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ORIGINAL ARTICLE

Ameliorative Effect of Camel's Milk and *Nigella Sativa* Oil against Thioacetamide-induced Hepatorenal Damage in Rats

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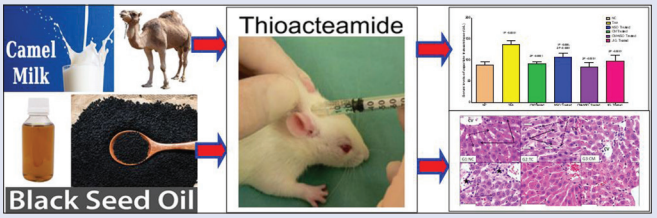
ABSTRACT

Background: Camel milk (CM) and *Nigella sativa* (NS) have been traditionally claimed to cure wide range of diseases and used as medicine in different part of world, particularly in Saudi Arabia. Several research studies have been published that proved beneficial effects of CM and NS. **Objective:** This study was undertaken to investigate the antihepatotxic potential of CM and NS oil (NSO) against thioacetamide (TAA)-induced hepato and nephrotoxicity in rats. **Materials and Methods:** Thirty female Albino Wistar rats were randomly divided in to six groups having five rats in each group. A single subcutaneous injection of TAA (100 mg/kg b. w.) was administered to all the rats in Group-II to VI on 1st day to induce hepatorenal damage. Group I served as a normal control while Group II served as toxic control for comparison purpose. Experimental animals in Group III, IV, and V were supplemented with fresh CM, (250 mL/24 h/cage), NSO (2 mL/kg/day p. o.), and NSO + fresh CM, respectively. Group VI was treated with a polyherbal hepatoprotective Unani medicine Jigreen (2 mL/kg/day p. o.) for 21 days. TAA-induced hepatorenal damage and protective effects of CM and NSO were assessed by analyzing liver and kidney function tests in the serum. Histopathology of liver and kidney tissues was also carried out to corroborate the findings of biochemical investigation. **Results:** The results indicated that the TAA intoxicated rats showed significant increase in the alanine transaminase, aspartate transaminase, gamma-glutamyl transpeptidase, alkaline phosphatase, lipid profile, urea, creatinine, uric acid, sodium, and potassium levels in serum. Treatment of rats with CM, NSO, and CM plus NSO combination and Jigreen significantly reversed the damage and brought down the serum biochemical parameters and lipid profile toward the normal levels. The histopathological studies also support the hepato and nephroprotective effects of CM and NSO. **Conclusion:** This study demonstrated the ameliorative effects of CM, NSO, and CM plus NSO combination against TAA-induced hepatorenal toxicity in rats.

Key words: Camel's milk, hepatorenal toxicity, kidney, liver, *Nigella sativa* oil, thioacetamide

SUMMARY

- The antihepatotxic potential of Camel's Milk (CM) and Nigella sativa oil (NSO) against thioacetamide (TAA) induced hepatorenal toxicity was evaluated in rats
- The oral administration of fresh CM (250 mL/24h/cage), NSO (2 mL/kg/day) and NSO+fresh CM and Jigreen (2 mL/kg/day) for 21 days significantly decreased the hepatorenal toxicity as evidenced from analyzed biochemical parameters in serum and histopathological studies of liver and kidney tissues
- This study demonstrated the ameliorative effects of CM and NSO against TAA induced hepatorenal toxicity.



Abbreviations used: CM: Camel milk; NS: Nigella sativa; NSO: Nigella sativa Oil; TAA: Thioacetamide; S.C.: Subcutaneous; Jig: Jigreen; b.w.: Body Weight; mL: Milli liter; mg: Milli gram; g: Gram; Kg: Kilo gram; ALT: Alanine transaminase; AST: Aspartate transaminase; GGT: Gamma-Glutamyl Transpeptidase; ALP: Alkaline Phosphatase; TC: Total Cholesterol; HDL-C: High Density Lipoprotein Cholesterol; LDL-C: Low Density Lipoprotein Cholesterol; TG: Triglyceride; TB: Total bilirubin; K^+: Potassium; Na^+: Sodium; CCl_4: Carbon Tetrachloride; °C: Degree Celsius; p.o.: Per Oral; RPM: Revolutions per minute; H&E: Hematoxylin and Eosin; SEM: Standard Error of Mean; ANOVA: The one-way analysis of variance.

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INTRODUCTION

The liver is a vital organ and the largest gland of the human body responsible for the metabolism of all foreign substances. Exposure to environmental pollutants, chemicals such as alcohol, carbon-tetrachloride, thioacetamide (TAA), D-galactosamine, and chronic use of drugs, for example, paracetamol, rifampicin, isoniazid, etc., can damage the liver cells leading to hepatotoxicity.[1] Hepatotoxicity by chemicals and various drugs happens to be the most common type of iatrogenic disease, and the situation is further worsened by the absence

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of reliable and specific treatment.[2] The rate of morbidity and mortality due to hepatotoxicity or liver dysfunction is on the rise which makes it a major health problem throughout the world posing a big challenge to health-care professionals, drug regulatory agencies, and pharmaceutical industry to find an adequate, suitable treatment.[3]

TAA is a potent centrilobular hepatotoxic agent and is widely used to induce liver toxicity in experimental animal model. It causes acute liver toxicity by interfering in the transfer of RNA to cytoplasm from the nucleus, which leads to injury to the membrane. Basically, microsomal CYP2E1 converts TAA through two steps to "TAA-s-oxide or sulfoxide" and then to a bioactive metabolite TAA-*S*, *S*-dioxide which acts as a direct hepatotoxicant and causes centrilobular necrosis.[4] TAA causes decrease in the viable hepatic cells count and oxygen utilization rate. It also found to decrease the bile volume and contents of bile such as cholic acid, deoxycholic acid, and bile salts. TAA at a dose of 100 mg/kg, subcutaneously (*sc*) causes hepatorenal toxicity. TAA-induced cirrhosis is very much similar to human cirrhosis.[5]

Nigella sativa (NS) is commonly known as black cumin in English, and Habat-ul-Sauda or Habbat-ul-Barakah (seed of blessing) in Arabic belongs to the Ranunculaceae family. *N. sativa* seeds are very useful in the treatment of several diseases. In Islam, it is said to be the best healing medicine. The daily use of black seeds has been recommended in *Tibb-e-Nabwi*.[6] It has been traditionally used as analgesic, antidiarrheal, appetite stimulant, antimicrobial, antihypertensive, digestive, diuretic, liver tonic, and in skin diseases. It has been revealed by numerous research studies that *N. sativa* possesses broad spectrum pharmacological properties such as antimicrobial, antioxidant, antidiabetic, analgesic, anti-inflammatory, anticancer, antihypertensive, antioxytocic, anticonvulsant, bronchodilator, diuretics, gastroprotective, hepatoprotective, immunomodulator, pulmonary-protective, renal protective, and spasmolytic properties, etc., *N. sativa* has received special attention and is currently one of the top ranking research priorities of evidence-based herbal medicines which is primarily due to its amazing potential of healing.[7-10] The hepatoprotective potential of *N. sativa* seed oil was reported against different animal's models of hepatotoxicity. The immunological and hepatoprotective properties of *N. sativa* seed oil was recently published.[11]

Camel (*Camelus dromedarius*) is an excellent source of food such as meat and milk. The Camel's milk (CM) is a rich source of important and well-balanced nutrients. It contains the highest amount of minerals such as sodium, potassium, copper, iron, magnesium, zinc, Vitamins A, B_2, C and E. It also contains a high level of insulin concentration. CM is known for its nonallergic properties in lactase-deficient individuals.[12-14] CM has been reported to possess some useful pharmacological properties such as antibacterial and antiviral properties which might be because of the presence of high quantity of lactoferrin in the camel's milk.[15] CM is used as home remedy in the management of asthma, anemia, dropsy, diabetes, hepatitis, jaundice, tuberculosis, pile, and spleen disorders. Korish in 2014 demonstrated that CM is capable of decreasing the elevated blood sugar level in diabetic rats.[16] Some reports have attributed the beneficial effects of CM to its free radicals and reactive oxygen scavenger activities. There is a customary faith in the Middle East, particularly in Saudi Arabia that regular consumption of CM can prevent and control diabetes. Prompted by these finding, the antidiabetic potential of CM was investigated and it was proposed that the hypoglycemic effects might be due to the presence of insulin-like protein that exerts immunomodulatory action on β-cells of pancreas.[17] CM is also reported to posses' hepatoprotective potential against various models of hepatotoxicity. The hepatoprotective activity of CM on carbon tetrachloride (CCl_4)-induced hepatotoxicity in rats was investigated by Althnaian in 2012. This study concluded that CM treatment may help in improving the histological changes against CCl_4-induced liver toxicity and improve liver enzyme activities in rats. Therefore, authors recommended to use CM for protection against toxicity of CCl_4 and other liver toxicants.[18] In another study, CM reported to alleviate the liver injury caused by alcohol in rats, and thus, it was concluded that CM consumption might be useful in the treatment of alcohol-associated liver toxicity.[19] CM has also shown useful effects on treating gentamicin-induced alterations in rat's liver. The pretreatment of rats with CM showed protection against gentamicin-induced hepatotoxicity. The authors attributed the mechanism of liver protection to its antioxidant, antiapoptotic, and anti-inflammatory properties.[20]

The unavailability of satisfactory and adequate number of synthetic drugs for prevention and treatment of liver disorders leads to further damage to the liver. Therefore, there is an urgent need of effective natural drugs and or foods for the prevention and treatment of liver diseases. Since CM has multiple medicinal properties while *N. sativa* seeds cure all diseases except death according to prophetic Hadith;[6] hence, this study was designed and undertaken to evaluate the effect of CM and *N. sativa* seeds oil (NSO) on hepatorenal toxicity. The present research study aimed to investigate the pharmacological and biochemical evaluation of CM and NSO alone and in combination for potential therapeutic activities against TAA-induced liver and kidney toxicities in rats. The serum levels of alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), total bilirubin (TB), uric acid, urea, creatinine sodium, and potassium were measured and histopathological studies from the section of liver and kidneys from the experimental rats were performed.

MATERIALS AND METHODS

Drugs and chemicals

TAA purchased from Sigma Co., USA, was administered as a single SC injection (100 mg/kg b. w.) to induce hepatorenal toxicity in rats.[1] The rest of the chemicals used in this experimental study were of analytical grade and pure. The NSO was purchased from the local market of Jeddah. Jigreen® was procured from Hamdard (Wakf) laboratories, New Delhi, India. The CM was freshly collected twice daily in the morning and evening from the shepherd of camels from the remote area of Jeddah. CM was obtained by hand milking from camels as a routine method by the camel farmers. The CM was collected in the sterile bottles tightly covered with screw and kept in ice cool boxes for the safe transportation to the laboratory. Duration of the transit of CM was 30–40 min. The fresh CM was transferred in the rat's feeding bottle without any additional treatment and given as per dosing schedule outlined in methods.

Animals

Healthy female Wistar Albino rats (100–200 g) were obtained from Faculty of Pharmacy, King Abdulaziz University-Jeddah, Saudi Arabia, and the study was carried out as per the institutional guidelines on animal care. All the rats were kept in the laboratory for 1 week before the commencement of the dosing for the adaptation of laboratory conditions. The 12 h light and dark cycle at 25 ± 2°C was provided to maintain standard laboratory conditions for all experimental rats. The rats were fed with standard pellet diet and water *ad libitum* was provided throughout the study.

Experimental design

Thirty female Albino Wistar rats were selected and randomly segregated in to six groups having five rats in each group. A single subcutaneous injection of TAA (100 mg/kg b. w.) in the form of 2% w/v solution was

given to all animals from Group-II to Group-VI on the 1st day to induce acute hepatorenal toxicity. The rats in all the groups except normal control (Group I) and toxic control (Group II) were then treated for 21 days as per the following dosing plan.

- Group I: Normal control (NC) group: given normal saline (1 mL, p. o.) only throughout the study period
- Group II: Toxic control (TC) group: TAA and given normal saline (1 mL, p. o.)
- Group III: TAA and treated with fresh CM (250 mL/24 h/cage)
- Group IV: TAA and treated with NSO (2 mL/kg/day p. o.)
- Group V: TAA and treated with combination of NSO (2 mL/kg/day p. o.) and fresh CM (250 mL/24 h/cage)
- Group VI: TAA and treated with a polyherbal preparation, Jigreen (2 mL/kg/day p. o.)

All the experimental animals were sacrificed on the 22nd day.

Collection of blood, liver, and kidney tissues

All animals were fasted overnight at the termination of the study and then sacrificed on 22nd day. The blood was directly withdrawn into centrifuge tubes from retro-orbital plexus under ether anesthesia. The collected blood was kept aside at room temperature to clot for 30 min. The clotted blood was centrifuged at 3000 rpm for10 min to obtain the serum and the separated serum was transferred into aliquots and kept at-80°C for biochemical investigations. Liver and kidney tissues were isolated by dissection of each rat and kept in 10% formalin solution after cleaning with normal saline. These preserved tissues were used for histopathological investigations.

Serum biochemistry (Estimation of the liver and kidney functions)

The biochemical parameters such as serum levels of AST, ALT, GGT, ALP, TC, HDL-C, TGs, LDL-C, TB, uric acid, urea, creatinine, sodium, and potassium were estimated using commercial diagnostic kits (Cayman Chemical and bioVision incorporated, USA) with the help of an auto analyzer (Chemistry Analyzer (CA 2005), B4B Diagnostic Division, China).

Histopathological examination

Histopathological assessment of liver and kidney tissues were carried out as per the standard method to check the histopathological changes. Small pieces of both liver and kidney tissues from all groups were immediately transferred and fixed in 10% formalin for 24 h. The sections (4–5 mm thick) of both liver and kidney tissues were prepared. These sections were properly stained using hematoxylin-eosin (H and E) dye. The microphotograph of stained sections of both liver and kidneys were taken and examined for expected pathological alterations in these tissues.

Statistical data analysis

The results of all data are presented as mean ± standard error of the mean The one-way analysis of variance was adapted to calculate the total variation in a set of data. $P < 0.05$ value was regarded as statistically significant.

RESULTS

Evaluation of liver biochemical parameters

The administration of a single dose of TAA (100 mg/kg) by SC injection to the rats significantly ($P < 0.05$) elevated the serum level of the ALT, AST, ALP, and GGT in comparison to the normal control rats, which confirmed the induction of hepatotoxicity. Treatment of experimental animals with CM, NSO, and combined CM + NSO reversed the TAA-induced hepatotoxicity and restored the elevated levels of AST, ALT, and ALP biomarkers toward normalcy. The serum level of AST was found to be significantly higher in TAA toxic control group in comparison with normal control, CM- treated, NSO-treated, CM plus NSO-treated, and JIG-treated group ($P = 0.0001$ for all) and in NSO treated versus normal control ($P = 0.006$). The serum levels of ALT was significantly higher in TAA toxic control group as in comparison with normal control, CM-treated, NSO-treated, CM plus NSO-treated, and JIG-treated group ($P = 0.0001$, $P = 0.001$, $P = 0.001$, $P = 0.0001$ and $P = 0.001$, respectively). The serum levels of ALP was significantly higher in TAA toxic control group as compared with normal control, CM-treated, NSO-treated, CM + NSO-treated, and JIG-treated group ($P = 0.0001$ for all). There was no significant change observed in the AST/ALT ratio and serum level of GGT [Table 1 and Figures 1-5].

Evaluation of lipid profile

Administration of TAA to toxic control group showed diminution in the serum levels of TC, HDL-C, and elevation in the levels of LDL-C and TG in comparison to normal control group. However, the serum levels of TC were noted to be significantly decreased in TAA toxic control group in comparison with normal control, CM-treated, NSO-treated, and JIG-treated groups ($P = 0.003$, $P = 0.011$, $P = 0.001$, and $P = 0.001$, respectively) and in NSO-treated and JIG-treated groups versus normal control group ($P = 0.0001$ for both). The serum levels of HDL-C were significantly decreased in TAA toxic control group in comparison with normal control, CM-treated, NSO-treated, and JIG-treated groups ($P = 0.006$, $P = 0.008$, $P = 0.001$, and $P = 0.0001$, respectively) and in NSO-treated and JIG-treated groups versus control ($P = 0.0001$ for both). The serum levels of LDL-C were significantly elevated in TAA toxic control group in comparison with CM-treated, NSO-treated, and CM + NSO-treated groups ($P = 0.006$, $P = 0.0001$, and $P = 0.034$, respectively) but was significantly lowered in NSO-treated group versus control ($P = 0.006$). The serum levels of TG was significantly higher in TAA toxic control; CM treated and JIS treated groups in comparison with normal control group ($P = 0.007$, $P = 0.001$, and $P = 0.019$, respectively). There was no significant effect noted on the bilirubin level as compared with TAA-treated toxic control group [Table 1 and Figures 6-10].

Evaluation of biochemical parameters of kidney

The administration of a single dose of TAA (100 mg/kg) by SC injection to the rats significantly ($P < 0.05$) elevated the serum level of the urea, uric acid, creatinine, sodium and potassium in comparison with the rats of normal control group which indicated the acute renal toxicity [Table 2].

The serum levels of urea were found to be increased significantly in the TAA toxic control group compared with control, CM-treated, NSO-treated, CM + NSO-treated and JIG-treated group ($P = 0.0001$, $P = 0.001$, $P = 0.0001$, $P = 0.0001$, and $P = 0.0001$, respectively) and in NSO-treated and JIG-treated groups versus control ($P = 0.006$ and $P = 0.031$). The serum levels of uric acid was found to be elevated significantly in TAA toxic control group in comparison to the JIG-treated group ($P = 0.0001$ for all) and NSO treated versus control ($P = 0.031$).

The serum levels of creatinine was decreased significantly in CM-treated, NSO-treated, CM + NSO-treated, and JIG-treated group versus normal control group ($P = 0.018$, $P = 0.0001$, $P = 0.001$, and $P = 0.011$, respectively) and TAA toxic control group ($P = 0.004$, $P = 0.0001$, $P = 0.001$, and $P = 0.002$, respectively).

The serum levels of sodium (Na+) were significantly lower in CM-treated, NSO-treated, CM + NSO-treated, and JIG-treated group versus normal control group ($P = 0.038$, $P = 0.001$, $P = 0.0001$, and

Table 1: Comparison of the serum levels of measured liver enzymes in different studied groups versus normal control and toxic group

Variable	NC	TAA	CM treated	NSO treated	CM + NSO treated	JIG treated
AST (U/L)	88.72±8.62	137.30±9.96	92.28±4.79	107.76±9.38	84.12±11.18	98.46±14.06
Significance		1*P*=0.0001	1*P*=0.581; 2*P*=0.0001	1*P*=0.006; 2*P*=0.0001	1*P*=0.477; 2*P*=0.0001	1*P*=0.139; 2*P*=0.0001
ALT (U/L)	40.68±4.39	62.36±8.54	45.89±2.10	46.46±12.30	41.52±2.48	45.42±6.31
Significance		1*P*=0.0001	1*P*=0.251; 2*P*=0.001	1*P*=0.204; 2*P*=0.001	1*P*=0.851; 2*P*=0.0001	1*P*=0.295; 2*P*=0.001
AST/ALT	2.20±0.29	2.25±0.44	2.01±0.10	2.43±0.58	2.02±0.22	2.18±0.28
Significance		1*P*=0.831	1*P*=0.414; 2*P*=0.305	1*P*=0.308; 2*P*=0.417	1*P*=0.443; 2*P*=0.329	1*P*=0.935; 2*P*=0.768
ALP (U/L)	92.80±8.90	153.20±6.87	99.40±5.86	94.00±9.22	94.60±5.46	95.60±5.59
Significance		1*P*=0.0001	1*P*=0.157; 2*P*=0.0001	1*P*=0.793; 2*P*=0.0001	1*P*=0.694; 2*P*=0.0001	1*P*=0.542; 2*P*=0.0001
GGT (U/L)	1.00±0.00	1.20±0.45	0.90±0.22	0.70±0.27	0.90±0.65	1.10±0.55
Significance		1*P*=0.457	1*P*=0.709; 2*P*=0.268	1*P*=0.268; 2*P*=0.071	1*P*=0.709; 2*P*=0.268	1*P*=0.709; 2*P*=0.709
TC (mg/dL)	90.96±7.59	71.98±11.10	87.52±7.89	49.34±5.23	82.46±13.66	54.18±4.53
Significance		1*P*=0.003	1*P*=0.548; 2*P*=0.011	1*P*=0.0001; 2*P*=0.001	1*P*=0.145; 2*P*=0.076	1*P*=0.0001; 2*P*=0.004
HDL-C (mg/dL)	76.86±5.52	62.30±6.06	76.26±7.04	44.46±8.28	71.34±12.15	42.40±4.28
Significance		1*P*=0.006	1*P*=0.902; 2*P*=0.008	1*P*=0.0001; 2*P*=0.001	1*P*=0.265; 2*P*=0.074	1*P*=0.0001; 2*P*=0.0001
LDL-C (mg/dL)	2.60±1.14	3.20±0.45	1.80±0.45	1.20±0.45	2.20±1.10	2.80±0.45
Significance		1*P*=0.213	1*P*=0.101; 2*P*=0.006	1*P*=0.006; 2*P*=0.0001	1*P*=0.402; 2*P*=0.043	1*P*=0.674; 2*P*=0.402
TG (mg/dL)	35.44±1.03	55.58±9.55	61.26±12.48	41.80±11.05	47.48±10.90	52.58±14.40
Significance		1*P*=0.007	1*P*=0.001; 2*P*=0.413	1*P*=0.360; 2*P*=0.054	1*P*=0.090; 2*P*=0.246	1*P*=0.019; 2*P*=0.664
TB (mg/dL)	0.100±0.00	0.100±0.00	0.100±0.00	0.500±0.84	0.280±0.40	0.100±0.00
Significance		1*P*=1.000	1*P*=1.000; 2*P*=1.000	1*P*=0.109; 2*P*=0.109	1*P*=0.461; 2*P*=0.461	1*P*=1.000; 2*P*=1.000

Data are express as mean±SD. 1*P*: Significance versus NC; 2*P*: Significance versus toxic group using one-way ANOVA test (LSD). AST: Aspartate transaminase; ALT: Alanine transaminase; ALP: Alkaline phosphatase; GGT: Gamma-glutamyl transferase; HDL-C: High density lipoprotein cholesterol; TC: Total cholesterol; LDL-C: Low-density lipoprotein cholesterol; TG: Triglyceride; TB: Total bilirubin; NC: Normal control; TAA: Thioacetamide; CM: Camel milk; NSO: *Nigella sativa* oil; CM plus NS: Camel milk + *Nigella sativa* oil; JIG: Jigreen; SD: Standard deviation; LSD: Least significant difference; ANOVA: Analysis of variance

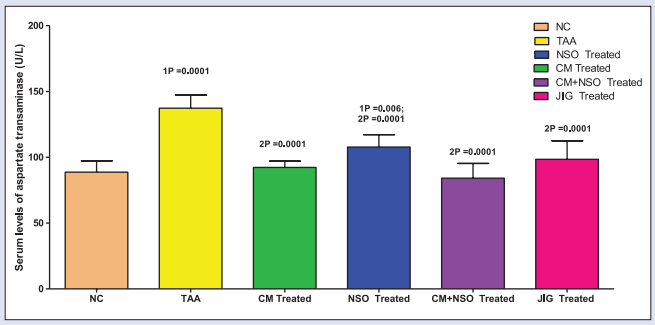

Figure 1: Serum levels of aspartate transaminase (U/L) in different studied groups

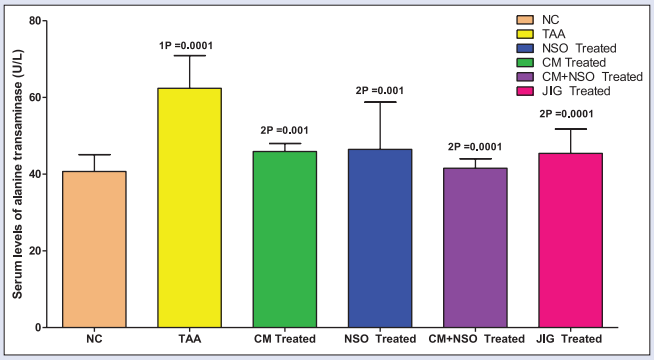

Figure 2: Serum levels of alanine transaminase (U/L) in different studied groups

P = 0.0001, respectively) and TAA group (*P* = 0.0001for all); but was significantly higher in TAA toxic control group versus normal control group (*P* = 0.0001). The serum levels of potassium (K+) was significantly higher in TAA toxic control group as compared with normal control, CM-treated, NSO-treated, CM + NSO-treated and JIG-treated groups (*P* = 0.001, *P* = 0.001, *P* = 0.0001, *P* = 0.0001, and *P* = 0.0001, respectively) [Table 2].

CM-treated, NSO-treated, CM + NSO-treated, and JIG-treated groups significantly ($P < 0.05$) brought down the elevated serum level of urea, uric acid, creatinine, sodium, and potassium level in comparison with toxic TAA control group, suggesting the nephroprotective activity of CM and NSO alone, CM plus NSO combination and Jigreen. The results of the nephroprotective potential of CM, NSO alone, and CM plus NSO combination and Jigreen against TAA (100 mg/kg)-induced nephrotoxicity are shown in Table 2.

Histopathological studies

Histopathological studies of the section from liver and kidney tissues were also carried out to confirm the outcome of the biochemical serum analysis. Histopathological examination of normal control rat liver showed normal structure with hepatocytes arranged in plates and disseminating from the central vein (CV) to the periphery of hepatic lobules. Hepatocytes were observed to have slightly basophilic cytoplasm with rounded central euchromatic nuclei. The plates were separated by thin-wall blood sinusoids lined by endothelial cells, and occasionally, Vonkuppder cells could be seen [G1:NC; Figure 11]. Administration of TAA through SC injection markedly altered the histological structure of rat liver focal regions of hepatocytes necrosis along with hemorrhage. Hepatocytes in other regions looked shrunken, dark stained with small pyknotic nuclei (signs of apoptosis), and blood sinusoids appeared to be heavily infiltrated with mononuclear cells (G2:TC). Administration of CM (G3:CM) showed potential protection from the alterations as compared with G2:TC, but the microphotograph still showed focal degenerative regions with monocytes cell infiltration (white arrow). Treatment group no IV which was supplemented with NSO (G4:NSO) showed nearly normal hepatocytes with euchromatic nuclei (black arrows) and slight monocytic cell infiltration (black stars) around CV. Blood sinusoids showed numerous lymphocytes (white arrows).(G5:NSO plus CM) A combination of NSO + CM revealed marked the preservation of

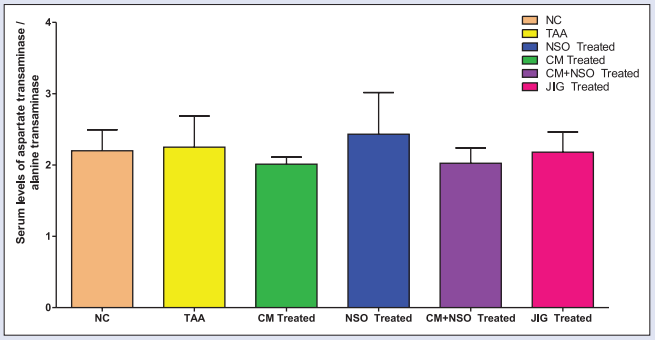

Figure 3: Serum levels of aspartate transaminase/alanine transaminase ratio in different studied groups

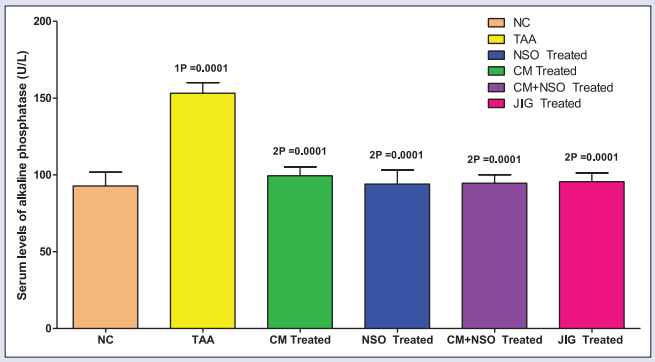

Figure 4: Serum levels of alkaline phosphatase (U/L) in different studied groups

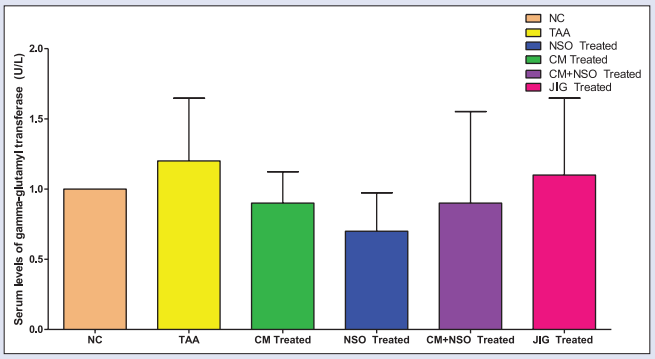

Figure 5: Serum levels of gamma-glutamyl transferase (U/L) in different studied groups

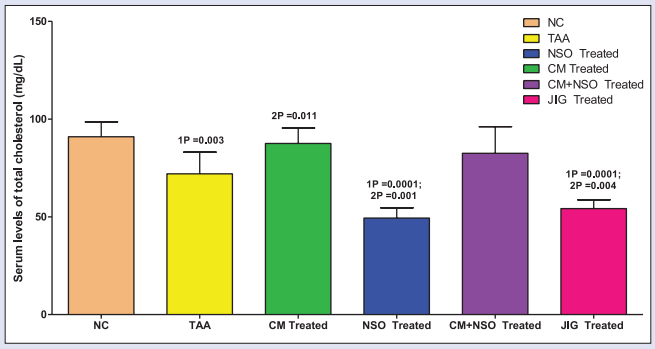

Figure 6: Serum levels of TC (mg/dL) in different studied groups

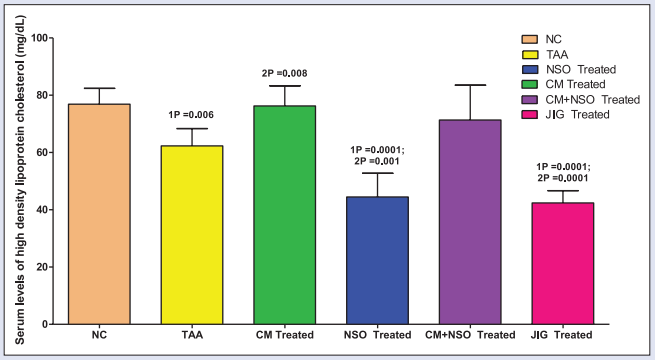

Figure 7: Serum levels of high density lipoprotein cholesterol (mg/dL) in different studied groups

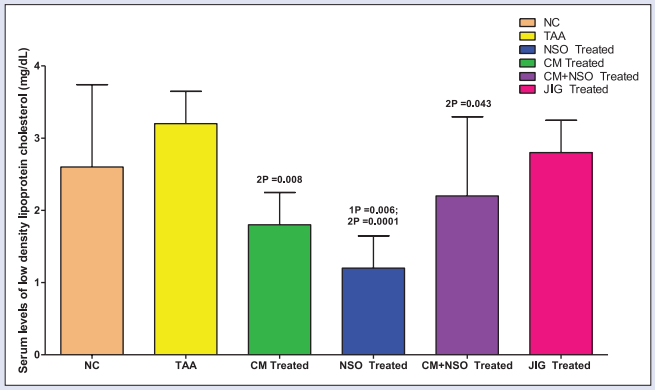

Figure 8: Serum levels of low-density lipoprotein cholesterol (mg/dL) in different studied groups

hepatic architecture except for few hepatocytes which showed dark cytoplasm and nuclei (arrows). The best protection was observed in the standard group supplemented with Jigreen (G6:JIG) wherein hepatocytes and sinusoids looked almost similar to the normal control group (H and E, ×400).

The histopathological examination of the kidneys of rats treated with TAA showed glomerular and blood vessel congestions, epithelial desquamation tubular casts, in comparison to the kidneys of Group I normal control rats. Treatment with CM and NSO combination has shown nearly entire normalization of the kidney tissues which pointed out a synergistic nephroprotective potential of CM plus NSO combination. Results are shown in Figures 11-13.

DISCUSSION

This study was planned to investigate the curative effects of supplementation with CM alone, NSO alone, and combination of CM plus NSO against TAA-induced hepatorenal toxicity with reference to a standard hepatoprotective drug Jigreen. The results are presented in the Tables 1 and 2 and Figures 1-13.

TAA is a well-known, potent hepatorenal toxicant, and carcinogenic agent in rats. TAA administration at a dose of 100 mg/kg *sc* is reported to cause hepatorenal toxicity. The increased oxidative stress is considered as the main cause of TAA-induced hepatotoxicity.[1,5] TAA is also reported to cause kidney and thymus toxicities.[21] and chronic

Table 2: Comparison of the serum levels of measured kidney enzymes in different studied groups versus normal control and toxic group

Variable	NC	TAA	CM treated	NSO treated	CM+NS treated	JIG treated
Uric acid (mg/dL)	1.04±0.14	1.28±0.18	1.22±0.41	1.02±0.24	1.26±0.05	0.90±0.30
Significance		1*P*=0.134	1*P*=0.253; 2*P*=0.706	1*P*=0.920; 2*P*=0.111	1*P*=0.167; 2*P*=0.900	1*P*=0.468; 2*P*=0.031
Urea (mg/dL)	41.30±1.83	56.60±2.67	38.06±4.71	47.98±4.72	39.04±3.06	46.36±2.90
Significance		1*P*=0.0001	1*P*=0.154; 2*P*=0.001	1*P*=0.006; 2*P*=0.0001	1*P*=0.315; 2*P*=0.0001	1*P*=0.031; 2*P*=0.0001
Creatinine (mg/dL)	0.43±0.05	0.45±0.03	0.37±0.05	0.33±0.04	0.34±0.04	0.36±0.02
Significance		1*P*=0.544	1*P*=0.018; 2*P*=0.004	1*P*=0.0001; 2*P*=0.0001	1*P*=0.001; 2*P*=0.0001	1*P*=0.011; 2*P*=0.002
Sodium (mmol/L)	144.20±1.10	151.40±2.51	141.20±1.92	139.00±1.87	136.60±2.70	139.00±2.45
Significance		1*P*=0.0001	1*P*=0.038; 2*P*=0.0001	1*P*=0.001; 2*P*=0.0001	1*P*=0.0001; 2*P*=0.0001	1*P*=0.001; 2*P*=0.0001
Potassium (mmol/L)	4.95±0.64	6.13±0.17	4.84±0.46	4.80±0.64	5.18±0.26	4.79±0.53
Significance		1*P*=0.001	1*P*=0.718; 2*P*=0.001	1*P*=0.621; 2*P*=0.0001	1*P*=0.457; 2*P*=0.0001	1*P*=0.603; 2*P*=0.0001

Data are express as mean±SD. 1P: Significance versus NC; 2P: Significance versus toxic group using one-way ANOVA test (LSD). NC: Normal control; TAA: Thioacetamide; CM: Camel milk; NSO: *Nigella sativa* oil; CM plus NS: Camel milk + *Nigella sativa* oil; JIG: Jigreen; SD: Standard deviation; LSD: Least significant difference; ANOVA: Analysis of variance

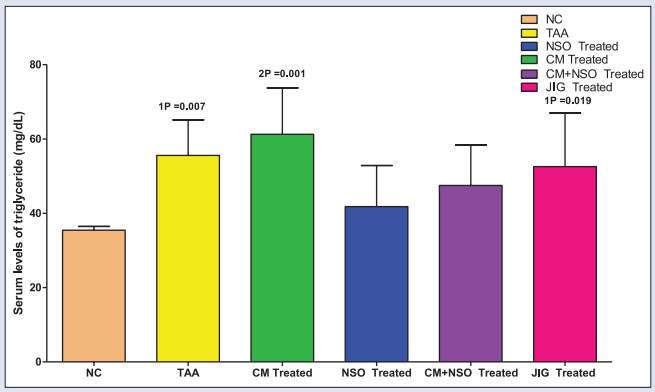

Figure 9: Serum levels of triglyceride (mg/dL) in different studied groups

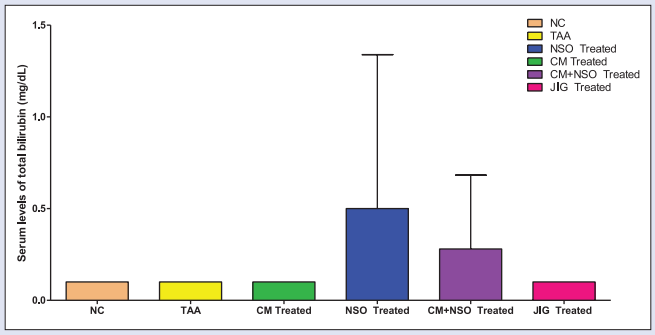

Figure 10: Serum levels of total bilirubin (mg/dL) in different studied groups

exposure of TAA may cause liver cirrhosis in rats.[22] The mechanism of hepatorenal toxicity of TAA is due to interference of the RNA movement to cytoplasm from the nucleus, which results in injury to the membrane. The TAA-s-s-dioxide is the reactive metabolite of TAA which is mainly responsible for hepatorenal toxicity. This metabolite causes an increase in the concentration of intracellular calcium, alteration in cell permeability, karyomegaly with increased nuclear volume, and mitochondrial inhibition which ultimately results in hepatic and renal cell death. The cellular enzymes leakage from hepatic cells into plasma is an important biomarker of hepatic injury. The enzymatic activities of ALT, AST, GGT, and ALP are the most reliable tests used in the investigations of the liver diseases. The serum level of ALT activity directly linked to the damage to the hepatocytes. ALT is highly regard as sensitive and important biomarker of liver toxicity. However, an increased serum ALT activity is also associated with other organ toxicities. ALT is mainly found in the liver and ALT is a more specific indicator of liver damage than AST. Apart from liver, AST is also found in other tissues such as brain, heart, kidneys, and skeletal muscle. The serum level of AST is another important biomarker of liver functions. Increased level of serum GGT activity is another important and reliable biomarker of liver toxicity. ALP occurs in almost all tissues in the body but is predominantly present in the liver and kidneys. Elevated levels of ALP are an important indicator of liver and kidney injury. A single dose of TAA cause necrosis along with increased level of serum transaminases and bilirubin concentrations in rats.[23-24] Significant increase in the concentrations of serum transaminases is considered as an index of liver damage and well-known biomarker of cellular leakage and damage of the cell membrane of liver.[25]

In the current study, TAA intoxicated rats showed significant alterations in the level of serum biomarker enzymes of liver, kidneys, and lipid profile. The administration of a single dose of TAA (100 mg/kg) by SC route to the rats significantly ($P < 0.05$) increased the serum level of the AST, ALT, ALP and GGT in comparison with rats of normal control group, which indicated the hepatotoxicity [Table 1 and Figures 1-5] which could be due to the leakage from damaged tissues. The TAA-induced elevation in the serum levels of the AST, ALT, and ALP was significantly reduced by the posttreatment with CM alone, NSO alone and CM plus NSO combination, but no significant effect was observed on GGT level. This suggested the antihepatotoxic potential of CM and NSO alone and in combination which could be due to their antioxidant properties as reported earlier. CM is rich source of proteins Vitamins A, B, C and E along with minerals like copper, sodium, potassium, magnesium, iron, manganese, etc. These vitamins and minerals have been shown to act as free radical scavengers and antioxidants which might contribute to the protective effects of CM in the prevention of TAA-induced liver injury. Our findings are in agreement with data obtained by the Hamad *et al.*, 2011 and Althnaian *et al.*, 2013.[26,27] On the other hand, NSO contains a bioactive phytochemical thymoquinone which have been explored and investigated for its numerous pharmacological properties including antioxidant effects. It has been reported to exert protective effects on many vital organs such as liver, kidneys, heart, brain, lungs, and many other organs against wide range of toxicants in animal models.[9] In the present study, NSO treatment also exhibited protective effects on liver which might be attributed to membrane stabilizing and antioxidant properties of thymoquinone. This finding is in agreement with the published data by Nehar and Kumari, 2013.[28]

The impairment of lipid metabolism is usually found with liver toxicity and liver diseases. Therefore, lipid profiles (TC (mg/dL), HDL-C (mg/dL), LDL-C (mg/dL), and TG (mg/dL), TB (mg/dL)) were measured for the assessment and their correlation with the severity of liver damage. The lipid profile results of TAA intoxicated rats showed a

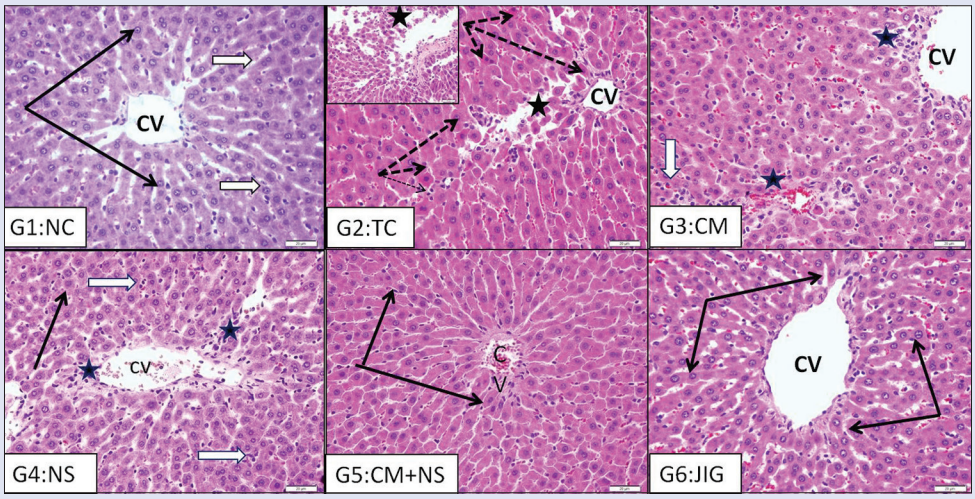

Figure 11: Sections from rats liver from the region of CV H and E: G1:NC showing hepatocytes cell cord radiating from the CV; hepatocytes have central euchromatic nuclei and homogeneous cytoplasm (black arrows), blood sinusoids showed normal lining cells (white arrows). G2:TC showing focal necrotic or hemorrhagic regions (black stars), the rest of hepatocytes looked shrunken, dark stained with small pyknotic nuclei (dotted arrows), Monocytic cell infiltrate could be seen (white arrow). G3:CM showing potential protection from degenerative changes as compared with G2:TC; There is still focal degenerative regions with monocytes cell infiltration (white arrow). G4:NS showing nearly normal hepatocytes with euchromatic nuclei (black arrows), slight monocytic cell infiltrate (black stars) around CV. Blood sinusoids showed numerous lymphocytes (white arrows). G5:NS + CM showing marked preservation of hepatic architecture except of few hepatocytes showing dark cytoplasm and nuclei (arrows). G6:JIG showing marked protection, hepatocytes and sinusoids looked almost similar to normal control group (H and E, ×400)

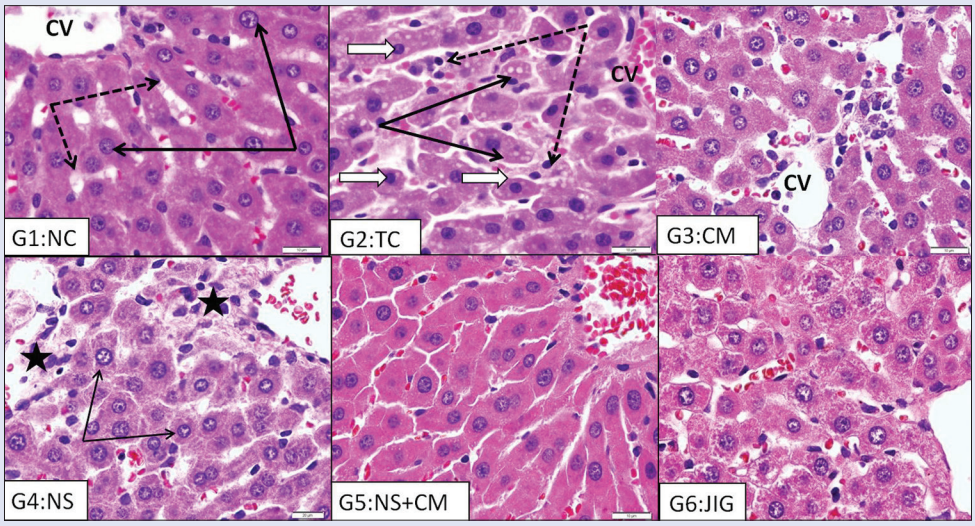

Figure 12: Magnified powers of rat liver to show hepatocytes (black arrows) and the intervening blood sinusoids (dotted arrows) in G1:NC. Administration of TAA (G2:TC) result in marked disruption of liver architectures, Hepatocytes (white arrows) appeared shrunken dark stained with pyknotic nuclei (signs of apoptosis). The cytoplasm of others showed tiny lipid accumulation (black arrows). Sinusoids showed monocyte infiltrate (dotted arrows). Treated groups (G3-G6) showed marked protection against changes induced by TAA. With more better response, the G5 which administrated mixture of NS + CM (H and E, ×1000)

significant increase in the serum levels of TG and LDL-C while serum levels of HDL-C and TC were significantly reduced in in comparison with normal control group. Similar results were also obtained by Kabiri *et al.*, 2014.[29] It was interesting to note that treatment of animals with CM, NSO, CM plus NSO combination, and JIG significantly reduced the increased level of TG, and LDL-C, while significantly raised the reduced serum level of HDL-C and TC, surprisingly, no significant effect was noted on the bilirubin level as compared with TAA-treated toxic control group. These findings are in line with El-Dakhakhny *et al.*, 2000; Shafiq *et al.*, 2014; Abdel-Daim and Ghazy, 2015[30-32] where in, they reported that NSO could significantly decrease the serum TG, LDL levels and significantly improve the diminished serum HDL level [Table 1 and Figures 6-10].

To investigate the nephroprotective effects of CM, NSO, and Jigreen on TAA-induced renal toxicity; the serum level of urea, uric acid, creatinine, sodium (Na+), and potassium (K+) were measured. The administration of a single dose of TAA (100 mg/kg) by SC injection to the experimental rats significantly ($P < 0.05$) enhanced the serum level of the urea, uric acid, creatinine, sodium, and potassium in comparison to the rats of normal control group, which indicated an acute kidney toxicity.[26,32] [Table 2].

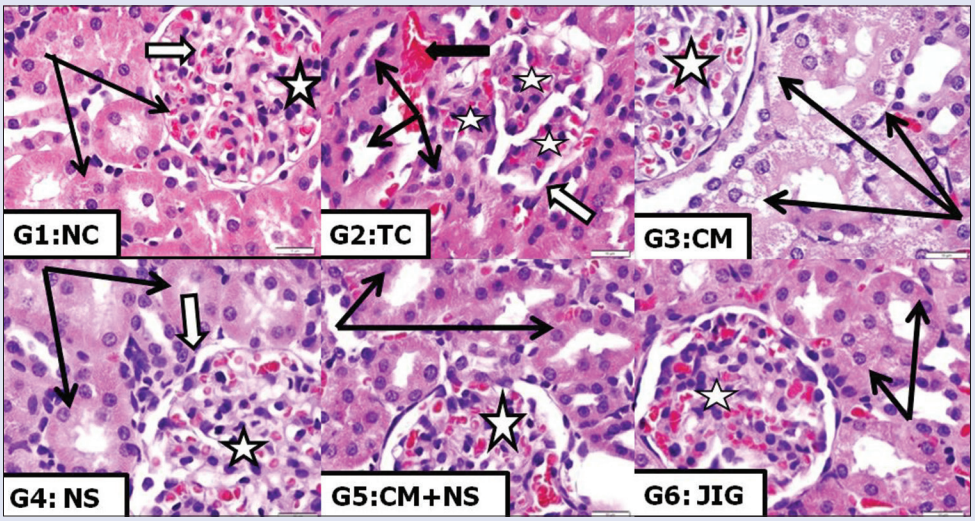

Figure 13: Sections from renal cortex of rat kidney stained by H and E showing: G1:NC with normal renal corpuscles and glomerulus (white star), renal tubules showing normal lining epithelium (black arrows). G2:TC showing disorganization and atrophy of tubular epithelium (black arrows) the nuclei of lining epithelium are dark and small (pyknosis), glomerular capillaries showed lobulation or atrophy (white stars) with widening of Bowman's space (black stars), Bowman capsule showed damaged epithelium (white arrows), Capillary congestion is evident (thick black arrow), G3:CM, Showed preservation of renal corpuscles and glomerular structure (white stars), renal tubules (Black arrows) are organized but still showed basal degenerative changes (Unstained regions). G4:NS showing marked preservation of both renal corpuscles and glomerulus (white stars), and renal tubules (black arrows). G5:CM + NS showing marked preservation of both tubular (black arrows) and renal corpuscles and glomerular (white stars). G6:JIG showing normal histological features of both renal tubules (black arrows) and renal corpuscles and glomerulus (white stars), Note: In all treated groups cellular nuclei of tubular epithelium looked more active compare to control group base on euchromatic appearance (H and E, ×1000)

Our results showed that posttreatment of rats with CM alone, NSO alone, CM plus NSO combination and standard marketed drug Jigreen significantly lowered the elevated levels of above kidney biomarkers toward the normal values. Therefore, the results of the current study provided evidence to support the traditional claim that the CM, NSO, and their combination have the potential to reverse the renal toxicity [Table 2]. The observed renal protective effects of CM and NSO again might be due to their antioxidant, free radical scavenging, membrane stabilizing properties, and ability to prevent the cellular leakage.

The overall findings of this study demonstrated that posttreatment of the rats with CM, NSO, and combined CM plus NSO regimen possess curative potential against TAA-induced hepatorenal toxicity. The mechanism of protection might be due to their alleviative effect on oxidative stress and inflammation. Further, it might be possible that CM and NSO exerted their curative effect against TAA-induced hepatorenal toxicity by their immune-modulating properties. Further detailed studies are required for the evaluation of mechanism of action of CM and NSO at molecular level for their hepatorenal protective effects. Finally, it can be recommended to consume CM and NS seeds or its oil as a supplement or home remedy along with the prescribed treatment for hepatorenal toxicity.

CONCLUSION

This study demonstrated the ameliorative effects of CM, NSO, and CM + NSO combination against TAA-induced hepatorenal toxicity in rats. The outcome of this study might contribute in the development of a novel complementary alternative medicine in combating and therapeutic intervention of TAA-induced hepatorenal toxicity.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Ahmad A, Pillai KK, Najmi AK, Ahmad SJ, Pal SN, Balani DK, *et al.* Evaluation of hepatoprotective potential of Jigrine post-treatment against thioacetamide induced hepatic damage. J Ethnopharmacol 2002;79:35-41.
2. Amir M, Khan MA, Ahmad S, Akhtar M, Mujeeb M, Ahmad A, *et al.* Ameliorating effects of *Tamarindus indica* fruit extract on anti-tubercular drugs induced liver toxicity in rats. Nat Prod Res 2016;30:715-9.
3. Ghabril M, Chalasani N, Björnsson E. Drug-induced liver injury: A clinical update. Curr Opin Gastroenterol 2010;26:222-6.
4. Chen TM, Subeq YM, Lee RP, Chiou TW, Hsu BG. Single dose intravenous thioacetamide administration as a model of acute liver damage in rats. Int J Exp Pathol 2008;89:223-31.
5. Aftab A, Pillai KK, Shibli JA, Balani DK, Najmi AK, Marwah R, *et al.* Evaluation of the hepatoprotective potential of jigrine pre-treatment on thioacetamide induced liver damage in rats. Indian J Pharmacol 1999;3:416-21.
6. Al-Bukhari MI. Division 71 on Medicine. In: Sahi Al-Bukhari, editor. The Collection of Authentic Sayings of Prophet Mohammad (Peace be upon him). 2nd ed. Ankara, Turkey: Hilal Yayinlari; 1976.
7. Abdel-Zaher AO, Abdel-Rahman MS, Elwasei FM. Protective effect of *Nigella sativa* oil against tramadol-induced tolerance and dependence in mice: Role of nitric oxide and oxidative stress. Neurotoxicology 2011;32:725-33.
8. Abel-Salam BK. Immunomodulatory effects of black seeds and garlic on alloxan-induced diabetes in albino rat. Allergol Immunopathol (Madr) 2012;40:336-40.
9. Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, *et al.* A review on therapeutic potential of *Nigella sativa:* A miracle herb. Asian Pac J Trop Biomed 2013;3:337-52.
10. Ahmad A, Husain A, Mujeeb M, Siddiqui NA, Damanhouri ZA, Bhandari A, *et al.* Physicochemical and phytochemical standardization with HPTLC fingerprinting of *Nigella sativa* L. Seeds. Pak J Pharm Sci 2014;27:1175-82.

11. Al-Suhaimi EA. Hepatoprotective and immunological functions of *Nigella sativa* seed oil against hypervitaminosis A in adult male rats. Int J Vitam Nutr Res 2012;82:288-97.
12. Konuspayeva G, Faye B, Loiseau G, Narmuratova M, Ivashchenko A, Meldebekova A, *et al*. Physiological change in camel milk composition (*Camelus dromedarius*) 1. Effect of lactation stage. Trop Anim Health Prod 2010;42:495-9.
13. Abdoun KA, Amin AS, Abdelatif AM. Milk composition of dromedary camels (*Camelus dromedarius*): Nutritional effects and correlation to corresponding blood parameters. Pak J Biol Sci 2007;10:2724-7.
14. Agrawal RP, Jain S, Shah S, Chopra A, Agarwal V. Effect of camel milk on glycemic control and insulin requirement in patients with type 1 diabetes: 2-years randomized controlled trial. Eur J Clin Nutr 2011;65:1048-52.
15. el Agamy EI, Ruppanner R, Ismail A, Champagne CP, Assaf R. Antibacterial and antiviral activity of camel milk protective proteins. J Dairy Res 1992;59:169-75.
16. Korish AA. The antidiabetic action of camel milk in experimental type 2 diabetes mellitus: An overview on the changes in incretin hormones, insulin resistance, and inflammatory cytokines. Horm Metab Res 2014;46:404-11.
17. Malik A, Al-Senaidy A, Skrzypczak-Jankun E, Jankun J. A study of the anti-diabetic agents of camel milk. Int J Mol Med 2012;30:585-92.
18. Althnaian T. Protective effect of camel milk against carbon tetrachloride hepatotoxicity in rats. Glob Vet 2012;9:564-70.
19. Darwish HA, Abd Raboh NR, Mahdy A. Camel's milk alleviates alcohol-induced liver injury in rats. Food Chem Toxicol 2012;50:1377-83.
20. Al-Asmari AK, Abbasmanthiri R, Al-Elewi AM, Al-Omani S, Al-Asmary S, Al-Asmari SA, *et al*. Camel milk beneficial effects on treating gentamicin induced alterations in rats. J Toxicol 2014;2014:917608.
21. Barker EA, Smuckler EA. Nonhepatic thioacetamide injury. II. The morphologic features of proximal renal tubular injury. Am J Pathol 1974;74:575-90.
22. Chieli E, Malvaldi G. Role of the microsomal FAD-containing monooxygenase in the liver toxicity of thioacetamide S-oxide. Toxicology 1984;31:41-52.
23. Hajovsky H, Hu G, Koen Y, Sarma D, Cui W, Moore DS, *et al*. Metabolism and toxicity of thioacetamide and thioacetamide S-oxide in rat hepatocytes. Chem Res Toxicol 2012;25:1955-63.
24. Koen YM, Sarma D, Hajovsky H, Galeva NA, Williams TD, Staudinger JL, *et al*. Protein targets of thioacetamide metabolites in rat hepatocytes. Chem Res Toxicol 2013;26:564-74.
25. Mujeeb M, Khan SA, Aeri V, Ali B. Hepatoprotective activity of the ethanolic extract of *Ficus carica* Linn. Leaves in carbon tetrachloride-induced hepatotoxicity in rats. Iran J Pharm Res 2011;10:301-6.
26. Hamad EM, Abdel-Rahim EA, Romeih EA. Beneficial effect of camel milk on liver and kidneys function in diabetic Sprague-Dawley rats. Int J Dairy Sci 2011;6:190-7.
27. Althnaian T, Albokhadaim I, El-Bahr SM. Biochemical and histopathological study in rats intoxicated with carbon tetrachloride and treated with camel milk. Springer Plus 2013;2:57.
28. Nehar S, Kumari M. Ameliorating effect of *Nigella sativa* oil in thioacetamide-induced liver cirrhosis in albino rats. Indian J Pharma Edu Res 2013;47:135-9.
29. Kabiri N, Darabi MA, Rafieian-Kopaei M, Setorki M, Doudi M. Protective effect of Kombucha tea on liver damage induced by thioacetamide in rats. J Bio Sci 2014;14:343-8.
30. El-Dakhakhny M, Mady NI, Halim MA. *Nigella sativa* L. Oil protects against induced hepatotoxicity and improves serum lipid profile in rats. Arzneimittelforschung 2000;50:832-6.
31. Shafiq H, Ahmad A, Masud T, Kaleem M. Cardio-protective and anti-cancer therapeutic potential of *Nigella sativa*. Iran J Basic Med Sci 2014;17:967-79.
32. Abdel-Daim MM, Ghazy EW. Effects of *Nigella sativa* oil and ascorbic acid against oxytetracycline-induced hepato-renal toxicity in rabbits. Iran J Basic Med Sci 2015;18:221-7.