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Research Article

Ameliorative Effects of *Moringa oleifera* Leaf Extract on the Cobalt Chloride-Induced Liver Damage in Adult Wistar Rats

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ABSTRACT

Cobalt is a natural element found throughout the environment. Cobalt is an essential trace element being an integral part of vitamin B12. Cobalt is toxic to virtually all organs of the body and has been shown to have significant debilitating effects on the nervous, renal, hepatic and hemopoietic systems. The liver is considered as one of the target organs affected by cobalt toxicity owing to the site of storage after exposure. Conversely, the leaf extract of *Moringa oleifera* is hepatoprotective amongst other medicinal and nutritional benefits. The present study therefore evaluated the effects of Moringa extract on cobalt chloride-induced liver damage in adult male wistar rats.

40 healthy male wistar rats weighing 90-120 g were used for the study and were divided into 5 groups of 8 animals per group. Group A served as control and was given normal feed and distilled water, Group B was the cobalt-treated group(45 mg/kg), Group C received cobalt (45 mg/kg) and low dose of *Moringa* extract (250 mg/kg) body weights, Group D received cobalt (45 mg/kg) and high dose of *Moringa* extract (500 mg/kg) body weights. The rats were orally administered in their respective dosages for 52 days before sacrifice. The weights of the wistar rats were recorded on weekly basis. On the 53rd day, the wistar rats were sacrificed by cervical dislocation. The liver of each rat was removed and weighed and fixed in 10% normal saline. Blood samples were obtained and used for the analyses of some hepatic marker enzyme. Statistical analysis was done and tested for significance using student's t-test and histological assessment using H and E techniques. The result showed that the final body weights of rats in group C and E increased significantly (p<0.05) and a significant (p<0.05) decrease in the final body weights of rat in group D when compared with the control. There was no significant difference between the control and the treated in the liver weights. The biochemical analysis of hepatic enzymes showed a significant (p<0.05) increase in the activity of Alanine Transaminase (ALT) in group B and D, and significant (p<0.05) decrease in group E when compared with the control. The level of Aspartate Transaminase (AST) showed a significant (p<0.05) increase in group B, C, and D when compared with the control. The level of Alkaline Phosphatase (ALP) showed a significant (p<0.05) increase in group B and D, and decrease in group E when compared with the control. Histological study of the liver revealed that liver parenchyma appeared grossly infiltrated and distorted in group B and C, while group D and E revealed preserved hepatic histoarchitecture similar to that of control group A.

The study concluded that cobalt chloride caused alterations in hepatic tissue consistent with observed changes in enzyme activities. Administration of leaf extract of *Moringa oleifera* ameliorated the deleterious effects of cobalt chloride induced hepatic damage in wistar rats. It is recommended that further studies aimed at corroborating these findings should be carried out.

Keywords: Cobalt chloride, *Moringa oleifera*, Liver, ALT (Alanine Transaminase), AST (Aspartate Transaminase), ALP (Alkaline Phosphatase)

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INTRODUCTION

Cobalt chloride is an inorganic compound of cobalt and chlorine, with the formula $CoCl_2$. It is a sky blue crystalline solid. The compound forms several hydrates $CoCl_2.H_2O$, for n=1,2,6, and 9. The di-hydrate is purple, and hexahydrate is pink. It is usually supplied as hexahydrate, which is one of the most used cobalt compounds in the laboratory (Greenwood NN and Earnshaw A, 1997). Cobalt chloride can be found in nature especially in rocks and minerals but also can be found in soil. It can act a weakly acidic salt; thus, it can be used to react and neutralize weak bases. This reaction generally is exothermic, so that generate heat and should be made carefully. Cobalt chloride is molecule relatively poor to react in REDOX reactions because it does not have enough electrons to lose or gain. Cobalt chloride decomposes at 400°C on long heating in air sublimes at 500°C in HCL gas forming iridescent, fluffy, colourless crystal turns pink on exposure to moist air (O'Neil MJ ,2013).

Cobalt is one of the most common toxic metals affecting the environment present in the wastewater generated from nuclear power plants, mining activities, metallurgical, electroplating industries, painting, pigment and electronic industries (Manohar, *et al.*, 2006). High concentrations of cobalt may cause several health problems such as paralysis, low blood pressure, diarrhoea, bone defects, lung irritation and bone defects (Kudesia VP, 2003). Toxic effects of cobalt in humans were observed after ingestion of cobalt as cobalt sulfate in beer or cobalt chloride in the treatment of anaemia, included cardiomyopathy, gastrointestinal effects, visual disturbances and thyroid effects (ATSDR, 2020).

Moringa is a multipurpose tropical tree. It is a small to medium evergreen or deciduous tree that can grow to a height of 10-12 m. It has a spreading open crown typically umbrella shaped. The roots are deep. The bore is crooked, generally one-stemmed but sometimes forked from the base. The barks are corky and grey (Bennett RN, *et al.*, 2003). The taxonomic description for *Moringa oleifera* was retrieved from the Integrated Taxonomic Information System (ITIS, 2016) an online database. The seeds contain proteins which are essential in antioxidant properties and water purification. It possesses the capability to remediate water pollution and numerous arrays of illnesses (Amagloh FK and Benang A, 2009). *Moringa oleifera* is valuable for its medicinal value. Different part of this plant such as the bark, leaves, immature pods, roots, fruit, flowers and seeds serve as cardiac and circulatory stimulants, possess antitumor, antipyretic, antiepileptic, choles-

terol lowering, antihypertensive, anti-inflammatory, antispasmodic, antiulcer, diuretic, antifungal, antibacterial hepatoprotective, antioxidant, antidiabetic activities. Traditionally, they serve for the treatment of different ailments in medical system. *Moringa's* seeds are antipyretic, acrid, bitter (Oliveira, *et al.*, 1999). Recent research demonstrated that they can also protect humans from diseases. So, when consumed in the diet, they may reduce the risk of age-related chronic diseases such as high blood pressure, various types of cancer, diabetes, coronary heart disease (Krishnaveni Y and Sathish A, 2010).

The liver is a large, meaty organ that sits on the right side of the belly. Weighing about 3 pounds, the liver is reddish-brown in color and feels rubbery to the touch. Normally you can't feel the liver, because it's protected by the rib cage. The liver has two large sections, called the right and the left lobes. The gallbladder sits under the liver, along with parts of the pancreas and intestines. The liver and these organs work together to digest, absorb, and process food. The liver's main job is to filter the blood coming from the digestive tract, before passing it to the rest of the body. The liver also detoxifies chemicals and metabolizes drugs. As it does so, the liver secretes bile that ends up back in the intestines. The liver also makes proteins important for blood clotting and other functions (Hoffman M, 2008).

MATERIALS AND METHODS

Experimental animals

40 healthy male wistar rats weighing 90-120 g were used for the study. The animals were purchased from the animal holding unit of the Department of Human Anatomy of LadokeAkintola University, Ogbomoso, and Oyo State. They were then housed in cages in a well-ventilated place. The animals were then acclimatised for two weeks. A 12-h light and dark cycle was maintained, and the animals were sustained on water and standard rat pellets from commercial feed supplier (Glory vet feeds).

The weight of the animals was taken and recorded every week using weighing scale during both the acclimatization and administration period in order for accuracy in the results gotten.

Plant material preparation

The fresh *Moringa oleifera* leaves were harvested during December 2019, with the permission from the owners of the land in both Ogbomoso and Ibadan. The plant specimen was authenticated by Professor Oyegoke, Faculty of Pure and Applied Biology, Ladoke Akintola University.

The fresh leaves of *Moringa oleifera* were immediately cleaned, cut into small pieces and it was dried in a well-ventilated place in Ogbomoso. The dried plant material was ground into powder on Waso market, Ogbomoso. The ground powder *Moringa* was extracted in Food Science Laboratory Department, Ladoke Akintola University, Ogbomoso. The weight of the *Moringa* been extracted was 400 g, in which 100 g of grounded *Moringa* was dissolved in 1000 ml of distilled water, therefore 400 g of grounded *Moringa* was dissolved in 4000 ml of distilled water. The extract for the *Moringa* gotten was 1 litre, 100 ml. The extract was preserved in a refrigerator.

Chemicals

The chemical used was Cobalt chloride which was first weighed and dissolved in distilled water. It was weighed in the Department of Human Anatomy Laboratory, Ladoke Akintola University, Ogbomoso.

Experimental design and grouping

40 male rats were randomly assigned to 5 groups based on their weights. Their last weights on the last day of acclimatization ranged from 110-200 g. The animals were labelled to enable identification.

Group A-Control group

Group B-Cobalt chloride only

Group C-Cobalt chloride+low dose of *Moringa*

Group D-Cobalt chloride+high dose of *Moringa*

Group E-*Moringa* only

The rats were orally administered in their respective dosages. The dosages are as follows;

Group A-They were given distilled water only

Group B-Orally received 45 mg/kg of cobalt chloride

Group C-Orally received 45 mg/kg of cobalt chloride and 250 mg/kg of *Moringa* extract

Group D-Orally received 45 mg/kg of cobalt chloride and 500 mg/kg of *Moringa* extract

Group E-Orally received 500 mg/kg of *Moringa* extract.

Collection of blood and organs

The overnight fasted animals were sacrificed by cervical dislocation which made the animals unconscious. Their body weights were recorded. Blood was collected by cardiac puncture. Serum was separated and stored for biochemical estimations. Liver was excised immediately, cleaned in normal saline, blotted and weighed on digital balance. It was fixed in 10% buffered formalin for histopathological study.

Tissue processing

The normal histological method of fixation, dehydration, and clearing, impregnation, embedding, sectioning and staining (with H and E) was used to produce the sections. The micrographs of the relevant stained sections were subsequently taken with the aid of a binocular light microscope.

Enzyme assay

Serum levels of Aspartate transaminase (AST), Alanine transaminase (ALT) and Alkaline phosphatase (ALP) were determined using colorimetric methods using Randox Diagnostic kits.

The assay of AST followed a modification of the colorimetric method reported by Varley *et al.*, 1980. 0.1 m aliquot of haemolyzate was added to 0.5 m substrate mixture containing 100 mm phosphate buffer (pH 7.4), 2 mml 2-oxoglutarate and 100 mml-Aspartate. Following incubation of the final mixture at 37 degrees celsius for 30 minutes, the reaction was terminated by the addition of 0.5 ml of 1 mm 2, 4-dinitrophenylhyrazine in 1 mm hydrochloric acid. After allowing the mixture to stand at room temperature for 20 minutes at 546 nm against a blank solution (no incubation at 37 degree Celsius and termination solution added before addition of the haemolyzate). Pyruvate solution of varied millimolar concentrations were used to prepare a standard curve from which AST activities were computed

Alanine transaminase (ALT) assay was carried out as described for AST except that 200 mm DL-alanine replaced L-aspartate in the procedure. The activities of AST and ALT were expressed in international unit/liter (IU/L).

Alkaline phosphatase (ALP) activity determination followed the procedure reported by Haussament, using p-nitrophenyl phosphate as substrate in 1 M di-ethanolamine buffer (pH 9.8) containing 0.5 mm magnesium chloride. A 0.02 ml of aliquot of haemolyzate was added to 1 ml of buffered substrate solution and incubated at 30 degrees Celsius. The absorbance of the reaction was read at 405 nm at 30 intervals for 3 minutes.

Photomicrography

The digital micrographs of the required liver sections were obtained to show the morphological changes that occurred in the treated groups as

compared to the control group. The photomicrographs were taken at the Department of Anatomy, Ladoke Akintola University of Technology, Ogbomosho, Oyo State, using digital Am scope (MD 900) photomicroscope.

Statistical analysis

All data are presented as Mean ± Standard error of mean (SEM). Statistical analysis of the data in this study was carried out appropriately and tested for significance using the student's-t-test (t-distribution). $p<0.05$, value greater than 0.05 were considered insignificant while values less than 0.05 are considered significant. Statistical programme for the social science (SPSS) software version 11.0 was used for the statistical analysis.

RESULTS

Significance: $p<0.05$, value greater than 0.05 were considered insignificant while values less than 0.05 were considered significant (*). Values are expressed as mean ± Standard error of mean *(Table 1)*.

Table 1: Table showing the mean ± S.E.M of the body weights of wistar rats before and during administration

Significance: $p<0.05$, value greater than 0.05 were considered insignificant while values less than 0.05 are considered significant (*). Values were expressed as mean ± Standard error of mean *(Table 2)*.

The weight analysis for the liver shows an insignificant decrease ($p>0.05$) in weight, comparing control group A to group B. Also, there was an insignificant decrease ($p>0.05$) in the weight of liver in groups C and D respectively when they were compared with control group A. There was an insignificantly decrease ($p>0.05$) in the weight of liver in group E when compared to control group A.

Figure 1 showed the effect of cobalt chloride and *Moringa oleifera* extract on the liver weights. The figure showed that rats in group B (those given cobalt chloride only) have insignificantly increased ($p>0.05$) liver weights compared to the liver weights of group A (control). There was an insignificantly increased ($p>0.05$) in liver weights of rats in group C compared to group A. There was a slightly insignificant decreased ($p>0.05$) liver weights of rats in group D compared to group A. There was an insignificantly increased ($p>0.05$) liver weights of rats in group E compared to group A.

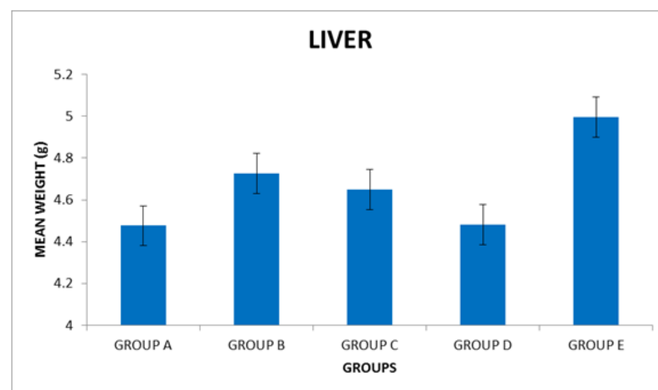

Figure 1: Histogram showing the mean ± S.E.M weight of liver

Biochemical evaluation

Significance: $p<0.05$, values greater than 0.05 were considered insignificant while values less than 0.05 were considered significant (*). Values were expressed as mean ± Standard error of mean *(Table 3)*.

Table 1: Table showing the mean ± S.E.M of the body weights of wistar rats before and during administration

Period/week	Group A	Group B	Group C	Group D	Group E
Week 0	148.8 ± 9.342	156.3 ± 2.631	168.8 ± 5.489	181.3 ± 5.806*	178.8 ± 6.105*
Week 1	136.3 ± 5.324	151.3 ± 3.504*	130.0 ± 6.172	143.8 ± 2.631	162.5 ± 8.399*
Week 2	162.5 ± 7.258	153.8 ± 3.239	145.0 ± 7.638	145.7 ± 4.286	168.8 ± 10.21
Week 3	163.1 ± 7.254	153.1 ± 4.324	149.2 ± 8.604	149.3 ± 9.413	169.4 ± 13.24
Week 4	175.6 ± 5.625	173.1 ± 4.719	176.7 ± 8.819	183.3 ± 7.601	210.0 ± 4.880***
Week 5	171.3 ± 5.154	175.0 ± 5.000	178.3 ± 9.804	185.0 ± 6.191	205.7 ± 4.809***
Week 6	150.8 ± 5.354	163.5 ± 5.392	180.4 ± 7.243**	177.7 ± 4.232**	185.1 ± 3.924***
Note: $p<0.05$, value greater than 0.05 were considered insignificant while values less than 0.05 were considered significant (*).					

Table 2: Showing the mean ± s.e.m of liver after treatment

Groups	Liver weight (mean ± s.e.m)(g)	Relative weight of liver (%)
A	4.48 ± 0.3001	3
B	4.73 ± 0.3901	2.9
C	4.65 ± 0.2543	2.6
D	4.50 ± 0.1363	2.5
E	5.00 ± 0.1307	2.7

Table 3: Showing the mean ± sem of Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphate (ALP)

Groups	ALT	AST	ALP
A	48.62 ± 4.253	86.92 ± 11.93	13.54 ± 0.8790
B	81.84 ± 4.245***	171.7 ± 9.821***	15.83 ± 0.2250*
C	50.07 ± 5.672	145.0 ± 7.367**	14.44 ± 0.5524
D	63.98 ± 1.446**	136.3 ± 11.28*	15.73 ± 0.1863*
E	36.77 ± 2.827*	109.0 ± 3.543	12.76 ± 0.3992

The above table revealed a significant increase (p<0.05) in the level of ALT in the cobalt-treated rats when compared with the control, it increased from 48.62 ± 4.253 to 81.84 ± 4.245 in group B. There was an insignificant increase (p>0.05) in group C, a significant increase (p<0.05) in group D and a significant decrease (p<0.05) in group E in the *Moringa*-treated rats, it decreased from 48.62 ± 4.253 to 36.77 ± 2.827.

Similarly, the level of AST increased significantly (p<0.05) in the treated groups B, C and D compared with the control group. It increased from 86.92 ± 11.93 in group A to 171.7 ± 9.821 in group B, 145.0 ± 7.367 in group C, 136.3 ± 11.28 in group D. There was an insignificantly increase (p>0.05) in group E which was 109.0 ± 3.543.

There was also a significant increase (p<0.05) in the level of ALP in the cobalt-treated group B compared with control group, it increased from 13.54 ± 0.8790 to 15.83 ± 0.2250. There was an insignificant increase (p>0.05) in group C which was 14.44 ± 0.5524, and a significant increase (p<0.05) in group D which was 15.73 ± 0.1863. There was an insignificantly decrease (p>0.05) in group E, it decreased from 13.54 ± 0.8790 to 12.76 ± 0.3992 *(Figure 2)*.

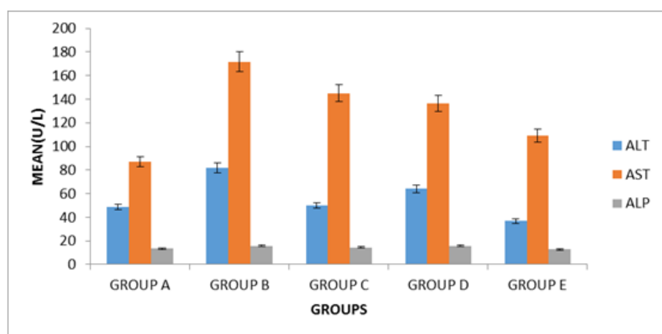

Figure 2: Histogram showing the mean ± SEM of Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphatase (ALP)

Graph revealed the effect of cobalt and *Moringa oleifera* on the activities of ALT, AST, ALP in the liver in all treated groups B to E compared to the control group A.

Histogram showed a significant increase (p<0.05) in the activity of ALT in group B, insignificant increase (p>0.05) in group C, significant increase (p<0.05) in group D, and a significant decrease (p<0.05) in group E. There was a significant increase (p<0.05) in the activity of AST in group B, C, and D, and an insignificant increase (p>0.05) in group E. There was a significant increase (p<0.05) in the activity of ALP in group B, insignificant increase (p>0.05) in group C, significant increase (p<0.05) in group D, and an insignificant decrease (p>0.05) in group E.

Histological analysis

Plate A: Panoramic views of a liver micromorphological section demonstrated by Haematoxylin and Eosin staining at both low and high magnification, shows normal central venules without congestion, the morphology of the hepatocytes appear normal, the sinusoids appeared normal without infiltration, no significant observable pathological lesion seen *(Figure 3)*.

Plate B: Panoramic views of a liver micromorphological section demonstrated by Haematoxylin and Eosin staining at both low and high magnification, shows dilated and distorted central venules with congestion, severe hemorhage and fibrosis, the morphology of the liver cells (hepatocytes) appear altered, the sinusoids appear congested with some infiltrations, liver parenchyma appear grossly infiltrated. Portal triad appear hemorrhagic, morphology of the hepatocytes show severe steatosis *(Figure 4)*.

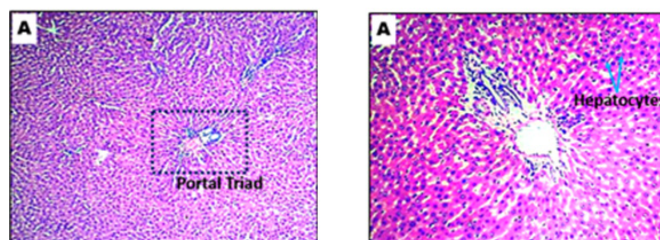

Figure 3: Histological analysis of group A: Control group (H and E)

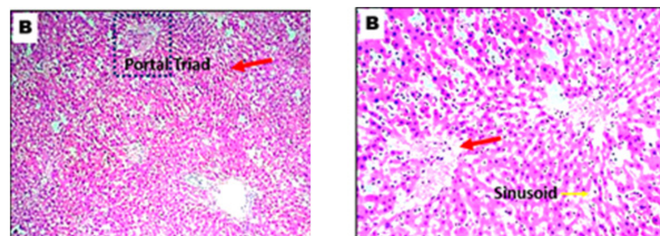

Figure 4: Histological analysis of group B: Treated with cobalt chloride 45 mg/kg for 52 days

Plate C: Panoramic views of a liver micromorphological section demonstrated by Haematoxylin and Eosin staining at both low and high magnification, shows dilated and distorted central venules with congestion, severe hemorhage and fibrosis, the morphology of the liver cells (hepatocytes) appear altered, the sinusoids appear congested with some infiltrations, liver parenchyma appear grossly infiltrated *(Figure 5)*.

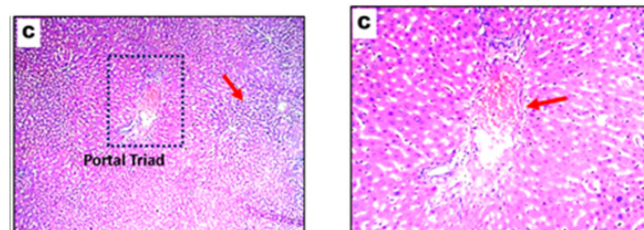

Figure 5: Histological analysis of group C: Treated with cobalt chloride (45 mg/kg) and low dose of *Moringa* extract (250 mg/kg) for 52 days

Plate D: Panoramic views of a liver micromorphological section demonstrated by Haematoxylin and Eosin staining at both low and high magnification, shows normal central venules without congestion, the morphology of the hepatocytes appear normal, the sinusoids appear normal and not infiltrated, no significant observable pathological lesion seen *(Figure 6)*.

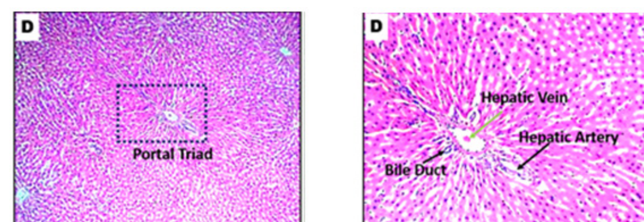

Figure 6: Histological analysis of group D: Treated with cobalt chloride (45 mg/kg) and high dose of *Moringa* extract (500 mg/kg) for 52 days

Plate E: Panoramic views of a liver micromorphological section demonstrated by Haematoxylin and Eosin staining at both low and high magnification, shows normal central venules without congestion, the morphology of the hepatocytes appear normal, the sinusoids appear normal and not infiltrated, no significant observable pathological lesion seen *(Figure 7)*.

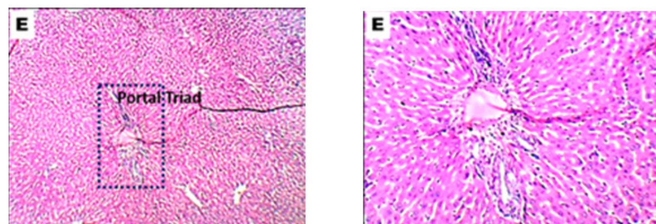

Figure 7: Histological analysis of group E: Treated with *moringa* exftracft (500 mg/kg) for 52 days

DISCUSSION

The study was designed to observe the effects of *Moringa oleifera* extract on cobalt chloride-induced liver of adult male wistar rats and to find the effects of cobalt chloride on body weights of rats, mean and relative organ weights in the adult wistar rats.

Comparing the first week of administration to the last week of administration the body weights of rats in group B (cobalt-treated group) which were exposed to 45 mg/kg of cobalt chloride increased insignificantly (p>0.05) when compared with the body weights of animals in group A. The body weights of animals in group C which were exposed to 45 mg/kg of cobalt chloride and 250 mg/kg of low dose of *Moringa* extract increased significantly (p<0.05) when compared with the body weights of animals in group A. The body weights of animals in group D which were exposed to 45 mg/kg of cobalt chloride and 500 mg/kg of high dose of *Moringa* extract decreased significantly (p<0.05) when compared with the body weights of animals in group A. The body weights of animals in group E (*Moringa*-treated group) which were exposed to 500 mg/kg of *Moringa* extract increased significantly (p<0.05) when compared with the body weights of animals in group A. There was reduction in the body weights of animals in group D compared with the other groups that increased and this could be attributed to the decrease in food consumption and absorption or may be due to the degeneration of lipids and proteins as a direct effect of cobalt (Galbraith RA and Kappas A, 1989).

An insignificant decrease (p>0.05) in the liver weights in proportion to the body weights of animals was observed when the rats in group B which were treated with 45 mg/kg of cobalt chloride were compared with the control of non-exposed rats (group A). There was also an insignificant decrease (p>0.05) in the liver weights of group C which were treated with 45 mg/kg of cobalt and 250 mg/kg of *Moringa* extract (low dose), and also in group D which were treated with 45 mg/kg of cobalt and 500 mg/kg of *Moringa* extract (high dose). Likewise, there was also an insignificant decrease (p>0.05) in liver weights of group E which were treated with *Moringa* extract only. It has been observed that decrease in absolute liver weights which was demonstrated in the study is not only a consequence of decreased food consumption, but also from direct toxicity of the cobalt chloride, perhaps by malabsorption of nutrients from toxic effects of the gastrointestinal tract or by inhibition of protein synthesis (Hammond PB, *et al.*, 1990). Cobalt toxicity has a stronger effect in the presence of iron, to which it is linked by similar atomic properties (Ryuko, *et al.*, 2012). Due to its quite high solubility in systemic fluids, it becomes more mobile and also more harmful to all internal organs (Cihan YB, *et al.*, 2011).

In biochemical analysis of hepatic enzymes, there was significant increase (p<0.05) in the activity of ALT in group B and D, insignificant increase (p>0.05) in group C and significant decrease (p<0.05) in group E when compared to control group A. In the level of AST, there was a significant increase (p<0.05) in group B, C, and D and an insignificant increase (p>0.05) in group E when compared to control group A. In the level of ALP, there was a significant increase (p<0.05) in group B and D, insignificant increase (p>0.05) in group C, insignificant decrease (p>0.05) in group E when compared to control group A. The liver enzymes (AST and ALT) are considered as important biomarkers for the detection of cobalt hepatoxicity. Toxic injury to the liver is associated with the release of some marker enzymes into circulation (Batzakis KG and Briere RO, 1979; Jaeschke, *et al.*, 2013). In the present study, hepatic damage was assessed by the assay of liver specific enzymes (AST, ALT and ALP). Administration of cobalt chloride caused a significant (p<0.05) increase in the activity of these enzymes. This observation is in agreement with other reports which demonstrated that exposure to cobalt chloride induced necrosis of the liver with elevation in the activities of liver specific enzymes (Wahab WMA, 2012; Okerenta OBM and Anacletus FC, 2016; Yakubu OE, *et al.*, 2016). AST and ALT are amino transferase enzymes that are usually released into plasma as a consequence of hepatic damage (Naik P, 2010). ALP is a membrane bound enzyme (Larkshmi R, *et al.*, 1991). The increase in serum activity of ALP has been attributed to membrane damage consequent upon cobalt chloride intoxication (Nehru B and Anand P, 2005; Wahab WMA, 2012). *Moringa* extract given to rats in group C and D helps to reduce the effect of cobalt chloride in the activity of AST, ALT and ALP when compared to group B which makes the *Moringa* to serve as a protective agent. Administration of extract of *Moringa oleifera* restored the activities of AST, ALT and ALP towards normal. This is an indication of improved liver function and protection against the hepatotoxicity of cobalt chloride. This observation is in consonance with other studies which reported that the leaf extract of *Moringa oleifera* significantly reduced the elevated activities of liver enzymes induced by toxicants (Saalu, *et al.*, 2012; Greenwood NN, Earnshaw A, 2013; Shiekh et al, 2014; Toppo *et al.*, 2015). The hepatoprotective effects of *Moringa oleifera* leaves have been observed to follow the antioxidant mediated mechanism provided by various bioactive compounds (Fakurazi S, *et al.*, 2012; El-bakry K *et al.*, 2016).

Histological findings in the control group A, panoramic views of a liver micromorphological section demonstrated by Haematoxylin and Eosin staining at both low and high magnification, shows normal central venules without congestion, the morphology of the hepatocytes appear normal, the sinusoids appeared normal without infiltration, no significant observable pathological lesion seen. In group B which is the cobalt treated group shows dilated and distorted central venules with congestion, severe hemorhage and fibrosis, the morphology of the liver cells (hepatocytes) appear altered, the sinusoids appear congested with some infiltrations, liver parenchyma appear grossly infiltrated. Portal triad appear hemorrhagic, morphology of the hepatocytes show severe steatosis. Likewise in group C(cobalt+low dose of *Moringa* extract) shows dilated and distorted central venules with congestion, severe hemorhage and fibrosis, the morphology of the liver cells (hepatocytes) appear altered, the sinusoids appear congested with some infiltrations, liver parenchyma appear grossly infiltrated. Also, group D (cobalt+high dose of *Moringa*) and group E (*Moringa* only) shows normal central venules without congestion, the morphology of the hepatocytes appear normal, the sinusoids appear normal and not infiltrated, no significant observable pathological lesion seen.

Cobalt chloride caused degenerative and necrotic changes in hepatic tissues. Similar or more advanced changes in the liver histology and

function under cobalt influence have been reported by others (Gonzales S, *et al.*, 2005). The cellular damage observed in the study may have been caused by the cytotoxic effect of cobalt on the liver. This obviously will adversely affect the normal detoxification processes and other functions of the liver. Group C which were given low dose of *Moringa* shows that the *Moringa* did not have a protective effect on it because of the hepatic damage that occured while group D and E shows that *Moringa oleifera* ameliorated the deleterious effects of cobalt chloride induced hepatic damage. The histopathological results of the present study confirm hepatotoxic effects of cobalt chloride and recovery consequent upon treatment with leaf extract of *Moringa oleifera*. Similar histopathological results have been obtained with the use of other bioactive substances against cobalt hepatotoxicity (Mahmoud ME and Elsoadaa SS, 2013; Shrivastava S, 2013).

CONCLUSION

The study concluded that oral administration of Cobalt chloride to male rats at a dose of 45 mg/kg body weight daily for a period of 52 days induced hepatic dysfunction and injury to biliary cells as evident in significant alterations in some statistical, biochemical, and histological parameters. The use of leaf extract *Moringa oleifera* in combination with Cobalt chloride was observed to attenuate some of the harmful effects of this element. Therefore, supplementation with *Moringa oleifera* leaves may prove useful as protective therapy against hepatotoxic effects of cobalt chloride.

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