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E.A.S.A designed the study; E.A.S.A, N.M.H.A, B.Y.H.A, S.A.A performed the experiments; F.S.M, A.M.A.M, collected data; E.A.S.A, H.M.I wrote the first draft of the manuscript; H.M.I, F.A.M.Q, performed the statistical analysis; E.A.S.A, H.M.I reviewed the draft of the manuscript; all authors approved the manuscript for publication.

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Histoprotective Potential of *Moringa peregrina* Methanolic Extract against CCl_4-induced Toxicity in the Liver, Kidney, and Testis of White Male Rats

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Abstract:
The study aimed to assess the protective effects of methanolic *Moringa peregrina* leaf extract against CCl_4- induced tissue damage in rats, focusing on liver, kidney, and testicular health. Twenty-five adult male albino rats were randomly assigned to five groups (n=5). Group I (control) received no treatment. Group TI received an intraperitoneal injection of CCl_4 (1 mL/kg). Group TII received olive oil (3 mL/kg). Group TIII was given *M. peregrina* ethanolic extract orally (500 mg/kg). Group TIV received CCl_4 (1 mL/kg, intraperitoneally, on alternate days) followed by oral *M. peregr*ina extract (500 mg/kg). At the end of the experiment, rats were sacrificed, and blood and organs were collected for biochemical and histological assessment. In the Moringa group TIII, serum creatinine (32.12 mg/dL), ALT (51.92 U/L), ALP (480.6 U/L), and AST (153.2 U/L) increased, indicating biochemical alterations. At the same time, initial body weight (IBW, 155.4 g) and relative testis weight (RTW, 1.05 g) decreased, reflecting tissue effects. The CCl_4 group showed elevated ALT (59.3 U/L), ALP (598.2 U/L), AST (189 U/L), and final body weight (FBW, 192.1 g), with reduced body weight gain (24 g) and relative kidney weight (RKW, 0.4 g) compared with control, TII, and TIV groups. In the CCl_4 + *M. peregrina* group, FBW (201.6 g), IBW (155.2 g), BWG (30.92 g), and ALP (441.8 U/L) decreased versus control and Moringa-only groups, while creatinine rose to 33.2 mg/dL, demonstrating Moringa's potential to mitigate tissue damage. Histologically, CCl_4 caused liver fibrosis, necrosis, hydropic degeneration, and vascular congestion; kidney fibrosis, inflammation, tubular casts, hemorrhage, and glomerular degeneration; and in testes, oligospermia, degeneration and shrinkage of spermatocytes, hydropic changes, abnormal cell proliferation, increased space between tubules, shrinkage of seminiferous tubules, and exfoliated seminiferous tubules. The observed tissue improvements suggest that *M. peregrina* extract has potential for therapeutic applications in tissue protection and repair.
Keywords: Liver, Kidney, Testes, CCL_4, *Moringa peregrina*, enzymes, Rat.

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INTRODUCTION

Carbon tetrachloride (CCl_4) is a chemical compound, a nonflammable, dense, colorless liquid. It is an important agent for multiple purposes, such as cleaning and industrial applications. CCl_4 can cause several adverse effects (Smuckler, 1976). Chronic exposure to CCl_4 has been shown to cause nephrotoxicity and hepatotoxicity in rodent models characterized by elevated serum urea, creatinine, ALT, and AST levels and also caused histopathological changes through the generation of reactive oxygen species (ROS), lipid peroxidation, and inflammatory processes in Wister rats (Weber *et al.,* 2003; Unsal *et al.,* 2020). Also, human toxicity is usually caused by accidental inhalation of its vapors, dermal absorption following direct skin contact, or ingestion, leading to cellular damage in multiple organs, mostly the liver, kidneys, and lungs (Weber *et al.,* 2003; Atef *et al.,* 2017; Teschke, 2018). Many studies confirm that CCl_4 remains a standard and reproducible model for investigating oxidative stress–mediated liver and kidney injury, as well as for assessing the efficacy of novel therapeutic and protective agents (Weber *et al.,* 2003; Atef *et al.,* 2017; Muriel and Ramos-Tovar, 2021; Adel *et al.,* 2024).

M. peregrina is a tree belonging to the family Moringaceae. It is an exceptionally rapid-growing tree that can reach heights of 5 to 15 meters and possess a diameter of 20 to 40 centimeters, characterized by its grayish-green bark. The leaves of this species range from 20 to 70 cm in length and are composed of numerous small leaflets that tend to fall as the leaf matures (Robiansyah *et al.,* 2014).

The *Moringa* genus comprises 13 species that are distributed across northeastern Africa, southwestern Africa, Southwest Asia, Madagascar, the Red Sea region, Arabia, and Northeast Africa (Abd Rani *et al.,* 2018). Phytochemical analyses of various *Moringa* species reveal an array of compounds, including alkaloids, saponins, tannins, steroids, phenolic acids, glucosinolates, flavonoids, and terpenes with several applications (Abd Rani *et al.,* 2018; Aernan *et al.,* 2023; Iqbal and Ashraf, 2023; Iqbal, 2023). Specifically, *M. peregrina* is indigenous to the Arabian Peninsula (Lopez-Rodriguez *et al.,* 2020) and is prevalent in regions such as Yemen, Somalia, Syria, Palestine, and Jordan (Somali *et al.,* 1984; Al-Dabbas *et al.,* 2010).

Moringa species are rich in diverse phytochemicals, including alkaloids, saponins, tannins, steroids, phenolic acids, glucosinolates, flavonoids, and terpenes (Abd Rani *et al.,* 2018). Previous studies have demonstrated that Moringa leaves are abundant in essential nutrients, including vitamin C, potassium, calcium, protein, vitamin B, and iron (Abdalla *et al.,* 2022; Qadir *et al.,* 2022). Furthermore, Moringa contains specific plant pigments that exhibit potent antioxidant properties, including carotenoids, lutein, alpha-carotene, and beta-carotene, as well as xanthins and chlorophyll. Additional phytochemicals known for their robust antioxidant capabilities include kaempferol, quercetin, rutin, and caffeoylquinic acids, alongside powerful antioxidant vitamins such as C, E, and A, and essential micronutrients with antioxidant activity, including selenium and zinc (Fuglie, 1999). These constituents contribute to its strong antioxidant, anti-inflammatory, and antimicrobial properties (Siddhuraju and Becker, 2003).

Extracts from various parts of the Moringa plant have been employed as anti-cancer agents (Guevara *et al.,* 1999; Iqbal, 2023), anti-trypanosomal treatments (Mekonnen *et al.,* 1999), antimicrobial agents (Caceres *et al.,* 1991; Aernan *et al.,* 2023), and for their anti-inflammatory and hepatoprotective properties (Singh *et al.,* 2014; Abushal, 2020). Moreover, leaf extracts have demonstrated the ability to modulate thyroid function (Tahiliani and Kar, 2000) and regulate cholesterol levels in rats (Ghasi *et al.,* 2000).

Studies have shown that ethanolic extracts of *M. peregrina* leaves and seeds possess high levels of phenolic and flavonoid compounds, which play a critical role in neutralizing free radicals and preventing oxidative stress (Al-Owaisi *et al.,* 2014). When administered to animals exposed to carbon tetrachloride (CCl_4), *M. oleifera* extract

significantly reduced serum liver enzyme activities, including SGOT, SGPT, GGT, LDH, ALP, ACP, and total bilirubin, indicating protection of hepatocellular integrity. Additionally, the extract had a great protective role in mice against CCl_4-stimulated liver fibrosis (Singh *et al.,* 2014; Ali, 2022; Adeiza *et al.,* 2025). Furthermore, Histological examination confirmed the biochemical and molecular findings. Severe fibrosis, necrosis, and inflammation observed in the CCl_4-injured group were significantly reduced in the Moringa-treated groups. The preservation of liver architecture suggests that Moringa protects against hepatic fibrosis and promotes tissue repair (Supriono *et al.,* 2020). The antioxidant mechanism is attributed to the enhancement of endogenous defense systems, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), as well as the reduction of lipid peroxidation markers such as malondialdehyde (MDA) (Ighodaro and Akinloye, 2018). These findings suggest that *Morenga olifera* exerts significant nephrotoxicity, hepatoprotective, and antioxidant effects against CCl_4-induced toxicity, likely due to its rich content of bioactive phytochemicals (Elbakry *et al.,* 2019). Since CCl_4 is a widely used industrial solvent and cleaning agent known for its severe hepatotoxic and nephrotoxic effects, as well as its persistence in the environment, it contributes to ecological pollution and public health risks. Continuous exposure to CCl_4 can lead to oxidative stress, lipid peroxidation, and cellular damage in vital organs such as the liver and kidneys (Boll *et al.,* 2001a,b; Guo *et al.,* 2022; Cinar *et al*., 2024). Therefore, there is an increasing demand for natural antioxidant sources that can counteract CCl_4-induced toxicity.

Eljaafari et al. (2024) recorded that CCL_4 caused marked degeneration of Spermatogenic layers, mild disturbance of the seminiferous tubule structure with occasional loss of germ cells and dilation of the interspaces between seminiferous tubules, congestion in testicular blood vessels and interstitial capillaries. Hence, the present study was designed to evaluate the hepatoprotective and nephroprotective effects of *M. peregrina* ethanolic extract against CCl_4-induced toxicity in male rats. Specifically, the study aimed to investigate the extract's ability to attenuate biochemical, oxidative, and histopathological alterations in hepatic, renal, and testis tissues. The findings are expected to provide scientific evidence supporting the potential use of *M. peregrina* as a natural therapeutic and environmental protective agent against chemical-induced oxidative damage.

MATERIALS AND METHODS

Plant collection

Leaves of *M. peregrina* were harvested from the Taiz Governorate in Yemen. The plant was authenticated by Dr. Hassan Ibrahim, Professor of Plant Taxonomy in the Biology Department at Sana'a University, Yemen. The plant was identified in comparison to the voucher specimen number (BHSS 1466) in the Faculty of Science Herbarium, Sana'a University.

Preparation of plant extract:

The collected leaves of *M. pregraenia* were meticulously washed and air-dried in a shaded environment, then ground into a fine powder and stored in opaque containers until required. A total of 100 grams of the powdered plant material was subjected to extraction using 80% ethanol for duration of 48 hours at 25°C (El-Sherbiny *et al.,* 2024). The resultant crude filtrate was evaporated in an oven, and the necessary doses were subsequently reconstituted in 5% dextrose normal saline.

Ethical approval

The study protocol was approved by the Animal Ethics Committee of the Biological Science Department, Sana'a University (ethical code: BAHSS101).

Animals

Twenty-five adult male albino rats with an average weight of 155.6 g were obtained from the Department of Biology, Faculty of Sciences, Sana'a University, Yemen. The animals were kept in plastic cages under controlled room

temperature (23-25°C) with a 12-hour light/ dark cycle. Animals had free access to laboratory pellet foods and tap water. The animals were acclimatized for a period of one week before the commencement of the experiment. Standard pellet consists of corn (30%), soy bean meal (8%), wheat bran (7%), wheat grain (25%), and dried fish (10%), Sorghum stover 20%, and 1teaspoonful/3.5 kg of the above stock of vegetable oil. The ingredients were ground, mixed, and then supplemented with multivitamins and minerals (Ikese *et al.,* 2019; Sakphisutthikul and Sanchaisuriya, 2020). The experimental protocol was approved by the Animal Ethics Committee of the Department of Biological Sciences, Faculty of Science, Sana'a University (ethical code: BAHSS101).

Experimental design

Twenty-five adult male rats were utilized in this study. Rats were divided into five groups of 5 animals each as follows:

Group C: Received a standard diet and 0.5 ml of distilled water and served as a control.

Group TI: Received 1 ml/kg b.w. CCl_4 + 3 ml/kg b.w olive oil.

Group TII: Received 3 ml/kg b.w. olive oil only.

Group TIII: Received 500 mg/kg b.w. *M. peregrina* ethanolic extract.

Group TIV: Received 500 mg/kg b.w. *M. peregrina* ethanolic extract + 1 ml/kg b.w. CCl_4.

All treatments were administered orally using a gastric tube. Before the commencement of the experimental period, the weight of rats of all groups was recorded.

Body and Organ Weights

At Autopsy, the final body weight (FBW) of each rat was recorded, and body weight gain (BWG) was calculated. The liver, kidneys, and testes were excised, weighed, and measured using a single-pan electronic balance. Relative organ weights (RW) were calculated as (organ weight /body weight) × 100.

Biochemical Analysis

Blood samples were collected from the retro-orbital plexus using microhematocrit capillary tubes. Liver function markers, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), as well as kidney function markers (creatinine), were analyzed at Al-Thobhany Laboratories, Al Zubari Street, Sana'a.

Histopathological Examination

Samples of the liver, kidneys, and testes were fixed in 10% formalin for 24 hours. Then, dehydrated in ascending grades of ethanol, cleared in xylene, and embedded in paraffin wax. Sections of 3 µm thickness were prepared using a microtome and stained with hematoxylin and eosin (H&E). Finally, the slides were examined microscopically for histopathological alterations (Bancroft *et al.*, 1996; El-Sayyad *et al*., 2009).

Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way ANOVA with Tukey's multiple comparison test in GraphPad Prism 8.02. Differences were considered statistically significant at $p < 0.05$ (Almansory *et al.*, 2021; Al-Shaibani *et al.*, 2023). Furthermore, the relationship between the studied groups (Control, TI, TII, TIII, and TIV) was illustrated via a dendrogram (TWCA) using PC-ORD for Windows, version 7.09 (Alhadi *et al*., 2025; Aqlan *et al.,* 2025).

RESULTS

Body weight and relative organ weight

No treatment-related clinical signs were observed in all animals during or after administration of the ethanolic extracts of *Moringa pregraenia* and CCL_4 of different experimental groups.

Table (1) showed that there was no significant decrease or increase in the final body weight of

rats treated with *M. peregrina* + CCl_4 (TIV). In contrast, that of other treated groups did not show any significant difference when compared to the control group. The relative liver and testes weights of rats in all treated groups did not differ significantly from those of the control group.

Table 1. Effects of *M. peregrina* against CCL_4-induced toxicity on body weight gain and relative organ weights in white male rats of all experimental groups.

Weights	Treatment Groups				
	Control	TI	TII	TIII	TIV
Final Body weight (g)	222.8± 37.6	192.1± 25.2	201.6 ± 19.7	220.6 ±15.28	191.24 ± 26
Body weight gain (g)	45.2± 31.4	23.9 ± 7.5	30.9 ± 15.6	43.406±14.22	25.03 ± 11.7
Relative Liver weight (g)	4.1 ± 0.3	3.7 ± 0.5	4 ± 0.6	4 ± 0.8	3.5 ± 0.4
Relative Kidney weight (g)	0.4 ± 0.04	0.4 ± 0.2	0.47 ± 0.03	0.5 ± 0.05	0.43 ± 0.05
Relative Testes weight (g)	1.25 ± 0.22	0.99 ± 0.1	1.14 ± 0.3	1.1 ± 0.3	1.01 ± 0.1

Mean values in each column were compared by one-way ANOVA followed by Tukey's multiple comparison tests. Values with the same superscript letters are not significantly different, whereas those with different superscript letters are significantly different. *p < 0.05; **p < 0.01; and *** p< 0.001.

TI: 1 ml/kg b.w. CCl_4+ 3 ml/kg b.w olive oil; **TII:** 3 ml/kg b.w. olive oil; **TIII:** 500 mg/kg b.w. *M. peregrina* extract; **TIV:** 500 mg/kg b.w. *M. peregrina* extract + 1 ml/kg b.w. CCl_4

Liver and Kidney biochemical markers

Table 2 showed a significant decrease in the mean serum ALT level in rats treated with *Moringa peregrina* + CCl_4 (TIV) compared with the TI group (59.3 ± 8.1 U/L). In contrast, that of other treated groups did not show any significant difference when compared to the control group. Also, the mean ALP results showed a significant decrease in the TII group (441.8 ± 56 U/L) and the TIV group (364 ± 38.9 U/L) compared with the TI group (598.2 ± 129 U/L). Moreover, the results for AST showed a significant increase in the TI group (189 ± 37.4 U/L) compared with the Control group (127.1 ± 33 U/L). In the Moringa group TIII, the level of serum creatinine (32.12 mg/dL) increased, indicating biochemical alterations. In the CCl_4 + *M. peregrina* group, creatinine rose to 33.2 mg/dL, demonstrating Moringa's potential to mitigate tissue damage.

Table 2. Effects of *M. peregrina* against CCL_4- induced toxicity on ALT, AST, ALP, and creatinine serum levels in male rats of all experimental groups.

Treatment Groups	Parameters			
	ALT (U/L)	AST (U/L)	ALP (U/L)	Creatinine (mg/dL)
Control	43.3 ± 15.3	127.1 ± 33	461 ± 37.7	30.5 ± 2.3
TI	59.3 ± 8.1	189 ± 37.4 a*	598.2 ± 129	32.5 ± 1.2
TII	45.32 ± 11.2	139±18.9	441.8 ± 56 b*	33.2 ± 5.7
TIII	51.92 ± 1.6	153.2 ± 21.1	480.6 ± 83.6	32.1 ± 1.3
TIV	35.8 ± 2.7 b**	164.6±38.1	364 + 38.9 b***	29.5±5.3

Mean values in each column were compared by one-way ANOVA followed by Tukey's multiple comparison tests. Values with the same superscript letters are not significantly different, whereas those with different superscript letters are significantly different. *p < 0.05; **p < 0.01; and *** p< 0.001.

TI: 1 ml/kg b.w. CCl_4 + 3 ml/kg b.w olive oil; TII: 3 ml/kg b.w. olive oil, TIII: 500 mg/kg b.w. *Moringa peregrina* extract, TIV: 500 mg/kg b.w. *Moringa peregrina* extract + 1 ml/kg b.w. CCl_4

Body weight, relative organ weight, and biochemical markers

According to the finding presented in Figure (1), the studied groups were divided into two categories at a relative similarity level of 95.05 % based on RLW, RKW, CREA, ALT, ALP and AST, where first category (C1) includes TI, which showed the decrease in the mean amount of RKW and BWG (0.4g and 24 g respectively), and the increase in the mean amount of ALT, ALP, and AST (59.3, 598.2, and 189 (U/L),

correspondingly). In contrast, the other category (C2) includes the four remaining groups (control, TII, TIII, and TIV). Moreover, Category 2 was subdivided into two subcategories (A&B) based on FBW, BWG, RLW, RTW, CREA, ALT, ALP, and AST at a relative similarity level of 95.77%, where subcategory A included the TIV group, which showed a decrease in the mean amount FBW (191.2 g), BWG (25.03 g), RLW(3.53g), RTW(1.01g), CREA (29.48), ALT (35.8 U/L), and ALP (364 U/L) and an increase in the mean AST (164.6 U/L) compared with subcategory B, which included the three remaining groups: TII, TIII, and control).

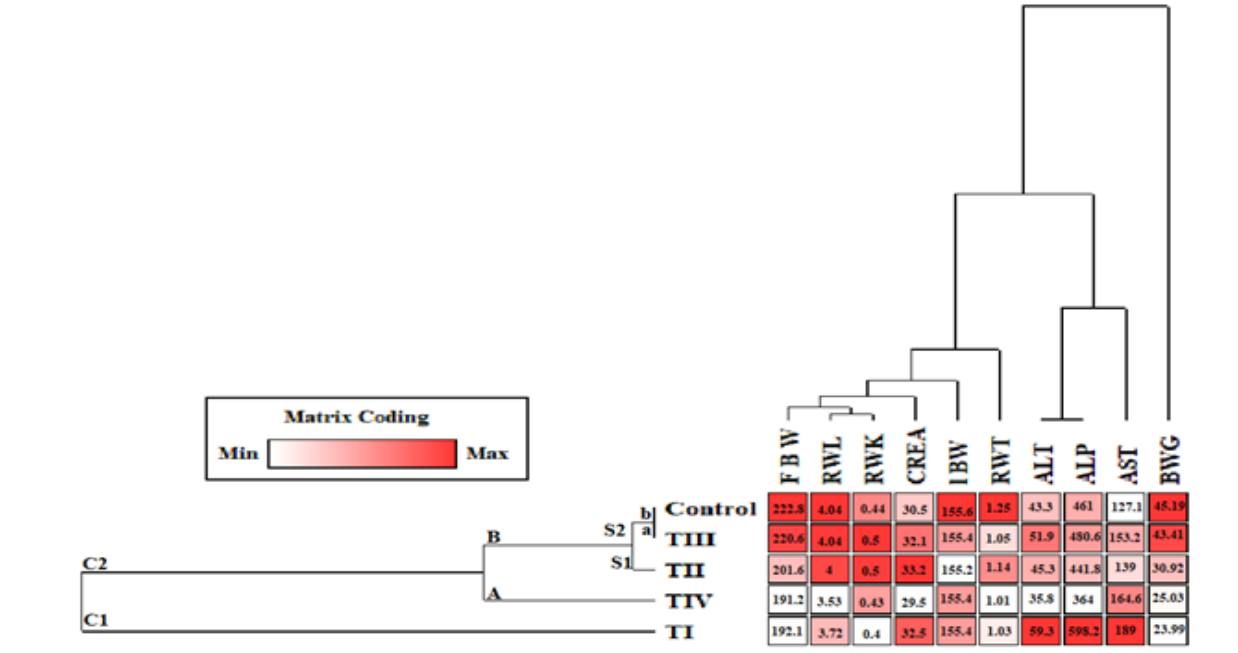

Fig. 1. Cluster analysis illustrates the relationship among the Five investigated groups based on 10 (6 Body Weights and 4 biochemical analyses) characters by using the Two-Way Cluster Analysis (TWCA) - Group average.

Furthermore, subcategory B was divided into two sections (S1 & S2) based on FBW, IBW, CREA, ALP, and BWG at a relative similarity level of 97.84%, where S1 includes the TII group, which exhibited a decrease in the mean amount of FBW (201.6 g), IBW (155.2 g), BWG (30.92 g), ALP (441.8), and displayed an increase in the mean amount of CREA (33.2) when compared with S2, which includes the control and TIII groups. On the other hand, section 2 is subdivided in two subsections (a & b) based on IBW, RTW, CREA, ALT, ALP and AST at relative similarity level of 98.41% where subsections a includes TIII group which showed the increase in mean amount of CREA (32.12), ALT (51.92), ALP (480.6) and AST(153.2) and decrease in the mean amount of IBW (155.4 g), RTW (1.05g) when compared with subsections b which includes the control group with a mean amount of 30.52, 43.28, 461, 127.1 and 155.6 g respectively.

Macroscopic Features of Liver

The liver in the control, TII, and TIII groups appeared well lobulated without any lesion (Figure 2C, TII, and TIII), while the liver in the TI group (CCL_4-treated group) showed that the lobules were irregular and rough. The color changed from tan to pink (Figure 2), while the liver in the TIV group (rats treated with *M. peregrina* extract) showed moderate roughness and an improvement in color and lobes.

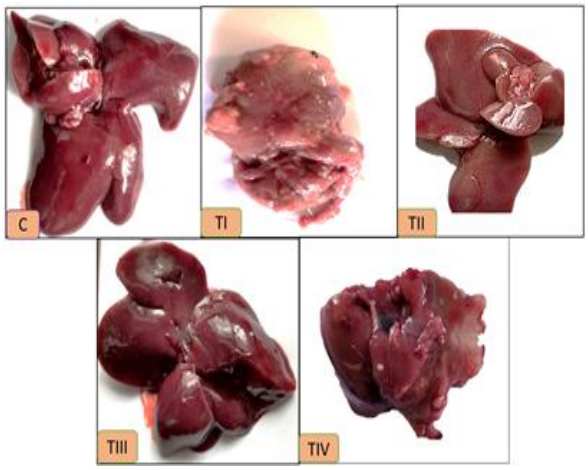

Fig. 2. Macroscopic changes in the white rat liver. C. control, TII, and TIII groups show a normal structure. TI and TIV showed that the lobules were irregular and rough, and the color changed from tan to pink.

Histopathological Examination

The Liver

Figure (3) showed that the histopathological examination of liver tissues revealed that the control group, as well as groups TII (olive oil-treated rats) and TIII (rats treated with *M. peregrina* extract), showed normal hepatic lobular architecture. Hepatocytes were arranged radially around the central vein, separated by blood sinusoids, and exhibited clear Kupffer cells. In contrast, group TI (CCl_4-treated rats) showed histopathological alterations, including a large area of fibrosis, hydropic cells, congested blood vessels and necrosis and group TIV (rats treated with *M. peregrina* extract + CCl_4) also showed histological changes, such as mild necrotic area and hydropic cells, such changes were less severe compared with TI treated group (CCl_4-treated rats).

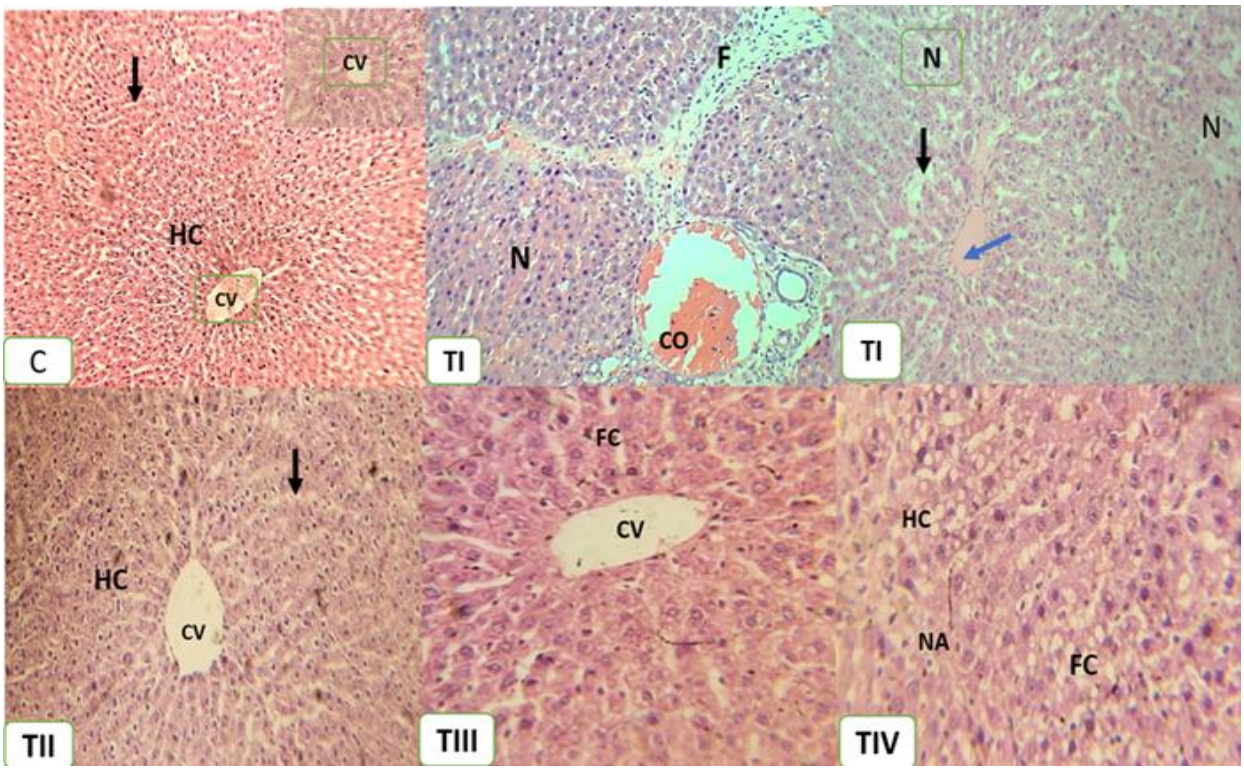

Fig. 3. Photomicrograph sections in the liver of white rats in all the experimental groups**.**
C (control group): shows a normal structure of hepatocytes **(HC)**, central vein **(CV)**, and sinusoid **(black arrow)**. **TI:** CCl4 group showed fibrosis **(F)** and necrosis **(N)**, hydropic cells **(black arrow),** and congested blood vessels **(CO)**. The **TII** group showed a normal structure of hepatocytes. **TIII (*M. peregrina* extract group):** showed normal structure of hepatocytes and central vein. **TIV (*M. peregrina* extract + CCl_4 group):** showed mild necrotic area (**NA)**, hydropic cells **(HC),** and fatty changes **(FC),** H & E (X 100 & 400).

The Kidney

Figure (4) showed that the histopathological examination of kidney tissues revealed that the control group, TII (olive oil-treated rats), and TIII (rats treated with *M. peregrina* extract), showed normal morphology of cortex and medulla, normal glomeruli, tubular cells, and vascular structure. While group TI (CCl_4-treated rats) showed fibrosis, inflammation, casts in tubules, hemorrhage, degenerated glomerulus, and thickness of blood vessels, and shrinking glomerulus, group TIV (rats treated with *M. peregrina* extract + CCl_4-treated rats) showed degenerated glomerulus and degenerated tubules.

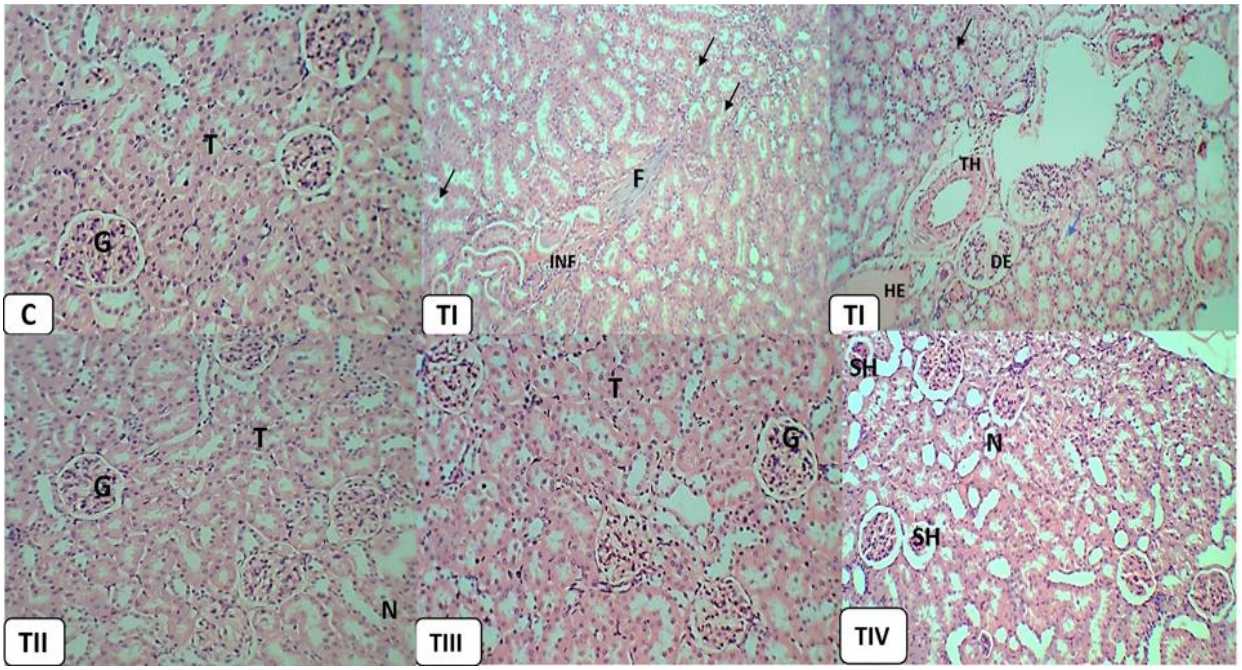

Fig. 4. Photomicrograph of Cross sections in the kidney of white rats in all the experimental groups.
C (control group): showed normal structure of glomerulus and renal tubules, **TI (CCl_4 treated group):** showed fibrosis (**F**), inflammation (**INF**), casts in tubules(**black arow**) hemorrhage (HE) degenerated glomerulus (**DE**) and thickness blood vessel (**TH**) and degenerated and shrinking glomerulus, **TII (Olive oil treated group):** showed normal structure of glomerulus and renal tubules, and mild necrosis (**N**) **TIII (*M. peregrina* extract group):** showed normal structure of glomerulus and renal tubules and casts in tubules, **TIV (*M. peregrina* extract + CCl_4 group):** showed shrinkage in glomerulus (SH) and mild necrosis (N). H & E (X 100 & 400).

The Testes

The histopathological examination of testes tissues reported that the control and ethanolic extract plant groups showed no noticeable histopathological alterations in orderly arranged germinal epithelium at different stages of spermatogenesis resting on clear basal laminae with interspersed Sertoli cells and interstitial cells of Leydig (Figure 5C+TIII). While the group treated with CCl_4 showed increased spaces between seminiferous tubules, oligospermia, degenerated and shrunken spermatocytes, hydropic cells, abnormal growth cells, Leydig cells disappeared, and record exfoliated tubules (Figure 5TI). On the other hand, the TII group showed a slight space and a hydropic area between spermatocytes and normally formed germinal epithelial cells (Figure 5TII). Furthermore, the group was treated with CCl_4 plus the ethanolic extract of the plant, which showed normally formed germinal epithelial cells, exfoliated tubules, and exhibited improved seminiferous tubule architecture (Figure 5 TIV).

DISCUSSION

It is widely recognized that acute and chronic toxicity from carbon tetrachloride (CCl_4)–derived free oxygen radicals can damage multiple organs, including the liver, heart, testes, lungs, kidneys, brain, and blood (Anatolia, 2014). The present study showed that *Moringa peregrina* exerted a protective effect against CCl_4-induced injury in the liver, kidneys, and testes. Throughout the experimental period, no clinical signs of toxicity were observed in any treated animals. This agrees with previous reports by Nagano et al. (2007) and Abd-Elhakim et al. (2020), who likewise noted an absence of clinical signs in the CCl_4-treated group of rats under similar experimental conditions.

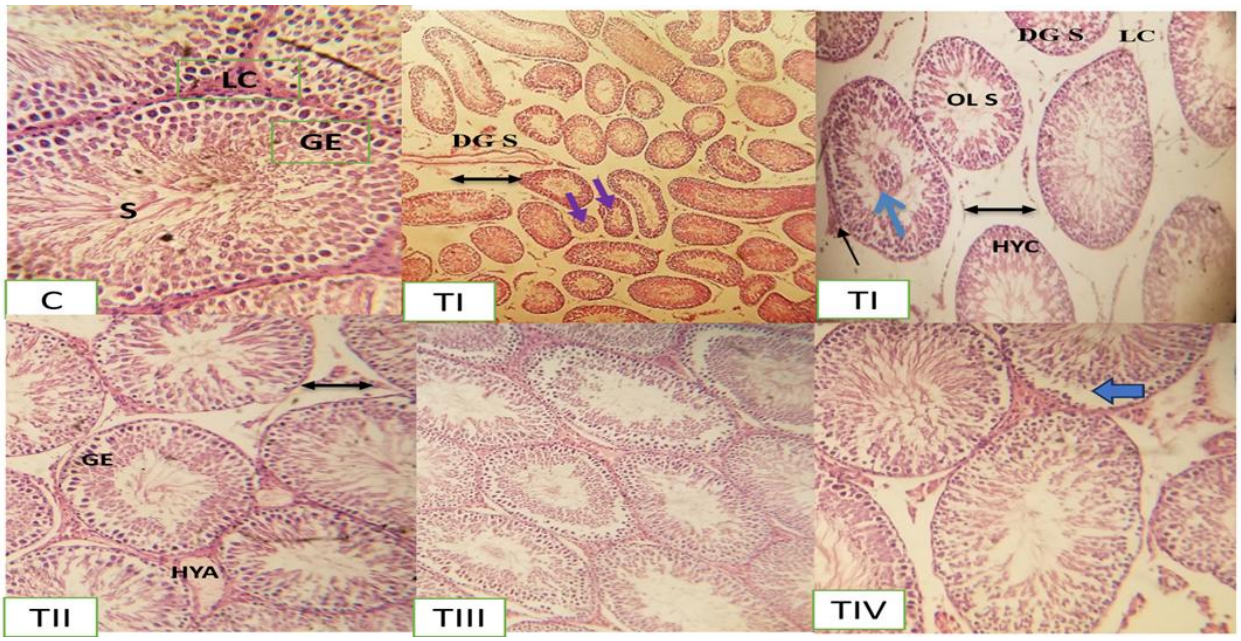

Fig. 5. Photomicrograph of Cross sections in the testes of white male rats in all the experimental groups.
C (control group): showed a normal structure in seminiferous tubules. Germinal epithelium (**GE**) sperms (s) and Leydig cells (**LC**). **TI (CCl_4 treated group):** showed oligospermia (**OLS**) degenerated Spermatocytes (**DGS**), Shrinkage spermatocytes (**black arrow**), Hydropic cells (**HYC**), and abnormal growth cells (**green arrow**), decrease of Leydig cells (**LC**), and increased space between tubules. (**black arrow**) and shrinkage of seminiferous tubules (**purple arrow**) and exfoliated tubule (**black arrow**). **TII (Olive oil treated group):** mild hydropic area (**HYA**) and space between tubules. (**black arrow**). **TIII (*M. peregrina* extract group):** showed normal structure in seminiferous tubules. **TIV (*M. peregrina* extract + CCl_4 group):** showed hypoplasia (**HP**) exfoliated tubule (**blue arrow**), H&E. (X 100 & 400).

In the present study, a decrease in the FBW and BWG of rats treated with TI and TIV treatment groups was observed when compared with other groups. This result was confirmed by Nagano et al. (2007), who found that CCL_4 caused a significant decrease in body weight at 810 ppm in exposed male rats. On the other hand, the relative kidney weight of the TI groups showed a decrease when compared with the control and other treatment groups. This result disagreed with Nagano et al. (2007), who found an increase in relative kidney weight exposed to 10 ppm of CCL_4.

The results for testis and liver weight indicate that exposure to CCl_4 does not result in a statistically significant change in tissue weight compared to the control group. The application of *M. peregrina* after CCl_4 did not cause a significant amelioration in testis and liver tissue weight; however, the values were not significantly different from those obtained for the control group either. This result was contradicted by Dunjic et al. (2022), who found a significantly

decreased testicular tissue weight in CCl_4-exposed animals. The current study showed there was a significant decrease in the main serum levels of Alanine aminotransferase (ALT) in the group TIV when compared with the other groups. This result agrees with the findings by Ujah et al. (2013), who recorded a decrease in the level of ALT enzyme in rats treated with ethanolic leaves extract of *M. oleifera*. However, these enzymes remain elevated CCl_4 treated, which did not receive the extract. A Similar trend was observed for Alkaline Phosphatase (ALP). The marked decrease in the activities of these marker enzymes ALT and ALP agrees with studies carried out by other workers on CCl_4 hepatotoxicity of other herbal plants such as *Hibiscus rosasinenis* (Obi *et al.*, 1998; Ulicna *et al.*, 2003). But an increase was observed for Aspartate aminotransferase (AST); this result disagreed with the findings by Ujah et al. (2013). On the other hand, the T1-treated group with CCL_4 showed an increase in the serum content of ALT, AST, and ALP. These results agree with the study of Ujah et al. (2013) and Kumar et al. (2018). This indicates liver injury, especially the rise in ALT activity (Ngaha *et al*., 1989). Hence, serum or plasma enzyme levels have been used as indices for monitoring chemically induced tissue damage.

In the present study, the macroscopy examination result of the liver showed normal appearance in the control, TII, and TIII groups. On the other hand, in group TI, the liver showed that the lobules were irregular and rough, and the color changed to tan to pink; also, the TIV group showed improvement in lobules and color. This result agreed with the findings by Supriono et al. (2020), who recorded that the liver of the CCL_4 group was paler and more wrinkled than the *M. oleifera* group.

Histopathological studies demonstrated CCl_4-induced fibrosis, necrosis, hydropic cells, and congested blood vessels. These results agree with previous studies (Ramdas, 2010; Mirshahvalad *et al.,* 2016; Kumar et al., 2018; Ezeonwumelu *et al.,* 2025). These could be due to biochemical changes in liver cells. The present study found no significant changes in liver enzymes (ALT, ALP, and AST) in the CCL_4 group. The section of liver from the TIV group showed improvement in liver cells; these results were similar to those of Ujah et al. (2013). It has been reported that the Moringa plant is rich in compounds containing the simple sugar rhamnose, as well as several vitamins and minerals (Lowell, 1989). Furthermore, it is noteworthy that the *M. peregrina* extract and olive oil group showed a normal liver cell structure, as in the normal control plate. Histological examination showed normal renal architecture in the control group, whereas the CCl_4 group showed fibrosis, inflammation, tubular casts, hemorrhage, degenerated glomeruli, thickened blood vessels, and a degenerated, shrinking glomerulus. These results agree with previous studies (Abd-Elhakim *et al*., 2020; Habashy *et al.,* 2021).

The present study showed a normal structure of testicular cells in a control and *M. peregrina* group. This result is similar to that of Eljaafari et al. (2024), and the histological alteration in the CCL_4 group also agrees with the findings of Abd-Elhakim et al. (2020) and Eljaafari et al. (2024), which reported histological alterations in the testes. On the other hand, in group TII, a few hydropic areas and spaces between tubules were observed. These results differ from those of Abd-Elhakim et al. (2020), who found that corn oil-treated rats showed normal histology, an orderly-arranged germinal epithelium at different stages of spermatogenesis resting on clear basal laminae with interspersed Sertoli cells and interstitial cells of Leydig.

CONCLUSION

In conclusion, the ethanolic leaf extract of *Moringa peregrina* markedly enhanced the enzymes of the liver and kidney and improved liver, kidney, and testes tissue damage induced by CCl_4 in rats by restoring normal liver, kidney, and testes architecture and improving serum enzymes and tissue integrity. These findings suggest that *M. peregrina*, with its strong antioxidant potential, may facilitate tissue recovery following chemical-induced injury, likely through the action of its bioactive and regenerative phytoconstituents.

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CONFLICT OF INTEREST

The authors hereby declare that they have no conflict of interest.

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