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Anti-oxidative Potential of Essential Oil of *Rosmarinus officinalis* in Cadmium-induced Neurotoxicity on the Cerebellum of Adult Wistar Rats

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ABSTRACT

The cerebellum connects the brain's sensory areas to its motor areas and is required to coordinate fine movements. However, it is vulnerable to neurotoxic agents and poisoning. This study aimed to determine the role of rosemary essential oil in cadmium-induced toxicity in the cerebellum of adult Wistar rats. Twenty-five (25) male Wistar rats weighing between 120 and 160 g were separated into five (5) groups, consisting of five (5) rats in each group. Group A (Control), Group B (6 mg/kg of Cadmium chloride), Group C (6 mg/kg of $CdCl_2$ + 200 mg/kg of Essential oil of *Rosmarinus officinalis* (EORO), Group D (6 mg/kg of $CdCl_2$ + 300 mg/kg of EORO), and Group E (6 mg/kg of $CdCl_2$ + 400 mg/kg of EORO). Neuro-behavioural assessment studies (open field test), brain's histological studies, and biochemical assessment of total cholesterol (TC), triacylglycerol (TG), high-density lipoprotein (HDL-C), and low-density lipoprotein (LDL-C) were carried out. Using one-way ANOVA and paired student's t-test, both the statistical differences in the group's mean and its comparison were analysed with the generated result reported as mean ± standard error of mean (SEM). Cadmium chloride caused locomotive impairment. These were demonstrated by distorted pia matter, molecular layer, and granular layer and haemorrhage around the neuronal cells. Neuro-behavioural analysis showed line crossing (LC) and freezing duration (FD) as (46.50±1.50, 17.50±2.50), (24.00±12.42, 181.00±56.07), (43.00±3.00, 72.50±2.50), (34.67±6.36, 66.67±8.82), and (34.67±8.82, 79.00±14.64) for groups A, B, C, D and E respectively. However, concurrent administration of rosemary essential oil ameliorates the neurotoxic effects induced by cadmium.

***Keywords*:** Cerebellum, Cadmium, Neurotoxicity, Rosemary Essential Oil, Neurobehavioural.

Introduction

The cerebellum (central portion of the main circuitry) connects the brain's sensory areas to its motor areas and facilitates fine movement coordination. It also provides links amid movement, which are the premise for accuracy and precision, and thus, it is involved in modifying reflex actions and motor learning.[1] The cerebellum is usually vulnerable to neurotoxic agents and poisoning(s); it's the major target of drug exposure and environmental toxins.[2,3] Cadmium (Cd), a carcinogenic heavy metal, is detrimental to humans' health and has become a significant ecological and occupational concern. It has been assessed that around 25,000 tons of Cd are deposited annually into the ecosystem,[4] which could enter and aggregate in humans and animals via drinking water, breathing, and food. Cadmium, having a lengthy biological half-life, is because of its excretion from the body at a low rate. Its prolonged exposure causes toxic effects, accumulating in various tissues, such as the kidneys, Central Nervous System (CNS), liver, and peripheral neuronal systems (PNS).[4,5]

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Exposure to cadmium has a significant negative impact on the functions of the nervous system, causing symptoms such as headache, Parkinsonian-like symptoms, slowed vasomotor activity, peripheral neuropathy, encephalopathy, impaired balance, vertigo, and diminished capacity for concentration, bleeding, olfactory dysfunction, and cognitive impairments;[5] and thus, oxidative stress in cadmium has been connected to its poisoning, to which the central nervous system is specifically vulnerable, causing these neurological disruptions and modifications to the typical neurochemistry of the brain.[6] In the brain, cadmium has been proven to cause oxidative stress via reactive oxygen species (ROS) induction.[6] This occurs when cadmium interacts with mitochondrial sites, breaking down the potentials of the mitochondria and lowering intracellular glutathione levels as a result. The brain produces more free radicals when cadmium is present. It modifies the antioxidant defence mechanism, which alters lipids' structural integrity and subsequently impacts membrane-bound enzymes.[6] Thus, the antioxidant enzymes in the cerebellum were infiltrated by cadmium exposure of rat cerebellar granule neurones, which led to neuronal death.[6] According to Wahdan *et al.*, the cerebellum was seen to have a greater concentration of cadmium than the cerebrum, with the vermis exhibiting the greatest accumulation.[7] The study revealed varied vascular alterations, oedema, capillary dilatation and distortion due to loss of their epithelial lining, blood vessel congestion with perivascular oedema, and vacuolations in all layers of the cerebellum after cadmium was administered.[7] However, longer periods of cadmium exposure resulted in more severe vascular alterations. Following these aforementioned disturbances in the biological system, this research employed histological, biochemical, and neurobehavioral analyses to ascertain the cellular, neurological, and lipid profile alteration, respectively, as well as the curative role of the dietary supplement used. Many studies have demonstrated that essential dietary supplements are vital in protecting against Cadmium-induced neurotoxicity by competing for enzymatic protein binding sites and triggering the production of the CNS-specific metallothionein III, which binds to Cd

and detoxifies it.[8] Of these natural medicinal products which promote human health, rosemary oil has been considered for its medicinal use.

Rosemary (*Rosmarinus officinalis L.*), family Lamiaceae, is an important aromatic and therapeutic plant that is used extensively as a spice and herb and has been grown for ages. Anthropological and archaeological evidence suggests that herbs from rosemary were utilised in preparations of traditional medicines, culinary products, and cosmetics in ancient China, Egypt, India, and Mesopotamia.[9] Plant breeders are now interested in it since it offers a global view on plant culture.[10, 11] Planting of rosemary is done so that its rich oil can be extracted when the plants are harvested, even before the flowers bud.[9] Aromatherapy, the fragrance and flavour industry, antioxidant activity, antibacterial, and antitumor characteristics are all applications for rosemary essential oils.[12, 13, 14] Additionally, it is used for culinary flavouring, cosmetics, and local medicine for hepatoprotective, choreatic, and antitumorigenic activity.[15] The essential oil soothes neuralgic pain, improves limb blood circulation and has antirheumatic properties. Research and reports on rosemary essential oil constituents can be found in different literatures.[16] The need for *R. Officinalis* is rising due to its application in agro, pharmaceutical sectors, and traditional medicine.[17] Research has indicated that a synergistic antioxidant effect is produced by the combination of various tocopherols, products of the Maillard reaction, peptides, flavonoids, phenolic acids, ascorbic acid, catalase, superoxidase dismutase, and reduced glutathione.[18, 19, 20] Rosemary essential oil has been known respectively to exhibit an anti-oxidative effect.[13, 14] However, there is limited or no information on the effect of rosemary essential oil on cadmium-induced neurotoxicity in the cerebellum. Hence, the researchers seek to investigate the anti-oxidative potential of rosemary oil in cadmium-induced neurotoxicity in the cerebellum of adult Wistar rats.

Materials and Methods

Materials
Twenty-five (25) male Wistar rats, normal saline, stopwatch, methanol, filter paper, grinding machine, 5 ml syringes, plan container, funnel, Pasteur pipette, conical flask, weighing balance, universal containers, glass slides, coverslip, 10% formalin, Bouin's fluid, cotton wool, tissue cassette, graded alcohol (70 %, 90 % and absolute alcohol), hot plate, water bath, enzymatic colourimetric test kit (AssayGenie, Ireland; Model BA0084), water bath, hematoxylin, and eosin stain, etc.

Experimental Animals
For the experiment, a total of twenty-five (25) male Wistar rats weighing between 120 and 160 g were procured from the animal house of the Faculty of Basic Medical Sciences, Alex Ekwueme Federal University Ndufu Alike Ikwo, AE-FUNAI. Two (2) weeks of acclimatisation preceded the start of the experiment. The rats were sheltered in conventional settings and given access to water and regular rats' pellets. With a temperature range of 27–290C and a 12-hour day/light photoperiod, the animal chamber was adequately ventilated.

Rosemary Essential Oil
Pure (100%) rosemary essential oil, brand name Aroma Palace, with a net fluid ounces (1 fl. Oz)- 30 ml, extracted by steam distillation (based on the manufacturer's description) and produced from India, was purchased from Margret Umahi International Market, Abakaliki; Ebonyi State. Description based on the manufacturer: An evergreen shrub with numerous branches, ash-coloured scaly bark, and leathery, thick leaves, which are lustrous and dark green above, and white underneath.

Cadmium Chloride ($CdCl_2$)
Cadmium Chloride ($CdCl_2$), of the highest purity grade, was obtained from Sigma-Aldrich Chemical Company (St. Louis, MO, USA).

Experimental Design
Twenty-five (25) male Wistars from AE-FUNAI were housed under conventional settings and given access to feed and water *ad libitum*. Randomly, the rats were selected and divided into five (5) groups, consisting of five (5) rats per group after seven (7) days of acclimatization. Group A served as the control and received normal saline, feed, and water *ad libitum*. Group B was the untreated cadmium, which received 6 mg/kg of cadmium chloride, feed, and water. Group C received 6 mg/kg of $CdCl_2$ + 200 mg/kg of essential oil of *Rosmarinus officinalis* **(**EORO) and feed and water. Group D, the medium dose received 6mg/kg of $CdCl_2$ + 300 mg/kg of EORO. Finally, group E was induced 6mg/kg of $CdCl_2$ + 400 mg/kg of EORO. All administrations were done orally using oral gavage. Cadmium chloride induction lasted for 7 days before treating animals in Groups C, D, and E with EORO. Group B, which served as cadmium untreated, only received cadmium without treatment.

Neurobehavioural studies
The neuro-behavioral study of the experimental animals (Wistar rats) was carried out using an open field test. This method was used to ascertain the depression, anxiety, and locomotor activities of the adult Wistar rats. The training period (pre-test) was done before administration, and the testing was done after the experimental period before the animals were sacrificed.

Assessment of locomotor activities and Procedure of the Open Field Test
The open field apparatus was made of plywood and had 40 by 40 cm walls. Its floor could be detached, and a marker was used to draw blue lines that divided the floor into sixteen 18 by 18 cm squares, each of which was smooth and polished in white and grey.[21] The rats were released and placed in a random pattern in the middle of the open field, facing the centre. They were then given five (5) minutes to explore the apparatus, during which time their movements were watched and captured on camera. Following the five (5) minutes, the rats were put back in their home cages, and cleaning of the open field was done using water and left to dry between tests.[22, 23]

Animal Sacrifice and Sample Collection
During the end of the experimental period, all the animals were subjected to overnight fasting, anaesthetized using diethyl ether, and sacrificed through cervical dislocation. Blood samples were collected using hematocrit tubes inserted into the superior orbital vein and complemented with a cardiac puncture for a sufficient blood volume prior to sacrifice for biochemical analysis. The animals were decapitated and skinned, and the skulls were fixed in Bouin's fluid. The rats' skulls were excised, and after 48 hours the cerebellum was harvested and further refixed in 10% formalin for histological tissue processing.

Analysis of Biochemical Parameters
After the rats were sacrificed, blood samples were taken and centrifuged at 1000 rmp for 10 mins and the serums were used for the estimation of the serum lipid profile parameters.

Total Cholesterol (TC) Estimation
The enzymatic colorimetric test kit according to the Siedel method of total cholesterol (TC) estimation.[24]

Serum Triacylglycerol (TG) Estimation
The triglyceride concentrations were estimated by the method of Negele.[25]

HDL-cholesterol (HDL-C) Estimation
HDL-cholesterol estimations were carried out according to Siedel method for total –cholesterol estimation.[24]

VLDL-cholesterol Concentration (VLDL-C) Estimation
Equation 1 shows how the concentrations of VLDL-C were determined with the ratio of VLDL to total plasma triglycerides as 1:5 in subjects who are fasting and have a triglyceride content of 400 mg/dl.

$$\text{VLDL} - \text{C (mg/dl)} = \frac{\text{Triglyceride (TG)}}{5} \text{. equation 1}$$

LDL-Cholesterol Concentration (LDL-C) Estimation
According to Friedewald's relationship, the difference between the blood total cholesterol (TC) and the total cholesterol of HDL and VLDL was used to calculate LDL cholesterol.[26]

LDL-C = TC - (HDL-C + VLDL-C). equation 2

Histological Study
Using a 10 % buffered formaldehyde, the brain tissues underwent a fixation process for 24 hours. Then, the tissues were dehydrated in graded levels of ethanol, viz., 70 %, 90 %, and absolute alcohol. For each alcohol level, the duration was 2 hours (for the absolute, three changes of absolute alcohol for 2 hours were observed too).

Next, the tissues were cleared in three (3) changes of xylene for 2 hours each, followed by infiltration in molten paraffin wax in the oven at 58 ºC for 2 hours. The tissues were embedded in paraffin wax using Leukhart embedding moulds, and blocked-out serial sections of 5µm thick were cut from a paraffin-embedded block. At 250 µm from the preceding section, cut sections were fixed on a clean grease-free slide coated with 10% ethanol to help cement the cut section properly. Cut sections were stained with hematoxylin & eosin (H & E), after they which were hydrated in decreasing grades of ethanol (three changes of 100 % ethanol, 90 %, and 70 % ethanol), passed through water, cleared in xylene and mounted using DPX. The tissue sections were oven-dried between 35 ℃ and 40 ℃ after being cleared in xylene, as previously mentioned by Sheehan and Hrapchak.[27] Following a qualitative and quantitative assessment of the slides using a research microscope attached to a computer monitor, photomicrographs were taken and analysed.

Statistical Analysis
The generated data were reported as mean ± standard error of mean (SEM). Furthermore, one-way ANOVA and paired student's t-test was utilised in determining the statistical differences in the groups' mean and also in comparing the results, respectively. The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL) software, version 23, was used to state the statistical significance level at $p < 0.05$.

Results and Discussion

It has been observed in biological systems that some cellular organelles and components, such as the cell membrane, mitochondria, lysosomes, endoplasmic reticulum, and enzymes related to metabolism, detoxification, and repair of tissue damage, are target sites of heavy metals, Cd in particular.[28] However, this study aimed to ascertain the neuro-curative role of rosemary essential oil on cadmium-induced locomotive disorder in the cerebellum of adult Wistar rats, and the results are discussed below.

Changes in Body Weight
Body weight changes of the adult Wistar rats that were induced with cadmium and treated with different doses of rosemary essential oil are shown in Table 1.

Body weight changes are used as a significant indicator for checking rats' health status, and reduction in body weight reveals worsening of the experimental rats during the experimental period.[29] A decrease in body weight during the toxicity of cadmium indicates its toxic effects.[30] In this study, there was a statistically significant decrease in body weight in group B (untreated) when compared to group A (control) at $p < 0.05$, as shown in Table 1. A similar finding is associated with the works of Poli and Renuka.[30, 31] Nevertheless, it has been noted that tissue damage and a decline in their activities are due to Cd accumulation, which upsets rats' overall body weight.[32] A visible low food intake (anorexia, food avoidance, or post-meal palatability) is caused by Cd toxicity, which includes the induction of oxidative stress and alterations in antioxidant status that lead to loss of weight and severe metabolic disorders.[30, 31] Additionally, the administration of Cd causes severe inflammation, distress, and pain, which in turn impairs the animals' movement and appetite. Contrarily, Young *et al.* showed that administering cadmium increased body weight.[33] This variation can be linked to the differing routes of exposure as well as the cadmium dosages used in the studies. However, previous studies involved administration of cadmium in water, while this study targeted full dose administration of cadmium directly to the rats' stomachs using the oral gavage method.

Furthermore, there was a statistically significant increase in body weight of the curative groups C, D and E which were given 200 mg/kg, 300 mg/kg, and 400 mg/kg of EORO as compared to group B untreated at $p < 0.05$ as shown in table 1. A similar finding was observed in the work of Seyidoglu *et al.*[34] However, Contradictory evidence was reported by Basheer *et al.*[35] in which rosemary oil extract treatments dosed at 250, 500, and 1000 mg/kg resulted in an increased mortality rate in newborns compared to the control group, as well as a significant ($P<0.05$) decrease in average maternal birth weights; weights between periods 7, 14, and 21 days, and birth weights. While this study used lower dosages (200, 300, and 400 mg/kg) and shorter administration period, it is possible that these changes were caused by increasing the dose of rosemary oil extract, which led to an increased number of neonatal deaths. Additionally, Abou-hashem reported that the use of an alcoholic extract of rosemary oil resulted in a reduction in the body weight of the white rats due to a decrease in appetite.[36] Finally, compared to rats treated with Cd, administration of rosemary essential oil significantly reduced the changes in body weight caused by Cd. Rosemary essential oil modulated the intracellular antioxidant systems by inducing the initiation of NRF2 target genes and boosting the glutathione level, which was depleted after consumption of cadmium with an increased GSH compared with its oxidized form, GSSG.[49, 50]

Table 1: Body weight changes in adult Wistar rats administered Cadmium chloride for 7 days and treated with varying dosages of Rosemary essential oil for 14 days

Group	Initial weight	Final weight	Weight diff.
A	120.33±0.88	152.00±1.53	31.67±0.65
B	133.00±1.20	129.00±0.58	-4.00±0.62*
C	112.00±0.58	125.00±2.65	13.00±2.07**
D	130.00±1.16	140.00±1.16	10.00±0.00**
E	150.33±2.60	159.67±1.76	9.34±0.84**

*Significant decrease at P<0.05 compared to A; **Significant increase at P<0.05 compared to B

Neurobehavioural Analysis
Learning and memory deficits associated with neurotoxic symptoms of anxiety and depression-like behaviour were caused following exposure to cadmium chloride. The open field test is frequently used to measure anxiety, mobility/locomotion, and exploratory behaviour.[40] The number of lines crossed, freezing, and frequency of rearing are typically used in this test to ascertain locomotor activity, while grooming, amounts of faecal pellets, centre square duration/entries, and time closed to the wall are markers of anxiety.[41] In this research, rats exposed to cadmium chloride were seen to show locomotive impairments and exploration when compared to the control, evident with a significant decrease in line crossing (LC) and an increase in freezing duration (FD) in group B (untreated) as compared to A (control) as shown in table 2. Similar findings were observed by Adaze and Churchill, Moreira *et al.* and

Montgomery *et al.* demonstrating that heavy metal poisoning affects the brain regions involved in coordination and motor control.[42, 43, 44] Numerous studies have shown susceptibility of the cerebellum to intoxication and poisoning.[2] The cerebellum is an anatomical component that regulates and coordinates both the motor and non-motor processes. However, lesions in the cerebellum cause dysfunction in motor coordination and balance, and Purkinje cells are considered frail upon exposure to heavy metals [45]. Contrastingly, Dogra *et al.* and Kouadria *et al.* have shown that oral administration of cadmium dramatically increased the number of lines/cells crossed and recoveries, indicating locomotor hyperactivity in rats that have been poisoned as opposed to rats that have not been inebriated. They attributed the increase to heavy metals (cadmium) actions on the dopaminergic systems, leading to increased release of the neurotransmitter, synthesis, and hyper-functioning postsynaptic receivers.[46, 47] No statistical differences were observed in the number of grooming (NG), number of rearing (NR) and center square entries (CSE) across the groups. However, there were statistical decrease in the rearing duration (RD) and grooming duration (GD) in groups D and E when compared to group B untreated. This clearly shows the antioxidant action of rosemary essential oil to attenuate the locomotive impairment following cadmium exposure.

Table 2: Effect of 14 days treatment with Rosemary essential oil on locomotive activity and anxiety following 7 days Cadmium induction on adult Wistar rats

Groups	A	B	C	D	E
LC	46.50±1.50	24.00±12.42*	43.00±3.00^	34.67±6.36^	34.67±8.82^
CSE	3.50±1.50	0.33±0.33	2.00±0.00	2.33±0.33	1.00±0.00
CSD	7.00±0.00	0.33±0.33*	1.50±0.50	4.33±0.88^	1.33±0.33
NR	7.00±1.00	7.33±3.71	8.50±3.50	7.67±1.45	6.00±1.52
NG	6.00±1.00	5.67±2.96	4.00±0.00	3.00±1.00	3.00±1.00
RD	11.50±1.50	13.33±6.67	14.00±6.00	9.00±0.58**	8.67±1.76**
GD	8.50±1.50	11.67±6.01	11.00±1.00	5.33±1.20**	5.67±1.76**
FD	17.50±2.50	181.00±56.07#	72.50±2.50**	66.67±8.82**	79.00±14.64**
TSCW	27.50±2.50	203.33±45.86#	77.50±2.50**	75.00±2.89**	81.67±10.93**

*Significant decrease at P<0.05 compared to A; **Significant decrease at P<0.05 compared to B; ^Significant increase at P<0.05 compared to B; #Significant increase at P<0.05 compared to A

Also, the level of anxiety of the animals was observed, which showed a significant increase in time spent close to the wall (TSCW) and decrease in center square duration (CSD) in group B (cadmium untreated) as compared to group A (control). This indicates that administration of cadmium exerts an anxiogenic effect on the rats according to Adaze and Churchill.[42] Similar findings were observed by Lamtai *et al.*, Moreira *et al.* and Montgomery *et al.* in which cadmium administration led to a significant increase and decrease in time spent in the periphery and centre square duration, respectively, as compared to the control.[48, 43, 44] Contradicting findings were observed by Kouadria *et al.* [47] This study's evaluation of a variety of fear-related behaviours, including locomotion, freezing time, exploring activity, and emotional state, showed a marked reduction in anxiety-like behaviours after administering rosemary essential oil. It has been suggested that rosemary essential oil regulates intracellular antioxidant systems by inducing the initiation of target genes for nuclear transcription factor (NTF)2 and raising glutathione levels, with an increase in reduced glutathione (GSH) relative to oxidised glutathione (GSSG).[49, 50] This outcome further supports that Limonene, a major constituent of rosemary essential oil, enhances anxiolytic effects in a variety of animal models according to Neha *et al.* and Eddin *et al.*,[51, 52] indicating limonene's distinct beneficial effects of having both antioxidant and anti-inflammatory potentials, which can be used in treating or preventing neurodegenerative diseases (NDs). While the precise mechanism underlying its anxiolytic activity is still up for debate, research has indicated a possible correlation between antioxidants and a reduction in oxidative stress in the brain.

Lipid Profile Analysis

Several studies have documented the role of cadmium-induced oxidative stress in the alteration of lipids and lipoproteins. This is associated with the generation of free oxygen radicals that leads to the oxidative deterioration of proteins and lipids. [37] According to table 3, no statistically significant differences were seen in lipid profile parameters across the groups in this study. Conversely, oral administration of cadmium was found to significantly mitigate the adverse metabolic effects in rats after 28 days and 90 days (3 months), as reported by Samarghandian *et al.* and *Chen et al.* The exposed rats' serum levels of Cd were higher in TC, HDL-C, LDL-C, and TG than those of the control animals.[37, 38] This incongruence in lipid profile parameters seen across the groups in this study might be attributed to cadmium administration over a short-term frame compared to previous studies that had a 28-day and 90-day (3 months) administration of cadmium, and also might be due to the dose of cadmium intake. However, in the case of assessing the effects of EORO in diabetic rats, Selmi *et al.* recorded a significant reduction of TC, TG, and LDL levels, while there were no changes in HDL levels.[39] These results correspond with this study, where changes in the level of HDL were insignificant in most groups.

Table 3: Lipid Profile Parameters in adult Wistar rats after 7 days Cadmium induction and 14 days treatment with Rosemary essential oil

Groups	TC	TG	HDL	LDL
A	3.43 ± 0.24	0.78 ± 0.5	0.63 ± 0.12	2.50 ± 0.21
B	2.67 ± 0.18	0.67 ± 0.84	0.50 ± 0.02	1.96 ± 0.26
C	2.72 ± 0.25	0.57 ± 0.21	0.70 ± 0.04	1.76 ± 0.20
D	3.55 ± 0.15	0.65 ± 0.02	0.71 ± 0.05	3.03 ± 0.30
E	2.53 ± 0.17	0.56 ± 0.05	0.59 ± 0.03	1.82 ± 0.14

TC: total cholesterol**, TG:** triglyceride**, HDL:** High-density lipoprotein**, LDL:** Low-density lipoprotein

Histology of the cerebellum

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The haematoxylin and eosin-stained sections of the cerebellum were evaluated under light microscopy as shown (Figure 1-5). As shown, the three layers viz., molecular layer (ML), Purkinje's cell layer (PL), and granular cell layer (GL)—as well as pia matter (triple thick arrows) and healthy neuronal cells are depicted in Fig. 1 in the control group. The cerebellum's normal architecture was shown to be disrupted in rats treated with cadmium after seven (7) days of administration. As illustrated in Fig. 2 these changes were demonstrated as distorted pia matter (red arrow), the molecular layer and granular layer, indicating Purkinje cell depletion and degeneration, and haemorrhage around the neuronal cells (blue arrow). Additionally, rats treated with cadmium had degenerating cells in the molecular layer of their cerebellum, alongside hemorrhagic and necrotic areas (black arrow). Similar observations were noted in the work of Karoui-Kharrat *et al.* and Wahdan *et al.*; presence of hemorrhages in all the parts of the cerebellum seven days post initiation of cadmium administration, leading to complete haemorrhagic cerebellum with increased size and number.[6, 7] Contrarily, Mohamed *et al.* reported the absence of haemorrhage following cadmium administration.[53] In this research, alterations were seen in the granular and molecular neurones of the cerebellum following cadmium exposure. The cells were loose, with a present of necrosis. These findings agree with the work of Wahdan *et al.* who reported developmental retardation and necrosis to neuronal cells caused by cadmium in the cerebellar neurones of developing albino rats—neuronal cells, which were regarded as the most vulnerable to cadmium toxicity.[7]

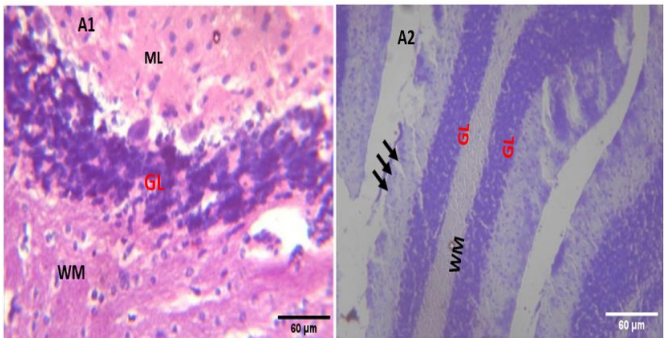

Figure 1: A section of cerebellum showing normal cerebellar cortex with pa matter (triple thick arrows). White matter (WM), molecular layer (ML), granular layer (GL), and healthy neuronal cells. Control group A1 X400 (H & E), and A2 X100 (crystal violet).

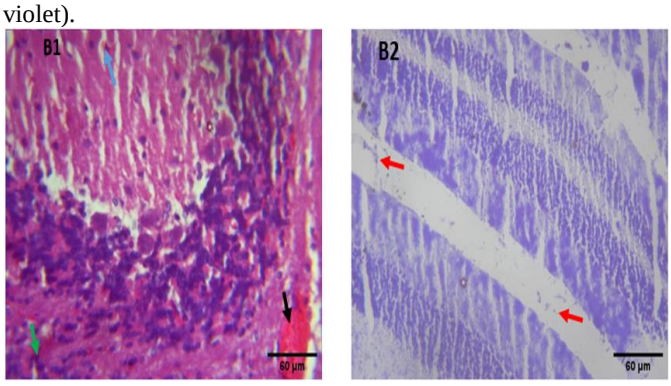

Figure 2: A section of cerebellum showing distorted pia matter (red marrow), hemorrhage around neuronal cells (blue arrow), necrotic area (green arrow), hemorrhagic area (black arrow), B1 X400 (H & E), and B2 X100 (Crystal violet).

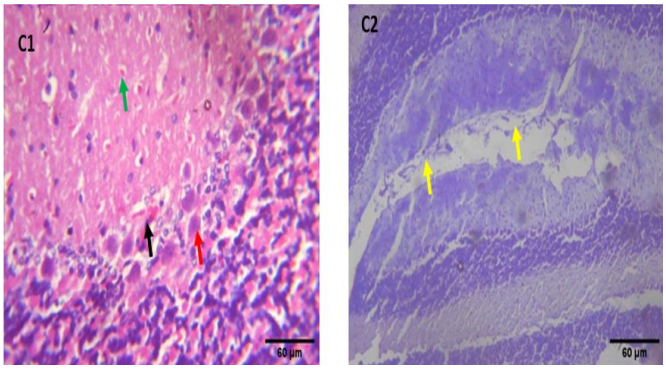

Figure 3: A section of cerebellum showing pia matter (yellow arrows). Distorted nuclei (green arrow), hemorrhage (black arrow), and pyramidal cell (red arrow). C1 X400 (H & E), and A2 X100 (crystal violet).

However, rosemary essential oil was found to ameliorate the alternations occurring in the cerebellum by cadmium, and this may be attributed to its anti-oxidative property by boosting the glutathione level with an increase in its reduced form,[49, 50] as shown in the photomicrographs labelled Fig 3, Fig 4, and Fig 5. Though, in a dose-dependent manner. Group C, photomicrograph Fig. 3 which received 200 mg/kg of rosemary essential oil, showed distorted nuclei (green arrow), hemorrhage (black arrow), and pyramidal cell (red arrow), Group D (fig 4) which received 300 mg/kg of rosemary essential oil showed near normal cerebellar cortex with white matter (WM) and normal/healthy neuronal cells while Group E (fig 5) which received 400mg/kg of rosemary essential oil showed normal histo-architecture presented as normal cerebellar cortex with pia matter (red arrow) and healthy neuronal cells.

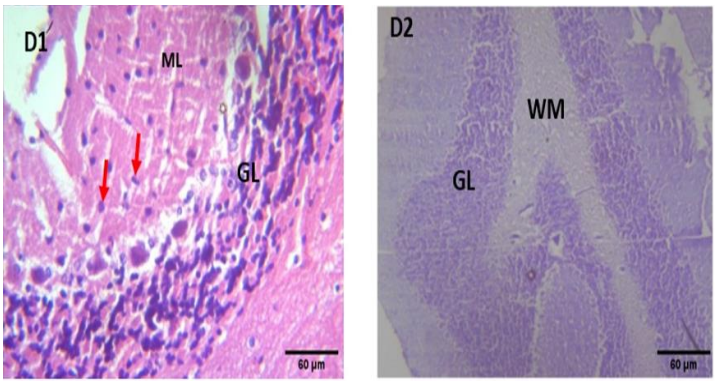

Figure 4: A section of cerebellum showing near normal cerebellar cortex with white matter (WM), molecular layer (ML), granular layer (GL) and healthy neuronal cells. D1 X400 (H & E), and D2 X100 (Crystal violet).

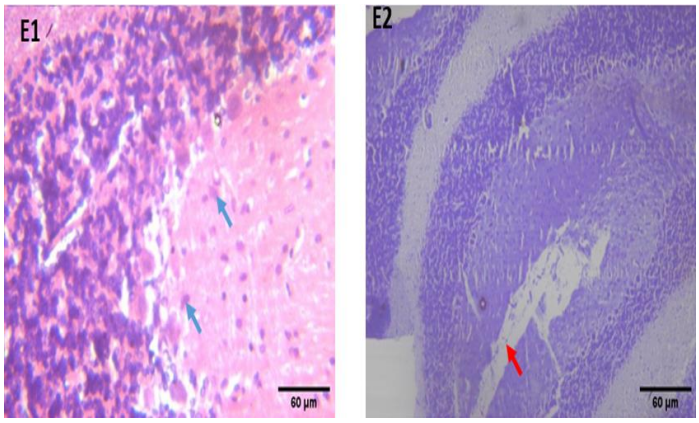

Figure 5: A section of cerebellum showing normal cerebellar cortex with pia matter (red arrow), and healthy neuronal cells. E1 X400 (H & E), and E2 X100 (crystal violet).

Conclusion

Learning and memory deficits associated with neurotoxic symptoms of anxiety and depression-like behaviour were caused following exposure to cadmium chloride as shown in our study. However, administration of rosemary essential oil ameliorates the neurotoxic effects induced by cadmium, though in a dose-dependent manner seen in the photomicrographs: 400 mg/kg rosemary essential oil, which served as the high dose had a better result, followed by 300 mg/kg (medium dose). The use of the essential oil is encouraged in populations prone to toxic levels of cadmium. Also, further studies need to be carried out to ascertain if rosemary essential oil, when administered for longer periods, can totally eliminate toxicity caused by cadmium.

Conflict of Interest

The authors declares no conflict of interests.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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