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Effect of *Moringa oleifera* Ethanolic Leaf Extract for the Management of Hepatotoxicity and Nephrotoxicity in Mice

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Authors' contributions

This work was carried out in collaboration between all authors. Author AT designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors US and MSJ managed the analyses of the study. Authors ZA and AR managed the literature searches. Author PNC did the statistical analysis and manuscript writing. All authors read and approved the final manuscript.

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ABSTRACT

Moringa oleifera is a valued medicinal plant. It is a rich source of nutrients, amino acid, antioxidants, anti-ageing and anti-inflammatory compounds. This study was carried out to assess the effect of the ethanolic extract of *Moringa oleifera* leaves on tetrachloride CCL4 induced liver damage and gentamycin induced kidney damage in albino mice. Twenty-eight (28) mice were divided into seven groups subjected to the different treatments. Results from the serum enzyme assay revealed that the *Moringa oleifera* leaf ethanolic extract reduced the activities of the hepatic

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and renal enzymes voz: ALP, AST, ALT, blood urea nitrogen and creatinine. *Moringa oleifera* leaf extracts showed significant amelioration of experimentally induced hepatotoxicity and nephrotoxicity which enhanced the recovery from hepatic damage induced by CCL4 and renal damage induced by gentamycin.

Keywords: Moringa oleifera; hepatotoxicity; nephrotoxicity; serum enzyme assay.

1. INTRODUCTION

Since ancient times, medicinal plants have been used by all civilizations as a source of medicines. Recently, there has been increasing interest in exploiting the biological activities of different ayurvedic medicinal herbs, owing to their natural origin, cost effectiveness and lesser side effects [1]. *Moringa oleifera* is one of the best plants of medicinal value. It is used in many tropical countries for medicinal purposes. It is a soft wooded deciduous tree belonging to the *Moringaceae* family [2]. Moringa is the most nutrient-rich plant yet discovered. It provides a rich and rare combination of nutrients, amino acids, antioxidants, anti-aging and anti-inflammatory compounds [3]. Leaves of *M. oleifera* contain compounds with antioxidants properties such as flavnoid, phenolic acid, phenolic diterpenes, lignane. Extract of different Moringa tissues have been used as anti-trypanosomal [4], anticancer, antimicrobial [5], anti-inflammatory and hepatoprotective [6] agents. Moreover, leaf extract has been shown to regulate thyroid level [7] and cholesterol level in rats [8]. The antioxidant activity of *Moringa oleifera* extracts is due to the presence of different bioactive compounds such as rutin, querceting lucoside, chlorogenic acid, and glucoside. In addition, the extract of Moringa leaves and other plant parts have been shown to have strong antioxidant action *in vivo*.

Liver protects our body from many injurious substances and toxic metabolic by products which have been absorbed from intestinal tract [9]. Carbon tetrachloride (CCl_4) is commonly used for experiments to induce liver damage. The mechanism of carbon tetrachloride induction is causing hepatic damage by lipid peroxidation and production of free radicals and reduced the activities of antioxidant enzymes [10]. Liver diseases remained one of the severe health problems. Medicinal plants have been used for the treatment of these diseases in the Indian traditional system of medicine [8]. The medicinal use of various plants with hepatoprotection activities like *Azardiracta indica*, *Cassia fistula*, *Andrographis paniculata* and *Moringa oleifera* has been reported in the literature [11]. Recently, herbal drugs have been used increasingly for the treatment of liver diseases all over the world. The herbal drugs are considered to be harmless and are free from serious adverse reactions as they are easily available and obtained from nature. There are about 600 commercial herbal formulations, which have hepatoprotective activity and several of them are being sold in the market worldwide [12].

Fakurazi et al. [13] conducted the antihepatotoxic study which showed that the moringa leaf and flower extracts (200 mg/kg and 400 mg/kg, i.p) which were administered an hour after APAP administration also reduced liver damage. N-Acetylcysteine was used as the positive control against APAP-induced hepatotoxicity.

Meanwhile, nephrotoxicity is one of the widespread kidney problems and arise when body is exposed to toxins and drugs [14]. Moringa controls lipid peroxidation and reactive oxygen species in nephrotoxicity [15]. Glutathione (GSH) is necessary in the removal of toxic agents. GSH is a strong scavenger of free radicals which also causes oxidation of GSH to oxidised glutathione which contributes to the depletion of stored GSH [16,17]. In addition, aqueous ethanolic extract of leaves of *Moringa oleifera* are capable of scavenging superoxyl, peroxyl, and 1,1-diphenyl 2-picrylhydrazyl radicals [18]. Different investigations recommended that the antioxidant activity is the major mechanism for the nephron protection by Moringa. Future researches are essential to analyse the promising protective effects of Moringa against renal injury, which will possibly contribute to a substantial impact in developing clinically reasonable strategies to treat patients suffering from renal failure.

Rakesh and Jai Singh, [19] conducted a research which assessed the different concentration of ethanolic (alcoholic) and crude aqueous extracts of *Moringa oleifera*. Pods and leaves were used to study the protective activity against CCl4 induced hepatocytes injury of mice in vitro and correlated with the standard silymarin. Results

showed that these extracts were efficient in reducing the CCl4 induced toxicity, improving the activities of GLU, SOD, lipid peroxidation, catalase and % viability. CCl4 treatment reduced SOD, catalase, glutathione and peroxidase while increasing the lipid oxidation as observed from the increased concentration of MDA. The study showed that hepatocellular damage caused by CCl4 and its recovery by pretreatment with the crude extract of leaves and pods suggests that moringa might be considered as a potential source of natural antioxidant agent, which could be related to the free radical scavenging properties of various components present in varying concentration in the extract which is evident from the free radical measurement.

Ouedraogo et al. [20] investigated the nephron protective effect of aqueous-ethanolic extract of *Moringa oleifera* leaves (150 and 300 mg/kg) against gentamicin-induced renal injury in rabbits. Creatinine levels and serum urea were assessed as the indicator of renal nephrotoxicity. The kidneys of rabbits were cut out for histological analysis and the lipid peroxidation levels were determined in the end of the experiment. Creatinine levels and serum urea were decreased in the rabbits subjected to *M. oleifera* (150 and 300 mg/kg) compared with the gentamicin treated groups. The histological analysis of the kidney of intoxicated rabbits group which were subjected to *M. oleifera* extracts showed promising results. There was a significant increase in lipid peroxidation (LPO) level in the kidneys of gentamicin-intoxicated rabbits subjected to *M. oleifera* while the gentamicin group showed a highly significant reduction in LPO.

The present study showed that aqueous-ethanolic leaf extract of *M. oleifera* leaves decreased both liver and renal injury in mice.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Materials

Fresh leaves of *M. oleifera* were collected from Layyah (Punjab) and were brought to the Institute of Molecular Biology and Biotechnology, The University of Lahore, Pakistan for further processing. The leaves were dried for three days were homogenized using an electric grinder to pulverize the leaves. The powdered leaves (500 g) was macerated in 70% ethanol at room temperature for 24 h. It was filtered and concentrated in a rotary evaporator at 60ºC. One gram of the extract residue was dissolved in minimum amount of DMSO and diluted with distilled water to make 100 ml solution. The extract solution was kept in air tight bottle in a refrigerator.

2.2 Laboratory Animals

Twenty eight albino mice (15 to 30 g) were obtained from the University of Veterinary and Animal Sciences, Lahore Pakistan which is a reputable experimental animal producing laboratory. They were provided with good source of water and food with ample amount of bedding during transport. The transit containers were escape-proof, comfortable, and with good ventilation for the animals. The animal handling instructions were found at the side of the containers used during transport. The transport was done in the early morning hours around 6 AM. The species were authenticated and certified by the university veterinarian. The newly received animals were quarantined and diagnosed for any possible infectious disease and were acclimatized for a period of 30 days in a well-ventilated room with a temperature and relative humidity of 29±2°C and 70%, respectively. They were individually caged and placed inside the animal house. The lighting was kept at 12 h: 12 h light and dark ratio. The presence of noise was minimized with provision of soft background music. The mice were maintained with water *ad libitum*.

Throughout the experiment, a veterinarian from the university was present to oversee procedures to the animals and check the overall health of the animals. There was a health keeping record provided by the veterinarian. The food for the mice was free from microbial contaminants and was stored in the 20-30°C. The water supplies were treated with HCL (6N per ml of water) to minimize contamination.

The disinfection and changing of animal bedding was done weekly and the animal restrainers were used on the most minimal time possible. The experimentation was done under the supervision of an expert veterinarian. During the experiment, the animals were euthanized. After the experiment, the animals were disposed properly by cremation.

2.3 Experimental Design

Twenty eight healthy albino mice weighing 15–30 g, were used in the present study and were

divided into 7 groups with four animals in each group (n=4):

- Group A (normal control).
- Group B (CCl4 at the dose of 0.5 ml CCl4 in 0.5ml olive oil (1:1)/Kgb.wt)
- Group C (0.5 ml Ccl4/Kg b.wt+*Moringa oleifera* leaf extract 250 mg/Kgb.wt)
- Group D (0.5 mlCcl4/Kg b.wt + *Moringa oleifera leaf extract* 500 mg/Kgb.wt)
- Group E (Gentamicin at the dose of 20 mg/Kgb.wt)
- Group F (20 mg Gentamicin /Kg b.wt + *Moringa oleifera* leaf extract 250 mg/Kgb.wt)
- Group G (20 mg Gentamicin/ Kg b.wt + *Moringa oleifera* leaf extract 500 mg/Kgb.wt)

Hepatotoxicity was induced by the administration of CCl4 at the dose of 0.5 ml CCl4 + 0.5 ml olive oil (1:1)/Kg intraperiotenially for eight consecutive days. Nephrotoxicity was induced in mice by intraperitoneal administration of gentamicin for 18 consecutive days at the dose of 20 mg/ Kg b.wt. After CCl4 gentamicin treatment, ethanolic leaf extract of *M. oleifera* was orally administered with a dosage of 250 mg/Kg b.wt and 500 mg/Kg b.wt for a period of 30 consecutive days.

One ml blood sample were taken from treated animals and subjected to centrifugation at 3000-4000 rpm for 10-15 minutes for the separation of serum. The estimation of Aspartate aminotransferase (AST) was done using the formula ∆A/min x 1745 (at 37°C), where ∆A = Change in absorbance (Anonymous 1996) and reference values are 15-40 IU/L for male and 13-35 IU/L for females. Alanine aminotransferase (ALT) was calculated by having a reference value of 10-40 IU/L for male and 07-35 IU/L for female. Alkaline phosphate (ALP) (Ochoa, 1968) was estimated using ∆A/min x 3433 formula and reference values used was 64-306 IU/L for serum / plasma. Commercially available Bio Merux and Randox kits were used.

Renal Function Test (RFT) included Blood Urea Nitrogen (BUN) and creatinine. Salicylate and hypochlorite in the reagent reacted with the ammonium ions to form a green complex. For BUN, 1000 µl of reagent was transferred to the tubes by pipette and 10 µl of each serum sample was added to the tubes. Contents of the tube was mixed and then incubated at 37^0C for 5 min. After incubation, 200 µl of sodium hypochlorite from the kit was added to all the tubes. The contents were again mixed for at least 5 min at 37°C, and then the absorbance of the sample was measured against standard blank within 2 hours. Creatinine level was estimated by the rate of change in absorbance using alkaline picrate`1.0 ml reagent. The 0.1 ml of each serum sample was used and mixed with the reagent. Contents of the tube were mixed after every 30 sec and the absorbance was taken right away (A_1) while the next absorbance was taken after 2 minutes as A_2. Absorbance was taken at 510 nm with 0.6-1.1 mg/dL for male and 0.5-0.9 mg/dL as reference values. Serum albumin (ALB) in the presence of bromocresol green at a slight acidic pH produces a color change of the indicator from yellow-green to green-blue. Absorbance of sample was measured at 600 nm and was calculated. The commercial kit (BioCor) was used to determine the serum total protein (TP) and was calculated using the formula:

Total Protein (g/dl) = {(Abs of Sample/Abs of Standard) X Standard Concentration}

The mice were dissected to remove the kidney and liver. Kidney and liver sections were preserved in 10% formalin solution. The desired sections of liver for each group were cut and dipped in concentrated formalin for two hours. The sections of each group were labeled as M-1 for group A (Control), M-2 for group B (CCL4 induced mice), M-3 for group C (Ccl4 +*Moringa* 250 mg/kg of body weight), M-4 for group D (Ccl4 + *Moringa*500 mg/kg of body weight.), M-5 for group E (gentamicin induced mice), M-6 for group F (gentamicin + *Moringa* 250 mg/kgof body weight) and M-7 for group G (gentamicin +*Moringa*500 mg/kg of body weight). Each specimen was processed in an automatic tissue processor. Automatic tissue processor contains 12 jars, 2 of Formalin, 4 jars for Acetone, 4 jars of Xylene and 2 jars Paraffin. The specimen was placed for approximately 2 hours in each jar and this process was completed in 24 hours. The specimens from tissue processor were blocked. For blocking purposes, the specimen were placed in block shape boxes and filled with wax. The blocks were cooled in the refrigerator for better cutting in microtome. The blocks (specimen + wax) were cut into a section of 5µm by microtome. The section was first placed in cool water and then placed in hot water and fixed on the glass slides. The slide was coated with the film of mixture of egg albumin and glycerin. This coating helps tissue to fix on slide. The slides were placed in the oven with a temperature of 50°C. The slides were placed for

10 to 15 minutes. Then the slides were dried. A drop of DPX mutant was placed on each slide and covered with cover slip. These prepared slides were examined under microscope for histological evaluation. The histological changes were evaluated randomly with a magnification of 200x.

2.4 Statistical Analysis

Results were expressed as mean±SD (Standard Deviation). Statistical significance was determined by (DMRT) Duncan's Multiple Range Test. Spearmans Rh,o and Pearson correlation (Two Tailed) were used to correlate the different variables. The differences were considered significant at $p<0.05$.

3. RESULTS

The present study was designed to evaluate the nephrotoxicity and hepatotxicity of *Moringa oleifera* on gentamicin and carbontetrachloride induced liver and kidney damage in mice. A total of 28 mice were used which were divided into 7 groups as A, B, C, D, E, F and G. Group A was the control group treated with normal chicks diet. Group B was subjected to CCl4 at the dose of 0.5 ml CCl4 in 0.5 ml olive oil (1:1)/Kg b.wt). Group C was subjected to CCl4+*Moringa oleifera* leaf extract (250 mg/Kg b.wt) while Group D was subjected to CCl4+*M. oleifera* leaf extract (500 mg/Kg b.wt). Group E was subjected to gentamicin at the dose of 20 mg/Kgb.wt while Group F was subjected to gentamicin+*M. oleifera* leaf extract (250 mg/Kgb.wt). Lastly, Group G was subjected to gentamicin+*M. oleifera* leaf extract (500 mg/Kgb.wt) of body weight. Blood samples were taken after slaughtering each mouse for the estimation of AST, ALT, ALP, TP, ALB, BUN and Creatinine in serum. Only 1% of the animal weight of blood was extracted.

Statistical analysis revealed that for AST levels, significant ($p>0.05$) changes in group B (117.22±9.71) was observed when compared with the control (28.75±1.28) due to CCL4 hepatotoxicity. However, significant reduction in elevated levels of AST was observed in *Moringa* treated Groups C and D ($p<0.05$). The extract recorded no significant ($p>0.05$) changes in Groups C (35.50±4.19) and D (30.76±0.58a) when compared with the control (28.75±1.28). Furthermore, similar trend was observed for serum ALT and ALP levels, in which significant elevation in serum ALT (108.22±11.40) and serum ALP (146.62±22.31) levels was observed in group B as compared to group A. Moreover, significant ($p<0.05$) decreased in serum ALT (34.75±5.06, 31.00±0.96) and serum ALP (84.75±9.11, 82.50±8.18) levels was observed in moringa-treated Groups C and D when compared with Group B (CCl4 treated).

Statistical analysis further revealed that ethanolic extract of *M. oleifera* leaves showed significant elevation in renal markers BUN (38.03±5.85) and creatinine (3.07±0.21) in CCl4 treated Group B as compared to control Group A with BUN of 14.75± 0.95 and creatinine of 1.05 ± 0.12. Significant reduction ($p<0.05$) in serum BUN (20.5±3.31, 15.5±1.29) and creatinine (2.72±0.35, 2.5±0.45) was observed in *Moringa*-treated Groups C and D. Furtherrmore, a significant ($p>0.05$) reduction in protein markers Albumin (3.29±0.18) and TP (2.69± 0.96) levels was observed in Group B as compared to control group. The normal levels of serum Albumin (3.67± 0.02, 4.23 ±0.02) and TP (6.62±0.91, 4.75±0.53) were recorded in *Moringa*-treated Groups C and D.

Table 1. Effect of ethanolic leaf extract of *M. oleifera* on serum enzyme activities of CCl4 induced hepatotoxic mice

Groups	Treatments	Enzyme activity		
		AST (U/l) MEANS±SD	ALT (U/l) MEANS±SD	ALP(U/l) MEANS±SD
A (Control)	……	28.75±1.28	27.93±3.01	80.14±8.12
B	0.5 ml CCl4/Kg b.wt	117.22±9.71**b**	108.22±11.40 **b**	146.62±22.31 **b**
C	0.5 ml CCl4/Kgb.wt+ 250 mg extract/Kgb.wt	35.50±4.19**a**	34.75±5.06 **a**	84.75±9.11 **a**
D	0.5 ml CCl4/Kg b.wt+ 500 mg extract/Kgb.wt	3076±0.58**a**	31.00±0.96 **a**	82.50±8.18 **a**

a= values are not significantly different from control at P≤ 0.05
b= values are significantly different from control at P≤0.05

Table 2. Effect of ethanolic leaf extract of *M. oleifera* on renal and protein biomarkers of CCL4 induced hepatotoxic mice

Groups	Treatments	Renal markers		Protein marker	
		BUN (mgl/dl) Means±SD	Creatinine (mg/dl) Means±SD	ALB(µmol/l) Means±SD	TP(mg/dl) MEANS±SD
A (Control)	……..	14.75± 0.95	1.05 ± 0.12	4.66 ± 0.32	6.15± 0.84
B	0.5 ml CCl4/Kgb.wt	38.03± 5.85 **b**	3.07± 0.21 **b**	3.29±0.18 **b**	2.69± 0.96 **b**
C	0.5 ml CCl4/Kgb.wt+ 250 mg extract/Kgb.wt	20.5±3.31 **a**	2.72±0.35 **a**	3.67± 0.02 **a**	6.62±0.91 **a**
D	0.5 ml CCl4/Kgb.wt+ 250 mg extract/Kgb.wt	15.5±1.29 **a**	2.5±0.45 **a**	4.23 ±0.02 **a**	4.75±0.53**a**

a= values are not significantly different from control at P≤ 0.05
b= values are significantly different from control at P≤0.05

Table 3. Effect of ethanolic leaf extract of *M. oleifera* on serum enzyme activities of gentamicin induced nephrotoxicity in mice

Groups	Treatments	Enzyme activity		
		AST (U/l) Means±SD	ALT (U/l) Means±SD	ALP(U/l) Means±SD
A (Control)	……	28.75±1.28	27.93±3.01	80.14±8.12
E	20 mg gentamicin/kgb.wt	40.33±3.34**b**	50.74±7.8 **b**	116.04±4.49 **b**
F	20 mg gentamicin/kgb.wt+ 250 mg extract/Kg b.wt	29.52 ± 1.25**a**	35.01±5.19 **a**	110.52±1.31 **a**
G	20 mg gentamicin/kgb.wt+ 500 mg extract/Kg b.wt	22.74±1.91**a**	23.03 ±4.40 **a**	77.25±5.35**a**

a= values are not significantly different from control at P≤ 0.05
b= values are significantly different from control at P≤0.05

Table 4. Effect of ethanolic leaf extract of *M. oleifera* on renal and protein biomarkers of gentamicin induced nephrotoxicity in mice

Groups	Treatments	Renal markers		Protein marker	
		BUN(mgl/dl) MEANS±SD	Creatinine (mg/dl) MEANS±SD	ALB (µmol/l) MEANS±SD	TP (mg/dl) MEANS±SD
A (Control)	……	14.75± 0.95	1.05 ± 0.12	4.66 ± 0.32	6.15± 0.84
E	20 mg gentamicin/Kgb.wt	31.00 ± 0.81**b**	2.42 ± 0.25 **b**	2.52± 0.09 **b**	2.69± 0.96 **b**
F	20 mg gentamicin/Kgb.wt+250m gextract/Kg b.wt	11.75± 1.71**a**	2.45 ± 0.47 **a**	3.56 ± 0.09**a**	5.51± 0.93**a**
G	20 mg gentamicin/kgb.wt+500m gextract/Kg b.wt	12.25± 2.62**a**	2.15 ± 0.34**a**	3.53 ± 0.10**a**	6.51± 0.89 **a**

a= values are not significantly different from control at P≤ 0.05
b= values are significantly different from control at P≤0.05

Statistical data were presented in Table 1 which showed that gentamicin had a significant effect on serum level. The administration of gentamicin in mice induced toxicity as seen in the increased levels of AST (40.33±3.34) which was observed in group E as compared to group A (28.75±1.28) which is control group. However, significant reduction in the level of AST was observed in *M. oleifera*-treated Group E and G (P<0.05) when compared with other treatments. The leaf extract recorded no significant changes (p>0.05) changes in group E (29.52 ± 1.25) and G (22.74±1.91**)** when compared with control **(**28.75±1.28). Moreover, similar trend was observed for serum ALT and ALP levels, where significant elevations in serum ALT (50.74±7.8)

and serum ALP (116.04±4.49) levels was observed in Group E as compared to Group A. A significant ($p<0.05$) decreased in serum ALT (35.01±5.19, 23.03 ±4.40) and serum ALP (110.52 ±1.31, 77.25 ± 5.35) levels was observed in *Moringa*-treated group F and G.

The histological examination showed normal morphology of liver in control animals. Hepatocytes were arranged in trabecules running radiantly from the central vein. Control mice showed normal echotexture, with normal sinusoidal structure as compared to the liver of the mice treated with CCL4 (group B) which showed that the normal structural organization of the hepatic lobules was impaired and the characteristic cord-like arrangement of the normal liver cells was lost. The central and portal veins were congested. In case of CCl4 and moringa (250 mg) extract treated mice, results showed that most of these histopathological changes were diminished and the portal inflammation is mild while the limiting plates were intact. In the central vein, congestions are prominent. Congestions also present in hepatocytes but absence of fibrosis was observed. The treatment of moringa *(*500 mg) extract showed that the liver tissue restored most of its normal structure and was able to reduce the fibrosis, congestion, incidence of inflammatory cells, infiltration, centrilobular hepatocytes swelling, hepatocytes vacuolisation, fatty changes and hemorrhagic clots (see Fig. 1).

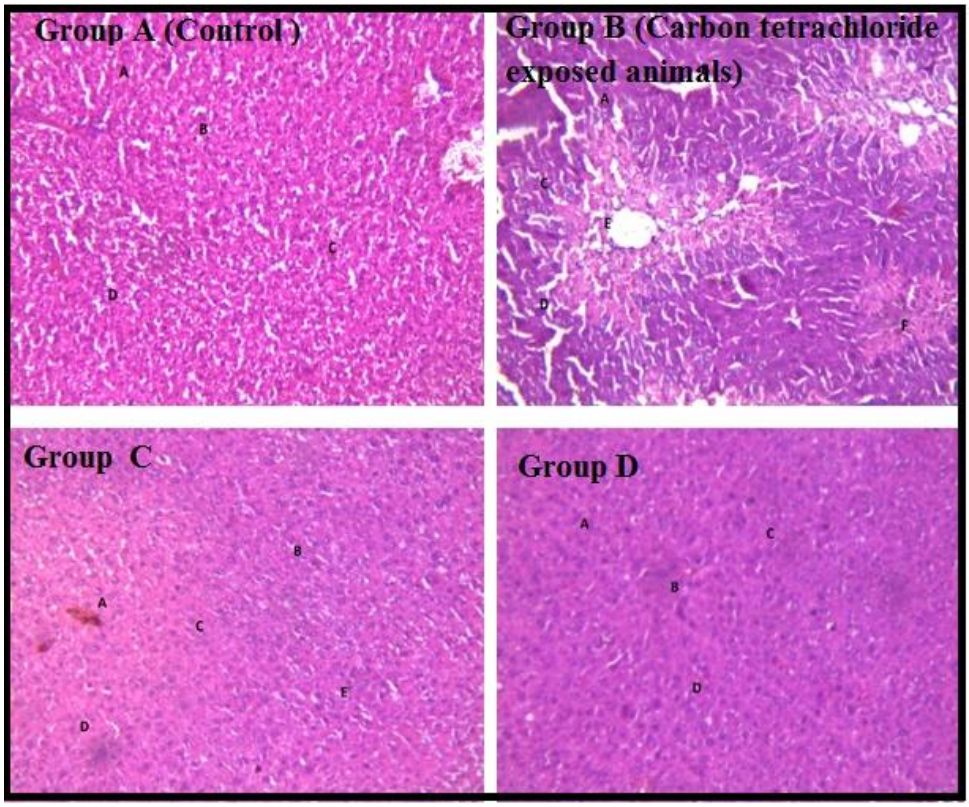

Fig. 1. Histological examination of the transverse sections of liver of the different Groups of animals using H and E stain (200x). Group A (control) showing the (A) normal sinusoid; (B) nucleus; (C) kupffer cells; and (D) normal hepatocyte. Group B is Carbon tetrachloride exposed showing the (A & E) ballooning degeneration with fatty degeneration or steatosis, (B & F) centrilobularhepatic congestion, (C) massive necrosis, and (D) inflammation. Group C (CCl4 + *Moringa oleifera* leaf extract 250 mg/ kg b.wt) (A) centrilobular hepatic congestion (B) kuppfer cells (C) hepatocyte nuclear diameter increase, (D) less ballooning degeneration, mild fatty change and less increase in inflammatory cells in portal tract, and (E) lower diameter of hepatocytes. Group D (CCl4 + *Moringa oleifera* leaf extract 500 mg/ kg b.wt) (A & B) regeneration of hepatocytes, (C) mild to moderate improvement of central vein with mild fatty change, and (D) less vacuole.

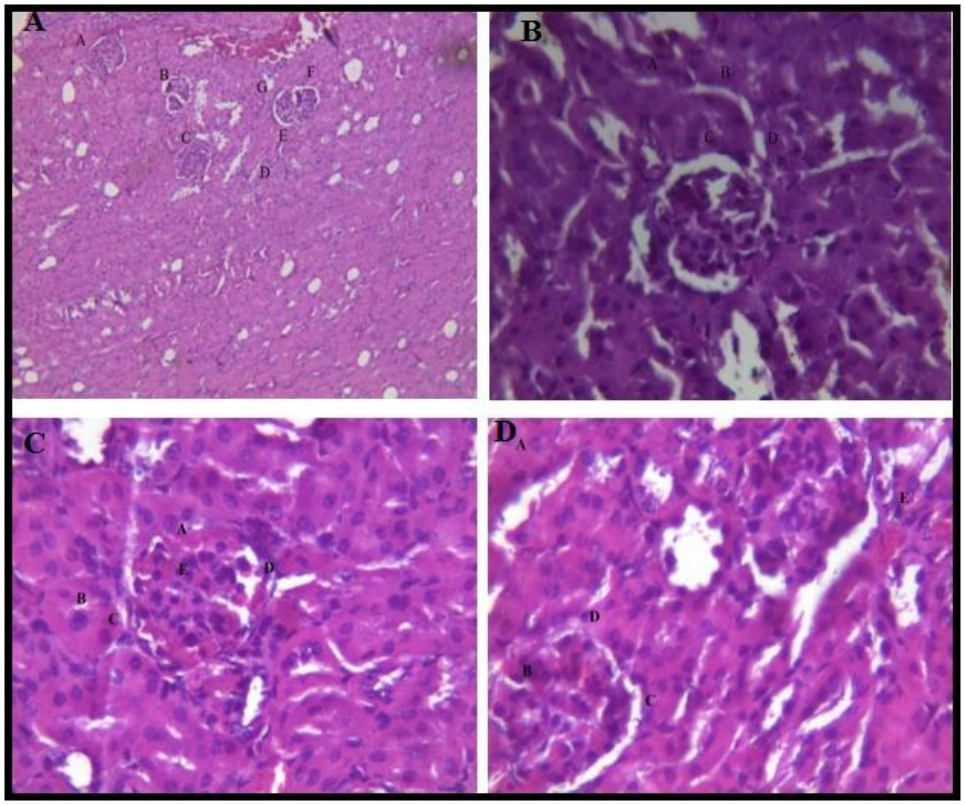

Fig. 2. Transverese sections of kidney from different groups using H and E stain (200x). Group A (control) showing the (A) glomerulus, (B) visceral layer, (C) parietal layer, (D) proximal convoluted tubule, (E) visceral layer, (F) parietal layer, (G) glomerulus lined with cuboidal epithelium and intact basement membrane (BM). Group B (Gentamicin exposed animals) showing the (A) damaged and dilated tubule with desquamating epithelium having cytoplasmic vacuolations, (B & C) karyolitic nuclei, and (D) intraluminal cellular debris. Group C (Gentamicin+ *Moringa oleifera* leaf extract 250 (mg/ kg b.wt) showing the (A) cortical tubules containing luminal cellular debris, (B) dropping out of cell, (C) lined epithelial cells with brush borders, (D) red blood cells, and (E) glomerulus. Group D (Gentamicin+ *Moringa oleifera* leaf extract 500 (mg/ kg b.wt) showing the (A) lined cuboidal epithelium and intact basement membrane, (B) glomerulus, (C) visceral layer (D) parietal layer, and (E) proximal convoluted tubule

Transverse section of kidney of the control mice (Group A) as seen in Fig. 2 showed normal structure of both the renal corpuscles and tubules. Control mice showed normal rounded glomeruli and did not show any signs of damage. Renal tubules are lined with typical thick cubic epithelium. In case of Group B (Gentamicin exposed animals), kidney parenchyma showed decreased number of glomeruli with karryohexis and necrosis of mesengial endothelial cells. The tubules show flattening with activation and edema. The pattern which emerges in the gentamycin exposed mice includes the dilation of tubules, sloughing of epithelium which implied an advanced disintegration of tubules. There were also casts (remains of dead tubules) were also seen. Glomeruli showed shrinkage and widened urinary space of the Bowman's capsule which further resulted to complete disintegration.

The renal parenchyma of mice treated with Gentamicin+*Moringa oleifera* leaf extract 250 mg/ kg b.wt showed little change with decreased tubular activation and some proliferation of glomeruli. Most of the cells of the convoluted tubules were mildly swollen. The tubules had a relatively regular distinct lumen. For higher concentration of moringa (500 mg), the renal parenchyma showed proliferation of glomeruli but

still lower than the control. The mesengial cells showed normal features with no karryohexis while the tubules showed decreased flattening and activation.

4. DISCUSSION

It has already been proven that chemicals including CCL4 have health hazardous effects such as inducing fatty changes, degeneration, vacuolation of cytoplasm, distended hepatocytes and compression of sinusoids [21,22]. In present study, the gentamicin administration in albino mice increased the level of serum creatinine and blood urea nitrogen which caused renal damage and dysfunction. *Moringa oleifera* enhanced the recovery from renal damage by repairing the tubules and glomeruli [23]. Results showed that CCL4 caused an elevation in serum contents of AST, ALT and ALP which confirmed the liver injury especially the increased level of ALT activity (Ashok and Pari, 2003: Ragesh and Latha, 2004). *Moringa oleifera* significantly contributed to the activation of total protein contents, blood urea nitrogen (BUN) and creatinine production as well as activation of three marker enzymes ALT, AST and ALP. These results conformed to the results of the previous studies [24].

CCL4 induced hepatotoxicity while gentamicin induced nephrotoxicity. *Moringa oleifera* leaves at low (250 mg) and high dosage (500 mg) showed significant results in the repair of both liver and kidney damage. The extracts reduced the oxidative damage in the liver as well as the histopathological changes. The kidney of the mice subjected to *Moringa oleifera* ethanolic leaf extract showed normal tubules which confirmed that moringa has a protective effect against gentamicin toxicity in the kidney. These results establish the potential of moringa to manage both hepatotoxicity and nephrotoxicity in humans.

5. CONCLUSION

Moringa oleifera leaves at low and high dose (250 and 500 mg /kg b.wt) is proven to activate total protein contents, blood urea nitrogen (BUN), creatinine production and three marker enzymes ALT, AST and ALP. *Moringa oleifera* ethanolic leaf extract has significant effect to slow down the oxidative damage while preventing the histopathological changes in the liver. It also repairs kidney damage by protecting the structure of kidney tubules.

ETHICAL APPROVAL

The authors sought the ethical and IACUC approval as a protocol before the research started. The main animal welfare regulations in Islam include considering to the natural needs of the animals, such as water, food and a suitable place to live, their living and mental condition, good health and avoidance of causing them pain, distress, or harm and unnecessary termination of their lives. These were considered carefully by the authors while working with animals in this study.

COMPETING INTERESTS

Authors declared that there was no competing interest among them.

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