



Hepatoprotective Activity of *LuffaCylindrica* Leaf Extract on Paracetamol Induced Liver Toxicity in Rats

Kumbhar P.B^{1,2}, V.P.Patil¹, Nanjappaiah HM¹ and Shivakumar Hugar^{1*}

¹P.G. Dept. of Pharmacology, B.L.D.E.A's College of Pharmacy, B.L.D.E.University campus, Bijapur-586103, Karnataka. India.

²Vilasrao Deshmukh Foundation, Group of Institutions, VDF School of Pharmacy, Latur.

*Correspondence author

E. Mail: shivkumarhugar@yahoo.com

Cell: +919448404102

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Abstract

Problems:The major purpose of study is to investigate the therapeutic efficacy of *Luffacylindrica* leaves in the treatment of liver disease.

Experimental approach:The 70% hydro-alcoholic leaf extract of *Luffacylindrica* was evaluated in liver toxicity. In present study liver toxicity was produced using 750 mg Paracetamol i.p. in male Wistar rats. The assessment of hepatoprotective activity of title plant was done by estimation of serum marker enzymes such as serum glutamate pyruvate transaminas (SGPT), serum glutamate oxaloacetate transaminase (SGOT), serum alkaline phosphatase (ALP), serum total bilirubin (TB) and serum direct bilirubin (DB).

Findings:The Paracetamol challenged rats exhibited significant elevation of serum marker enzymes indicating hepatic damage when compared with normal control group. There was dose dependent significant reduction in elevated levels of SGPT, SGOT, ALP, TB and DB observed in rats subjected to pre and post treatment with test extract at the dose 100 mg/kg and 200 mg/kg.

Conclusion:The results indicate that the alcoholic extract of *Luffacylindrica* possess potential preventive and curative hepatoprotective effects.

Key words:*Luffacylindrica*, hepatoprotective, Paracetamol, SGPT and SGOT.

Introduction

In recent years many researchers have examined the effects of plants used traditionally by native practitioners and herbalists to support liver function and treat liver disorders. However, only few of them have been fairly well researched. According to the United States acute liver failure study group, drug-induced liver injury accounts for more than 50% of acute liver failure, including hepatotoxicity caused by overdose of acetaminophen (39%) and idiosyncratic liver injury triggered by other drugs (13%)¹. Towards these pathologies, modern medicine does not find any curative treatments. Hence, searching the safe and potent remedies from the medicinal plants for the treatment of hepatic disorders has become desired area of research for the pharmacologists. Literature review showed that some of the Indian medicinal plants used traditionally in the management of liver disorders have been scientifically evaluated and reported for their hepatoprotective efficacy against various experimental animal models. The present research work was designed to explore the possible preventive and curative hepatoprotective efficacy of 70% hydro-alcoholic leaf extract of *Luffacylindrica* on Paracetamol rendered rat liver injury.

Materials and Methods

Preparation of extract:

Fresh leaves of the *Luffacylindrica* were collected from the gardens of Latur, Maharashtra, after the plant material authentication. The leaves were shade dried at room temperature, coarse powdered and extracted with 70% hydro-alcohol by Soxhlet's extraction method. Thereafter, the extract was concentrated using rotary flash evaporator to get semisolid crude extract. The extract was stored in airtight container in refrigerator below 10° C. Appropriate concentration of stock solutions of extract were prepared using distilled water and used for the studies.

Preliminary phytochemical screening:

Preliminary phytochemical tests were performed for the leaf extract of title plant to detect the presence of phytochemicals by following the standard methods described in the Practical Pharmacognosy by Kokate and Khandelwal^{2,3}.

Experimental animals:

Male albino Wistar rats (150-200 g) and albino Swiss mice (20-25 g) were used in the experiment. They were procured from Shri Venkateshwara enterprises, Rajajinagar, Bangalore. After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 10 days. Animals were housed in polypropylene cages and maintained under standard environmental conditions such as temperature (26 ± 2°C), relative humidity (45-55%) and 12 hr. dark/light cycle. The animals were fed with rodent pellet diet and water *ad libitum*. The study protocol was approved from the Institutional Animal Ethics Committee (IAEC) of B.L.D.E.A's College of Pharmacy, Bijapur, before commencement of experiment.

Determination of acute toxicity:⁴

The acute toxicity of test extract was determined in female albino mice following fixed dose method of organization for economical and co-operation development (OECD) guideline No. 423. The mice weighing between 20-25 g were fasted overnight prior to experiment. 1/5th, 1/10th and 1/20th lethal dose₅₀ (LD₅₀) cutoff value of the extract was selected as screening doses for the hepatoprotective study.

Evaluation of hepatoprotective activity:⁵

Albino rats of Wistar strain weighing 150–200 g were allocated to 10 groups of six animals each as shown below. Group 1 served as normal control given orally (Vehicle, 5% ethanol) 5 ml/kg body weight (b.w.) for 7 consecutive days. Group 2 served as hepatic control given orally (Vehicle, 5% ethanol) 5 ml/kg for 7 consecutive days and Paracetamol 750 mg/kg b.w. intraperitoneal (i.p.) on 7th day. Group 3 received standard drug Silymarin orally 100 mg/kg for 7 consecutive days and Paracetamol 750 mg/kg b.w. i.p. on 7th day (**In preventive study**). Group 4 received standard drug Silymarin orally 100 mg/kg for 7 consecutive days and Paracetamol 750 mg/kg b.w. i.p. on 1st day (**In curative study**). Groups 5, 6 and 7 were given orally 50, 100 and 200 mg/kg respectively for 7 consecutive days and Paracetamol 750 mg/kg body wt. i.p. on 7th day (**In preventive study**). Groups 8, 9 and 10 were given orally 50, 100 and 200 mg/kg respectively for 7 consecutive days and Paracetamol 750 mg/kg body wt. i.p. on 1st day (**In curative study**).

Assessment of hepatoprotective activity:

During the period of treatment the rats were maintained under normal diet and water. Twenty four hours after the last treatment i.e. on 8th day, the blood samples were collected by direct cardiac puncture under the influence of light ether anesthesia. Plasma was separated by centrifugation at 3000 rpm for 15 min and used for estimation of biochemical parameters such as SGPT, SGOT, ALP and Bilirubin (Total and Direct) using ready Erba Diagnostics Mannheim GmbH – Germany kits by Semi autoanalyser (ErbaChem).

Statistical analysis:

The results were expressed as the mean \pm S.E.M. The results obtained from the present study were analyzed using one-way ANOVA followed by Dunnett's multiple comparison tests. Data was computed for statistical analysis using Graph Pad Prism Software. Differences between the data were considered significant at $p < 0.05$.

Results

Preliminary phytochemical screening:

The preliminary phytochemical investigation of leaf extract of the title plant showed the presence of tannins, flavonoids and carbohydrates etc.

Acute toxicity:

In acute toxicity studies, it was observed that test extract of title plant found to be toxic

(produced mortality 2/3 of the animals) at the dose of 2000 mg/kg b.w. but, did not cause any mortality (found safe i.e. 0/3 animals died) at dose of 300 mg/kg. Hence, 1000 mg/kg dose was fixed as LD₅₀ cutoff value as per fixed dose method of OECD. The screening doses selected for the preventive and curative effects of the extract were: 200 mg/kg (1/5th dose of 1000 mg/kg), 100 mg/kg (1/10th dose of 1000 mg/kg), 50 mg/kg (1/20th dose of 1000 mg/kg).

Effect of pre treatment of *Luffacylindricaleaf* extract (preventive effect) on biochemical markers in Paracetamol induced rat liver injury:

The Paracetamol challenged rats exhibited significant elevation of serum marker enzymes SGPT, SGOT, ALP and increased concentration of bilirubin (Total and Direct) indicating hepatic damage when compared with normal control group. There was dose dependent significant reduction in elevated levels of SGPT, SGOT, ALP, TB and DB observed in rats subjected to pre treatment with test extract at higher doses only (100 and 200 mg/kg). However, extract at dose of 50 mg/kg has not shown statistically significant reduction in elevated biochemicals. The reference standard drug, Silymarin did reverse the Paracetamol induced elevated levels of biochemical parameters and indicating its preventive hepatoprotective activity. The results are presented in table No 2.

Effect of post treatment of *Luffacylindricaleaf* extract (curative effect) on biochemical markers in Paracetamol induced rat liver injury:

Significant increase in serum marker enzymes and elevated bilirubin levels in rats intoxicated with Paracetamol indicates the liver toxicity in hepatic control groups. The test extract post treated groups demonstrated significant decrease in elevated biochemical parameters in a dose related manner at higher doses only (100 and 200 mg/kg). However, extract at dose of 50 mg/kg has not shown statistically significant reduction in elevated biochemicals. The reference standard drug, Silymarin showed significant reduction in the Paracetamol induced elevated levels of biochemical parameters and indicating its curative hepatoprotective property. The results are presented in table No 2.

Discussion

Liver disorders are mainly caused by toxic chemical and drugs e.g. Paracetamol, anti-tubercular, anticancer agents or alcohol, some natural toxins such as peptides of *Amanita phalloides*, pyrrolidizines and the toxin of cycad nut⁶. Most of the hepatotoxic chemicals damage liver cells by inducing lipid peroxidation and others by oxidative cell damage. Paracetamol is the most versatile and widely used analgesic and antipyretic drug worldwide. Its potential hepatotoxicity was not suspected until the first clinical reports of severe and fatal liver damage following over dosage was reported by David and Eastham (1966). PCM taken in over doses can induce severe hepatotoxicity in men and in experimental animal⁷. Excessive administration of Paracetamol can cause over production of reactive oxygen species (ROS) during formation of N-acetyl-pbenzoquinoneimine (NAPQI) by cytochrome P450. This mechanism has been suggested

Table No. 2

Preventive & curative effects of *Luffacylindricaleaf* extract on paracetamol damaged rat liver

Groups	SGPT IU/l	SGOT IU/l	ALP IU/l	TB mg/dl	DB mg/dl
Normal control	32.53 ± 1.00	265.88 ± 14.47	353.46 ± 11.69	0.44 ± 0.01	0.52 ± 0.03
Hepatic control	113.09 ± 2.93 [#]	405.57 ± 13.36 [#]	563.77 ± 14.15 [#]	2.55 ± 0.18 [#]	2.93 ± 0.09 [#]
Preventive					
Silymarin	40.68 ± 3.61	292.79 ± 10.62	382.67 ± 9.14	0.74 ± 0.02	0.65 ± 0.07
50 mg	113.20 ± 4.53 ^{ns}	384.67 ± 14.62 ^{ns}	557.54 ± 12.33 ^{ns}	2.17 ± 0.13 ^{ns}	2.76 ± 0.15 ^{ns}
100 mg	87.84 ± 5.74 ^{**}	340.30 ± 11.46 [*]	499.53 ± 8.83 ^{**}	1.96 ± 0.10 ^{**}	2.46 ± 0.15 [*]
200 mg	76.53 ± 7.29 ^{***}	334.12 ± 11.30 ^{**}	405.57 ± 13.36 ^{***}	1.56 ± 0.06 ^{***}	1.72 ± 0.08 ^{***}
Curative					
Silymarin	56.31 ± 1.86	283.22 ± 9.71	392.41 ± 8.52	0.61 ± 0.03	0.73 ± 0.03
50 mg	105.92 ± 6.19 ^{ns}	369.05 ± 12.73 ^{ns}	530.88 ± 12.46 ^{ns}	2.28 ± 0.17 ^{ns}	2.74 ± 0.09 ^{ns}
100 mg	91.37 ± 2.60 ^{**}	332.75 ± 11.25 ^{**}	507.45 ± 12.24 [*]	1.91 ± 0.06 ^{**}	2.28 ± 0.22 [*]
200 mg	79.27 ± 5.94 ^{***}	311.05 ± 8.13 ^{***}	395.68 ± 8.85 ^{***}	0.72 ± 0.04 ^{***}	1.40 ± 0.16 ^{***}

Results are Mean ± SEM, n = 6, # p < 0.001 compare to normal control
* p < 0.05, ** p < 0.01 and *** p < 0.001 compared to Hepatic control.

to participate in the development of oxidative stress and injury in Paracetamol induced hepatotoxicity⁸.

The aim of current study was to explore preventive and curative effects of *Luffacylindricaleaf* extract against Paracetamol mediated liver damage in rats. The dose dependent significant reduction in Paracetamol induced increased plasma concentrations of SGPT, SGOT, ALP, total and direct Bilirubin in animals pre and post treated with 70% hydro-alcoholic leaf extract of *Luffacylindricaleaf* demonstrated their ability to restore the normal functional status of the poisoned liver, and also to protect against subsequent paracetamol liver damage. Thereports documented in the literature demonstrated significant hepatoprotective activity of the fruit and leaf extracts of the title plant against

Paracetamol⁹ and erythromycin¹⁰ induced liver toxicity respectively. The results obtained from the present investigation also suggest that leaf extract of the *Luffacylindrica* possess significant curative effects against Paracetamol poisoned rat liver. Hence, the findings of the present study are in agreement with previous literature reports on title plant regarding hepatoprotective efficacy. Literature survey indicated that flavonoids and tannins present in the plant extract were known to exhibit hepatoprotective property^{11, 12, 13, 14}. In the present investigation also preliminary phytochemical screening revealed the presence of flavonoids and tannins in the extract of title plant and this could be the reason for observed significant preventive and curative hepatoprotective property of the test extract.

Conclusion

The findings of the present study suggest that leaf extract of the *Luffacylindrica* possesses both preventive and curative hepatoprotective efficacy against paracetamol rendered liver injury in rats and justifies its folklore use in the treatment liver diseases.

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Authors Column



Dr. Shivakumar Hugar, Professor and Head, P.G. Dept. of Pharmacology, BLDEA's College of Pharmacy, Bijapur, Karnataka, is having 16 years of experience in teaching and research. He guided 31 M. Pharm and 1 Ph.D. students. Presently he is guiding 03 Ph.D. students. Dr. Hugar Published 28 research papers in national and 18 in international journals. He has presented 8 oral and 50 posters in national and International conferences and bagged GOT BEST REASERCH PAPER AWARD for 3 oral presentations. He attended International conference held in Malaysia in the year 2011. He is Editorial board member and reviewer for few Journals. He was the member of International Organizing Committee of 12th International congress of Ethnopharmacology held in Jadavpur University, Kolkata, India on February 17-19, 2012. He is life member of APTI, American Botanical Council and ACP.



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