tests on the morning of the 15th day while the novel object recognition and object location test followed from the 15th to the 17th day of the experiment:

Open field tests: An open field test was carried out in a large cubic box, of about 1m long ×1m wide x 1m high[37] where the top was typically left uncovered. A rat was placed in the middle of the bottom surface, and its movements were recorded over the course of five minutes as it moved around and explored its environment. The floor of the box was cleaned with 70% ethyl alcohol and permitted to dry between tests to eliminate olfactory bias in-between one rat and another. The following parameters were measured: center square duration, center square entry, line crossing, grooming and rearing[38].

Novel object recognition: The novel object recognition paradigm assesses an animal's innate ability to distinguish an old from a new object. Rodents are naturally curious and will take time to explore novel objects. They were subjected to the procedures of habituation, training and testing. The test phase was preceded by habituation and training phases during which the rats were exposed to the test medium (an open field box). This ensured the rats got used to the medium and also reduced stress and avoided a potential neophobic response. Habituation was done for 2 minutes. A day after the rats were trained by exposing them to two objects of the same size and color for 3minutes each for 3 days, the rats were then tested for recognition by replacing one of the objects with a novel object (a new object entirely different from the previous

object in shape and colour) and the rats were then exposed for 5minutes. When presented with both the new and the previously explored objects, rodents remember the old object and spend relatively more time exploring the new object[37]. The time spent exploring the old object and the novel object was recorded and discrimination index was calculated using the formula below:

$$\text{Discrimination Index (DI)} = \frac{\text{Time spent with novel object}}{\text{Total time spent exploring the two objects}} \times 100$$

Object location test (OLT) procedure: This test comprised three successive trials with an inter-trial interval of 60 minutes. The first trial comprised placing the rat in the center of an empty open field box 1m long×1m wide×1m high[37] and allowing the rat to freely explore the box for 5 minutes (i.e., the habituation phase). The second trial commenced 60 minutes after the first trial and involved placing the rat in the center of the same open field box having two identical objects on opposite sides of the box and allowing the rat to freely explore the objects for 5 minutes. Following an inter-trial interval of 60 minutes, third trial was performed for 5 minutes through placement of the rat in the center of the same open field box with one of the objects remaining in the same location as in trial 2 and the second object moved to a new location in the open field box (i.e., the testing phase of OLT). A rat is considered to be exploring an object when its nose is within 2 cm of the object. The movement of rat in the third trial (test phase) was continuously recorded. The apparatus was cleaned with methylated spirit prior to the commencement of each trial for every rat. Data such as times spent in exploring the object moved to a novel place, the object remaining in the familiar place, and total time spent in the object exploration were measured. Furthermore, the place discrimination index was calculated by using the formula:

$$\text{Discrimination Index (DI)} = \frac{\text{Time spent with novel location}}{\text{Total time spent exploring the two locations}} \times 100$$

Then, the percentages of object exploration time spent with the object moved to a novel place and the object remaining in the familiar place were compared within each group. The novel place discrimination index was also directly compared across the group.

Histological studies: After the completion of the behavioral tests on day 17 of the experiment, the animals were sacrificed using Ketamine hydrochloride (100 mg/kg) followed by endocardial perfusion after which the brains were harvested and preserved for histological study by applying the Avwioro[39] procedure/technique. Tissue processing was done by subjecting the fixed brain tissue to grossing, dehydration and embedding. Paraffin blocks were thereafter sectioned at 6 μm thickness with a Rotary Microtome (Leica RM2125RTS, Germany). The ribbons were floated, dried and the slides stained using Haematoxylin and Eosin (H&E) technique according to the method of Bancroft and Gamble[40] to demonstrate general histology of the brain and possible microscopic alterations.

Histomorphometric Study: Histological images were obtained from the slides using Leica DM 500 digital light microscope (Germany) and the images captured with Leica ICC50 E digital camera (Germany). Histomorphometric analyses were done using Image motic 2000 (China) and the Pyknotic index was then calculated using the formula according to Taveira et al[41].

Data analysis: Histomorphometric analyses were done using Image motic 2000 (China) and the Pyknotic index was calculated using the formula according Taveira et al[41]:

Pyknotic Index = Number of pyknotic cells / Total number of cells/sq. mm.

Statistical analysis: Data derived from various analyses were presented as mean ± SD and analysed using one-way ANOVA (Analysis of Variance) test followed by Tukey Post hoc for multiple comparison. All analyses were carried out using GraphPad® prism version 5.0 windows (GraphPad® software, SanDiego, Califonia USA). The statistical significance was set at $p \leq 0.05$.

RESULTS

General observations and effects of treatment on the body weight and relative brain weight of rats. Rats in the CIS and CTD+CIS groups experienced loss of appetite, watery stool, loss of weight and reduced motor activities following administration of CIS on day 7 of the experiment. This was more pronounced in the CIS group as the animals in CTD+CIS group regained appetite progressively before the end of the study. There was significant ($p<0.05$) reduction in body weight of animals in the CTD+CIS and CIS groups at the end of the experiment relative to control (Figure 1). Brain-body weight index significantly reduced only in the animals in CIS group relative to the control (Figure 2).

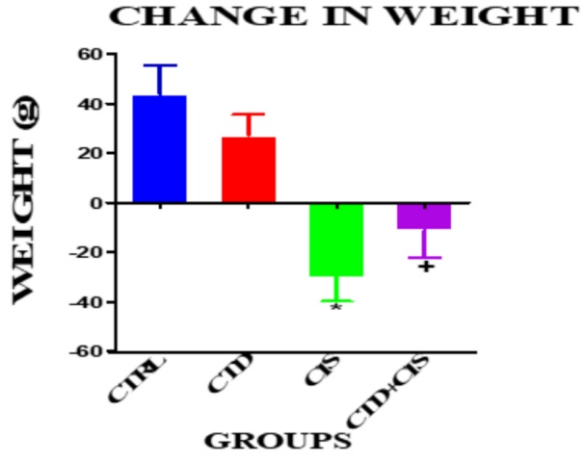


Figure 1: Effect of CTD on the body weight changes in rats treated with CIS**.** CTRL- Control, CTD- Cimetidine only, CIS- Cisplatin only. Values are presented as Mean ± SD (n= 10). **P<0.05 versus CTRL and CTD, +P<0.05 versus CTDL and CIS.*

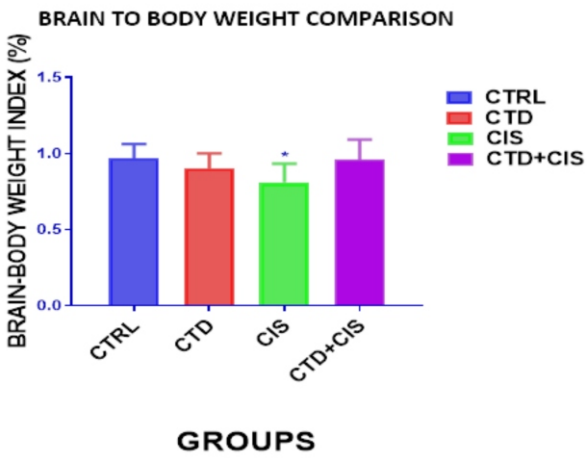
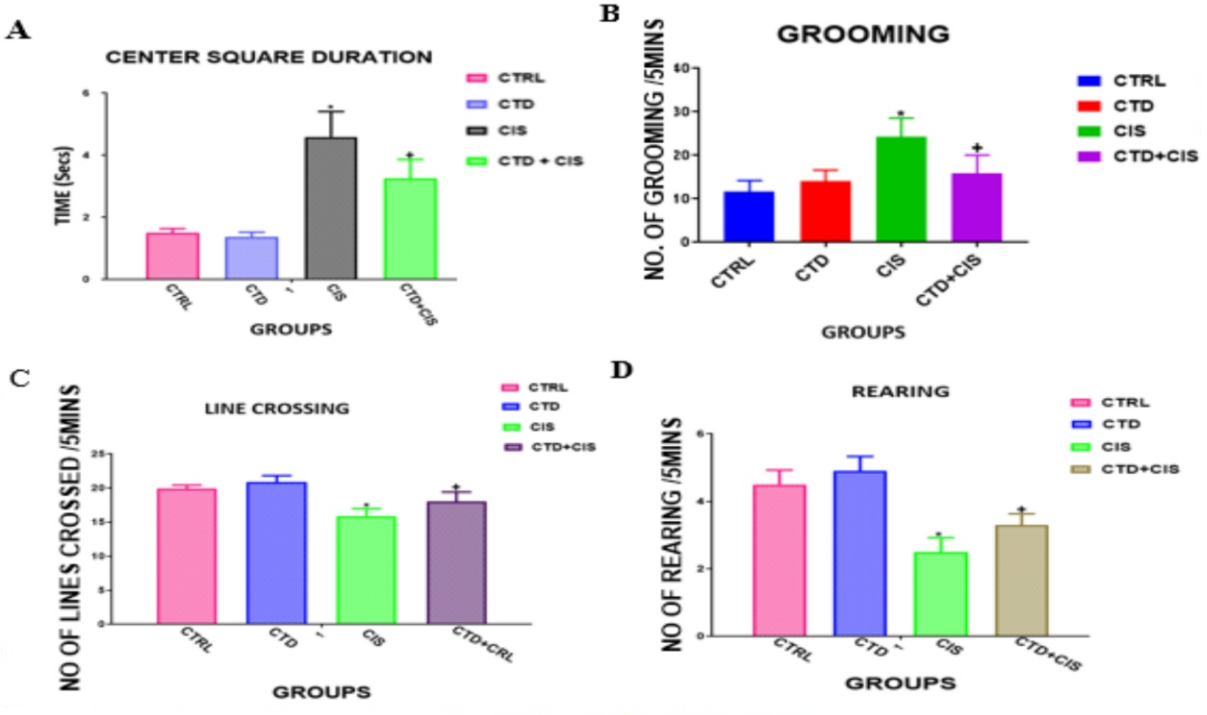


Figure 2: Relative brain weight change across the groups. CTRL- Control, CTD- Cimetidine only, CIS- Cisplatin only. Values are presented as Mean ± SD (n= 10). **P<0.05 versus CTRL.*

Effects of CTD on behavioural tests in rats treated with CIS*:* The center square duration and grooming increased significantly ($p<0.05$) in the CIS group when compared with the CTRL as shown in Figures 3A and B, suggesting some form of anxiety in the CIS-treated rats. A significant ($p<0.05$) reduction was observed in numbers of lines crossed and rearing when compared with control (Figure 3C and D) which suggested depression or sedation in the rats treated with CIS which co-treatment with CTD ameliorated in both cases.



Effects of CTD on memory tests in rats treated with CIS: Both the object location and object recognition tests were significantly ($p<0.05$) reduced in the CIS group when compared with CTRL and CTD groups which suggests an impairment of the memory of these rats. However, the combination of CIS and CTD ameliorated this effect when compared with the CIS group suggesting an improvement in the memory of rats in this latter group of rats as shown in Figure 4A and B.

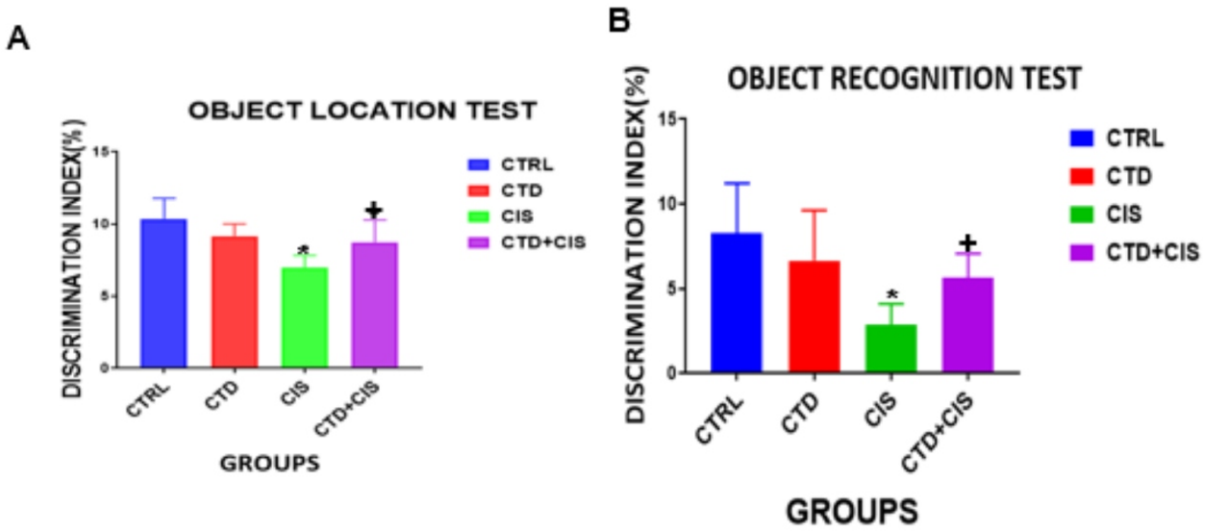


Figure 4: Effects of CTD on memory tests in rats treated with CIS. CTRL- Control, CTD- Cimetidine only, and CIS- Cisplatin only. Values are presented as Mean ± SD (n= 10). * *P<0.05 versus CTRL; + P<0.05 versus CIS.*

Effects of CTD on the histology of the cerebral cortex and cerebellar cortex in rats treated with CIS: The histology of the cerebral cortex shows normal cortical neurons in Figures 5 A, B and D, while those in C are shrunken and pyknotic. Similarly, the cerebellar cortex shows large and basophilic Purkinje cells (Figs 6 A, B and D) whereas those of the CIS group (Fig 6C) are shrunken (green arrows) while some exhibited features of karyolysis (black arrows) when compared with the CTRL group.

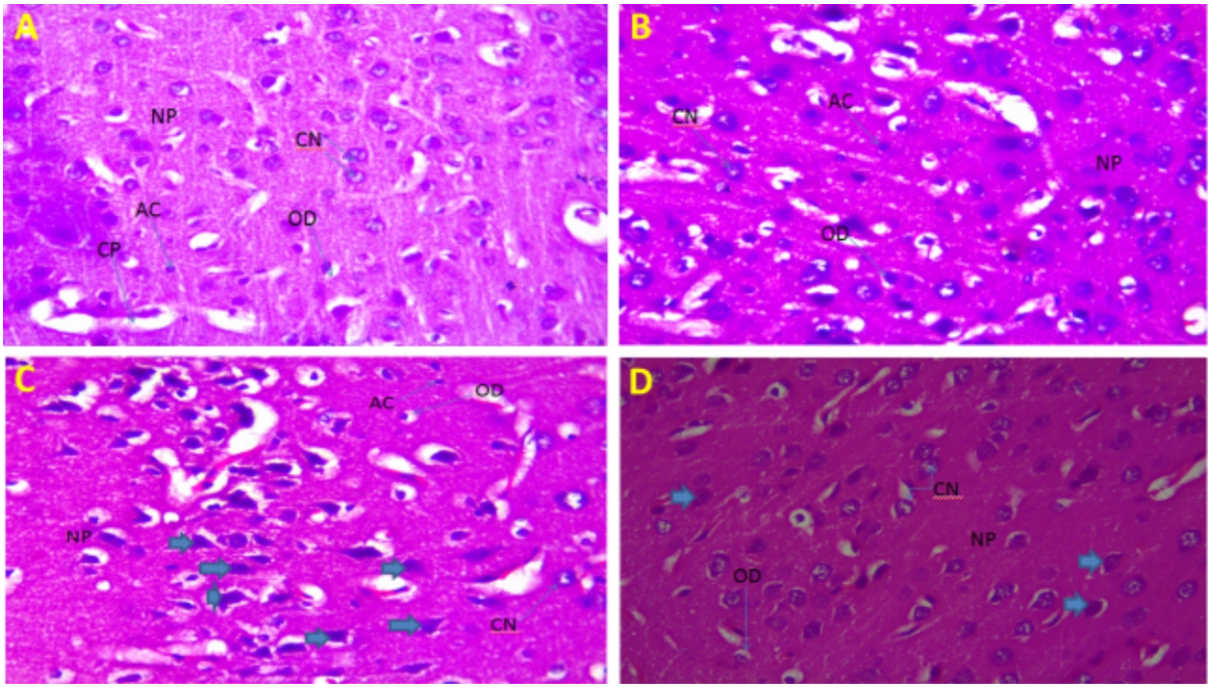


Figure 5: Representative stained sections of the cerebral cortex of rats. (A) CTRL, (B) CTD, (C) CIS, (D) CTD + CIS. Pyknotic cerebral neurons are indicated with blue arrows in the group CIS cells. CTRL- Control, CTD- Cimetidine only, CIS- Cisplatin only, NP- Neuropil, CN- Cortical neuron, OD- Olidodendrocyte, CP- Capillary, AC- Astrocyte. H&E stain, X 400.

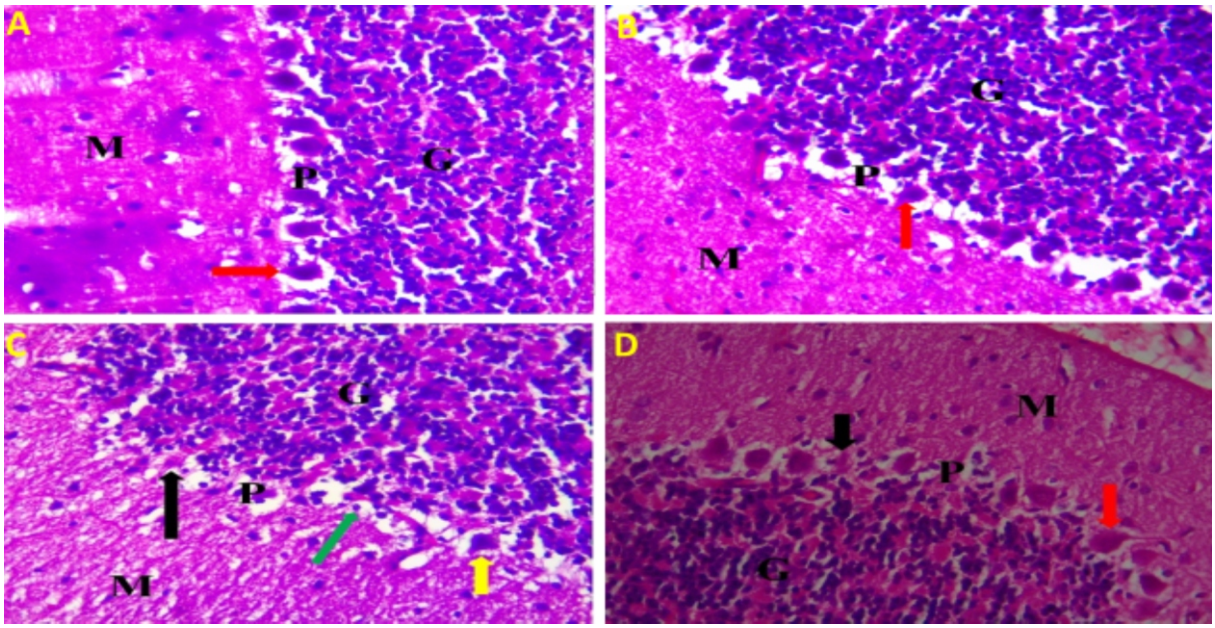


Figure 6: Representative stained sections of the cerebellum of rats. (A) CTRL, (B) CTD, (C) CIS, (D) CTD + CIS. Purkinje cells are large, normal and deeply basophilic in Figs 6 A, B and D (red arrows). The Purkinje cells in Fig 6C were observed to be shrunken (yellow arrow) with some showing features of karyolysis (green arrow) and some oesinophilic (black arrow). CTRL- Control, CTD- Cimetidine only, CIS- Cisplatin only. M- Molecular Layer, P- Purkinje Layer, G- Granular Layer. H&E stain, X 400.

Effects of CTD on the histology of the hippocampal formation in rats treated with CIS: The histology of the dentate gyrus was unaltered in all the groups as the granule neurons in all the layers show similar histological features (Figs 7). However, in both the CAI and CA3 the pyramidal neurons in the CIS group were shrunken and pyknotic (Figs 8C and 9C) when compared with the larger rounded pyramidal neurons with vesicular nuclei and some of which exhibit nucleoli in the other groups (Figs 8 A, B and D; Figs 9A, B and D) respectively.

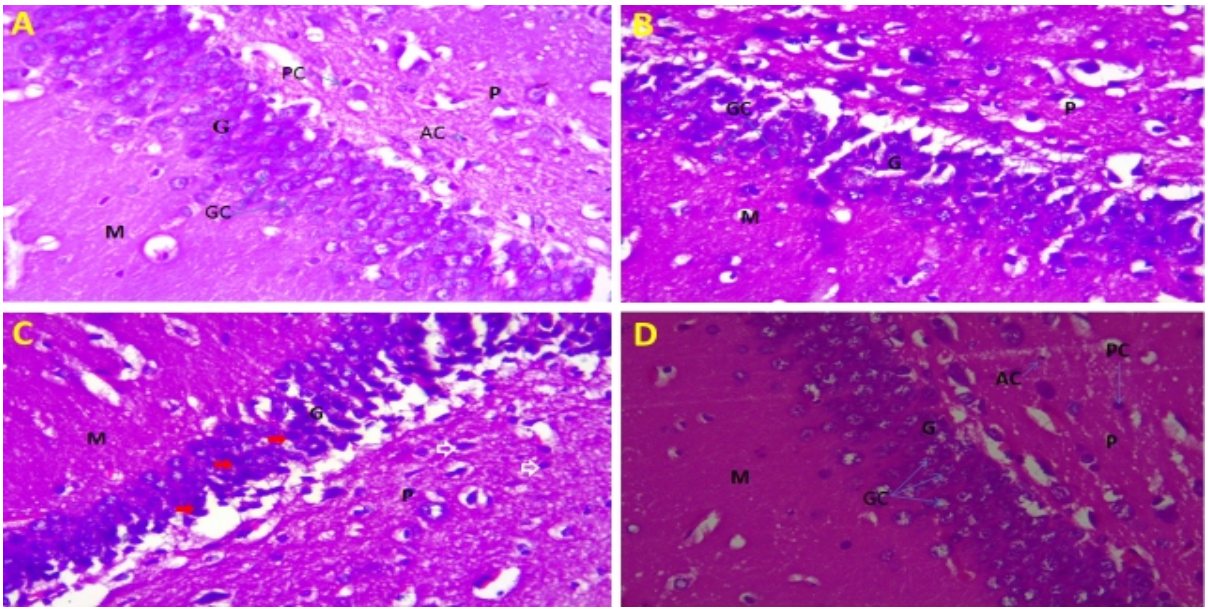


Figure 7: Representative stained sections of the dentate gyrus of hippocampal formation of rats. (A) CTRL, (B) CTD, (C) CIS, (D) CTD + CIS. There were no histological differences noted in the granule neurons in the treated groups when compared with control. CTRL- Control, CTD- Cimetidine only, CIS- Cisplatin only; M- Molecular Layer, GC- Granular Cell, G- Granular Layer, P- Polymorphic layer, AC- Astrocyte. H & E stain, X 400.

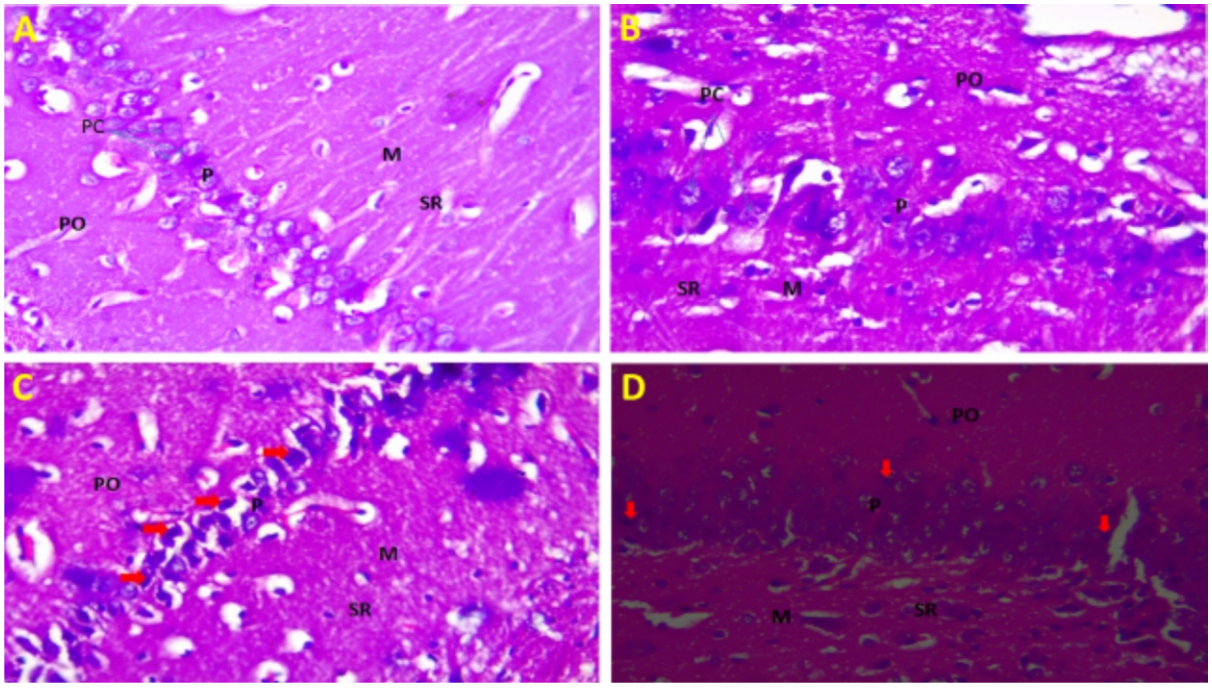


Figure 8: Representative stained sections of the Cornu ammonis 1 (CA1) field of hippocampal formation of rats. (A) CTRL, (B) CTD, (C) CIS, (D) CTD + CIS. The Pyramidal neurons in Figs 8 A, B and D appear normal whereas those in Fig 1C were shrunken with some showing features of pyknosis. CTRL- Control, CTD- Cimetidine only, CIS- Cisplatin only. PO- Stratum oriens, PC- Pyramidal Cell, SR- Stratum Radiatum, M- Lacunosum-Moleculare Layer. H & E stain, X 400.

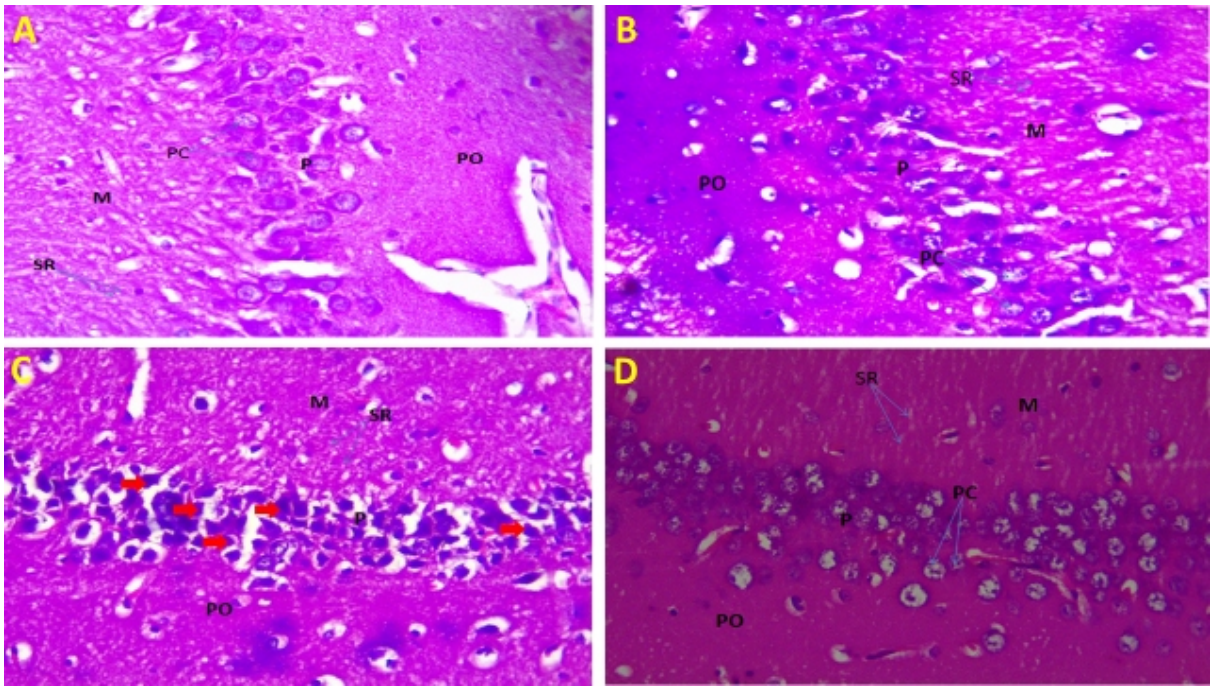


Figure 9: Representative stained sections of the Cornu ammonis3 (CA3) of hippocampal formation of rats. (A) CTRL, (B) CTD, (C) CIS, (D) CTD + CIS. The Pyramidal neurons in Figs 9 A, B and D appear normal whereas those in Fig 1C were shrunken with some showing features of pyknosis indicated by red arrows. CTRL- Control, CTD- Cimetidine only, CIS- Cisplatin only, PO- Stratum oriens, PC- Pyramidal Cell, SR- Stratum Radiatum, M- Lacunosum-Moleculare Layer. H & E stain, X 400.

Effect of CTD on the pyknotic indices in rats treated with CIS: The pyknotic index indicated the quotient of dividing number of pyknotic cells by the total number of cells/sq mm. The value reflects the number of dead neurons in population of total neurons counted. Results show that the index was significantly higher ($p<0.05$) in the CIS only group when compared with control. Treatment with CTD+CIS showed a reduction in the pyknotic index when compared with CISA as shown in Figure 10.

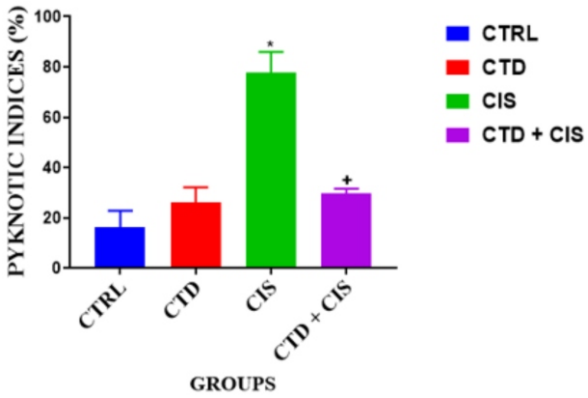


Figure 10: The pyknotic indices on CTD in rats treated with CIS. CTRL- Control, CTD- Cimetidine only, and CIS- Cisplatin only. Values are presented as Mean ± SD (n= 10). ** P<0.05 versus CTRL; + P<0.05 versus CIS.*

DISCUSSION

The administration of cisplatin (CIS) on day 7 of the experiment elicited anorexia and diarrhoea in rats in CIS and CTD+CIS groups, a finding supported by the report of Hattori *et al*[42]. The passing of watery stool observed may be considered as chemotherapy-induced diarrhoea which is also reported in patients treated with CIS where the incidence could be up to 50-80%[43].

Cisplatin has also been reported to impair fluid and electrolyte absorption in rat small intestine[44], which may have resulted in weight loss in CIS and CTD+CIS groups, in addition to the anorexia and diarrhoea[42,45].

However, food intake by rats in CTD+CIS group increased as the study continued, which is in agreement with previous report[46]. Literature has shown CIS treatment being associated with weight loss due to alteration in lipid metabolism which causes lipodystrophy commonly associated with cachexia[47], this may alter brain weight as it is majorly composed of lipid. Prevention of brain weight loss in the CTD+CIS group may be due to restorative effect of cimetidine on lipid peroxidation[48] suggesting that cimetidine as co-treatment might have prevented brain weight loss in the CTD+CIS group.

The open field test is used to evaluate the emotional and locomotor activity of an animal[49]. Rats in both CIS and CTD+CIS groups crossed fewer number of lines (used to assess horizontal movement) than the control group, although CTD+CIS group crossed more lines than the CIS suggesting central nervous sedation since the number of lines crossed is a behavioural index for locomotory activity[50]. Rearing (vertical movement) frequency of animals in the CIS and CTD+CIS group was significantly lower than those of the other groups, which possibly was due to central nervous sedation. The sedation or weakness might have resulted from sedation or depression due to or possible electrolyte loss that might have accompanied fluid loss referred to above. The result of significantly higher grooming activity in animals in CIS group when compared with animals in control group is an indication of anxiety[49].

The result of object location and recognition test, which is an assessment for spatial and recognition memory[37] showed that cisplatin caused significant impairment in spatial and recognition memory as the animals in CIS group spent less time exploring novel location and novel object, hence lower discrimination indices, an observation supported by the findings of Elbatsh[51].

Interestingly, cimetidine co-treatment increased exploratory behaviour of animals in CTD+CIS group, thereby preventing reduction of the indices, suggesting the ameliorative effect of cimetidine on cisplatin-induced recognition memory impairment.

Photomicrographs of rat brain revealed more neuronal degeneration in the cerebral cortices of animals in CIS group, while few neurons degenerated in the those of rats in CTD+CIS group, suggesting that cimetidine treatment ameliorated cisplatin induced neuronal cell death in the cerebral cortices of CTD+CIS group animals. The pyknosis observed in the granular cells of the dentate gyrus of CIS treated animals were not evident in photomicrographs of the animals in CTD+CIS as these appeared normal. Similarly, pyknosis of the pyramidal cells was also observed at the hippocampal regions CA3 and CA1 in the CIS group suggesting brain cell death the consequence of which is

reduction in short term memory storage. This finding conforms to published reports of Adrienne et al[52], Lomeli et al[53] and Owoeye et al[12]. However, cimetidine treatment appeared to improve the alterations caused by CIS on pyramidal neurons in CTD+CIS group as exemplified by the normal neurons observed in the hippocampus of CA1 and CA3 areas (figure 8D and 9D). Similarly, the pyknotic index from the histomorphometry of the pyramidal cells of the hippocampal region of the animals was higher for the CIS group when compared with the control and CTD+CIS groups suggesting some form of protection by cimetidine from cisplatin toxicity. Comparatively, cimetidine even at 3 mg/kg and up to 18 mg/kg was toxic to transgenic leukaemia infested mice[54] unlike healthy experimental Wistar rats.

The multiple forms of insult ranging from pyknosis, degeneration, karyolysis and irregularly shaped Purkinje cells observed in the photomicrographs of the cerebellar cortices of the CIS group animals indicated cisplatin injury. This observation agrees with the study of Abeer et al[55], Owoeye et al[12] and Imosemi et al[56] where they reported alterations in the microanatomy of the Purkinje cells of rats treated with cisplatin. Evidently, as seen on figure 6D, cimetidine co-treatment in CTD+CIS group reduced the insults on Purkinje cells compared to CIS group. The observed alterations in the Purkinje cells in animals of the CIS group was likely part of the reasons for the fewer number of lines crossed by animals in the group since cisplatin causes motor in-coordination by affecting the growth and morphology of Purkinje and granule cells[57]. The implication of death of cerebral cortical and Purkinje neurons in the CIS animals is possible perturbations in the coordinating functions of the cortex and cerebellar involvement in motor activity since cortical neurons in conjunction with cerebellum, thalamus and basal ganglia control motor functions.

The observed effects of cisplatin as demonstrated histologically on the different parts of the brain implies that the functions of the cerebral cortex, the hippocampus and the cerebellum might be affected[4,5]. However, the histological amelioration of these micro-anatomical alterations by cimetidine as demonstrated in animals of the CTD+CIS group suggested that the perturbations would be minimized while the functions of such parts might be restored to near normalcy.

CONCLUSION

In conclusion, cimetidine exhibited ameliorative effect against body weight loss, behavioural abnormalities and neuronal damage by protecting the integrity of the cytoarchitecture of rat brain components against cisplatin-induced insults.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, funding and/or publication of this article.

REFERENCES

1. Butters, D.J., Ghersi, D., Wilcken, N., Kirk, S.J. and Mallon, T. Addition of drug/s to a chemotherapy regimen for metastatic breast cancer. *Cochrane Database Syst Rev. In press.* 2010.
2. McWhinney, S.R., R.M. Goldberg and H.L. McLeod. Platinum neurotoxicity pharmacogenetics. *Mol. Cancer Ther,*2009; 8: 10–16.
3. Bottone, M.G., Santin, Piccolin, G., V.M., Bo,V.D. and Bernocchi, G. Cisplatinneurotoxicity 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. 2012; 123-140.
4. Gulec M., E. Oral, O.B. Dursun, A. Yucel, A. Hacimuftuoglu, F. Akcay, and H. Suleyman. Mirtazapine protects against cisplatin-induced oxidative stress and DNA damage in the rat brain. *Psychiatry and Clinical Neurosciences*.2013; 67: 50–58.
5. Turan, M.I., Turan,I.S., Mammadov, R., Altinkaynak, K. and Kisaoglu, A. The Effect of Thiamine and Thiamine Pyrophosphate on Oxidative Liver Damage Induced in Rats with Cisplatin. *BioMed Research International.*2013; 783809: 1-6
6. Karavelioglu, E., Mehmet,G.B., Nejdet, S., Mehmet,A.S.,Rabia,K.V. et. al. Selenium protects cerebral cells by cisplatin induced neurotoxicity. *ActaCirúrgicaBrasileira*. 2015;30 (6)
7. DeVita, V.T. Jr, Hellman, S. and Rosenberg, S.A. Principles and practice of oncology. Lippincott Williams & Wilkins. *Philadelphia*. 2001; 2964-76
8. Altun, Z.S., D. Gunes, S. Aktas, Z. Erbayraktar, and Olgun, N. 2010. Protective effects of acetyl L-carnitine on cisplatin cytotoxicity and oxidative stress in neuroblastoma. Neurochem. Res, 35:437–443.
9. Jessica P., Cyril, D., Govender, M.P. and Sewram,V. Cisplatin-Associated Ototoxicity: A Review for the Health Professional. *Journal of Toxicology*. 2016:13.
10. Gill, J. S. and Windebank, A. Cisplatin-induced apoptosis in rat dorsal root ganglion neurons is associated with attempted entry into the cell cycle. *Journal of Clinical Investigation*.1998; 101(12): 2842–2850.
11. Cersosimo, R. J. Cisplatin neurotoxicity. *Cancer Treatment Reviews*. 1989; 16 (4):195- 211.
12. Owoeye, O., Akinlosotu, O.M. and S.A. Adejuwon. Launaeataraxacifolia aqueous extract attenuates cisplatin-induced neurotoxicity by decreasing oxidative stress and neuronal cell death in rats. *Archives of Basic and Applied Medicine.* 2015; 3: 71-78.
13. Owoeye, O. and S.K. Onwuka. Tomato pomace powder ameliorated cisplatin-induced microanatomical alterations in brain of Wistar rats. *International Journal of Biological and Chemical*

*Sciences.*2015; 9 (1):1-11.
14. Valko, M., Rhodes, C.J., Moncol, J., Izakovic, M. and Mazur, M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *ChemBiol Interact.*2006; 160(1):1-40.
15. Almutairi, M.M., AlanaziW.A., Musaad, A.A., Alotaibi,M.R. and Ali, R.A. Neuroprotective effect of rutin against Cisplatin-induced neurotoxic rat model. *BMCComplementary and Alternative Medicine.* 2017; 17:472
16. Zhu, C., Raber, J. and L.A. Eriksson. Hydrolysis process of the second generation platinum-based anticancer drug cis-ammine dichlorocyclohexylamineplatinum(II). *J PhysChem B Condens Matter Mater Surf Interfaces Biophys.*2005; 109:12195–12205.
17. Chvalova, K., Brabec, V. and Kasparkova, J. Mechanism of the formation of DNA-protein cross-links by antitumor cisplatin. *Nucleic acids research.* 2007; 35:1812–1821
18. Dunlap, B. and Paice, J. A. Chemotherapy-induced peripheral neuropathy: A need for standardization in measurement. *J Support Oncol*. 2006; 4: 398–399.
19. Giusi, G., R. Alo, M. Crudo, A. D. Vito, R. M. Facciolo, and Canonaco, M. Environmental stressors and neurobiological features of marine teleosts: Histamine receptors as targets. *Critical Reviews in Toxicology*. 2010; 40(7): 620–632.
20. Sudo, N. Biogenic Amines: Signals Between Commensal Microbiota and Gut Physiology Front. *Endocrinol*. 2019; https://doi.org/10.3389/fendo.2019.00504. Accessed on 19/06/2021 from: https://www.frontiersin.org/articles/10.3389/fendo.2019.00504/ful.
21. Visciano, P., M. Schirone and A. Paparella. An Overview of Histamine and Other Biogenic Amines in Fish and Fish Products. *Foods*. 2020; 9, 1795; 90/foods9121795
22. Durak-Dados, A., Michalski, M. and Osek, J. Histamine and other biogenic amines in food. *J Vet Res*. 2020; 64, 281-288, DOI:10.2478/jvetres-2020-0029.
23. Criado, P.R., R.F.J. Criado, C.W. Maruta and M. Filho. *An Bras Dermatol*. 2010; 85 (2):195-210.
24. Parsons, M.E. and C. R. Ganellin. Histamine and its receptors. *British Journal of Pharmacology*. 2006; 147, S127–S135.
25. Tiligada, T. and Ennis, M. Histamine Pharmacology: from Sir Henry Dale to the 21st century. *British Journal of Pharmacology*. 2020; 177: 469–489
26. Lambat, Z., Limson, J.L. and Daya, S. Cimetidine: antioxidant and metal binding properties; *Journal of pharmacy and pharmacology JPP*. 2002; 54: 1681–1686
27. Nowis, D., M. Legat, J. Bil, Z. Kurzaj, T. Issat, T. Staklosa, B. Mioduszewska, L. Kaczmarek, M. Jakobisiak and J. Golab. Erythropoietin reduces cisplatin-induced neurotoxicity without impairment of cytotoxic effects against tumor cells. *Int. J. Oncol*. 2007; 31(6): 1547–1552.
28. Brouwers, E.E.M., Huitema, A. D. R., Willem, B. J., Beijnen, H. and Schellens, J.H.M. Persistent neuropathy after treatment with cisplatin and oxaliplatin. *Acta Oncologica*. 2009; 48: 832-841.
29. Vichaya, E.G., Chiu, G.S., Krukowski, K., Lacourt, T.E., Kavelaars, A., Dantzer, R., Heijnen, C.J. and Walker, A.K. Mechanisms of chemotherapy-induced behavioural toxicities. *Front Neurosci*. 2015; 21;9:131. doi: 10.3389/fnins.2015.00131.
30. Mendonça, L.S., Antonucci, G.A., Santos, A., Bianchi, M. L. and L. Antunes. Evaluation of the cytotoxicity and genotoxicity of curcumin in PC12 cells. *Mutation research*. 2009; 675: 29-34.
31. Serpeloni, M. Moraes C.B., Muniz, J.R., Motta, M.C., Ramos, A.S., Kessler, R.L., et. al. An essential nuclear protein in trypanosomes is a component of mRNA transcription/export pathway. *PLoS One*. 2011; 6 (6): 20730
32. National Institutes of Health (US). Office for Protection from Research Risks. Public Health Service policy on humane care and use of laboratory animals. Office for Protection from Research Risks (OPRR), *National Institutes of Health*. 1986.
33. Kiwon J., An, J.M., Dae-Woon, E., Kang, K.S. and Su-Nam, K. Preventive effect of fermented black ginseng against cisplatin-induced nephrotoxicity in rats. *J Ginseng Res.* 2017; 41:188-194
34. Sima J., A. T, and M. Nematbakhsh. Cisplatin Alters Sodium Excretion and Renal Clearance in Rats: Gender and Drug Dose Related. *Adv Biomed Res.* 2018; 7: 54.
35. Adedoyin, A., Aarons, L. and Houston, J.B. Dose-dependent pharmacokinetics of cimetidine in the rat. *J. Xenobiotica*. 2008; 17: 595-604.
36. Monia D., Carmen, F., Lotfi, G., Maria, V., Simona, D., Hichem, B.J. and B. Abderrahman. Anti-inflammatory and analgesic activities with gastroprotective effect of semi-purified fractions and isolation of pure compounds from Mediterranean gorgonian Eunicellasingularis. *Asian Pacific Journal of Tropical Medicine*. 2015; 8(8):412-420
37. Matt, C. and J. Shieh. Animal behaviour; Guide to Research Techniques in Neuroscience. *Academic press.* 2015; 2: 39-71.
38. Mohammad, S., Shahrnaz, P., Masoud, N., Moazamehosadat, R., Nasser, Z., Khadije, E. and A. Fatemeh. Walnut consumption protects rats against cisplatin - induced neurotoxicity. *Neurotoxicol*. 2010. oi:10.1016/j.neuro.2012.08.004.
39. Avwioro, O.G. 2010. Histochemistry and tissue pathology, principles and techniques. Nigeria: Claverianum, Jpcs 1 (5), 24-34.
40. Bancroft, J.D. and Gamble, M. Theory and Practice of Histology Techniques, 6th edition. *Churchill*

Livingstone Elsevier, Philadelphia: 2008; 83 -134
41. Taveira, K.T., Carraro, K.T., Catalão, C.H. and Lopes, L.S. Morphological and Morphometric Analysis of the Hippocampus in Wistar Rats with Experimental Hydrocephalus. *Pediatric neurosurgery*. 2012; 48. 10.1159/000345959.
42. Hattori, T., Yakabi, K. and H. Takeda. Cisplatin-induced anorexia and ghrelin. *Vitam Horm*. 2013; 92:301-317.
43. Alexander, S., Wieland, V. and J. Karin. Chemotherapy-induced diarrhea: pathophysiology, frequency and guideline-based management. *Therapeutic Advances in Medical Oncology.* 2010; 2 (1) 51-63.
44. Bearcroft, C.P., Domizio, P., Mourad, F.H., André, E.A. and Farthing, M.J. Cisplatin impairs fluid and electrolyte absorption in rat small intestine: a role for 5 hydroxytryptamine. *Gut*. 1999; 44(2): 174–179.
45. Amber, E., Henderson, R., Adeyanju, J. and Gyang, E. Single Drug Chemotherapy of canine transmissible venereal tumor with Cyclophosphamide, Methotrexate, or Vincristine. *Journal of veterinary internal medicine*. 1990; 4: 144-7.
46. Singh, K. and P.I. Singh. Effect of cimetidine on weight gain in rats. *Indian J Med Sci*. 1995; 49(11):257-60.
47. Jose, M. G., Thomas, S., Ji-an, C., Bobby, G., Anriada, N., Victor, P., Joana, S., Mark, A., Christoph, B. and G.S. Roy. Inhibition of Cisplatin-Induced Lipid Catabolism and Weight Loss by Ghrelin in Male Mice. *Endocrinology.* 2013; 154(9): 3118–3129.
48. Ardestani, S. K., Janlow, M.M., Kariminia, A. and Tavakoli, Z. Effect of cimetidine and ranitidine on lipid Profile and lipid peroxidation in -irradiated mice. *ActaMedicaIranica.* 2004; 42(3): 198-204.
49. Ajao, M.Y. and Akindele, A.J. Anxiolytic and sedative properties of hydroethanolic extract of Telfairia occidentalis leaves in mice. *Brazilian Journal of Pharmacognosy*.2013; 23(2): 301-309.
50. Walsh, R.N and R.A. Cummins. The open field test: a critical review. *Psychological Bulletin.* 1975; 83: 42-504.
51. ElBatsh, M. Antidepressant-like effect of simvastatin in diabetic rats. *Canadian Journal of Physiology and Pharmacology*. 2015; 93. 150324143455000.
52. Adrienne, L. A., Gong, X., Di, K. and Bota, D.A. Low-doses of cisplatin injure hippocampal synapses: a mechanism for 'chemo' brain? *Exp Neurol*. 2014; 255: 137–144.
53. Lomeli, N., Kaijun, D., Czerniawski, J. Guzowski, F. J. and Daniela, A.B. Cisplatin-induced mitochondrial dysfunction is associated with impaired cognitive function in rats. *Free RadicBiol Med.* 2017; 102: 274–286.
54. Dorr, R.T. and Soble, M. J . Cimetidine enhances cisplatin toxicity in mice. *J Cancer Res Clin Oncol*. 1988; 114(1):1-2. doi: 10.1007/BF00390477.
55. Abeer, F., Mohsen, A., Ahmed, N.A., Altaib, Z.M. and S.M. Zaher. Effect of Cisplatin on Cerebellar Cortex of Albino Rat and Possible Protective Role of Granulocyte Colony Stimulating Factor versus Citrullus lanatus Juice. A Histological Study. *EJH*. 2019; 43(3): 1110-0559.
56. Imosemi, I.O., Adekanmbi, A.J. and F.A. Atiba. Telfairiaoccidentalis Leaf Extract Protects the Cerebellar Cortex against Cisplatin-Induced Oxidative Damage in Wistar Rat. *Arch. Bas. App. Med.* 2017; 5: 119 – 124.
57. Pisu, M.B., Roda, E., Avella, D. and G. Bernocchi. Developmental plasticity of rat cerebellar cortex after cisplatin injury: inhibitory synapses and differentiating Purkinje neurons. *Neuroscience.* 2004; 129:655–664.