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Preventive and curative effect of methanolic extract of *Gardenia gummifera* Linn. f. on thioacetamide induced oxidative stress in rats

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ABSTRACT

Objective: To evaluate the antioxidant and antihepatotoxic effect of methanolic extract of *Gardenia gummifera* Linn. f. root (MEGG) on thioacetamide (TAA) induced oxidative stress in male Wistar rats. **Methods:** In the preventive study, rats were administered with 125 and 250 mg/kg of MEGG for 9 days prior to TAA administration (100 mg/kg s.c.). In post-treatment groups, rats were treated with MEGG at doses of 125 and 250 mg/kg, 2, 24 and 48 h after TAA intoxication. Silymarin was used as a standard drug control (100 mg/kg). Hepatotoxicity was assessed by quantifying the serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH). The antioxidant potential of MEGG was evaluated by the estimation of catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST), reduced glutathione (GSH) and lipid peroxidation [thiobarbituric acid reactive substances (TBARS)] in hepatic and renal tissues. Histopathological changes were also evaluated. **Results:** MEGG significantly ($P \leq 0.05$) prevented the elevation of serum AST, ALT, ALP, LDH and tissue malondialdehyde levels in both experimental groups, when compared to the TAA alone treated groups. The rats receiving TAA plus MEGG exhibited significant ($P \leq 0.05$) increases in hepatic and renal antioxidant activities including GSH, GST, GR, GPx and CAT levels. Quantification of histopathological changes also supported the dose dependent protective effects of MEGG. **Conclusions:** These observations suggest that MEGG has dose dependent hepatoprotective and antioxidant effect against TAA induced oxidative stress.

1. Introduction

Reactive oxygen species (ROS) are generated ubiquitously in the human body from either endogenous or exogenous sources. Excessive generation of ROS causes oxidative stress, a deleterious process leading to the oxidation of biomolecules such as proteins, lipids, carbohydrates and DNA. Oxidative stress is known to play a major role in the development of several chronic ailments such as cardiovascular diseases, different types of cancer, arthritis, diabetes, autoimmune and neurodegenerative disorders and aging[1]. Antioxidants play an important role in inhibiting and scavenging radicals, thus providing protection against infections and degenerative diseases. Antioxidants

can either directly scavenge or prevent generation of ROS. Recently, interest in finding naturally occurring antioxidants has increased considerably to replace synthetic antioxidants. The two most commonly used synthetic antioxidants, *i.e.*, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have begun to be restricted because of their toxicity and DNA damage induction. Therefore, natural antioxidants from plant extracts have attracted increasing interests due to their safety[2].

Gardenia gummifera (*G. gummifera*) Linn. f. belonging to the family Rubiaceae is a large medicinal shrub with resinous buds. The resin is acrid, bitter, thermogenic, cardiogenic, carminative, antispasmodic, stimulant, diaphoretic, antihelminthic, antiseptic and expectorant. It is traditionally used in conditions of cardiac debility, obesity, lipolytic disorders, bronchitis, neuropathy and splenomegaly and is given to children in nervous disorders and diarrhoea due to dentition. The gum yielded flavones, including gardenin, de-Me-tangeretin and nevadensin; wogonins, isoscutellarein, apigenin and de-MeO-sudachitin[3-5]. Oleoic aldehyde, sitosterol, D-mannitol,

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erythrodiol and 19-hydroxyerythrodiol were isolated and characterized from *G. gummifera* stem bark[6]. In the present investigation, the antioxidant and antihepatotoxic activity of methanolic extract of *G. gummifera* Linn. f. (MEGG) was conducted in acute liver injury model against thioacetamide (TAA) induced oxidative stress.

2. Materials and methods

2.1. Chemicals

TAA was purchased from Loba Cheme, Mumbai, India. Assay kits for serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were purchased from Agappe Diagnostic, India. All other chemicals were of analytical grade.

2.2. Collection of plant material and preparation of plant extracts

G. gummifera roots were collected from its natural habitat (Kottayam, Kerala, India) and authenticated. A voucher specimen (SBSBRL05) was maintained in the institute. Roots were cleaned, chopped, shade-dried and powdered. Dried powder (50 g) was soxhlet extracted with 400 mL of methanol for 48 h. The extracts were concentrated under reduced pressure using a rotary evaporator and were kept under refrigeration. The yield of methanolic extract was 10.3% (w/w). The concentrate was suspended in 5% Tween 80 for *in vivo* studies.

2.3. Animals and diets

Male Wistar rats weighing (150–160 g) were used in this study. The animals were housed in polypropylene cages and given standard rat chow (Sai Feeds, Bangalore, India) and drinking water *ad libitum*. The animals were maintained at a controlled condition of temperature of (26–28 °C) with a 12 h light: 12 h dark cycle. Animal studies were followed according to Institute Animal Ethics Committee (IAEC) regulations approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Reg. No. B 2442009/3) and conducted humanely.

2.4. TAA induced oxidative stress

TAA suspended in normal saline was administered (100 mg/kg bw) subcutaneously to induce the oxidative stress in rats[7]. Silymarin at an oral dose of 100 mg/kg bw was used as standard control in the experiment[8]. Different doses (125 and 250 mg/kg) of MEGG suspended in 5% Tween 80 were also prepared for oral administration to the animals.

The animals were divided into pre-treatment and post-treatment groups. In each experiment, five groups of animals were included. Group I was vehicle control. Group II was TAA control. Groups III–V received silymarin at an oral dose of 100 mg/kg and MEGG at oral doses of 125 and 250 mg/kg

bw, respectively. All the groups except group I received a single dose of TAA (100 mg/kg s.c.). Group I animals treated as vehicle control received 5% Tween 80 and normal saline instead of drug and TAA, respectively.

2.4.1. Pre-treatment evaluation

In pre-treatment animals[9], groups II–V received TAA on the 9th day of the experiment. Groups III–V received silymarin and MEGG for 9 days before TAA challenge. Animals were sacrificed 24 h after TAA administration.

2.4.2. Post-treatment evaluation

In post-treatment study[10], groups II–V received a single dose of TAA on the first day of the experiment. Groups III–V received silymarin and MEGG 2, 24 and 48 h after TAA challenge. Animals were sacrificed 72 h after TAA administration. Blood was collected from the neck blood vessels under mild ether anesthesia and kept for 30 min at 4 °C. Serum was separated by centrifugation at 2500 rpm at 4 °C for 15 min. Dissected livers and kidneys were washed with normal saline and cut into separate portions for antioxidant estimation and for histopathological examination.

2.5. Serum enzyme analysis

Hepatotoxicity was assessed by quantifying the serum levels of AST (EC 2.6.1.1), ALT (EC 2.6.1.2), ALP (EC 3.1.3.1) and LDH (EC 1.1.1.27) by kinetic method using the kit of Agappe Diagnostic Ltd., India. Activities of these serum enzymes were measured by using semi autoanalyzer (RMS, India).

2.6. Tissue analysis

Liver and kidney were excised, washed thoroughly in ice-cold saline to remove the blood. Ten percent of homogenate was prepared in 0.1 M Tris HCl buffer (pH–7.4). The homogenate was centrifuged at 3000 rpm for 20 min at 4 °C and the supernatant was used for the estimation of catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST), reduced glutathione (GSH), lipid peroxidation product [thiobarbituric acid reactive substances (TBARS)] and total protein.

Tissue CAT (EC 1.11.1.6) activity was determined from the rate of decomposition of H₂O₂[11]. GPx (EC 1.11.1.9) activity was determined by measuring the decrease in GSH content after incubating the sample in the presence of H₂O₂ and NaN₃[12]. GR (EC 1.6.4.2) activity was assayed at 37 °C and 340 nm by following the oxidation of NADPH by GSSG[13]. GST (EC 2.5.1.18) activity was determined from the rate of increase in conjugate formation between GSH and CDNB[14]. GSH was determined based on the formation of a yellow colored complex with DTNB[15]. The level of lipid peroxidation was measured as malondialdehyde (MDA), a TBARS, using 1'1'3'3' tetramethoxypropane as standard[16]. Protein content in the tissue was determined[17] using bovine serum albumin (BSA) as the standard.

2.7. Histopathological studies

Small pieces of liver fixed in 10% buffered formalin were processed for embedding in paraffin. Sections of (5–6 μ m) were cut and stained with hematoxylin and eosin and examined for histopathological changes under the microscope (Motic AE 21, Germany). The microphotographs were taken using Moticam 1000 camera at original magnification of 100 \times .

Liver sections were graded numerically to assess the degree of histological features in acute hepatic injury. Centrilobular necrosis is the necrosis around the central vein characterized by prominent ballooning, swollen granular cytoplasm with fading nuclei. Bridging hepatic necrosis is a form of confluent necrosis of liver cells linking central veins to portal tracts or portal tracts to one another^[18]. A combined score of centrilobular necrosis, bridging hepatic necrosis and lymphocyte infiltration was given a maximum value of 6 and descriptive modifiers such as mild, moderate, and severe were applied to activity and staging. The parameters were graded from score 0 to 6, with 0 indicating no abnormality, 1 to 2 indicating mild injury, 3 to 4 indicating moderate injury and 5 to 6 representing severe liver injury.

2.8. Statistical analysis

Results were expressed as mean \pm SD and all statistical comparisons were made by means of one-way ANOVA test followed by Tukey's *post hoc* analysis and *P*-values less than or equal to 0.05 were considered significant.

3. Results

3.1. Serum analysis

3.1.1. Effects of MEGG on the changes in serum enzyme levels of rats pre-treated with TAA

The serum levels of AST, ALT, ALP and LDH in group II were significantly ($P\leq 0.05$) elevated by the administration of

a single dose of TAA, when compared to normal control. The treatment of MEGG at doses of 125 and 250 mg/kg showed a significant decrease ($P\leq 0.05$) of AST, ALT, ALP and LDH. Standard control drug, silymarin at a dose of 100 mg/kg also prevented the elevation of serum enzymes (Figure 1). Treatment with 250 mg/kg methanolic extract and silymarin exhibited a protection of 75.6% and 72.4% in AST levels, 68.1% and 62.6% in ALT levels, 72.7% and 71.6% in ALP levels and 55.1% and 52.2% in LDH levels, respectively. The preventive effect of the extract in decreasing the elevated levels of serum enzymes was in a dose dependent manner.

3.1.2. Effects of MEGG on the changes in serum enzyme levels of rats post-treated with TAA

There was a significant ($P\leq 0.05$) rise in the serum levels of AST, ALT, ALP and LDH after administration of TAA in post-treated animals. In contrast, treatment with MEGG (125 and 250 mg/kg) exhibited an ability to counteract the TAA induced hepatotoxicity by decreasing the serum enzymes levels ($P\leq 0.05$) when compared to TAA control. MEGG at a dose of 205 mg/kg showed a protection of 92.1%, 77.9%, 82.7%, and 59.5% for AST, ALT, ALP and LDH, respectively. Silymarin also showed a remarkable protection of 82.1%, 64.9%, 81.8% and 53.2% for AST, ALT, ALP and LDH, respectively towards TAA intoxication ($P\leq 0.05$) (Figure 1).

3.2. Tissue analysis

3.2.1. Estimation of GSH

In the pre-treatment groups, rats administered with TAA alone were found significantly ($P\leq 0.05$) lower level of GSH. Treatment with MEGG exhibited significant increase ($P\leq 0.05$) in both hepatic (Table 1) and renal (Table 2) glutathione levels. In liver and kidney, 250 mg/kg of MEGG showed a protection of 82.4% and 77.3%, respectively. Silymarin-treated rats also prevented the lowering of GSH and the percentage of protection was 73.6% and 67.9%, respectively for liver and kidney.

Table 1

Preventive effects of MEGG against TAA induced changes in the liver antioxidant status (mean \pm SD) ($n=6$).

Treatment groups	GSH (A)	GST (B)	GR (C)	GPx (D)	CAT (E)	MDA (F)
Normal control	18.5 \pm 0.4	47.9 \pm 0.5	19.1 \pm 0.5	9.9 \pm 0.5	24.5 \pm 0.4	1.7 \pm 0.2
TAA (100 mg/kg s.c.)	9.4 \pm 0.6*	17.8 \pm 0.6*	8.1 \pm 0.6*	3.1 \pm 0.4*	9.5 \pm 0.3*	9.2 \pm 0.5*
Silymarin (100 mg/kg) + TAA	16.1 \pm 0.4**	42.1 \pm 0.5**	16.3 \pm 0.4**	8.1 \pm 0.4**	20.8 \pm 0.5**	3.8 \pm 0.3**
MEGG (125 mg/kg) + TAA	13.6 \pm 0.3**	39.6 \pm 0.6**	13.8 \pm 0.5**	7.6 \pm 0.5**	19.9 \pm 0.4**	4.2 \pm 0.3**
MEGG (250 mg/kg) + TAA	16.9 \pm 0.3**	45.2 \pm 0.5**	16.8 \pm 0.4**	8.5 \pm 0.5**	22.2 \pm 0.5**	3.1 \pm 0.2**

A: nmol/mg protein; B: μ mol CDNB-GSH conjugate formed/min/mg protein; C: nmol of GSSG utilized/min/mg protein; D: nmol of GSH oxidized/min/mg protein; E: U/mg protein; F: nmol/g tissue; *: $P\leq 0.05$ vs normal control; **: $P\leq 0.05$ vs TAA control.

Table 2

Preventive effects of MEGG against TAA induced changes in the kidney antioxidant status (mean \pm SD) ($n=6$).

Treatment groups	GSH (A)	GST (B)	GR (C)	GPx (D)	CAT (E)	MDA (F)
Normal control	12.8 \pm 0.4	30.9 \pm 0.4	15.8 \pm 0.5	8.5 \pm 0.5	10.6 \pm 0.4	1.2 \pm 0.2
TAA (100 mg/kg s.c.)	7.5 \pm 0.3*	9.4 \pm 0.6*	5.7 \pm 0.6*	1.4 \pm 0.4*	4.3 \pm 0.5*	5.3 \pm 0.3*
Silymarin (100 mg/kg) + TAA	11.1 \pm 0.4**	26.2 \pm 0.5**	12.3 \pm 0.4**	5.9 \pm 0.5**	8.7 \pm 0.4**	2.7 \pm 0.3**
MEGG (125 mg/kg) + TAA	10.8 \pm 0.5**	25.1 \pm 0.6**	11.2 \pm 0.5**	5.2 \pm 0.4**	8.4 \pm 0.4**	3.1 \pm 0.3**
MEGG (250 mg/kg) + TAA	11.6 \pm 0.4**	27.8 \pm 0.5**	13.1 \pm 0.5**	6.3 \pm 0.4**	9.2 \pm 0.5**	2.2 \pm 0.2**

A: nmol/mg protein; B: μ mol CDNB-GSH conjugate formed/min/mg protein; C: nmol of GSSG utilized/min/mg protein; D: nmol of GSH oxidized/min/mg protein; E: U/mg protein; F: nmol/g tissue; *: $P\leq 0.05$ vs normal control; **: $P\leq 0.05$ vs TAA control.

Table 3

Curative effects of MEGG against TAA induced changes in the liver antioxidant status (mean±SD) (n=6).

Treatment groups	GSH (A)	GST (B)	GR (C)	GPx (D)	CAT (E)	MDA (F)
Normal control	19.9±0.3	47.8±0.6	22.1±0.5	11.6±0.6	25.8±0.4	1.1±0.2
TAA (100 mg/kg s.c.)	7.1±0.5*	19.6±0.4*	7.4±0.3*	3.5±0.4*	8.5±0.4*	8.5±0.4*
Silymarin (100 mg/kg) + TAA	17.6±0.3**	44.2±0.4**	18.7±0.4**	10.2±0.6**	22.4±0.3**	2.6±0.3**
MEGG (125 mg/kg) + TAA	14.8±0.4**	42.5±0.5**	16.1±0.4**	9.7±0.4**	20.6±0.3**	3.2±0.5**
MEGG (250 mg/kg) + TAA	18.1±0.4**	45.1±0.5**	20.5±0.3**	10.6±0.5**	23.2±0.4**	2.2±0.3**

A: nmol/mg protein; B: μ mol CDNB–GSH conjugate formed/min/mg protein; C: nmol of GSSG utilized/min/mg protein; D: nmol of GSH oxidized/min/mg protein; E: U/mg protein; F: nmol/g tissue; *: P<0.05 vs normal control; **: P<0.05 vs TAA control.

Table 4

Curative effects of MEGG against TAA induced changes in the kidney antioxidant status (mean±SD) (n=6).

Treatment Groups	GSH (A)	GST (B)	GR (C)	GPx (D)	CAT (E)	MDA (F)
Normal control	14.6±0.3	32.7±0.6	16.5±0.6	10.8±0.8	15.4±0.5	0.9±0.2
TAA (100 mg/kg s.c.)	5.8±0.4*	11.2±0.4*	6.4±0.4*	2.8±0.4*	4.2±0.3*	6.2±0.5*
Silymarin (100 mg/kg) + TAA	12.6±0.4**	28.2±0.6**	13.6±0.5**	8.9±0.6**	12.6±0.3**	2.2±0.2**
MEGG (125 mg/kg) + TAA	10.8±0.5**	24.3±0.4**	11.8±0.4**	6.3±0.5**	9.8±0.3**	2.7±0.2**
MEGG (250 mg/kg) + TAA	12.8±0.5**	29.6±0.5**	14.2±0.6**	9.3±0.7**	13.4±0.4**	1.8±0.2**

A: nmol/mg protein; B: μ mol CDNB–GSH conjugate formed/min/mg protein; C: nmol of GSSG utilized/min/mg protein; D: nmol of GSH oxidized/min/mg protein; E: U/mg protein; F: nmol/g tissue; *: P<0.05 vs normal control; **: P<0.05 vs TAA control.

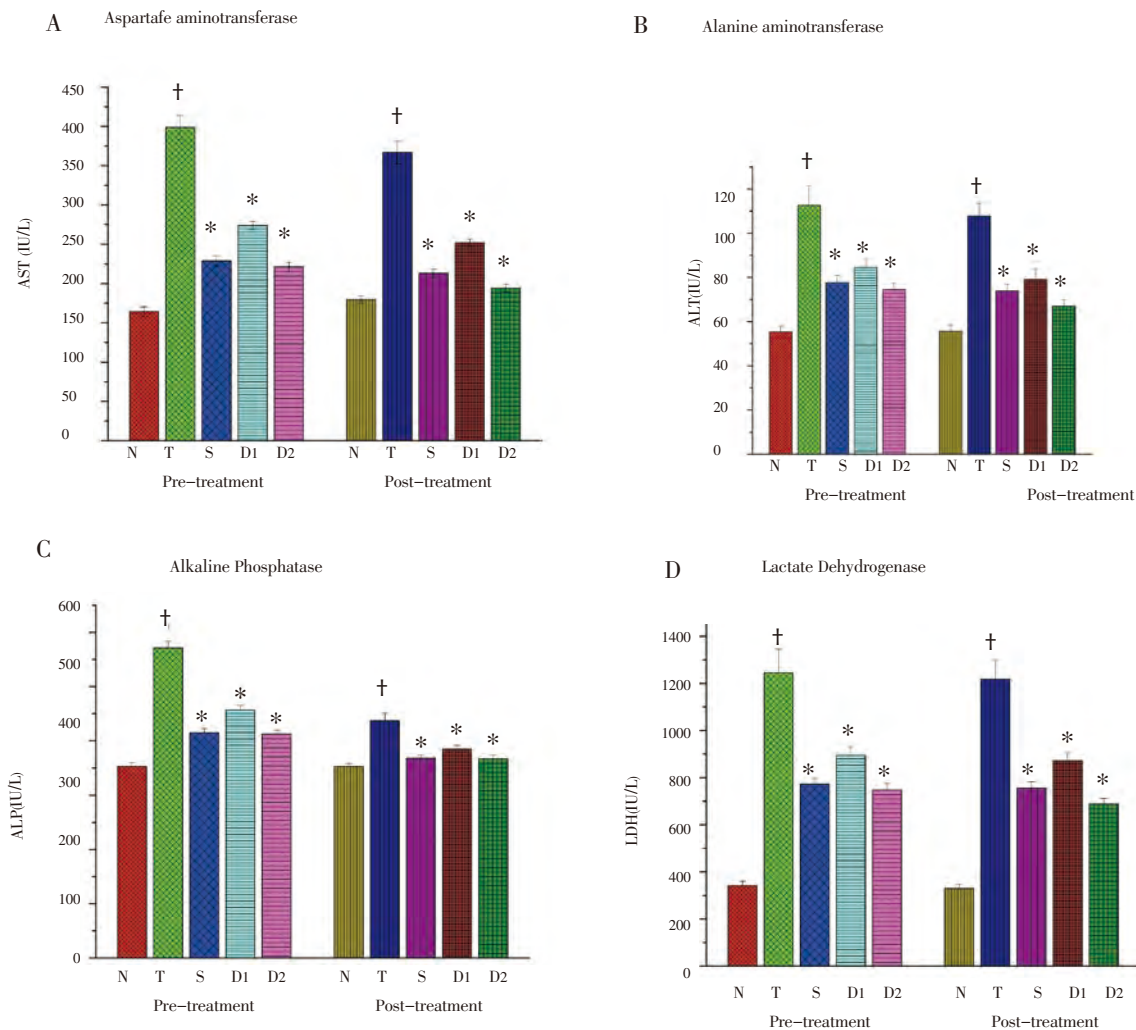


Figure 1. Effects of MEGG on changes in serum enzyme levels of rats treated with TAA.

A: Effects of MEGG on AST; B: Effects of MEGG on ALT; C: Effects of MEGG on ALP; D: Effects of MEGG on LDH; N: Normal control; T: TAA control; S: Silymarin; D1: MEGG at a dose of 125 mg/kg; D2: MEGG at a dose of 250 mg/kg; †: P<0.05 vs normal control; *: P<0.05 vs TAA control. Values are mean±SD, error bar indicating the standard deviation, (n=6).

In the post-treatment groups, rats treated with 125 and 250 mg/kg of MEGG significantly ($P \leq 0.05$) restored the decreased

glutathione levels in liver (Table 3) and kidney (Table 4). The results were comparable with silymarin. In hepatic tissue,

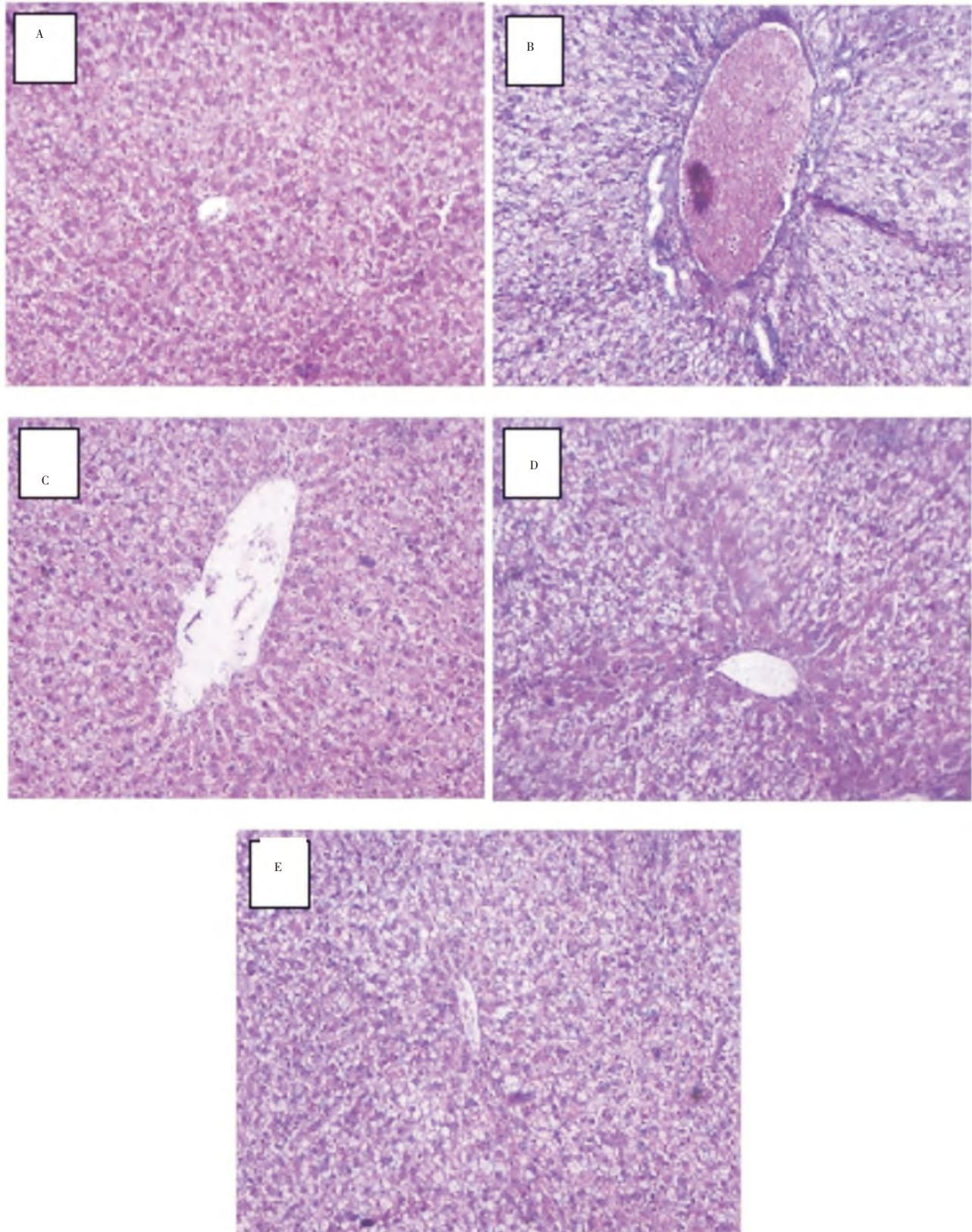


Figure 2. Histopathological changes occurred in pre-treatment groups after TAA intoxication and prevention by the treatment with MEGG (hematoxylin and eosin, 100 \times).

A: Normal control; B: TAA control (100 mg/kg s.c.); C: Silymarin (100 mg/kg) + TAA; D: MEGG (125 mg/kg) + TAA; E: MEGG (250 mg/kg) + TAA.

85.9% reversal in GSH level shown by 250 mg/kg of MEGG was comparable with 82.1% exhibited by 100 mg/kg of silymarin. In

renal tissue, 250 mg/kg of MEGG and 100 mg/kg of silymarin restored the GSH level by 79.5% and 77.2%, respectively.

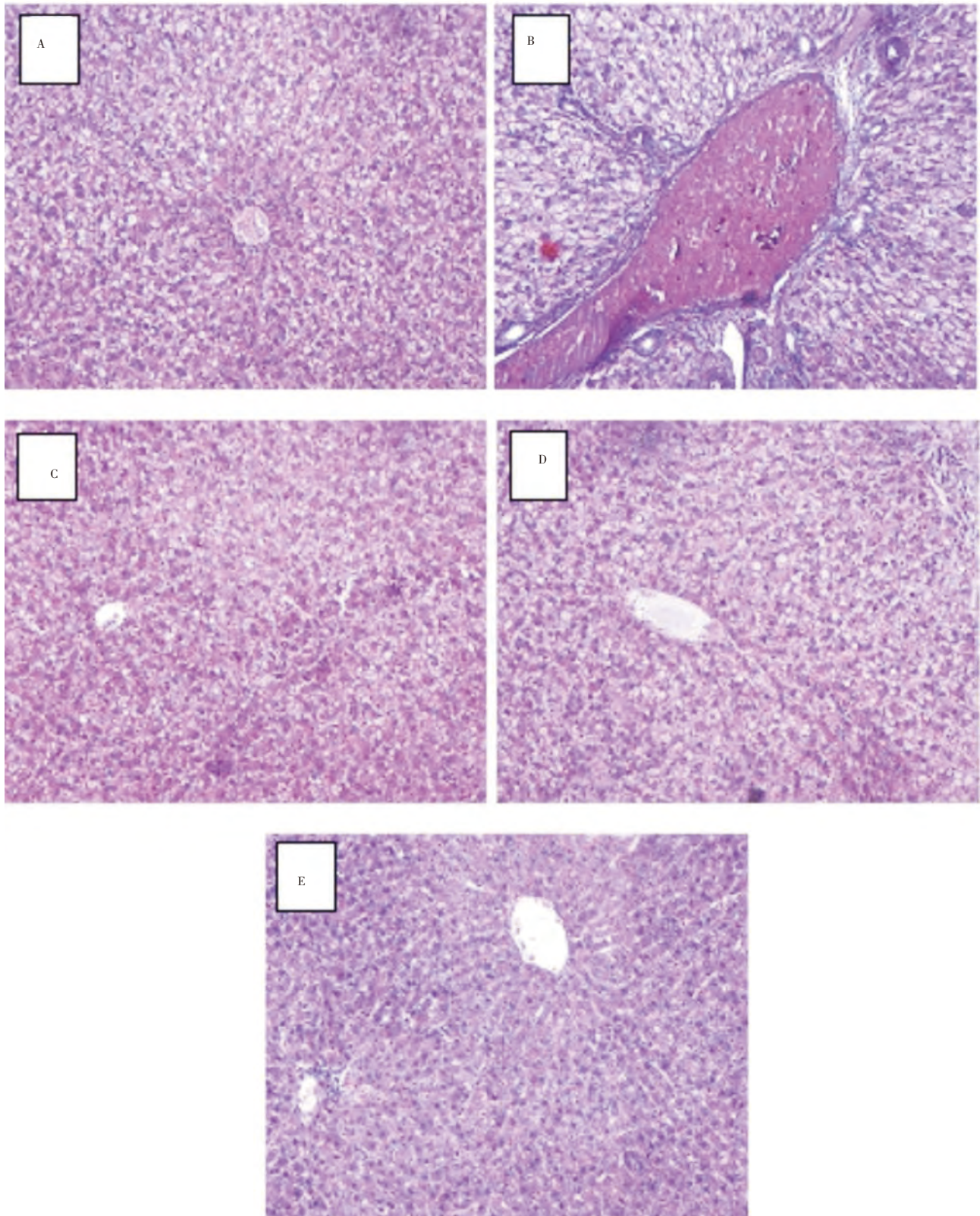


Figure 3. Histopathological changes occurred in post-treatment groups after TAA intoxication and recovery by the treatment with MEGG (hematoxylin and eosin, 100×).
 A: Normal control; B: TAA control (100 mg/kg s.c.); C: Silymarin (100 mg/kg) + TAA; D: MEGG (125 mg/kg) + TAA; E: MEGG (250 mg/kg) + TAA.

3.2.2. Estimation of GST

The GST activity of liver and kidney tissues was significantly ($P \leq 0.05$) reduced in TAA intoxicated rats of pre-treatment groups when compared to normal control. The MEGG dose dependently increased ($P \leq 0.05$) the activity of GST in both the hepatic and renal tissues (Table 1 and 2). Treatment with 250 mg/kg of MEGG exhibited significant increase *i.e.*, 91.1% and 85.5%, respectively in hepatic and renal GST levels. In addition, silymarin treated rats also prevented the TAA induced decrease in GST activity by 80.7% and 78.1% in hepatic and renal tissues, respectively.

Rats administered with TAA alone showed significant ($P \leq 0.05$) reduction in hepatic and renal GST level in post-treatment groups. Treatment with MEGG at doses of 125 and 250 mg/kg showed significant reversal ($P \leq 0.05$) of TAA induced toxicity. Silymarin (100 mg/kg) also markedly ($P \leq 0.05$) inhibited the TAA induced decrease in GST activity. Rats treated with 250 mg/kg of MEGG and 100 mg/kg of silymarin restored the decrease of GST levels by 90.4% and 87.2% in the liver and 85.5% and 79.1% in the kidney, respectively (Table 3 and 4).

3.2.3. Estimation of GR

GR activity was significantly decreased ($P \leq 0.05$) in TAA treated animals when compared to control in the pre-treatment groups. A significant increase ($P \leq 0.05$) in the level of GR was observed in MEGG (125 and 250 mg/kg) and silymarin (100 mg/kg) treated rats intoxicated with TAA. Both hepatic and renal tissues showed the same pattern of GR activity in all groups treated with MEGG and silymarin (Table 1 and 2). The percentages of protection in liver and kidney were 79.1% and 73.2%, respectively for 250 mg/kg of methanolic extract. Silymarin restored the GR activity by 74.5% in liver and 65.3% in kidney.

In the post-treatment groups, rats administered with TAA alone significantly ($P \leq 0.05$) reduced the activity of GR. Treatment with MEGG exhibited significant increase ($P \leq 0.05$) in both hepatic (Table 3) and renal (Table 4) GR activity. In liver and kidney, 250 mg/kg of MEGG restored the activity of GR by 89.1% and 77.2%, respectively. Silymarin-treated rats also prevented the lowering of GR activity and the percentages of restoration were 76.8% and 71.2%, respectively for liver and kidney.

3.2.4. Estimation of GPx

Activities of hepatic and renal GPx in pre-treatment groups were significantly ($P \leq 0.05$) lowered in TAA treated rats (Table 1 and 2). MEGG dose dependently prevented the lowering of GPx in both the organs when compared to TAA alone treated groups. In liver and kidney, 250 mg/kg of methanolic extract showed a protection of 79.4% and 69.0%, respectively. Silymarin-treated rats also prevented the lowering of GPx by 73.5% in hepatic and 63.3% in renal tissues.

In the post-treatment groups, rats treated with 125 and 250 mg/kg of MEGG significantly ($P \leq 0.05$) restored the decreased GPx activity in liver (Table 3) and kidney (Table 4). In hepatic tissue, 87.6% reversal in GPx activity shown by 250 mg/kg of MEGG was comparable to 82.7% exhibited by 100 mg/kg of

silymarin. In renal tissue, 250 mg/kg of MEGG and 100 mg/kg of silymarin reinstated the GPx activity by 81.2% and 76.2%, respectively.

3.2.5. Estimation of CAT

The CAT activity in liver and kidney showed a significant ($P \leq 0.05$) reduction in TAA intoxicated rats of pre-treatment groups when compared to normal control. The MEGG dose dependently increased the activity of CAT in both hepatic and renal tissues (Table 1 and 2). Treatment with 250 mg/kg of MEGG exhibited significant increase *i.e.*, 84.9% and 82.1%, respectively in liver and kidney. In addition, silymarin treated rats also prevented ($P \leq 0.05$) the TAA induced decrease in CAT activity by 80.3% and 75.1% in hepatic and renal tissues, respectively.

Animals injected with TAA alone showed significant ($P \leq 0.05$) reduction in hepatic and renal CAT activity in post-treatment groups. Treatment with MEGG at doses of 125 and 250 mg/kg showed significant reversal ($P \leq 0.05$) of TAA induced hepatotoxicity. Silymarin (100 mg/kg) also markedly ($P \leq 0.05$) restored the TAA induced decrease in CAT activity. Rats treated with 250 mg/kg methanolic extract and 100 mg/kg silymarin restored the decrease of CAT levels by 84.9% and 80.3% in the liver and 82.1% and 77.6% in the kidney, respectively (Table 3 and 4).

3.2.6. Estimation of MDA

In pre-treatment animals, a significant increase ($P \leq 0.05$) in tissue MDA level was observed in TAA alone treated rats. However, TAA induced elevation of MDA concentration was lowered ($P \leq 0.05$) by 81.3% and 75.6% in hepatic and renal tissues of rats treated with MEGG at a dose of 250 mg/kg. Silymarin also showed a protection ($P \leq 0.05$) of 72.1% in liver and 63.4% in kidney (Table 1 and 2).

In post-treatment animals (Table 3 and 4), a significant increase ($P \leq 0.05$) in tissue MDA level was shown in TAA alone treated animals when compared to normal control. MEGG and silymarin significantly ($P \leq 0.05$) reversed the elevation of hepatic and renal MDA formation. MEGG at a dose of 250 mg/kg reinstated the MDA formation by 85.1% in hepatic tissue and 83.7% in renal tissue. Silymarin exhibited 79.7% and 76.7% inhibition in MDA formation in liver and kidney, respectively.

3.3. Histopathological analysis

In pre-treatment groups, rats treated with TAA, the normal architecture of liver (Figure 2B) was completely lost with the appearance of centrilobular necrosis, bridging hepatic necrosis and lymphocyte infiltration with a score of (5.2 ± 0.5) (mean \pm SD, $n=3$). The animals administered with silymarin (100 mg/kg) and MEGG and at doses of 125 and 250 mg/kg showed a significant ($P \leq 0.05$) protection from TAA induced liver damage as evident from hepatic architectural pattern with mild to moderate hepatitis with scores of (2.4 ± 0.5) , (2.8 ± 1.0) , and (2.0 ± 0.5) (mean \pm SD, $n=3$, $P \leq 0.05$), respectively (Figure 2C–2E).

In the post-treatment group, TAA intoxicated rats showed a maximum score of (5.0 ± 1.0) (mean \pm SD, $n=3$) (Figure 3B).

Rats treated with silymarin and MEGG (125 and 250 mg/kg) after the establishment of toxic injury showed recovery from centrilobular necrosis, bridging necrosis and lymphocyte infiltration with scores (1.8 ± 0.6), (2.2 ± 0.5) and (1.1 ± 0.5) (mean \pm SD, $n=3$, $P \leq 0.05$), respectively (Figure 3C–3E).

4. Discussion

TAA is a potent hepatotoxic agent[19]. It is also reported that the chronic TAA exposure produced cirrhosis in rats. The cytochrome-P450 system is known to metabolise TAA in rat liver. The mechanism of TAA toxicity is due to the formation of TAA-S-oxide, which is responsible for the change in cell permeability, increased intracellular concentration of Ca^{++} , increase in nuclear volume and enlargement of nucleoli, and also inhibits the mitochondrial activity which leads to cell death[20].

In the present study, administration of a single dose of TAA significantly elevated the serum transaminases, ALP and LDH activities when compared to the normal rats. There was a significant ($P \leq 0.05$) restoration of these enzyme levels on administration of the MEGG in a dose dependent manner and also by silymarin at a dose of 100 mg/kg. The increase in the activities of AST and ALT in plasma of rats treated with TAA is mainly due to the leakage of these enzymes from the liver cytosol into the blood stream[21]. Serum levels of transaminase return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes[22,23]. The increase in the activities of ALP and LDH in plasma might be due to the increased permeability of plasma membrane or cellular necrosis and this showed the stress condition of the TAA treated animals[24]. Marked decrease in serum transaminases, ALP and LDH levels demonstrates the preventive and curative effect of MEGG in TAA intoxication.

Oxidative stress is the state of imbalance between the level of antioxidant defense system and production of the oxygen derived species[25]. Antioxidants have been linked with the prevention of ROS[26]. The intracellular antioxidant system comprises different free radical scavenging antioxidant enzymes along with some non-enzyme antioxidants like GSH and other thiols. CAT, GST, GPx, and GR constitute the first line of cellular antioxidant defense enzymes. Treatment with MEGG and silymarin significantly ($P \leq 0.05$) enhanced the hepatic and renal antioxidant activity including the GSH level when compared to the TAA alone treated animals in both pre and post treatment groups. Glutathione is the major endogenous antioxidant, which forms an important substrate for other enzymes which are involved in the free radical scavenging[27,28] and detoxifies toxic metabolites of drugs, regulates gene expression, apoptosis and transmembrane transport of organic solutes and it is essential to maintain the reduced status of the cell/tissue. Pretreatment with MEGG prior to TAA intoxication significantly ($P \leq 0.05$) enhanced the GST activity, a phase II enzyme. This was attributed to the decreased bioactivation of TAA caused by the MEGG pre-treatment. In post-treatment rats also the GST level was significantly ($P \leq 0.05$) lowered in TAA

treated animals and upward reversal was observed after treatment with MEGG and silymarin. GST offers protection against lipid peroxidation by promoting the conjugation of toxic electrophiles with GSH[29]. GR is also essential for the maintenance of GSH levels *in vivo*[30]. The significantly ($P \leq 0.05$) elevated level of GR activity in the hepatic and renal tissues of pre and post-treatment groups shows the role of MEGG to maintain the GSH level in these tissues. GPx catalyzes the GSH dependant reduction of H_2O_2 and other peroxides and protects the organism from oxidative damage[31]. The significant ($P \leq 0.05$) restoration of GPx activity after pre and post-treatment with MEGG and silymarin is also due to the antioxidant activity by detoxifying the endogenous metabolic peroxides generated after TAA injury in hepatic and renal tissues.

CAT is responsible for breakdown of H_2O_2 , an important ROS, formed during the reaction catalyzed by SOD[32]. Reduced activity of CAT after exposure to TAA in the present finding could be correlated to increased generation of H_2O_2 . Presumably, a decrease in CAT activity could be attributed to cross-linking and inaction of the enzyme protein in the lipid peroxides. The pre-treatment and post-treatment of MEGG significantly ($P \leq 0.05$) aided to maintain the CAT activity near to normal level in both hepatic and renal tissues. This evidently shows the antioxidant property of the extract against oxygen free radicals. The concentration of MDA in tissues of TAA alone exposed group was significantly ($P \leq 0.05$) differed from that of normal control. MDA is a major oxidation product of peroxidized polyunsaturated fatty acids and increased MDA content is an important indicator of lipid peroxidation[33]. Treatment of rats with the MEGG protected the liver and kidney from increasing MDA formation in pre and post-treatment groups. This demonstrates the antilipid peroxidative effect of the extract. The increased MDA content might have resulted from an increase of ROS as a result of stress condition in the rats with TAA intoxication. Histopathological studies also provide supportive evidence for biochemical analysis. Therapy of MEGG significantly improved cellular morphology in a dose dependent manner. The improvement of histological scores proved the efficacy of the extract as an antihepatotoxic agent.

The result of serum biochemical parameter, level of hepatic lipid peroxides, tissue antioxidants and histopathological studies in the pre-treatment and post-treatment groups together support the highly potent hepatoprotective and antioxidant activity of methanolic extract of *G. gummifera* root. Phytochemical analysis revealed the presence of flavonoids, alkaloids, tannins, phenolics and steroids in MEGG[34]. β -sitosterol, oleanonic aldehyde, erythrodiol, *etc* have also been reported from *G. gummifera*[6]. β -sitosterol is a component reported as a hepatoprotective agent from *Phoenix dactylifera* against carbon tetrachloride-induced hepatotoxicity[35]. The identified class of components in single or in combination with other components present in the extract might be responsible for the antioxidant activity and reduction of hepatotoxicity in both of the treatment groups.

Conflict of interest statement

We declare that we have no conflict of interest.

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