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Hepatoprotective Activity of *Litchi chinensis* Leaf Against Paracetamol-Induced Liver Damage in Rats

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Abstract: In the present study, the chloroform and methanol extracts from *Litchi chinensis* leaf were evaluated for their protective effects on paracetamol-induced liver damage in Wistar albino rats. Serum biochemical parameters viz. serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), total protein, bilirubin, cholesterol, triglycerides; and liver biochemical parameters such as lipid peroxidation, reduced glutathione (GSH) content and catalase (CAT) activities were evaluated. All biochemical observations indicated that both the test extracts exerted significant hepatoprotective efficacy against paracetamol-induced hepatic damage in rats. The methanol extract was found to be more effective than chloroform extract. Therefore, from the present study it can be concluded that *L. chinensis* leaf had remarkable hepatoprotective activity in rats.

Key words: *Litchi chinensis* · Hepatoprotective · Leaf · Biochemical

INTRODUCTION

Litchi (*Litchi chinensis* Sonn., Sapindaceae) is a medium sized evergreen tree indigenous to China, Vietnam, Indonesia and Philippines and is cultivated commercially for its palatable sweet fruits all over the tropical and subtropical world [1]. Previous researchers reported several phytochemcial and pharmacological studies on fruits of this plant. However, experimental studies on its leaf are comparatively scanty. Previous pharmacological studies showed the petroleum ether extract of leaves of the plant possess anti-inflammatory, analgesic and antipyretic activity without toxicity [2]. The present study was aimed to investigate the hepatoprotective activity of chloroform and methanol extracts of *Litchi chinensis* leaf against paracetamol induced hepatic damage in Wistar albino rats.

MATERIALS AND METHODS

Plant Material: The leaves of *Litchi chinensis* Sonn. (Sapindaceae) were collected during September 2011 from North 24-Paraganas, West Bengal, India. The plant material was taxonomically identified at Botanical Survey of India, Central National Herbarium, Howrah, West Bengal, India. The voucher specimen [CNH/97/2001/Tech II/593] was maintained in our laboratory for future reference. The leaves were shade-dried with occasional shifting and then powdered with mechanical grinder passing through sieve no. 40 and stored in an air-tight container.

Drugs and Chemicals: Bovine serum albumin from Sigma Chemical Co., St. Louis, Mo, USA; Trichloroacetic acid (TCA) from Merck Ltd., Mumbai, India; Thiobarbituric acid (TBA), Paracetamol, 5,5′-dithio *bis*-2-nitro benzoic acid (DTNB), Phenazonium methosulphate (PMS), Nicotinamide adenine dinucleotide (NADH) and reduced glutathione (GSH) from SISCO Research Laboratory, Mumbai, India. Silymarin, potassium dichromate and glacial acetic acid from Ranbaxy, Mumbai. All the other reagents used were of analytical reagent grade obtained commercially.

Preparation of Extract: The powdered plant material (450 g) was extracted successively with chloroform and methanol for 72 h in the cone shaped percolator. The solvents were distilled off in reduced pressure and

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resulting semisolid mass was vacuum dried to yield the dry chloroform and methanol extracts (yields 3.0% and 5.62% w/w respectively). Preliminary phytochemical studies revealed the presence of triterpenoids and steroids in chloroform extract; steroids, alkaloids, tannins, carbohydrates and glycosides in methanol extract [3].

Experimental Animals: Adult male Wistar albino rats weighing 170-200 g were used for the present investigation. They were housed in a clean polypropylene cage and maintained under standard laboratory conditions (temperature 25±2°C with dark/light cycle 12/12 h). They were fed with standard pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The animals were acclimatized to laboratory conditions for one week prior to experiment. All procedures described were reviewed and approved by the Institutional Animal Ethics Committee.

Treatment Protocol: The rats were divided into five groups ($n = 6$). A single dose of 650 mg/kg paracetamol in 2% methyl cellulose was administered orally to each animal in group II, III, IV and V. Group I served as normal (vehicle) control and group II served as paracetamol control and both received normal saline (5 ml/kg b.w., p. o.) daily for 14 days. After administration of paracetamol suspension, the chloroform and methanol extracts were administered orally (p. o.) at the dose of 200 mg/kg body weight (b. w.) to groups III and IV respectively daily for 14 days. Group V received reference drug silymarin (25 mg/kg b.w; p. o.) daily for 14 days. After 24 h of last dose, blood was collected from overnight fasted rats of each group by cardiac puncture for estimation of serum biochemical parameters. Then the rats were sacrificed by cervical dislocation for the study of liver biochemical parameters [4].

Body Weight, Liver and Kidney Weights: The body weight of rats of each group were measured just before and 14 days after treatment. Liver and kidney weights of all rats were measured after post treatment sacrifice.

Serum Biochemical Parameters: The collected blood was used for the estimation of serum biochemical parameters viz. serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), total bilirubin, total cholesterol and triglycerides contents were estimated by using commercially available reagent kits (Span Diagnostic Ltd., Surat, India). Serum total protein was estimated according to the reported method [5].

Liver Biochemical Parameters: Lipid peroxidation i.e. thiobarbituric acid reactive substances (TBARS) was estimated by the reported method [6]. Reduced glutathione (GSH) was determined by the standard method [7]. Catalase (CAT) was assayed according the method described by previous workers [8].

Statistical Analysis: All results were expressed as the mean ± standard error of mean (SEM). The results were analyzed for statistical significance by one-way ANOVA followed by Dunnett's *post hoc* test of significance. $P < 0.05$ was considered as statistically significant.

RESULTS

Body Weight, Liver and Kidney Weights: The body weight, liver and kidney weights of rats from paracetamol control group (after 14 days) were significantly ($p < 0.001$) decreased when compared with normal control group. Both the extracts at 200 mg/kg b.w. significantly ($p < 0.001$) maintained the body weight, liver and kidney weights towards normal as compared to paracetamol control (Table 1).

Serum Biochemical Parameters: Serum biochemical parameters like SGOT, SGPT, SALP, bilirubin, total cholesterol and triglycerides in the paracetamol control group were significantly ($p < 0.001$) elevated as compared to the normal saline group. Treatment with the test

Table 1: Effect of the *L. chinensis* leaf extracts on body weight, liver weight and kidney weight of normal and paracetamol-treated rats

Group	Dose	Initial body wt (g)	Final body wt (g)	Final liver wt (g)	Final kidney wt (g)
I (Normal saline)	5 ml/kg	168.76±7.8	174.54±5.2	6.55±2.9	1.46±1.3
II (PCM)	650 mg/kg	171.68±7.2	164.54±4.5*	3.25±2.3*	0.94±1.1*
III (PCM + Chloroform extract)	200 mg/kg	163.11±5.7	157.84±1.5**	5.98±3.6**	1.27±1.3**
IV (PCM + Methanol extract)	200 mg/kg	176.53±4.9	168.76±3.6**	6.37±3.6**	1.29±1.8**
V (PCM + Silymarin)	25 mg/kg	177.53±4.5	169.76±3.3**	6.36±3.1**	1.29±1.6**

Values are expressed as mean ± SEM ($n = 6$); *$p < 0.001$ compared with normal control and

** $p < 0.001$ compared with paracetamol control group. PCM: Paracetamol.

Table 2: Effect of the *L. chinensis* leaf extracts on serum biochemical parameters of normal and paracetamol-treated rats

Group	Dose	SGOT(IU/L)	SGPT(IU/L)	SALP(IU/L)	Total Bilirubin (mg/dL)	Total Protein (mg/dL)	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)
I(Normal saline)	5 ml/kg	19.15±1.05	21.73±1.66	77.17±2.71	1.05±0.10	7.96±0.18	121.57±2.09	97.97±6.76
II (PCM)	650 mg/kg	36.13±1.31*	38.85±1.14*	134.81±3.88*	2.78±0.25*	4.07±0.27*	162.21±1.88*	171.51±7.67*
IV (PCM + Chloroform extract)	200 mg/kg	21.29±1.59**	25.33±0.29**	89.58±4.21**	1.15±0.16**	6.85±0.27**	129.18±3.53**	118.95±6.93**
IV (PCM + Methanol extract)	200 mg/kg	19.98±0.98**	22.81±0.72**	81.63±1.53**	1.09±0.29**	7.90±0.15**	124.91±1.95**	108.45±7.63**
V (PCM + Silymarin)	25 mg/kg	19.38±0.68**	21.91±0.72**	79.33±1.43**	1.08±0.09**	7.93±0.13**	123.31±1.65**	105.15±7.33**

Values are expressed as mean ± SEM (n = 6);

*p < 0.001 compared with normal control and

** p < 0.001 compared with paracetamol control group. PCM: Paracetamol

Table 3: Effect of the *L. chinensis* leaf extracts on liver biochemical parameters of normal and paracetamol-treated rats

Group	Dose	µM of MDA/mg of wet liver tissue	GSH (µg/mg of wet liver tissue)	CAT (µM of H_2O_2 consumed/min/mg of wet liver tissue)
I (Normal saline)	5 ml/kg	0.87±0.06	105.15±4.60	11.55±0.58
II (PCM)	650 mg/kg	6.55±0.08*	35.42±2.15*	1.87±0.41*
III (PCM + Chloroform extract)	200 mg/kg	2.24±0.06**	102.81±7.45**	8.66±0.82**
IV (PCM + Methanol extract)	200 mg/kg	1.98±0.05**	103.33±5.63**	9.93±0.87**
V (PCM + Silymarin)	25 mg/kg	0.91±0.04**	104.63±5.83**	10.33±0.77**

Values are expressed as mean ± SEM (n = 6); *p < 0.001 compared with normal control and

** p < 0.001 compared with paracetamol control group. PCM: Paracetamol

extracts at the dose of 200 mg/kg significantly ($p < 0.001$) reduced their levels towards the normal values. The total protein content was found to be significantly decreased in the paracetamol control group as compared with the normal saline group ($p < 0.001$). Administration of both the extracts in paracetamol-intoxicated rats significantly ($p < 0.001$) increased the total protein content as compared with the paracetamol control (Table 2).

Liver Biochemical Parameters: The levels of TBARS were significantly ($p < 0.001$) increased in paracetamol control animals as compared to normal control group. Treatment with the test extracts at 200 mg/kg b.w. significantly ($p < 0.001$) reduced the TBARS levels when compared with paracetamol control animals. The level of reduced glutathione (GSH) was significantly ($p < 0.01$) depleted in paracetamol control group as compared with normal control group. Reduced GSH level was found to be significantly ($p < 0.001$) elevated towards normal level on administration of the extracts as compared with paracetamol control group. There was significant ($p < 0.001$) reduction in catalase activity in paracetamol control group compared with normal group. Administration of both the test extracts significantly ($p < 0.001$) recovered the CAT activity towards normal when compared with paracetamol control animals (Table 3).

DISCUSSION

Paracetamol is a widely used antipyretic and analgesic drug which is safe in therapeutic doses but can cause fatal hepatic damage in human and animals at higher toxic doses [9]. Bioactivation of paracetamol by hepatic cytochrome P-450 leads to formation of a highly reactive and toxic metabolite N-acetyl-*p*-benzoquinone imine (NAPQI). NAPQI is normally detoxified by conjugation with reduced glutathione (GSH) to form mercapturic acid which is excreted in urine. Toxic overdose of paracetamol depletes hepatic reduced glutathione (GSH) content so that free NAPQI binds covalently to cellular macromolecules causing acute hepatocellular necrosis. The NAPQI then causes acylation or oxidation of cytosolic and membrane proteins and generation of reactive oxygen species (ROS). This leads to further oxidation of protein thiols, lipid peroxidation, DNA fragmentation and ultimately cell [10].

It has been well established that elevated levels of SGOT, SGPT and SALP are indicative of cellular leakage and loss of functional integrity of the hepatic cell membranes implying hepatocellular damage. Serum total protein and bilirubin levels on the other hand are related to the function of the hepatic cells revealing the functional status of the hepatic cell [4]. Elevated serum cholesterol and triglyceride levels in paracetamol challenged rats indicated impaired fat metabolism due to hepatic damage. Both the extracts decreased the elevated

serum enzyme activities, bilirubin and lipid contents with elevation of total protein content in the paracetamol treated rats which are comparable to the normal control group. It appears that the extracts preserved the structural integrity of the hepatocellular membrane which is evident from the significant reduction in paracetamol-induced rise in serum marker enzymes in rats. The methanol extract was found to be more active. The test extracts also showed marked effect in controlling the loss of body weight, liver and kidney weights of paracetamol-intoxicated rats.

Lipid peroxidation is a phenomenon involved in peroxidative loss of unsaturated lipids, thus bringing about cellular lipid degradation and membrane disordering. Reactive oxygen species (ROS) results in lipid peroxidation and subsequently increase in thiobarbituric acid reactive substances (TBARS) levels. Elevated lipid perixodation causes degradation of cellular macromolecules leading to tissue damage [11]. A marked increase in the concentration of TBARS in paracetamol-intoxicated rats indicated enhanced lipid peroxidation leading to tissue injury and failure of the antioxidant defense mechanisms to prevent overproduction of ROS. Both the extracts showed ability to prevent paracetamol induced elevation of TBARS level, suggesting that they inhibited hepatic lipid peroxidation in paracetamol intoxicated rats. This implies the reduction in free radical production and subsequent decrease in damage to the hepatocellular membranes.

Glutathione, the most abundant tripeptide thiol, is the endogenous non-enzymatic antioxidant in our body system and it is protective against chemically induced hepatic damage and oxidative stress [12]. Depleted GSH level with elevated level of lipid peroxidation in paracetamol-induced rats indicated that the experimental dose of paracetamol 650 mg/kg was highly hepatotoxic. It was confirmed from the present study that the test extracts significantly restored hepatic GSH content towards normal in paracetamol intoxicated rats indicating decreased free NAPQI level in the blood.

Enzymatic antioxidant mechanisms play an important role in the elimination of free radicals (ROS). Catalase (CAT) is a haem containing enzyme catalyzing the detoxification of H_2O_2 to water and oxygen [13]. The suppression of CAT activity as a result of hepatic damage was reported [4]. Similar findings were observed in our present results in paracetamol control rats. The administration of both the test extracts significantly recovered the CAT activity towards normal.

Preliminary phytochemical studies revealed the presence of triterpenoids and steroids in chloroform extract; steroids, alkaloids and tannins in methanol extract. The extracts because of the presence of these constituents, must have exerted protective action against paracetamol-induced hepatic damage, plausibly by ameliorating the extent of oxidative stress mediated hepatocellular damage caused by paracetamol. Higher activity in methanol extract may be due to presence of tannins, well known polyphenolic natural antioxidant.

CONCLUSION

From the present investigation, it can be concluded that the chloroform and methanol extracts of *Litchi chinensis* leaf offered potential hepatoprotection against paracetamol-induced hepatic damage, normalizing biochemical parameters in rats plausibly by augmenting endogenous antioxidant defense mechanisms. The methanol extract was found to be more active.

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