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Protective Effect of *Luffa acutangula* (var) *amara* against Carbon Tetrachloride-Induced Hepatotoxicity in Experimental Rats

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Abstract: Hepatoprotective and anti-oxidant activity of Ethanol Extract of *Luffa acutangula* (var) *amara* (EELA) and were evaluated against carbon tetrachloride (CCl_4) induced hepatic damage in rats. The extract were administered orally at dose dependent manner of 200, 400, 600 mg kg^{-1}, b.wt, p.o. The substantially elevated serum enzymatic levels of Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Serum alkaline Phosphatase (ALP), total bilirubin, total cholesterol and total protein were restored towards normalization significantly by the extract. Silymarin was used as standard reference and exhibited significant hepatoprotective activity against carbon tetrachloride induced haptotoxicity in rats. The biochemical observations were supplemented with histopathological examination of rat liver sections. The results of this study strongly indicate that *Luffa acutangula* (var) *amara* leaves extract have potent hepatoprotective action against carbon tetrachloride induced hepatic damage in rats. Ethanolic extract was found more potent hepatoprotective. Mean while *in vivo* antioxidant activities were also screened which were positive for EELA extract. This study suggests that possible mechanism of this activity may be due to free radical scavenging and antioxidant activities which may be due to the presence of flavanoids in the extract.

Key words: *Luffa acutangula* (var) *amara*, carbon tetrachloride, hepatoprotective activity, antioxidant activity, silymarin, histopathology

INTRODUCTION

Liver diseases are a serious health problem. In the absence of reliable liver protective drugs in allopathic medical practices, herbs play a role in the management of various liver disorders. Numerous medicinal plants and their formulations are used for liver disorders in ethno medical practices and in traditional system of medicine in India. However, we do not have satisfactory remedy for serious liver disease; most of the herbal drugs speed up the natural healing process of liver. So, the search for effective hepatoprotective drug continues. In spite of tremendous advances in modern medicine, no effective drugs are available which stimulate liver functions and offers protection to the liver from the damage or help to regenerate hepatic cells (Chattopadhyay, 2003). In absence of reliable liver-protective drugs in modern medicine, a large number of medicinal preparations are recommended for the treatment of liver disorders (Chatterjee, 2000) and quite often claimed to offer significant relief. Attempts are being made globally to get scientific evidences for traditionally reported herbal drugs.

Carbon tetrachloride (CCl_4) is a selective hepatotoxic chemical agent. CCl_4 induced reactive free radicals initiate cell damage through two different mechanisms of covalent binding to the membrane proteins and cause lipid Peroxidation (Kanter *et al*., 2005). Many investigators have utilized this chemical to induce liver cirrhosis in experimental animals (Parola *et al*., 1992). Liver cirrhosis is a major public health problem all over the world. Now-a-days, the prevention of liver cirrhosis and fibrosis is the major and vital concern of the therapy in hepatology. *L. acutangula* (var) *amara* belonging to the family-cucurbitaceae is an annual herb found in all parts of India, especially the western peninsula and ceylon (Chopra *et al*., 1996).

This plant is used as laxative, carminative digestable, alixteric a tonic to the intestines, used to cure vata, kapha, biliousness, anemia, liver complaints, leucoderma, piles, bronchitis, acites, tumors and tuberculous glands, uterine and vaginal tumors. It is said to be useful in asthma. The kernel of seeds is used to treat dysentery (Kirtikar and Basu, 1999; Chopra, 1958). Isolation and structure elucidation of seven new oleanane type triterpene saponins-acutosides A-G from whole plant, seeds

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showed presence of two hepatoglycosides-acutoside H and acutoside 1 (Nagao *et al*., 1991). Crystalline bitter principle Cucurbitacin B and oleanolic acid has been reported (Barua *et al*., 1958). An unnamed alkaloid has been reported (Willaman and Li, 1970). The alcoholic extract of seeds yielded a crude saponin which on hydrolysis gave oleanolic acid. The ether extract of fruit mesocarp yielded Cucurbitacin B and Cucurbitacin E whereas, the ethanolic extract gave oleanolic acid (Mallavarapu and Rao, 1979). The fruits have been reported to have oxalate, fluoride, oxalic acid, calcium, phosphorus, iodine and fluorine (Gopalan *et al*., 1984). The plant has pharmacological actions like demulcent, diuretic, bitter tonic, nutritive and expectorant (Aswal *et al*., 1984).

Hypoglycemic action and abortificient activity of this plant performed in female rats (Singh *et al*., 1978). The leaves of the plant support the growth and development of the larvae of the pest *Diacrisia oblique* (Deshmukh *et al*., 1979). The seed contains an enzyme saponin which produces vomiting and purging in dogs (Greta and Kocha, 1943). It also contains ribosome activating proteins which have immunomodulatory and anti-tumor activities (Ng *et al*., 1992). There were no reports on hepatoprotective and antioxidant activity for this plant. Hence in the present study, the possibility of hepatoprotective and anti-oxidant activity of leave extracts of *Luffa acutangula* (var) *amara* were evaluated in carbon tetrachloride induced liver injury model in rats.

MATERIALS AND METHODS

Animals: Colony inbred strains of wistar rats of both sex weighing (180-200 g) and mice (20-25 g) were used for the pharmacological studies. The animals were kept under standard conditions (day/night rhythm) 8.00 am-8.00 pm, 22±2°C room temperature, standard pelleted diet (Hindustan Lever, Bangalore) and water *ad libitum*. The animals were housed for one week in polypropylene cages prior to the experiments to acclimatize to laboratory conditions. Rats and mice were divided into groups. Each groups containing six animals and were kept in different cages. Animals were selected at random and both sexes were used. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC).

Plant collection and identification: The fresh leaves of *Luffa acutangula* (var) were collected from the forest in Courtallum, Tirunelvelli dist during the month of July to August 2005. The plant was identified and authenticated by Dr. S. Jayaraman, Botanist, Plant anatomy Research Centre, West Tambaram, 600045 Chennai. A voucher specimen was deposited in the Department of Pharmacology, C. L. Baid Metha College of Pharmacy, Chennai, Tamil Nadu (Voucher no. 207/2006).

Preparation of extract: Leaves were collected, dried under shade, coarsely powdered and extracted with ethanol using soxhlet extractor. Extracts were dried under reduced pressure using a rotary flash evaporator and stored between 0-4°C protected from sunlight. The percentage yield of ethanol (EELA) extract was found to be 11% w/w. The extract was used for the pharmacological studies by dissolving in 4% v/v tween 80.

Phytochemical study: The EELA leaves were subjected to evaluate the preliminary phytochemical screening for various plant constituents (Kokate, 1991; Horbone, 1998).

Acute toxicity studies: Adult albino mice of either sex with the body weights ranging from 18-30 g were selected. Since the compound was insoluble in water, it was mixed evenly in 4% v/v tween 80 solution and administered orally in the doses of 50, 100, 250, 500, 1000, >2000 mg kg^{-1} in different groups having three mice each. One group was treated with the vehicle which serves as solvent control. Observations for toxic symptoms and mortality were made >72 h.

Chronic CCl_4 induced liver injury: Chronic administration of CCl_4 to rats leads to several disturbances of hepatic function together with histological observed liver cirrhosis (Augusti *et al*., 2005; Recknagel, 1983). The rats were divided into six groups; each group consists of six animals. The groups were administered with drug and extract as follows:

Group 1: Animals were administered with vehicle alone (10 mL kg^{-1}, b.wt, p.o).

Group 2: Animals were administered with vehicle (10 mL kg^{-1}, b.wt, p.o) for 30 days and CCl_4 (1 mL kg^{-1}, b.wt, p.o) was administered twice a week.

Group 3-5: Animals were administered EELA (200, 400 and 600 mg kg^{-1}, b.wt, p.o) and CCl_4 (1 mL kg^{-1}, b.wt, p.o) was administered twice a week.

Group 6: Animals were administered silymarin (25 mg kg^{-1}, b.wt, p.o) and CCl_4 (1 mL kg^{-1}, b.wt, p.o) was administered twice a week.

All the animals were observed daily and a change in body weight was noted once in a week. All the animals were kept 24 h fasting after sacrificed by cervical

decapitation under light ether anesthesia at the end of the experiment. Blood was collected, allowed to clot and serum was separated.

Liver was dissected, weighed and used for biochemical and histopathological studies. Serum was separated and the dissected liver was washed in ice-cold 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 15 min and the supernatant was used for the following investigations. Assay of enzymes were carried out at particular nm using schimadzu UV spectrophotometer 1601 model.

Biochemical estimation: The biochemical parameters like serum enzymes aspartate aminotranferase (GOT) Glutamate Pyruvate Transaminase (GPT) (Reitman and Frankel, 1997), Serum Alkaline Phosphatase (SALP) (Kind and King, 1954), total cholesterol (Richardson *et al*., 1971), total bilirubin (Malloy and Evelyn, 1937) and total protein (Gornall and Bardawil, 1949) were assayed using assay kit.

Determination of Glutathione Peroxidase (GPx): Glutathione peroxidase of liver homogenate was assayed according to the method (Rotruck *et al*., 1973). The reaction mixture contained 0.2 mL of 0.4 M Tris-HCl buffer pH, 7.0, 0.1 mL of 10 mM sodium azide, 0.2 mL of tissue homogenate (homogenised in 0.4 M, Tris-HCl buffer, pH 7.0), 0.2 mL glutathione and 0.1 mL of 0.2 mM hydrogen peroxide. The contents were incubated at 37°C for 10 min. The reaction was arrested by 0.4 mL of 10% TCA and centrifuged.

Supernatant was assayed for glutathione content by using Ellmans reagent (19.8 mg of 5, 5'-Dithio-bisnitro Benzoic acid (DTNB) in 100 mL of 0.1% sodium nitrate). Glutathione peroxidase activity was expressed as μg of GSH consumed/min/mg protein.

Estimation of Glutathione-S-Transferase (GST): Glutathione-s-transferase of liver homogenate was assayed according to the method (Habig *et al*., 1974). The reaction mixture 3 mL contained 1.0 mL of 0.3 mM phosphate buffer (pH 6.5), 0.1 mL of 30 mM 1-Chloro-2, 4-Dinitrobenzene (CDNB) and 1.7 mL of distilled water. After pre-incubating the reaction mixture at 37°C for 5 min, the reaction was started by the addition of 0.1 mL of tissue homogenate and 0.1 mL of glutathione as substrate. The absorbance was followed for 5 min at 340 nm. Reaction mixture without the enzyme was used as blank. The activity of GST is expressed as μ moles of GSH-CDNB conjugate formed/min/mg protein.

Determination of reduced glutathione (GSH): Reduced glutathione activity of liver homogenate was measured by the method (Moron *et al*., 1979). About 1 mL of tissue homogenate was precipitated with 1 mL of 10% TCA and was removed by centrifugation. To an aliquot of the supernatant, 4 mL of phosphate solution and 0.5 mL of DTNB were added. The colour developed was read at 420 nm. Reduced glutathione activity was expressed as $\mu g\ mg^{-1}$ of tissue.

Assay of Superoxide Dismutase (SOD): Superoxide dismutase was assayed according to the method (Marklund and Marklund, 1974). To 1 mL of the sample, 0.25 mL of absolute ethanol and 0.15 mL of chloroform were added. After 15 min of shaking in a mechanical shaker, the suspension was centrifuged and the supernatant obtained constituted the enzyme extract. The reaction mixture for auto-oxidation consisted of 2 mL of buffer, 0.5 mL of 2 mM pyrogallol and 1.5 mL of water. Initially, the rate of auto-oxidation of pyrogallol was noted at an interval of 1 min for 3 min. The assay mixture of the enzyme contained 2 mL of 0.1M Tris-HCl buffer, 0.5 mL of pyrogallol, aliquots of the enzyme preparation and water to make up 4 mL. The rate of inhibition of pyrogallol auto-oxidation after the addition of the enzyme was noted. The enzyme activity was expressed in terms of units/min/mg protein.

Assay of Catalase (CAT): Catalase activity was assayed according to the method (Sinha, 1972). About 0.1 mL of the homogenate was taken to which 1 mL of phosphate buffer and 0.5 mL of H_2O_2 were added. The reaction was arrested by addition of 2 mL dichromate acetic acid reagent. Standard H_2O_2 in the range of 4-20 μ moles were taken and treated similarly. The tubes were heated in a boiling water bath for 10 min. The green colour developed was read at 570 nm. The enzyme activity in tissue homogenate was expressed as μ moles of H_2O_2 consumed/min/mg protein at 37°C.

Estimation of Lipid Peroxidation (LPO): Lipid peroxidation in the liver homogenate was determined by measuring the amounts of malondialdehyde produced primarily according to the method (Ohkawa *et al*., 1979). About 0.2 mL of tissue homogenate, 1.5 mL of 20% acetic acid and 0.2 mL of sodium dodecyl sulphate finally 1.5 mL of thiobarbituric acid were added. The volume of the mixture was made up to 4 mL with distilled water and then heated at 95°C in a water bath for 60 min. After incubation, the tubes were cooled to room temperature and final volume was made to 5 mL in each tube. About

5 mL of n-butanol: pyridine (15: 1) mixture was added and the contents were vortexed thoroughly for 2 min. After centrifugation at 3000 rpm for 10 min, the organic upper layer was taken and its OD read at 532 nm against an appropriate blank without the sample. The levels of lipid peroxides were expressed as n moles of MDA/mg protein in liver homogenate.

Vitamin E: Vitamin E was determined by the method (Desai, 1984). To the 3 mL of liver homogenate, add 3 mL of ethanol and 3 mL of xylene, the mixture was centrifuged at 3000 rpm for 5 min. From the centrifugate, 2 mL of xylene layer was separated and mixed with 2 mL of bipyridyl reagent. Then, 3 mL of aliquot was taken and read at 460 nm. To this, 0.66 mL of $FeCl_3$ reagent was added and the OD was measured at 520 nm after 1.5 min. Vitamin E concentration was expressed as n moles/g wet tissue in liver homogenate.

Vitamin C: Vitamin C was determined by the method (Omaye *et al*., 1979). To 1.5 mL of liver homogenate, 0.5 mL of water and 1 mL of 10% TCA were added and mixed thoroughly. The mixture was centrifuged at 3000 rpm for 5 min. To 1 mL of the supernatant 0.2 mL of DTC reagent was added and incubated at 37°C for 3 h. Then 1.5 mL of sulphuric acid was added, mixed well and the solution was allowed to stand at room temperature for another 30 min. The colour developed was read at 520 nm. Vitamin C concentration was expressed as n moles/g wet tissue in liver homogenate.

Histopathological studies: The liver pieces was collected and immediately fixed in 10% formalin, dehydrated in ethanol, cleared in xylene and embedded in paraffin. Sections (4-5 μm) were prepared and then stained with Hematoxylin and Eosin (HE) dye for photo microscopic observation.

Statistical analysis: The data represents mean±SEM. Results were analysed statistically by one way ANOVA followed by Dunnet's t-test. The difference was considered significant when $p<0.05$.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of the EELA showed the presence of alkaloid, carbohydrate, protein, tannins, phenols, gums and mucilage, flavanoids, glycoside, saponin, terpenes. The compound did not show any acute toxic effects and death up to a dose of 2000 mg kg^{-1}, b.wt. Therefore, the lethal dose could not be determined. Change in body weight was observed once in a week in the chronic study for 30 days. In group 2 animals showed significant ($p<0.001$) reduction in body weight when compared to normal animals. The group 3 and 4 treated animals showed significant ($p<0.01$, $p<0.001$) protection, respectively in body weight changes when compared to group 2 treated animals. The group 5 and 6 treated animals showed significant ($p<0.001$) protection when compared to the group 2 animals (Table 1). In chronic liver injury, the group 2 treated animals showed a significant ($p<0.001$) increase in wet weight of the liver when compared to group 1. There was a significant ($p<0.001$, $p<0.05$) decrease in wet weight of liver in group 3 and 4 treated animals in chronic model, respectively when compared with the group 2 treated animals. The group 5 and 6 treated group also showed significant ($p<0.001$) reduction when compared with the group 2 treated animals (Table 1). GOT and GPT levels of serum and liver homogenate were significantly ($p<0.001$) increased in animals challenged with group 2 induced chronic liver injury when compared to group 1 animals.

A significant ($p<0.001$) reduction in GOT and GPT levels were observed in animals treated with group 3 and 4 in chronic model when compared to CCl_4 treated animals. The group 5 and 6 treated group also produced a significant ($p<0.001$) reduction in chronic model when compared to group 2 treated animals (Table 2). The levels of alkaline phosphatase in serum were significantly ($p<0.001$) increased in animals challenged with group 2 in chronic liver injury when compared to group 1 animals. A significant ($p<0.001$) reduction in alkaline phosphatase levels was observed in animals treated with group 3-6 in chronic models when compared to group 2 treated animals (Table 2).

Table 1: Comparison of mean changes in body weight and organ weight in Ccl_4 induced liver injury in animals and control, drug treated animals

	Body weight changes in weeks interval (g) (a)				
Groups	1	2	3	4	Weight of liver (mg/100 g of rat) (b)
1	2.16±0.30	2.66±0.42	3.83±0.47	4.83±0.48	34.36±0.91
2	16.00±0.57^{a***}	25.33±0.55^{a***}	37.16±0.60^{a***}	53.83±0.56^{a***}	58.90±0.02^{a***}
3	7.33±1.33^{b**}	16.66±0.66^{b**}	27.00±0.57^{b**}	31.33±0.55^{b**}	52.60±1.06^{b**}
4	2.93±0.21^{b***}	8.30±0.25^{b***}	14.60±0.47^{b***}	15.60±0.54^{b***}	43.50±0.65^{b***}
5	2.83±0.30^{b***}	7.16±0.06^{b***}	12.33±1.05^{b***}	14.00±0.85^{b***}	42.60±0.90^{b***}
6	2.33±0.21^{b***}	3.00±0.25^{b***}	6.16±0.47^{b***}	10.16±0.54^{b***}	37.95±0.65^{b***}

Comparisons were made between; ^{a}group 1 vs. 2; ^{b}group 2 vs. 3-6. Values are expressed as mean±SEM of six animals in each group. Symbols represent statistical significance; ***$p<0.001$, **$p<0.01$

Table 2: Mean changes in biochemical parameters in serum of CCl_4 induced liver injury in rats

Groups	SGOT (a)	SGPT (b)	Alkaline (c) phosphatase	Total bilirubin (d)	Total (e) cholesterol
1	63.30±1.04	61.08±0.55	103.70±0.84	0.22±0.004	70.88±0.79
2	211.50±3.74^{a***}	188.50±6.19^{a***}	315.16±2.28^{a***}	2.65±0.060^{a***}	58.90±0.24^{a***}
3	103.00±1.22^{b***}	118.25±3.44^{b***}	168.20±1.20^{b***}	0.69±0.010^{b***}	60.50±0.76^{b***}
4	95.65±1.44^{b***}	98.63±0.67^{b***}	142.00±2.14^{b***}	0.57±0.090^{b*}	61.80±0.50^{b***}
5	81.17±0.94^{b*}	86.70±0.67^{b***}	138.50±0.99^{b***}	0.40±0.006^{b***}	68.40±0.18^{b***}
6	86.80±0.75^{b***}	89.53±1.48^{b***}	139.20±1.45^{b***}	0.31± 0.01^{b***}	64.18±0.39^{b***}

Comparisons were made between; ^{a}group 1 vs. 2; ^{b}group 2 vs. 3-6. Values are expressed as mean±SEM of six animals in each group. Symbols represent statistical significance; ***p<0.001, **p<0.01

Table 3: Changes in total protein in serum and liver homogenate in Ccl_4 induced liver injury in rats

	Total protein (mg dL^{-1})	
Groups	Serum	Liver
1	6.82±0.13	7.56±0.08
2	3.49±0.16^{a***}	2.64±0.11^{a***}
3	5.22±0.17^{b***}	6.67±0.06^{b***}
4	6.20±0.08^{b***}	6.95±0.04^{b***}
5	6.63±0.14^{b***}	6.98±0.06^{b***}
6	6.71±0.05^{b***}	6.82±0.13^{b***}

Comparisons were made between; ^{a}group 1 vs. 2; ^{b}group 2 vs. 3-6. Values are expressed as mean±SEM of six animals in each group. Symbols represent statistical significance; ***p<0.001, **p<0.01

In chronic model, the group 2 treated animals showed a significant (p<0.001) increase in bilurubin levels when compared to group 1. There was a significant (p<0.001) protection in bilurubin levels in group 3-6 treated animals in chronic model, respectively when compared with the group-2 treated animals (Table 2). In chronic liver injury, the group 2 treated animals showed a significant (p<0.001) decline in cholesterol levels when compared to group 1. The group 3-6 showed significant (p<0.001)protection in cholesterol levels when compared with the group 2 treated animals (Table 2). The total protein levels in serum and liver were significantly decreased (p<0.001) in animals challenged with group 1 in chronic model when compared to group 2 animals (Table 3).

There was a significant (p<0.001) protection in serum protein level with group 3-6 when compared to group 2 treated animals (Table 4). The GPx activity was significantly (p<0.001) reduced in group 2 treated animals in chronic model when compared to group 1. There was a significant (p<0.01) protection in GPx level in group 3 when compared to group 2 treated animals. Group 4-6 treated animals also showed a significant protection (p<0.001) in chronic model when compared to group 2 treated animals (Table 4).

There was a significant (p<0.001) depletion of GSH in group 2 treated animals in chronic model when compared to group 1. There was a significant (p<0.01) protection in the enzyme level in group 3 and 4 treated animals when compared with the group 2 treated animals. The group 5 and 6 treated group also showed significant (p<0.001) reduction when compared to group 2 treated animals (Table 4).

The GST activity was significantly (p<0.001) reduced in group 2 treated animals in chronic model when compared to group 1. There was a significant p<0.05) protection in the enzyme level in group 3 treated animals in chronic model when compared with the CCl_4 treated animals.

Group 4 treated animals also showed a significant (p<0.01) protection when compared to group 2 treated animals. The group 5 and 6 treated group also showed significant (p<0.001) reduction in chronic model when compared to group 2 treated animals (Table 4). The SOD activity was significantly (p<0.001) reduced in CCl_4 treated animals in chronic model when compared to control. There was a significant (p<0.001) protection in the SOD level in group 3-6 animals when compared with the group 2 treated animals (Table 4).

The LPO level was significantly (p<0.001) increased in group 2 treated animals in chronic model when compared to group 1. There was a significant (p<0.05) protection in the LPO level in group 3 treated animals in chronic model when compared with the group 2 treated animals. The group 4 treated animals also showed a significant (p<0.01) protection when compared with the group 2 treated animals in chronic models. The group 5 and 6 treated group also showed significant (p<0.001) reduction when compared with the group 2 treated animals in chronic model (Table 4).

The CAT activity was significantly (p<0.001) reduced in group 2 treated animals in chronic model when compared to group 1. There was a significant (p<0.05) protection in the CAT level in group 3 treated animals in chronic model when compared with the group 2 treated animals.

Group 4 treated animals also showed a significant protection in chronic (p<0.01) model. The group 5 and 6 treated group also showed significant (p<0.001) reduction in chronic model when compared to group 2 treated animals (Table 4).

The vitamin E level was significantly (p<0.001) decreased in group 2 treated animals in chronic model when compared to group 1. There was a significant protection (p<0.01) in the vitamin E level in group 3 treated animals in when compared with the group 2 treated animals. The group 4 treated animals also showed

Table 4: Effect of *Luffa acutangula* (var) *amara* on the activities of antioxidant activities in liver homogenate of control and experimental rats

Groups	GPx (a)	RGSH (b)	GST (c)	SOD (d)	LPO (e)	CAT (f)
1	16.85±0.72	3.55±0.11	98.64±0.33	1.48±0.06	141.9±0.57	136.0±1.46
2	10.05±0.21^{a***}	1.71±0.06^{a***}	70.25±0.47^{a***}	0.61±0.04^{a***}	289.1±1.43^{a***}	75.9±1.81^{a***}
3	12.35±0.28^{b**}	2.01±0.03^{b**}	76.43±0.32^{b*}	0.88±0.02^{b***}	227.5±0.94^{b*}	88.4±0.55^{b*}
4	14.41±0.30^{b***}	2.66±0.1^{b**}	84.05±0.45^{b**}	1.04±0.03^{b***}	183.7±2.95^{b**}	99.7±1.37^{b**}
5	16.15±0.25^{b***}	3.18±0.09^{b***}	88.50±1.05^{b***}	1.15±0.08^{b***}	169.2±1.21^{b***}	109.5±2.90^{b***}
6	16.25±0.18^{b***}	3.35±0.11^{b***}	91.11±013^{b***}	1.30±0.05^{b***}	160.7±3.72^{b***}	113.6±2.72^{b***}

Comparisons were made between; ^{a}group 1 vs. 2; ^{b}group 2 vs. 3-5. Values are expressed as mean±SEM of six animals. Symbols represent statistical significance; **p<0.01, ***p<0.001

Table 5: Effect of *Luffa acutangula* (var) *amara* on the activities of vitamin levels in liver homogenate of control and experimental rats

Groups	Vit. E (a)	Vit. C (b)
1	1.88±0.01	1.150±0.01
2	1.09±0.02^{a***}	0.720±0.02^{a***}
3	1.24±0.01^{b**}	0.830±0.01^{b*}
4	1.43±0.02^{b***}	0.920±0.01^{b**}
5	1.57±0.03^{b***}	0.956±0.01^{b***}
6	1.64±0.03^{b***}	1.070±.002^{b***}

Comparisons were made between; ^{a}group 1 vs. 2; ^{b}group 2 vs. 3-5. Values are expressed as mean±SEM of six animals. Symbols represent statistical significance; **p<0.01, ***p<0.001

a significant (p<0.001) protection when compared with the group 2 treated animals in chronic model. The group 5 and 6 treated group also showed significant (p<0.001) reduction when compared with the group 2 treated animals in chronic model (Table 5). The vitamin C level was significantly (p<0.001) decreased in group 2 treated animals in chronic model when compared to group 1. There was a significant (p<0.05 and p<0.01) protection in the vitamin C level in group 3 and 4 treated animals in chronic model when compared with the group 2 treated animals. The group 5 and 6 treated group also showed significant (p<0.001) protection in chronic model (Table 5).

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 evidence the chronic damage induced by the CCl_4. The ethanol extract of *Luffa acutangula* (var) *amara* showed normal architecture, mild inflammation and mild hepatocellular damage against liver damage induced by CCl_4, the hepatotoxicant. A significant correlation of hepatoprotective activity was observed in terms of biochemical indices and histopathological observation.

The plant *Luffa acutangula* (var) *amara* has a folklore claim that it is used in jaundice. Thus, it paved an interest to evaluate scientifically using standard experimental animal model (CCl_4 induced liver damage). This is supported by various biochemical findings and histopathological studies. The present study revealed the hepatoprotective nature of the plant *Luffa acutangula* (var) *amara* leaf extracts against CCl_4 induced liver damage in chronic model. CCL_4 is being used extensively to investigate hepatoprotective activity on various experimental animals (Bhathal *et al*., 1983). A major defence mechanism included the antioxidant enzymes including SOD, CAT and GPx which converted into active oxygen molecules into the non-toxic compounds. CCL_4 is a universal hepatotoxicant inducing chronic liver injury. It is metabolized by the mixed-function oxidase system in the endoplasmic reticulum of the liver. Cleavage of the carbon-chloride bond results in the formation of free trichloromethyl radicals (CCl_3) which are highly unstable and immediately react with membrane components. This free radical causes lipid and protein peroxidation thus, responsible for the cellular damage (Plaa, 2000). The liver damage in rat is evidenced by increase in marker enzyme levels like GOT, GPT, ALP and bilirubin in the serum. The marker enzymes are the important index for the diagnosis of liver diseases and it indicates the damage of hepatic cells, cellular leakage and loss of functional integrity of cell membrane in liver. Treatment with the extracts lowered the elevated serum enzyme levels. Thus, the serum levels of transaminases return to normal with healing of hepatic parenchyma and the regeneration of hepatocytes (Wolf, 1999). ALP 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 but also present in many tissues. The extract reduced the elevated ALP levels thus, it is able to stabilize the dysfunction in chronic liver injury.

The liver regulates fats in the blood stream. It does this by converting excess amount of carbohydrates and proteins into fatty acids. The liver also synthesizes cholesterol from this fat. Decrease in cholesterol is seen in liver dysfunction. The extracts treated animals protected the decline of cholesterol. The liver is known to play a significant role in the serum protein synthesis, being the source of plasma albumin and fibrinogen and also the other important components like α, β and γ globulin. The serum albumin level is low in hepatic diseases (Venukumar and Latha, 2002). The present results reveal that when animals pretreated with extracts of *Luffa acutangula* (var) *amara* prior to the challenge with CCl_4, the liver biosynthesis of protein continues to be unaffected thus, bringing to normal. Bilirubin, an endogenous organic anion binds reversibly to albumin is

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 (Venukumar and Latha, 2002). Ample experimental and epidemiological studies support the involvement of oxidative stress in the pathogenesis and progression of several chronic diseases (Tewari *et al*., 2000). It is now known that oxygen, indispensable for maintaining life, sometimes becomes toxic and results in the generation of most aggressive agents such as Reactive Oxygen Species (ROS). The high reactivity of ROS may trigger a host of disorders in body resulting in tissue damage and necrosis in many instances (Prasad *et al*., 1999). Glutathione peroxidase (Gpx) is a seleno-enzyme two third of which (in liver) is present in cytosol and one-third in the mitochondria. It catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide and the reduction product of the hydro peroxide (Pari and Latha, 2004). GPx activity was significantly declined after CCl_4 treatment when compared to control.

Depression in Gpx activity was also observed in liver during liver damage. GPx has been shown to be an important adaptive response to condition of increased peroxidative stress.

The reversal of the GPx activity to normal after pretreatment with the plant extracts reveals the antioxidant activity of the extracts in scavenging/detoxifying the endogenous metabolic peroxides generated after Ccl_4 injury in the tissues (Zhang *et al*., 1999). The depletion of GPx content may also lower the GSH activity. GSH level was significantly reduced in CCl_4 treated animals and upward reversal was observed after the treatment with both extracts of the plant.

Enzymatic antioxidants like superoxide dismutase, catalase and reduced glutathione synergistically defense against reactive oxygen species. The steady state levels of superoxide, catalase and glutathione peroxidase are involved in removal of H_2O_2. Glutathione-s-transferase enhances the detoxification of electrophilic and lipophilic compounds through conjugation with GSH and forming GSH conjugate (Eaton, 1991). In the present study the superoxide dismutase activity is significantly reduced in CCl_4 intoxicated rats.

The SOD activity was brought to near normal after treatment with the extracts in CCl_4 intoxicated rats. Decreased activity of catalase was observed in animals treated with CCl_4. Presumably, a decrease in catalase activity could be attributed to cross linking and inactivation of the enzyme protein in the lipid peroxides. Decreased catalase activity is linked up to exhaustion of the enzyme as a result of oxidative stress caused by CCl_4 (Sreejayan, 1994). The catalase activity was restored to normal after treatment with extracts evidently shows that anti-oxidative property of the 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, the elevation of lipid peroxidation is seen in CCl_4 treated animals. The increase in MDA levels in liver suggests enhanced lipid peroxidation leading to tissue damage and thus antioxidant defense mechanism fails to prevent formation of excessive free radicals. A significant decrease in the levels of lipid peroxides in *Luffa acutangula* (var) *amara* extracts pre-treated rats suggests that the extracts may have the ability to protect the liver from free radical injury induced by carbon tetrachloride. There was also depletion of nonenzymic antioxidants like vitamin E and C in the CCl_4 treated groups when compared to the control. These antioxidants act to overcome oxidative stress being a part of the total antioxidant system. Vitamin E is the most important lipophilic antioxidant and resides mainly in the cell membranes and thus helps to maintain membrane stability (Omaye *et al*., 1979).

The vitamin C is hydrophilic and is a very important free radical scavenger in extra cellular fluids, trapping radicals in the aqueous phase and protecting bio-membranes from per-oxidative damage. In addition to its antioxidant effects, vitamin C is also involved in the regeneration of tocopherol from tocopheroxyl radicals in the membrane (Desai, 1984). Since, the preliminary phytochemical analysis of the extracts has shown the presence of flavonoids and phenolic compounds which have been known for its antioxidant and hepatoprotective activities (Di Carlo *et al*., 1999).

The preliminary phytochemical analysis of extract of *Luffa acutangula* (var) *amara* showed the hepatoprotective activity against CCl_4 induced hepatic dysfunction may be attributed due to the presence of phytoconstituents like flavanoids, alkaloids and glycosides in extract. The biological activities of plant constituents are complex. However, reports on the therapeutic potential of flavanoids as natural antioxidants are available in literature. Hence, the hepatoprotective activity of extracts of *Luffa acutangula* (var) *amara* may be attributed to the complex pharmacological action of phytoconstituents present in the extract, particularly the flavanoids.

Histological section of control animals group 1 showed normal hepatic cells with well-preserved cytoplasm, prominent nucleus and nucleolus and conspicuous central vein (Fig. 1a). Histological section of group 2 animals showed high degree of damage characterized by Inflammation is seen, fatty changes,

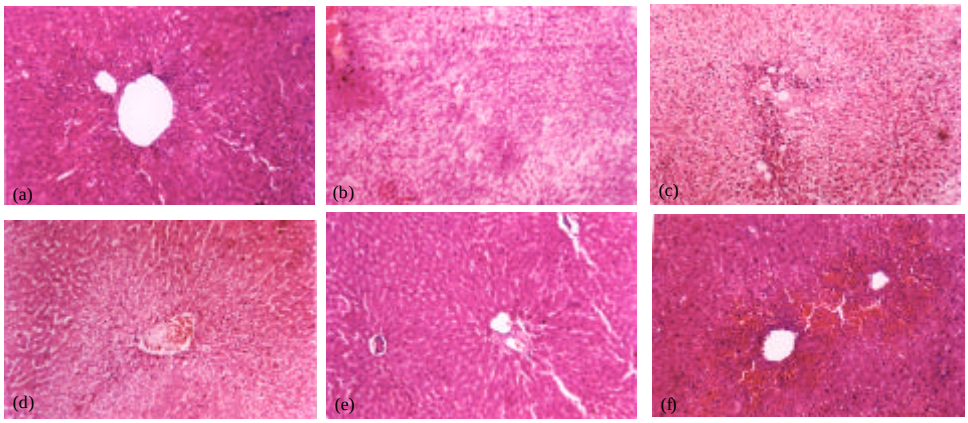

Fig. 1: Histopathological screening of carbon tetra chloride induced hepatotoxicity in liver

gross necrosis (Fig. 1b). Histopathological profile of the group 3 showed the recovery against the CCl_4 induced damage as compared to control (Fig. 1c). Whereas, the histological section of the group 4 showed that nuclei are not clear as in normal hepatocytes but as compared to the CCl_4 damage ones, the hepatocytes with normal nucleus are more. Endothelium was disrupted at places but in lesser number than, CCl_4 intoxicated rats. Hepatic cells adjoining to interlobular vein showed atrophy. Inflammation is seen, fatty changes, gross necrosis observed to be low. There seemed to be a satisfactory recovery (Fig. 1d). Histopathological screening were showed in group 5 having central vein seen some mild hepatocytic changes are present in (Fig. 1e). The standard drug showed in normal central vein with mild dilation is protect against liver damage induced CCl_4 the hepatotoxicant (Fig. 1f). Significant correlations of hepatoprotective activity were observed in terms of biochemical parameters and histopathological observation.

CONCLUSION

The action of EELA against hepatotoxic index at 600 mg kg^{-1}, b.wt, p.o also confirms the effectiveness of the EELA in carbon tetrachloride induced liver injured condition. The potent hepatoprotective effect of EELA at 600 mg kg^{-1} b.wt, p.o was confirmed by biochemical parameters studies. These changes were reverted back to near normal upon EELA treatment. This is further supported by histopathological changes in liver injury and EELA treated (600 mg kg^{-1} b.wt.) rats. This favorable effect of EELA as hepatoprotective drug is strongly expressed at 600 mg kg^{-1} b.wt as shown in Table 2 since, total protein, serum bilirubin, SGOT, SGPT, total cholesterol and alkaline phosphatase is present only in lysosomes and its activity was taken as a measure of lysosomal stability in order to arrive at an effective dose of EELA.

Further studies are required to isolation and characterization of the active compounds present in *Luffa acutangula* (var) *amara* and evaluate its pharmacological activity which may give a potential hepatoprotective drug.

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