EFFECTS OF ETHANOL Moringa oleifera LEAVES EXTRACT ON COBALT- CHLORIDE INDUCED HISTOMORPHOLOGICAL AND OXIDATIVE-STRESS DAMAGE ON CEREBELLAR CORTEX OF MALE WISTAR RATS.

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ABSTRACT

Cobalt is an essential cofactor in the body, found in nutrients like vitamin B12. It has been reported that occupational exposure to cobalt chloride leads to neurodegeneration. Presently, neurodegenerative diseases have remains problems of global health concern which necessitates the search for appropriate treatment. *Moringa olefeira* has been shown to possess great amount of flavonoid which established its neuroprotective potential but there is a dearth of information on its effects on Cobalt chloride induced neurotoxicity.

Aims: This study evaluated the effects of *Moringa olefeira* ethanoic extract on cobalt chloride-induced cerebellar cortex damage on adult male Wistar rats.

Study design: Sixty (60) adult male Wistar rats weighing about 120-150g were divided into six groups (A-F) of ten animals each for oral administration for 50days.

Group A (Control): fed with rat chops and water.

Group B: Received 50mg/kg of cobalt chloride

Group C: Received 50 mg/kg cobalt chloride and 200 mg/kg of moringa extract

Group D: Received 50 mg/kg cobalt chloride and 400 mg/kg of moringa extract

Group E and F: Received 200 mg/kg and 400mg/kg of moringa extract only respectively.

Place and Duration of Study: Department of anatomy, Ladoke Akintola University of technology, Ogbomoso, Oyo state Nigeria. Between January 2024 and June 2024.

Methodology: The body weight of the experimental animals were taken weekly and at the 51st day of the experiment the animal were euthanized, the cerebellum was taken out, separated into two halve and one section was homogenized for biomedical analysis [lipid peroxide (MDA) and glutathione (GSH)] while the other half was fixed in formal calcium and processed further for histological study staining with Hematoxylin and Eosin stain

Results: The result revealed insignificant decrease (*P*=.07) in body weight of Group B (cobalt only treated group) conversely the body weight increased significantly (*P*=.01) with groups C, D and E when compared to control, Biochemical analysis shows significant increase (p>0.01) in MDA level of group B while there was a significant decreased in group C and D compared to control whereas the levels of GSH decreased significantly (p=.01) in Group B and increased significant in Group C and D compared with group A, Histological observation shows normal histo-morphology of group A,E and F while there was cortical neurodegenerative changes in Group B, while group C and D showed preserved cerebellar histo-architecture.

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Conclusion: According to this study, *Moringa Oleifera ethanoic* extract has potential Ameliorative effect on cobalt chloride induced cerebellar neurodegeneration in male adult wistar rats.

Keywords: Cortical neurodegenerative, cobalt chloride, glutathione (GSH), Malondialdehyde (MDA)

1. INTRODUCTION

Reactive oxygen species are produced during oxidative stress, which lowers the body's antioxidant defense system and causes lipid peroxidation, disruption of the cell membrane, oxidation of nucleic acids, and ultimately cell destruction. Numerous studies have demonstrated that oxidative stress in several bodily organs and systems, including the kidney, liver, neurological system, and cardiovascular system, may be the mechanism behind the toxicity of medications and some other chemical molecules.(Liu and Pessayre, 2001)Thus, there is a growing interest in learning more about the mechanism and effectiveness of using natural antioxidant compounds to treat toxicity lately, a lot of natural plants and food supplements have been used as antioxidant agents in the different studies to prevent or treat toxicities in the various body systems that are induced by diverse toxicants. The safety, efficacy, availability and affordability of *Moringa oleifera* in comparison with other therapeutic agents make it an excellent choice in the prevention and treatment of toxicities, findings of other investigator have shown that *Moringa* extract administered to experiment rat was reported to reduce MDA levels in acetaminophen induced oxidative stress (Pari and Kumar, 2002) and (Hamza, 2010) Cobalt chloride is frequently used in laboratory study, this makes it a valuable tool for scientists and researcher and it has been established that occupational exposure to cobalt chloride can leads to several health issues including neuronal degeneration (Kuehn *et al.,* 2017)

Oxidative Stress

Oxidative stress is known as an imbalance between the generation of free radicals and their removal by an organism's anti-oxidative systems. Electron transport, which is necessary for energy release, is the foundation of oxidative phosphorylation and other catabolic processes. Electrons travels in the inner mitochondrial membrane from one protein complex to the next. (Sinha *et al*, 2013) As a result, radicals are naturally intermediates in this reaction (Kudryavtseva et al, 2016). Nevertheless, later processes degrade these intermediates. The last electron acceptor in the electron transport chain is oxygen, which leads to the formation of water, which is not a radical. Therefore, it is essential that these cycles of reactions continue without interruption. Issues such as a lack of oxygen in the reactions cause oxidative stress (mitochondrial), which initiates the tissue's antioxidant mechanism (Kagan and Tyurina, 1998).

There have been attempts to classify oxidative stress, ranging from physiological oxidative stress to excessive and toxic oxidative overload, due to the vast range and magnitude of pro- and anti-oxidative compounds (Sies, 2015) Numerous health conditions are significantly influenced by oxidative stress which includes reduction in antioxidant mechanisms brought on by a deficiency of essential nutrients but frequently disregarded mechanisms that perpetuates oxidative stress (Margaritelis, 2018) The opposing process would be a rise in the production of free radicals, which can occur from external sources like inflammation. Oxidative stress has wide-ranging effects on numerous biological functions. All significant macromolecules are harmed by oxidative stress. Apoptosis may be initiated as a result of several cell signaling effects caused by lipid peroxidation, protein oxidation, and DNA

fragmentation (Shirley and Ord, 2014) the mitochondria are the main location where ROS are generated. Through the release of cytochrome C, they can trigger cell death by activating the intrinsic apoptotic pathway (Kirkland *et al*, 2002)

Moringa oleifera

Phytochemical of moringa oleifera

The tropical tree *Moringa* (*Moringa oleifera* Lam.) has many uses. It has several industrial, medicinal, and agricultural purposes, including feeding animals, but its primary purpose is food. This ancient plant, which is drought-tolerant, nutrient-rich, and grows quickly and possessing phytochemicals such as flavonoids, terpenoids, phenolic acids carotenoids and alkaloids,(Ahmadifar et al 2020) was rediscovered in the 1990s.and since then it has gained popularity in Asia and Africa as one of the most commercially useful crops. The media has referred to it as the "tree of life" or the "miracle tree" (Bosch, 2004 and Orwa, 2009).

Medicinal and Pharmacological use of Moringa

Several studies have proven the health benefits of Moringa in both medical research and pharmacological applications. These studies have established that various extracts prepared for moringa oleifera have a number of pharmacological actions, which includes Oxidative Stress (Zhou , *et al*, 2018)Neuroprotective effect (Ray and Guba, 2005) Anti-Venom (Adeyi *et al*, 2020) Antimicrobial agents (Mishra *et al*, 2011)anti-fungal (Upadhyay , *et al* 2015)anti-inflammatory (Abdel-Daim *et al*, 2020)antioxidant (Singh and Navneet, 2018) anticancer (Upadhyay , *et al* 2015)fertility and anti-fertility activity (Attah *et al*, 2020 wound healing (Mishra *et al*, 2011), hepatoprotective activity (Sharifudin et al, 2013)cardiovascular activity (Nandave *et al*, 2009)anti-ulcer (Mallya *et al* 2017), antipyretic activity (Martínez-Gonzálezb, *et al*, 2017), and anti-obesity activity (Bais, *et al* 2014). Activity against Allergies (Bhattacharya *et al,* 2018) Diuretic Activity (Tahkur *et al*, 2016), Cytotoxicity Effect (Parvathy *et al* 2007), Anti-Diabetic Activity Villarruel-(López *et al,* 2018)

Moringa is one of the tremendous plants that has been used since ancient times to treat diseases. Traditionally, the plant's leaf, pod, bark, gum, flower, seed, seed oil, and root have been used to prevent or treat several kinds of illnesses (Stohs and Harman, 2015), including those related to hypertension (Aekthammarat et al., 2019), diarrhea (Misra et al., 2014), and anxiety (Bhat and Joy, 2014). Additionally, it has been claimed that moringa leaves have a protective effect against inflammations, such as glandular inflammation, headaches, and bronchitis (Posmontier, 2011). According to Gothai et al. (2016), the leaves has also been used for wound treatment and insomnia (Liu et al., 2022).According to Gopalakrishnan et al. (2016), the pods are utilized to treat hepatitis and aching joints. Moringa root is used to cure kidney stones (Karadi et al., 2006), liver diseases (Ghasi et al., 2000), inflammation (Paliwal et al., 2011), ulcers (Debnath and Guha, 2007), and health conditions associated with pain in ear and tooth (Mahajan et al., 2007). Additionally, is stated that skin infections and wounds can be treated with the bark of the moringa stem (Rathi et al, 2006). Moringa seeds laxative qualities and ability to reduce oxidative stress (Meireles et al., 2020) that explained its anti-tumor properties on organs like prostate and bladder (Pandey et al., 2012). In both the ancient Egyptian and modern cosmetic industries, moringa is used to make skin ointments

Cobalt chloride

Cobalt dichloride can be found in nature, especially in rocks and minerals but also can be found in soil Cobalt (II) chloride, sometimes called cobaltous chloride or muriate of cobalt, is an inorganic salt that is primarily utilized as a cobalt source in organic synthesis techniques

One of the more colorful salt compounds is cobalt (II) chloride (Cocl2), which has the ability to absorb moisture from the air. Depending on the degree of hydration, it can exist in three different forms: the anhydrous form maintains its blue color, while the hexahydrate form has a pink monoclinic crystal. They serve as reagents in the initial stages of cobalt-related processes (Wojakowska *et al*, 2007).

In relation to cobalt (II) chloride, it's melting and boiling points are as follows: anhydrous melts at 735 °C, dehydrates at 100 °C, hexahydrates at 86 °C, and boils at 1049 °C. Cobalt (II) chloride dissolves in methanol (38.5 g/100 mL), water (52.9 g/100 mL at 20 °C), and diethyl ether (acetone) with a minor solubility. the densities of anhydrous, dehydrate, and hexahydrate are 3.356 g/cm3, 2.477 g/cm3, and 1.924 g/cm3, respectively (Wojakowska *et al*, 2007).

Uses of cobalt chloride

Cobalt dichloride is used by the chemical industry to create certain precursors that are needed to produce other cobalt compounds, whereas cobalt chloride can be used as an indicator to check for the presence of water or to watch chemical reactions. For instance, cobalt dichloride can react with amines or ammonia to generate a large number of cobalt (II) complexes. In addition, it finds application as a constituent of materials with magnetic, thermoelectric, and oxidation-resistant attributes. Water in desiccants is indicated by cobalt (II) dichloride or other cobalt (II) salts. It is an established chemical that induces hypoxia-like responses, including erythropoiesis, is cobalt chloride (Lippi and Franchini, 2015).

Oxygen sensors are essential for keeping an eye on oxygen levels in a variety of settings, such as industrial settings and medical equipment. These sensors use cobalt chloride because of its capacity to change color in response to oxygen content. This characteristic makes oxygen detection precise and trustworthy (Lippi and Franchini, 2015).

Mechanism of toxicity of cobalt chloride

Cytotoxic hydroxy radicals may form when cobalt ions interact with reactive oxygen species. Hydroxy radicals may then cause the production of further free radicals which reduce cellular glutathione concentrations and NADPH activity. The resulting oxidative stress leads to DNA and cellular protein damage (Barceloux, 1999,Maxwell and Salnikow, 2004).

Cerebellum

Cerebellum is a word from latin that connote little brain (Hodos 2009)., it is a structure of the central nervous system and the largest part of the hindbrain, cerebellum is derived from the alar plates (rhombic lips) of the metencephalon with 150g in weight.It lies between the temporal and occipital lobes of cerebrum and the brainstem in the posterior cranial fossa (Standring *et al*., 2008).. It is attached to the posterior surface of the brainstem by three large white fibre bundles.

Histologically, Cerebellum consists of outer gray matter and inner white matter. Cerebellar cortex is the outer gray matter covering mainly the surface of cerebellum while medulla is formed by the inner white matter that made up of central part of cerebellum. Cerebellar cortex is area with highly convoluted and numerous transversely oriented folium. This area is covered neuronal bodies, dendrites, and various synapses. It is histologically divided into three distinct layers (*Llinas et al, 2004)*.

Molecular layer is the outermost layer of the cerebellar cortex and fibres rich portion of the cortex, found adjacent to the pia matter and contains two types of neurons; outer stellate cells and inner basket cells, which are spreads among dendritic arborisation of purkinje cells and numerous parallel fibres of granules cells. Purkinje cell layer (Ganglionic layer) is situated in-between the molecular layer and the granule cell layer (*Llinas et al, 2004)*.

It is a layer of a single row of Purkinje cells bodies in which their dendrites extends into the molecular layer (outer). Meanwhile H&E micrographs show only the cell bodies in a pear shape, there is need of special staining method to make visible the extended branching of

dendrites in the molecular layer. The cerebellar cortex neuronal output is only done by axons of Purkinje cells, Axons of the Purkinje cells has their endings connected to the four cerebellar nuclei (dentate, emboliform, globose, fastigial) and vestibular nuclei. (Schweighofer *et al*.., 2004). The nuclei has an inhibitory effect on purkinje cells (gama-aminobutyric acid, GABA) and facilitates through the inhibition of the cells of deep cerebellar nuclei.Granule cell layer; It is layer between the Purkinje cell layer and the white mater of cerebellum, it consists small granule cells with dark-staining nuclei and scanty cytoplasm. Each cell posse four to five dendrites, their dendrites formed cerebellar glomeruli found in this layer, the parallel fibres of granule cells excite Purkinje cells, basket cells, stellate cells, Golgi cells, Golgi tenson axon and mossy fibre rosette. The Input pathway of cerebellar cortex is through mossy fibers and climbing fibers. Mossy fibers come in to granular layer and form synaptic junction with the granule cells. This synaptic area formed by mossy fibers and granule cell dendrites is within the cerebellar glomeruli. Also in the cerebellar glomeruli located the terminals of Golgi cells. Climbing fibers reach the molecular layer, where one fiber "climbs" the dendrites of the Purkinje cell, winding around them (*Llinas et al, 2004)*.
The cerebellum lies under the occipital and temporal lobes of the cerebral cortex, it is an integral structure in transmitting sensory signals to the motor portion of the brain. It has an important role in motor control, with cerebellar dysfunction often presenting with motor signs (Wolf *et al*, 2009). In particular, it is active in the coordination, precision and timing of movements, as well as in motor learning. Most importantly, the cerebellum is responsible for receiving signals from other parts of the brain, the spinal cord, and senses (Fine and Lohr, 2002). Therefore, damage to this part of our brain often leads to tremors, speech problems (Schmahmann and Jeremy, 2019). , lack of balance, lack of movement coordination, and slow movements. Poor muscle control, irregular eye movements, and poor mobility are results of various cerebellum damages and disorders. Those can be caused by a stroke, inborn anomalies, toxins, or cancer. Cerebellum may also have non-motor functions such as cognition (acquisition of knowledge) and language processing. Damage to the cerebellum can result in a loss of ability to coordinate.

Significance of study

This study was to advance our knowledge of the neurotoxicity of cobalt chloride in male Wistar rats, the histo-morphological effect and oxidative effect of cobalt chloride on the cerebellar cortex in adult male Wistar rats, and the effect of ethanoic moringa oleifera leaves extract on cobalt chloride induced cerebellar cortex damage of male Wistar rats.

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2.0 MATERIAL AND METHODS

2.1 Materials

Experimental cage, Oral cannula, Distilled water, Measuring scale, Syringes, Dissecting set, Hand gloves, Fixative, Laboratory coat, Cover slip, Wood shaving, Mortar and Pestle, Feeding bowl, Drinker, Surgical Gloves, Glass specimen bottle, Digital weighing balance, Glass slides, Paraffin wax, Cotton wool and staining jars, Freezer, Water bath, and Microscope

2.2 Plant material

The fresh *Moringa* leaves were harvested from Mr. /Mrs. Olaniyan's land in Ogbomoso Oyo State, Nigeria in the month of January, 2024

2.2.1 Preparation of ethanol extract of moringa leaves

The leaves were identified using voucher numbers LHO-887 at Ladoke Akintola University of Technology, Ogbomoso's Department of Pure and Applied Biology. After drying the leaves at room temperature and grinding them into a pounder form, 1 kilogram of Moringa powder was measured and left to soak for 48 hours in 5 liters of ethanol and then filtered twice by a sterile filter paper (2-µm pore size). A rotary evaporator set at 50 °C was used to condense

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the resulting ethanol extract. Ugwu *et al*. (2013), the residual yield was 50g per 1 kg of dried powder (5%).

2.3 Acclimatization of the experimental animals

Sixty (60) male wistar rats, weighing of 120-150g, were obtained from Calvary breeds animal house ogbomoso, oyo state. The rats were acclimatized for two weeks and the body weight of the experimental animal was obtained weekly. They were provided with standard rat feed and water ad libitum.

2.4 Experimental design

The acclimated animal were divided into six (6) groups of ten (10) animals each
Group A: was given proper care and had access to water and food
Group B: The rats were given Cobalt chloride at the dose of 50mg/kg
Group C: received 50mg/kg of cobalt chloride and 200mg/kg of *Moringa* extract.
Group D: received 50mg/kg of cobalt chloride and 400mg/kg of *Moringa* extract.
Group E: The group received 200mg/kg of *Moringa* extract
Group F: The group received 400mg/kg of *Moringa* extract
The administration of cobalt chloride and *Moringa* extract were done simultaneously orally with the aid of oral cannula for 50days

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Animal sacrifice and collection of organs

The experimental animals were sacrificed via cervical dislocation. The cerebellum was taken out, examined, and split into two halves. One section was homogenized, and used to assay Glutathione (GSH) and lipid peroxidation (MDA). The other half was fixed with formal calcium fixative. Cerebellar cortices were sectioned at 5 µm, and processed for routine histological staining with H&E

2.5 Statistical analysis

Chris Rorden's ANOVA was used to analyze the data collected in one way analysis of variance while comparing within and between groups post-hoc test (Tukey HSD) was used. The results were expressed as mean ± S.E.M. and $p < 0.05$ was taken as the accepted level of significant difference from control.

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3. RESULTS AND DISCUSSION

RESULTS

Table 1: Data analysis of body weights of experimental rats before and during treatment (data presented as the (Mean ± S.E.M)

weeks	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E	GROUP F
week 0	120±1.24	156±1.37*	132±2.5*	146±2.47*	124.6±3.28	142.4±2.24*
week 1	121.5±3.69	156.6±4.68*	135.8±4.03*	142±2.62*	132.5±3.13*	154.8±3.77*
week 2	131.5±5.86	167±3.72*	135.7±5.09	141±3.98	146.2±4.08	161.6±3.96*
week 3	140±7.76	172.4±3.01*	146±1.74	149.7±3.91	151.2±3.43	170.8±5.17*
week 4	155.6±4.33	173±4.49*	147±4.36	152±3.48	150.5±7.62	177±4.56*
week 5	170.2±8.67	175.2±5.72	154.2±7.05	156±4.52	151.5±11.94	189±5.87
week 6	172.6±9.61	163.75±4.6	158.5±9.34	164.3±3.42	169.6±8.11	191±5.77
week 7	184.25±2.24	174±4.88*	180±6.96	172.8±3.99*	180.2±5.19*	198.4±6.42

Significance: P < .05, value was considered significant (*) while value greater than 0.05 was considered insignificant. Values were expressed as Mean ± SEM

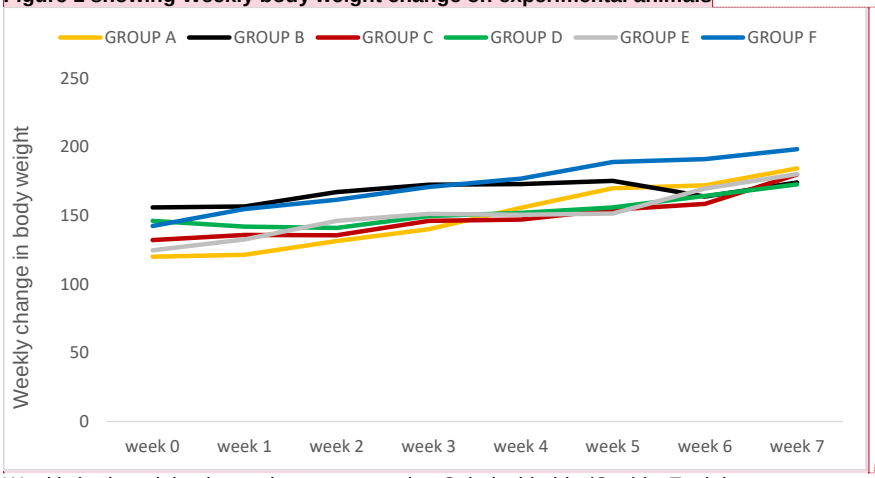
TABLE 2: Showing the initial and final body of the experimental animal (data presented as the (Mean ± S.E.M)

GROUPS	INITIAL WEIGHT(g)	FINAL WEIGHT(g)	WEIGHT GAIN (g)
A	120±1.24	184.25±2.24	64
E	124.6±3.28	180.2±5.19*	56
F	142.4±2.24*	198.4±6.42	56
B	156±1.37*	174±4.88*	18
C	132±2.5*	180±6.96	48
D	146±2.47*	172.8±3.99*	27

Level of significance, P< .05. All values less than 0.05 are statistically significance (*)

The Table 1 shows the body weight gain of the experimental groups, the body weight of the experimental animal increase across.

Figure 1 showing Weekly body weight change on experimental animals



Weekly body weight change in rats exposed to Cobalt chloride ($Cocl_2$). Each bar represents Mean ± S.E.M,

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Table 2: Demonstrates the action of Moringa ethanoic extract on Malondialdehyde (MDA) and Glutathione (GSH) in experimental rats.

GROUPS	MDA (µmol/L)	GSH (µmol/L)
A (CON)	26.87±1.59	1.57±0.1
E (M200)	21.43±1.94$^{\#}$	1.63±0.14$^{\#}$
F (M400)	18.39±2.23*$^{\#}$	1.9±0.12*
B ($COCL_2$)	48.22±2.71*	0.89±0.07*
C ($COCL_2$+M200)	46.31±2.41*	1.32±0.13$^{\#}$
($COCL_2$+M400)	38.42±1.55*$^{\#}$	1.45±0.08$^{\#}$

Presented in Mean ± S.E.M, $^{*}p < 0.05$ against control, $^{\#}p<0.05$ from $Cocl_2$, treatment animal per group =10. CON-control, $COCL_2$-cobalt chloride, $COCL_2$+M200- cobalt chloride+moringa 200mg, $COCL_2$+M400- cobalt chloride+moringa 400mg, M200- moringa 200mg and M400- moringa 400mg

Table 2 Demonstrates the action of Moringa ethanoic extract on Malondialdehyde (MDA) and Glutathione (GSH) in experimental rats.

Malondialdehyde (MDA) levels decreased significantly with Group E and insignificantly with Group F while increased significantly with Group B,C,and D compared to Group A (control). Compared to Group B, MDA levels decreased significantly with Group F and insignificantly with Group E.

Glutathione (GSH) levels increased significantly with Group F and insignificantly with E while decreased significantly with Group B then, decreased insignificantly with C and D compared to control. Compared with Group B, the levels of GSH increased significantly with Group C and D.

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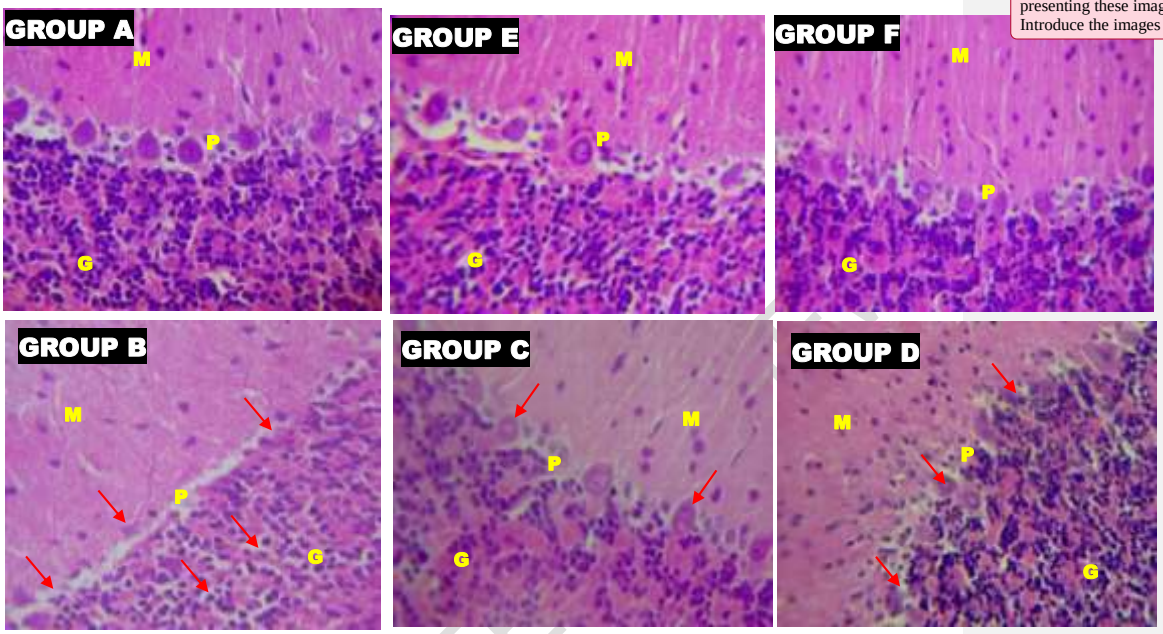

Plate 1: Photomicrographs showing effects of *Moringa oleifera* ethanoic extract on cerebellar morphology in cobalt chloride ($cocl_2$)-administered rats (H &E). The cortical layers; Molecular layer (M), Purkinje cell layer (P), Granule cell layer (G) are demonstrated, The Cortical layer appeared normal in **A,E** and **F** characterized by presence of Purkinje cells and numerous Granule cells. Degenerated Purkinje cells, granular neurons with large open-faced nuclei seen in **B** while a preservation against neuronal degeneration was observed in the **C** and **D**. (Mag.X400)

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4. CONCLUSION

According to this study, *Moringa Oleifera ethanoic* extract has potential Ameliorative effect on cobalt chloride induced cerebellar neurodegeneration in male adult wistar rats.

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ETHICAL APPROVAL

All procedures were carried out in compliance with the approved protocols of the ethical committee Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, and within the guidelines for animal care and use prescribed in the European Council Directive (EU2010/63) for scientific procedures on living animals. Research ethical approval was obtained with identification code (ERC/FBMS/039/2024).

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