Hepatoprotective Activity of Fruit Pulp Extract of Litchi chinensis Sonner on Carbon tetrachloride Induced Hepatotoxicity in albino Rats

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Abstract

Purpose: To evaluate aqueous and alcoholic extract of fruit pulp of Litchi chinensis for hepatoprotective activity on carbon tetrachloride induced hepatotoxicity in rats

Methodology: Fruit pulp of Litchi chinensis was pulverized, first batch was extracted with alcohol (90% v/v) and second batch was extracted with distilled water. Both the extracts were concentrated and dried separately under vacuum. Extracts were screened for hepatoprotective activity using albino rats (250-300gms) of either sex. Control group was treated with normal saline. Hepatotoxicity was induced by administering carbon tetrachloride, LIV-52 a marketed product was taken as standard and other groups were treated with alcoholic and aqueous extracts. After nine days the serum was analyzed for Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxalate Transaminase (SGOT), Alkaline Phosphatase (ALP) and serum bilirubin. Livers were isolated, weighed and subjected for histopathological studies.

Results: Carbon tetrachloride administration in rats elevated the level of SGPT, SGOT, ALP and bilirubin. Administration of LIV-52, alcoholic and aqueous extract significantly prevented this increase. Aqueous extract was found to be more effective than the alcoholic extract. Histopathological studies also confirmed the above investigation.

Conclusion: Both alcoholic and aqueous extract of fruit pulp of Lichi chinensis has shown significant ($p<0.05$) hepatoprotective activity in carbon tetrachloride induced hepatotoxicity and aqueous extract is found to be more effective than the alcoholic extract.

SOURCE OF SUPPORT

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INTRODUCTION

Litchi chinensis Sonner. (Sapindaceae) plant is evergreen shrub or tree, 10-12 meter high with broad round-topped crown of glossy green foliage. Fruits are globose or oblong to ovate, indehiscent carpels about 3.8 cm long by 2.5 cm diameter. Red when ripe (brown as usually seen), pericarp dry, thin, brittle, sharply tuberculate, containing fleshy white translucent, juicy and edible aril.

Figure 1

Fruit pulp contains sugars (12.1-14.8%), reducing sugars (0.9-13.7%), non reducing sugars, 1.3-4%, citric acid (0.22-0.33%), Vit. C (34.5-45.4mg/100gm), isobutyl acetate, Cis-rose oxide, 2-geraniol, isovaleric acid, guaiacol, vanillin, 2-acetyl-2-thiazosine and 2-phenyl ethanol. Fruit has a sweet odour of rose and traditionally the fruits are said to be used as tonic to heart, brain, liver, allys thirst, very wholesome to the body (Unani)[1,2] Impairment of vital organs like liver, heart and brain leads to serious consequences on the health of an individual and in majority of cases it is life threatening. Management of these diseases is still a challenge to the modern medicine. Various natural products are available for the treatment of liver disorders. Litchi is one such plant claimed to possess liver tonic activity, hence, was selected with the aim to establish scientific data for its traditional claim. The plant has already been investigated for its nutritive composition[2], anti-inflammatory[3] and antioxidant (in vitro) [4] activities.

OBJECTIVES

- Collection and authentication of Litchi chinensis Sonner
- Extraction of fruit pulp of Litchi with alcohol (90%) and distilled water
- Qualitative chemical Analysis of the extracts
- Acute toxicity studies
- Evaluation of extracts for Hepatoprotective activity against carbon tetrachloride induced hepatotoxicity

MATERIALS AND METHODS

Collection and authentication: Fruits of Litchi chinensis were collected from the surrounding areas of Delhi and the plant was authenticated by Anil K Goel, Scientist E-II, National Botanical Research institute, Lucknow. A voucher specimen (01 PG 502) has been in the museum of K.L.E.'s College of Pharmacy, Hubli.

Preparation of extracts: Fruit pulp was shade dried and pulverized to coarse powder I conventional grinder and was extracted with 95% ethanol in Soxhlet extractor, concentrated and under vacuum (yield 52.3% w/w). Aqueous extract was prepared by simple maceration method. Fruit pulp was macerated with distilled water. Extract was filtered and dried under vacuum (yield 49.02% w/w).

Acute toxicity studies: Acute oral toxicity was performed according to Up and Down procedure[5].

Evaluation for Hepatoprotective activity: Hepatoprotective activity was evaluated using hepatic injury models induced by carbon tetrachloride. Adult, healthy, wistar rats of either sex weighing between 180-250g housed under standard conditions and fed with standard rodent diet with water ad libitum were used. The experimental protocol was approved by the Institutional Animal Ethics Committee.

Carbon tetrachloride (CCl4) induced hepatotoxicity: The CCl4 (0.7ml/kg body weight, oral) diluted with liquid paraffin (1:1) before administration. The experimental animals were divided into seven groups of five each. The animals were then subjected to the treatments as mentioned in Table no: 1.

Figure 2

Table 1

Group 1: Treated with distilled water (1ml/kg, per oral (P.O))

Group 2: Treated with distilled water for 9 days + CCl_4 (0.7ml/kg, I.P) administered on 9th day.

Group 3: Treated with **LIV-52** (Hepato protective preparation manufactured by Himalaya herbal health care, India) 1ml/kg, P.O for 9 days+ CCl_4 (0.7ml/kg, I.P) administered on 9th day

Group 4: Treated with alcoholic extract of *L. chinensis* (250mg/kg, P.O) for 9 days+ CCl_4 (0.7ml/kg, I.P) administered on 9th day

Group 5: Treated with aqueous extract of *L. chinensis* (250mg/kg, P.O) for 9 days+ CCl_4 (0.7ml/kg, I.P) administered on 9th day

Group 6: Treated with alcoholic extract of *L. chinensis* (500mg/kg, P.O) for 9 days+ CCl_4 (0.7ml/kg, I.P) administered on 9th day

Group 7: Treated with aqueous extract of *L. chinensis* (500mg/kg, P.O) for 9 days+ CCl_4 (0.7ml/kg, I.P) administered on 9th day

During the experiment all the animals were fed with normal standard diet and water ad libitum. All the animals were sacrificed by cervical dislocation, 24hrs.after the

administration of CCl4. Blood sample were collected, serum was separated and used for the assay of marker enzymes such as Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Alkaline Phosphatase (ALP) and serum bilirubin. Livers were isolated immediately and washed with normal saline, blotted with filter paper and weights were determined and then were subjected to histopathological studies[6, 7].

STATISTICAL ANALYSIS

The statistical significance was assessed using One Way Analysis Of Variance (ANOVA) followed Student's' t' test as shown in Table no:2. $P < 0.05$ was considered as significant, CCl4 control.

Figure 3

Table 2

TREATMENT	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	SERUM BILIRUBIN (MG/DL)	LIVER WEIGHT (G/100GM BODY WEIGHT)
VEHICLE CONTROL	70.31±3.73	175.70±6.5	186.51±7.75	0.25±0.03	4.08±0.10
CCL_4CONTROL (UNTREATED)	172.06±8.03	323.25±11.98	328.00±13.45	0.61±0.07	5.23±0.10
LIV 52 (1 ML/KG, PO) + CCL_4	73.02±6.80*	177.45±8.94*	190.75±8.56*	0.30±0.03*	4.12±0.15*
ALC.EXT (250MG/KG.PO)+ CCL_4	76.08±3.99*	184.40±8.20*	202.10±3.43*	0.34±0.02*	4.54±0.21
AQ.EXT (250MG/KG.PO)+ CCL_4	73.85±2.50*	180.55±10.12*	200.20±5.76*	0.34±0.04*	4.18±0.29*
ALC.EXT (500MG/KG.PO)+ CCL_4	74.22±2.74*	182.22±6.88*	194.88±10.72*	0.32±0.06*	4.20±0.04*
AQ.EXT (500MG/KG.PO)+ CCL_4	72.82±1.08*	178.88±8.24*	192.42±6.76*	0.30±0.04*	4.12±0.62*

Effect of Alcoholic and Aqueous Extracts of Liltchi chinensis fruit pulp and LIV.52 on Carbon tetrachloride induced hepatotoxicity in rats

All the values are expressed ad Mean ± SEM

*P values <0.05, considered significant compared to untreated group

RESULTS AND DISCUSSION

Preliminary phytochemical investigation: Qualitative chemical investigation of the alcoholic extract showed the presence of carbohydrates, flavanoids, triterpenoids and tannins; aqueous extract showed the presence of carbohydrates, flavanoids and tannins proteins and amino acids

Acute Oral toxicity Studies: Aqueous and alcoholic extracts of Litchi chinensis fruit pulp did not produce any behavioral changes and mortality up to the dose of 5000mg/kg body weight. Hence, $1/10^{th}$ and $1/20^{th}$ of this dose i.e. 250mg/kg P.O and 500mg/kg P.O of both the extracts were used for the study.

Carbon tetrachloride induced toxicity: Both the extracts showed dose dependant activity. Alcoholic and aqueous extract at higher dose i.e. 500mg/kg body weight P.O and LIV-52 (1 ml/kg P.O) produced a significant reduction ($p<0.05$) in the marker enzymes (SGPT, SGOT, ALP and serum bilirubin). Lower dose of 250mg/kg P.O also showed significant reduction in marker enzyme levels but the effect was lesser compared to higher dose. Administration of CCl4 produced moderately significant increase in the liver weights. Higher dose of alcoholic and aqueous extract, LIV-52, and lower dose of aqueous extract produced significant decrease in the liver weights. But lower dose of alcoholic extract did not significantly affect the liver weight even though to lesser extent the decreased weight was observed (Table 1). Histopathological examination of the liver tissues from CCl4 intoxicated animals showed intense inflammation, congestion in the sinusoids, pyknosis of nucleus and necrosis of the liver cells. Pretreatment of animals with LIV-52 alcoholic and aqueous extracts of Litchi have shown reduction in inflammation and significantly prevented degeneration of hepatocytes.

CONCLUSION

The aqueous extract and alcoholic extract of Litchi chinensis fruit pulp has shown promising hepatoprotective activity at the administered dose of 250mg/kg and 500mg/kg body weight, orally. Alcoholic extract at the dose of 300mg/kg was not so effective compared to others. Both the extracts showed dose dependant activity. Higher dose of aqueous extract has shown the protective activity comparable with the reference drug LIV-52. CCl4 a well known hepatotoxin produces liver toxicity due to generation of free radicals. The protective activity shown by the of Litchi fruit extracts may by due to their antioxidant activity, since the extracts have shown the presence of flavanoids and also the fruits are known to be rich in Vit C, one of the well known antioxidant. Increase in the liver weights shown by CCl4 induction is due to the blocking of hepatic triglycerides secretion into plasma and the extracts might have prevented this blockade which might be one of the reasons to show the decrease in liver weights. Finally, it can be concluded that

aqueous and alcoholic extracts of fruit pulp of Litchi chinensis possess hepatoprotective activity which may be due to its antioxidant effect.

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