Neuroprotective Effects of Moringa oleifera Extract Against Cobalt Chloride-Induced Oxidative Damage in the Cerebellum 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 male Wistar rats weighing between 120g-150g were randomly separated into six groups of ten animals each.

Group A (Control): served as control

Group B: administered 50 mg/kg cobalt chloride

Group C: administered50mg/kg cobalt chloride and 200 mg/kg of moringa extract

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

Group E and F: were administered 200 mg/kg and 400mg/kg of moringa extract 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>.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.

Conclusion: According to this study, *Moringa Oleifera* ethanol leavesextract 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

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 meanwhile complications such as a lack of oxygen in the reactions cause mitochondrialoxidative stress that initiates the tissue's antioxidant mechanism

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 that can results from deficiency of essential nutrients, though lack of essential nutrients is usually disregarded as one of mechanisms that perpetuates oxidative stress (Margaritelis, 2018), the opposing process of this, occurs when there is rise in the production of free radicals that might comefrom external sources like inflammation. These two mechanism are leading the lists of conditions that initiates oxidative stress. 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 therefore, oxidative stress has wide-ranging effects on numerous biological functions and all macromolecules are significantly 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 (Reactive oxygen species) are generated. 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.(Jiang *et al*, 2023)Thus, there is a growing interest in learning more about the mechanism and effectiveness of using natural antioxidant compounds to treat toxicities and oxidative stress 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 (Mthiyane*et al*, 2022)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 (Chen and lee, 2023), oxidative stress is first initiated and accomplished by other health complications such as neuronal degeneration.

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" (Khan and Ali, 2023)

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 (Azlan, *et al*, 2023) 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 (Dhiraj *et al,* 2020).
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 (Tepebaşı *et al*,2023).

Cerebellum

Cerebellum is a word from latin that connote little brain.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(Lara-Aparicio, et al 2022). It is attached to the posterior surface of the brainstem by three large whitefibrebundles.
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 (Kalanjati, *et al,* 2017*)*.
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 adverse effects of cobalt chloride on cerebellum of male wistar rats as well as neuroprotective effects of moringa ethanol extract on cobalt chloride induced oxidative stress on cerebellar cortex of male Wistar rats.

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

2.2Plant 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 inDepartment of Pure and Applied Biology at Ladoke Akintola University of Technology, Ogbomoso. The leaves were air-drying inside a room (room temperature 37^{o}) for two (2) weeks and grounded with mortar and pestle 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 the resulting ethanol extract. The residual yield was 50g per 1 kg of dried powder (5%) (Ugwu*et al*, 2013).

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 and 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

2.5 Dose preparation

The stock solution of the cobalt chloride was prepared by mixing 1g of the substance with 100ml of distilled water.
The crude moringa extract obtained was reconstituted by mixing with distill water according to the two dosages (200mg and 400mg/kg)used in this study, each rat received 1.2mls of the extract. These preparations are done daily to make sure the experimental animals received freshly prepared cobalt chloride and Moringa extract.

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

ezANOVA (Software) was used to analyze the data obtained from the study. Data were analyzed using analysis of variance followed by Tukey's post hoc test. The results were expressed as mean ± S.E.M. and $p < 0.05$ was taken as the accepted level of significant difference fromcontrol

2.6Tissue histology

The harvested brain tissues were fixed in 10% formal-calcium after sacrifice to arrest autolysis, prevent putrefaction and preserve the tissue in a life state as close as possible.

2.6.1 Dehydration

Progressive alcohol grades, ranging from 70% to absolute alcohol, were used to dry the tissues, with the tissues spending at least 1 hour in each grade of alcohol. This was done to remove excess water present in tissues. The tissues were dehydrated in 70%, 80%, 90%, and 95% alcohol for one hour, as well as 99% alcohol for one hour (Aziz and Zeman, 2022).

2.6.2 Clearing

Alcohol was removed from section of tissue by immersing them in ante-medium. The tissues were cleared using Xylene to remove the alcohol and allow the miscibility with wax. (Aziz and Zeman, 2022)

2.6.3Infiltration

Hot paraffin wax that greater than 30 degrees Celsius in melting point was used to saturate the tissues. In order to make the tissues rigid and water-resistant. The automated tissue processing device known as "Histokinette" was used for the procedures (Carson, 2007 and MD ALK, 2019).

2.6.4 Embedding

The tissues were embedded in wax at 71^{o}C using automated embedding machine. The tissues were in blocks of wax at the end of this process as ice was used to solidify the tissue blocks at freezing point. Embedding is done to give the tissues the strength to withstand the squeezing effect of the microtome blade during sectioning. At this stage, the tissues were enclosed in block of wax and this process is referred to as blocking out. The procedure was as follows: The mould was smeared with glycerol to avoid sticking of the wax to the mould. The mould was then filled with molten paraffin wax and the tissue samples were picked with forceps from the cassette and then oriented in the moulds. The corresponding cassette which was placed on the mould after the blocks were solidified; they were subsequently placed on ice blocks to ease removal after which they were gently removed from the mould. Afterwards, the edges of the block were trimmed with hot knife to attain smoothness and then became ready for sectioning (Aziz and ZemanPocrnich, 2022).

2.6.5Sectioning

The tissues were sectioned using a microtome (either using a Rotary or rocking type) at micron (μ) to allow light to pass through them (transparency) during microscopy. After cooling, the cassette blocks were fixed in position on the microtome. A clean albumen-seared slide was dipped obliquely into the water to attach the section after the tissue was serially sectioned using a microtome set to 5 microtomes. The resulting ribbons were then carefully placed on the surface of warm water in a water bath (at a temperature about 10oC lower than the melting point of wax) to allow them to spread out of the tissue with the surrounding wax. The slide was then taken out of the water, blotted dry, and labeled with a diamond pencil (Aziz and Zeman-Pocrnich, 2022).

2.6.7 Dewaxing

The sections were dewaxed using xylene. This was done to allow miscibility with the stains to be used and to ensure clear optical reflection of the tissues when viewed on the microscope (Aziz and Zeman-Pocrnich, 2022).

2.6.8 Staining

Sections were de-paraffilized in two xylene changes for two minutes each. Sections were run through two changes of absolute alcohol for four minutes each in order to eliminate xylene. To enable staining with an aqueous dye, the sections were subsequently hydrated using a sequence of decreasing alcohol concentrations until water was utilized.

2.6.9 Haematoxylin and Eosin

The slides were placed in a metal staining rack then immersed in the filtered Harris Hematoxylin for 10 seconds. The rack removed to a water filled beaker, the water was exchange until the water is clear, after this process, sections were then immersed in EOSIN stain for 30 seconds. Then remove to water until is clear just as the previous procedure. These was followed by Dehydration of the section in ascending alcohol solutions Sheehan (Feldman and Wolfe, 2014)

2.6.10 Photomicrography

The processed histologically slides were viewed with photomicroscope and photomicrograph was taken by attached Camera in the photomicroscope.

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*	125±3.28	142±2.24*
week 1	122±3.69	156±4.68*	135±4.03*	142±2.62*	133±3.13*	155±3.77*
week 2	132±5.86	167±3.72*	135±5.09	141±3.98	146±4.08	162±3.96*
week 3	140±7.76	172±3.01*	146±1.74	150±3.91	151±3.43	171±5.17*
week 4	156±4.33	173±4.49*	147±4.36	152±3.48	151±7.62	177±4.56*
week 5	170±8.67	175±5.72	154±7.05	156±4.52	152±11.94	189±5.87
week 6	173±9.61	164±4.6	158±9.34	164±3.42	170±8.11	191±5.77
week 7	184±2.24	174±4.88*	180±6.96	173±3.99*	180±5.19*	198±6.42

Significance: P <0.05, value was considered significant (*) while value greater than 0.05 was considered insignificant.

TABLE 2: Showing the initial and final body of the experimental animals (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

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

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

Table 3: Demonstrates the action of Moringa ethanoic extract on Malondialdehyde (MDA) and Glutathione (GSH) on cobalt chloride induced 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$^{\#}$
D ($COCL_2$+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.

Histological observation

Photomicrograph (Hematoxylin and Eosin)

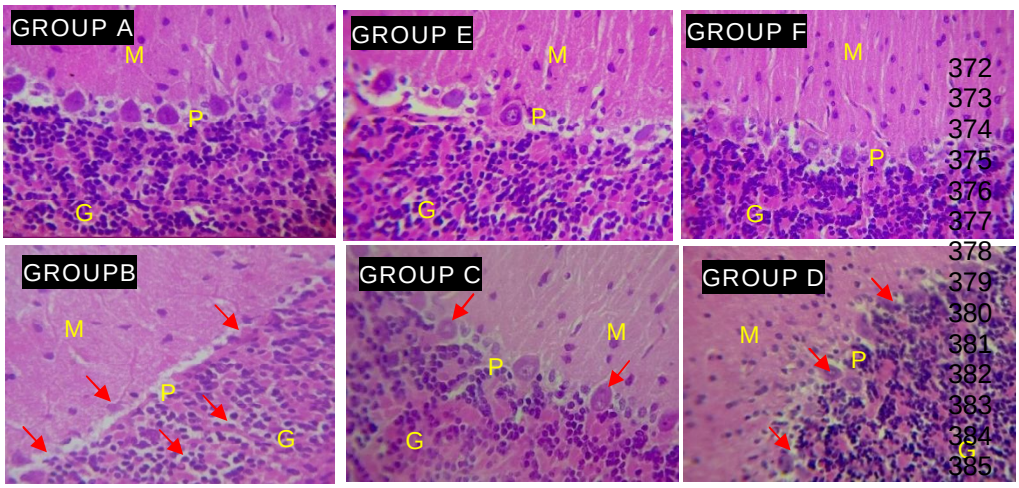

Plate 1: Photomicrographs showing effects of *Moringa oleifera* ethanoic extract on cerebellar morphology in cobalt chloride ($cocl_2$)-administered rats (H &E). The cerebellar cortical layers; Molecular layer (M), Purkinje cell layer (P), Granule cell layer (G) are demonstrated, The Cortical layer appeared 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)

Discussion

This study examined the potential ameliorative effects of Moringa oleifera ethanol extract on alterations in biochemical and histomorphological indicators of cerebellum integrity, oxidant–antioxidant status, and the results showed that Moringa oleifera ethanol extract protected against Cobalt chloride-induced cerebellar damage in Wistar rats.

In this study, weekly body weight decreased in cobalt chloride treated groups compared to control, the effects of cobalt chloride on body weight seen in this study are in line with findings from several other studies that also showed that administration of cobalt chloride resulted to significant reduction in body weight which include findings of sharma and kumar (2014),the reduction in weight must have result from toxic effect and increased metabolism induced by cobalt chloride as suggested by Leggett, (2008). This study show that, Moringa oleifera ethanoic extract mitigated Cobalt chloride -induced weight loss in co-administered groups with group C administered 50mg/kg cobalt chloride and 200mg of Moringa ethanol extract being more effective dosage, the antioxidant capacity of Moringa could explain this, in addition Moringa stimulates appetite (Adedapo et al, 2009) which increased food intake and weight gain. Moringa is rich in nutrients, which could contribute to weight gain or prevent weight lost.

MDA (Malondialdehyde) which is an index of lipid peroxidation (Draper and Hadley 1990) and GSH (Glutathione) were used as oxidative-stress parameters in this study, the level of MDA increased significantly with cobalt chloride treated group while GSH level decreased significantly when compared to control, this confirmed that administration of chloride induced oxidative stress and can deplete antioxidants levels such as glutathione (GSH) that essentially protects neuronal cells from oxidative damage, These findings are in agreement with the reports that exposure to cobalt ions induced oxidative stress "Neurotoxicity of cobalt chloride in rats" by Sharma and kumar (2014) and research done by Akinrinde, et al. (2024) "Protective effect of cholecalciferol against cobalt-induced neurotoxicity in rats". on the other

hand, administration of Moringa extract result to significant decreased in MDA levels and increase in GSH levels in Moringa treated groups compared to control which proved the antioxidant capacity of Moringa and also able to mitigate the neurotoxic effect induced by cobalt chloride on co-administered (Cobalt chloride and Moringa extract) by evidence of significant reduction in the levels of MDA and increase in GSH level.

The histological analysis with Hematoxylin and Eosin staining showed normal morphological presentations of the cerebellar cortex in control (group A) and Moringa only groups (group E and F)characterized by Purkinje cells with conspicuous cell bodies and dendrites that are projecting deep into the molecular layers, the granule layer in these groups consist of compactly disposed small granule neurons. In contract to these, there are loosely arranged and cryptic cells in the granule layers and Purkinje cells degenerated completely with pyknotic cell bodies and short dendritic processes can be seen around the indistinctly demarcated cerebellar layers in Group B(received cobalt chloride only) while in the group C and D (co-treated group) there were preservations against neuronal degeneration damage, showed a mild degenerative changes in Group D (administered 50mg/kg cobalt chloride and treated with 400mg/kg Moringa ethanol extract) when compared to group B and presence of distorted Purkinje cells and clustered granule cells, histological section of Group C (administered 50mg/kg cobalt chloride and treated with 200mg/kg Moringa ethanol extract) was close to normal with a well demarcated cerebellar cortical layers.
The following histological findings, shows that the administration of Moringa oleifera ethanol leaves extract preserved thehisto-architecture of cerebellar cortical layers in group C and D by preventing loss of Purkinje cellsand damage of other cortical tissue that was seen in Group B.This is in line with the findings of Ajibade*et al*..(2021) that Moringa prevents against cobalt induced cerebral damage. These shows that, moringa active components such as flavonoids, phenolic acid, tannins and saponins which established antioxidant capacity of moringa(Vergara-Jiménez et al, 2017) potentiate moringa to have neuroprotective effects on cobalt chloride induced oxidative stress on cerebellum of male Wistar rat.

4. CONCLUSION

This study concluded that oral administration of Cobalt chloride to male rats at a dose of 50mg/kg body weight daily for a period of 50days induced oxidative stress on cerebellum of the experimental rats with the evidence of histological and biochemical damage. The study reveals that administration of Moringa oleifera leavesethanol extract in dosage of 200mg/kg and 400mg/kg has neuroprotective effects on cobalt chloride induced oxidative stress on Wistar rat and preserved against neuronal degeneration caused by administration of cobalt chloride.

COMPETING INTERESTS

Authors have declared that no competing

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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1.
2.
3.

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