

ORIGINAL RESEARCH ARTICLE

Protective Effect of *Luffa cylindrica* L. Fruit in Paracetamol Induced Hepatotoxicity in Rats

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ABSTRACT

In present study to evaluate the hepatoprotective activity of the ethanol and aqueous extracts (100 and 200 mg/kg) of fruit of *Luffa cylindrica* were studied on paracetamol (2 gm/kg) treated albino rats. The hepatoprotective effect was evaluated on the basis of various biochemical parameters which includes aspartate aminotransferase (AST) and alanine aminotransferase (ALT), serum alkaline phosphatase (ALP), total bilirubin (TB), total cholesterol (TC) and total protein (TP). Treatment with ethanol and aqueous extracts (100 and 200 mg/kg) of fruit of *Luffa cylindrical* shown significant hepatoprotective effect and also supported by histopathological study.

Keywords: *Luffa cylindrica*, Paracetamol, Silymarin, Hepatoprotective

INTRODUCTION

Liver diseases are the most serious ailment and are mainly caused by toxic chemicals (Excess consumption of alcohol, high doses of paracetamol, carbon tetrachloride, chemotherapeutic agents, peroxidised oil, etc.). In spite of the tremendous advances made in allopathic medicine, no effective hepatoprotective medicine is available. Plant drugs are known to play a vital role in the management of liver diseases in India. Around 87 medicinal plants and 33 patented herbal formulations claimed to have hepatoprotective activities^[1,2].

Luffa cylindrica L. (cucurbitaceae) commonly called sponge gourds and the fruits have a network of fibers surrounding a large number of flat blackish seeds. It has been reported to possess both medicinal and nutritional properties and its seed have been used in the treatment of asthma, sinusitis, fever^[3]. In the present study we have evaluated the hepatoprotective activity of ethanol and aqueous extracts of fruit of *Luffa cylindrica* against paracetamol induced hepatotoxicity in rats.

MATERIALS AND METHODS

Collection and Identification

The fruits of *Luffa cylindrical* L. was collected from the Panna, Madhya Pradesh in the month of December 2010 and authenticated at Motilal Vigyan Mahavidyalaya, Bhopal, Madhya Pradesh, India. The voucher specimen (1133.77-309) was

deposited in department of Pharmacognosy, Technocrats Institute of Technology-Pharmacy, Bhopal, Madhya Pradesh for further reference.

Preparation of Plant Extract

The collected cleaned powder of fruits of *Luffa cylindrica* were used for the extraction process 500 gm of powder of fruit were evenly packed in the soxhlet apparatus and extracted with various solvent increasing polarity including petroleum ether, chloroform, ethyl acetate, ethanol by hot continuous extraction process for about 26 hrs and the aqueous extraction was carried out by cold maceration process after each solvent extraction process.

Animal

Swiss albino mice (20-25 g) and Wistar rats (125-200 g) of either sex and of approximate 9-12 week old used in the present study and were procured from National Central Laboratory for animal science, Hyderabad. The experimental protocol approved by Institutional animal ethics committee (Reg. no. TIT/IAEC/831/P' cog/2011/10).

Hepatoprotective activity

The rats were randomly divided into various groups of six rats each (n = 6)^[4].

Group I (Control): Normal control rats were received 1ml/100gm of 0.5% sodium CMC using for 7days.

Group II (Toxic control): Toxic control rats were received 1ml/100gm of 0.5% sodium CMC

using for 7 days and paracetamol 2 g/kg, p.o. on 6th day.

Group III (Standard): Rats were received Silymarin (100 mg/kg, p.o.) for 7 days and paracetamol 2 g/kg, p.o. on 6th day.

Group IV and V: Rats were received aqueous extract of *Luffa cylindrica* (100 and 200 mg/kg) once daily for 7 days and paracetamol 2g/kg, p.o. on 6th day, respectively.

Group VI and VII: Rats were received ethanol extract of *Luffa cylindrica* (100 and 200 mg/kg) once daily for 7 days and paracetamol 2g/kg, p.o. on 6th day, respectively.

On the 7th day of the start of respective treatment the rats were anaesthetized by light ether anaesthesia and the blood was withdrawn by making intra-cardiac puncture to the rats and it was allowed to coagulate for 30 min and serum was separated by centrifugation at 2500 rpm. The biochemical parameters such as aspartate aminotransferase (AST)^[5], alanine aminotransferase (ALT)^[6], serum alkaline phosphatase (ALP)^[7], total bilirubin (TB)^[8], total cholesterol (TC) and total protein (TP)^[9] were determined to find out the hepatoprotective activity of aqueous and ethanol extracts of fruit of *Luffa cylindrica*.

Histopathological Study

Small pieces of liver tissue were collected in 10% formaldehyde solution for histopathological study. Paraffin sections (5-10 μ) were prepared and stained with haematoxylin eosin, finally mounted in neutral DPX medium. The histopathological examinations were performed using compound microscope.

Drugs and Chemicals

All the chemicals and solvents were of analytical grade. The various reagents and solvent purchased from the local dealer V.K. Traders, Bhopal. Paracetamol from Cipla, India and ethanol from Chang shu yang yuan chemicals, China. Silymarin from Himalaya Drug company, Bangalore.

Statistical Analysis

The values were expressed as mean \pm SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet's test. P values <0.05 were considered significant.

RESULT AND DISCUSSION

In this preliminary phytochemical screening of aqueous and ethanol extracts of fruit of *Luffa cylindrica* showed presences of various phytochemicals including flavonoids, tannin, alkaloids and steroids and the result is presented in (Table 1).

The present study was showed the hepatoprotective effect of aqueous and ethanol extracts of fruit of *Luffa cylindrica* on paracetamol induced hepatotoxicity in rats, which confined through various biochemical parameters (Table 2) and histopathological study (Fig 1). Paracetamol is a widely used antipyretic and analgesic drug and produces acute hepatic damage on accidental over dosage. It is established that a fraction of acetaminophen is converted via the cytochrome P₄₅₀ pathway to a highly toxic metabolite such as acetyl-p-benzoquinamine (NAPQI)^[10].

In the assessment of liver damage by paracetamol the determination of enzyme level such as AST, ALT, ALP and biochemical parameters such as TB, TP and TC are largely used. Necrosis or membrane damage releases the enzymes into systemic circulation and hence it can be measured in the serum. High levels of these parameters indicate liver damage that is caused by viral hepatitis as well as cardiac infarction and muscle injury. Elevated levels of serum enzymes and biochemical parameters are indicative of cellular leakage loss of functional integrity of cell membrane in liver^[11].

The levels of AST and ALT in normal control group were found to be 45.17 and 57.33 respectively, while the levels of AST and ALT were elevated in paracetamol treated rats and found to be 118.5 and 114.00 respectively. The aqueous and ethanol extracts of fruit of *Luffa cylindrica* (100 and 200 mg/kg) were decreased the levels of AST and ALT, when compared with toxic group. The aqueous and ethanol extracts of fruit of *Luffa cylindrica* showed more significant activity at a dose level of 200 mg/kg. The level of ALP in normal control group was found to be 16.13 and elevated value was found to be 32.24 in paracetamol treated rats. The aqueous and ethanol extracts of fruit of *Luffa cylindrica* (100 and 200 mg/kg) were decreased the level of ALP, when compared with toxic group. The aqueous and ethanol extracts of fruit of *Luffa cylindrica* (200 mg/kg) showed significant reduction in level of ALP, when compared to toxic group. The level of TB in normal control group was found to be 1.60 and paracetamol treated rats was found to be 6.20 the aqueous of fruit of *Luffa cylindrica* (100 and 200 mg/kg) significantly decreased the level of TB, when compared with toxic group. The TB value of 100 and 200 mg/kg of aqueous extract were 1.06 and 1.44. The ethanol extract of stem bark at dose of 100 and 200 mg/kg were decreased the level of TB to 1.02 and 0.92 respectively. The aqueous and ethanol extracts of fruit of *Luffa*

cylindrica (100 and 200 mg/kg) were significantly reduced the level of TB, when compared with toxic group.

The level of TC in normal control group was 89.8 and elevated level was found to be paracetamol treated rats 166.5. The aqueous and ethanol extracts of fruit of *Luffa cylindrica* 100 and 200 mg/kg were decreased the levels of TC, when compared with toxic group. The aqueous and ethanol extracts of fruit of *Luffa cylindrica* 200 mg/kg showed more significant activity than the 100 mg/kg. The decrease the level of TP was found to be paracetamol treated rats 5.06), where the level of TP in normal rat group was found to be 7.91 the aqueous and ethanol fruit of *Luffa cylindrica* 100 and 200 mg/kg significantly increase the level of TP, when compared with normal control group rats. The TP level of 100 and 200 mg/kg of aqueous and ethanol extracts were found to be 3.36, 2.95 and 2.68, 2.18 respectively. The aqueous and ethanol fruit of *Luffa cylindrica* 100 and 200 mg/kg increase the level of TP, when compared to normal group.

The normal liver tissue shows normal hepatic cells with central vein and sinusoidal dilation (**Fig.1a**). In paracetamol treated group shows severe hepatotoxicity as well as severe necrosis with disappearance of nuclei (**Fig.1b**). The liver taken from animals treated with standard drug Silymarin

showed the normal hepatic cells with portal vein and portal artery (**Fig.1c**). Mild degree of necrosis with normal cells and mild degree of inflammation adjacent to necrosed area was observed in the animals treated with aqueous extract 100 & 200 mg/kg. (**Fig.1d & Fig.1e**). While normal hepatocytes with regenerating hepatocytes and mild inflammation in the portal area observed with group treated with Ethanol extract 100 and 200 mg/kg (**Fig.1f & Fig 1g**).

The terpenoids and flavonoids are well known for their hepatoprotective potential [12]. The observed hepatoprotective activity of *Luffa cylindrica* may be due to the presence of flavonoids and terpenoids. The aqueous and ethanol extracts of fruit of *Luffa cylindrica* showed adequate hepatoprotective activity on albino rats and histopathological examination of the liver section of the rats treated with toxicant showed intense centrilobular necrosis and vacuolization. The rats treated with extracts along with toxicant showed sign of protection against this toxicant to considerable extent as evident from absence of necrosis and vacuoles. Thus it was concluded that the extracts exhibited significant dose dependent hepatoprotective activity. Further studies to characterize the active principles and to elucidate the mechanism are in progress.

Table 1: Preliminary phytochemical screening of various extracts of *Luffa cylindrica* L. fruit

Phytochemicals	Pet.ether	Chloroform	Ethyl acetate	Ethanol	Water
Flavonoide	-	-	+	+	+
Glycoside	-	+	-	-	-
Tannin	-	-	+	+	+
Alkaloid	-	-	-	+	+
Saponin	-	-	-	-	+ve
Steroid	+	+	+	+	+ve

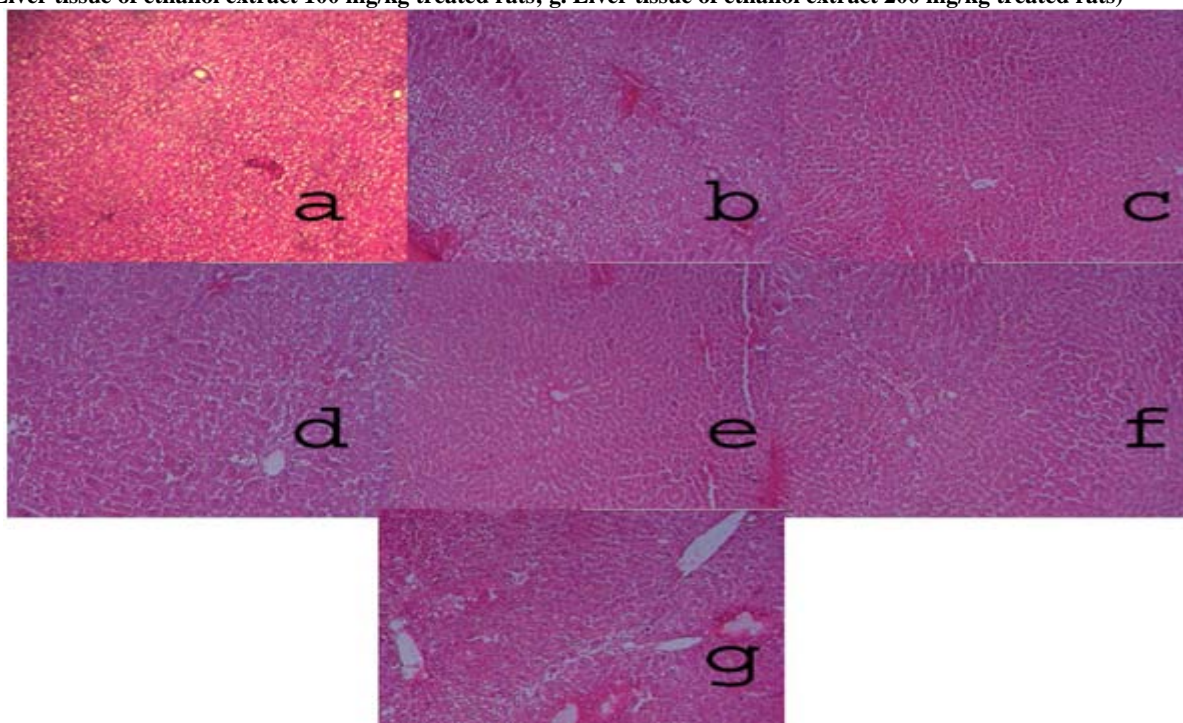
- = Negative; += Positive

Table 2: Effect of aqueous and ethanol extracts of fruit of *Luffa cylindrica* L. on the serum transminase (AST, ALT), ALP, TB, TP and TC in paracetamol induced hepatic damage in rats.

Treatment	AST (IU/dl)	ALT (IU/dl)	ALP (IU/dl)	TB (mg/dl)	TP(g/dl)	TC (mg/dl)
Normal	45.17 ±2.40	57.33 ±2.33	16.13 ±1.36	1.60±0.14	7.91±0.49	89.8 ±6.8
Toxic	118.50 ±1.72*	114.00 ±2.67*	32.24 ±1.96*	6.20±0.63*	5.06±0.20*	166.5 ±10.6*
Standard silymarin	55.50 ±1.61**	58.67 ±2.29**	17.43±1.63**	1.97 ±0.22**	7.99±0.41**	98.7 ± 9.2**
Aqueous extract 100 mg/kg	83.67 ±3.16**	77.50 ±1.87**	22.92±1.11**	1.06 ±0.45**	3.36 ±0.16**	112.8±8.2**
Aqueous extract 200 mg/kg	68.17 ±1.74**	67.50 ±1.48**	19.71±1.27**	1.44 ±0.22**	2.95 ±0.35**	101.6±2.2**
Ethanol extract 100 mg/kg	80.67 ±2.25**	68.33 ±2.06**	20.39±1.99**	1.02 ±2.42**	2.68 ±0.35**	124.7 ± 10.4**
Ethanol extract 200 mg/kg	58.33 ±2.00**	59.50 ±1.99**	17.11±1.34**	0.92 ±0.03***	2.18 ±0.15**	108.2 ± 9.5**

Values are Mean ± SEM; n = 6 animals in each group; *, <0.01 is considered significant when compared with control group; **P<0.05 is considered significant when compared with toxic group.

Fig 1: Photomicrographs of liver tissues (a. Normal liver tissue; b. Liver tissue of paracetamol treated rats; c. Liver tissue of silymarin treated rats; d. Liver tissue of aqueous extract 100 mg/kg treated rats; e. Liver tissue of aqueous extract 200 mg/kg treated rats; f. Liver tissue of ethanol extract 100 mg/kg treated rats; g. Liver tissue of ethanol extract 200 mg/kg treated rats)



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