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Efficacy of Moringa Oil and Flaxseed Oil against Carbendazim Toxicity in Hepatorenal Organs of Male Rats: A Physiological and Histological Study

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ABSTRACT

Environmental pollution spreads pollutants, and their toxicities have remained significant global health concerns for decades. The level of toxicity exhibited by pesticides is influenced by multiple factors, including the duration and method of exposure as well as the overall physical condition of the individuals exposed. Carbendazim (CBZ) is used to control multiple types of fungal attacks on different crops. Experimentally, CBZ has been observed to cause physiological, biochemical and histopathological alterations. Medicinal plants, natural products, and nutrition remain vital to mitigate these toxicities. This research aimed to examine the protective effect of moringa oil and flaxseed oil on hepatorenal toxicity and changes in the activity of antioxidants induced by exposure to CBZ in male rats. The rats were grouped into six groups (G1-G6) consisting of a total of 60 rats, with each group containing 10 rats. The groups were designated as follows: G1 (Control), G2 (CBZ intoxicated at a dose of 200 mg/kg BW), G3 (moringa oil at a dose of 800 mg/kg BW plus CBZ), G4 (flaxseed oil at a dose of 800 mg/kg BW plus CBZ), G5 (moringa oil at a dose of 800 mg/kg BW), and G6 (flaxseed oil at a dose of 800 mg/kg BW). The rats were observed for a period of 6 weeks. In rats treated with only CBZ (G2), an elevation in the levels of alanine transaminase, aspartate transaminase, alkaline phosphatase and total bilirubin was observed. On the other hand, a decline in serum albumin and total protein was recorded. The CBZ-exposed group expressed some histological alterations in liver tissues. Moreover, elevations in serum creatinine, blood urea nitrogen and uric acid were noted with histological alterations in kidney structure. Additionally, a significant decrease in the levels of catalase, superoxide dismutase and reduced glutathione was noticed with an increase in malondialdehyde level. Treatments with moringa oil (G3) and flaxseed oil (G4) in rats exposed to CBZ showed remarkable reducing and protecting effects of physiological and histopathological changes. In conclusion, the obtained results indicate that the protective influences of moringa oil and flaxseed oil are attributed to their antioxidant activities. Finally, this study provides valuable information about the potential protective role of moringa oil and flaxseed oil against CBZ-induced hepatorenal toxicity.

Keywords: Carbendazim, Hepatorenal Toxicity, Moringa Oil, Flaxseed Oil, Antioxidant, Rats

1. Introduction

Environmental pollution resulting from the use of pesticides is a significant concern with widespread implications for ecosystems and human health. While their intended purpose is to increase crop yield and protect human health, the improper use and disposal of pesticides can lead to adverse environmental consequences. Exposure to pesticides through contaminated food and water or occupational contact can have detrimental effects on human well-being. Prolonged exposure or high doses of certain pesticides have been linked to various health issues, including cancer, organ toxicity, neurodevelopmental disorders, reproductive problems, endocrine disruption, and physiological alterations (Mostafalou and Abdollahi, 2017; Deremeaux *et al*., 2020; Ottenbros *et al*., 2023).

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Carbendazim (CBZ), a broad-spectrum systemic widely used fungicide in agriculture, is widely recognized for its effectiveness in controlling various fungal diseases in crops. Its chemical name is methyl benzimidazole-2-ylcarbamate, and it belongs to the benzimidazole class of chemicals (Singh *et al*., 2016; Hashim *et al.,* 2023). CBZ is reported to cause hematological alterations including, hormonal, oxidative, liver, kidney and testicular malfunction functions. In addition, it is responsible for multiorgan toxicities confirmed by histological studies in exposed animals (Zari and Al Attar, 2011; Sharma *et al*., 2022).

CBZ leads to modifications in crucial organs' homeostasis, potentially leading to compromised organ operation and the emergence of ailments. It has been noted to initiate harm to the liver, identifiable by the death of liver cells, inflammation, and the infiltration of fat. These shifts often coincide with increased levels of liver enzymes like alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). In relation to the kidneys, there have been documented instances of structural distortions such as the withering of tubular structures, the presence of fibrous tissue in the interstitial spaces, and injury to the glomeruli. These consequences can impede proper kidney function and contribute to the accumulation of harmful substances within the body. High serum creatinine, uric acid, and blood urea nitrogen (BUN) levels have been reported in in rats treated with CBZ. Moreover, CBZ has been shown to induce degeneration of the seminiferous tubules in the testes, resulting in reduced sperm production and impaired spermatogenesis. Consequently, male fertility may decline due to these impacts (Zari and Al Attar, 2011; Abolaji *et al.,* 2017; Patil *et al.,* 2022).

A range of structural changes in the kidneys of CBZ-exposed rats, including the narrowing of the glomerulus, degeneration of the tubules, and reduced Bowman's capsule have been observed. Moreover, CBZ exposure increased the levels of certain markers of oxidative stress in the kidneys, such as malondialdehyde (MDA) and superoxide dismutase (SOD), suggesting potential harm caused by CBZ (Selmanoglu *et al.,* 2001; Nwozo *et al.,* 2017). CBZ exposure is evident in the deterioration of the liver, as indicated by elevated levels of markers associated with liver damage, including, AST, ALT, ALP, and total bilirubin in the serum. CBZ exposure is also accountable for diminishing levels of crucial antioxidants like GSH and SOD, as well as a decrease in total antioxidant capacity (TAC). Conversely, there is an observed rise in levels of lactate dehydrogenase (LDH), MDA and myeloperoxidase (MPO), indicating an increase in oxidative stress, cardiac malfunction, and inflammation. (Yousef *et al*., 2022). Several prior investigations demonstrated that using different botanicals, such as olive (*Olea europaea*) leaves, licorice (*Glycyrrhiza glabra*), fenugreek (*Trigonella foenum-graecum Linn*.), *Gingo bilola*, ginger (*Zingiber officinale*), Jaft internal layer of *Quercus brantii*, *Nigella sativa*, *Foeniculum vulgar* seeds*,* Quercetin, Vitamin C., Banana peel and *Adiantum Capillus-Veneris L.* (Zari and Al-Attar, 2011; Lamfon, 2012; Mahboub and Lamfon, 2013; Sakr and Shalaby, 2014; Mirzaei *et al.* 2015; Salihu *et al.*, 2016; Abou Zaid *et al*. 2018; Hashem *et al.*, 2018; Alghamdi, 2020; Lalhriatpuia *et al*., 2021; Abdel-Rahman *et al.* 2022; Kiran, 2022; Seif *et al.,* 2023) can mitigate CBZ toxicities in different animals.

Medicinal plants have long been recognized for their therapeutic properties in treating various toxicities in humans and animals. These plants contain bioactive compounds that can help counteract the effects of toxic substances and promote improving biochemical, physiological, and structural disruptions in multi-organ system such as liver and kidney (Abenavoli *et al.,* 2010; Akram *et al*., 2017). *Moringa oleifera* (*M. oleifera*) belonging to the Moringaceae family, comprises a total of 13 species distributed globally. *M. oleifera* sometimes referred to as moringa is cultivated in more than 80 countries for its nutritional and phytochemical significance (Mahmood *et al.,* 2010). Moringa has 119 physiologically active constituents belonging to 8 different species, including proteins, flavonoids, saponins, phenolic acids, tannins, isothiocyanates, lipids, minerals, and vitamins. Pharmacologically, it helps in treating many health issues and acts as hepatoprotective, anti-inflammatory, anti-cancer, treats diabetes, full of antioxidants, regulates blood chemistry and detoxifies toxic chemicals in the body (Meireles *et al.,* 2020; Abdel Shakour *et al.,* 2023).

Linum usitatissimum L. is called by different local names like flaxseed, linseed, or oil seed. It belongs to *Linaceae* which is called the flax family. Widely distributed, with 14 genera and 230 species. Despite greater diversity, flax is the only cultivated species in the family (Verma and Mishar, 2013; CABI, 2018). Flaxseeds are an important source of functional foods due to their high nutritional content of omega-3 fatty acids, phytohormone lignans, dietary fibre, high quality essential proteins, and oils. It contributes to maintaining physiological and biochemical requirements. Flaxseed’s bioactive components are of great interest to pharmacists. It is used to treat antioxidant stress, toxicity problems,

diabetes, cholesterol management, cancer, osteoporosis, cardiovascular problems, and healthy hair and skin protecting agents (Wang *et al.,* 2017; Shim *et al*., 2022; Sangiorgio *et al*., 2023). In the realm of natural solutions to CBZ toxicity, moringa oil and flaxseed oil emerge as guardians, offering their protective potential in mitigating hepatorenal toxicity, and restoring balance to disrupted organs in rats, both physiologically and histologically. This study focused on specific conditions, doses, and animal responses toward CBZ exposure. Moreover, the present study was conducted to investigate the influence of moringa oil and flaxseed oil on hepatorenal disturbances induced by CBZ in male rats. Histopathological examination of the liver and kidney, and the physiological markers such as ALT, AST, ALP, total bilirubin, total protein, albumin, creatinine, BUN, uric acid, catalase (CAT), SOD, reduced glutathione (GSH) and MDA were evaluated in this study.

2. Materials and Methods

2.1. Experimental Animals

Male albino rats of the Wistar strain (*Rattus norvegicus*), weighing 110-149 g, were utilized in the current study. The experimental animals were acclimatized to the laboratory conditions for one week prior to the initiation of the experiments. Rats were housed in well-aerated standard plastic cages and maintained under controlled laboratory conditions of humidity (55% ±10), constant room temperature (20±1 °C), and 12:12 h light: dark cycle each day. Rats were fed with a standard laboratory chow diet *ad libitum* with free access to water. The animal ethics guidelines released by the Animal Care and Use Committee (ACUC) of King Abdulaziz University were followed for conducting the experiments. Moreover, all experiments were conducted in compliance with the Arrive guidelines and in accordance with the EU Directive 2010/63/EU for animal experiments.

2.2. Animal Groupings and Treatment

A total of sixty male rats were divided into six experimental groups, ten of rats each. The experimental groups were treated as follows:

Group 1: Untreated rats and served as normal controls.
Group 2: Rats were orally given 200 mg /kg body weight of CBZ (Sigma-Aldrich Corp., St. Louis, MO, USA), daily for six weeks.
Group 3: Rats were orally supplemented with moringa oil at a dose of 800 mg/ kg body weight/ day.

Moreover, after 3 h they were exposed to CBZ at the same dose given to group 2 for six weeks.
Group 4: Rats were orally given flaxseed oil at a dose of 800 mg/ kg body weight/ day. Moreover, after 3 h they were exposed to CBZ at the same dose given to group 2 for six weeks.
Group 5: Rats were orally administered daily with moringa oil at a dose of 800 mg/kg body weight for six weeks.
Group 6: Rats were orally given flaxseed oil daily at a dose of 800 mg/kg body weight for six weeks.

2.3. Analysis of Blood Serum

At the end of the experimental period, rats were fasted for 12 hours, water was not restricted, and they were then anesthetized with diethyl ether. Blood samples were collected from orbital plexus veins in non-heparinized tubes, and then centrifuged at 2500 rpm for 15 minutes for serum separation. The blood sera were then collected, frozen at -80 °C and stored until use for the biochemical analysis. The levels of serum ALT, AST, ALP, total bilirubin, total protein, albumin, creatinine, BUN, uric acid, CAT, SOD, GSH and MDA were measured using assay kits following the manufacturer's instructions.

2.4. Histopathological Examination

Rats from each experimental group were sacrificed for their liver and kidney tissues. These tissues underwent processing and were sectioned into thin pieces measuring 4-5µm using a microtome. Subsequently, the sections were stained using the hematoxylin and eosin (H&E) method, following the procedures outlined in the referenced study (Suvarna *et al.,* 2018). All of the sections of the liver and kidney were examined using a light microscope (Zari and Al Attar, 2011).

2.5. Statistical Analysis

The statistical analysis was performed using SPSS version 22.0. One-way analysis of variance (ANOVA) was used to assess group differences, and Dunnett's test for post hoc comparisons was used. The statistical significance was set at $P \leq 0.05$, and the data were presented as mean ± standard deviation (S.D.).

3. Results

3.1. Assessment of Liver Functions

3.1.1. Serum ALT

The serum ALT levels for the following groups of rats: control rats, rats exposed to CBZ, rats treated with moringa oil plus CBZ, rats treated with flaxseed oil plus CBZ, rats treated with only moringa oil, and rats treated only with flaxseed oil are presented in Figure 1. Rats exposed to CBZ (+441.9%, $P \leq 0.000$), treated with moringa oil CBZ (+166.7%, $P \leq 0.000$) and treated with flaxseed oil and CBZ (+323.8%m $P \leq 0.000$) all had significantly higher serum ALT levels than control rats. Rats supplemented with moringa oil (group 5) and flaxseed oil (group 6) both had a non-significant change in the levels of ALT compared with control rats (Figure 1A).

3.1.2. Serum AST

Regarding serum AST levels, rats treated with flaxseed oil and CBZ exhibited significant declines (-19.1%, $P \leq 0.001$) compared to control rats, while rats exposed to CBZ experienced significant increases (+30.2%, $P \leq 0.000$). Rats from groups 3, 5, and 6 had negligible changes in their serum ALT levels (Figure 1B).

3.1.3. Serum ALP

In the context of serum ALP levels, the levels across all experimental groups were demonstrated in Figure 1C. Administration of CBZ to normal rats led to a significant increase in serum ALP levels (+67.8%, $P \leq 0.000$) compared to control rats. A statistical increase in serum ALP levels was observed in rats treated with moringa oil plus CBZ (+7.9%. $P \leq 0.01$) compared to control rats from group 1. However, no statistically significant differences were observed in the serum ALP levels of rats treated with flaxseed oil plus CBZ (group 4), moringa oil (group 5) and flaxseed oil (group 6) compared to control rats.

3.1.4. Serum total bilirubin

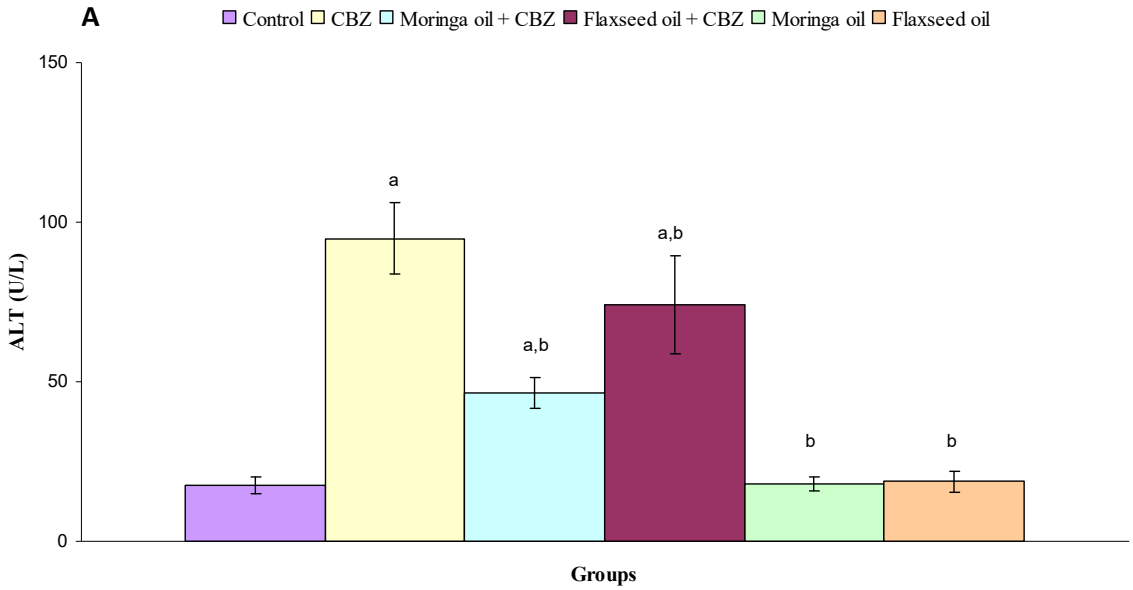
Serum total bilirubin levels were statistically enhanced in rats exposed to CBZ (+ 100.0%, $P \leq 0.000$), moringa plus CBZ (+ 38.9%, $P \leq 0.01$), and flaxseed oil plus CBZ (+ 53.8%, $P \leq 0.01$) compared with control rats. Rats supplemented with moringa oil (group 5) and flaxseed oil (group 6) showed insignificant changes in the level of serum total bilirubin (Figure 1D).

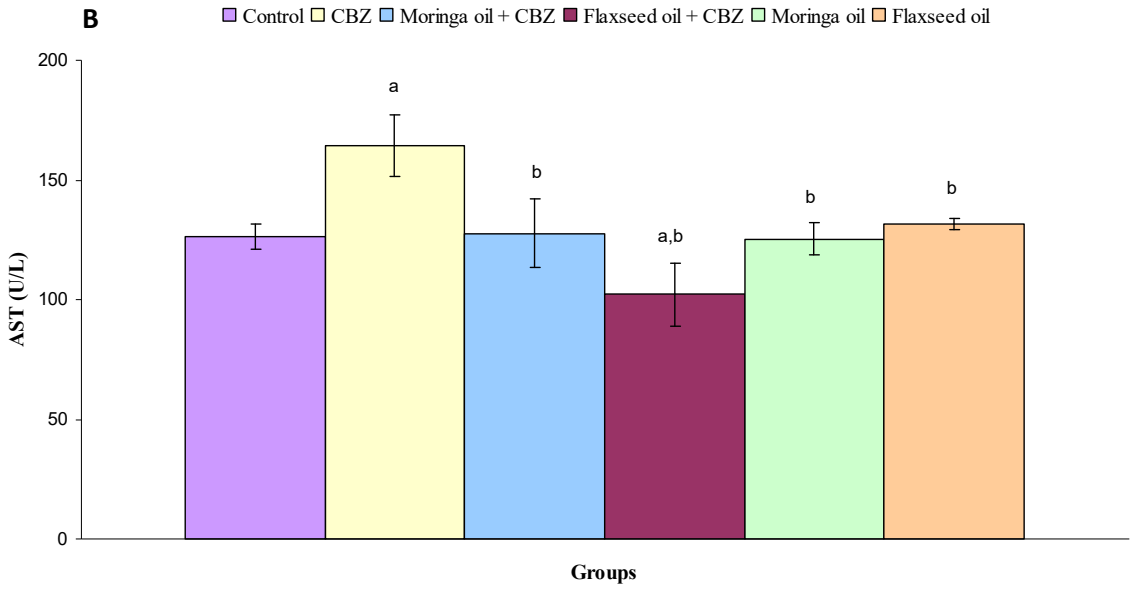
3.1.5. Serum total protein

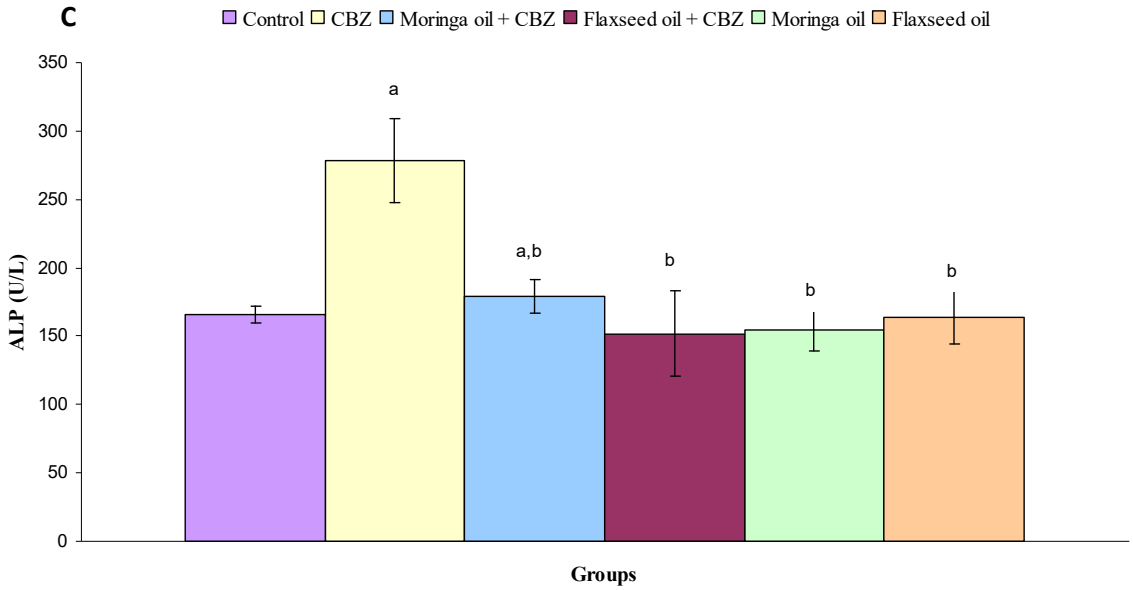
Notably, rats treated with moringa oil plus CBZ (group 3), flaxseed oil plus CBZ (group 4), moringa oil (group 5) and flaxseed oil (group 6) showed no significant differences in serum total protein when compared to control rats, while rats exposed to CBZ (group 2) showed a decrease in serum total protein (-14.5%, $P \leq 0.001$) (Figure 1E).

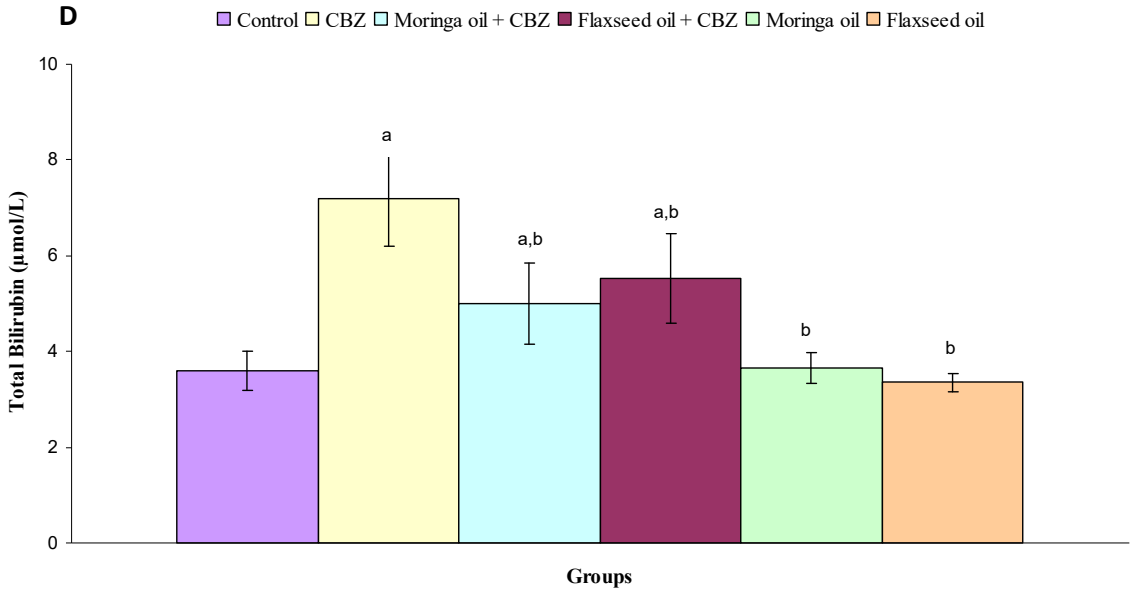
3.1.6. Serum albumin

CBZ-treated rats (group 2) showed a statistically significant decrease in serum albumin levels (-13.4%, $P \leq 0.001$) compared to control rats. Rats from groups 3, 4, 5, and 6 did not exhibit any appreciable changes in their serum albumin levels, though (Figure 1F).


A
Control
CBZ
Moringa oil + CBZ
Flaxseed oil + CBZ
Moringa oil
Flaxseed oil
ALT (U/L)
150
100
50
0
a
a,b
a,b
b
b
Groups


B
Control
CBZ
Moringa oil + CBZ
Flaxseed oil + CBZ
Moringa oil
Flaxseed oil
AST (U/L)
200
150
100
50
0
a
b
a,b
b
b
Groups


C
Control
CBZ
Moringa oil + CBZ
Flaxseed oil + CBZ
Moringa oil
Flaxseed oil
ALP (U/L)
350
300
250
200
150
100
50
0
a
a,b
b
b
b
Groups


D
Control
CBZ
Moringa oil + CBZ
Flaxseed oil + CBZ
Moringa oil
Flaxseed oil
Total Bilirubin (µmol/L)
10
8
6
4
2
0
a
a,b
a,b
b
b
Groups

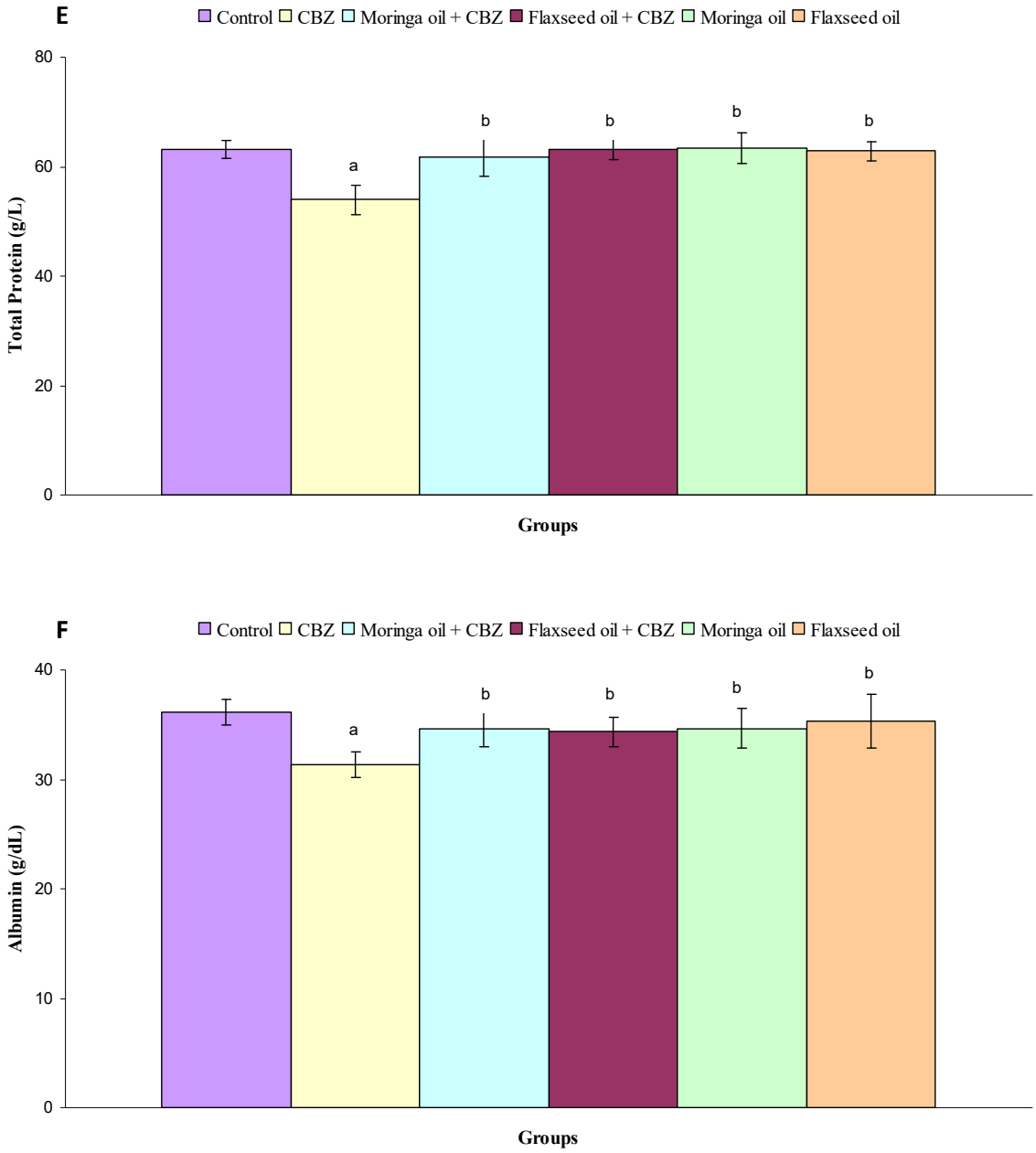


Fig. 1 (A-F): Levels of serum ALT (A), AST (B), ALP (C), total bilirubin (D), total protein (E) and albumin (F) in group 1: control; group 2: CBZ; group 3: moringa oil plus CBZ; group 4: flaxseed oil plus CBZ; group 5: moringa oil; and group 6: flaxseed oil-treated rats. **a**Indicates a significant difference between control (group 1) and treated groups (2, 3, 4, 5, and 6). **b** Indicates a significant difference between rats treated with CBZ (group 2) and groups 3, 4, 5 and 6.

3.2. Assessment of Kidney Functions

3.2.1. Serum creatinine

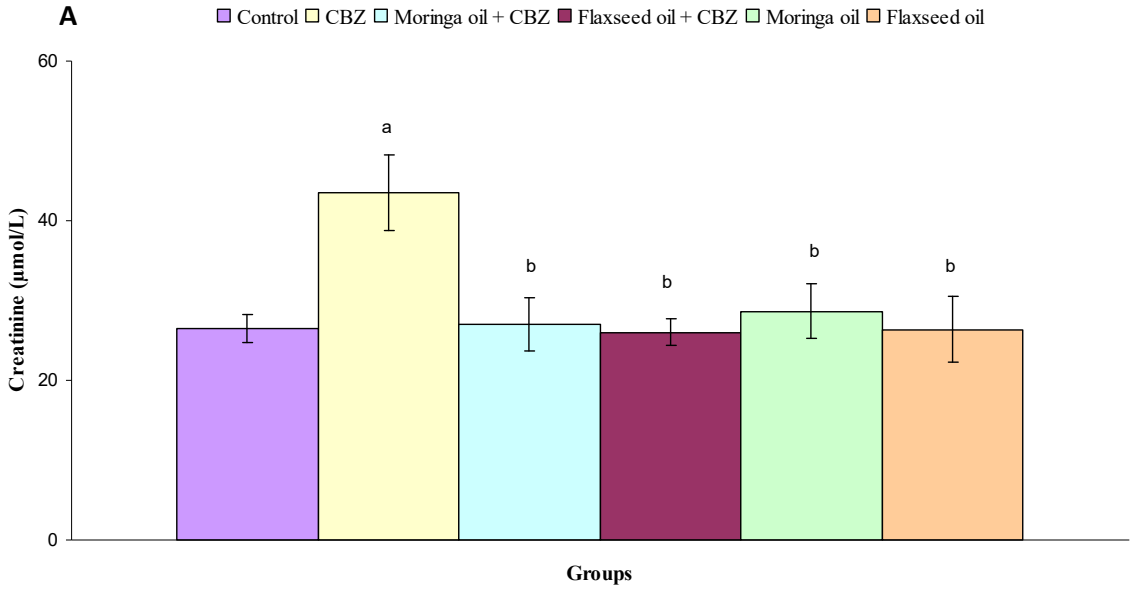
A significant rise in serum creatinine levels was observed in rats that were subjected to CBZ exposure, showing an increase of +64.2% ($P \leq 0.000$). Furthermore, there were no substantial variances in serum creatinine levels among rats treated with moringa oil plus CBZ (group 3), flaxseed oil plus CBZ (group 4), as well as rats administered moringa oil (group 5) or flaxseed oil (group 6), when compared to the control rats (Figure 2A).

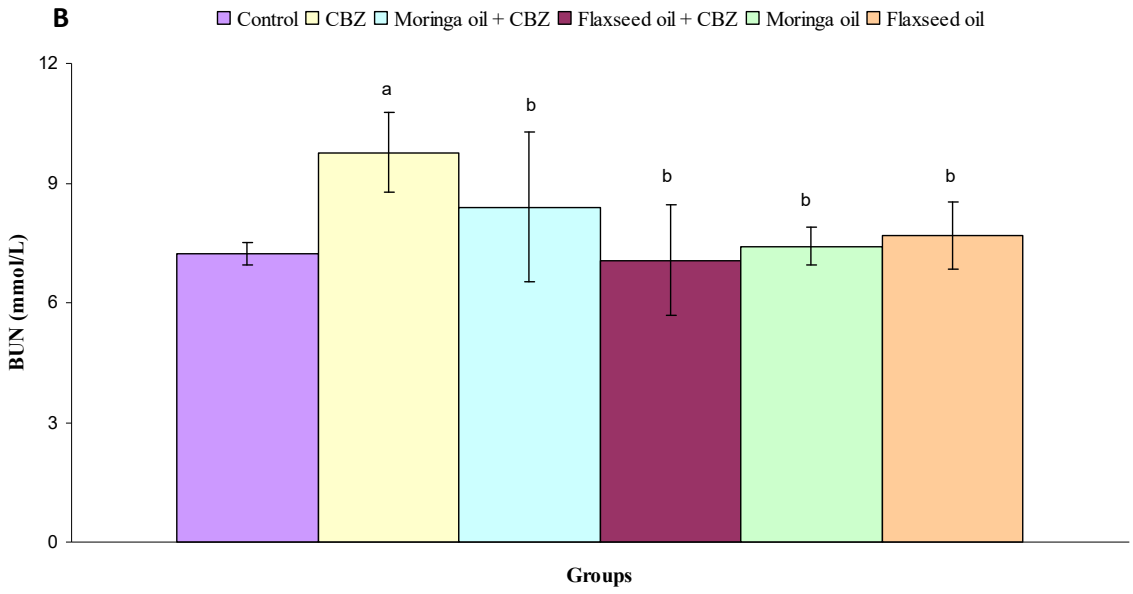
3.2.2. Serum BUN

Serum BUN levels displayed a notable increase in rats exposed to CBZ intoxication, indicating a rise of +35.3% ($P \leq 0.001$) in comparison to the control rats. Conversely, there were no meaningful changes in the BUN levels observed among rats belonging to groups 3, 4, 5, and 6 when compared to the control rats (Figure 2B).

3.2.3. Serum uric acid

In contrast to the control rats, there was a significant rise in serum uric acid levels observed in rats that were exposed to CBZ (group 2), indicating an increase of +50.8% ($P \leq 0.001$). However, negligible alterations were detected in the serum uric acid levels of rats receiving moringa oil plus CBZ (group 3), flaxseed oil plus CBZ (group 4), and those administered with moringa oil (group 5) or flaxseed oil (group 6), when compared to the control rats (Figure 2C).





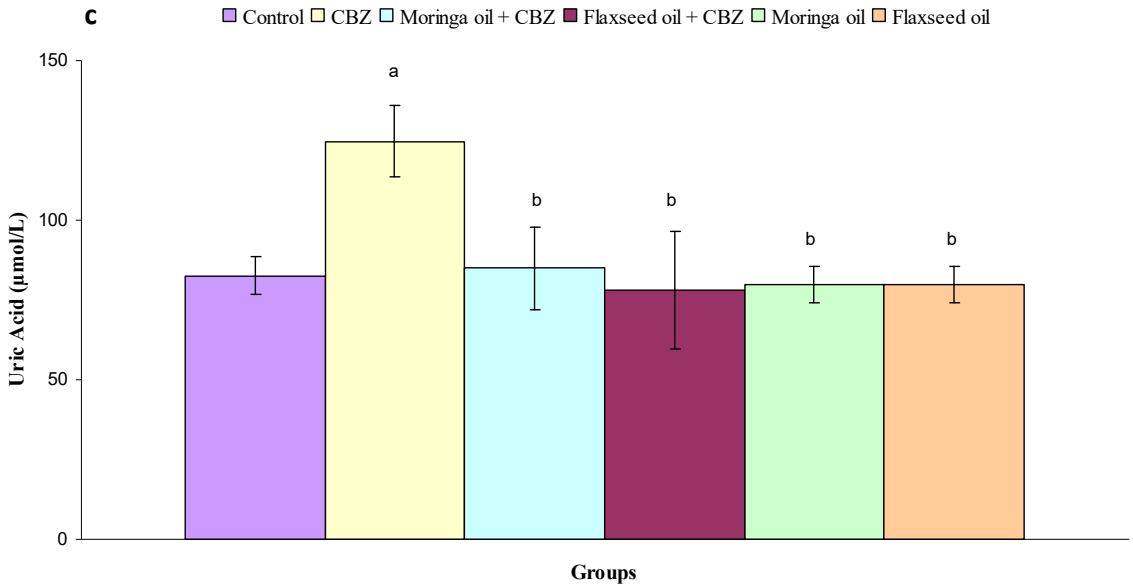


Fig. 2: (A-C). Levels of serum creatinine (A), BUN (B) and uric acid (C) in group 1: control; group 2: CBZ exposed; group 3: moringa oil plus CBZ; group 4: flaxseed oil plus CBZ; group 5: moringa oil and group 6: flaxseed oil-treated rats. **a**Indicates a significant difference between control (group 1) and treated groups (2, 3, 4, 5, and 6). **b** Indicates a significant difference between rats treated with CBZ (group 2) and groups 3, 4, 5 and 6.

3.3. Assessment of Oxidative Stress Markers

3.3.1. Serum CAT

The concentration of serum CAT showed a notable reduction in rats that were exposed to CBZ, with a decrease of -49.0% ($P \leq 0.000$), as compared to the rats in the control group. Similarly, in rats that were treated with a combination of moringa oil and CBZ, there was a decrease of -30.4% ($P \leq 0.000$), and in those treated with a combination of flaxseed oil and CBZ, there was a decrease of -38.7% ($P \leq 0.001$), all of which demonstrated statistically significant decreases in serum CAT levels. In contrast, there were no statistically significant variations in serum CAT levels observed between rats that received supplementation with either moringa oil (group 5) or flaxseed oil (group 6) when compared to the control rats (Figure 3A).

3.3.2. Serum SOD

A significant reduction in the levels of serum SOD was evident in rats that underwent treatment with CBZ (group 2), with a decrease of -61.1% ($P \leq 0.000$). Similarly, in rats subjected to a combination of moringa oil and CBZ, there was an observed decrease of -45.5% ($P \leq 0.000$), and in those treated with a combination of flaxseed oil and CBZ, the decrease amounted to -57.3% ($P \leq 0.000$). These outcomes demonstrated statistically significant decreases in serum SOD levels when compared to the control group of rats. Conversely, there were no statistically significant disparities in serum SOD levels noted between rats that were given supplementation with either moringa oil (group 5) or flaxseed oil (group 6) and the control rats. (Figure 3B).

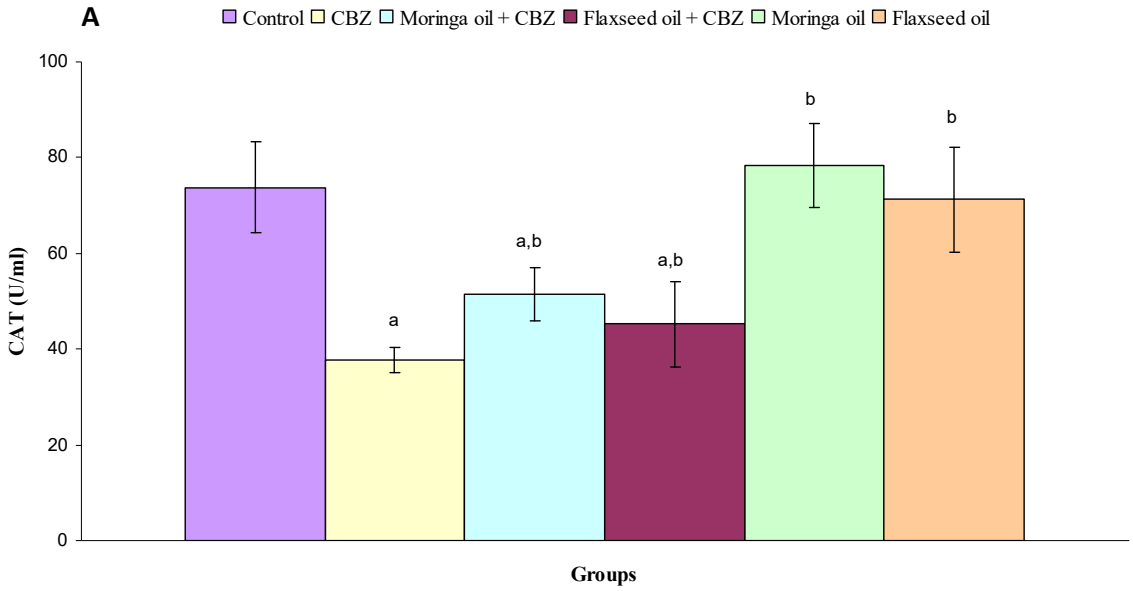
3.3.3. Serum GSH

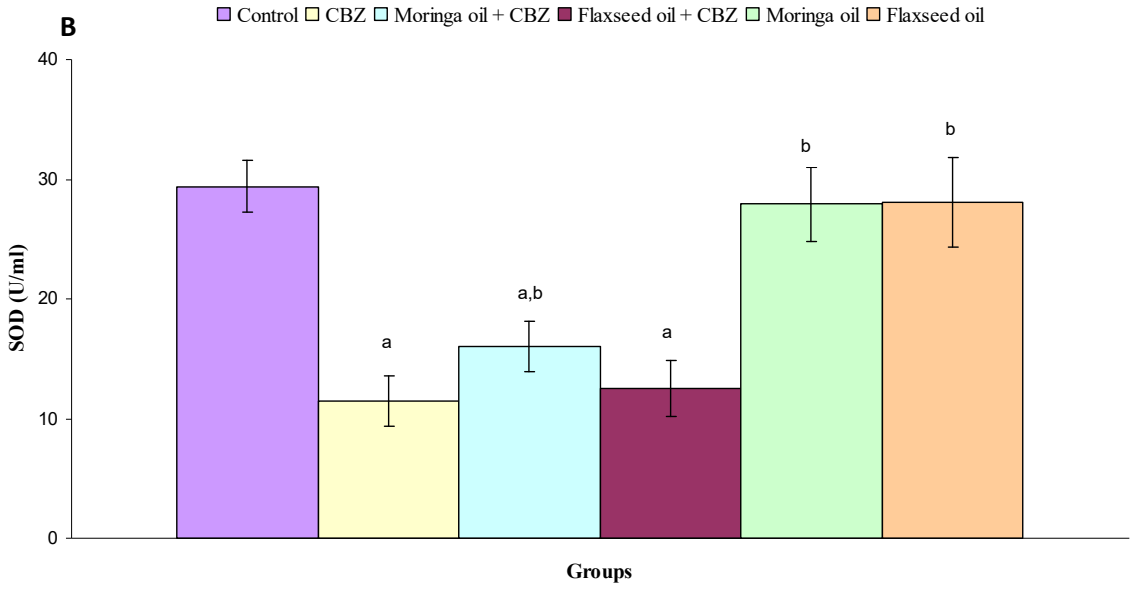
Significant reductions in the levels of serum GSH were noted among rats subjected to various treatments. In the case of rats treated with CBZ alone, there was an observed decrease of -49.2% ($P \leq 0.000$). Similarly, when rats were administered a combination of moringa oil and CBZ, the decrease was measured at -30.1% ($P \leq 0.007$), and for those treated with a combination of flaxseed oil and CBZ, the decrease reached -24.6% ($P \leq 0.003$). These findings indicated statistically significant decreases in

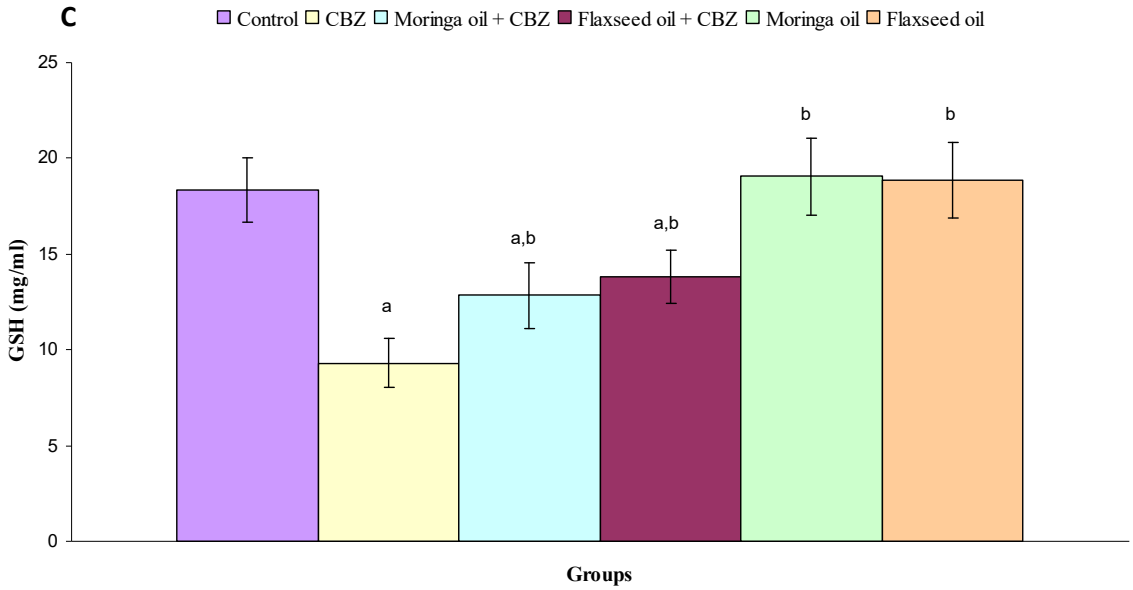
serum GSH levels as compared to the control group of rats. Nevertheless, there were no statistically significant variations in serum GSH levels between rats that received supplementation with either moringa oil (group 5) or flaxseed oil (group 6), and the control rats (Figure 3C).

3.3.4. Serum MDA

Serum levels of MDA exhibited a noteworthy increase in rats treated with CBZ, showing an elevation of +87.7% ($P \leq 0.001$) in comparison to the control group of rats. Conversely, rats from groups 3, 4, 5 and 6 did not display any evident changes in serum MDA levels when compared to the control rats. (Figure 3D).







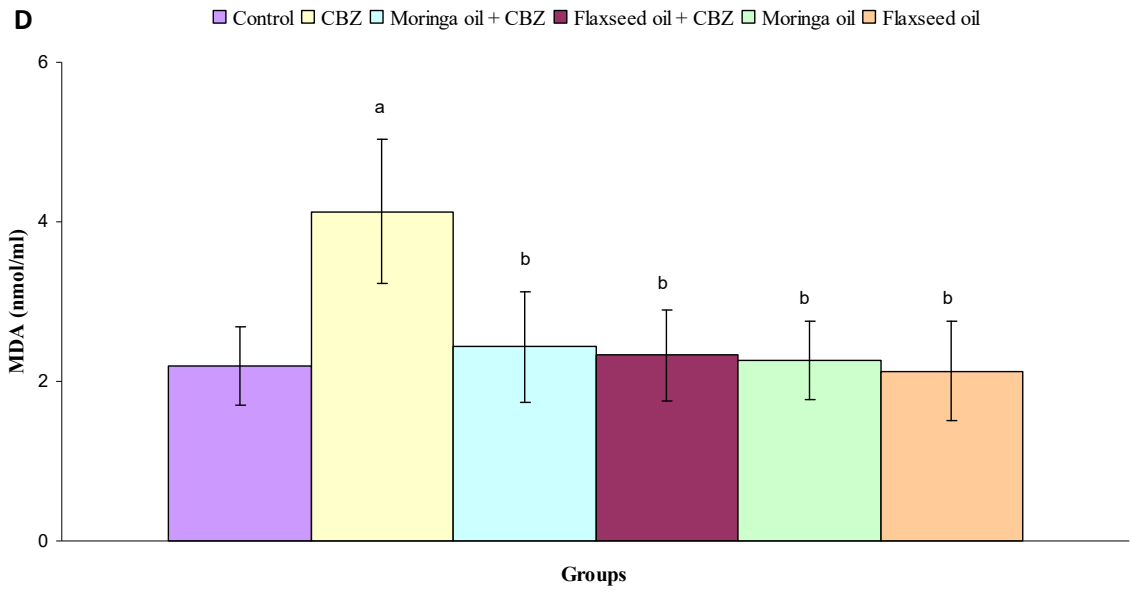


Fig. 3 (A-D): Level of oxidative stress markers in serum (A: CAT level), (B: SOD level), (C: GSH level) and (D: MDA level) in group 1: control, group 2: CBZ exposed, group 3: moringa oil plus CBZ, group 4: flaxseed oil plus CBZ, group 5: moringa oil and group 6: flaxseed oil-treated rats. **a**Indicates a significant difference between control (group 1) and treated groups (2, 3, 4, 5 and 6). **b** Indicates a significant difference between rats treated with CBZ (group 2) and groups 3, 4, 5 and 6.

3.4. Histopathological 0bservations

3.4.1. Liver

Liver tissues obtained from groups 1, 3, 4, 5 and 6 displayed a typical hepatic structure characterized by a central vein and extending hepatic cords. The liver sections of these groups exhibited regular hepatocytes with intact cytoplasm, distinct nuclei and nucleoli, and a well-defined central vein.

These hepatocytes appeared as cuboidal epithelial cells arranged in interconnected plates and cords, as demonstrated in (Figure 4A, C, D, E and F). Liver sections from rats subjected to CBZ exposure alone (Figure 4B) exhibited an atypical appearance marked by disrupted hepatic structures, sinusoidal enlargement, and the formation of vacuoles.

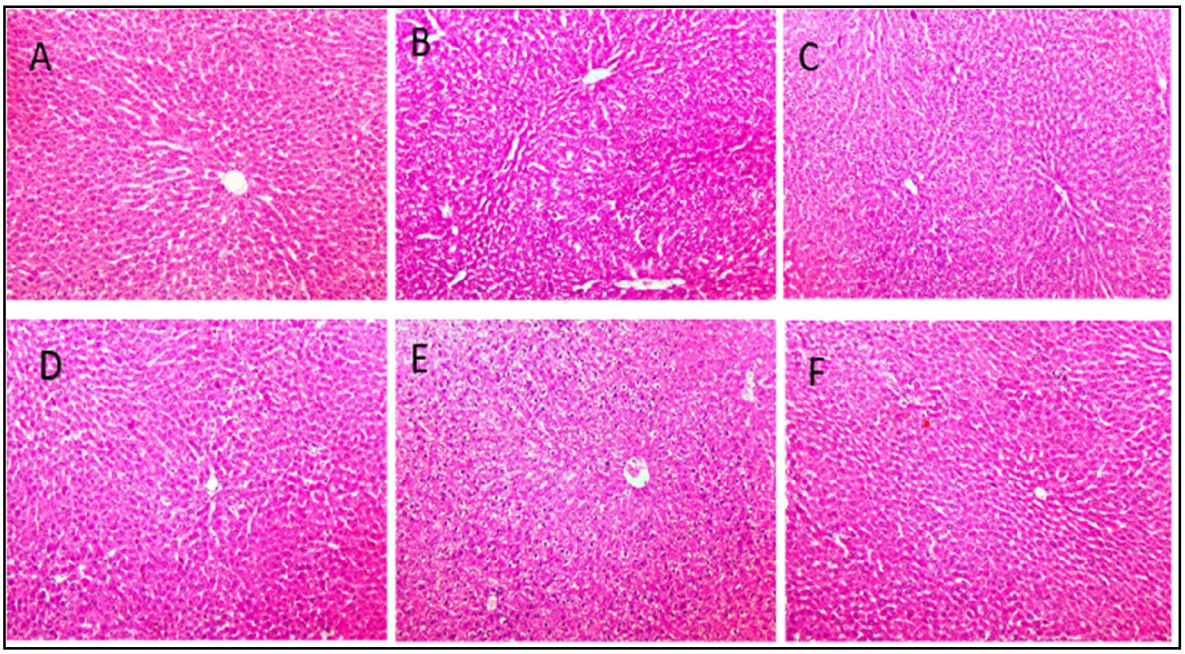


Fig. 4 (A-F): Liver histological structures: Section A from control, C from moringa oil plus CBZ, D from flaxseed oil plus CBZ, E from moringa oil and F from flaxseed oil treated groups show normal histological structures. Section B from CBZ treated group exhibited abnormal morphology, disarrangement of hepatic strands, enlarged sinusoids and vacuoles formation. Original magnification X 200.

3.4.2. Kidney

The tissues retrieved from groups 1, 3, 4, 5 and 6 displayed a regular renal corpuscle configuration, comprised of a cluster of capillaries known as the glomerulus, encircled by a double-layered epithelial capsule referred to as Bowman's capsule (Figure 5A, C, D, E and F). Histopathological analysis of renal sections taken from rats exposed solely to CBZ (group 2) revealed various changes in the configuration of the majority of renal corpuscles, exhibiting glomerular degeneration and Bowman's capsule (Figure 5B).

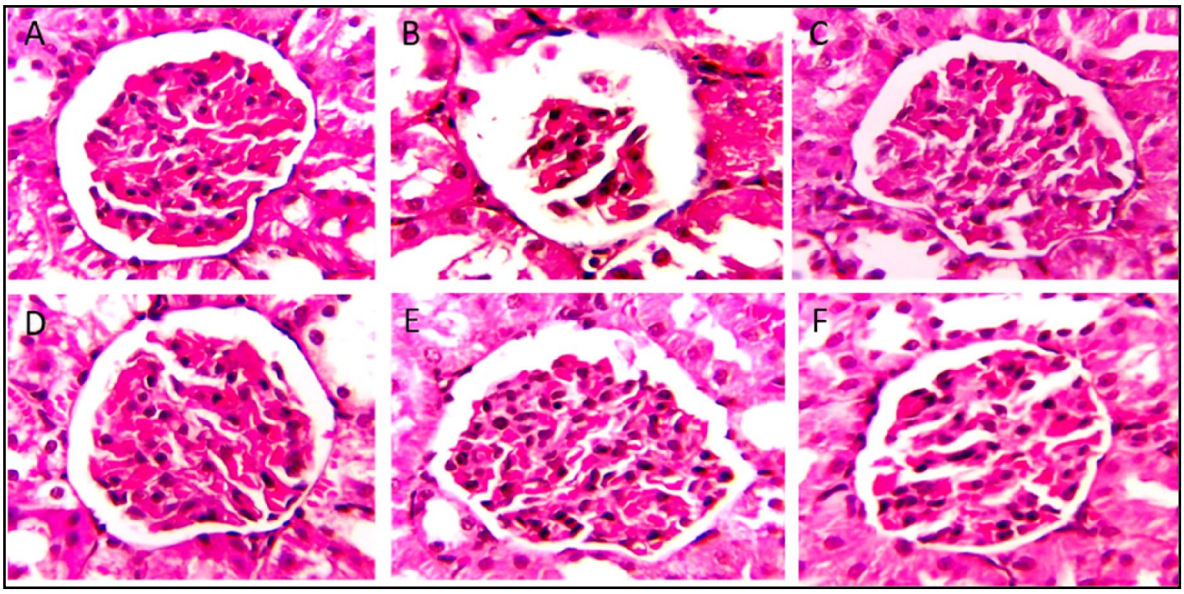


Fig. 5 (A-F): Kidney histological structures: Section A from control, C from CBZ plus moringa oil, D from CBZ plus flaxseed oil, E from moringa oil and F from flaxseed oil treated groups show normal histological structure. Section B from CBZ treated group exhibited alterations in most of the renal corpuscles, including degeneration of glomeruli and Bowman's capsules. Original magnification X400.

4. Discussion

This is the first experimental investigation that explains the protective effect of moringa oil and flaxseed oil against CBZ-induced hepatorenal toxicity and oxidative stress. In the current investigation, CBZ exposure induced elevations of serum ALT, AST, ALP and total bilirubin, while decline in serum total protein and albumin for liver function with histopathological alterations were noticed. While in kidney function, a rise in creatinine, BUN and uric acid was observed with histopathological alterations. Additionally, a significant decrease in the levels of CAT, SOD and GSH was noticed with increase in MDA levels. Similar observations were noted in previous experimental studies (Zari and Al Attar, 2011; Nwozo *et al*., 2017; Yousef *et al*., 2022). CBZ acts as a multiorgan toxicant that induces physiological, biochemical and histopathological changes in the testes, liver and kidney (Zari and Al-Attar, 2011). CBZ toxicity can generate reactive oxygen species (ROS) and cause oxidative stress in hepatorenal tissues which was confirmed by histopathological alterations. This oxidative stress disrupts the balance between antioxidants and pro-oxidants, leading to cellular damage and organ dysfunction (Nwozo *et al.,* 2017; Ebedy *et al*., 2022; Seif *et al.,* 2023).

The findings of present study are pioneering in explaining that moringa oil and flaxseed oil have a protective role in treating and normalizing liver and kidney functions. Additionally, both of these have curative capabilities for histopathological alterations induced due by CBZ. As a natural and sustainable method to reduce the negative effects of chemical substances of pesticides, medicinal plants have been recognized for their possible protective benefits against pesticides. These plants include a variety of bioactive substances, including terpenoids, alkaloids, flavonoids, and phenolic compounds, which have antioxidant, anti-inflammatory, and detoxifying activities. The defensive actions of medicinal plants include, inhibiting pesticide metabolism enzymes, improving detoxification procedures, scavenging free radicals and regulating inflammatory responses (Suthar *et al.,* 2020).

The plant *M. oleifera*, also called the drumstick tree, has been thoroughly investigated for its pharmacological properties and traditional medical use. It demonstrates a broad spectrum of pharmacological properties, including hepatoprotective, anti-inflammatory, anticancer, and antioxidant effects. According to the phytochemical investigation, the plant contains bioactive substances that contribute to its therapeutic effects, including phenolic compounds, flavonoids, alkaloids, and glucosinolates (Pareek *et al.,* 2023). The flaxseed is rich in lignans, dietary fiber, omega-3 fatty acids, and other bioactive components. The omega-3 fatty acids included in flaxseed have been linked to cardiovascular advantages, such as lowering cholesterol and blood pressure. Flaxseed's high fiber

content encourages digestive health and might aid with constipation. Flaxseed lignans have additionally demonstrated potential in lowering the risk of a number of hormone-related malignancies, including breast and prostate cancer. flaxseed may have anti-inflammatory, antioxidant, and neuroprotective effects (Nowak and Jeziorek, 2023).

Sharifudin *et al.* (2013) investigated that exposure to acetaminophen caused liver damage in rats, as evidenced by increased levels of liver enzymes ALT and AST, and kidney markers BUN and creatinine. However, treatment with moringa extract moderates all these pathological effects. In a study, Toppo *et al.* (2015) reported that treatment with moringa leaves extract significantly reduced the damage caused by cadmium (Cd), as evidenced by reduced levels of the liver enzymes ALP, AST, ALT and lipid peroxidation while increased levels of antioxidant enzymes. A significant reduction in ALT and AST was also recorded by Fathy and Mahmoud (2021) when mice were supplemented with moringa leaf extract against carbon tetrachloride (CCl_4)-induced hepatic toxicity. Elhamalawy *et al.* (2022) reported that moringa leaf extract has an ameliorating effect against thiamethoxam-induced disorder of liver functions such as ALP, AST, ALT and MDA. Abou El-Naga *et al.* (2022) reported that bisphenol A induces alterations in ALT, AST, ALP, total bilirubin and lipid profile. All these malfunctions were restored by nanoparticles prepared from moringa. Wijayanti *et al*. (2023) reported that the moringa leaves extract demonstrated hepatoprotective and nephroprotective properties. It helps in maintaining the histopathological structural integrity of the liver and kidneys, lowering oxidative stress, enhancing antioxidant enzyme activity, and controlling the liver MDA level to normal.

Another study by Reda *et al.* (2023) revealed that abamectin induceed a significant increase in serum ALT, AST and total protein, as well as decreasing albumin levels in exposed fish. While moringa leaf extract supplementation to fish normalize all these biochemical alterations in liver functions and notable improvements in MDA and GSH levels in brain and liver samples, as well as enhanced SOD, glutathione peroxidase (GPx) and glutathione S-transferases (GST) levels specifically in liver samples, were also recorded. According to Aljazzaf *et al*. (2023) findings, exposure to alloxan leads to elevated liver enzymes, disruptions in lipid profiles, decreased antioxidant levels, and histopathological disruptions in the liver, kidney, and pancreas in all diabetic-induced mice. However, supplementation with moringa leaves and seeds extract contributes to maintaining lower levels of liver enzymes like ALP, AST, and ALT, and serum creatinine. Moreover, moringa leaves and seed extract supplementation shows a positive impact on oxidative stress biomarkers, reducing MDA, nitric oxide (NO) and protein carbonyl (PC) levels. Additionally, it leads to sustained increases in antioxidant activities, specifically in GSH and CAT.

Rizwan *et al.* (2014) reported a protective effect of flaxseed oil on renal toxicity induced by arsenic (As). It causes an increase in BUN and creatine kinase (CK), alters lipid peroxidation activities, changes brush border membrane (BBM) enzymes, and weakens the defense system by interfering with antioxidant enzyme activities, and metabolic enzyme function which is supported by histopathological evidence as well. These alterations could be attenuated by supplementation of flaxseed oil. Shaikh Omar (2018) investigated the influence of flaxseed oil on renal toxicity induced by thioacetamide (TAA) in male rats. The levels of serum creatinine, BUN and uric acid were significantly increased in rats exposed to TAA. Histopathologically, the renal structures from TAA-treated rats showed severe changes. Treatment with flaxseed oil showed significant improvement in biochemical and histopathological changes induced by TAA exposure. The results suggested that the protective effect of flaxseed oil might be due to its antioxidant activity against TAA-induced renal injury.

Histopathological analysis revealed severe abnormalities in the renal corpuscles, including glomerular degeneration and Bowman's capsule alterations, in the rats. Treatment with flaxseed oil demonstrated a protective effect against the observed biochemical and histopathological changes. Lead (Pb) induces disturbance in normal liver and kidney biomarkers, including serum ALT, AST, ALP, gamma glutamyl transferase (GGT), total protein, albumin, and globulin, urea and creatinine (Mohamed *et al.,* 2020). All these alterations could be treated with flaxseed protein isolate (FPI) incorporated in lemon juice. Similar chemoprotective biochemical potencies of flaxseed oil supplementation were reported by Al-kadhi *et al*. (2020) against bicalutamide-induced toxicity. Arafat and Baghdadi (2021) reported that acetaminophen caused significant liver damage in the rats as evidenced by increased levels of liver enzymes ALT, AST and ALP, and markers of oxidative stress. However, treatment with flaxseed oil significantly reduced the levels of these markers and improved liver function. Histologically, flaxseed oil supplementation improves liver architecture and reduces the

expression of genes involved in inflammation and cell death in the liver. Aljedaani *et al.* (2021) reported that the administration of flaxseed oil exerted a protective role against Pb toxicity in the liver tissue architecture. It also resulted in a significant reduction in markers of liver damage, including serum levels of ALT, AST, ALP and GGT, and increased levels of total protein and albumin, and decreased level of bilirubin.

In another study, Gurumallu *et al.* (2022) suggested that the combination of Indian flaxseed and sesame seed oils, rich in ω-3 and ω-6 fatty acids, offers significant protection against CCl_4-induced liver damage. The authors reported that the administration of either flaxseed oil or sesame seed oil alone significantly reduced the levels of liver damage markers such as ALT, AST and ALP and both pre and post-treatments notably enhanced the *in vivo* activities of antioxidant enzymes including CAT, SOD and peroxidase (POX) in the liver and kidneys. In contrast, the activity of lipid peroxidation (LPO) showed a significant reduction in a manner that depended on the dosage applied. Alsoudany *et al.* (2023) demonstrated that cisplatin-induced liver damage by disturbing levels of AST and ALT in the serum. Flaxseed oil and alpha lipoic acid treatments demonstrated a significant ameliorative effect by normalizing liver functions and antioxidant level including CAT, GSH and MDA.

5. Conclusion

The study's conclusions highlight the value of medicinal plants and offer future approaches for resolving toxicity problems utilizing moringa oil and flaxseed oil. Both moringa oil and flaxseed oil could treat liver and renal functions as well as ameliorate histopathological alterations. This study demonstrates that hepatorenal toxicities brought on by CBZ may be treated with moringa and flaxseed oils. Finally, the data of present study suggest that the protective properties of these oils are attributed to the antioxidant activity of their chemical components.

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