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Original Research Article

Protective Role of *Commiphora molmol* Extract against Liver and Kidney Toxicity Induced by Carbon Tetrachloride in Mice

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Abstract

Purpose: *To explore the protective role of Mirazid® (MRZ), a mixture extracted from Commiphora molmol Engler (Burseraceae), against toxicity induced by carbon tetrachloride (CCl_4).*
Method: *Forty male Swiss albino mice were divided into 4 groups. Group 1 was control and included mice which were injected with normal saline; group 2 was positive control and included mice that had no treatment for 17 days followed by intraperitoneal (i.p) injection of 200 mg/kg/day MRZ for 7 consecutive days; groups 3 and 4 included mice which were injected intraperitoneally (i.p) with 0.8 mL/kg of 30 % CCl_4 on days 1, 4, 7, 10, 13 and 16; animals of group 4 were then post treated with 200 mg/kg/day MRZ for 7 consecutive days. At the end of the experiment, the mice were euthanized and subjected to a complete necropsy. Hematological and biochemical parameters were assessed. To estimate the histological changes, liver and kidney sections were exposed to microscopic examination.*
Results: *The levels of leucocytes, granulocytes, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, urea, creatinine, cholesterol and triglycerides showed a significant increase ($p < 0.05$) while the levels of lymphocytes, platelets counts, total protein and albumin showed a significant decrease ($p < 0.05$) in CCl_4-injected mice when compared with the control groups, respectively. Histological observation of the liver and kidney showed necrotic areas with cellular infiltration and atrophied renal glomerulei with degenerated renal tubule lining, respectively. Mice that were treated with MRZ after CCl_4 showed that the levels of lymphocytes, ALT, AST and albumin had insignificant change ($p > 0.05$) compared with the control groups, respectively.*
Conclusion: *MRZ partially ameliorates the toxicity induced by CCl_4 in mice by improving ALT, AST and protein profiles. Therefore, further investigations are required to figure out its antioxidant potential in order to ascertain if it can be used as an antioxidant drug.*

Keywords: *Carbon tetrachloride, Mirazid®, Toxicity, Biochemical, Heamatological, Lipid profile, Liver and kidney functions*

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INTRODUCTION

Carbon tetrachloride (CCl_4) is a common industrial solvent characterized by its toxic effects on the liver [1]. However, the liver is not the only targeted organ for CCl_4; it also affects the kidney as well [2]. It has been reported that CCl_4 activates Kupffer cells in liver tissue to release harmful cytokines that lead to hepatocyte death [3]. Chronic exposure to CCl_4 can cause fatty degeneration and liver fibrosis [3]. The toxicity of CCl_4 resulted from the bio-activation of CCl_4 into trichloromethyl free radical by cytochrome P450 system in the liver microsomes and consequently this causes lipid peroxidation of membranes that leads to liver injury [4]. Exposure to CCl_4 also causes kidney damage that may finally lead to cancer [5]. Several approaches have shown how CCl_4 could cause this damage. One of these approaches showed that CCl_4 intoxication may lead to hypo-methylation of cellular elements [6]. In addition, the cellular infiltration of activated neutrophils may increase the inflammatory response, which leads to the death of the cells due to superoxide and other toxic mediators release [7].

Oleo-gum-resin of *Commiphora molmol* (Burseraceae) is known as myrrh and the commercial extract is known as Mirazid® (MRZ) in the Egyptian pharmacies. Myrrh is collected from trees in Somalia and the Arabian Peninsula [8]. It contains 2-8 % volatile oils, terpenes, sesquiterpenes, and cuminic aldehyde [9]. Currently, MRZ is used to treat several parasites, for instance, it is used to treat fascioliasis and antischistosomal drug since 2001 [10]. MZR is used also to treat hepatic coccidiosis in domestic rabbits and *Giardia lamblia* infection in rats [11]. Myrrh-extract possesses antipyretic, antihistaminic and anti-inflammatory effects [12], hypocholesteremic, antiartheroscerotic effects [12], antigastric ulcer and cytoprotective effects [13]. Furthermore, myrrh-extract showed hypolipidemic effects [14] with reduction of cholesterol and triglycerides [15]. Controversial studies showed that myrrh-extract has no efficacy against tumor while others showed a potential antitumor activity [16] and antiarthritic effects [17]. Recently, MRZ is described as a hypoglycemic and antioxidant agent via enhancing the antioxidant activity in diabetes [18]. However, it is still unclear if MRZ can be used as antioxidant agent to protect liver and kidney from the injury induced by CCl_4. Therefore, the present study was conducted to address the protective role of MRZ against hepatic and nephritic injury induced by CCl_4 in mice.

EXPERIMENTAL

Animals

The experiment was performed on 40 healthy male Swiss albino mice aged between 6 and 8 weeks, weighing approximately 20 g each. The mice were obtained from the Faculty of Science, King Saud University, Kingdom of Saudi Arabia (KSA). The animals were housed in Biology Department, College of Science, Aljouf University, KSA. They were kept in clean and dry plastic cages (5 mice per cage) in 12 h dark/light cycle under normal laboratory condition of temperature and humidity, fed with commercial rodent pellets purchased from Sakaka City, Aljouf, KSA and tap water ad libitum. The animals were acclimatized for one week before running the experiments. The animals were maintained in accordance with the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals [19]. Furthermore, the anesthetic procedures and handling of animals complied with the ethical guidelines of the Aljouf University's Ethical Committee, Aljouf University, KSA (approval ref no. JU- 25/2013).

The mice were divided into 4 groups of 10 mice each. Group 1 (G1): normal control group included mice which were received normal saline (200 ul/mouse i.p.), group 2 (G2): positive control group included mice which had no treatment for 17 days followed by intraperitoneal (i.p) injection of 200 mg/kg/day MRZ for 7 consecutive days, groups 3 and 4: (G3& G4) included mice which were injected i.p with 0.8 mL/kg CCl_4 on days 1, 4, 7, 10, 13 and 16; the mice in G4 were then post injected with 200 mg/kg/day MRZ for 7 consecutive days after 24 h from the last injection of CCl_4 [20]. At the end of the experiment, the mice were euthanized via i.p. injection with sodium pentobarbital and subjected to a complete necropsy.

Chemicals and drugs

CCl_4 was kindly provided by Dr Ahmad Tantawy, College of Applied Sciences, Aljouf University, and dissolved in olive oil. Mirazid®, a drug containing 300 mg purified resin extract of *Commiphora molmol* was obtained in the form of soft gelatin capsules from Pharco Pharma-ceuticals Company, Egypt. MRZ was dissolved in normal saline.

Estimation of hematological and biochemical parameters

For the hematological parameters, blood samples were obtained from the orbital plexus of the eyes and then used to estimate red blood cells (RBCs) counts, hemoglobin content (Hb g/dl), hematocrit (Hct %) and the total white blood cell (WBCs) counts using an electronic blood counter (Auto hematology analyzer; BC-3200, Mindray, China). Differential WBCs was determined using blood smears from all groups. For biochemical parameters, blood samples were collected from the inferior vena cava in heparinized glass tubes. Serum was separated by centrifugation at 3000 rpm for 15 min. The enzymatic activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) were measured by commercial kits [21].Total protein and albumin levels were estimated by Biuret method [22]. Creatinine level was determined by kinetic method as described by Larsen [23]. Urea level was measured as described by Fawcett and Scott [24]. Total triglycerides (TG) and total cholesterol (TC) levels were assessed according to the commercial kits. Total bilirubin was determined using diagnostic kits.

Histological investigation

Livers and kidneys were immediately removed from the mice, sliced and fixed in Bouin's fixative for 2 to 4 h. The specimens were then dehydrated, cleared and embedded in paraffin. Serial sections of 5 µm thick were cut by rotary microtome and processed for haematoxylin and eosin staining [25].

Statistical analysis

Numerical data obtained from each experiment were expressed as mean ± standard deviation (SD). Statistical differences between experimental groups were assessed using one-way ANOVA and $p < 0.05$ indicated statistically significant difference. All statistical analyses were performed using SPSS version 16 software package (SPSS® Inc, USA).

RESULTS

Effect of MRZ treatment on hematological profile

The data showed that there was insignificant difference ($p > 0.05$) in the total number of RBCs, Hb and Hct % while there was a significant decrease ($p < 0.05$) in the platelets count in the mice that received MRZ alone (G2) compared with the mice of the normal control group (G1). Mice in group 3 (G3) which were received CCl_4 alone showed insignificant change ($p > 0.05$) in the total RBCs, mean hematocrit (Hct %) and hemoglobin content (Hb) while showed a significant decrease ($p < 0.05$) in the platelets counts compared with the control groups, respectively. Remarkably, mice that were injected with MRZ after CCl4 showed a significant decrease ($p < 0.05$) in all the previous parameters compared with the control groups (G1 & G2), respectively (Table 1).

Effect of MRZ treatment on the total and differential leucocytes

The data showed that there was an insignificant difference ($p > 0.05$) in the mean% of the lymphocytes, granulocytes and monocytes between the normal and positive control groups (G1 & G2) although the total number of the WBCs in G2 showed a significant ($p < 0.05$) increase compared with the control group (G1). Mice that were treated with CCl_4 (G3) showed a significant increase ($p < 0.05$) in WBCs and mean % granulocytes and a significant decrease ($p < 0.05$) in the mean % lymphocytes while showed insignificant increase ($p > 0.05$) in mean % monocytes compared with the control groups, respectively. Interestingly, mice that were treated with MRZ after CCl_4 (G4) showed a significant decrease ($p < 0.05$) in WBCs and mean % granulocytes and a significant increase ($p < 0.05$) in mean % monocytes while showed ($p > 0.05$) insignificant increase in mean % lymphocytes compared with the control groups, respectively (Table 2).

Effect of MRZ treatment on biochemical parameters

The enzymatic activities of ALT, AST, urea and creatinine in showed an insignificant change ($p > 0.05$) between normal and positive control mice (G1 & G2). Only total bilirubin level was significantly decreased ($p < 0.05$) in the mice of the positive control group (G2) compared with the normal control group (G1). All of the previous parameters were significantly increased ($p < 0.05$) in the mice that were treated with CCl_4 (G3) compared with the control groups, respectively. Successfully, mice that were treated with MRZ after CCl_4 (G4) showed insignificant change ($p > 0.05$) in the levels of AST and ALT while still showed a significant increase ($p < 0.05$) in the levels of total bilirubin, urea and creatinine comparing with the control groups, respectively (Table 3).

Table 1: Effect of MRZ administration on hematological profile in CCl_4-treated mice

Groups	RBCs ($x10^6$)	Platelets ($x10^3$)	Hb (g/dl)	Hct%
G1	6.5±0.2	1055.0±48	12.5±0.2	35.0±0.2
G2	7.0±0.8	890.0±44[a]	11.5±1.5	35.5±2.0
G3	6.3±0.8	725.5±96[b]	12.4±0.9	33.5±3.0
G4	5.7±0.4[b]	480.0 ± 82[b]	10.7±1.3[b]	30.5±3.2[b]

The values represent mean ± SD, number of animals is 10 mice per group; [a]p < 0.05: versus normal control group (G1); [b]p < 0.05: versus control groups (G1& G2); (G1): normal control group includes mice that received no treatment, (G2) includes mice that were injected with MRZ, (G3) includes mice that were injected with CCl_4,(G4) includes mice that were injected with both CCl_4 and MRZ; (RBCs): red blood cells, (Hb): hemoglobin content and the (Hct %): hematocrit

Table 2: Effect of MRZ administration on the total and the differential leucocytes in the CCl_4 treated mice

Groups	WBCs ($x 10^3$)	Mean % lymphocytes	Mean % granulocytes	Mean % monocytes
G1	4.3± 0.2	55.0 ± 1.7	43.0± 1.5	2.5±0.5
G2	6.6±0.5[a]	54.0± 2.6	43.0±2.0	3.0±0.5
G3	7.3±0.3[b]	43.8±1.8[b]	51.8±1.0[b]	4.0±0.5
G4	5.5±0.5b	56.0±4.1	38.0±1.5[b]	6.9±2.5[b]

The values represent mean ± SD, number of samples is 10 mice per group; [a]p < 0.05: versus normal control group (G1) [b]p < 0.05: versus control groups (G1& G2): normal control group includes mice that received no treatment, (G2) includes mice that were injected with MRZ, (G3) includes mice that were injected with CCl_4, (G4) includes mice that were injected with both CCl_4 and MRZ. (WBCs): Total white blood cells.

Table 3: Effect of MRZ administration on the liver and kidney functions in the CCl_4 treated mice

Groups	ALT (IU/ml)	AST (IU/ml)	T. bilirubin (mg/dl)	Urea (mmol/l)	Creatinine (mg/dl)
G1	24.0±1.80	44.4±1.20	0.37±0.08	5.0±0.55	0.68±0.04
G2	26.0±2.10	42.5±2.30	0.25±0.05[a]	5.3±0.30	0.64±0.03
G3	49.0±2.50[b]	79.0±11.40[b]	0.94±0.03[b]	10.5±1.30[b]	2.78±0.05[b]
G4	27.5±4.90	49.5±4.50	0.50±0.05[b]	7.1±0.70[b]	0.82±0.02[b]

Values represent mean ± SD, number of samples is 10 mice per group; [a]p < 0.05: versus normal control group (G1); [b]p < 0.05: versus control groups (G1and G2); (G1): normal control group includes mice that received no treatment, (G2) includes mice that were injected with MRZ, (G3) includes mice that were injected with CCl_4, (G4) includes mice that were injected with both CCl_4 and MRZ; (ALT): alanine aminotransferase and (AST): aspartate aminotransferase.

The levels of the total protein and albumin showed an insignificant change ($p > 0.05$) while cholesterol and triglycerides levels showed a significant change ($p < 0.05$) in the positive control group (G2) compared with the normal control group (G1), respectively. All of those parameters were significantly changed ($p < 0.05$) in the mice that were treated with CCl_4 (G3) compared with control groups (G1 & G2). In the mice that were treated with both of CCl_4 and MRZ (G4), the albumin level returned nearly to its normal level as in the control groups (G1 and G2). However, the total protein and the cholesterol levels still showed a significant decrease ($p < 0.05$) compared with their levels in the control groups. Triglycerides level showed a significant decrease ($p < 0.05$) compared with the normal control group (G1) while an insignificant decrease ($p > 0.05$) was observed compared with the mice in the positive control group (G2) (Table 4).

Histopathological features of mice liver and kidney

Histological examination of the liver sections of the normal and positive control groups (G1 & G2) showed normal hepatic architecture with radial arrangement of hepatocytes that contain pronounced nuclei and obvious nucleoli (Figure 1A). Examination of the liver sections of the mice that were treated with CCl_4 (G3) showed pale stained hepatocytes with swollen nuclei, undergoing lytic necrosis, portal area associated with cellular infiltration (Figure 1B). Examination of the liver sections of the mice that were treated with both CCl_4 and MRZ (G4) showed partial improvement, pale stained hepatocytes with

Table 4: Effect of MRZ administration on the protein and lipid profiles in the CCl_4 treated mice

Groups	Total protein (g/dl)	Albumin (g/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)
G1	7.6±0.26	3.0±0.09	54.0±2.00	44.0±3.00
G2	7.4±0.36	3.2±0.20	33.0±3.20[a]	31.6±2.50[a]
G3	4.5±0.43[b]	2.4±0.40[b]	72.2±6.00[b]	50.2±7.00[b]
G4	5.5±0.80[b]	3.3±0.20	25.0±2.00[b]	27.6±3.20[a]

The values represent mean ± SD, number of samples is 10 mice per group; [a]p < 0.05: versus normal control group (G1), [b]p < 0.05: versus control groups (G1& G2); (G1): normal control group includes mice that received no treatment, (G2) includes mice that were injected with MRZ, (G3) includes mice that were injected with CCl_4, (G4) includes mice that were injected with both CCl_4 and MRZ.

some cytoplasmic degeneration and well developed nuclei (Figure 1C).

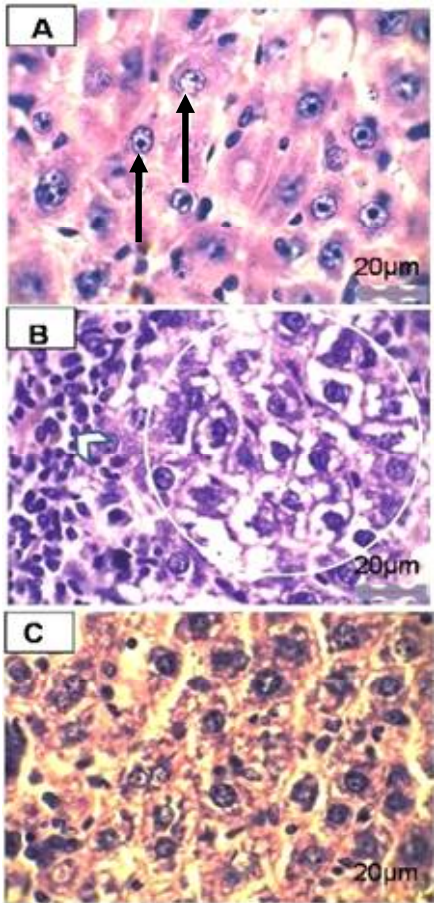

Figure 1: Light photomicrographs of the mice liver sections stained with haematoxylin and eosin showing. (A) Control mice showing normal hepatic architecture with radial arrangement of hepatocytes contain pronounced nuclei and obvious nucleoli (arrows). (B) Mice that were treated with CCl_4 showing pale stained cells with swollen nuclei, undergoing lytic necrosis (area inside circle), and portal area associated with cellular infiltration (arrow head). (C) Mice that treated with both of CCl_4 and MRZ showing pale stained hepatocytes with well-developed nuclei and some cytoplasmic vacuolation.

Examination of the transverse sections of the kidney of the normal and positive control groups (G1 & G2) showed normal renal cortex with normal renal tubules (Figures 2A, B, 3A). Kidney sections of mice that were treated with CCl_4, showed a distinct vacuolated and degenerated epithelial lining cells of the renal tubules accompanied with glomerular vacuolation, widening of the renal tubular lumen. Also some of the renal tubules appeared with an eosinophilic hyaline substance, high degree of cellular infiltration and vascular congestion. Also, obstruction of the renal spaces was noticed. (Figures 2C, D, 3B). The mice that were treated with both CCl_4 and MRZ showed cellular degeneration in both of the epithelial lining of the renal tubules and the glomeruli together with appearance of hemorrhagic areas (Figures 2E, F, 3C).

DISCUSSION

The present investigation addressed the effect of MRZ treatment on liver and kidney toxicity induced by CCl_4 administration in mice. The results revealed that treatment with CCl_4 showed a significant change in the total leucocytes number, the mean percentage of both lymphocytes and granulocytes and platelets counts. Significant changes in the liver enzymes (ALT and AST), T. bilirubin, urea, creatinine, total protein and albumin were also recorded. CCl_4 injection led to an increase in the total cholesterol and triglycerides levels. Furthermore, dramatic histological alternations in the kidney and liver tissues were recorded like the appearance of the necrotic areas, cellular infiltration, atrophied renal glomeruli and degenerated renal tubule lining with widening of the renal lumens and appearance of the eosinophilic substance. The present data is consistent with the previous study reporting that exposure to CCl_4 causes hepatic injury, including hepatocytic necrosis and inflammation [26]. The authors added that low-dose and long-term exposure to CCl_4 induces hepatic fibrogenesis, which leads to hepatic fibrosis. It has also reported that chronic exposure of CCl_4 generates free radicals that trigger a cascade of events resulting in the appearance of fibrosis [27].

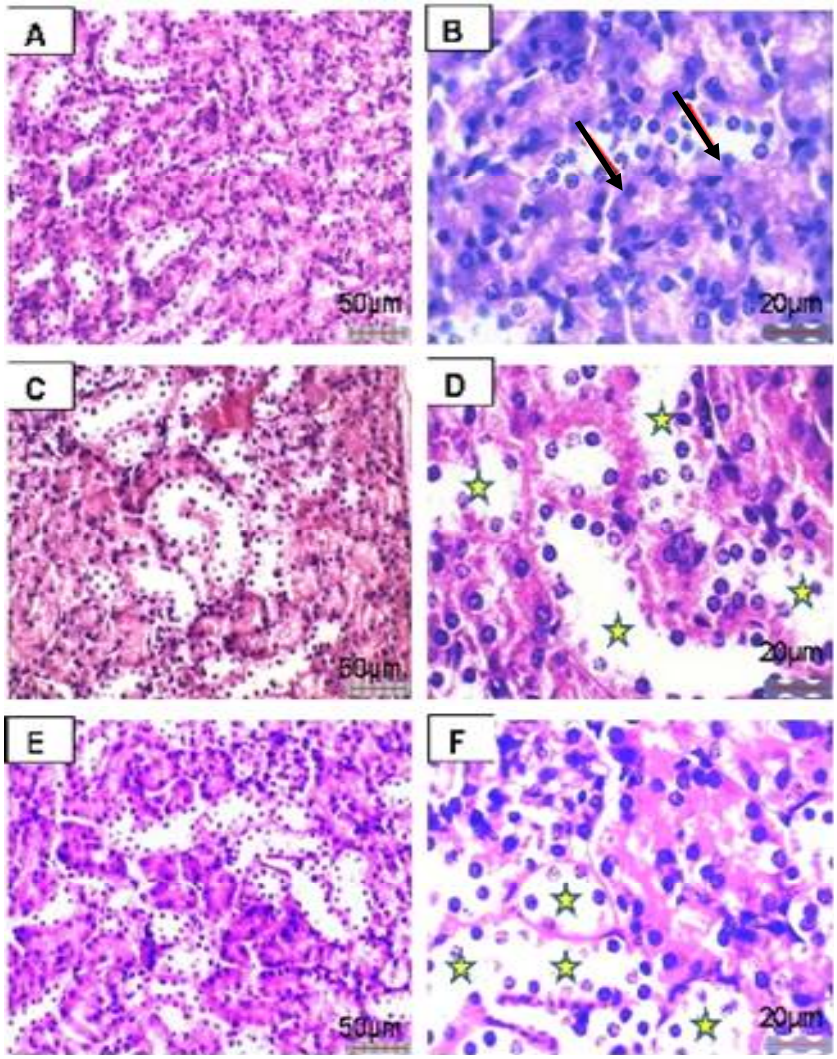

Figure 2: Light photomicrographs of the transverse sections in the mice kidney stained with haematoxylin and eosin showing renal cortex stained with haematoxylin and eosin. (A and B) control mice normal renal tubules (arrows). (C and D) treated mice with CCl_4 revealed cellular degeneration of the renal epithelial lining cells and widening of the renal lumen (star). (E and F) mice that were treated with both CCl_4 and MRZ showing less widening , highly degenerated epithelial lining cells (arrow), appearance of an eosinophilic hyaline substance (arrow head) (star).

Furthermore, a variety of enzymes such as AST and ALT are released into blood after hepatocytes damage, both which are considered as potential indicators of hepatocytes disorder [28].

On the other side, it was discovered that not just the liver is affected by CCl_4 intoxication, but also the kidneys are damaged and this could lead to cancer [5]. The kidney is sensitive to CCl_4 [2,5].Therefore, less urine may be formed, leading to a buildup of water and waste products in the body and blood, respectively. Recently, it was reported that there is an increase in the reactive oxygen species production, oxidative stress and a significant decrease in the antioxidant enzymes after CCl_4 injection [4,6]. Furthermore, a significant increase in the lipid peroxidation which causes damage in the cell membrane that leads to pathological changes in acute and chronic renal injuries has been reported by Khan *et al* [4,6].

Treatment with MRZ showed small morphometric deviations in some measured parameters compared with the control mice. The current findings are in agreement with several experimental and pre-clinical studies on myrrh extract which proved its safety to use [18].The safety of MRZ administration has been tested on adult male albino rats by determination of the serum levels of ALT, AST and T. bilirubin and an insignificant change in their levels was reported [10].

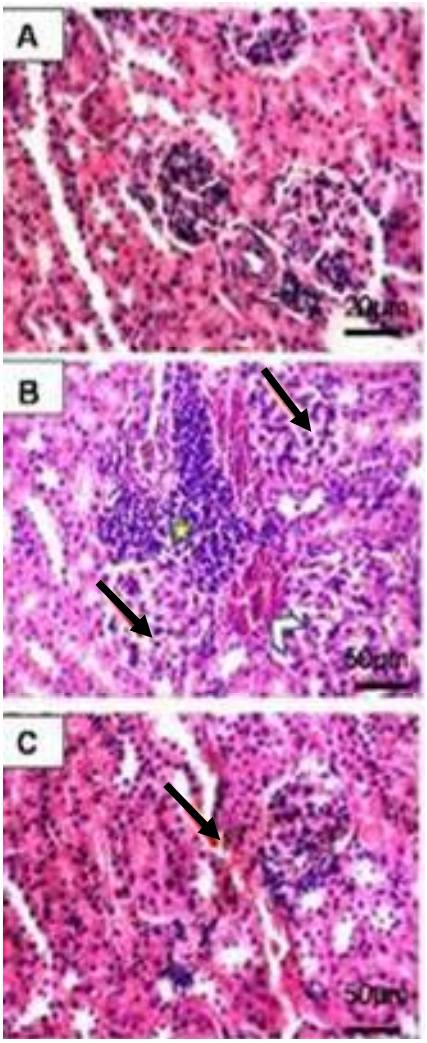

Figure 3: Light photomicrographs of transverse sections in the mice renal tubules stained with haematoxylin and eosin showing renal glomeruli. (A) control mice showing a normal structure of the renal glomeruli. (B) mice that were treated with CCl_4 showing glomerular vacuolation (arrows), obstruction of the glomerular renal space, vascular congestion (arrowheads) and cellular infiltration (star) (C) mice that were treated with both CCl_4 and MRZ showing glomerular degeneration with hemorrhage areas (arrow)

Supplementation of MRZ after CCl_4 administration in our study could only return the mean percentage of the lymphocytes, liver function as indicated by ALT, AST levels and the lipid profiles to its normal levels. However, impairment of kidney architecture and renal function, as indicated by the levels of serum creatinine and urea, were recorded following MRZ treatment. The improvements recorded in the previous parameters may be explained by the ability of MRZ to function as antioxidant, immuno-stimulatory and anti-inflammatory agent [12]. The current data is consistent with Malhotra [14] who showed the ability of myrrh-extract to act as a hypolipidemic agent. It has been documented that myrrh-extract can reduce the high levels of cholesterol and triglycerides [15]. Myrrh extract containing polyphenolic groups induces a protective effect against reactive oxygen species [18]. *Commiphora molmol* had a protective effect on gastric ulcer due to its free radical- scavenging [13]. Recently, it was recorded that Myrrh considered a potent antioxidant exerts its activity through increasing the total antioxidant activity of the serum and tissues [18]. However, further future studies should be done to explain why renal architecture and function damaged by CCl_4 injection in the present study would not be improved after MRZ treatment.

CONCLUSION

MRZ partially ameliorates the toxicity induced by CCl4 in mice by improving ALT, AST and protein profiles. Therefore, further investigation is required to ascertain its antioxidant potential and thus determine if it can be used as an antioxidant drug.

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CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

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