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# Effects of Ethanol Extract of *Rauwolfia vomitoria* Leaf on Lipid Profile and Cerebellar Histology in Cisplatin-induced Oxidative Stress

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## ABSTRACT
*Rauwolfia vomitoria* has been observed to ameliorate and prevent cisplatin-induced neural loss due to its antioxidant and anti-inflammatory properties. To investigate the effects of ethanol extract of *R. vomitoria* (RV) leaf on lipid profile, oxidative stress biomarkers, and the cerebellar histology using cisplatin-induced oxidative stress in Wistar albino rats. Thirty-six (36) rats were randomly shared into four groups (*n*=9). Group1 (positive control) received drinking water and rats feed. Group 2 (negative control) received cisplatin (5mg/kg body weight) via intraperitoneal (IP) injection. Group 3 received cisplatin (5mg/kg body weight) and 100mg/kg/day (RV). Group 4 received cisplatin (5mg/kg body weight) and 200mg/kg/day (RV). RV was given for 20 days via oral gavage while the single dose of cisplatin was given intraperitoneally on the first day. On day 21, lipid profile was analyzed, oxidative stress biomarkers activities were determined, and the cerebellum was prepared using Haematoxylin and Eosin (H&E) and Cresyl Violet (CV) staining techniques. Cisplatin elevated total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), and low density Cisplatin is a platinum-based chemotherapy drug used to treat various types of cancers. lipoprotein (LDL) ($p>0.05$). The TC, HDL, and LDL in the RV-treated groups reduced slightly ($p>0.05$), while TG was further elevated ($p>0.05$). The cisplatin group had an insignificant rise ($p>0.05$) in lipid peroxidation malondialdehyde, glutathione, catalase, and superoxide dismutase. RV further elevated these values. Cisplatin distorted the cerebellar cortex histology by causing altered Purkinje cell bodies, infiltration of the pyknotic Purkinje cells into the granular layer, fatty changes and vacuolation. The cerebellar histology of rats in groups 3 and 4 showed considerable improvement. The administration of ethanol extract of *Rauwolfia vomitoria* significantly suppressed oxidative stress, increased antioxidative capacity, ameliorated the histological alterations of the cerebellum, and demonstrated neuroprotective ability against cisplatin-induced neurotoxicity.

**Keywords**: *Rauwolfia vomitoria,* cisplatin, oxidative stress, lipid profile, cerebellar histology, neurotoxicity

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# INTRODUCTION

Cisplatin is a platinum-derived chemotherapy drug. Like other platinum-derived chemotherapy drugs, it is used for the treatment of some types of cancers such as sarcoma, leukaemia, neuroblastoma, lymphoma, germ cell tumours, as well as testicular, breast, ovarian, cervical, oesophageal, lung, head and neck, bladder, gastrointestinal, and prostate cancers [5, 47]. The drug has a side effect of inducing peripheral neuropathy, a common side effect that leads to dosage reduction or discontinuation in treatment [14, 39]. Various mechanisms have been assumed for the neurotoxicity of cisplatin, including oxidative injury, activation of pro-inflammatory cytokines, and apoptosis [25]. Another side effect is that it creates links between DNA and RNA strands, thereby interfering with their performances and causing increased uric acid in the blood (hyperuricaemia), anaemia, and nephrotoxicity [34].

The administration of antineoplastic (chemotherapy) agents during cancer chemotherapy creates a physiological imbalance between the levels of pro-oxidants and antioxidants in favour of the oxidants [21, 40] resulting in oxidative stress [27, 39, 40]. These oxidants are reactive oxygen species (ROS) in the form of free radicals that contain one or more unpaired electrons which makes them highly reactive or non-radicals that share their unpaired electrons [22]. Major free radicals that are of physiological significance are superoxide anion, hydroxyl radical, and hydroperoxyl radical, while non-radical is hydrogen peroxide [10, 48]. Reports suggest that ROS have an essential function in certain chemotherapy-induced side effects, for instance cisplatin-induced nephrotoxicity, ototoxicity and neurotoxicity [14, 39]. More so, lipid peroxidation that produces numerous electrophilic aldehydes, such as malondialdehyde that can attack many cellular targets is a result of oxidative stress [14, 39].

The link between oxidative stress and hyperuricaemia is still being studied. Positive correlative relationships exist between asymptomatic hyperuricemia, increased albuminuria and a decrease in glomerular filtration rate (GFR), dyslipidemia, systolic blood pressure (SBP), glycated haemoglobin ($HbA_1C$), inflammatory processes and kidney damage, which indicate the multifactorial processes of renal pathogenicity in such patients [49, 50, 51, 52, 54, 56, 58, 59, 61, 62, 64, 65, 67].

Oxidative stress is evaluated by the presence of major biomarkers like malondialdehyde, glutathione, superoxide dismutase, catalase, isoprostane, nitrotyrosine, hydrogen peroxide ($H_2O_2$), glutathione S-transferase, and glutathione disulphide [24, 40]. Meanwhile, some of these biomarkers are antioxidants [40].

*Rauwolfia vomitoria* (RV) is a tropical shrub found primarily in Senegal in the west of Africa, Sudan and Tanzania in the east of Africa, and Angola in the southern part of Africa [31]. It is called "poisonous devil's-pepper", "swizzle-stick", and locally called "akanta",

"sofeyeji", "wadda", "mmoneb", and "utoenyin" in Igbo, Yoruba, Hausa, Efik, and Ibibio languages respectively in Nigeria [17]. The plant belongs to the family of *Apocynaceae* used locally in the treatment of snake bites, nervous disorders, jaundice, cerebral cramps, and gastrointestinal disorders. Moreover, the plant has been documented to act as an analgesic and anticonvulsant [11, 35] and also in treating malaria, leprosy, skin infections, and high blood pressure [26]. RV extract is enriched with anti-inflammatory, antipyretic, and anti-cancer properties [4]. RV and *Gongronema latifolium* have been proven to be antioxidants and antidiabetics [11, 13, 37, 43], and the combination of the former with viable synthetic drugs may be evaluated for health management including the prevention of neurodegenerative diseases.

Oxidative stress and hyperuricaemia can damage the kidneys. Dapagliflozin, a Sodium-Glucose Linked Transporter-2 (SGLT-2) inhibitor improves renal functions, reduces hyperglycaemia [53, 55, 57, 60, 63, 66]. An increased intake of antioxidant-rich foods and reduced exposure to free radicals will enhance the body's potential to minimize the risk of free radical-related health problems [33]. Antioxidants such as polyphenols, vitamins A & C, α-lipoid acid, thioredoxin, glutathione, melatonin, β-carotenoids, α-tocopherol as well as antioxidant enzymes including superoxide dismutase, catalase, glutathione peroxidases, and isoprostane have been widely investigated for prevention and treatment of diseases resulting from oxidative damage [23, 36]. These antioxidants are potent scavengers of free radicals and they repair radical-induced damage which prevents diseases [6, 16]. Therefore, this work aimed to evaluate the effects of ethanol extract of *R. vomitoria* on oxidative stress biomarkers, lipid profile, and cerebellar histology of Wistar rats treated with cisplatin.

## MATERIALS AND METHOD

### Materials

#### Chemicals and reagents

Cisplatin and other reagents used were obtained from a registered pharmacy in Abakaliki, Ebonyi State, Nigeria. Cisplatin was freshly prepared by dissolving it in 0.9% saline.

#### Experimental animals

36 albino Wistar rats of weights between 150g-180g were purchased from the animal house of the Department of Anatomy, Ebonyi State University (EBSU), Abakaliki, Nigeria. The rats were handled as described by Udeh *et al* [42]**.** They were kept in well-ventilated cages and kept under a controlled environment condition - 12 hours of light/dark cycle and a room temperature of 25.0±5.0˚C. The rats acclimatized for a week and were fed with standard rat feed and clean water *ad libitum.*

#### Plant collection and identification

A bulk of fresh *R. vomitoria* was collected from the “offia Izzo” forest in Ezza North Local Government Area of Ebonyi State, Nigeria where the plant is abundant. The plant was collected in the morning hours because of the scientific evidence that supports the fact that the yield of some plants’ chemical constituents differs with the time of the day due to the inter conversions of compounds [20]. The plant was identified and authenticated in the Department of Applied Biology of EBSU whereas herbarium exists.

**Methods**

**Extraction of the plant material**

The leaves were air-dried at room temperature and blended into a fine powder using a kitchen blender. The blended sample was soaked in ethanol for 24 hours and the extract was filtered and evaporated to obtain the crude extract [2].

**Administrations**

The 36 albino Wistar rats were randomly divided into four experimental groups (*n*=9) and treated as follows:

**Group 1**: **Normal control** (received clean drinking water and rat feed)

**Group 2: Negative control** (injected with 5mg/kg BW of cisplatin, single dose)

**Group 3:** injected with 5mg/kg BW of cisplatin, single dose and 100mg/kg BW of ethanol extract of *R. vomitoria*, oral administration with oral gavage for 20 days after cisplatin injection

**Group 4:** injected with 5mg/kg BW of cisplatin, single dose and 200mg/kg BW of ethanol extract of *R. vomitoria*, oral administration with oral gavage for 20 days after cisplatin injection

**Sample collection**

On the $21^{st}$ day, the animals were anaesthetized with diethyl ether and blood samples were collected through orbital puncture into plane tubes. The rats were sacrificed by cervical dislocation for the collection of the blood for biochemical analyses and the cerebellum for histological processing.

**Biochemical analyses**

The blood samples were centrifuged (3,000 rpm) for 15 minutes to obtain the serum. For lipid profile, total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL) levels were analyzed using RANDOX kits. The spectrophotometric method was used to measure the oxidative stress biomarkers such as malondialdehyde (MDA), glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD).

1. **Analysis of lipid profile:** The lipid profiles were determined by the standard homogenous enzymatic method using an automatic chemistry analyzer (Hitachi 902,

Roche). TC was measured enzymatically in a series of coupled reactions. The produced $H_2O_2$ in the second reaction was measured quantitatively by a peroxidase-catalyzed reaction that produced a colour change. Finally, absorbance was measured at 500nm [32]. For TG measurement, TG was hydrolyzed to produce glycerol using a series of coupled reactions. Then, glycerol was oxidized using glycerol oxidase and the produced $H_2O_2$ was measured as described for TC [32]. The serum was treated with a blocking reagent to remove Apolipoprotein B (ApoB) from the assay, and then sulphated alpha-cyclodextrin, polyethylene glycol-coupled cholesteryl esterase and cholesterol oxidase were used to measure HDL directly [32]. Finally, LDL was initially separated from the chylomicrons, and then very low density lipoprotein (VLDL) and HDL, after which LDL was measured using an enzymatic colourimetric assay [29].

2. **Determination of MDA level:** Lipid peroxidation was determined spectrophotometrically in the serum by measuring the malondialdehyde levels as a thiobarbituric acid reactive substance. Briefly, trichloroacetic acid and thiobarbituric acid reactive substance reagents were added to the serum samples, then mixed and incubated at 100˚C for 60 minutes. After cooling on ice, the samples were centrifuged at 3000 rpm for 20 minutes and the absorbance of the supernatant was read at 55nM [45].
3. **Determination of GSH level:** Glutathione was measured according to the Ellman method where thiol interacts with 5,5-dithiobis-(2-nitrobenzoic acid) and forms a coloured anion [19].
4. **Measurement of CAT activity:** Catalase assay was measured by a spectrophotometric procedure measuring peroxide removal and was measured by the method of Beers and Sizer [8].
5. **Measurement of SOD activity:** This was determined using the method of Xin *et al* [46]. Superoxide dismutase reduces superoxide to $H_2O_2$ [40].

**Tissue Processing**

The cerebella of the Wistar rats were fixed in 10% normal saline and then prepared for microscopy. The following histological procedures were followed to get the tissue ready for microscopy. The tissues were fixed for 5 days after which organs were dehydrated in ascending graded ethanol (50%, 70%, 90% and absolute ethanol) for 2 hours. The tissues were cleared in 2 changes of xylene for 1 hour and then impregnated in a hot oven with three changes of molten paraffin wax for 20 minutes each. The tissues were embedded and allowed to solidify. The paraffin blocks were trimmed and later sectioned to form 3µm thick ribbons using a rotary microtome machine. The sections were mounted on glass slides and stained in

Haematoxylin and Eosin (H&E) stains and Cresyl Violet (CV) special stain. These sections were viewed under a microscope and photomicrographs were snapped.

**Statistical analysis**

Statistical Package for Social Science version 25.0 was used for the data analysis. All data were expressed as mean ± standard error of the mean (SEM). The results were analyzed by one-way analysis of variance (ANOVA) and Tukeys' post hoc test for data comparison. The level of significance was drawn when $p<0.05$.

**Ethical approval**

This was obtained from the Research and Ethics Committee of the Faculty of Basic Medical Sciences, EBSU, with the reference number: EBSU/FBMS/REC/002/012.

## RESULTS

**Lipid Profile Activities of Animals Administered with Cisplatin and Treated with Ethanol Extract of *Rauwolfia Vomitoria* (RV)**

The result of the lipid profile is illustrated in Table 1. The mean ± SEM TC levels for groups 1, 2, 3, and 4 are 4.10±0.50 mmol/l, 5.35±0.25 mmol/l, 4.95±0.45 mmol/l and 5.05±0.15 mmol/l respectively. The table shows that TC level increased in the negative control (group 2), as a result of the cisplatin injection when compared to the control group. The TC level is higher in group 4 than in group 3, while the values in both groups are lesser than the value in group 2. However, the values in the experimental groups are not significantly different ($p>0.05$) from the control group's TC value.

The TG mean ± SEM for groups 1-4 are 1.10±0.10 mmol/l, 1.25±0.25 mmol/l, 1.35±0.15 mmol/l, and 1.45±0.15 mmol/l respectively. A stepwise increase in the levels of TG is seen in groups 2, 3, and 4, although with no significance ($p>0.05$) in comparison to group 1.

HDL has the mean ± SEM of 2.58±0.42 mmol/l, 3.20±0.10 mmol/l, 3.03±0.37 mmol/l, and 3.26±0.18 mmol/l for groups 1, 2, 3, and 4 respectively. It can be seen that the HDL level is high in group 2 in comparison to group 1. The HDL level in group 3 was reduced when compared to the HDL level in group 2, while the HDL level in group 4 was the highest. The difference in the values of HDL is not significant ($p>0.05$) when compared to the control group.

Finally, LDL mean ± SEM in groups 1, 2, 3, and 4 was 1.30±0.10 mmol/l, 1.90±0.10 mmol/l, 1.65±0.05 mmol/l, and 1.50±0.30 mmol/l respectively. The LDL level was elevated in group 2 when compared to group 1 and a stepwise reduction with an increment in dosage of RV in groups 3 and 4 was obvious. However, this increase and decrease in the LDL level in the experimental groups are not significantly different ($p>0.05$) when compared with the control group.

**MDA, GSH, CAT, and SOD Levels in Animals Administered with Cisplatin and Ethanol Extract of RV**

Table 2 indicates the values for all the above parameters. The mean ± standard error of the mean (SEM) of MDA were 3.37±0.54, 3.63±0.55, 4.03±0.16, and 4.08±0.08 for groups 1, 2, 3, and 4 respectively. A stepwise insignificant ($p$>0.05) rise in the level of MDA can be observed in groups 2 to 4 when compared with group 1.

The mean ± SEM of GSH are 6.11±0.03, 9.07±0.41, 7.57±0.06, and 7.63±0.94 for groups 1, 2, 3, and 4 respectively. This stepwise increase in groups 2 to 4 as seen in Table 2, shows no significant difference ($p$≥0.05) when compared with group 1.

Table 2 shows that the mean ±SEM of CAT in the groups were 3.57±0.47, 3.82±0.28, 4.78±0.23, and 3.46±0.35 for groups 1, 2, 3, and 4 respectively. This study revealed that catalase in group 1 was not significantly low ($p$>0.05) when compared with group 2 which had a higher value. Moreover, from the table comparing the catalase level in groups 3 and 4 to group 1, the former is not significantly higher ($p$>0.05).

The SOD mean ± SEM was 10.41±0.03, 11.46±0.02, 11.41±0.00, and 10.33±0.03 for groups 1, 2, 3, and 4 respectively. This study revealed that SOD in Group 2 was not significantly higher ($p$>0.05) than the SOD value in Group 1. In the comparison of the values in groups 3 and 4 with group 1, the former was significantly higher ($p$=0.00).

**Table 1: Result of Lipid Profile Activities in Animals Administered with Cisplatin and Ethanol Extracts of RV**

| Groups | TC (mmol/l) | | TG (mmol/l) | | HDL (mmol/l) | | LDL (mmol/l) | |
|---|---|---|---|---|---|---|---|---|
| | Mean ± SEM | *p*-value | Mean ± SEM | *p*-value | Mean ± SEM | *p*-value | Mean ± SEM | *p*-value |
| 1 | 4.10±0.50 | | 1.10±0.10 | | 2.58±0.42 | | 1.30±0.10 | |
| 2 | 5.35±0.25 | 0.216** | 1.25±0.25 | 0.921** | 3.20±0.10 | 0.527** | 1.90±0.10 | 0.193** |
| 3 | 4.95±0.45 | 0.452** | 1.35±0.15 | 0.743** | 3.03±0.37 | 0.725** | 1.65±0.05 | 0.524** |
| 4 | 5.05±0.15 | 0.378** | 1.45±0.15 | 0.539** | 3.26±0.18 | 0.464** | 1.50±0.30 | 0.833** |

SEM = standard error of the mean; level of significance at $p$<0.05; ** = not significantly different

**Source:** Field Work, 2022

**Table 2: Results of Lipid Peroxidation Malondialdehyde (MDA) Level, Glutathione (GSH) Level, Catalase (CAT) Activities, and Superoxide Dismutase (SOD) Activities in Animals Administered with Cisplatin and Ethanol Extract of RV.**

| Parameters | Groups | Mean ± SEM | A | B | *p*-value |
|---|---|---|---|---|---|
| MDA | G1 | 3.37±0.54 | | G2 | 0.962** |
| (mg/l) | G2 | 3.63±0.55 | G1 | G3 | 0.658** |
| | G3 | 4.03±0.16 | | G4 | 0.618** |
| | G4 | 4.08±0.08 | | | |
| GSH | G1 | 6.11±0.03 | | G2 | 0.318** |
| (mg/l) | G2 | 7.57±0.41 | G1 | G3 | 0.291** |

| Parameter | Group | Mean±SEM | A | B | p-value |
|---|---|---|---|---|---|
| | G3 | 7.63±0.06 | | G4 | 0.050** |
| | G4 | 9.07±0.94 | | | |
| CAT | G1 | 3.57±0.47 | | G2 | 0.948** |
| (u/l) | G2 | 3.82±0.28 | G1 | G3 | 0.198** |
| | G3 | 4.78±0.23 | | G4 | 0.996** |
| | G4 | 3.46±0.35 | | | |
| SOD | G1 | 10.41±0.03 | | G2 | 0.230** |
| (u/l) | G2 | 10.33±0.03 | G1 | G3 | 0.000* |
| | G3 | 11.41±0.00 | | G4 | 0.000* |
| | G4 | 11.46±0.02 | | | |

SEM = standard error of the mean; A and B = indices groups; level of significance at $p<0.05$; * = significantly different; ** = not significantly different

**Source:** Field Work, 2022

**Histological Results**

The H&E staining showed that the histological architecture of the cerebellar cortex of the Wistar rats in the normal control group (group 1) was normal. Their photomicrographs in Figure 1 show normal cerebellar cortex histology (outer molecular layer containing dendrites of Purkinje cells, axons of granule cells, small cells, basket cells, and neuroglia cells; middle Purkinje cell layer containing the somas of the Purkinje cells; and inner granular layer containing granule cell bodies). The histological architecture of group 2 rats was severely distorted as seen in figure 2. The granule cells' size shrunk and appeared degenerated with necrotised nuclei, and then fatty changes or steatosis at the Purkinje cell layer as against that of group 1. Figures 3 and 4 show the photomicrographs of the rats treated with 100mg/kg of RV (group 3) and 200mg/kg of RV (group 4) respectively. The photomicrographs show not many observable differences in the histological architecture because both showed protective/regenerative effects and restoration of normal cerebellar histological arrangement and reduction in fatty changes. Therefore, the histology revealed that RV treatment in groups 3 and 4 reduced neurotoxic-related cerebellar damage compared with the negative control group (group 2).

Under Cresyl Violet (CV) staining, the photomicrographs show the trilaminar appearance of the cerebellar cortex in all the groups. Photomicrographs for group 2 reveal that the cerebellum was severely injured as the Purkinje cell layer was gradually vacuolated and the pyknotic Purkinje cells were gradually invading the inner granular cells, shown in figure 6. Photomicrographs for group 3 show that there was mild healing with moderate vacuolation and a moderate clumping of granular cell layer by pyknotic Purkinje cells (figure 7). In group 4, the photomicrographs show mild vacuolation and a mild decrease in the number of Purkinje cells invading the granular cells, as shown in figure 4.

**Histological Findings and Interpretation of Cerebellar Cortex Architecture Using H&E Stains**

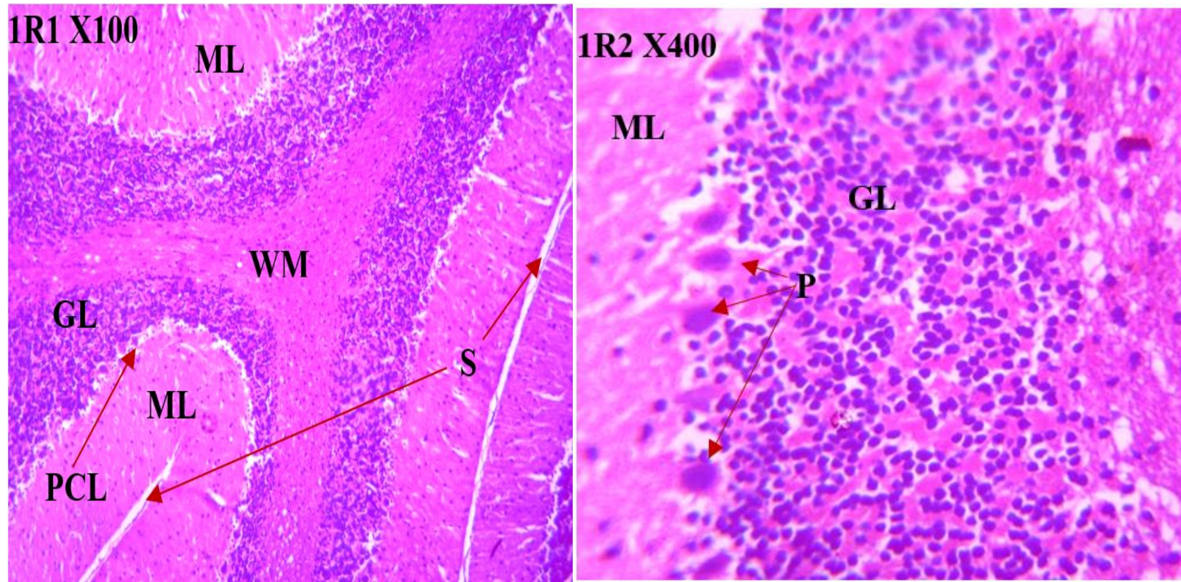
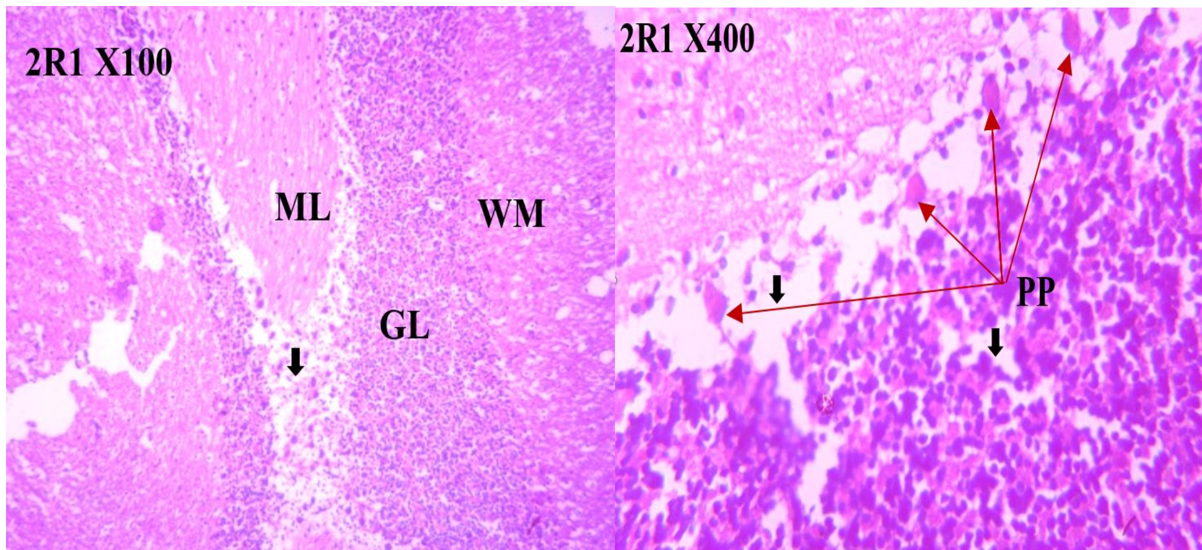



**Figure 1: Photomicrographs of sections of the cerebellum of group 1 R1 and R2 showing normal cerebellar cortex with outer molecular layer (ML) containing the dendrites of Purkinje cells and the axons of granule cells, inner granular layer (GL) containing granule cell bodies (G), and Purkinje cell layer (PCL) containing the Purkinje somas (P) with centrally placed nucleus. There is also the underlying white matter (WM) and then the sulci (S) between the folia of the cerebellum. H&E: magnifications are X100 and X400 respectively.**



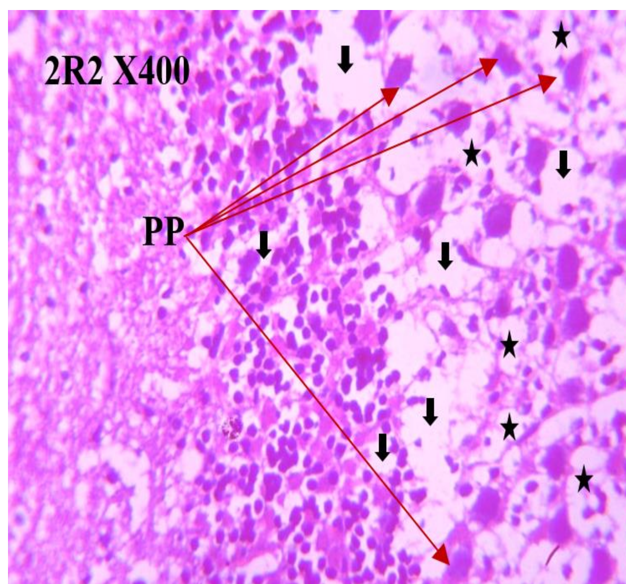



**Figure 2: Photomicrographs of group 2 R1 and R2 section of the cerebellar cortex, induced with 5mg/kg of cisplatin without treatment. Photomicrographs show severe vacuolation (↓), fatty changes (*), and infiltration of Purkinje cells with necrotized nuclei (PP). H&E: magnifications are X100, X400 and X400 respectively.**

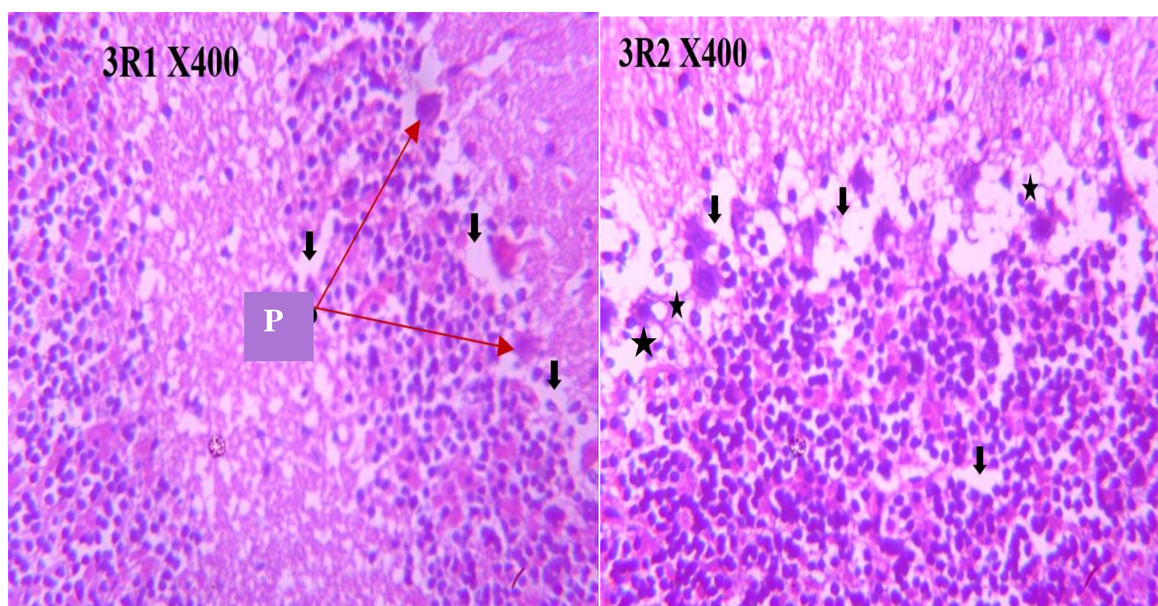



**Figure 3: Photomicrographs of group 3 R1 and R2 sections of the cerebellar cortex, induced with 5mg/kg of cisplatin and treated with 100mg/kg dose extract of *R. vomitoria*. These show moderate healing characterized by narrowing of the vacuoles in the layers (↓), moderate fatty changes (*), and moderate appearing Purkinje (P) cell bodies with obvious nuclei. Moreover, the granular cells appear normal. H&E: magnifications are X400 and X400 respectively.**

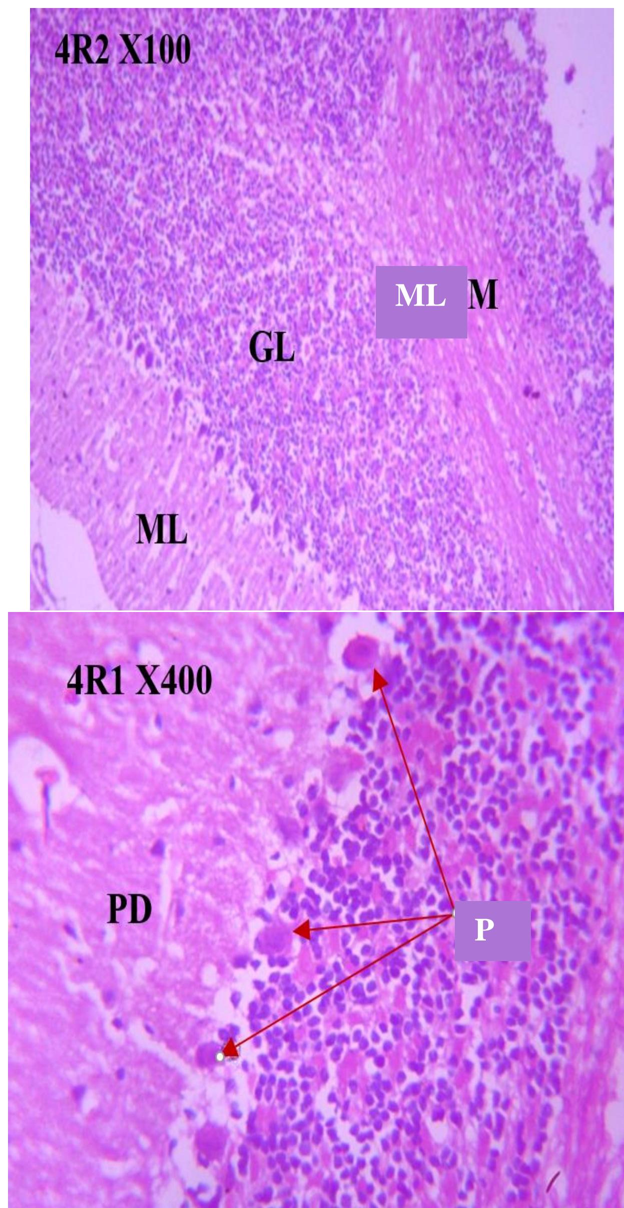



**Figure 4: Photomicrographs of group 4 R1 and R2 sections of the cerebellar cortex, induced with 5mg/kg dose of cisplatin and treated with 200mg/kg dose extract of *R. vomitoria.* These show moderate healing with milder vacuolation. The Purkinje cell bodies (P) at the Purkinje cell layer, the dendrites of the Purkinje cells and the axons of granule cells at the molecular layer (ML) appear normal. H&E: magnifications are X100 and X400 respectively**.

**Histological Findings and Interpretation of Cerebellar Cortex Architecture Using Cresyl Violet Stain**

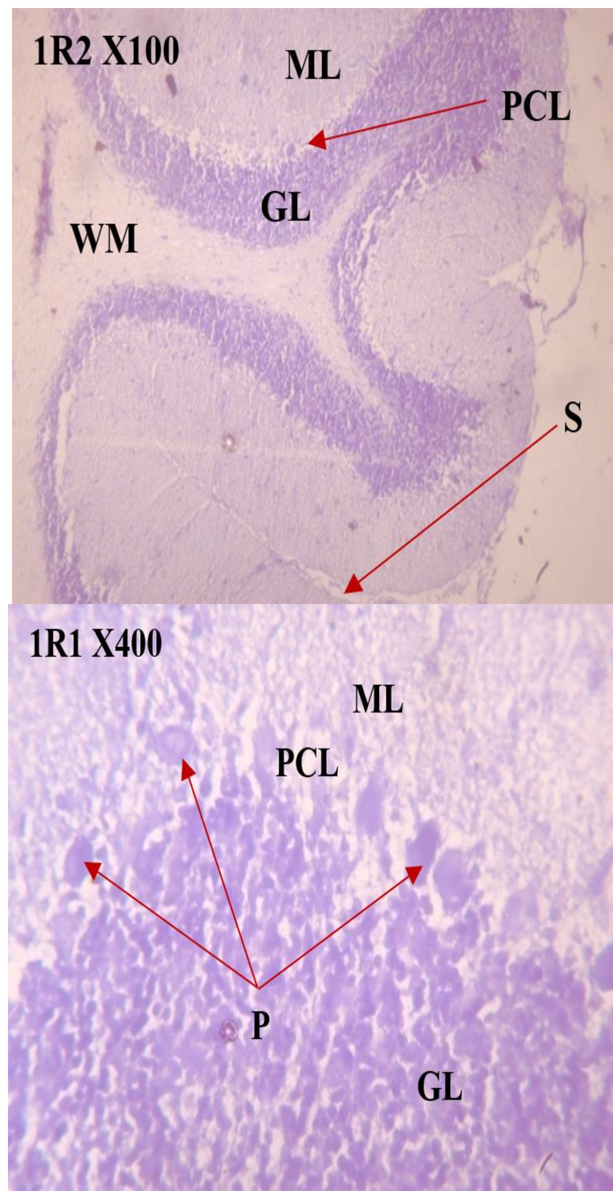



**Figure 5: Photomicrographs of group 1 R1 and R2 sections of the cerebellar cortex, showing normal cerebellar cortex with outer molecular layer (ML), inner granular layer (GL) containing granule cells bodies (G), and the Purkinje cell layer (PCL) containing the Purkinje somas (P) with centrally placed nuclei. There is the underlying white matter (WM), and sulci (S) between the folia of the cerebellum. Cresyl Violet (CV): magnifications are X100 and X400 respectively.**

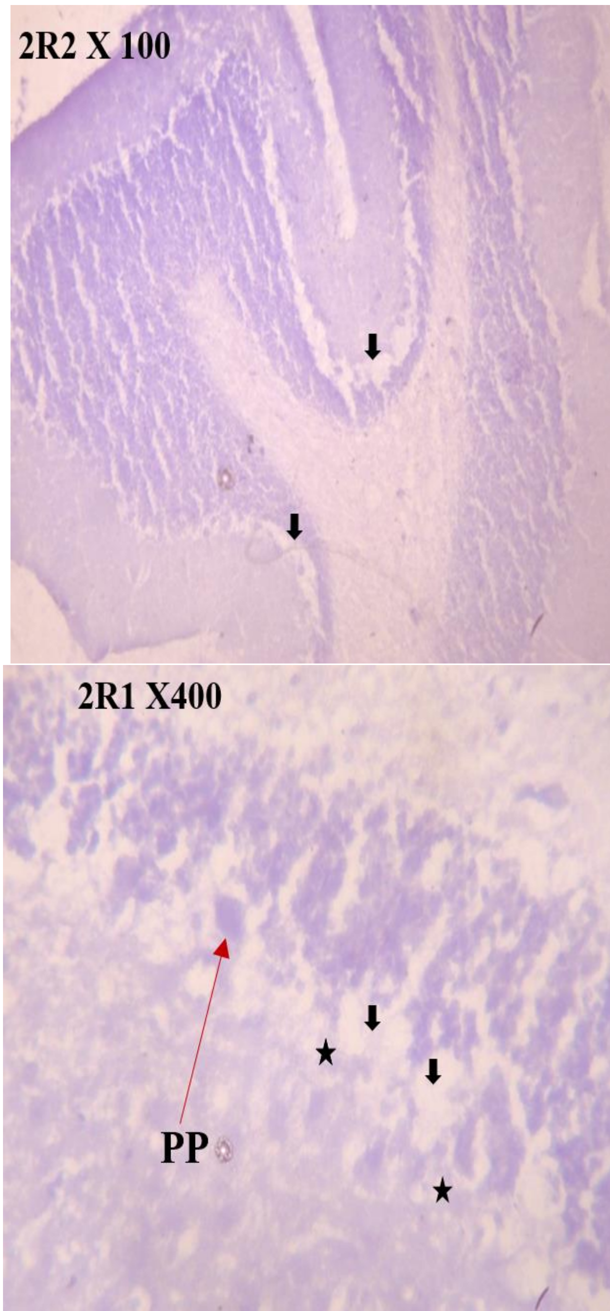



**Figure 6: Photomicrographs of group 2 R1 and R2 sections of the cerebellar cortex, induced with 5mg/kg of cisplatin. These show severe vacuolation (↓) in the Purkinje layer, fatty changes (∗), and severe infiltration of pyknotic Purkinje (PP) cell bodies into the granular layer in R1 as the cell bodies were less seen on the Purkinje cell layer (PCL). CV: magnifications are X100 and X400 respectively.**

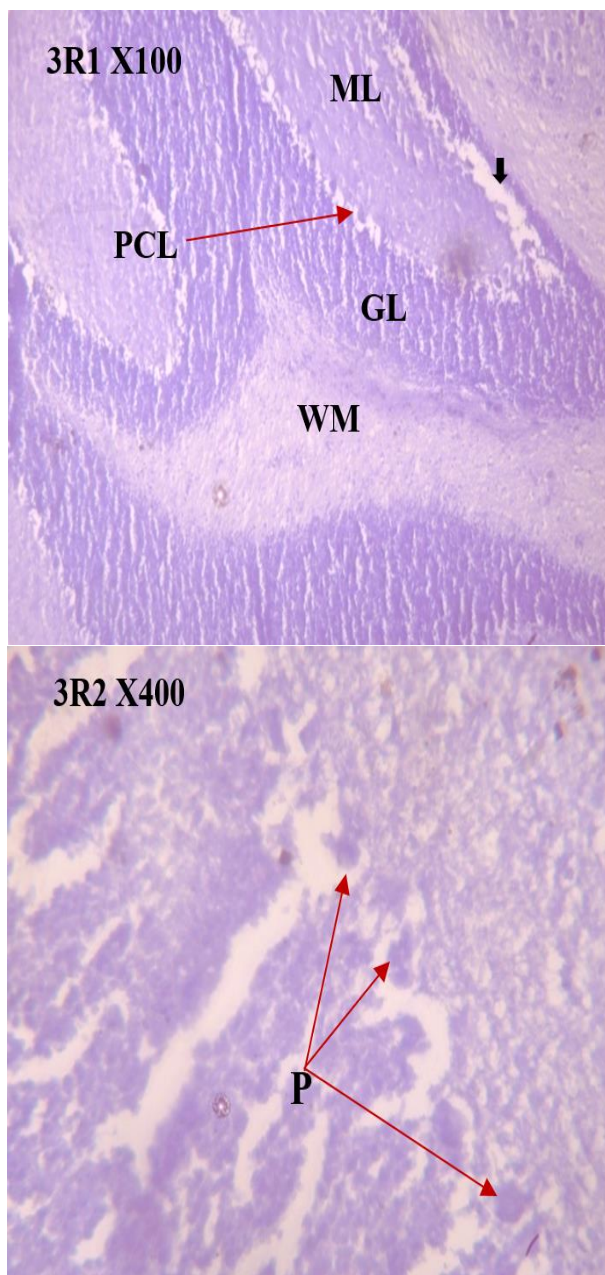



**Figure 7: Photomicrographs of group 3 R1 and R2 sections of the cerebellar cortex, induced with 5mg/kg of cisplatin and treated with 100mg/kg dose extract of *R. vomitoria*. These show moderate healing: moderate vacuolation (↓), moderate fatty changes, and lessened clumping of Purkinje (P) cell bodies. CV: magnifications are X100 and X400 respectively.**

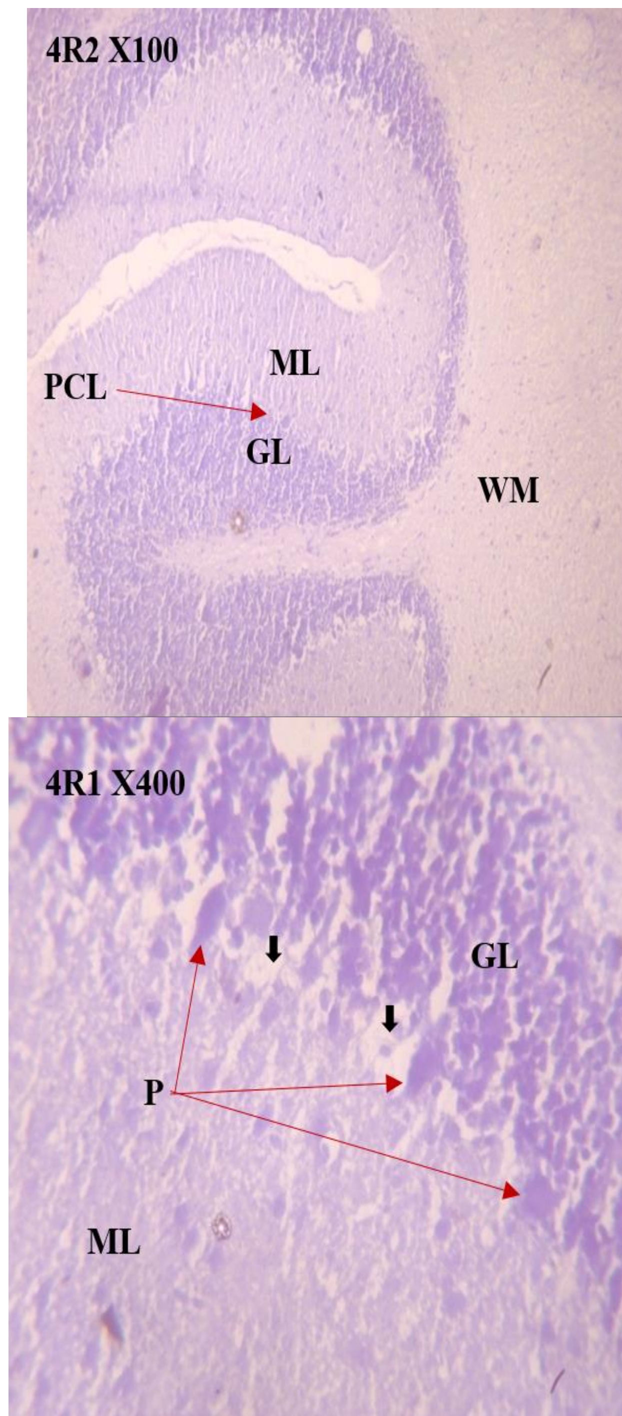



**Figure 8: Photomicrographs of group 4 R1 and R2 sections of the cerebellar cortex, induced with 5mg/kg of cisplatin and treated with 200mg/kg dose of *R. vomitoria* ethanol extract. These show moderate healing: milder vacuolation (↓) in the granular layer, and Purkinje cell bodies appearing normal in the PCL. CV: magnifications are X100 and X400 respectively.**

## DISCUSSION

Oxidative stress keeps gaining wide attention due to excessive exposure of humans to varieties of stressors like cigarette smoke, alcohol consumption, ultraviolet radiation, antineoplastic agents, and non-steroidal anti-inflammatory drugs (NSAIDs). The resultant effect of this exposure is the formation of ROS which have a high tendency to bond with

healthy compounds in the body, and then form harmful molecules [9, 22, 48]. However, the body has antioxidants to nullify the effect of these ROS (oxidants) but in some cases, the antioxidants are overwhelmed thereby creating an oxidant/antioxidant imbalance termed "oxidative stress" [40]. The result of this stress is the manifestation of disease conditions like cancers, inflammation, ageing, cardiovascular diseases, and neurodegenerative disorders [7, 14, 39, 56, 58].

In this study, it was found that cisplatin elevated serum lipid profile parameters - total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL). However, the increase was not significant ($p$>0.05). Vardin, *et al* observed that cisplatin caused a lowered TC which was insignificant, increase in TG which was significant, as well as insignificant increases in HDL and LDL [44]. Abdel-Gayoum, *et al* found a significant increase in TC and TG ($p$<0.05), but no significant difference in HDL and LDL ($p$>0.05) [1]. Ellis, *et al* noted that cisplatin caused no significant difference in lipid profile upon administration of cisplatin in patients undergoing chemotherapy [18].

Upon the induction of oxidative stress in the Wistar rats, this study found that *R. vomitoria* was able to slightly reduce the TC, HDL, and LDL, but could not reduce TG. Rather, it elevated TG slightly. Statistically, these differences were not significant. In a similar study on the effects of RV on lipid profile, Akanji, *et al* found that RV significantly reduced TG and LDL, but a dose-dependent increase was found in HDL [3]. HDL is generally known as the good cholesterol while LDL is generally known as the bad cholesterol because when LDL is oxidized, it forms plaques on the tunica intima of arteries thereby initiating hypertension and atherosclerosis (cardiovascular diseases) [30, 49, 50, 51, 52, 54, 59, 61, 62, 64, 65, 67]. Flavonoids have been reported to act as antioxidants, antidepressants, and anti-inflammatory. RV has been phytochemically analyzed to contain flavonoids and other compounds like alkaloids, phenols, and saponins [3, 38]. This could be why RV lowered LDL in this study with a little dose as compared to other studies where the dosage was much greater than 200mg/kg of BW. This could be the reason for the insignificant difference.

Serum malondialdehyde (MDA), a product of lipid peroxidation slightly increased ($p$>0.05) in group 2. The values of other biomarkers of oxidative stress also changed insignificantly upon cisplatin administration - glutathione (GSH) increased, catalase (CAT) increased, but superoxide dismutase (SOD) decreased. Vardin, *et al* noted that CAT reduced significantly, together with glutathione peroxidase (GPx). This was contrary to the findings of this study. But then, the Vardin, *et al* finding of a significant increase in MDA and SOD was very close to the findings in group 2 of this study [44].

In addition, this study revealed that *R. vomitoria* treatment further elevated the levels of MDA, GSH, and SOD slightly ($p$>0.05), but insignificantly reduced ($p$>0.05) the level of

CAT at a dose of 200mg/kg BW. This can be interpreted as ethanol extract of RV acting positively in enhancing the production of more antioxidants to neutralize the harmful products (oxidants) released due to the cisplatin injection, and with the reduction in lipid profile parameters (TC, HDL, and LDL). A study on the hepatoprotective and antioxidant effects of aqueous extract of *R. vomitoria* stem root on Wistar rats by Djanche, *et al* showed a similar result in which RV increased the enzyme activity of MDA, CAT, and SOD [15]. Okolie, *et al* found that RV reduced MDA [37], while Oyeniran, *et al* study on phenolic constituents and inhibitory effects of the leaf of *R. vomitoria* on free radicals showed that RV did not show any significant difference in inhibiting lipid peroxidation that produces MDA [38].

Concerning the histological architecture (also known as histo architecture) of the cerebellar cortex, cisplatin was found to induce severe vacuolation in the granular and Purkinje cell layer, fatty changes (fat impregnation), pyknosis and nuclear degeneration of the Purkinje cells, and the infiltration of pyknotic Purkinje cells' somas into the granular layer. These features are in line with study of Kamisli, *et al*, as seen in the photomicrographs produced [28]. Upon administration of the ethanol extract of *R. vomitoria*, these histopathological features were better seen with H&E stains, to have been slightly reversed as the Purkinje cells' nuclei became visible. Moreover, the cellular infiltration, vacuolation and fat impregnation in the middle and outer layers of the cerebellar cortex were slightly reversed.

Cresyl violet stain produced less appreciated photomicrographs. Nevertheless, the images obtained support the fact that *R. vomitoria* ameliorates the effects of cisplatin-induced oxidative stress. With the positive findings seen in the histological analysis of this study, supported by the biochemical analyses, it was obvious *R. vomitoria* slightly reversed or ameliorated the toxic effects of cisplatin on the cerebellum.

## CONCLUSION

The common use of cisplatin in chemotherapy results in deleterious neurotoxic effects. *Rauwolfia vomitoria* has a neuroprotective ability in cisplatin-induced cerebellar toxicity as it elevates antioxidants and suppresses lipid peroxidation. *Rauwolfia vomitoria* is a potential agent that can be used for the prevention of cisplatin-induced cerebellar neurotoxicity. Therefore, more studies need to focus on synergizing *Rauwolfia vomitoria* with other antioxidants (either of natural source or synthetic source) on ameliorating the effects of cisplatin-induced oxidative stress on the cerebellum.

## CONFLICT OF INTEREST

The authors assure the ingenuity of this manuscript and declare no conflict of interest. This manuscript was written from original research and has never been published, nor is it under

consideration for publication elsewhere.

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