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ORIGINAL ARTICLE

Effect of Carbon Tetrachloride-induced Hepatic Injury on *Luffa acutangula* (var.) Amara

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ABSTRACT

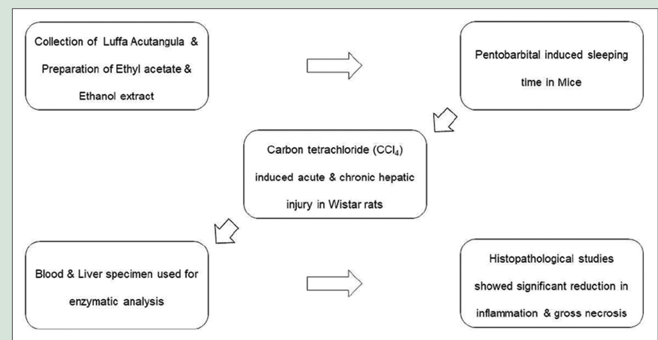
Objectives: To study the reversal effect of carbon tetrachloride (CCl_4)-induced hepatic injury on the ethyl acetate and ethanol extracts of *Luffa acutangula* var. amara in Wistar rats. **Materials and Methods:** The study included experimental mice groups in which pentobarbital-induced sleeping time and its prolongation by CCl_4 is assessed by righting reflex and recovery time. Chronic liver injury was induced by oral administration of CCl_4 and acute injury by a single intraperitoneal injection in experimental Wistar rats. Blood and liver specimens were used for analysis of enzymatic reactions. **Results:** It was noted that the mice groups treated with extracts decrease the sleeping time in and standard silymarin than the control group. Acute and chronic liver injury models showed increases in total protein and glutathione peroxidase, whereas there was a reduction in total bilirubin and serum alkaline phosphate level in extracts and standard silymarin-treated rats than control. Histopathological studies showed a significant reduction in inflammation, fatty change and gross necrosis in extracts and standard silymarin-treated animals than control. **Conclusion:** The study suggests that extracts of *L. acutangula* var. amara can be useful in treating acute and chronic hepatic injury.

Key words: Aspartate aminotransferase, carbon tetrachloride, hepatoprotective, *Luffa acutangula,* silymarin, Wistar rats

SUMMARY

- *Luffa acutangula* var. amara showed amazing hepatoprotective effect, which was evident in the results seen. Due to increase in number and mortality due to liver disorders, it is indeed to have a good plant-based drug which is effective and safe on humans. This plant throws some light on its hepatoprotective properties. On further investigations on this plant, using extraction of active components in aid to identifying the pathway the active drug work is mandatory which can have a best drug in future.

Abbreviations Used: CCl_4: Carbon tetrachloride; EELA: Ethanol extract of *L. acutangula*; EAELA: Ethyl acetate extract of *L. acutangula*; PARC: Plant Anatomy Research Centre; %: Percentage; v/v: Volume/Volume; w/w: Weight/Weight; G: Gram; °C: Degree centigrade; CPCSEA: The Committee for the Purpose of Control and Supervision of Experiments on Animals; IAEC: Institutional Animal Ethics Committee; ml: Milliliter; p. o.: Per oral; i. p.: Intraperitoneal; Mg: Milligram; Kg: Kilogram; Hrs: Hours; Rpm: Revaluation per minute; w/v: Weight/Volume; Tris-HCL: Tris-hydrochloric acid; pH: Potential of hydrogen ion; SGOT: Serum glutamic oxaloacetic transaminase; SGPT: Serum glutamic pyruvic transaminase; ALP: Alkaline phosphatase; GSH: Reduced glutathione; LPO: Lipid peroxidation; Nm: Nanometer; UV: Ultraviolet; µm: Micrometer; H-E: Hematoxylin and eosin; ANOVA: Analysis of variance; SPSS: Statistical Package for the Social Sciences; SD: Standard deviation; P: Probability; CCl_3*: Trichloromethyl radicals; MDA: Melondialdehyde; Min: Minute.



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INTRODUCTION

All the drugs were obtained only from the natural sources, thus in the earlier days the plants were exploited for medicinal usage which is evident from the history. It is accepted worldwide that proper research on plants can help them to come up with new discoveries on medicines which can provide proper way for the new coming diseases. As it is proven that the herbal medicines can be considered to be safer when compared to the synthetic drugs as the damage caused by the medicines are more while using synthetic drugs.[1] Our planet-earth (biosphere) contains more than 20 million species of organisms. However, of which, only 1.4 million species have been identified so far and each has its own metabolite. These metabolites are of potential medical interest. Thus, the selection of plants for phytotherapeutic investigation is very important and significant step. It is mainly based on traditional folklore claim, available chemical composition, and screening for a specific biological activity.[2]

In India, so many plants were screened to identify the pharmacological use of the plant, but still not even one successful drug or lead as been discovered though many lead molecules were obtained.[3] *L. acutangula* var. amara is also commonly known Ridge Gourd in English and

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Peerkankai in Tamil, is an annual herb belonging to the Cucurbitaceae family. It is found in the Western, Central and Southern India. The leaves are smaller, at first whitish and softly villous and scabrid at length. The flowers are smaller in size and the seeds are found in large numbers. The fruit is ovoid, obtusely conical at the ends, 5–10 cm long, 2–4 cm thick, 10 ribbed and bitter.[4,5]

The plant is slightly pungent, acrid, bitter and is known to act as a laxative, carminative and digestable tonic to the intestines, cures "vata," "kapha," biliousness, anemia, liver disorders, leukoderma, piles, inflammation, bronchitis, jaundice, tumors, tuberculous glands, uterine and vaginal tumors.[6] The fruit is cathartic and emetic and destroys bad taste in the mouth and cures urinary discharges and headache. The pulp of the fruit is given with water in dog bite and other animal bites and the dry fruit is used as a snuff in jaundice. The ripe seeds either in infusion or powder is used as emetic and cathartic. In small doses, they are used as expectorant and demulcent. The kernel of the seeds has control over dysentery.[7]

All these inherent medicinal properties of *L. acutangula* lead to the possible understanding of its hepatoprotective activity. Many studies have been conducted to prove the hepatoprotective nature of this plant and understand the possible mechanism behind. This study uses the alcoholic extracts of *L. acutangula* and has shown to possess protective potential against the free radicals' activity which is evident by *in vitro* antioxidant effect.[8] The hepatoprotective nature of the ethyl acetate extract of *Luffa acutangula* (EAELA) and ethanol extract of *Luffa acutangula* (EELA) in carbon tetrachloride (CCl_4)-induced hepatic damage in rats was studied and supported by the prolonged sleeping time induced by pentobarbital in mice. This study can be a new paver path in the treatment of hepatic failures and other liver disorders in the future with minimal side effects when compared to allopathic and other synthetic available drugs.

MATERIALS AND METHODS

Plant collection and identification

The *L. acutangula* var. amara, a climber that is usually found in tropical climates were collected from the local gardens which was authenticated by Plant Taxonomist Prof. P. Jayaraman, Ph.D., Plant Anatomy Research Centre, Chennai, Tamil Nadu, India. A specimen was deposited in the Department of Pharmacology in the same college for future reference.

Plant extracts preparation

The leaves were collected, dried under shade and coarsely powdered. The powder was then extracted using a Soxhlet extractor with ethyl acetate and ethanol. Both the extracts were dried under pressure using a rotary flask evaporator. The extracts thus obtained were used by dissolving each time with 4% v/v tween 80. The percentage yield of EAELA was 9% w/w and EELA was 41% w/w.

Phytochemical screening

Both EAELA and EELA were subject to phytochemical screening for the presence or absence of phytochemical constituents such as alkaloids, carbohydrates, steroids, proteins, tannins, phenols, flavonoids, mucilage, glycosides, saponins, and terpenes were done.[9]

Chemicals

Ethanol obtained from E. Merck (India) Ltd., and silymarin obtained from M/S. Micro Labs Ltd., Mumbai and all other analytical grade chemicals were obtained from S. D. Fine Chemical Ltd., Mumbai, India.

Experimental animals

Wistar rats weighing 200–250 g and Swiss albino mice (20–25 g) were used for performing the pharmacological experimental studies. The rats were obtained from Tamil Nadu Veterinary and Animal Sciences University, Chennai. The animals were kept under proper conditions (day/night rhythm) 8.00 AM to 8.00 PM, 23°C ± 2°C room temperature, 55% ± 5% relative humidity and standard pelleted diet and water *ad libitum*. The animals were housed for 1 week in polypropylene cages prior to the experiments to acclimatize to the laboratory conditions. It is randomly distributed into 5 different groups with 6 animals in each group under identical conditions throughout the experiments. All the experimental protocols were followed according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi, India. The experimental protocol was approved by the standing Institutional Animal Ethics Committee of the college (IAEC X-14/CLBMCP/2004-2005, Dated: 19/04/2004).

Acute toxicity study

Acute oral toxicity study of both extract of *L. acutangula* var. amara was carried out according to the Organization for Economic Cooperation and Development guidelines 423.[10]

Pentobarbital-induced sleeping time

The effect of plant extracts on pentobarbital-induced sleeping time and CCl_4-induced prolongation of pentobarbital-induced sleeping time was studied in mice.[11]

- Group I: Animals received 4% v/v Tween 80 (10 ml/kg, p. o.) and pentobarbital (75 mg/kg, i. p.)
- Group II: Animals received CCl_4 mixture (1.5 ml/kg, p. o. in 50% v/v Olive oil) followed by pentobarbital (75 mg/kg, i. p.)
- Group III: Animals received EAELA (250 mg/kg, p. o.) and CCl_4 mixture (1.5 ml/kg, p. o. in 50% v/v olive oil) 1 h posttreatment of the last dose of extract, followed by pentobarbital (75 mg/kg, i. p.)
- Group IV: Animals received EELA (250 mg/kg, p. o.) and CCl_4 mixture (1.5 ml/kg, p. o. in 50% v/v Olive oil) 1 h posttreatment of the last dose of extract, followed by pentobarbital (75 mg/kg, i. p.)
- Group V: Animals received silymarin (25 mg/kg, p. o.) and CCl_4 mixture (1.5 ml/kg, p. o. in 50% v/v Olive oil) followed after 24 h by pentobarbital (75 mg/kg, i. p.).

After administration of pentobarbital injection the mice were placed on their backs on a table and sleeping time was noted. The time between the loss of righting reflex and its recovery was taken as duration of pentobarbitone-induced sleeping time.

Acute liver toxicity induction method

Acute liver injury in rats was induced by a single dose intraperitoneal injection of CCl_4 mixture (3 ml/kg in 50% v/v olive oil)[12]

- Group I: Animals received 4% tween 80 v/v (10 ml/kg, p. o.)
- Group II: Animals received 4% tween 80 v/v (10 ml/kg, p. o.) and CCl_4 mixture (3 ml/kg in 50% v/v olive oil) intraperitoneal
- Group III: Animals received EAELA (250 mg/kg, p. o.) and CCl_4 mixture (3 ml/kg in 50% v/v OLIVE oil) intraperitoneal
- Group IV: Animals received EELA (250 mg/kg, p. o.) and CCl_4 mixture (3 ml/kg in 50% v/v olive oil) intraperitoneal
- Group V: Animals received silymarin (25 mg/kg, p. o.) and CCl_4 mixture (3 ml/kg in 50% v/v olive oil) intraperitoneal.

All the animals were treated with respective drugs and extracts for 5 days. The CCl_4 mixture was administered on the 4th day 1 h before drug

administration. On the 6th day, all the animals were sacrificed by using light ether anesthesia. Blood was collected, allowed to clot and serum was separated. Liver was dissected out and used for biochemical and histopathological studies. The wet weight of liver was noted as mg weight of liver/g body weight.

Chronic liver toxicity induction

Chronic administration of CCl_4 to rats leads to several disturbances of hepatic function together with histologically observed liver cirrhosis.[13]

- Group I: Animals received 4% tween 80 v/v (10 ml/kg, p. o.) for 8 weeks daily
- Group II: Animals received 4% tween 80 v/v (10 ml/kg, p. o.) for 8 weeks daily and CCl_4 mixture (1 ml/kg in 50% v/v olive oil) was administered orally twice a week
- Group III: Animals received EAELA (250 mg/kg, p. o.) for 8 weeks daily and CCl_4 mixture (1 ml/kg in 50% v/v olive oil) was administered orally twice a week
- Group IV: Animals received EELA (250 mg/kg, p. o.) for 8 weeks daily and CCl_4 mixture (1 ml/kg in 50% v/v olive oil) was administered orally twice a week
- Group V: Animals received silymarin (25 mg/kg, p. o.) for 8 weeks daily and CCl_4 mixture (1 ml/kg in 50% v/v Olive oil) was administered orally twice a week.

All the animals were observed daily and changes in body weight were noted once a week. All the animals were sacrificed using light ether anesthesia at the end of the experiment. Blood was collected and allowed to clot and serum was separated. Liver was dissected out and used for biochemical and histopathological studies. The wet weight of liver was noted as mg weight of liver/g body weight.

Biochemical assay

Serum was separated by centrifugation at 3000 rpm for 10 min. The liver samples were washed in saline and a 10% w/v homogenate was prepared using 0.1 M Tris-HCL buffer, pH 7.4 and centrifuged at 3000 rpm for 10 min using a centrifuge and the supernatant was used for the investigations of hepatic enzymes.

Serum glutamic oxaloacetic transaminase (SGOT),[14] serum glutamic pyruvic transaminase (SGPT),[14] alkaline phosphatase (ALP),[15] total bilirubin,[16] and total protein[17] were assayed using standard assay kits. From the liver homogenate reduced glutathione (GSH)[18] and lipid peroxidation (LPO)[19] levels were studied. All the enzyme assay was read at specified nm using Shimadzu UV spectrophotometer.

Histopathological studies

The liver pieces were immediately collected and fixed in 10% formalin, dehydrated in ethanol, cleared in xylene, and embedded in paraffin. Sections (4–5 μm) were prepared and stained with hematoxylin and eosin dye for microscopic observation.

Statistical analysis

The results were analyzed statistically by analysis of variance, which is followed by Dunnet's *t*-test using Statistical Package for the Social Sciences software statistics for window, version 20.0 (IBM Crop. Armonk, NY, USA). The data represented mean ± standard deviation and $P < 0.05$ was considered statistically significant.

RESULTS

Preliminary phytochemical screening

EAELA showed the presence of alkaloids, flavonoids, glycosides, saponins, carbohydrates and terpenes. However, steroids, phenols, gums, mucilage, protein, tannins, and glycosides were absent.

EELA showed the presence of alkaloids, carbohydrates, phenols, flavonoids, gums, mucilage, saponins, and terpenes. Steroids, proteins, tannins, and glycosides were absent.

Pentobarbital-induced sleeping time

Carbon tetrachloride-treated animals showed significant ($P < 0.001$) increase in pentobarbital-induced sleeping time when compared to control mice. However, prior treatment with EAELA and EELA showed significant reduction ($P < 0.01$) in sleeping time when compared to CCl_4-treated animals. Standard silymarin-treated animals also showed highly significant ($P < 0.001$) protection when compared to the CCl_4-treated animals [Figure 1].

Body weight reduction

Change in body weight was observed once a week in the chronic study for 8 weeks and the CCl_4-treated rats showed significant ($P < 0.001$) reduction when compared to normal rats. EAELA and EELA-treated animals showed significant ($P < 0.01$, $P < 0.001$) protection respectively in body weight changes when compared to the CCl_4-treated animals. Silymarin-treated animals showed significant ($P < 0.001$) protection when compared to the control animals [Figure 2]. The acute and chronic liver injury groups showed a significant increase in the wet weight of the liver compared to control while the EAELA and EELA groups showed a decrease in wet liver weight when compared with the CCl_4 group.

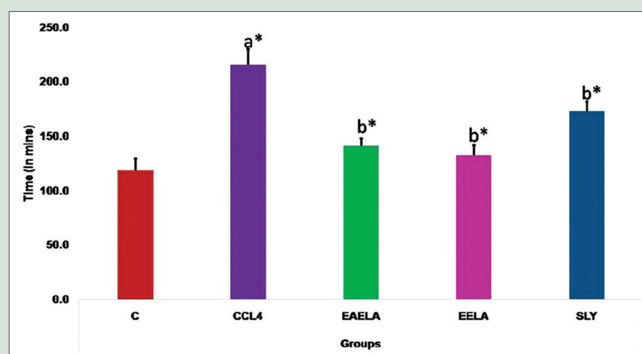

Figure 1: Pentobarbital-induced sleeping time in mice. Comparisons were made between: (a) Group I versus II, (b) Group II versus III, IV and V. The values are expressed as mean ± standard deviation (n = 6). Note: *P < 0.001

Table 1a: Serum glutamic oxaloacetic transaminase in serum (U/ml)

Treatment	*n*	Acute	Chronic
Normal	6	65.7±5.3	65.7±5.3
Control + CCl_4	6	166.7±9.9[a,*]	593.2±16.59[a,*]
EAELA (250 mg/kg)	6	97.2±8.4[b,*]	169.3±8.64[b,*]
EELA (250 mg/kg)	6	86.8±9.9[b,*]	103.2±4.83[b,*]
SLY (25mg/kg)	6	78.3±7.2[b,*]	82.2±3.76[b,*]

*P<0.001; Comparisons were made between: [a]Group I versus II; [b]Group II versus III, IV and V. The values are expressed as mean±SD (n=6). SD: Standard deviation; EAELA: Ethyl acetate extract of *Luffa acutangula*; EELA: Ethanol extract of *Luffa acutangula*; SLY: Silymarin

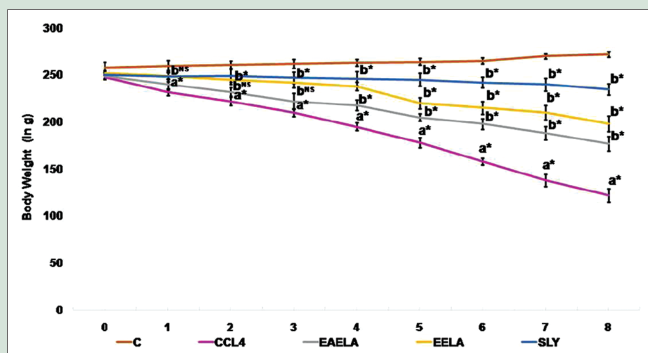

Figure 2: Change in body weight of rats in carbon tetrachloride-induced chronic toxicity. Comparisons were made between: (a) Group I versus II, (b) Group II versus III, IV and V. The values are expressed as mean ± standard deviation (n = 6). Note: *P < 0.001

Silymarin group also showed significant weight reduction in both the models.

Hepatic enzymes

SGOT [Tables 1a and b], SGPT [Tables 2a and b] and Alkaline phosphate [Table 3] levels increased significantly in chronic and acute liver injured group challenged with CCl_4 while a significant reduction was observed with the EAELA, EELA and silymarin groups when compared to the CCl_4 group.

A significant increase in bilirubin levels was noticed in the CCl_4-treated animals in both acute and chronic models when compared to control group. A significant protection was noticed in the ethanol, ethyl acetate and silymarin groups in both the models [Table 4].

Total protein levels in serum and liver were significantly decreased in the CCl_4 group in both acute and chronic models when compared to control models. A significant protection (P < 0.001) in serum protein [Table 5a] and liver protein [Table 5b] levels were observed in the ethanol and ethyl acetate and silymarin-treated groups when compared with the CCl_4 groups.

Reduced GSH activity was significantly reduced in CCl_4-treated animals in both models when compared to the control group. There was a significant protection observed in their activity with ethanol and ethyl acetate extract-treated groups when compared to the CCl_4 group. Standard silymarin group also showed a significant reduction in both the models [Table 6]. LPO level was significantly increased in CCl_4-treated animals in both models when compared to control. There was a significant reduction in lipid peroxide level in ethanol, ethyl acetate extract groups and silymarin group in both acute and chronic models [Table 7].

Histopathological studies

The histopathology of the CCl_4-induced liver damage showed inflammation and patches of cell necrosis in the acute damage. Severe hepatic lesions and cirrhotic nodules was evident in the chronic damage induced by CCl_4 hepatotoxicant. The EAELA and EELA extracts showed normal architecture, mild inflammation and mild hepatocellular damage against liver damage induced by CCl_4. A significant correlation of hepatoprotective activity was observed in terms of biochemical indices and histopathological observation [Figure 3].

DISCUSSION

Globally, the usage of traditional system of medicine and alternative medicine has increased over the past few eras. India is known for its traditional system of medicines including Ayurveda, Siddha and Unani. In traditional medicine, plants are most commonly used for

Table 1b: Serum glutamic oxaloacetic transaminase in liver (U/ml)

Treatment	n	Acute	Chronic
Normal	6	29.0±3.63	29.0±3.63
Control + CCl_4	6	176.8±8.61[a,*]	447.7±12.36[a,*]
EAELA (250 mg/kg)	6	68.3±3.44[b,*]	228.3±9.40[b,*]
EELA (250 mg/kg)	6	54.8±3.82[b,*]	182.3±10.09[b,*]
SLY (25 mg/kg)	6	39.7±5.24[b,*]	55.3±3.72[b,*]

*P<0.001; Comparisons were made between: [a]Group I versus II; [b]Group II versus III, IV and V. The values are expressed as mean±SD (n=6). SD: Standard deviation; EAELA: Ethyl acetate extract of *Luffa acutangula;* EELA: Ethanol extract of *Luffa acutangula*; SLY: Silymarin

Table 2a: Serum glutamic pyruvic transaminase in serum (U/ml)

Treatment	n	Acute	Chronic
Normal	6	50.2±2.79	50.2±2.79
Control + CCl_4	6	128.5±5.72[a,*]	413.0±14.89[a,*]
EAELA (250 mg/kg)	6	86.2±7.11[b,*]	118.7±11.69[b,*]
EELA (250 mg/kg)	6	78.7±6.12[b,*]	89.5±5.68[b,*]
SLY (25 mg/kg)	6	59.5±4.59[b,*]	69.3±4.37[b,*]

*P<0.001; Comparisons were made between: [a]Group I versus II; [b]Group II versus III, IV and V. The values are expressed as mean±SD (n=6). SD: Standard deviation; EAELA: Ethyl acetate extract of *Luffa acutangula*; EELA: Ethanol extract of *Luffa acutangula*; SLY: Silymarin

Table 2b: Serum glutamic pyruvic transaminase in liver (U/ml)

Treatment	n	Acute	Chronic
Normal	6	28.0±4.90	28.0±4.90
Control + CCl_4	6	128.5±9.31[a,*]	441.3±19.79[a,*]
EAELA (250 mg/kg)	6	81.3±4.97[b,*]	141.8±17.37[b,*]
EELA (250 mg/kg)	6	53.3±7.84[b,*]	128.8±6.68[b,*]
SLY (25 mg/kg)	6	38.3±4.63[b,*]	41.8±4.36[b,*]

*P<0.001; Comparisons were made between: [a]Group I versus II; [b]Group II versus III, IV and V. The values are expressed as mean±SD (n=6). SD: Standard deviation; EAELA: Ethyl acetate extract of *Luffa acutangula*; EELA: Ethanol extract of *Luffa acutangula*; SLY: Silymarin

Table 3: Alkaline phosphate in serum (U/ml)

Treatment	n	Acute	Chronic
Normal	6	102.7±4.55	102.7±4.55
Control + CCl_4	6	240.7±13.02[a,*]	661.7±29.10[a,*]
EAELA (250 mg/kg)	6	173.5±7.53[b,*]	199.5±12.77[b,*]
EELA (250 mg/kg)	6	165.8±15.09[b,*]	178.8±7.33[b,*]
SLY (25 mg/kg)	6	138.0±7.18[b,*]	156.0±7.87[b,*]

*P<0.001. Comparisons were made between: [a]Group I versus II; [b]Group II versus III, IV and V. The values are expressed as mean±SD (n=6). SD: Standard deviation; EAELA: Ethyl acetate extract of *Luffa acutangula*; EELA: Ethanol extract of *Luffa acutangula*; SLY: Silymarin

Table 4: Total bilirubin in serum (mg/dL)

Treatment	n	Acute	Chronic
Normal	6	0.2±0.04	0.2±0.04
Control + CCl_4	6	1.2±0.32[a,*]	2.6±0.43[a,*]
EAELA (250 mg/kg)	6	0.4±0.15[b,*]	1.2±0.36[b,*]
EELA (250 mg/kg)	6	0.3±0.11[b,*]	0.5±0.08[b,*]
SLY (25 mg/kg)	6	0.2±0.04[b,*]	0.3±0.06[b,*]

*P<0.001. Comparisons were made between: [a]Group I versus II; [b]Group II versus III, IV and V. The values are expressed as mean±SD (n=6). SD: Standard deviation; EAELA: Ethyl acetate extract of *Luffa acutangula*; EELA: Ethanol extract of *Luffa acutangula*; SLY: Silymarin

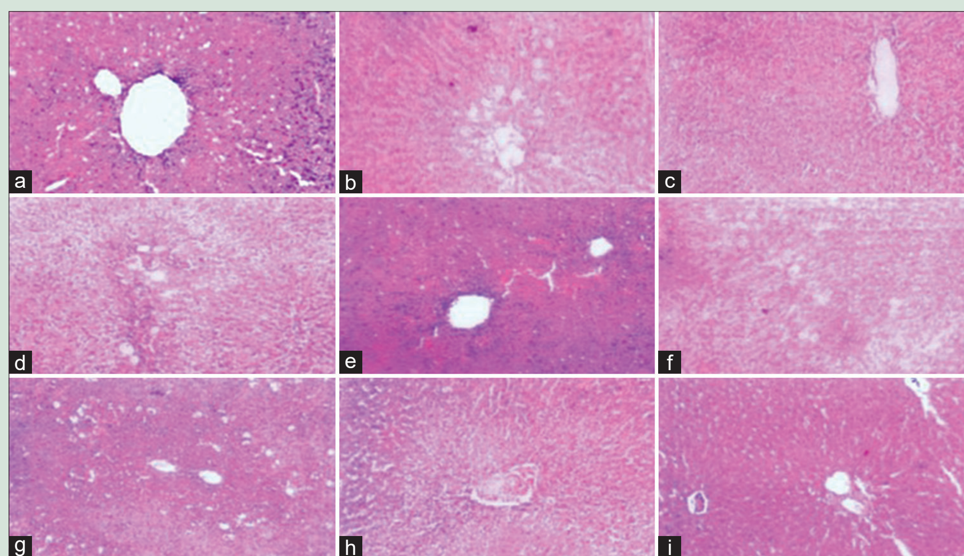

Figure 3: *Luffa acutangula* var. amara ameliorate carbon tetrachloride-induced hepatic injury rats model: Photomicrograph of rat liver cross section was showing. (a) Normal lobular architecture with central veins and radiating hepatic cords; (b) Inflammation is seen, fatty changes, gross necrosis in carbon tetrachloride acute model; (c) Mild inflammation and dilation of central vein in ethyl acetate extract of *Luffa acutangula* acute model; (d) Dilation of central vein and mild changes in centrilobular region in ethanol extract of *Luffa acutangula* acute model; (e) Normal central vein with mild dilation in silymarin acute model; (f) Severe hepatic lesions, cirrhotic nodules and collagen deposition is seen in carbon tetrachloride chronic model; (g) Inflammation with fatty changes and mild hepatocellular damage in ethyl acetate extract of *Luffa acutangula* chronic model; (h) Sinusoidal dilation and peripheral hepatocytic fatty changes in ethanol extract of *Luffa acutangula* chronic model; Central vein seen with some mild hepatocytic changes in SLY chronic model

Table 5a: Total protein in serum (g/dL)

Treatment	*n*	Acute	Chronic
Normal	6	7.1±1.13	7.1±1.13
Control + CCl_4	6	3.1±1.13[a,*]	2.05±0.82[a,*]
EAELA (250 mg/kg)	6	5.6±0.83[b,*]	3.48±0.98[b,#]
EELA (250 mg/kg)	6	6.3±0.71[b,*]	5.43±1.31[b,*]
SLY (25 mg/kg)	6	6.8±0.76[b,*]	6.28±1.07[b,*]

*P<0.001; #P<0.01; Comparisons were made between: [a]Group I versus II; [b]Group II versus III, IV and V. The values are expressed as mean±SD (n=6). SD: Standard deviation; EAELA: Ethyl acetate extract of *Luffa acutangula*; EELA: Ethanol extract of *Luffa acutangula*; SLY: Silymarin

Table 5b: Total protein in liver (mg/g of wet tissues)

Treatment	*n*	Acute	Chronic
Normal	6	239.0±20.11	239.0±20.11
Control + CCl_4	6	191.0±18.89[a,*]	170.8±19.20[a,*]
EAELA (250 mg/kg)	6	214.2±19.13[b,@]	199.5±17.27[b,@]
EELA (250 mg/kg)	6	221.3±19.24[b,#]	208.2±11.44[b,@]
SLY (25 mg/kg)	6	230.7±13.49[b,*]	230.5±12.84[b,*]

*P<0.001; #P<0.01; @P<0.05. Comparisons were made between: [a]Group I versus II; [b]Group II versus III, IV and V. The values are expressed as mean±SD (n=6). SD: Standard deviation; EAELA: Ethyl acetate extract of *Luffa acutangula*; EELA: Ethanol extract of *Luffa acutangula*; SLY: Silymarin

the treatment, and part of minerals is also used. Medicinal plants are generally used as substances to treat or prevent diseases. There are numerous types of drugs that are derived from plants such as analgesics, cardiotonic, antimalarial, antihypertensive, memory enhancing, muscle relaxant, anti-inflammatory, anthelmintic, antitussive, central nervous system stimulant, anti-parkinsonism, anti-cholinergic and anti-tumour.[20,21] Herbal drugs have appeared to exhibit new clinical effects as an alternative choice for the treatment of diseases. A further and deep research had been carried out by targeting at few chronic diseases such as hepatotoxicity which could lead to the new discovery of drugs for those diseases with improved efficacy. *L. acutangula* is one such medicinal herb that has cathartic, laxative, emetic, diuretic, demulcent, expectorant and hepatoprotective activities.[22,23]

Upon initial phytochemical screening the EAELA and EELA showed the presence of phytochemical constituents like alkaloids, carbohydrates, phenols, flavonoids, gums and mucilage, saponins and terpenes. The pilot study of EAELA and EELA demonstrated the hepatoprotective nature by prolonging the sleeping of mice on injection of pentobarbital. One of the possible mechanisms for the prolongation of pentobarbital-induced sleeping time is the inhibition of pentobarbital metabolism. This is an indicator of normalization of cytochrome P-450 and related hepatic mixed function oxidase enzymes system. The duration of pentobarbital-induced sleep in intact animals is considered as a reliable index for the activity of hepatic microsomal drug metabolizing enzymes. The pentobarbital is metabolized by hepatic microsomal drug metabolizing enzymes to inactive metabolites and any drug with an inhibitory effect on microsomal drug metabolizing enzymes is expected to prolong pentobarbital-induced sleep time.[24-26]

Acute and chronic injury can be made by the well-known hepato-toxicant Carbon tetrachloride. It is metabolized by the mixed function oxidase system in the endoplasmic reticulum of liver. Cleavage of the carbon-chloride bond results in the formation of trichloromethyl radicals (CCl_3•) which are highly unstable and immediately react with membrane components. This free radical causes lipid and protein peroxidation thus leading to cellular damage.[27]

The liver damage in rat is evident by increase in marker enzyme levels like SGOT, SGPT, ALP and bilirubin in the serum.[28] The marker enzymes are the important indices for the diagnosis of liver diseases and it indicates the damage of the hepatic cells, cellular leakage and loss of functional integrity of cell membrane in the liver. Treatment with both the extracts lowered the elevated serum enzyme levels, where the serum levels of transaminases return to normal with healing of hepatic parenchyma and regeneration of hepatocytes.[29] The probable mechanism behind

Table 6: Glutathione peroxidase (µg of glutathione consumed/g of wet tissues) in liver

Treatment	n	Acute	Chronic
Normal	6	17.8±2.79	17.8±2.79
Control + CCl_4	6	10.5±2.90[a,*]	7.7±1.18[a,*]
EAELA (250 mg/kg)	6	13.6±2.67[b,@]	12.8±1.58[b,@]
EELA (250 mg/kg)	6	15.6±2.07[b,*]	14.4±1.78[b,#]
SLY (25 mg/kg)	6	17.2±1.50[b,*]	16.4±1.90[b,*]

*$P<0.001$; #$P<0.01$; @$P<0.05$. Comparisons were made between: [a]Group I versus II; [b]Group II versus III, IV and V. The values are expressed as mean±SD (n=6). SD: Standard deviation; EAELA: Ethyl acetate extract of *Luffa acutangula*; EELA: Ethanol extract of *Luffa acutangula*; SLY: Silymarin

Table 7: Lipid peroxidation (nm of melondialdehyde/g of wet tissues) in liver

Treatment	n	Acute	Chronic
Normal	6	6.2±1.17	6.2±1.17
Control + CCl_4	6	8.2±0.75[a,*]	11.0±0.89[a,*]
EAELA (250 mg/kg)	6	6.7±0.82[b,@]	7.2±0.75[b,@]
EELA (250 mg/kg)	6	6.5±0.55[b,#]	6.7±0.52[b,@]
SLY (25 mg/kg)	6	6.2±0.75[b,*]	6.5±0.84[b,*]

*$P<0.001$; #$P<0.01$; @$P<0.05$; Comparisons were made between: [a]Group I versus II; [b]Group II versus III, IV and V. The values are expressed as mean±SD (n=6). SD: Standard deviation; EAELA: Ethyl acetate extract of *Luffa acutangula*; EELA: Ethanol extract of *Luffa acutangula*; SLY: Silymarin

this protective nature is either through enhanced protein synthesis or interference with the microsomal activation of CCl_4 or its accelerated detoxification (free radical scavenging activity) or excretion.

The alkaline phosphate level is elevated in many types of liver disease. It is an enzyme that is produced in the bile ducts and sinusoidal membranes of the liver and is also present in many other tissues. The experimental extracts are reduced the elevated alkaline phosphate levels thereby stabilizing the dysfunction in acute and chronic liver injury.[30]

The liver plays a significant role in serum protein synthesis, being the source of plasma albumin and fibrinogen and also other important components like α, β and γ globulin. The serum albumin level is low in hepatic diseases.[30] The present study reveals that in animals pretreated with the extracts of *L. acutangula* prior to challenge with CCl_4, the liver biosynthesis of proteins continues to be unaffected.

Bilirubin is an endogenous organic anion that binds reversibly to albumin, transported to the liver and then conjugated with glucuronic acid and excreted in the bile. Hepatobiliary diseases or hepatic injury is indicated when conjugated fraction of total bilirubin exceeds the upper limit of normal, even if the total serum bilirubin is normal. The increased levels of direct and indirect bilirubin due to CCl_4 exposure was significantly reduced by treatment with silymarin and the plant extracts of *L. acutangula*.

GSH is a seleno enzyme two thirds of which (in liver) is present in cytosol and one third in the mitochondria. It catalysis the reaction of hydroperoxides with reduced GSH to form GSH disulphide and the reduction product of the hydroperoxides.[31] Depletion of GSH peroxidase content may also lower the GSH activity. GSH level was significantly reduced in CCl_4-treated animals an upward reversal was observed after treatment with plant extracts.

The level of lipid peroxide is a measure of membrane damage and the alterations in structure and function of cellular membranes. In this study elevation of LPO is seen in CCl_4-treated animals. The increase in melondialdehyde levels in liver suggests enhanced LPO leading to tissue damage and thus antioxidant defense mechanism fails to prevent formation of excessive free radicals.[32] A significant decrease in the levels of lipid peroxides in *L. acutangula* var. amara extracts pretreated rats suggests that the extracts have the ability to protect the liver from free radical injury induced by carbon tetrachloride.

The preliminary screening of extracts of *L. acutangula* var. amara showed the hepatoprotective activity against CCl_4-induced hepatic dysfunction and this may be attributed to the presence of phytochemical constituents like flavonoids, alkaloids and glycosides in both extracts. The plant constituents are many as their activities are also complex. However, the literature proves the therapeutic potential of flavonoids as natural antioxidants. Hence the hepatoprotective activity of extracts of *L. acutangula* var. amara may be attributed to the complex pharmacological action of phytoconstituents present in the extract, particularly the flavonoids.

CONCLUSION

The present study on the plant *L. acutangula* var. amara against CCl_4-induced acute and chronic liver damage reveals the hepatoprotective nature of the plant. The presence of flavonoids could be responsible or hepatoprotective activity, is more likely to be involved in the reaction with the proteins of the layer tissues and there by showing the activity. Further studies need to done for identification, isolation, and purification of the active ingredients of this hepatoprotective plants and to examine their efficacy and safety through various animal models.

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Conflicts of interest

There are no conflicts of interest.

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