Ameliorative Effect of *Prosopis africana* Seed Extract on Cobalt Chloride Induced Cerebellar Toxicity: Neurobehavioural, Histomorphological and Biochemical Findings

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ABSTRACT

Cobalt toxicity from industrial exposure and medical metal prosthesis has been linked to neurological problems such as motor dysfunction. The goal of this study was to find out whether *Prosopis africana* (PA) seed extract had ameliorative effects on the cerebellum of adult Wistar rats exposed to cobalt chloride ($CoCl_2$). 60 male Wistar rats were grouped into 4 (n=15). Rats were treated with $CoCl_2$ or $CoCl_2$ in combination with PA seed extract (PAE) at 50mg or 100mg orally for 14 days. Control rats received distilled water for the same period. The findings showed that $CoCl_2$ caused neurobehavioural impairment in rats by reducing exploratory activities, increasing anxiety, and significantly ($p<0.05$) reducing hanging latency along with a low limb impairment score. Co-treatment with PAE on the other hand, enhanced these parameters to levels comparable to control, reduced hydrogen peroxide and malondialdehyde in cerebellar tissues, while also improving superoxide dismutase and glutathione peroxidase activities. Furthermore, PAE50mg or 100mg significantly ($p<0.05$) reduced proinflammatory biomarkers such as interleukin-1 beta and tumour necrosis factor. In the histology and immunohistochemistry, $CoCl_2$ treated groups showed severe cytoplasmic vacuolations and nuclei fragmentation in Purkinje neurons, as well as elevated astrocytic expression of glial fibrillary acidic protein, which was alleviated by PAE therapy. Thus, when PAE was administered, cerebellar Purkinje cell integrity was improved, antioxidant status was boosted, and lipid peroxidation in the cerebellum was suppressed. Hence, PAE ameliorated $CoCl_2$ induced alterations by reducing oxidative stress, enhancing anti-oxidant enzyme status and decreasing inflammation.

Key words: *Cobalt chloride; Prosopis africana; Neurotoxicity; Purkinje cell; Oxidative stress*

INTRODUCTION

Cobalt (Co) is a metal that can be found in the earth's crust and is located in a range of human tissues. It is extensively distributed in combination with cobalamin (vitamin B_{12}), which remians its core part, and is immensely beneficial in trace amounts (Scharf et al. 2015). Sluggish growth rate, pernicious anaemia, and damage to myelin sheaths are symptoms of Co deficiency (Olivieri et al. 2001). Heavy metals are associated with the aetiology of several neurological

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disorders (Jaishankar 2014): Known for its role in inducing toxicity in biological tissues by bolstering hypoxia inducible factor (HIF) 1, resulting in hypoxia and subsequent oxidative stress by the generation of reactive oxygen species. Co exposure remains a common health concern in prosthetic limb users, workers who use hard cutting tools, miners, and athletes (Mohamed et al. 2019). Furthermore, Co is abundant in the earth's crust, posing a risk of high concentrations in contaminated food and water for humans and animals. For domestic, agricultural, and industrial purposes, the majority of the residents rely on boreholes and shallow (hand-dug) wells to extract groundwater, as well as the few surface water bodies in the area. Consequently, the levels of Co in nearby soil and water bodies rise well above regional background concentrations (Cheyns 2014).

More specifically, degenerative joint diseases such as osteoarthritis have led to the replacement of the knee (Katchy et al. 2018) and hip (Nwadinigwe et al. 2012), with prostheses made of metals, particularly Co, as it provides pain relief and improved joint function. However, there is a risk of metallosis caused by corrosion, which yields soluble metal ions such as Co in nanoscopic sizes, with the lymph and systemic vascular circulation serving as the main channel of dissemination: This has been criticized as it causes neurological disorders including cognitive decline, depression, hearing and visual problems (Keegan et al. 2019). Overproduction of reactive oxygen species and the development of oxidative stress are also linked to Co poisoning (Tan et al. 2009; Kubrak et al. 2011). The pathogenesis of neuronal disorders and neurodegenerative diseases is heavily influenced by oxidative stress. Co exposure at elevated levels has a high risk of causing cell damage and metabolic disturbances in sensitive cells like neurons. There are data on cobalt chloride ($CoCl_2$) toxicity, including hepatotoxicity (Gonzales et al. 2005; Garoui et al. 2011), nephrotoxicity (Garoui et al. 2012), cardiotoxicity (Clyne et al. 2001), and reproductive toxicity (Garoui et al. 2011). Evidences have linked the main cause of these damages to the excessive production of reactive oxygen species (ROS). When ROS levels exceed those that can be decomposed by the body's natural antioxidant defense systems like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), or reduced glutathione (GSH), cellular damage is inevitable. A study also reported a low-cost, commonly available dietary methods to mitigate the harmful consequences of heavy metal exposure (Zhai et al. 2015).

North, Central, and West Africa are homes to *Prosopis africana* (African mesquite), whose fermented seeds are well-known to be a local seasoning (Achi 2005). Medicinally, almost every part of the plant is useful: Bronchitis, dermatitis, tooth decay, dysentery, malaria, and stomach cramps are all treated with the leaves, bark, twigs, and roots of this plant in Mali. In Nigeria, *Prosopis africana* seeds are used to make daddawa or okpeye, used as foods' condiment (Ajiboye et al. 2013). Phlobatannin, flavonoid, polyphenols, tannin, saponin, steroid, and alkaloid have all been found in *Prosopis africana* seed and pod extracts in previous studies (Ajiboye et al. 2013; Olajide et al. 2013). Flavonoids have been shown to protect against oxidative injury by scavenging oxygen radicals, lipid peroxidation, and metal ion chelation (Afanas'ev et al. 1989; Laughton et al. 1991; Erden-Inal et al. 2002), and are particularly beneficial as there possess a number of medicinal benefits, including anticancer, antioxidant, anti-inflammatory, and antiviral properties. They also have neuroprotective and cardio-protective effects (Patel at al. 2018; Zhao et al. 2018; Zhao et al. 2019). In addition, certain natural agents have been shown to protect against $CoCl_2$-induced toxicity. Cerebellar neurons are amongst the population of cells in the central nervous system that are vulnerable to hypoxia especially due to their high metabolic rate. The goal of this study was to investigate if *Prosopis africana* seed extract, a cheap and readily available plant in this part of the Africa can protect Wistar rats from $CoCl_2$-induced cerebellar damage.

MATERIALS AND METHODS

Plant Material
The pods were collected from *Prosopis africana* trees near the campus of Cross River State University's Faculty of Basic Medical Sciences in Okuku, Yala Local Government Area, Cross River State, Nigeria. To begin, the *Prosopis africana* seeds (600 g) were extracted from the pods and boiled in a gas cooker for 5-7 h to soften the hulls so that the cotyledons could be easily removed and separated, and allowed to cool to room temperature. The cotyledons were dehulled and then cooked in a little amount of water for 10 min before draining through a raffia basket. They were allowed to cool before being covered with plantain leaves and left to ferment for three days, according to Ugwu (2018). The fermented seeds were then mashed into flour after being sun-dried to a consistent weight (Yusuf et al. 2008). The flour was then stored at 4°C before use.

Ethical Approval
This study was approved by the Research and Ethics Committee of Faculty of Basic Medical Sciences, Cross River University of Technology, Okuku campus (CRUT/FBMC/REC/21/012). Animals were handled in line with the guidelines for animal research detailed in the NIH Guidelines for the care and use of laboratory animals (NIH Publication No. 85–23, revised 1996 (PHS 1996).

Animals and Experimental Design
Sixty male Wistar rats (150–200 g) used in this study were obtained from the Experimental Animal Unit, Faculty of Basic Medical Sciences, Cross River University of Technology, Okuku campus. They were housed in clean plastic cages in a clean environment of natural day/light cycle (12 h light and dark), at room temperature (28 ℃). Animals were allowed free access to standard laboratory rat chow and water *ad libitum*. The animals were randomly assigned to four groups of 15 rats each and were treated as follows:
Control group received water and food only; $CoCl_2$ only group received $CoCl_2$ (40 mg/kg) for 14 days; PAE50mg + $CoCl_2$ group: received $CoCl_2$ (40 mg/kg) + *Prosopis africana* seed extract (50 mg/kg) for 14 days; PAE100mg + $CoCl_2$ group received $CoCl_2$ (40 mg/kg) + *Prosopis africana* seed extract (100 mg/kg) for 14 days.
The dosages of $CoCl_2$ and *Prosopis africana* seed extract were based on previous studies (Ugwu et al. 2018; Mohamed et al. 2019). The body weights of the animals were measure at the beginning and end of treatment.

Neurobehavioural Tests
Neurobehavioural tests were conducted 24 h after the last administration. The tests were performed in the morning (7-11am) in the neurobehavioural laboratory and recorded live using a digital camcorder and later scored by at least two independent trained observers. The hanging wire and open field neurobehavioural tests were carried out to access motor coordination and locomotor activities, respectively.

Hanging Wire Tests
This was used to measure grip strength and limb impairment. On day 15, the rats were suspended on a steel wire (2 mm in diameter and 60 cm in length) and allowed to hold it with the forepaws at a height of 50 cm over cushion support. To prevent the animal from using all four paws, the hind limbs were gently covered with adhesive tape. The length of time the rat was able to hold the wire till it fell was recorded, with the cut-off time being 180 sec. This latency to the grip loss was the key endpoint used to assess grip strength (Tariq et al. 2005). More so, to access limb impairment, rats were scored 3 for gripping the wire with both hind paws, 2 for gripping the wire with 1 hind paw, and 1 for not gripping the wire with either hind paws. The results were expressed as the total score (Yi et al. 2007). The test was repeated for each rat after resting for 2 h.

Open Field Test (OFT)
The OFT is widely used to study locomotor and exploratory activities in experimental rats and mice. The apparatus consists of a box (72×72×36 cm) made of white plywood and the bottom divided into 18×18 square units with a black marker. The inside of the apparatus was painted white and the floor and one of the walls were covered with Plexiglas so that rats can be through. A central square (18×18 cm) was drawn in the middle of the open field. The animals were placed in the middle of the box and were allowed to explore the area freely for 5 min. The following parameters were obtained during the test; Frequency of locomotion (number of crossings from one square to another), frequency of rearing (frequency of standing only on hind paws), rearing up on the wall (frequency of standing only on hind paws, with fore paws on the wall), stretch attend posture (frequency of forward elongation of the head and shoulders followed by a retraction to the original position), faecal boli (number of faecal boli defecation), centre square duration (time spent within the centre of the OFT box) and freezing (time of complete stationary posture). The open field apparatus was cleaned between each trial with 70% ethyl alcohol to eliminate possible bias due to previous animal odours (Brown et al. 1999; Santiago et al. 2010). All behavioural assessments were then scored by an observer who was blind to the treatment conditions. At the beginning of each session, the rat was placed in the centre of the box, and the parameters were scored as reported by Brown et al. (1999).

Animal Sacrifice and Tissue Preparation
After completing all neurobehavioural tests, blood samples were collected from ten rats in each group via the retro-orbital plexus into plain sample bottles. The blood was allowed to coagulate for at least 1 h and was subsequently centrifuged at 3000 rpm for 5 min to separate the serum, which was later stored in Eppendorf tubes and frozen at −20 °C till use. Whole-brain samples from five rats were homogenized in 50 mM Tris–HCl buffer (pH 7.4) containing 1.15% potassium chloride and were later centrifuged at 10,000 rpm for 10 min at 4 °C using a cold centrifuge. The supernatant collected was used for the determination of biochemical parameters (Akinrinde and Adebiyi 2019).

Biochemical Assays
Lipid Peroxidation
The concentration of hydrogen peroxide (H_2O_2) was determined spectrophotometrically at 560 nm according to the method of Wolff (1994). MDA concentration as an index of lipid peroxidation was estimated using the method of Varshney and Kale (1990). MDA was quantified with a molar extinction coefficient of $1.56 \times 10^5 M^{-1} cm^{-1}$ and expressed as micromoles per gram of tissue.

Antioxidant Enzymes Activities
SOD activity was determined according to the method of Martin et al. (1987). Exactly 920 µL of phosphate buffer (pH 7.8) of 0.05M was added into a clean test tube containing 40 µL of sample; they were

mixed and incubated for 2 min at 25°C. Forty microlitre of haematoxylin solution was added, mixed quickly and the absorbance was measured at 560 nm. Auto-oxidation of haematoxylin as inhibited by SOD, whose percentage inhibition was linearly proportional to the amount of SOD present within a specific range (Martin et al. 1987). The activity of glutathione peroxidase (GPx) was determined with the method of Rotruck et al. (1973).

Determination of Serum Levels of Pro-Inflammatory Biomarkers

The serum concentrations of Interleukin-1 beta (IL-1β) and tumour necrosis factor (TNF-α) were determined according to the manufacturer's instructions using commercially available enzyme-linked immunosorbent assay kits (Elabscience®, UK).

Histological Studies

Rats were euthanized by cervical dislocation and the brain removed. The brain tissues were fixed in 10% neutral buffered formalin. After fixation, tissues were processed for routine tissue processing, and serial slices of 5 µm thickness were produced on a rotary microtome. Tissues were stained using haematoxylin and eosin (H&E) technique for general histological appearance, as described by Ijomone and Obi (2013).

Immunohistochemistry

Thin sections of 5 µm thickness were obtained from routine paraffin-embedded brain tissues. After deparaffinization, sections were subjected to heat mediated antigen retrieval in citrate buffer solution (pH 6.0). Endogenous peroxidase blocking was performed in 0.3% hydrogen peroxide. Sections were then incubated overnight at 4°C in rabbit anti-GFAP (Thermo Fisher, USA; #16825-1-AP) at 1:10000. Secondary antibody incubation was performed in ImmPRESSTM HRP Anti-Rabbit IgG (Peroxidase) Polymer Reagent, made in horse (Vector® #MP-
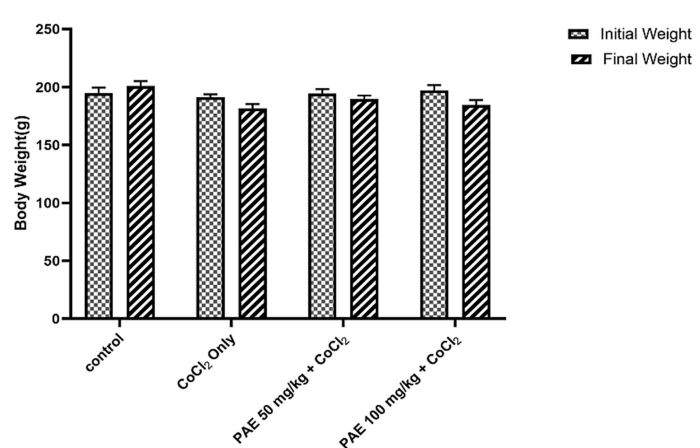
Fig. 1: Change in Weight of Rats Exposed to Cobalt Chloride (CoCl₂) and *Prosopis africana* Seed Extract (PAE)
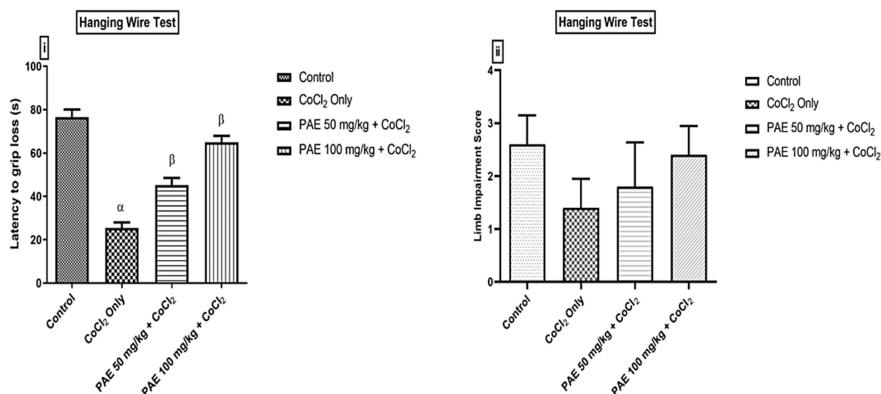
7401). DAB peroxidase (HRP) substrate kit (Vector® #SK-4100) was used for colour development, and sections were counter-stained in Harris haematoxylin.

Image Analysis and Cell Count

Sections were observed under a digital bright field microscope and photomicrographs were obtained at ×400 magnification. Image analysis was performed with Image J software (NIH, USA). Immunoreactivity was quantified by counting positive expressed cells using the cell counter tool of Image J software as previously described (Ijomone and Nwoha 2015; Akingbade et al. 2021).

Statistical Analysis

Statistical analysis was performed using the GraphPad Prism software (version 8.00, GraphPad Software, USA). Results of neurobehavioural tests, biochemical analyses and cell counts were expressed as mean ± standard deviation and analysed using one-way analysis of variance (ANOVA) followed by the Student Newman-Keuls (SNK) for multiple comparisons. The results were statistically significant at $p < 0.05$.

RESULTS

Weight Changes

There was no significant difference in the initial body weights between the groups, but there was body weight loss in all the groups at the end of the experiment. There was a slight change in weight between the initial and final body weight in the CoCl₂ group which was not significantly different ($p > 0.05$). The final body weight of animals in the PAE groups was reduced

Fig. 2: Hanging Wire Test. i) latency to Grip Loss. ii) Limb Impairment of Rats Exposed to Cobalt Chloride (CoCl₂) and *Prosopis africana* Seed Extract (PAE). α -significantly different from the control group ($p < 0.05$). β-significantly different from cobalt chloride only group ($p < 0.05$). Results are presented as Mean ± SD, (n=5)

compared with the initial body weights in the same groups, but the difference was insignificant. However, the control group gained weight at the end of the experiment which was not statistically significant ($p > 0.05$) (Fig. 1).

wire when compared with the $CoCl_2$ group (Fig. 2i). More so, the $CoCl_2$ group had a low score in the limb impairment test, but treatment with either PAE50mg or 100mg improved the score appreciably, although not significantly (Fig. 2ii).
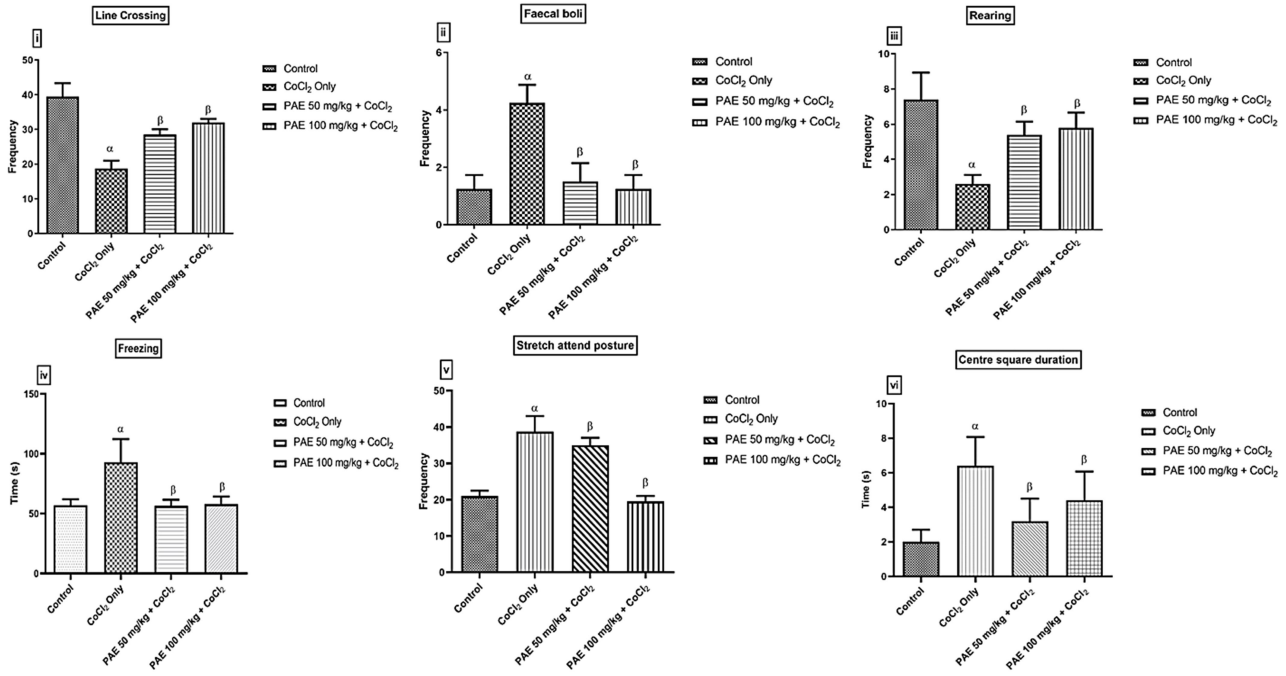

Fig. 3: The Number of Line Crosses, Faecal Boli, Rearing, Freezing Time, Centre Square Duration, and Stretch Attend Posture of Rats Exposed to Cobalt Chloride ($CoCl_2$) and *Prosopis africana* Seed Extract (PAE) in the Open Field Test. α - significantly different from the control group ($p < 0.05$). β - significantly different from cobalt only group ($p < 0.05$). Results are presented as Mean ± SD, (n=5)

Hanging Wire Test
In the $CoCl_2$ group the average hanging time on the wire was significantly decreased ($p < 0.05$) compared to the control group. However, co-treatment with PAE50mg or PAE100mg, produced a significant increase ($p < 0.05$) in the time spent on the hanging

Open Field Test
The result revealed significant differences between the $CoCl_2$ and the PAE50mg or PAE100mg treated groups (Fig. 3): The line crosses were significantly reduced ($p < 0.05$) in the $CoCl_2$ group when compared to the control and PAE50mg or PAE100mg treatment groups (Fig. 3i); likewise, the frequency of rearing was decreased ($p < 0.05$) in the $CoCl_2$ group compared to PAE50mg or PAE100 mg treated groups (Fig. 3iii). The number of faecal boli, freezing time, and stretched attend posture was significantly ($p < 0.05$) increased in the $CoCl_2$ only treated groups in contrast to the control and PAE50mg or PAE100mg treated groups (Fig. 3ii, iv, v). In addition, the $CoCl_2$ group spent more time in the centre of the box
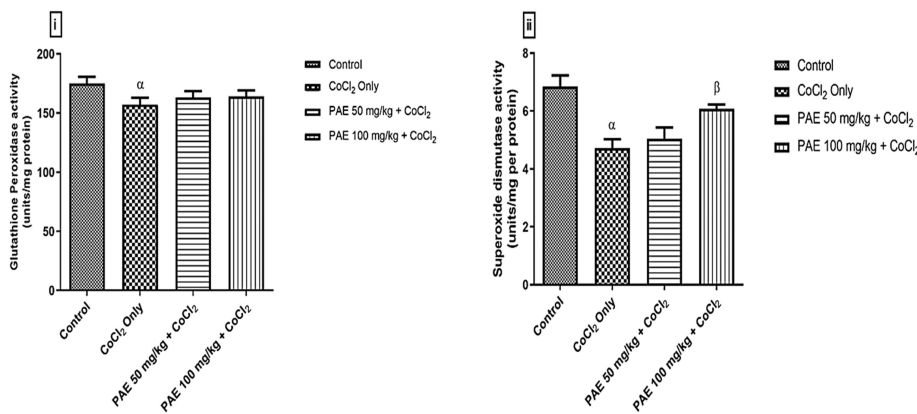
Fig. 4: Effect of *Prosopis africana* Seed Extract on Antioxidant Enzyme Activities on the Cerebellum of Rats Exposed to Cobalt chloride ($CoCl_2$) and *Prosopis africana* Seed Extract (PAE). α - significantly different from the control group ($p < 0.05$). β - significantly different from cobalt only group ($p < 0.05$). Results are presented as Mean ± SD, (n=5)

compared to those in the other groups (Fig. 3vi).

Antioxidant Enzymes

The administration of $CoCl_2$ resulted in a significant reduction ($p < 0.05$) in GPx and SOD activities when compared with the control group (Fig. 4i and ii). However, the simultaneous administration of $CoCl_2$ with either PAE50mg or 100mg resulted in marked increased SOD and GPx activities compared to the rats treated with $CoCl_2$ alone.

Oxidative Stress Markers

The results showed that $CoCl_2$ administration caused significant ($p < 0.05$) increased brain concentration of H_2O_2 and MDA when compared to the control group (Fig. 5i and ii). However, simultaneous administration with either PAE50mg or PAE100mg resulted in significant decreased H_2O_2 and MDA concentration when compared with rats exposed to $CoCl_2$ alone.

Pro-inflammatory Biomarkers

$CoCl_2$ administration produced significant ($p < 0.05$) increased serum levels of pro-inflammatory biomarkers, IL-1β, and TNF-α when compared with the control (Fig. 6i and ii). In contrast, co-administration with either PAE50mg or PAE100mg

resulted in a marked decrease in the levels of these cytokines to levels comparable to that of the control.

Histopathology

Cerebellar histology clearly showed the three distinct layers; molecular, Purkinje, and granular. The molecular layer comprised sparsely spaced small-sized neurons. Purkinje layer consisted of large flask-shaped Purkinje cells with large round nuclei and a basophilic cytoplasm arranged in a single row. The granular layer comprised of numerous small closely packed round granule cells with intensely stained nuclei. Glia cells were also visible in all layers. Control rats showed intact and normal histology of the cerebellum (Fig. 7A). $CoCl_2$ only group showed obvious features of degenerating neurons characterized by Purkinje cell cytoplasmic vacuolations and nuclei fragmentation (Fig. 7B). Also, within the granular layer, several areas of vacuolations in the neuropil were observed. Additionally, many granule neurons exhibited nuclear fragmentation, hence appearing lightly stained. Treatment with PAE50mg and 100mg showed obvious restoration of cerebellar histology (Fig. 7C and D).

GFAP Immunoreactivity

There was astrocytic GFAP expression in the cerebellum of all the groups. In the control group, these glial cells were few with fewer processes. However, after the administration of $CoCl_2$, the astrocytes became more ramified. Similar effects were noticeable in rats co-treated with PAE50mg or 100mg/kg, with PAE50mg showing most ramified, although this did not cut across all the groups. (Fig. 8 A–D). Quantification showed that the number of GFAP-positive cells was significantly higher in $CoCl_2$, and PAE treated rats compared to control (Fig. 8E).
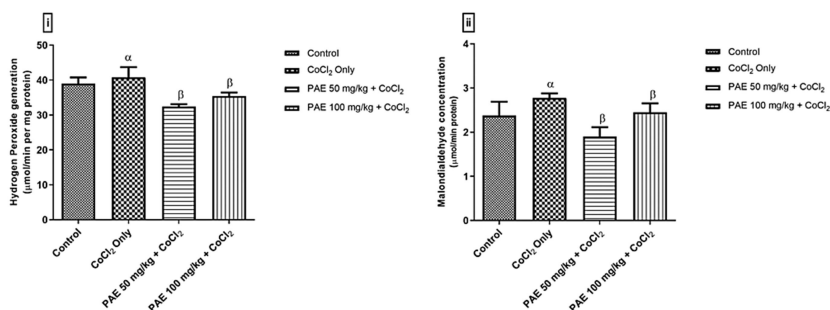
Fig. 5: Effect of *Prosopis* africana Seed Extract on Oxidative Stress Markers on the Cerebellum of Rats Exposed to Cobalt Chloride (CoCl₂) and *Prosopis africana* Seed Extract (PAE). α - significantly different from the control group ($p < 0.05$). β - significantly different from cobalt only group ($p < 0.05$). Results are presented as Mean ± SD, (n=5)
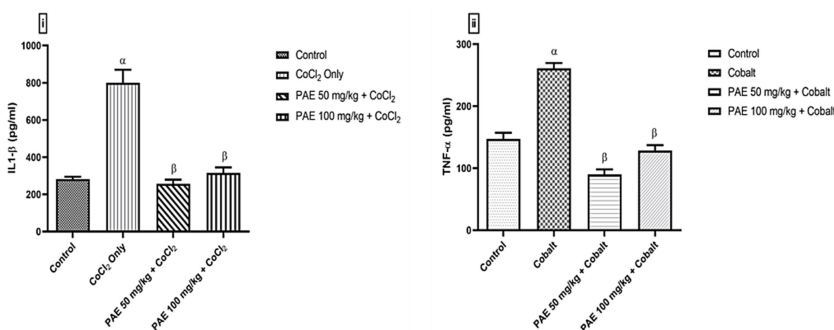
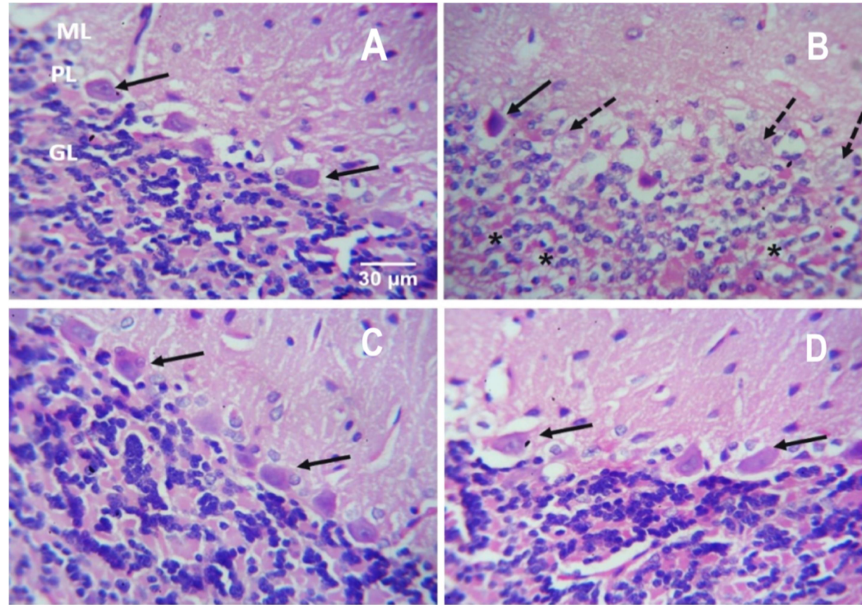**Fig. 6: Effect of *Prosopis africana* Seed Extract on Pro-Inflammatory Markers on the Cerebellum of Rats Exposed to Cobalt Chloride (CoCl₂) and *Prosopis africana* Seed Extract (PAE)**. α - significantly different from the control group ($p < 0.05$). β - significantly different from cobalt only group ($p < 0.05$). Results are presented as Mean ± SD, (n=5)

DISCUSSION

Due to their mutagenic (Jensen and Tüchsen 1990) and carcinogenic (Lison et al. 2001) effects, occupational exposure to various Co compounds is a matter of concern. The current study looked at a variety of toxicity targets in rats exposed to $CoCl_2$, as well as the mechanisms underlying the reversal of Co-induced neurotoxicity by *Prosopis africana* seed extract.

$CoCl_2$ induced cerebellar injuries to the experimental animals but treatment with *Prosopis africana* seed extract showed some ameliorative potentials.

Fig. 7: Histology of Cerebellum of Rats Exposed to Cobalt Chloride (CoCl₂) and *Prosopis africana* Seed Extract (PAE). A - Control group; B - $CoCl_2$ only group; C - PAE50mg + $CoCl_2$ group; D – PAE100mg + $CoCl_2$ group. Arrows – intact Purkinje neurons; Dashed arrows – degenerating Purkinje neurons characterized by cytoplasmic vacuolations and nuclei fragmentation; Asterisks – granular layer neuropil vacuolations; ML – molecular layer; PL – Purkinje layer; GL – granular layer. Scale bar - 30 µm.

Fig. 8: Distribution of GFAP-Positive Astrocytes and Count in the Cerebellum of Rats Exposed to Cobalt Chloride (CoCl₂) and *Prosopis africana* Seed Extract (PAE). A - Control group; B - $CoCl_2$ only group; C - PAE50mg + $CoCl_2$ group; D – PAE100mg + $CoCl_2$ group; E - The quantification of GFAP-positive astrocytes. Red arrows indicate GFAP-positive astrocytes ** $p<0.01$, *** $p<0.001$ compared to control. Scale bars - 30 µm
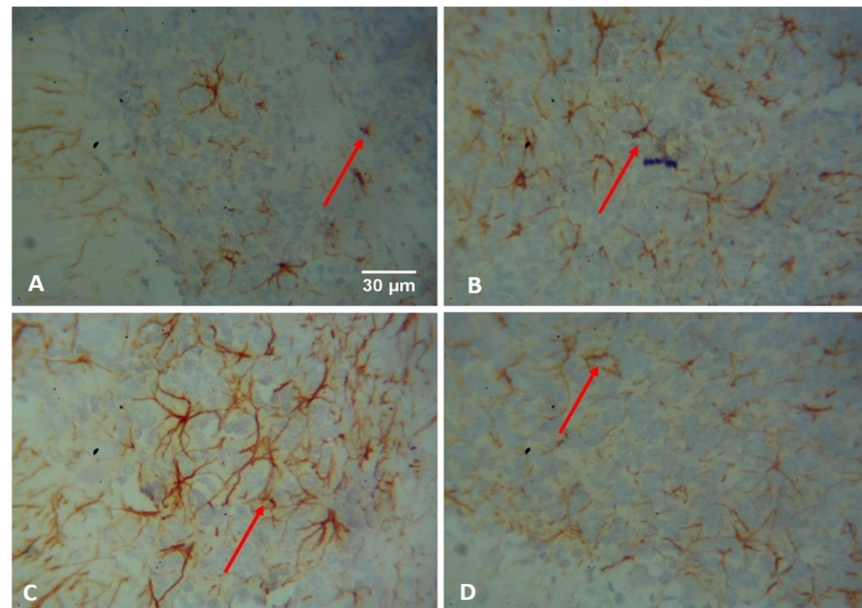
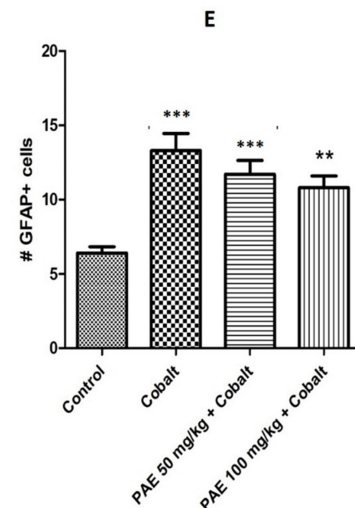The hanging wire test was used to assess grip strength, balance, and endurance. It also serves as a traction apparatus for accessing limb impairment. Exposure to $CoCl_2$ caused motor impairment in the forelimb and significantly decrease the hanging capability of the rats. The rats in the $CoCl_2$ only group had low scores in the limb impairment test as well. However, Co-treatment with PAE improved motor coordination in the forelimb as suggested by the extended duration in latency to fall and improved limb impairment test scores. The present finding agrees with that of Akinrinde and Adebiyi (2019) who reported motor impairment and reduced hanging ability in rats following $CoCl_2$ administration.

According to Prut and Belzung (2003), the open field test is used in rodents to assess motor function, standard exploration, locomotor activity, and anxiety. In the present study, both explorations, as well as motor activities of rats, were altered following exposure to $CoCl_2$ which was evident in the reduction of line crosses, enhanced center square duration and freezing. This corroborates with the report of Akinrinde and Adebiyi (2019) where rats had reduced grid crosses as well as enhanced freezing and centre square duration. Furthermore, the animals in the present study

had increased stretched-attend posture as well as feacal boli which have been linked with anxiety by previous researchers (Shabani et al. 2012; Adebiyi et al. 2018). Co-treatment with PAE50mg or 100mg, however, reduced the frequency of stretched-attend posture and feacal boli to values similar to that of control, indicative of the likelihood of anxiolytic potential of PAE, which also generally improve the locomotor activities of the rat in the open field arena.

The brain is very prone to oxidative damage due to its high consumption of oxygen, as it uses about 20% of blood oxygen, its comparatively low activity of antioxidant enzymes, and the terminally oriented nature of its neurons (Dringen et al. 2000). More so, the membranes of neurons are also rich in polyunsaturated fatty acids which are very susceptible to reactive oxygen species attack, leading to variations in neuronal integrity and function (Mates 2001). On the other hand, induction of oxidative stress in one generally adopted mechanism of cobalt toxicity (Catelas et al. 2005). The results of the current investigation revealed a marked increased production of H_2O_2 and MDA as well as a reduction in the levels of antioxidant enzymes including GPx and SOD. Earlier studies by Akinrinde and Adebiyi (2019) and Garoui et al. (2012) lend credence to our results as they both reported increased levels of H_2O_2 and MDA along with a concurrent reduction in GPx and SOD in the brain of rats following Co administration. However, treatment with PAE50mg or 100mg was effective in reversing the Co-induced oxidative stress by boosting the activities of the antioxidant enzymes, GPx and SOD, while simultaneously reducing the levels of H_2O_2 and MDA. This result corroborates reported antioxidant attributes of PAE (Ugwu et al. 2018).

Mou et al. (2012) reported that neuronal damage is often intensified by a metal-induced stimulation of some glial cells with the subsequent release of inflammatory mediators (cytokines and chemokines). In the present study, exposure to Co led to a significant rise in serum levels of the proinflammatory cytokines, IL-1β and TNF-α. These results agree with those of Mou et al. (2012) who reported a concentration and time-sensitive rise in the levels of TNF-α and IL-1β in N9 and primary mouse microglia following cobalt administration. Similarly, our results are coherent with the findings of Akinrinde and Adebiyi (2019) who also reported a marked increase in the serum levels of TNF-α and IL-1β after treatment with cobalt. Treatment with PAE50mg or 100mg significantly reversed the pro-inflammatory effects of cobalt administration.

Cobalt administration in the present study produced deleterious effects on the cerebellum, which is saddled with motor coordination in the brain. We also looked at the histomorphological features of the cerebellum after exposing the rats to $CoCl_2$. The Co treated group, on the other hand, revealed Purkinje cell cytoplasmic vacuolations and nuclei fragmentation, which are hallmarks of degenerating neurons. This is comparable with Akinrinde and Adebiyi (2019) findings of Purkinje cell disorientation. However, PAE treatment resulted in preservation of the cerebellar histology. Expression of astrocytes in the cerebellum was done using GFAP, a significant protein found in the glial intermediate filaments and widely used as a marker for astroglial cells. The potential role of glial cells in neural plasticity and higher brain functions has been demonstrated, and changes in astrocyte functionality are becoming recognized in an increasing variety of illness situations (Seifert et al. 2006; Allaman et al. 2011). Astrocytes frequently experience a dramatic metamorphosis known as reactive astrocytosis or astrogliosis, which is the most common cellular response to various types of brain insults. We found reactive astrogliosis in the rat brain, which is consistent with earlier research on Co neurotoxicity. After exposure to $CoCl_2$, GFAP immunoreactivity revealed reactive astroglia in the cerebellum. We also observed persistence in a few activated astrocytes even after co-treatment with PAE50mg or 100mg, leading to the hypothesis that reactive astrogliosis is a physiological reaction to the brain's exposure to heavy metals (Akinrinde and Adebiyi 2019).

Conclusion

In conclusion, our findings revealed that Co-induced impairments with motor coordination by altering the Purkinje cell morphology as supported by both histomorphological and neurobehavioural data. The suppression of damage to Purkinje neurons in the cerebellum, on the other hand, seems to be a pathway through which *Prosopis africana* seed extract mitigates the $CoCl_2$ induced alterations. The antioxidant and anti-inflammatory characteristics of *Prosopis africana* likely helped to preserve Purkinje cell integrity.

Conflict of Interest
None declared.

Authors Contribution
RO - conceptualization, experimental design and final draft of manuscript; IO, PM and GE: performed the experiments, resources and data collection; OK and

AE: Analysed the data; RO and IO: Supervision, review and editing.

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