# Hepatoprotective Activity of *Lagenaria siceraria* Leaf Extract against Carbon Tetrachloride-Induced Damage in Rats

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## Abstract

Traditionally, the juice and decoction of aerial part and leaves of *Lagenaria siceraria* was used for cure and management of hepatic disorders in South Asia. There is a scarcity of scientific details to justify the traditional claim for hepatoprotective potential of leaves of *L. Siceraria.* In the present research work, the hepatoprotective potential was evaluated for methanolic extract of *L. siceraria* leaves (LSME) against carbon tetrachloride induced hepatotoxicity in albino rats. The levels of hepatic biochemical markers were estimated in treated groups. The treatment with LSME (50 mg/kg) altered back the normal levels of biochemical markers as well as done the significant improvement in the damaged hepatatocytes. The levels of endogenous liver antioxidant enzymes, catalase, superoxide dismutase and glutathione contents were increased significantly. There was also recorded the significant ($P<0.001$) depletion in serum glutamic-pyruvic transaminase, serum glutamic-oxaloacetic transaminase, Alkaline- phosphatase and total bilirubin in LSME treated group. From these results, it is suggested that methanolic extract of *L. siceraria* leaves possesses hepatoprotective properties.

**Key Words** *Lagenaria siceraria*, carbon tetrachloride, Hepatoprotective effect, biochemical enzymes.

## Introduction

Liver is the largest vital organ to facilitate intense metabolism and excretion. It plays the vital role in the maintenance, performance and regulating homeostasis of the body. Various biochemical pathways of growth, immunity, energy and reproduction are passed through the liver [1]. The major functions of the liver are metabolism of carbohydrate, protein and fat, detoxification, bile secretions and storage of some vitamins. So, keeping the liver healthy is an

important factor for overall health and well being. The environmental toxins, abused by drug habits & alcohol and over the-counter drugs may lead to continuously and variedly exposure to liver unfortunately [2]. This can eventually lead to various liver ailments like fatty liver, hepatitis, cirrhosis and alcoholic liver disease. Now a day's the plant based preparations are employed for the treatment of liver disorders and alleviation of hepatic diseases [3]. Therefore, a lot of traditional remedies from plant origin are evaluated for its potential for antioxidant and hepatoprotective effect by using experimental rodent model [4]. Carbon tetrachloride ($CCl_4$)-induced hepatotoxicity is widely used model to evaluate hepatoprotective effects of herbs extracts [5].

*Lagenaria siceraria* (Bottle gourd) is an official drug in Ayurvedic Pharmacopoeia [6]. It is one of the most valuable fruit for human being made and gifted by the nature having composition of all the essential constituents that are required for normal and good human health [7]. Juice or decoction of aerial part and leaves of *L. siceraria* is used for cure of jaundice in India traditionally[8]. Leaves paste is used for treatment of alopecia and applied on the head for the headache by ancient people [9]. Leaves are also used as cathartic in constipation [10].The edible part of fruit of *L. siceraria* is rich source of ascorbic acid, β- carotene, vitamin B complex and pectin [11]. It is also good source of minerals [12]. Two sterols namely fucosterol and campesterol were identified and isolated from ethanol extract of dried fruit pulp [13]. HPLC analysis of extract of flowering plant of *L. siceraria* shows presence of flavone-C glycosides[14]. Fecal steroid excretion was reported on administration of semi purified dietary fibres isolated from the fruit of *L. Siceraria* [15]. In the present work hepatoprotective activity was investigated for methanolic extract of *L. Siceraria* leaves (LSME) in experimental rodents.

## Materials and methods

### Reagents and chemicals

Carbon tetrachloride was procured from Krishna Chemical Industry Vadodara Gujarat, India; Silymarin was received as a gift sample from Micro labs ltd. India; Kits of standards i.e. SGPT SGOT, ALP and bilirubin was obtained from Erba Diagnostics, India. All the reagents used in experimental work were of analytical grade.

### Collection and authentication of plant material

The leaves of *L. siceraria* plant were collected in the month of July from village area of district Bareilly, U.P, India**.** Leaves were authenticated by Department of Taxonomy, Hindu college (PG) Moradabad, Uttar Pradesh, where a voucher specimen (Taxonomy: HC.MBD/HAP-BK-2010-07-167) has been submitted.

### Preparation of extract

Leaves of *L. siceraria* were washed in tap water and dried in shade to avoid degradation of active constituents. Dried leaves were powdered to form coarse particles and stored in an airtight container. The powdered material was extracted by soxhlet apparatus in several batches using different solvents in increasing order of polarity. Petroleum ether, chloroform, acetone, methanol and distilled water were used as solvent. The extracts were concentrated by evaporating the solvent by rotary evaporator (Rotavapor® R-100). Preliminary phytochemical

screening was performed on all extracts of *L. siceraria* to confirm the presence of various phytochemical groups[16].

**Estimation of total phenolic content**

In the phytochemicaliy active extract, total phenolic content was analyzed by the Folin-Ciocalteu reagent method using gallic acid as a standard. In about 250 ml of methanol:water mixture (60:40 V/V, 0.3% HCl), 100 mg of extract was added and mixed well. Then the mixture was filtered through 0.45 μm milipore filter. Equimolar quantities (100 ml) of the filtrate and Folin-Ciocalteu reagent (50 %, V/V) were mixed followed by the addition of 2.0 ml of 2% sodium carbonate solution in water. The absorbance of the solution was recorded after 2 hours at 750 nm wavelength (UV spectrophometer, Shimadzu, UV-2600i). Quantification was based on the standard curve of gallic acid (0–1.0 mg/ml) dissolved in methanol/water (60:40, V/V, 0.3 % HCl). Phenolic content was expressed as milligrams per gram of gallic acid equivalent [17].

**Experimental animals**

Wistar albino rats of either sex, weighing 150 to 200 gm, were housed in groups of four per cage under controlled light (12:12 light: dark cycle) and temperature (25±2°C). Environmental and behavioral assessment was conducted during the light cycle. Food (Golden feed, New Delhi, India) and water *ad libitum* was provided. The animals were acclimatized to laboratory conditions for seven days before commencement of experiments[18].

All the procedure described in manuscript were reviewed and approved by Institutional Animal Ethical Committee (IAEC) of Teerthanker Mahaveer College of Pharmacy, Moradabad, India (Approval no- 1205/C/08/CPCSEA/2021/IAEC/12).

**Acute oral toxicity study**

Acute oral toxicity study was performed according to OECD guidelines 423 for LSME (2000 mg/kg body weight) in Wistar albino rats. Hematological parameters, laboratory parameters like body weight, consumption of food, urine examinations, ophthalmologic examinations and behavior were also evaluated during the experiment[19].

**Hepatoprotective Screening**

The study was conducted by using $CCl_4$ as toxicant to produce hepatotoxicity and Silymarin as a standard/reference drug [20]. LSME at a dose of 50 mg/kg was used as test and compared with the results obtained from standard and control. Animals were divided into 4 groups (n=6/group).

Group 1- Positive control: The animals received distilled water for 7 days and given $CCl_4$ in olive oil (1:9 ratio) single dose (SD), 10ml/kg orally on 8$^{th}$ day.

Group 2- Negative control: The animals received saline for 7 days.

Group 3- Standard: Pre-treated with standard drug Silymarin (100mg/kg body weight /day p.o.) for 7 days followed by SD of $CCl_4$ on 8$^{th}$ day.

Group 4- Test: Pre-treated with LSME (50 mg/kg/day p.o.) for 7 days followed by a SD of $CCl_4$ on 8$^{th}$ day.

After 16 hrs from the administration of last dose of treated groups, the blood samples were collected through retro orbital artery [21]. Serums were separated from the blood samples by centrifugation for 10 minutes at 2000 rpm [22]. The estimation of biochemical parameters was done for hepatoprotective effect of *L. Siceraria.* Alkaline-phosphatase (ALP), Serum glutamic-oxalo-acetic-transaminase (SGOT), serum glutamic-pyruvic-transaminase (SGPT), and tissue bilirubin level (TBL) were estimated by using standard kits [23]. Then animals were sacrificed and their livers were excised & washed in normal saline followed by 0.15 M Tris-HCL buffer. The estimation of tissue lipid peroxidase (LPO) was carried out [24]. A portion of homogenate after precipitating proteins with trichloroacetic acid (TCA) was used for the estimation of glutathione (GSH) [25]. The rest of the homogenate was centrifuged at 15000 rpm for 15 min at $4^0$C. The supernatant was separated for the estimation of super-oxide- dismutase (SOD) and catalase (CAT) [26].

**Histopathological study**

Tissue specimens of liver lobules were used for histopathological examination. Sections of 50 micron thickness were cut by embedding tissue in wax. Haematoxylin and eosin staining reagents were used to stain the sections. All stained sections were examined at 40x and 100x magnification power. Constitution of normal internal structure, swelling/inflammation and necrosis were considered at the time of observation.

**Statistical analysis**

The analysis of statistical significance was determined by one way analysis of variance (ANOVA) followed by Dunnett's Test. The values were expressed in terms of mean ± SEM and $P<0.05$ was considered significant.

## Results

The presence of carbohydrates, saponins, steroids, tannins, flavonoids and terpenoids in LSME was revealed through performing the preliminary phytochemical screening. Hence, LSME was found to be phytochemically active. Total phenolic content of LSME was estimated through standard curve equation (y = 0.0032x+0.0009) for gallic acid at 750 nm wavelength. The total phenolic content of *L. siceraria* leaves methanolic extract was found to contain 98.98 µg/mg of gallic acid equivalent.

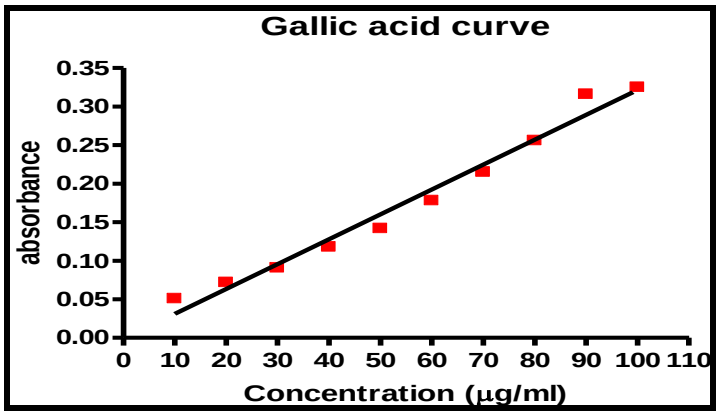


**Fig. 1** Standard curve of gallic acid for estimation of total phenolic content of LSME

**Acute toxicity of LSME**

Hematological parameters were reported normal at the end of the toxicity study. LSME did not produce any mortality and toxic effect up to the dose level at 2000 mg/kg body weight in rats, hence the extract was considered to be safe and non-toxic for further pharmacological screening.

**Estimation of biochemical parameters for hepatoprotective effect of LSME**

Estimation of the serum alkaline phosphatase activity and total bilirubin is the most widely used parameters for measuring the hepatic damage. There is a significant (*P<0.001*) increase in the levels of bilirubin, SGOT, SGPT and ALP was observed in $CCl_4$ intoxicated group of this study. LSME possesses significant (*P<0.001*) effect on $CCl_4$ induced hepatotoxicity (Table 1). The significant depletion of bilirubin, SGOT, SGPT and ALP in LSME pre-treated group revealed the hepatoprotective effect of LSME (50 mg/kg) against $CCl_4$ induced hepatotoxicity.

| Treated group | Dose (mg/kg) | SGPT (IU/L) | SGOT (IU/L) | ALP (IU/L) | Total bilirubin mg/dl |
|---|---|---|---|---|---|
| Positive control | Only vehicle | 123.8 ± 1.26 | 171.2 ± 1.61 | 122.4± 3.96 | 4.86 ± 0.21 |
| Normal control | Only vehicle | 50.32 ± 1.12*** | 58.8 ±1.37*** | 49.04±3.36 *** | 1.1 ± 0.17 *** |
| Standard | 100 | 55.2 ± 1.36*** | 64.47 ± 1.59*** | 54.32±3.91 *** | 1.94 ± 0.24 *** |
| LSME | 50 | 58.83 ± 1.11*** | 79.26 ± 1.66*** | 61.49±4.08 *** | 2.52 ± 0.21 *** |

**Table1 Effect of LSME on biochemical parameters in $CCl_4$-induced hepatotoxicity**

All values expressed as Mean ± SEM (n= 6), ****P<0.001* as compared with positive control group (One way ANOVA followed by Dunnett's test)

**Effect of LSME on lipid per-oxidation in carbon tetrachloride treated groups**

Lipid peroxidase (LPO) levels by thiobarbituric acid reaction showed a significant (*P<0.001*) increase in carbon tetrachloride treated rats. Treatment with LSME (50 mg/kg) significantly (*P*<0.001) inhibited the LPO level which was altered back to normal. The antioxidant effect of LSME was comparable to standard drug Silymarin.

$CCl_4$ treatment was resulted as a significant (*P<0.001*) decrease in the level of SOD, Catalase and GSH in hepatic tissue when compared with control group. The treatment with LSME at the doses of 50 mg/kg produced a significant increase (*P<0.001*) of SOD, Catalase and GSH levels in comparison of positive control group. The liver of animals treated with Silymarin also showed a significant increase in the levels of antioxidant enzymes when compared to positive control group (Table 2).

**Table 2 Antioxidant effect of LSME on $CCl_4$-induced hepatotoxicity in rats**

| Treated group | Dose (mg/kg) | CATALASE (U/mg protein) | SOD (%Inhibition of NBT) | LPO (nM/mg protein) | GSH (mM/gm tissue wt.) |
|---|---|---|---|---|---|
| Positive control | Only vehicle | 13.83 ± 0.10 | 24.38± 0.22 | 7.19 ± 0.09 | 7.92 ± 0.18 |
| Negative control | Only vehicle | 34.5 ± 0.12*** | 66.64 ± 0.49 *** | 2.61 ± 0.05*** | 28.14 ± 0.29 *** |
| Standard (Silymarin) | 100 | 32.68 ± 0.17*** | 61.33 ± 0.36*** | 2.89 ± 0.06*** | 24.57 ± 0.26* ** |
| LSME | 50 | 27.92 ± 1.01*** | 52.20 ± 0.25 *** | 3.23 ± 0.12*** | 16.32 ± 0.14 *** |

All values expressed as Mean ± SEM (n=6) ****P<0.001* as compared to positive control group (One way ANOVA followed by Dunnett's test)

**Histopathology of liver**

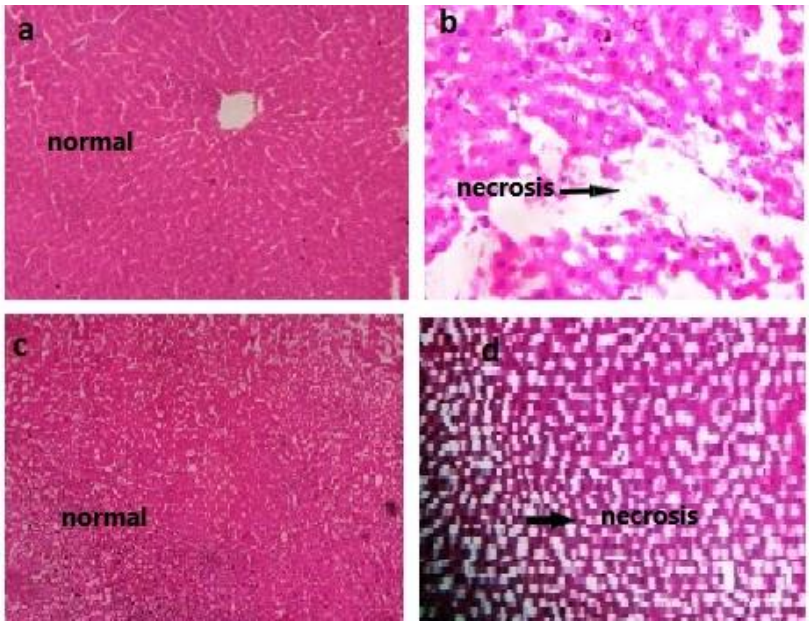


**Fig. 2** (a-d) Histopathological images rat liver: a) Negative control group, showing normal architecture; b) Positive control group; showing necrosis of central vein; c): Standard group; showing normal appearance with no evidence of necrosis; d) LSME treated groups; showing less evidence of necrosis compared to positive control group

Histological profile of the animals treated with LSME (50 mg/kg) supported the results obtained by the biochemical investigations. The improvements in tissue regeneration and recovery against $CCl_4$ induced necrosis of hepatocellular tissues were observed microscopically. The tissue section of liver of positive control group showed that extent of liver damage was more in magnitude as compared to test and standard groups of animals; Whereas test (LSME 50 mg/kg)

and standard (Silymarin 100 mg/kg) groups showed the comparable significant healing effect (Fig. 2) but there was so much difference in the effective dose of test and standard.

## Discussion

The organ liver plays a vital role in metabolism of all things including drug and nutrients. Liver has a central role in drug metabolism and it is a most vulnerable tissue for drug toxicity. According to the reports published by USFDA, herbs have been reported to cause liver injury, and 20-40% of all instances of hepatic failure. The asymptomatic elevation of liver enzymes to fulminant hepatic failure is a manifestation of hepatotoxicity due to various environmental toxicants, herbal remedies and clinically useful drugs, like paracetamol, NSAIDS, and gentamycin through the activation to highly reactive free radicals including the oxygen reactive species and super-oxides [27]. According to literature survey, various enzymatic and non-enzymatic regulations have been developed by the cell to protect from the oxidative stress and other free radicals generated in day to day lifestyle changes. Histopathological investigation along with estimation of SGOT SGPT, ALP, TBL, LPO GSH, Catalase and SOD are the most widely used methods in the diagnosis of status of healthy hepatocytes to confirm their normal functioning and regeneration of new hepatocytes[28]. The methanol extract of *L. siceraria* leaf reduced the elevated levels of all the biochemical parameters along with significantly inhibition of liver necrosis caused by $CCl_4$ induced hepatotoxicity.

## Conclusion

It can be concluded from this investigation that LSME (50 mg/kg) possess hepatoprotective potential. This effect may be due to the presence of polyphenols and other antioxidants in the extract. The present study submit the evidence for hepatoprotective potential of the leaves of *L. siceraria and* may give a lead to further investigation of active molecule responsible for promising effectiveness in the treatment of hepatic disorders in future.

## Declarations

### Conflict of Interest

The authors declare that they have no conflict of interest to disclose.

### Acknowledgement

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