

Moringa oleifera ameliorates renal, hepatic and testicular damages following chloramphenicol-induced toxicity in albino rats: a histological and biochemical study

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SUMMARY

Chloramphenicol is an antibiotic with wide range application in antimicrobial therapy; however, its toxicity is associated with liver, kidney and testicular damages. *Moringa oleifera* (MO) is used in the present study to determine its effect in ameliorating the toxic effect of chloramphenicol on the liver, kidney and testicular tissues. 25 albino rats were clustered into 5 groups: Group 1 was administered equal measures of distilled water; Group 2 administered 50 mg/kg chloramphenicol; Groups 3 and 4 were administered 250 mg/kg MO + chloramphenicol, and 5000 mg/kg MO + chloramphenicol 50 mg/kg, respectively; and Group 5 received 100 mg/kg Silymarin and chloramphenicol 50 mg/kg for a period of 28 days, after which the rats were sacrificed. Blood samples were collected through cardiac puncture and serum, which was analyzed for liver and kidney function enzymes. Liver, kidney and testes were harvested and weighed before being processed for routine histological analysis. Sperm count analysis was

carried out using Neubauer's hemocytometer under the light microscope at ×400 magnification.

MO had no significant effect on liver and kidney weight, or sperm count. It significantly decreased serum AST, ALT and ALP level, but had no effect on ALB. It decreased serum creatinine, bicarbonate and sodium levels at a low dose, and increased creatinine and bicarbonate ions at a higher dose. MO did not fully ameliorate the effects of chloramphenicol, as renal histology revealed dilated renal tubules and tubular epithelial cell degeneration and, in the liver, lymphocytic infiltration was observed in the sinusoids. In the testes, the germinal layers were improved with more sperm cells preserved with observed bleeding in the interstitial space when compared to the negative control group. MO presents a promising remedy in ameliorating the deleterious effects posed by chloramphenicol-induced toxicity.

Key words: *Moringa oleifera* – Kidney – Liver –

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Testes – Chloramphenicol

INTRODUCTION

Chloramphenicol, a broad-spectrum antibiotic, has been a mainstay in antimicrobial therapy for decades. Its efficacy against a wide range of bacteria, including both gram-positive and gram-negative organisms, has made it a valuable tool in treating various infections (Moffa and Brook, 2015). However, the emergence of antibiotic resistance and the association of chloramphenicol with severe adverse effects have led to a decline in its use in recent years (Oyagbemi et al., 2010). In living systems, chloramphenicol is hydrolyzed and absorbed completely. Its excretion is also at a high rate but is highly impaired in disorders associated with the liver and kidneys. It is metabolized in liver to chloramphenicol glucuronide (Shukla et al., 2011). Use of chloramphenicol was banned due to its adverse effects like aplastic anaemia and leukemia (Bale et al., 2023). However, even with its ban, traces were detected in several herb and grass samples from different geographic origins, leading to inadvertent exposure (Hanekamp and Bast, 2015).

Even though the drug is deemed as being highly toxic, it is still prescribed at a noticeable rate (Shivaji and Tanaji, 2023). It is recommended that it be prescribed only when there is no other alternative present with a monitoring of its concentration in patients' bodies (Hanekamp and Bast, 2015). Several studies show that chloramphenicol causes toxicity in the liver, small intestine, spleen and thymus of laboratory animals (Doshi and Sarkar, 2009, Saba et al., 2010, Oyagbemi et al., 2010). Chloramphenicol at doses of 25-112 mg/kg body weight per day has been shown to cause degeneration of the testes and effects sperm quality in rats (European Food Safety Authority (EFSA), 2014).

Some plants can help to reduce the harmful side effects of chloramphenicol, particularly those related to tissue damage and immune system effects including *Alium sativa* (Shalaby et al., 2006), *Nigella sativa* (Ebaid et al., 2011). This study attempts to investigate the protective ef-

fect of *Moringa oleifera* (MO) on Chloramphenicol-induced kidney, liver and testicular damage.

MO is a medicinal plant that has attracted a lot of scientific interest due to its varying biological properties (Vergara-Jimenez et al., 2017). It has an impressive range of nutritional components including proteins, amino acids (both essential and non-essential), carbohydrates, fats, fiber, vitamins, and phenolic compounds (Gul et al., 2025). Various parts of MO, including the roots, flowers, fruits, seeds, and leaves, have been traditionally employed to combat abdominal ulcers, heart diseases, liver damages, cancer, inflammation, wounds, paralysis, helminthic bladder issues, prostate problems, sores, and skin infections (Pareek et al., 2023, Gul et al., 2025). It can also be used as food ingredients, skin care, hair care and cosmetic ingredient (Abdelwanis et al., 2024).

MO leaves possess a wide range of additional biological activities including antioxidant, tissue-protective (liver, kidneys, heart, testes, and lungs), analgesic, antiulcer, antihypertensive, radioprotective, and immunomodulatory actions (Stohs and Hartmann, 2015). A wide variety of polyphenols and phenolic acids as well as flavonoids, glucosinolates, and possibly alkaloids is believed to be responsible for the observed effects (Singh et al., 2014; Osawe and Farombi, 2018). MO leaves, abundant in antioxidants and cytoprotective natural agents, position the administration of this plant as a promising future strategy in mitigating testicular toxicity, impaired spermatogenesis and increased apoptosis (Mthiyane et al., 2022). The current study investigates the protective role of MO on the liver, kidney and testes in chloramphenicol-induced tissue damage in albino rats.

MATERIALS AND METHODS

Plant collection, identification and authentication

MO leaves were obtained from a local garden in Shagari Low-cost Area, Maiduguri, Borno State. It was identified and authenticated by a taxonomist from the Faculty of Pharmacy, University of Maiduguri, Nigeria. Samples of the plant were

deposited and voucher number was specified and recorded (UMM/FPH/MOG/001).

Aqueous extraction of *Moringa oleifera*

The aqueous extraction of MO leaf was done using the maceration method (Dani et al., 2022). The leaves were collected and air dried for 7 days under room temperature. It was pulverized mechanically using a blender (SCB- 505 55000W, Germany). 497 g of pulverized MO leaf was dissolved into 6 Liters of distilled water and allowed to steep for 24 hours. It was then filtered, poured on a tray and evaporated within an oven to obtain the dry extract. The percentage yield was calculated using the formular below:

$$\frac{\text{Weight of extract obtained}}{\text{Weight of pulverized dried moringa oleifera} \times 100}$$

Animal Husbandry

A total of twenty-five (25) adult male Wistar rats were purchased from the Department of Animal Science, University of Jos, Plateau State. Following an acclimatization period of two weeks; the rats were individually marked for identification using a permanent marker and weighed. They were housed in well-ventilated plastic cages and fed with pelletized animal feed and water daily. They were maintained at a constant 12hr/12hr dark and light cycles.

Animal grouping and treatment protocol

The rats were randomly divided into five groups for the experimental study as displayed in Table 1 below. Rats in Groups 3 and 4 were administered with MO at concentrations of 250 mg/kg and 500 mg/kg respectively, and rats in Group 5 was administered silymarin (100 mg/kg). Group 1 was

administered only distilled water, while Group 2 served as the negative control and was administered chloramphenicol (50 mg/kg). Groups 2, 3, 4 and 5 were administered 50 mg/kg chloramphenicol one hour afterwards. All treatment were administered orally for a period of 28 days.

Animal Sacrifice

On the 29th day, the animals were anaesthetized by administering intra-peritoneal ketamine injection (100 mg/kg). Blood samples were collected through cardiac puncture and placed inside a plain container and centrifuged at 7000rpm for 10 minutes. The serum was collected and analyzed for liver and kidney function enzymes (Attah et al., 2022a).

The liver tissue was exposed by making a median incision on the anterior abdominal wall and carefully dissected out. The kidney tissue was harvested from the posterior abdominal wall and rinsed in normal saline and processed via routine histological processes to obtain tissue slides. The scrotal sac of each rat was dissected, and the testes of all animals were immediately excised. The epididymis was trimmed off the testis and placed in a glass petri dishes containing 2mls of normal saline. The tissue was then crushed by making small incisions and the spermatozoa was dispersed in the saline solution. The resulting fluid containing the sperm cells was preserved in fluoride tubes to maintain the sperm viability for sperm count analysis using Neubaur's hemocytometer under the light microscope at $\times 400$ magnification.

Histological analysis

Liver and kidney tissues were harvested and fixed in 10% neutral buffered formalin (NBF); dehydrated in ascending grades of alcohol (50%, 70%, 90% and 100%), cleared in xylene, embedded in paraffin wax, sectioned at 5 μ m and mount-

Table 1. The experimental design

Groups	Treatment
Group 1 (Normal control)	Distilled Water
Group 2 (Negative control)	Chloramphenicol 50 mg/kg
Group 3 (Low dose treatment)	MO 250 mg/kg + Chloramphenicol 50 mg/kg
Group 4 (High dose treatment)	MO 500 mg/kg + Chloramphenicol 50 mg/kg
Group 5 (Positive control)	Silymarin 100 mg/kg + Chloramphenicol 50 mg/kg

ed on glass slide and allowed to air dry. Sections were stained with Hematoxylin and Eosin (H and E) and mounted with DPX mountant. The testes were fixed immediately in Bouin’s fluid for routine histological analysis.

Determinations of weights

Animals were weighed and the records taken weekly for the entire duration of the study. The organ weights were obtained by weighing the organs using an electronic weighting balance after trimming off excess fats and connective tissue. The body-to-organ weight ratio was determined using the equation below:

$$\frac{\text{Organ weight}}{\text{Mean and weight of rat under investigation} \times 100\%}$$

Statistical Analysis

The data were analyzed using GraphPad Prism 10.0 (GraphPad software, San Diego, California, USA). One-way ANOVA followed by Tukey’s multiple comparison was carried out and statistical significance was considered at $P < .05$.

RESULTS

The effect of MO on the initial and final weights of the rats in all groups

There was an increase in weight observed in all groups at the end of the investigation as observed in Fig. 1. This is most marked in Groups 2, 4 and 5.

The effect of MO on the liver index

Administration of MO did not significantly affect the liver index in treated groups compared to the normal control – Group 1 (Fig. 2). There was no effect when compared with the reference drug silymarin (Group 5) and the negative control group (Group 2).

The effect of MO on the kidney index

MO did not cause a significant increase in the weight of left and right kidneys when compared to Groups 1 and 2 as observed in Fig. 3. In Group 5, silymarin did not also have an effect on the kidney index when compared to Groups 1 and 2.

The effect of MO on the relative index of the testes

Administration of low dose of MO caused a sig-

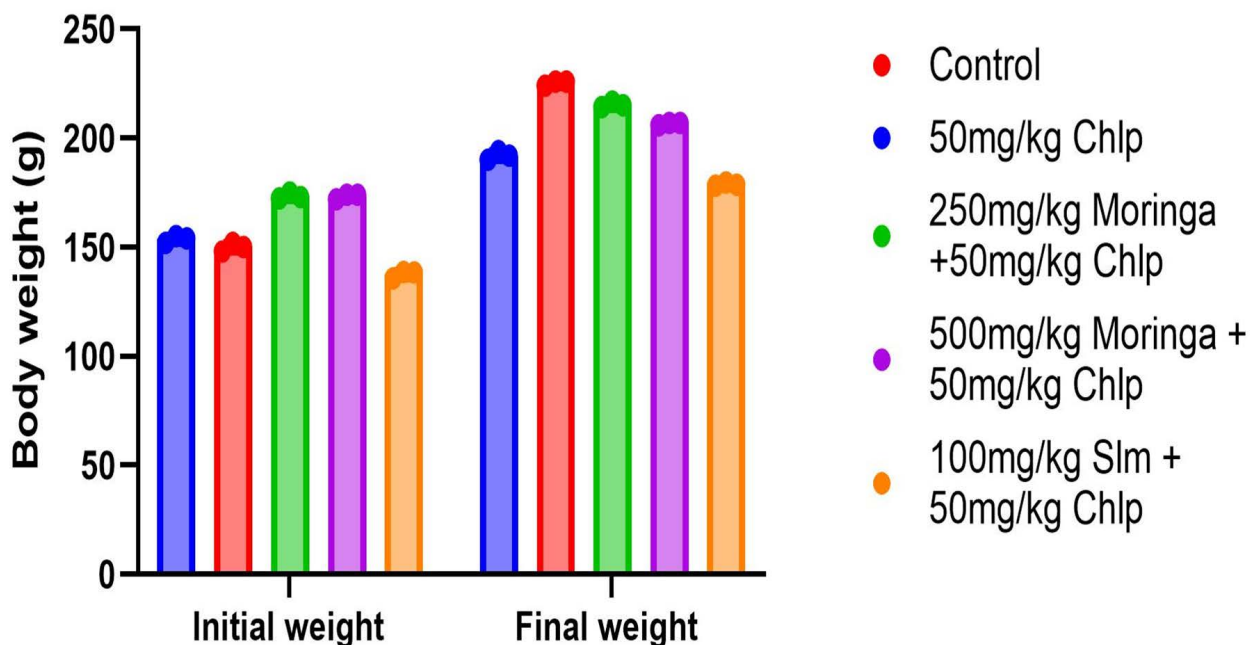


Fig. 1.- Initial and final body weight of rats in all groups. All figures are represented as an average of the mean ±SD. g – gram, mg – milligram, kg – kilogram, Chlp – chloramphenicol, SIm – Silymarin.

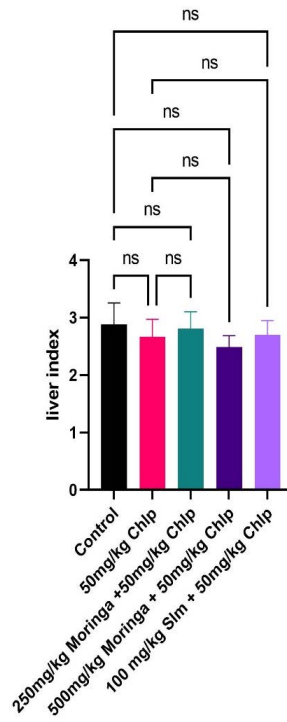


Fig. 2.- Effect of MO on the liver index in all groups. All figures are represented as an average of the mean \pm SD. ns – not significant, mg – milligram, kg – kilogram, Chlp – chloramphenicol, SIm – Silymarin.

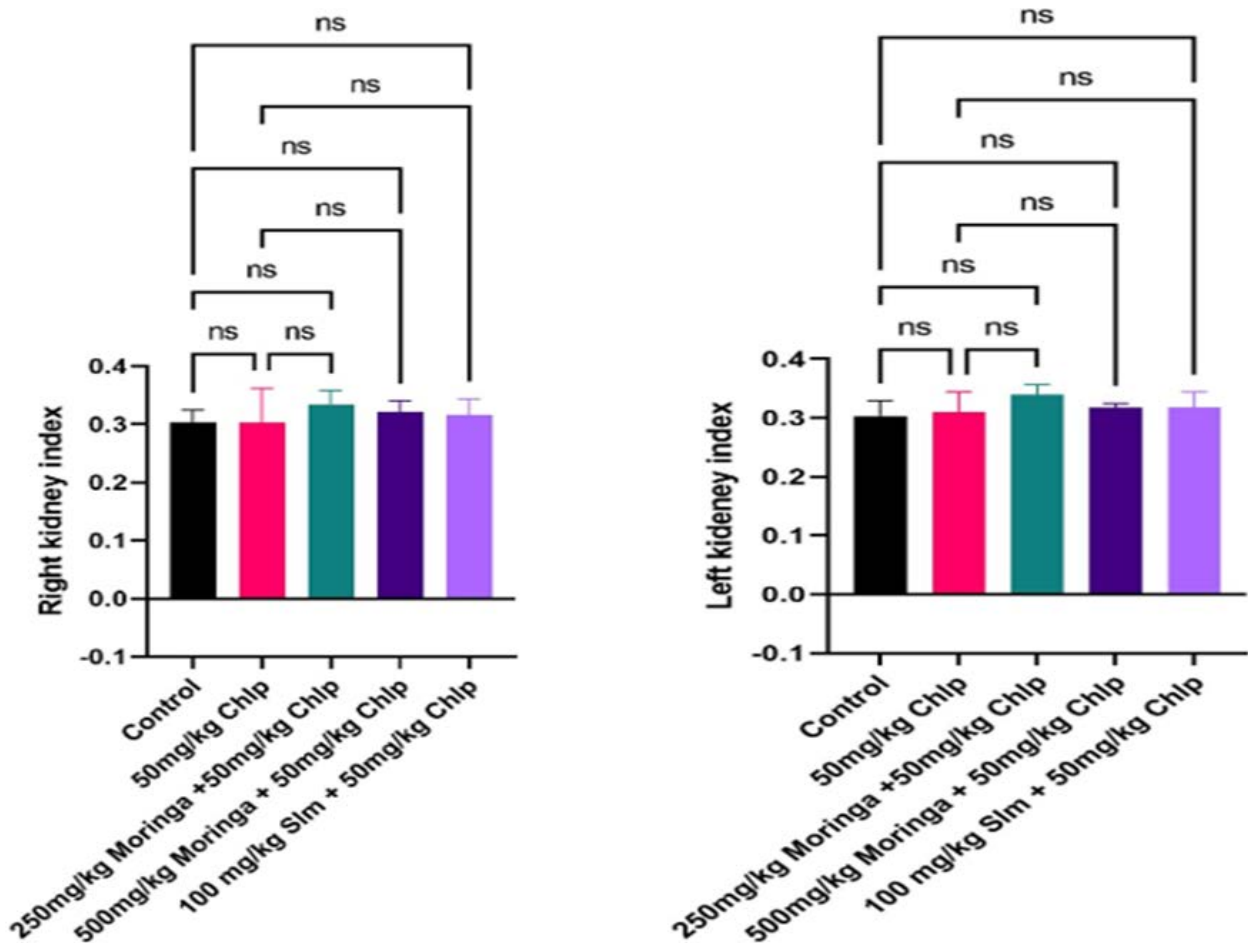


Fig. 3.- Effect of MO on the kidney index of the left and right kidneys. All figures are represented as an average of the mean \pm SD. ns – not significant, mg – milligram, kg – kilogram, Chlp – chloramphenicol, SIm – Silymarin.

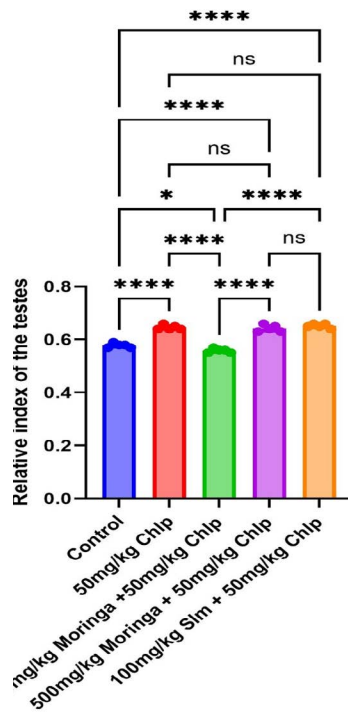


Fig. 4.- Effect of MO on the relative index of the testes. All figures are represented as an average of the mean ±SD. mg – milli-gram, kg – kilogram, Chlp – chloramphenicol, Slm - Silymarin – not significant, * - P<0.05, **** - P<0.001.

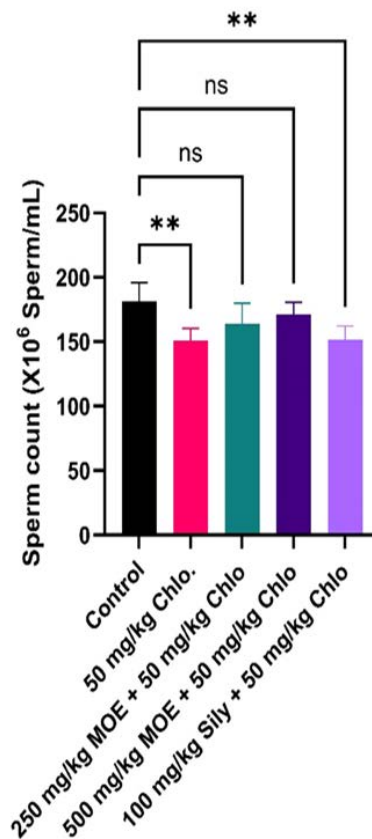


Fig. 5.- Effect of MO on sperm count. All figures are represented as an average of the mean ±SD. ns – not significant, mg – mil- ligram, kg – kilogram, Chlp – chloramphenicol, Slm – Silymarin, * - P<0.05, ** - P<0.01.

nificant ($P < 0.05$) decrease when compared with Group 1, and a significant decrease ($P < 0.001$) compared to Group 2. The higher dose of MO caused a significant ($P < 0.001$) increase compared weight of the testes in Group 1, and a non-significant difference compared to Group 2. Silymarin caused a non-significant change when compared to Group 2 and a significant ($P < 0.001$) increase when compared to the testes of rats in Group 1 (Fig. 4).

The effect of MO on the sperm count

MO had no significant effect on the sperm count at low and high concentrations in Groups 3 and 4 when compared to Group 1. In Group 5 there was a significant ($P < 0.01$) decrease in sperm count when compared to Group 1. There was also a significant ($P < 0.01$) decrease in Group 2 when compared to Group 1 (Fig. 5).

The effect of MO on serum liver parameters

MO in Groups 3 and 4 significantly increased the serum levels of aspartate aminotransferase (AST) when compared to the serum AST in the Group, 1 but decreased AST levels compared to

Group 2. However, in Group 5, there was no significant change in serum AST activity compared to Group 1. Serum concentration of alanine transaminase (ALT) was significantly decreased in the MO treated groups (Groups 3 and 4) compared to the negative control group (Group 2) and Group 5. Alkaline phosphatase (ALP) activity was also decreased in the serum of rats in Groups 3 and 4 when compared to Group 2, and significantly reduced when compared to Group There was no significant change in serum albumin (ALB) levels in all the groups (Fig. 6). The treatment appears to partially protect the liver from damage.

The effect of MO on serum kidney parameters

There was a non-significant increase in serum urea concentration in Groups 2,3,4 and 5 when compared with Group 1. MO reduced serum creatinine concentration in Group 3 when compared with Group 1, but elevated creatinine levels in Group 4 when compared to Group 1. Serum creatinine level was also elevated in Group 5 when compared to Group 1. Serum sodium level was non-significantly elevated in MO treated groups (3 and 4) and Group 5 when compared to Group 1.

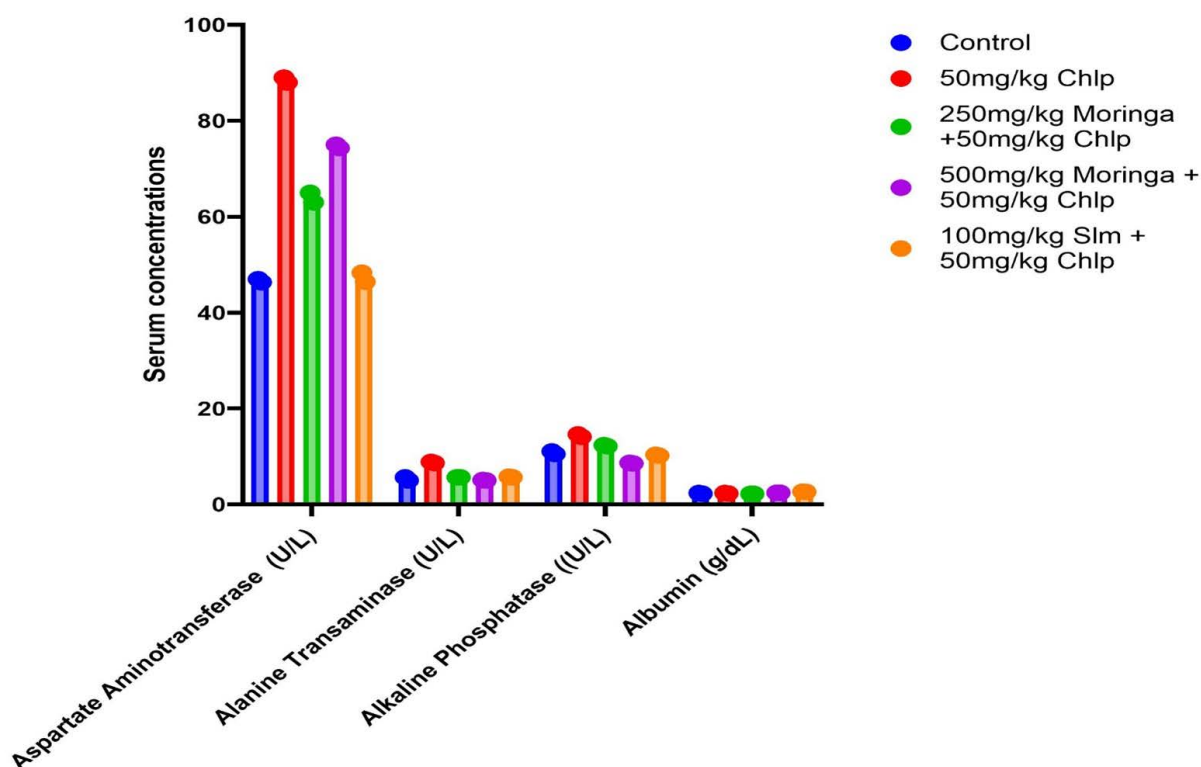


Fig. 6.- Effect of MO on liver functional parameters. All figures are represented as an average of the mean \pm SD. mg – milligram, kg – kilogram, Chlp – chloramphenicol, SIm – Silymarin, U/L – units/litre, g/dL – gram/decilitre.

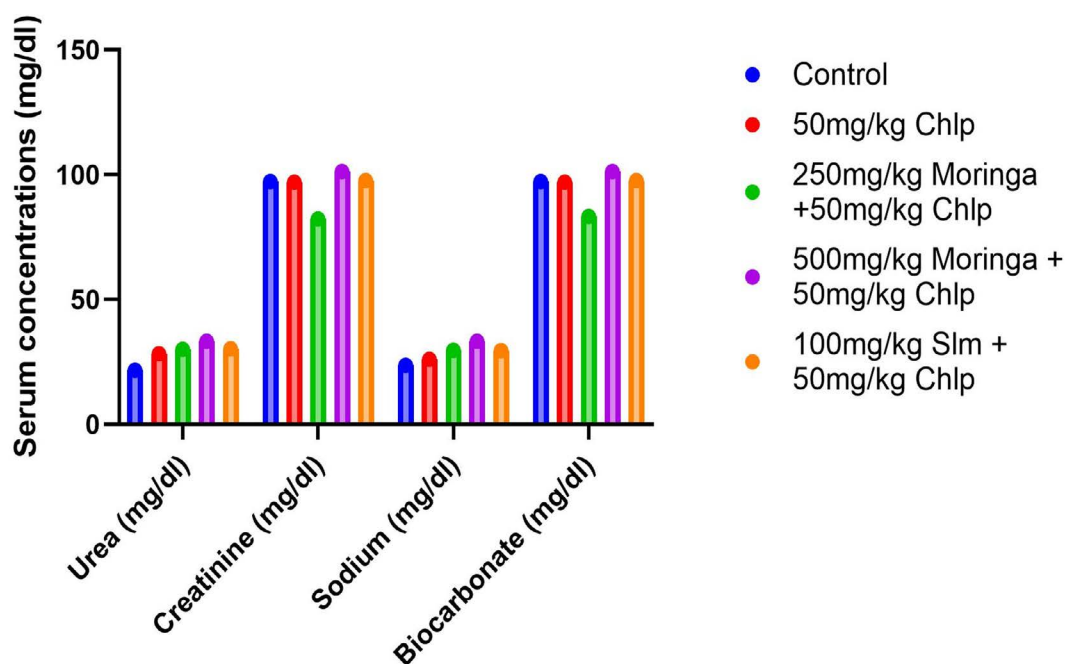


Fig. 7.- Effect of MO on the kidney functional parameters. All figures are represented as an average of the mean \pm SD. mg – milligram, kg – kilogram, Chlp – chloramphenicol, SIm – Silymarin, mg/dL – milligram/decilitre.

Serum bicarbonate level was reduced in group C when compared to all groups (Fig. 7).

The effect of MO on the histology of the liver tissue in chloramphenicol-induced toxicity

Figure 8A displays the histology of the liver of rats in Group 1, showing cords of hepatocytes radiating towards the central vein. The sinusoids appeared as white columnar spaces between branching rows of hepatic cells (Fig. 8A). The liver of rats in Group 2 showed slight ballooning of the hepatocytes, aggregation of lymphocytes around the central vein and immune cells in the sinusoidal spaces. The liver in Groups 3 and 4 had normal appearance of hepatocytes, which contained strongly eosinophilic granulated cytoplasm and spherical nuclei. Hepatic sinusoids were recognized as spaces between the strands of hepatocytes, with infiltration of immune cells into the hepatic sinusoids (Fig. 8C). Rats in Group 5 had disorganized arrangement of the hepatic cords and blood cells in the hepatic central vein (Fig. 8E).

The effect of MO on the histology of the kidney in chloramphenicol-induced toxicity

The kidney tissue in Group 1 showed normal

renal structure with the glomerular tuft located in Bowman's capsule, surrounded by Bowman's space. The simple cuboidal epithelial cells of the renal tubules were clearly observed, with the lumen blurred indicating the presence of brush border (Fig. 9A). The kidney of rats in Group 2 showed a reduced glomerular tuft and widened Bowman's space. The renal tubules were dilated and arrangement of the lining epithelial cells were distorted (Fig. 9B). Group 3 kidney tissue also had minimal distortion similar to Group 2 with dilated renal tubules, dilated epithelial cells with dispersed brush border layer (Fig. 9C). The kidney tissue in Group 4 rats was similar to the kidney of rats in Group 1 showed normal histology (Fig. 9D). The kidney of rats in Group 5 had similar appearance to the kidney in Groups 2, 3 and 5 (Fig. 9E).

The effect of MO on the histology of the testes in chloramphenicol-induced toxicity

The histology of the testes in Group 1 showed seminiferous tubules with a germinal layer that had sperm cells and supporting Sertoli cells at various levels of development. Mature sperm cells were located in the lumen. The interstitial space surrounded the seminiferous tubules, with interstitial cells present in the spaces (Fig. 10A). The

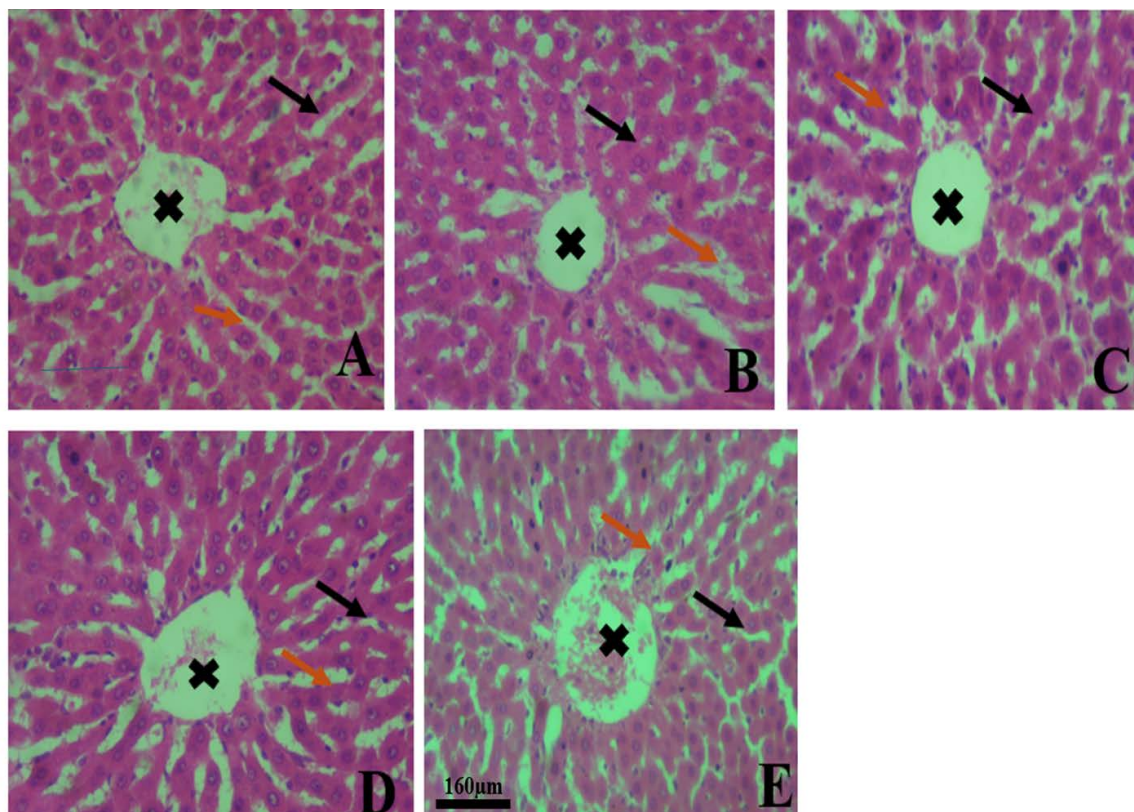


Fig. 8.- Effect of MO on the liver tissue of rats in all groups. Black arrow – cords of hepatocyte, orange arrow – hepatic sinusoid, black X – central vein. H&E, X 200, scale bar: 160 µm. A – Group 1, B – Group 2, C – Group 3, D – Group 4, E – Group 5.

testes in Group 2 had a greatly reduced germinal layer with just a layer of spermatogenic cells lining the seminiferous tubule. Very few maturing sperm cells were observed in the lumen of the seminiferous tubule. The interstitial space was filled with blood (Fig. 10B). The histology of the testes in Group 3 was similar to that in Group 2, with reduced spermatocytes and blood-filled interstitial spaces (Fig. 10C). In the seminiferous tubules of the rats of Group 4, there was a thicker germinal layer compared to the testes of rat in Group 2 and 3. There was still blood located in the interstitial space (Fig. 10D). In Group 5, there was also a reduced germinal layer when compared to the control group and similar to the other chloramphenicol-treated groups, there was blood in the interstitial space (Fig. 10E).

DISCUSSION

MO leaves, seeds, bark, roots, sap, and flowers have been widely studied and used in traditional medicine, and the leaves as food products in human nutrition. Leaf extracts of MO exhibit the greatest antioxidant activity, and various studies

in animals and human subjects involving aqueous leaf extracts indicate a high degree of safety with no reported adverse effects in either species (Stohs and Hartman, 2015). Several studies demonstrate that chloramphenicol causes severe histological damage in vital organs such as liver, kidney, and heart in animal models (EFSA, 2014).

In the current investigation, MO treatment of chloramphenicol-induced tissue injury increased total body weight in all treated groups but had no significant effect on the liver index and kidney of treated groups. The low dose of MO caused a significant decrease in the weight of the testes when compared to the control group, while the high dose caused a significant increase in testicular weight when compared with the control groups. Administration of MO had no significant effect on sperm count of treated rats, compared with the control groups. The result from this study, however, varied from studies carried out by Farombi et al. (2002), which showed that chloramphenicol administration at doses of 28 mg/kg, 57 mg/kg and 86 mg/kg body weight administered for 10 consecutive days in rats resulted in a dose-depen-

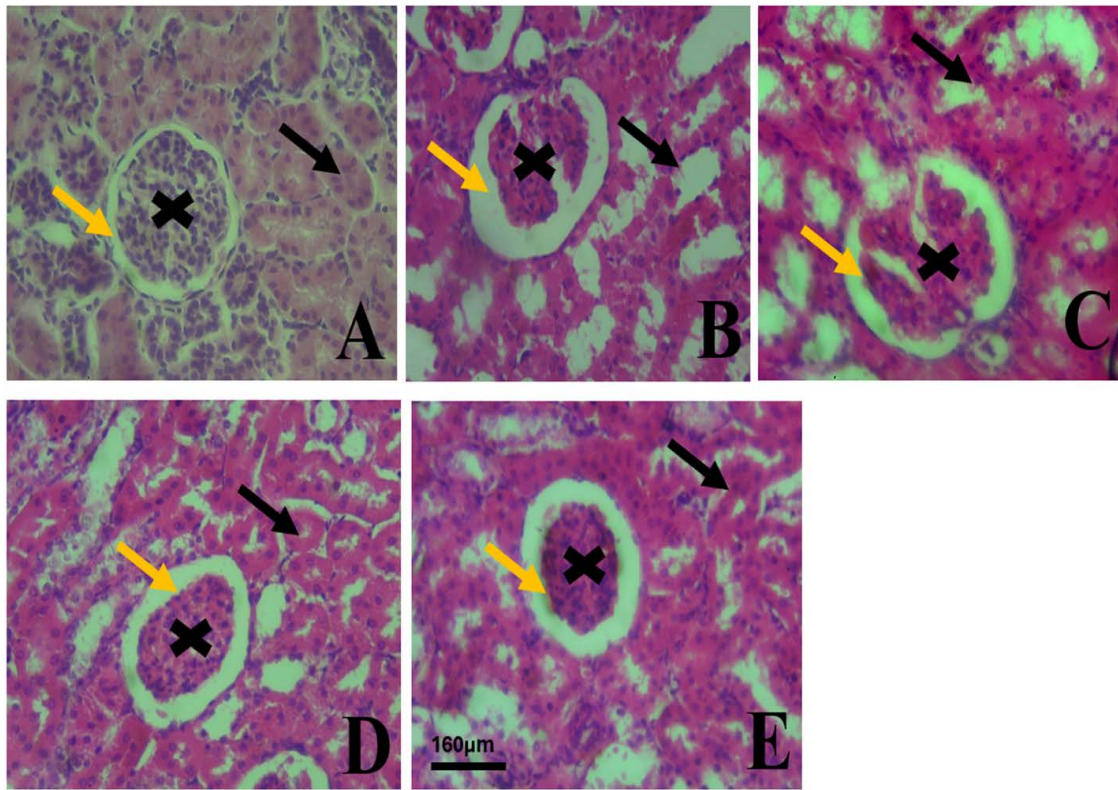


Fig. 9.- Effect of MO on the histology of the kidney tissue in all groups. Orange arrow – Bowman's space, black arrow: renal tubules, black X – glomerulus. H&E, X 200, scale bar: 160 µm. A – Group 1, B – Group 2, C – Group 3, D – Group 4, E – Group 5.

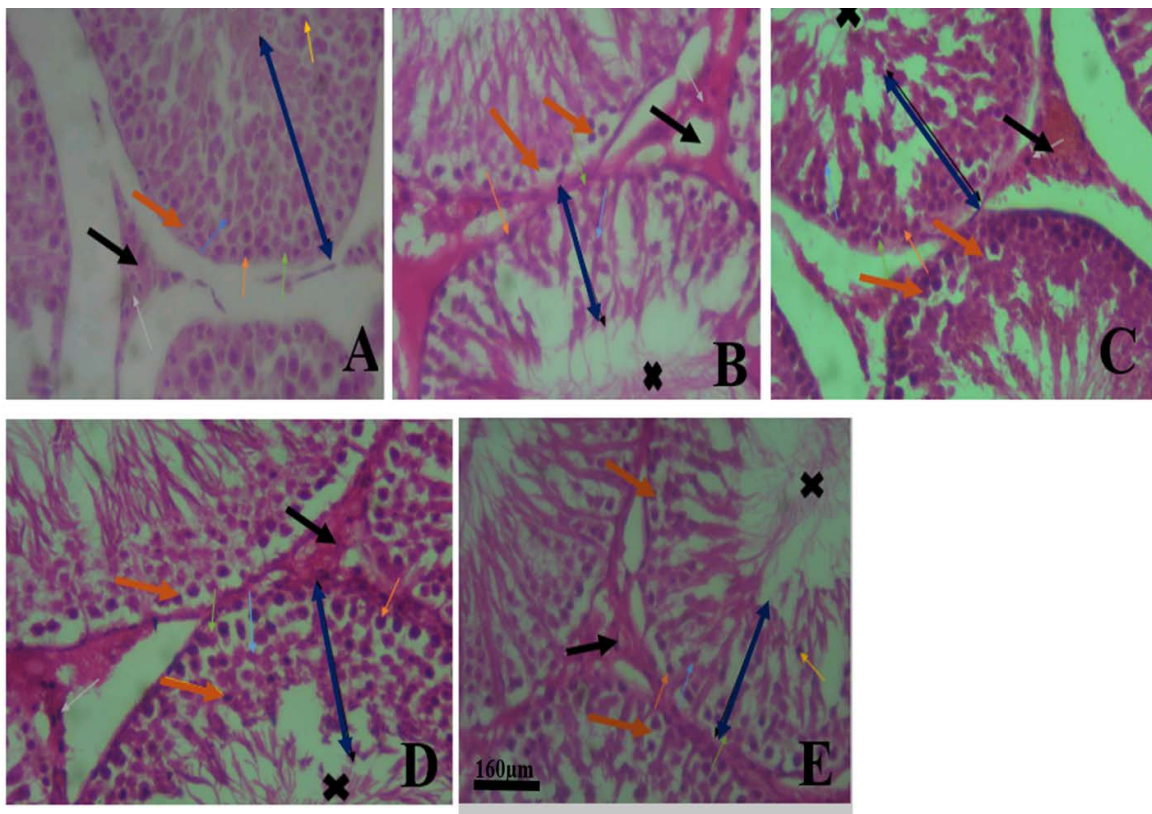


Fig. 10.- Effect of MO on the histology of the testes in all groups. Blue arrow – germinal layer, black arrow – interstitial space, black X – lumen of seminiferous tubule, orange arrow – spermatogonia. H&E, X 200, scale bar: 160 µm. A – Group 1, B – Group 2, C – Group 3, D – Group 4, E – Group 5.

dent decrease in body weight, liver weight, relative liver weight and protein content compared to control group. Turton et al. (1999) recorded that administration of 2000 and 3200 mg/kg chloramphenicol had no significant effect on the body weight in treated rats, but at 3600 and 4000 mg/kg it increased the mean body weight in treated rats (Turton et al., 1999; Kumangry et al., 2022).

Serum enzymes and levels of AST, ALT, ALP, ALB, serum sodium, bicarbonate, urea, and creatinine are evaluated to establish any hepatic or renal dysfunction (Husic-Selimovic et al., 2021). ALP is useful in the diagnosis of obstructive liver diseases. AST and ALT are indicators of hepatocellular injury. ALP is linked to biliary function and bone metabolism; ALB measures the synthetic function of the liver (Manu et al., 2022; Attah et al., 2022b). An increase in ALP, ALT and AST values would signify necrosis or myocardial infarction, which are all indicators of drug toxicity or harmful chemicals in the body (Egu et al., 2021).

In the current investigation, MO decreased serum AST and ALT activity in both groups when compared with the negative control group, but increased the activity of these enzymes when compared with the normal control group. It decreased serum ALP activity at both concentrations when compared to the negative and normal control groups, but had no significant effect on serum ALB levels. Increased AST/ALT, compared to the normal control, indicates some liver stress or injury due to the treatment; yet lower AST/ALT concentrations when compared to the negative control indicates less damage than in the negative control group, suggesting hepatoprotective properties of MO. The decrease in ALP concentrations when compared to the control groups suggests suppression or inhibition of biliary enzyme activity. In the current study, results obtained shows that MO played a protective role. MO helps in steering the amelioration of underlying liver cell necrosis and pertinent inflammatory changes; it also preserves normal-level hepatic structural composition.

In similar studies, Saba et al., (2010) revealed that MO significantly increased in AST and ALT serum levels in treated animals compared to the control groups. Contrary to the current investi-

gation, the increase in serum ALP was not statistically significant. In other studies, MO has been demonstrated to have hepatoprotective effects by decreasing the levels of liver enzymes AST, ALP, and ALT (Al-Sultan and Al-Sowayan, 2024). Hamza (2010) showed that administration of MO seed extract decreased the CCl₄-induced elevation of serum aminotransferase activities and globulin level. Aly et al. (2020) showed that MO caused a significant increase in liver enzymes (ALT, AST, ALP) in acetaminophen-induced toxicity; and Hassan et al. (2020) demonstrated that, at a concentration of 500-750 mg/kg, MO did not significantly affect serum ALT, ALP and AST concentrations. However, above 1000 mg/kg, it significantly increased serum ALT levels when compared to the control groups, and this increase may be attributed to possible damage to hepatocytes as a consequence of the MO extract intake. Similar to the current study, Abduljalil et al. (2024) MO nanoparticles significantly increased ALT and AST activity in acrylamide-induced toxicity; other studies also showed that MO caused a reduction of AST, ALT, and albumin in MO treatment groups (Ebrahim et al., 2022; Melebary et al., 2023).

Increased creatinine levels suggest impaired kidney function; elevated urea levels also indicate renal dysfunction. However, it is more sensitive to hydration and protein intake. Sodium concentrations reflect fluid balance and renal handling, while bicarbonate levels may indicate metabolic acidosis or alkalosis. Serum creatinine concentration was decreased in the low dose group when compared with the control groups but increased with administration of the high dose when compared to control groups. Serum urea concentration was not changed following administration of MO in treated groups; however, sodium concentration was elevated and serum bicarbonate levels were decreased in the low dose treatment groups and elevated in high dose treatment groups when compared to the control groups. Lower creatinine suggests improved renal clearance or kidney function at this dose and indicates a beneficial (nephroprotective) effect of low-dose MO. Increased creatinine levels in the high dose group indicates renal impairment or reduced filtration capacity at this concentration. No significant

change in urea concentration indicates that MO had no significant effect on protein metabolism or urea clearance. Increased sodium levels suggests that MO increased sodium reabsorption.

Decreased bicarbonate in the low dose group may reflect mild metabolic acidosis, potentially due to increased acid production or renal loss of bicarbonate. With high doses of MO, elevated bicarbonate suggests a metabolic alkalosis or electrolyte imbalance or renal tubular dysfunction at high doses. In a similar study, serum urea and creatinine levels were elevated in the test rats; this increase is only statistically significant for serum urea but not significant for creatinine (Saba et al., 2010; Akinrinde et al., 2020). Abduljalil et al. (2024) revealed that MO reduced serum creatinine and urea concentrations.

Administration of chloramphenicol revealed vascular congestion and foamy cytoplasm of hepatocytes at the centrilobular region of the liver but did not reveal any damage done to the renal tissue (Saba et al., 2000). The liver tissue of MO treated rats showed normal liver histology, but with lymphocytic infiltration into the sinusoidal spaces. The hepatocytes showed no signs of ballooning; the histoarchitecture was not altered either. Similar to the current result, studies carried out by Kazeem et al. (2019) showed that MO ameliorated hepatocellular degeneration and severe necrosis measured by histological analysis of liver in ethanol-extract-of-MO-treated rats. Treatment using MO nanoparticles demonstrated the proper structure of the liver lobule, with hepatocyte cords surrounding the central vein. MO nanoparticles have greatly reduced the harmful effects of acrylamide-induced toxicity (Abduljalil et al., 2024).

Other studies reported that MO caused significant reduction of cytoplasmic vacuolation, granulation in some cells, pyknosis of the nuclei, inflammatory cell infiltration, vessel congestion, and hepatocyte necrosis in lead-induced toxicity (Melebary et al., 2023). Treatment of tissues with varying high concentrations of MO revealed diffuse hemorrhages in the tubular structures; focal hyaline degeneration in both cortex and medulla were observed in kidneys; congestion of central vein and sinusoid with perivascular cuffing, hepatic nuclear vacuolation and haemorrhages

with cellular infiltration in liver may also be due to the effects of the crude extract on hepatic cells at concentrations above 1000 mg/kg (Hassan et al., 2020; Igwe et al., 2022). Bayu et al. (2020) reported that liver tissue treated with MO at 800 and 1600 mg/kg body weight induced portal cellular infiltration, periportal congestion and hydropic degeneration of hepatocytes in the liver. Phytochemical analysis of MO shows that it primarily comprises phenolic compounds, which are essential plant-derived micronutrients characterized by hydroxyl groups, which are directly attached to aromatic structures, encompassing phenolic acids, flavonoids, xanthenes, quinones, coumarins, tannins, and lignans (Phenolic compounds play significant roles in safeguarding plants against physical damage, ultraviolet radiation, oxidative stress, and similar factors) (Irawan et al., 2017).

In the current study, treatment with MO did not fully ameliorate the effects of chloramphenicol, as renal histology revealed dilated renal tubules and tubular epithelial cell degeneration. The glomerulus appeared normal, although Bowman's space was wider than observed in the control groups. In related studies, treatment with MO nanoparticles demonstrated normal renal corpuscle architecture and renal tubule appearance (Abduljalil et al., 2024). Akinrinde et al. (2020) showed that treatment with MO maintained several normal glomeruli with the interstitial spaces mostly appearing normal, although there were moderate degrees of tubular necrosis, dilation and inflammatory cell infiltration (Akinrinde et al., 2020). Bayu et al. (2020) showed that MO did not affect the histology of the kidney at a concentration below 2000 mg/kg; yet above that dosage, there were minor tubulointerstitial leukocytic infiltration, wider urinary space and congested glomeruli in some areas of both cortex and medulla of the kidney sections.

In another study, MO prevented distortion of the lining cuboidal epithelium, renal epithelial cell necrosis, and inflammatory infiltration in the renal interstitium of ethylene glycol-induced toxicity (Meles et al., 2025). These protective effects can be attributed to MO's anti-inflammatory and antioxidative components, particularly flavonoid, making MO a promising therapeutic agent for managing and preventing renal pathologies

(Osawe and Farombi, 2018).

In the testicular tissue, MO administration failed to protect the germinal epithelium of the seminiferous tubules, as the layer was greatly reduced compared with the normal control group, with fewer viable spermatocytes and supporting Sertoli cells observed in the low dose group. In the high dose group, more spermatocytes were observed, and the germinal layer was thicker and more cells were preserved. The interstitial space was also filled with blood in the MO-treated groups following chloramphenicol-induced injury. In a related study, MO nanoparticles dramatically enhanced spermatogenesis, with the exception of certain germinal cells (Abduljalil et al., 2024). Ragab et al. (2024) reported testicular histopathological changes, including excessive collagen deposition, and reduced glycogen content, which were evident following uranyl acetate exposure. However, supplementation with MO effectively countered these mentioned abnormalities. Flavonoids present in MO, such as luteolin, preserve seminiferous tubules and blood-testis barrier stability by enhancing the expression of numerous downstream antioxidant genes and increasing the protein expression of ZO-1, occluding claudin-11, and Cx4367, which possess the ability to permeate the lipid bilayer of membranes, providing direct protection for spermatozoa against peroxidative damage, while also stimulating the electron transport chain and oxidative phosphorylation to energize the germ cells (Osawe and Farombi, 2018).

CONCLUSION

There is a growing interest in uncovering, extracting, and enhancing phytochemicals due to their potential to serve as alternatives to synthetic drugs with fewer side effects. *Moringa oleifera* (MO) is one of these plants and has been proved to have preserved the testes of treated rats at a higher dose, and shown hepatoprotective, reno-protective effects in chloramphenicol-induced toxicity in albino rats.

Ethical Considerations

The rats were handled according to Animal Care Ethics as outlined in “Guide for Care and Use of Laboratory Animals”, and experiments conduct-

ed conferring to the National Institute of Health Guide for the Care and Use of Laboratory Animals and ARRIVE Guidelines. The research protocol with animal experimentation was approved by the Scientific Ethics Committee of University of Maiduguri (Protocol Number: UM/HA/UGP 23.24-019). All surgery was performed under ketamine hydrochloride anesthesia and every effort was made to minimize suffering to the mice.

Data Availability

The data used and/or analyzed in the present study are available from the corresponding author upon request.

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AUTHORS' CONTRIBUTIONS

All authors contributed to the study's concept and design. Helga Ishaya Bedan, Luteino Lorna Hamman, Nathan Isaac Dibal and Martha Orendu Oche Attah were involved in the material preparation. Data collection was done by Chablaty Japhet, Collins Ushie and Rasheeda Sambo, Sunday Joseph Manye. The first draft of the manuscript was written by Helga Ishaya Bedan, Nathan Isaac Dibal, and Martha Orendu Oche Attah, Luteino Lorna Hamman, Chablaty Japhet, Collins Ushie and Rasheeda Sambo. The research was supervised by Helga Ishaya Bedan, Luteino Lorna Hamman, Nathan Isaac Dibal and Martha Orendu Oche Attah. All authors commented on the final version of the manuscript; all authors read and approved the final manuscript.

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