

Hepatoprotective activity of *Luffa cylindrica* (L.) M. J. Roem. leaf extract in paracetamol intoxicated rats

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Received 27 July 2012; Accepted 6 February 2014

Methanolic extract of *Luffa cylindrica* (L.) M. J. Roem. leaves (MELC) was evaluated for its hepatoprotective potential against paracetamol intoxicated wistar rats. Administration of MELC and standard drug silymarin showed significant ($P < 0.05$) hepatoprotective protection in experimental animals. The study was conducted by estimating serum enzyme activities of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and bilirubin and by estimation of some serum antioxidant enzymes. Elevated serum marker enzymes of SGOT, SGPT, ALP and serum bilirubin were significantly ($P < 0.001$) reduced to near normal level in MELC treated animals. Lipid peroxidation level was decreased significantly by MELC 250 mg/kg and 500 mg/kg doses treatment groups. In case of antioxidant enzymes SOD, GSH and catalase levels were increased significantly ($P < 0.001$) after treatment with MELC. The extract showed a dose-dependent reduction of paracetamol induced elevated serum levels of enzyme activities indicating the extract could preserve the normal functional status of the liver. Results of this study demonstrated that *L. cylindrica* has good hepatoprotective potential.

Keywords: Hepatoprotective, *Luffa cylindrica*, Methanolic extract, Paracetamol.

IPC code; Int. cl. (2014.01)–A61K 36/00, A61P 1/16.

Introduction

Hepatic system is pivotal organ system and plays an astounding range of vital functions in the maintenance, performance and regulating homeostasis of the body by detoxification and excretion of many endogenous and exogenous compounds. Therefore, damage to the liver inflicted by hepatotoxic agents is of grave consequences¹. The liver is often abused by environmental toxins, poor eating habits, alcohol and over-the-counter drug use, which can damage and weaken the liver and eventually lead to several liver disorders^{2,3}. Unfortunately the conventional medicines used in the treatment of hepatotoxicity are inadequate. However, traditional system of medicine offers a number of medicinal preparations which play a vital role in the management of liver diseases. In the present context there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines to

explore the potential of the agents that are claimed to possess hepatoprotective activity.

Luffa [*Luffa cylindrica* (L.) Roem. syn *L. aegyptiaca* Mill.] commonly called sponge gourd, loofa, vegetable sponge, bath sponge or dish cloth gourd, is a member of Cucurbitaceae family, cultivated throughout the world and is distributed mainly in tropical to warm-temperate areas⁴. The plant is reported to have anti-tubercular and antiseptic properties^{5,6}. Usually, the skin of the gourd is peeled off when it is used as vegetable. Seeds and sponge of the old fruits are used in traditional medicine as anthelmintic, stomachic and antipyretic phytochemical drugs⁷. Its seeds have also been used in the treatment of asthma, sinusitis and fever⁸. Its seed oil is reported to possess antioxidant⁹, antimicrobial^{10,11}, anti-inflammatory¹² and bronchodilator properties¹³. An abortifacient protein luffaculin, isolated from seeds of *L. cylindrica* has ribosome inhibiting properties on the replication of HIV infected lymphocyte and phagocyte cells, explain its potential as therapeutic agent for AIDS^{14,15}. Its fruit is used in the treatment of hyperglycaemia¹⁶, ascites, hepatotoxicity¹⁷, biliary

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and intestinal colitis and also in enlarged spleen and liver. Juice extracted from its stem has been used in the treatment of several respiratory disorders¹⁸. It has been demonstrated from untimely studies that plant possesses a variety of pharmacological effects, viz. anticancer¹⁹, enzyme inhibitor²⁰, etc.

However, despite such interesting health virtue of the fruit, seeds and stems of this common vegetable, literature reveals that no scientific study has been carried out to determine the pharmacological activity of leaves of this plant in terms of hepatic effects. Therefore it was considered worthwhile to explore the prospect for hepatoprotective activity of methanolic extract of *L. cylindrica* (MELC) leaves in the present investigation.

Materials and Methods

Chemicals and reagents

Paracetamol was procured from E. Merck (India) Ltd. Mumbai; Silymarin was obtained from Ranbaxy Laboratories Limited, India. Standard kit of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and bilirubin was obtained from Centronic GmbH, Germany. The chemicals used for evaluation of oxidative stress parameters were obtained from Sisco Research Laboratories, India. Folin-Ciocalteu reagent was purchased from Sigma Chemicals (USA). All the other drugs and chemicals used in the study were of analytical grade.

Plant material

The leaves of *L. cylindrica* were procured locally from Pakbara village, District Moradabad of Uttar Pradesh, India and were authenticated by Dr. Beena Kumari, Taxonomist, Hindu College, Moradabad (India). A voucher specimen has been kept in the herbarium (HC.MBD/HAP/BK/2010/5/168) of the Department of Botany, Hindu college, Moradabad (India) for further references.

Preparation of extract

Leaves were washed with tap water and dried in shade. Dried leaves were ground to coarse powder and stored in an airtight container. The dried and coarsely powdered plant material was extracted with petroleum ether (60-80°) by hot percolation in soxhlet apparatus until it became colorless. The defatted plant material was also extracted with

methanol until it became colorless. The extract was concentrated under reduced pressure to yield a crude semi-solid mass. The last traces of the solvent were evaporated under reduced pressure in rotatory evaporator. The methanolic extract was subjected to preliminary phytochemical screening²¹ for detection of major phytoconstituents.

Determination of total phenolics

100 mL of the MELC was dissolved in 250 mL of methanol/ water (60:40, v/v, 0.3% HCl)²² and filtered through a 0.45 µm Millipore filter. To 100 mL of filtrate, 100 mL of Folin-Ciocalteu reagent (50%, v/v) and 2.0 mL of sodium carbonate (2%, m/v) were added and mixed completely. After 2 h, the absorbance of the solution was measured at 750 nm. 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 (GAE).

Animals

Wistar albino male rats weighing between 120-170g were used for the hepatoprotective study. The animals were housed in a group of four in clean polypropylene cages and maintained at 22 ± 2°C under 12 h light/dark cycle and were fed *ad libitum* with standard pellet diet (Golden feed, New Delhi, India) and had free access to water. The animals were acclimatized to laboratory condition for seven days before commencement of experiments. The study protocol was approved by the Institutional Animal Ethical Committee as per the requirements of Committee for the Purpose of Control and Supervision on Animals (CPCSEA), New Delhi.

Acute toxicity study

Acute toxicity study of *L. cylindrica* leaf extract was performed according to OECD Guideline 423. The different doses of leaf extract solution (5, 50, 100, 300, 1000 and 2000 mg/kg b.w.) were given orally. Animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 h (with special attention given during the first 4 h) and daily then after, for a total of 14 days. As there was no mortality rate found in animals, leaf extract was found to be safe in dose range of 5-2000 mg/kg. Consequently selection of dose was established as 250 mg/kg and 500 mg/kg.

Paracetamol induced hepatotoxicity

Animals were divided into five groups of six animals each. For the paracetamol induced hepatotoxicity studies, paracetamol (500mg/kg po) suspension was prepared using 1% gum acacia and was administered to all animals except the animals of the normal control group. Silymarin (100 mg/kg po) was used as standard. Group I, which served as vehicle control received 1% gum acacia suspension only. Group II served as paracetamol treated control. 48 h after paracetamol administration, groups III, IV & V received MELC extract 250, 500 and silymarin 100 mg/kg b.w., po, respectively, once daily for 5 consecutive days.

Hepatoprotective activity

Blood was collected by retro orbital artery bleeding, 16 h after administration of last dose of drugs. Blood samples were centrifuged for 10 minutes at 3000 rpm to separate the serum. The biochemical parameters like SGOT²³, SGPT²³, ALP²⁴ and bilirubin²⁵ were estimated using respective assay kits according to the methods described by the manufacturers.

The rats were sacrificed by ether anesthesia on day 6 and liver was excised, rinsed in 0.25 M sucrose solution and 10% w/v homogenate was prepared in 0.15M KCl, centrifuged at 1000 rpm for 10 min followed by centrifugation of the supernatant at 12000 rpm for 15 min. The supernatant obtained was used for estimation of various oxidative enzymes. Lipid peroxidation (LPO) was estimated by standard method²⁶ and expressed as nmol of malondialdehyde formed/min/mg protein. Superoxide dismutase (SOD) activity was assayed by method of Kakkar²⁷ and the results have been expressed as unit of SOD activity/mg protein. Catalase (CAT) and reduced glutathione (GSH) were determined according to respective methods^{28,29} and the results are expressed as units of CAT activity/mg protein and GSH formed nmol/mg protein/min, respectively.

The results of hepatoprotective activity were presented as the mean \pm SEM of 6 animals each group. Results were analyzed statistically using one way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test. Values of $P < 0.05$ were considered significant.

Histopathological analysis of liver

For histological studies, liver tissues were fixed with 10% phosphate-buffered neutral formalin, dehydrated in graded (50-100 %) alcohol and embedded in paraffin. Thin sections were cut and stained with hematoxylin and eosin stain for microscopic assessment. The initial examination was qualitative, with the purpose of determining histopathological lesions in liver tissue.

Results

The preliminary phytochemical screening of MELC indicated the presence of glycosides, terpenoids, steroids, flavonoids and tannins. The extract was found to contain 53.78 ± 1.01 μ g/mg total polyphenolics expressed as GAE (micrograms per milligram of GAE).

Effects on serum enzymes

The results of biochemical parameters revealed the elevation of enzyme level in paracetamol treated group, indicating that paracetamol induces damage to the liver. A significant reduction ($P < 0.001$) was observed in SGPT, SGOT, ALP and total bilirubin levels in the groups treated with silymarin and methanolic extract of *L. cylindrica*. The enzyme levels were almost restored to the normal after the said treatment (Table 1). It was found that the extract decreased the paracetamol induced elevated levels of the enzymes, indicating the production of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells by the extract.

Table 1—Effect of MELC on various biochemical parameters in rats with paracetamol induced hepatotoxicity

	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dl)
Vehicle control	44.01 \pm 0.18	34.74 \pm 2.28	41.70 \pm 1.95	1.232 \pm 0.081
PCM control	107.7 \pm 0.38 ^a	99.85 \pm 0.71 ^a	114.8 \pm 0.73 ^a	5.915 \pm 0.49 ^a
MELC (250 mg/kg)	76.32 \pm 1.15 ^a	65.70 \pm 0.66 ^a	78.04 \pm 0.49 ^a	4.007 \pm 0.38 ^a
MELC (500 mg/kg)	57.10 \pm 0.52 ^a	53.53 \pm 0.34 ^a	62.57 \pm 0.60 ^a	2.547 \pm 0.06 ^b
Silymarin (100 mg/kg)	53.43 \pm 1.07 ^a	48.53 \pm 0.95 ^a	57.02 \pm 0.68 ^a	2.307 \pm 0.15 ^b

Values represent mean \pm SEM of six animals in each group; ^a $P < 0.001$, ^b $P < 0.05$ compared to normal control group

Table 2—Effect of MELC on various parameters of oxidative stress in rats with paracetamol induced hepatotoxicity

Group	GSH (mM/gm)	Catalase (U/mg)	LPO (nM/mg)	SOD (% inhibition NBT)
Vehicle control	35.58±1.02	19.57±0.11	3.292±0.01	67.19±0.48
PCM control	9.505±0.05 ^a	7.823±0.02 ^a	7.930±0.17 ^a	22.80±0.11 ^a
MELC (250 mg/kg)	25.09±0.46 ^a	14.09±0.19 ^a	4.868±0.03 ^a	51.87±0.38 ^a
MELC (500 mg/kg)	29.06±0.32 ^a	15.69±0.07 ^a	4.212±0.02 ^a	58.48±0.81 ^a
Silymarin (100 mg/kg)	32.25±0.19 ^a	16.65±0.09 ^a	3.717±0.02 ^c	64.41±0.31 ^c

Values represent mean ± SEM of six animals in each group; ^a*P* <0.001, ^c*P* <0.01 compared to normal control group

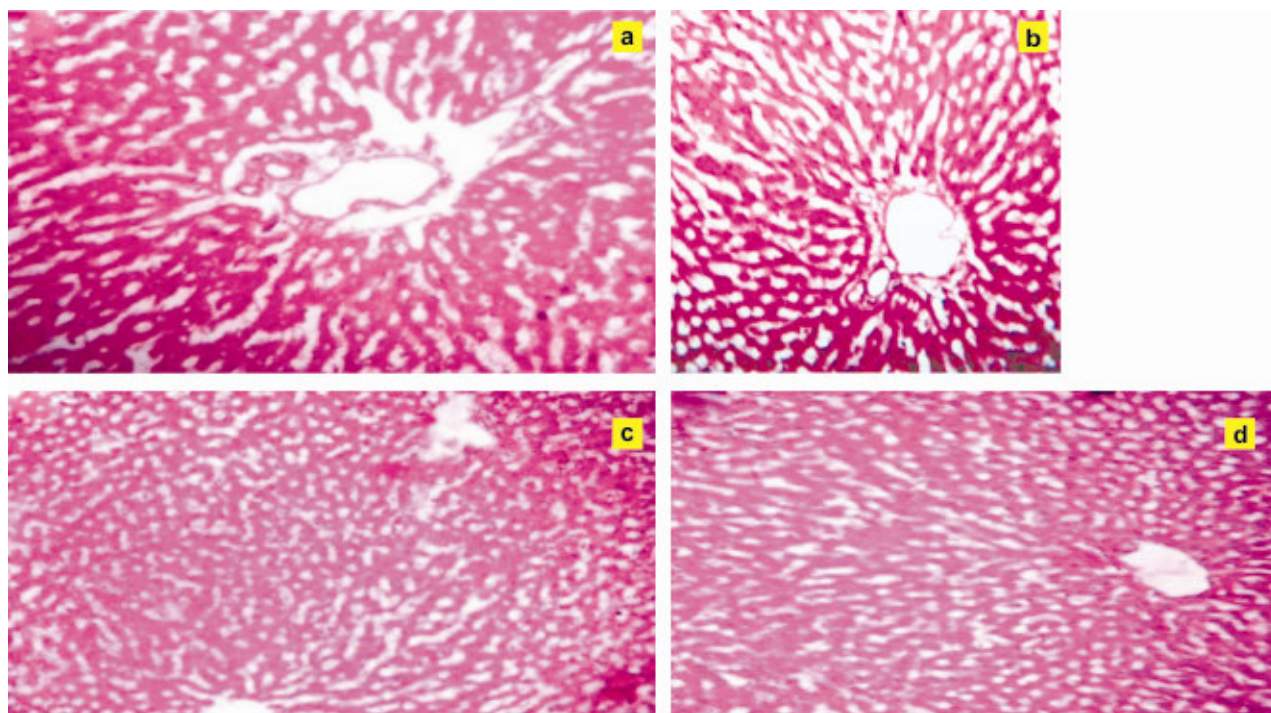


Plate 1—Histological images of treated animals (a) Paracetamol intoxicated group (b) MELC 250 mg/kg treated group (c) MELC 500 mg/kg treated group (d) Silymarin 100 mg/kg treated group.

Effects on lipid peroxidation, antioxidant enzymes

The effects of MELC extract on GSH, LPO, SOD and catalase in rats with paracetamol-induced hepatotoxicity are summarized in Table 2. There was a marked decrease in GSH level in rats treated with paracetamol, i.e. 9.50 mM/g compared to 35.58 mM/g in normal control rats. The GSH level was significantly increased to 25.09 mM/g (*P* <0.05) and 26.06 mM/g (*P* <0.001) by the treatment with MELC at 250 mg/kg and 500 mg/kg, respectively. The data obtained in the present study (Table 2) clearly shows an increase in the MDA levels of rats treated with paracetamol suggesting enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of

excessive free radicals. The increase in TBARS levels (7.93 nmol/mg compared to 3.29 nmol/mg in normal control) was significantly reduced by the treatment with 250 mg/kg and 500 mg/kg MELC (4.86 nmol/mg and 4.21 nmol/mg, respectively) and by the treatment with silymarin (3.717 nmol/mg). The decreased SOD level in paracetamol treated rats was also observed when compared to control. The level of SOD was increased again by the administration of MELC and silymarin.

Administration of MELC increased the CAT level in paracetamol treated rats with induced liver damage, thus preventing accumulation of excessive free radicals and protecting the liver from paracetamol intoxication (Table 2). The level of catalase (7.82 U/mg protein)

was significantly increased by the administration of 250 mg/kg MELC to 14.09 U/mg protein and by 500 mg/kg to 15.69 U/mg protein and by silymarin to a 16.65 U/mg protein value.

The decreased SOD level in paracetamol treated rats was also observed when compared to control. The level of SOD was increased again by the administration of MELC and silymarin. MELC causes a significant ($P < 0.001$) increase in hepatic SOD activity and thus suggests the reduction in reactive free radical induced oxidative damage to liver.

Histopathological observations

After histological examination of paracetamol treated animals, degeneration in centrilobular fatty section was revealed. Sinusoids were observed to be inflamed and flooded with inflammatory cells. Hepatocytes were observed to possess necrosis, degeneration and disarrangement. In treated groups significant protection was observed; as sign of necrosis disappeared although few of cells were found to be inflamed with sign of infiltration of macrophages. Extracts were found to be much effective in protecting hepatocytes at selected doses. Recovery against paracetamol induced necrosis in their compact arrangement of hepatic cells was observed; whereas the section of liver of animals treated with silymarin showed that extent of liver damage was lesser in magnitude as compared to the paracetamol treated animals (Plate 1).

Discussion

Paracetamol-induced liver injury is commonly used as models for investigation into the efficacy of hepatoprotective drugs³⁰. The toxicity of paracetamol is not due to paracetamol itself but is attributed to its reactive metabolite N-acetyl-p-benzoquinoneimine (NAPQI) which is capable of binding covalently to cellular macromolecules (proteins, DNA) to produce protein adducts. Higher doses of paracetamol and NAPQI results in oxidation and depletion of liver GSH pool and deviate it to augmented lipid peroxidation and liver damage³¹. This also results in the alteration in the level of other oxidative enzymes. In the present investigation a significant damage to the liver tissues was observed when animals were exposed to the paracetamol.

The raised serum liver enzymes such as ALT, AST and ALP in intoxicated rats can be attributed to the damage in the histostructural integrity of the liver

cells (hepatocytes). The crude extract of *L. cylindrica* leaves used in this study preserve the structural integrity of hepatocytes membrane. This was evident from the hepatoprotection provided by MELC to rats given paracetamol which reversed the rise in serum liver enzymes. The aspect of the observed alterations of serum enzymes levels to hepatic damage on health was confirmed by histopathological studies of liver which have shown that livers challenged with paracetamol have centrilobular necrosis, focal necrosis and ballooning. In animals treated with MELC no noticeable hepatocellular necrosis was observed. The preliminary phytochemical studies revealed the presence of flavonoids in methanolic extract of *L. cylindrica*. Flavonoids have been reported for their hepatoprotective activity³². So the hepatoprotective effect of *L. cylindrica* may be due to its flavonoid content.

Conclusion

The hepatoprotective effect of *L. cylindrica* leaves may be attributed to the presence of flavonoids, however, further studies to characterize the active principles and to elucidate the mechanism is required.

Acknowledgements

Authors are thankful to Shri Suresh Jain, Hon'ble chancellor, Teerthanker Mahaveer University for his invariable encouragement and endowing us with facilities necessitated for successful completion of the study.

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