Lagenaria siceraria (Mol.) Standley, commonly known as bottle-gourd (in English), belongs to cucurbitaceae family. The plant is widely available throughout India as an edible vegetable. It is a climbing or trailing herb, with bottle or dumb-bell shaped fruits. Both of its aerial parts and fruits are commonly consumed as vegetable. Traditionally the plant is used as medicine in India, China, European countries, Brazil, Hawaiian island etc. for its cardiotonic, general tonic, hepatoprotective, diuretic properties.[12] Further, antihepatotoxic, analgesic and anti-inflammatory, hypolipidemic, antihyperglycemic, immunomodulatory and antioxidant activities of its fruit extract have been evaluated.[13-17] Lagenaria siceraria fruits are good source of vitamin B complex, ascorbic acid, fibers, proteins, cucurbitacins, saponins, fucosterols and compesterols, polyphenolics, flavones-C-glycoside.[13,14,18-20] Methanol extract of its leaves showed the presence of sterols, polyphenolics, flavonoids, saponin, protein and carbohydrates.[21] A novel protein, Lagenin has also been isolated from its seeds and it possesses antitumor, immunoprotective and antiproliferative properties.[22] Although extensive studies have been carried out on its fruits and seeds, pharmacology of the aerial parts of L. siceraria however has not been explored yet. The present investigation was therefore carried out to evaluate the antioxidant and hepatoprotective potential of methanol extract of L. siceraria aerial part (MELS).

Antioxidant and Hepatoprotective Activity of Lagenaria siceraria Aerial parts

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Abstract

Introduction: Lagenaria siceraria is traditionally used in liver disorders and various free radicals induced diseases. The present study was carried out to evaluate the antioxidant and hepatoprotective activities of the aerial parts of L. siceraria methanol extract (MELS). Methods: DPPH, nitric oxide, superoxide, hydrogen peroxide, lipid peroxide free radical scavenging activity, reductive ability and total phenolic and flavonoid content of MELS were determined. Hepatoprotective activity of the extract was investigated against carbon tetrachloride induced hepatotoxicity in rats. Results: The results explored significant in vitro antioxidant activity of MELS. It also showed potent hepatoprotective activity in rat, which was evident from its significant effect on the levels of serum biomarker enzymes and total protein & bilirubin. Significant improvement of the endogenous antioxidant status by the treatment of MELS further reflects its hepatoprotective potential, which was finally substantiated by the histological studies of the liver tissues. Conclusion: The results reveal potent hepatoprotective activity of MELS which is probably attributed to its significant free radical scavenging activity and high polyphenolic and flavonoid contents.

Key words: Antioxidant, Cucurbitaceae, Carbon tetrachloride, DPPH, Free radical, Hepatoprotective, Lagenaria siceraria.
MATERIALS AND METHODS

Plant Material
The aerial parts of *L. siceraria* was collected in November 2008, from Madanpur, West Bengal, India and identified by the Botanical Survey of India, Howrah, India. A voucher specimen (P/LS/1/08) was retained in our laboratory for further reference.

Preliminary Phytochemical Screening
Preliminary phytochemical screening was carried out by following the standard procedures.[23]

Preparation of Plant Extract
The aerial parts were dried under shade and powdered in a mechanical grinder. The powdered material was extracted with methanol using soxhlet apparatus. This extract was filtered and concentrated in *suo* in a Buchi evaporator, R-114 and kept in vacuum desiccators until use. The yield was 18.13% w/w with respect to dried powder. Various concentrations of methanol solution of MELS was used for *in vitro* antioxidant studies, while, aqueous suspension of MELS was prepared using 2% (v/v) Tween-80 for oral administration.

Animals
Healthy Wistar albino rats (180 g ± 20) were used for the present study. They were maintained at standard laboratory conditions and fed with commercial pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The animals were acclimatized to laboratory condition for one week before commencement of experiment. The experiments were performed following the animal ethics guidelines of Institutional Animals Ethics Committee.

Acute Toxicity Study
Healthy rats were starved overnight and then were divided into five groups (*n* = 4). Group I-IV animals were orally fed with MELS in increasing dose levels of 0.5, 1.0, 1.5 and 2.0 g/kg b.wt, while group V (untreated) served as control. The animals were observed continuously for first 2 h for any gross change in behavioral, neurological and autonomic profiles or any other symptoms of toxicity and mortality if any, and intermittently for the next 6 h and then again after 24 h, 48 h and 72 h for any lethality or death. One-tenth and one-fifth of the maximum safe dose of the extract tested for acute toxicity, were selected for the experiment.[24]

*In vitro* Antioxidant Activity Study
Various concentrations of MELS (10-160 µg/ml in methanol) was used for *in vitro* antioxidant studies on different models, DPPH, NO, SO, H₂O₂ and LPO. For reductive ability study, 100-800 µg/ml concentration of the extract was used. Butylated hydroxyl toluene (BHT) was used as standard.

Determination of DPPH Radical Scavenging Activity
1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was measured using the method of Cotelle *et al*.[25] with some modifications. 3 ml of reaction mixture containing 0.2 ml of DPPH (100 µM in methanol) and 2.8 ml of test or standard solution of various concentrations was incubated at 37 °C for 30 min and absorbance of the resulting solution was measured at 517 nm using Beckman model DU-40 spectrophotometer. The percentage inhibition of DPPH radical was calculated by comparing the results of the test with those of the control (not treated with extract) using the formula:

\[
\text{Percentage inhibition} = \left( \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \right) \times 100
\]

Determination of Nitric oxide Scavenging Activity Assay
At physiological pH, sodium nitroprusside generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be measured by the Griess reaction.[26] 1 ml of 10 mM sodium nitroprusside was mixed with 1 ml of test or standard solution of different concentrations in phosphate buffer (pH 7.4) and the mixture was incubated at 25 °C for 150 min. From the incubated mixture, 1 ml was taken out and 1 ml of Griess’ reagent (1% sulphanilamide, 2% o-phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride) was added to it. Absorbance of the chromophore formed by the diazotization of nitrite with sulfanilamide and subsequent coupling with naphthyl ethylene diamine dihydrochloride was read at 546 nm and percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula.

Determination of Superoxide Radical Scavenging Activity
Superoxide anion scavenging activity was measured according to the method of Robak and Gryglewski[27] with some modifications. All the solutions were prepared in 100 mM phosphate buffer (pH 7.4). 1 ml of nitroblue tetrazolium (NBT, 156 µM), 1 ml of reduced nicotinamide adenine dinucleotide (NADH, 468 µM) and 3 ml of test/standard solution were mixed. The reaction was initiated by adding 100 µl of phenazine methosulphate (PMS, 60 µM). The reaction mixture was incubated at 25 °C for 5 min, followed by measurement of absorbance at 560 nm. The percentage inhibition was calculated from the above formula.

Determination of Hydrogen peroxide Scavenging Activity
The hydrogen peroxide scavenging ability of the extract was determined according to the method of Ruch *et al*.[28] A solution
of \( H_2O_2 \) (40 mM) was prepared in phosphate buffer (pH 7.4). Extract or standards (of different concentrations) in phosphate buffer (3.4 ml) was added to the \( H_2O_2 \) solution (0.6 ml, 40 mM). The absorbance of the reaction mixture was recorded at 230 nm after 10 min against a blank solution of phosphate buffer. Percentage of \( H_2O_2 \) scavenging was calculated using the above formula.

**Determination of Inhibition of Lipid Peroxidation**

Rat liver homogenate was used as the source of polyunsaturated fatty acids for determining the extent of lipid peroxidation. Reaction mixture (0.5 ml) containing rat liver homogenate (0.1 ml, 25% w/v) in Tris–HCl buffer (40 mM, pH 7.0), KCl (30 mM), ferrous ion (0.16 mM) and ascorbic acid (0.06 mM) were incubated at 37 °C for 1 h in the presence or absence of the extracts or standards. The lipid peroxide formed was measured by TBARS formation according to the method of Ohkawa et al.[29] Incubation mixtures were treated with sodium dodecyl sulphate (SDS; 8.1%, 0.2 ml), thiobarbituric acid (TBA; 0.8%, 1.5 ml) and acetic acid (20%, 1.5 ml). The total volume was then made up to 4 ml with distilled water and kept on water bath for 30 min. After cooling, 1 ml of distilled water and 5 ml of a mixture of n-butanol and pyridine (15:1 v/v) were added and centrifuged at 4000 rpm for 10 min. The absorbance of the organic layer, containing the colored TBA-MDA complex, was measured at 532 nm. The percentage inhibition of lipid peroxidation was determined by comparing the results of the test compound with those of the control, using the formula mentioned above.

**Determination of Reductive Ability**

Reducing power of the test samples was determined on the basis of the ability of their antioxidant principles to form colored complex with potassium ferricyanide, trichloroacetic acid (TCA) and FeCl₃, which is measured at 700 nm.[30] 1 ml of different concentrations of the extract or standard solution was mixed with potassium ferricyanide (2.5 ml, 1%) and 2.5 ml of phosphate buffer (pH 6.6). The mixture was incubated at 50 °C for 20 min. 2.5 ml TCA (10%) was added to it and centrifuged at 3000 rpm for 10 min. 2.5 ml of supernatant was taken out and to this 2.5 ml water and 0.5 ml FeCl₃ (0.1%) were added and absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated higher reducing power.

**Determination of Total Phenolic Compounds**

The amount of total phenolic compounds in MELS was determined using Folin–Ciocalteu’s reagent and sodium carbonate solution and the absorbence was measured at 760 nm.[31] A calibration curve of standard pyrocatechol was prepared and the results were expressed as mg of pyrocatechol equivalents/g of dry extract.

**Determination of Total Flavonoid Content in the Extract**

The total flavonoid content of MELS was determined spectrophotometrically.[32] Briefly 0.5 ml of 2% aluminium chloride in ethanol was mixed with same volume of extract (1.0 mg/ml). Absorption readings at 415 nm were taken after 1 h against a blank (ethanol). The total flavonoid content was determined using a standard curve with quercetin (0-50 mg/L). The mean of three readings was used and expressed as mg of quercetin equivalents/g of dry extract.

**Hepatoprotective Activity Study**

**Experimental Design**

After seven days of acclimatization, the healthy male rats were divided into five groups of six animals each. Treatment was done for 14 days. Group I served as control and received 2% Tween-80; 1 ml/kg. Group II-V received CCl₄ in liquid paraffin (1:2) (1 ml/kg i.p.) once in every 72 h. Group II was not treated with any drug and served as CCl₄ control. Group III and IV were orally administered 200 and 400 mg/kg MELS once daily. Group V received standard drug silymarin (25 mg/kg, p.o.).[33] After 24 h of the last dose, blood was collected from retro-orbital plexus under ether anesthesia. The blood samples were allowed to clot and the serum was separated by centrifugation at 2500 g at 37 °C and was used for biochemical estimation. All the animals were then sacrificed and liver tissues were collected for the evaluation of in vivo antioxidant status and histopathological examination.

**Estimation of Biochemical Parameters**

Serum was analysed for various biochemical parameters like serum glutamic pyruvate transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT)[34] and alkaline phosphatase (ALP)[35] activities. The total protein concentration and total bilirubin were also measured by the method of Lowry et al.[36] and Mallay and Evelyn[37] respectively. All the analysis were performed by using commercially available kits from Span Diagnostics Ltd.

**Evaluation of Antioxidant Properties**

For assessment of antioxidant activities, 25% (w/v) liver tissue homogenate for each animal was prepared using KCl solution (1.15% w/v) and centrifuged at 3000 g at 4 °C for 1 hr. The supernatant was used for the determination of lipid peroxidation (LPO)[29] (Ohkawa et al., 1979) and endogenous antioxidant systems such as reduced glutathione (GSH),[38] superoxide dismutase (SOD)[39] and catalase[40] (CAT).

**Histological Observation**

For histological study, the liver tissues were collected and immediately fixed in 10% formalin, dehydrated in
gradual ethanol (50-100%), cleared in xylene and embedded in paraffin. Sections (4-5 µm) were prepared and then stained with hematoxylin-eosin dye for photomicroscopic observations.

**Statistical Analysis**
Values were presented as mean ± S.E.M. Data were statistically evaluated by one-way analysis of variance (ANOVA) followed by post hoc Dunnett’s test using SPSS software. *P* < 0.01 were considered as statistically significant.

**RESULTS**

Preliminary phytochemical screening of MELS revealed the presence of polyphenolics, flavonoids, glycosides, triterpinoids and carbohydrates.

In acute toxicity study, MELS did not show any mortality or toxic effect upto the dose of 2 g/kg b.wt, accordingly 200 and 400 mg/kg b.wt were taken as low and high dose of MELS for the *in vivo* experiment.

**In vitro Antioxidant Study**

The methanol extract of *L. siceraria* aerial part was found to scavenge DPPH, nitric oxide, superoxide radical and hydrogen peroxide as well as inhibited lipid peroxidation *in vitro* in a concentration dependent manner. The concentrations of MELS needed for 50% scavenging of the ROS and lipid peroxidation (IC₅₀ values) have been given in table 1. Reductive ability of the extract was also found to be concentration dependent, as like the standard (Figure1). The total phenolic and flavonoid contents of MELS were found to be 95.7 ± 3.96 mg pyrocatechol/g dry extract and 25.32 ± 1.48 mg quercetin equivalent/g dry extract respectively.

**Table 1: Free radical scavenging activity of methanol extract of L. siceraria aerial parts (MELS) and Butylated hydroxyl toluene (BHT) on different in vitro models**

<table>
<thead>
<tr>
<th>In vitro models</th>
<th>IC₅₀ Values (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MELS</td>
</tr>
<tr>
<td>DPPH</td>
<td>25.70 ± 1.02</td>
</tr>
<tr>
<td>NO</td>
<td>52.20 ± 2.05</td>
</tr>
<tr>
<td>SO</td>
<td>62.50 ± 3.65</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>46.50 ± 4.55</td>
</tr>
<tr>
<td>LPO</td>
<td>43.85 ± 4.26</td>
</tr>
</tbody>
</table>

DPPH: Diphenyl-2-picryl hydrazyl; NO: Nitric oxide; SO: Super oxide; H₂O₂: Hydrogen peroxide; LPO: Lipid peroxide.

% Inhibition = (Absorbance of control – Absorbance of test) × 100/Absorbance of control

IC₅₀ Value: Concentration required for 50% inhibition of the free radical reactions

Values are mean ± SEM of triplicate determinations

![Figure1: Reductive ability of methanol extract of L. siceraria aerial parts (MELS) and Butylated hydroxyl toluene (BHT)](image)
Hepatoprotective Activity Study
Administration of \( \text{CCl}_4 \) to the animals resulted in a marked elevation of serum transaminases (SGOT and SGPT), serum alkaline phosphatase (ALP) and total bilirubin (TB), when compared with those of normal control animals. However serum total protein level was decreased. The rats treated with methanol extract of \( L. \) \textit{siceraria} and with silymarin showed a significant decrease \((p < 0.001)\) in all the elevated serum marker levels, SGOT, SGPT, ALP and TB, and significant increase \((p < 0.001)\) in total protein (Table 2) which showed the restoration of the level of liver function biochemistry to the near normal values.

Toxic dose of \( \text{CCl}_4 \) significantly reduced the activities of enzymic (CAT and SOD) and non-enzymic (GSH) antioxidant system and enhanced lipid peroxidation (LPO) level of liver tissue, as were found in group II animals. MELS treatment significantly raised both of the enzymic and non-enzymic antioxidant systems as was found in case of silymarin treated group, while the elevated LPO level were found to be reduced back to/towards the normal level in MELS as well as silymarin treated rats (Table 3).

Histological examination of liver sections of normal control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein (Figure 2A). Disarrangement of normal hepatocytes with centrilobuler necrosis, vacuolization of cytoplasm and fatty changes were observed in \( \text{CCl}_4 \) intoxicated rat livers (Figure 2B). The liver sections of the rats treated with MELS low and high dose (Figure 2C and Figure 2D) and silymarin (Figure 2E) showed a sign of protection against \( \text{CCl}_4 \) intoxication as evident by presence of normal hepatic cords and absence of necrosis with minimal inflammatory conditions around the central vein.

**DISCUSSION**
Excessive concentration of reactive oxygen species (ROS) and other radicals leads to oxidative stress in the body and that in turn is associated with a number of pathological conditions. Antioxidants can combat against this oxidative stress either by scavenging free radicals or by their potent reductive ability.[2] There are restrictions on the use of synthetic antioxidants, such as Butylated Hydroxy Toluene (BHT), Butylated Hydroxy Anisole (BHA), as they are suspected to be carcinogenic.[41,42] Natural antioxidants, therefore, have gained importance.

DPPH is a relatively stable nitrogen centered free radical, which is widely used to evaluate free radical scavenging property of natural antioxidants. An antioxidant can scavenge DPPH radical by donating H atom and forming corresponding non-radical hydrazine.[43] In the present study, MELS was found to scavenge DPPH in a concentration dependent manner. Further, MELS effectively scavenged superoxide, nitric oxide, hydrogen peroxide and lipid peroxidation.

### Table 2.
**Effect of Methanol extract of \( L. \) \textit{siceraria} (MELS) on serum enzyme levels and Total bilirubin and Total protein of \( \text{CCl}_4 \) intoxicated rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGPT (IU/L)</th>
<th>SGOT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>Total bilirubin (mg/dl)</th>
<th>Total protein (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>74.63 ± 1.43</td>
<td>113.21 ± 2.41</td>
<td>31.64 ± 1.57</td>
<td>0.83 ± 0.04</td>
<td>7.48 ± 0.06</td>
</tr>
<tr>
<td>( \text{CCl}_4 ) Control</td>
<td>199.08 ± 4.62*</td>
<td>247.85 ± 4.28**</td>
<td>90.01 ± 2.27*</td>
<td>2.66 ± 0.06*</td>
<td>4.19 ± 0.04*</td>
</tr>
<tr>
<td>MELS (200 mg/kg)</td>
<td>90.55 ± 2.33*</td>
<td>156.62 ± 2.40**</td>
<td>45.48 ± 1.75*</td>
<td>1.43 ± 0.04*</td>
<td>6.95 ± 0.05*</td>
</tr>
<tr>
<td>MELS (400 mg/kg)</td>
<td>72.30 ± 1.18**</td>
<td>127.23 ± 2.23**</td>
<td>32.89 ± 1.40**</td>
<td>1.24 ± 0.03**</td>
<td>7.35 ± 0.03**</td>
</tr>
<tr>
<td>Silymarin (20 mg/kg)</td>
<td>80.95 ± 1.51**</td>
<td>140.22 ± 2.17**</td>
<td>40.06 ± 1.84**</td>
<td>1.01 ± 0.04**</td>
<td>7.08 ± 0.04**</td>
</tr>
</tbody>
</table>

Values are Mean ± S.E.M.; \( n = 6 \) in each group. Drug treatment was done for 14 days.

*\( \text{CCl}_4 \) control group vs normal control group, \(^* p < 0.001;\)

**Treated groups vs \( \text{CCl}_4 \) control group, \(^{**} p < 0.001; \) where the significance was performed by One way ANOVA followed by post hoc Dunnett’s test.

### Table 3.
**Effect of Methanol extract of \( L. \) \textit{siceraria} (MELS) on LPO, GSH, SOD and CAT levels of \( \text{CCl}_4 \) intoxicated rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>LPO (nM/mg wet tissue)</th>
<th>GSH (µg/mg wet tissue)</th>
<th>SOD level (Units/mg wet tissue)</th>
<th>CAT level (µM of H(_2)O(_2) decomposed/min/mg wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>98.91 ± 1.91</td>
<td>64.05 ± 1.48</td>
<td>1.50 ± 0.03</td>
<td>3.90 ± 0.05</td>
</tr>
<tr>
<td>( \text{CCl}_4 ) Control</td>
<td>297.85 ± 5.12**</td>
<td>28.06 ± 1.62**</td>
<td>0.79 ± 0.02**</td>
<td>1.14 ± 0.03**</td>
</tr>
<tr>
<td>MELS (200 mg/kg)</td>
<td>122.08 ± 1.84**</td>
<td>58.72 ± 1.99**</td>
<td>1.01 ± 0.04**</td>
<td>1.99 ± 0.05**</td>
</tr>
<tr>
<td>MELS (400 mg/kg)</td>
<td>92.86 ± 1.54**</td>
<td>78.71 ± 1.68**</td>
<td>1.31 ± 0.03**</td>
<td>3.04 ± 0.05**</td>
</tr>
<tr>
<td>Silymarin (20 mg/kg)</td>
<td>100.32 ± 2.91**</td>
<td>70.07 ± 2.11**</td>
<td>1.45 ± 0.03**</td>
<td>3.00 ± 0.07**</td>
</tr>
</tbody>
</table>

LPO: Lipidperoxide; GSH: reduced Glutathione; SOD: Superoxide dismutase; CAT: Catalase.

Values are Mean ± S.E.M.; \( n = 6 \) in each group. Drug treatment was done for 14 days.

*\( \text{CCl}_4 \) control group vs normal control group, \(^* p < 0.001;\)

**Treated groups vs \( \text{CCl}_4 \) control group, \(^{**} p < 0.001; \) where the significance was performed by One way ANOVA followed by post hoc Dunnett’s test.
Figure 2: Histological observations of 14 days experimental rat liver
peroxide in vitro. These radicals are generated inside our body during the normal metabolism or in presence of xenobiotics and removed by the body’s innate antioxidant defense mechanism. However, under pathologic conditions, there is an imbalance between ROS and antioxidant defense, which leads to oxidative modifications in the cellular membrane or major intracellular molecules. In addition to ROS, the potential mechanism of cellular oxidative damage by nitric oxide is the nitration of the tyrosine residues of proteins, peroxidation of the lipids, degradation of DNA and oligonucleosomal fragments. Increased level of lipid peroxide can give rise to the formation of carbonyl compounds like malondialdehyde (MDA) which are highly cytotoxic. In the present study, the dose dependent scavenging of both ROS as well as nitric oxide radicals indicates the potent antioxidant property of MELS. The significant antioxidant activity of the extract thus suggests the possible therapeutic value of this plant.

Potent reductive ability of the extract implies its capability of donating hydrogen atom in a dose dependent manner. The high phenolic and flavonoid content of the extract may be the contributing factor towards its antioxidant activity. Because of the presence of the hydroxyl groups phenolic constituents can function as hydrogen donor. Antioxidant potential of flavonoids against oxidative stress is the interesting subject of many investigations. The hepatotoxicity induced by CCl₄ is due to its metabolite trichloromethyl free radical (CCl₃·), that alkylates cellular proteins and other macromolecules and finally result in cell death. Hepatocellular necrosis leads to elevation of the serum marker enzymes, which are released from the liver into the blood. The present study revealed a significant increase in the activities of SPT, SGOT ALP and total bilirubin levels as well as total lipid peroxides and caused a subsequent recovery towards normalization almost like that of silymarin treatment. Decreased total protein content in the CCl₄ control group was also restored to near normal value by MELS supplementation. Stabilization of serum total bilirubin and total protein levels by MELS treatment is a clear indication of the improvement of functional status of the hepatic cells.

The body’s innate defense mechanism consists of a set of endogenous antioxidant enzymes, including catalase (CAT), superoxide dismutase (SOD) and non-enzymatic system such as reduced glutathione. In CCl₄ induced hepatotoxicity, the balance between ROS production and this antioxidant system is lost, hence the oxidative stress results. This was reflected by the reduced level of SOD and CAT and the exhausted GSH content in CCl₄ control group animals. Improvement in the antioxidant status to/towards normal level by the treatment with MELS as like silymarin treated animals implies the hepatoprotective effect of the extract. The free radicals generated in the metabolism of CCl₄ react with the unsaturated lipid of the cell and initiates the chain reactions of lipid peroxidation, which can cause peroxidative tissue damage in inflammation, cancer, aging, ulcer, cirrhosis and atherosclerosis. Increased lipid peroxidation in CCl₄ control group indicates tissue damage and failure of antioxidant defense mechanisms. However significant decrease in lipid peroxidation in the treated groups further potentiates its antioxidant activity in vivo.

The protective effect of MELS in liver injury can further be concluded by the histological examinations of the liver tissues.

Hence the present investigation infers potent antioxidant and hepatoprotective activity of MELS, which may be due to its good amount of phenolic and flavonoid contents; however, further research is going on in our laboratory to isolate and purify the bioactive principle(s) from the methanol extract of L. siceraria aerial parts.

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**REFERENCES**

28. 839-841.
41. Wichi HP. Enhanced tumor development by butylated hydroxyanisole (BHA) from the prospective of effect on forestomach and oesophageal squamous epithelium. Food and Chemical Toxicology. 1988; 26:717-723.
43. Soares JR, Dins TCP, Almeida LM. Antioxidant activity of some extracts of Thymus zygis (BHA) from the prospective of effect on forestomach and oesophageal squamous epithelium. Food and Chemical Toxicology. 1988; 26:717-723.