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Hepatoprotective effect of *Solanum xanthocarpum* fruit extract against CCl_4 induced acute liver toxicity in experimental animals

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ABSTRACT

Objective: To investigate the hepatoprotective potential of *Solanum xanthocarpum* (Solanaceae) (*S. xanthocarpum*) in experimental rats to validate its traditional claim. **Methods:** 50% ethanolic fruit extract of *S. xanthocarpum* (SXE, 100, 200 or 400 mg/kg body weight) was administered daily for 14 days in experimental animals. Liver injury was induced chemically, by CCl_4 administration (1 mL/kg i.p.).The hepatoprotective activity was assessed using various biochemical parameters like aspartate aminotransferase (AST), alanine aminotransferase (ALT), Serum alkaline phosphatise (SALP) and total bilirubin. Meanwhile, in vivo antioxidant activities as lipid peroxidation (LPO), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) were screened along with histopathological studies. **Results:** Obtained results demonstrated that the treatment with SXE significantly (P<0.05- <0.001) and dose-dependently prevented chemically induced increase in serum levels of hepatic enzymes. Furthermore, SXE significantly (up to P<0.001) reduced the lipid peroxidation in the liver tissue and restored activities of defence antioxidant enzymes GSH, SOD and catalase towards normal levels. Histopathology of the liver tissue showed that SXE attenuated the hepatocellular necrosis and led to reduction of inflammatory cells infiltration. **Conclusions:** The results of this study strongly indicate the protective effect of SXE against acute liver injury which may be attributed to its hepatoprotective activity, and there by scientifically support its traditional use.

1. Introduction

Solanum xanthocarpum Schrad. & Wendl. (Family: Solanaceae) (*S. xanthocarpum*) commonly known as Yellow Berried Nightshade (syn: Kantakari), is a prickly diffuse bright green perennial herb, woody at the base, 2-3 m height found throughout India, mostly in dry places as a weed on road sides and waste lands. The fruits are of 1.3 cm diameter berry, yellow or white with green veins, surrounded by enlarged calyx[1]. The fruits are known for several traditional medicine uses like anthelmintic, antipyretic, laxative, antiinflammatory, urinary bladder, enlargement of the liver, antiasthmatic and aphrodisiac activities[2]. The stem, flowers and fruits are prescribed for relief in burning sensation in the feet accompanied by vesicular eruptions[3]. *S. xanthocarpum* has shown antiasthmatic, anti-nociceptive, anti-fungal and molluscicide activities[4]. The fruit paste of it applied externally to the affected area for treating pimples and swellings.

The fruits are reported to contain several steroidal alkaloids like solanacarpine, solanacarpidine, solancarpine, solasonine, solamargine and other constituents like caffeic acid, coumarins like aesculetin and aesculin, steroids carpesterol, diosgenin, campesterol, daucosterol and triterpenes like cycloartanol and cycloartenol were reported from the fruits[5]. The antispasmodic, antitumor, cardiotonic, hypotensive, antianaphylactic, arbuda tumour[6], Anti-urolithiatic and natriuretic activities were

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also reported[7]. To the best of our knowledge there was lack of scientific reports available in support of its traditional claim of hepatoprotective potential. So far, there has been only one research report on hepatoprotective effect against paracetamol[8]. in an animal model is available. However, its effectiveness in protection against acute liver injury caused by carbon tetrachloride (CCl_4) had not been previously established. Therefore, present study was designed to evaluate the effect of *S. xanthocarpum* fruit extract (SXE) against carbon tetrachloride induced acute liver injury in experimental animals.

2. Materials and methods

2.1. Chemicals

All the chemicals used were of analytical grade and procured from Sigma chemicals Co., USA and Qualigens fine chemicals, Mumbai, India.

2.2. Preparation of plant extract

Fresh and matured fruits were collected from campus garden of National Botanical Research Institute, Lucknow, India in December 2010. The plant material was identified and authenticated and the voucher specimen number NAB-79023 was deposited in the institutional herbarium. The freshly collected fruits (2 kg) of *S. xanthocarpum* were dried and powdered. The powdered plant a material (900 g) was macerated with petroleum ether, the marc was exhaustively extracted with of 50 % ethanol for three days. The extract was separated by filtraction and concentrated on rotavapour (Buchi,USA) and then dried in lyophilizer (Labconco,USA) under reduced pressure. The yield obtained was 198.40 g of solid residue (yield 22.04% w/w).). Preliminary qualitative phytochemical screening of SXE has given the positive testes for flavonoids, steroidal alkaloids, triterpenes, flavanoids, quercitrin and apigenin glycosides[9].

2.3. Animals

Sprague-Dawley rats weighing (150-170 g) and Swiss albino mice (25-30 g) of either sex were procured from CDRI, Lucknow. They were kept in departmental animal house in well cross ventilated room at (22±2) ℃, and relative humidity 44%-56 %, light and dark cycles of 12 h, for 1 week before and during the experiments. Animals were provided with standard rodent pellet diet (Amrut, India) and the food was withdrawn 18-24 h before the experiment though water was given ad libitum. All studies were performed in accordance with the guide for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee, CPCSEA, India (Reg. No. 222/2000/CPCSEA).

2.4. Acute oral toxicity studies

Acute toxicity study was performed according to Organisation for Economic Co-operation and Development guidelines No. 423[10]. Swiss albino mice of either sex were divided into six groups with six animals each. SXE was administered orally as a single dose to mice at different dose levels of 250, 500, 1 000, 1 500 and 2 000 mg/kg b.w. Animals were observed periodically for the symptoms of toxicity and death within 24 h and then daily for 14 days.

2.5. CCl_4 induced hepatotoxicity

The Sprague-Dawley rats were divided into six groups, each group had six animals. Group I (control) animals were administered a single daily dose of carboxymethyl cellulose (1 mL of 1%, w/v, p.o. body weight). Group II received carbon tetrachloride (1 mL/kg body weight, i.p. 1:1 v/v mixture of CCl_4 and liquid paraffin) alone while group III, IV and V received orally 100, 200 and 400 mg/kg body weight of SXE in (1 %, w/v, CMC) respectively along with carbon tetrachloride as in group II. Group VI received silymarin, the known hepatoprotective compound (Sigma Chemicals Company, USA), at a dose of 100 mg/kg, p.o., along with carbon tetrachloride. The SXE was given daily while carbon tetrachloride was given for every 72 h for 14 days. Animals were sacrificed 48 h after the last dose of the drug. The liver samples were dissected and blood was collected[11].

2.6. Assessment of hepatoprotective activity

The collected blood was allowed to clot and serum was separated at 2 500 rpm for 15 min and the biochemical parameters like serum enzymes: aspartate aminotransferase (AST, U/L), serum glutamate pyruvate transaminase (ALT, U/L)[12], serum alkaline phosphatase (ALP, U/L)[13] and total bilirubin (mg/dL)[14] were assayed using assay kits.

2.7. Assessment of antioxidant parameters

The dissected out liver samples were washed immediately with ice cold saline to remove as much blood as possible. Liver homogenized (5%) in ice cold 0.9% NaCl with a Potter- Elvenhjem glass homogenizer. The homogenate was centrifuged at 800 for 10 min and the supernatant was again centrifuged at 12 000 for 15 min and the obtained mitochondrial fraction was used for the estimation of LPO[15], catalase (CAT)[16]. Superoxide dismutase (SOD) activity was estimated by the inhibition of nicotinamide adenine dinucleotide (reduced)-phenazine methosulphate-nitrobluetetrazolium reaction system as described by Nishikimi[17]. The concentration of GSH was determined by the method of Anderson[18].

2.8. Histopathological studies

For histologic studies, the liver tissues were fixed with 10% phosphate buffered neutral formalin, dehydrated in graded (50%-100%) alcohol and embedded in paraffin. Thin

sections (5 M) were cut and stained with routine hematoxylin and eosin (H & E) stain for photo microscopic assessment. The initial examination was qualitative, with the purpose of determining histopathological lesions in liver tissue.

2.9. Statistical analysis

The values were represented as mean±S.E.M. for six rats. Analysis of variance (ANOVA) test was followed by individual comparison by Newman-Keuls test using Prism Pad software (version 3.0) for the determination of level of significance. The values of $P<0.05$ was considered statistically significant.

3. Results

3.1. Acute toxicity studies

Solanum xanthocarpum produces no mortality at 2 000 mg/kg. Therefore, one-tenth of the maximum no mortality dose of extract were selected as therapeutic middle dose (200 mg/kg) and just double as well as half dose of it as highest (400 mg/kg) and lowest dose (100 mg/kg) respectively, in this study.

3.2. Effect of SXE on AST, ALT, ALP and total bilirubin

The effect various doses of SXE were studied on serum marker enzymes and total bilirubin in CCl_4 intoxicated animal. Hepatic injury induced by CCl_4 caused significant changes in marker enzyme as AST by 268.94%, ALT by 383.68%, ALP by 134.72% and total bilirubin by 309.21% compared to control group. The percentage protection in marker enzyme of treated group at 100, 200 mg/kg as AST 29.11 ($P< 0.01$), 51.22 ($P< 0.001$), ALT 25.01 ($P< 0.05$), 54.66 ($P< 0.001$), ALP 28.50 ($P< 0.01$), 43.24 ($P< 0.001$) and total bilirubin 25.08 ($P< 0.01$), 62.37 ($P< 0.001$) compared to CCl_4 group while maximum percentage protection in marker enzyme at the dose of 400 mg/kg and silymarin (100mg/kg) as AST 67.71 ($P< 0.001$), 70.36 ($P< 0.001$), ALT 75.66 ($P< 0.001$), 77.40 ($P< 0.001$), ALP 54.52 ($P< 0.001$), 59.80 ($P< 0.001$) and total bilirubin 72.34 ($P< 0.001$), 73.31 ($P< 0.001$) which is almost comparable to the group treated with silymarin, a potent hepatoprotective drug used as reference standard (Table 1).

3.3. Estimation of LPO, GSH, SOD and CAT

The results in Table 2 showed clear significant percentage change in the levels of LPO in CCl_4 intoxicated rats as 251.28 ($P< 0.001$) compared to control group.

% Change (protection) in activity = (1 -T/C) × 100

Where: T = treatment groups II to VI (either test groups or toxic group), C = Normal control group I

Treatment with SXE at the doses of 100, 200 and 400 mg/kg significantly prevented this heave in levels and the percentage protection in LPO were 31.38 ($P< 0.05$), 52.28 ($P< 0.01$) and 67.88 ($P< 0.001$) respectively. The GSH, SOD and CAT content had significantly increased in SXE treated groups whereas CCl_4 intoxicated group had shown significant decrease in these parameters compared to control group. The percentage changed of GSH, SOD and CAT in CCl_4 intoxicated group were as 124.32 ($P< 0.001$), 64.63 ($P< 0.001$) and 40.87 ($P< 0.001$) respectively. The percentage protection in GSH as 37.83 (ns), 81.08 ($P< 0.01$), 110.81 ($P< 0.001$) and SOD 47.97 (ns), 101.86 ($P<0.05$), 145.17 ($P<0.001$) while in CAT 18.28 (ns),

Table 1
Effect of SXE on serum AST(U/L), ALT (U/L), ALP (U/L) and Total bilirubin level (mg/dL) against CCl_4 induced liver toxicity in rats.

Groups	AST	ALT	ALP	TBL
Control	104.26±18.13	47.81±8.22	67.13±7.88	0.76±0.15
CCl_4	384.66±36.21†	231.25±24.87†	157.57±14.55†	3.11±0.39†
SXE 100	272.65±23.62b	173.41±18.24a	112.66±11.21b	2.23±0.14b
SXE 200	187.63±19.87c	104.83±14.46c	89.43±8.61c	1.17±0.11c
SXE 400	124.17±16.54c	56.27±9.33c	71.65±7.54c	0.86±0.13c
Silymarin	113.99±12.43c	52.24±6.72c	69.34±7.11c	0.83±0.12c

Values are mean±S.E.M. of 6 rats in each group. *P* values: $^{\dagger}<0.001$ compared with respective control group I; *P* values: a<0.05, b<0.01, c<0.001 compared with group II (CCl_4).

Table 2
Effect of SXE on liver LPO (MDA nmole/min/mg of protein), GSH (nmole/mg of protein), SOD (unit/mg of protein) and CAT (units/mg of protein) against CCl_4 induced liver toxicity in rats.

Groups	LPO	GSH	SOD	CAT
Control	0.39±0.08	0.83±0.07	27.23±2.82	56.51±5.52
CCl_4	1.37±0.17†	0.37±0.04†	9.63±1.62†	33.41±2.37†
SXE 100	0.94±0.13a	0.51±0.05n	14.25±1.21	39.52±2.57
SXE 200	0.64±0.17b	0.67±0.08b	19.44±2.63a	46.43±3.11a
SXE 400	0.44±0.12c	0.78±0.05c	23.61±2.51c	53.4±3.45b
Silymarin	0.42±0.09c	0.80±0.06c	24.32±2.32c	54.9±2.51b

Values are mean±S.E.M. of 6 rats in each group. *P* values: $^{\dagger}<0.001$ compared with respective control group I; *P* values: a<0.05, b<0.01, c<0.001 compared with group II (CCl_4) .

38.97 (P<0.05), 59.83 (P<0.01) at the doses levels 100, 200 and 400 mg/kg, respectively. In different doses level of SXE, 400 mg/kg has shown maximum protection which were almost comparable to those of the normal control and silymarin

3.4. Histopathological observations

The histological observations basically support the results obtained from serum enzyme assays. Histopathology of liver section is well described in Figure 1 ligand.

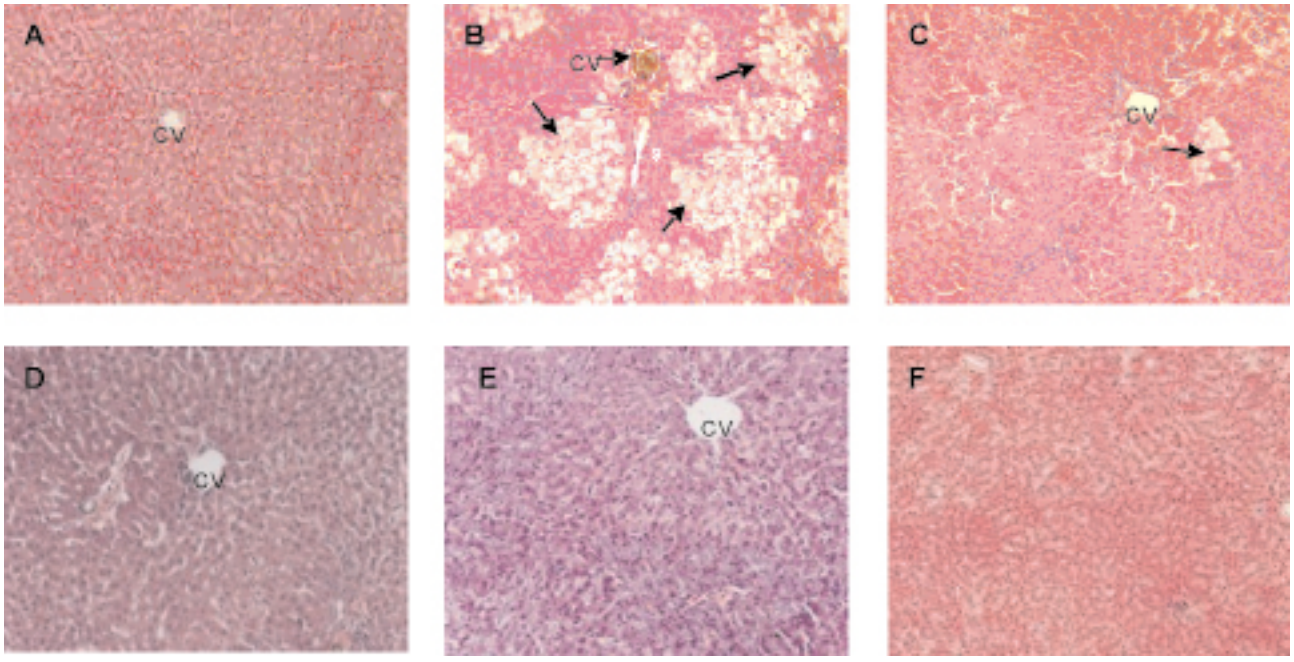

Figure 1. Histopathology of liver tissues.
A: Liver section of normal control rat shows central vein surrounded by hepatic cord of cells (normal architecture).
B: Liver section of CCl_4 treated rats showing massive fatty changes along with congestion in central vein, necrosis, ballooning degeneration and the loss of cellular boundaries (indicated by arrow).
C: Liver section of rats treated CCl_4 and 100 mg/kg of SXE showing inflammatory collections around central vein and focal necrosis with sinusoidal dilatation.
D: Liver section of rats treated CCl_4 and 200 mg/kg of SXE showing less inflammatory cells around central vein, absence of necrosis.
E: Liver section of rats treated CCl_4 and 400 mg/kg of SXE showing regeneration of hepatocytes around central vein toward near normal liver architecture.
F: Liver section of rats treated CCl_4 and 100 mg/kg of silymarine showing normal liver architecture.

4. Discussion

In the present investigation, SXE was evaluated for the hepatoprotective activity using CCl_4 induced hepatotoxicity in rat. The hepatotoxicity induced by CCl_4 is due to its metabolite $CCl_3^{\bullet}$, a free radical that alkylates cellular proteins and other macromolecules with a simultaneous attack on polyunsaturated fatty acids, in the presence of oxygen, to produce lipid peroxides, leading to liver damage[19]. Hepatocellular necrosis leads to elevation of the serum marker enzymes, which are released from the liver into blood[20-23].

The present study revealed a significant increase in the activities of AST, ALT, ALP and serum bilirubin levels on exposure to CCl_4, indicating considerable hepatocellular injury. Administration of SXE at different doses level (100, 200 and 400 mg/kg) attenuated the increased levels of the serum enzymes, produced by CCl_4 and caused a subsequent recovery towards normalization comparable to the control groups animals. The hepatoprotective effect of the SXE was further accomplished by the histopathological examinations. SXE at different dose levels offers hepatoprotection, but 400 mg/kg is more effective than all other lower doses.

In CCl_4 induced hepatotoxicity, the balance between ROS production and these antioxidant defences may be lost, 'oxidative stress' results, which through a series of events deregulates the cellular functions leading to hepatic necrosis. The reduced activities of SOD and catalase observed point out the hepatic damage in the rats administered with CCl_4 but the treated with 100, 200 and 400 mg/kg of SXE groups showed significant increase in the level of these enzymes, which indicates the antioxidant activity of the Solanum xanthocarpum. Regarding non enzymic antioxidants, GSH is a critical determinant of tissue susceptibility to oxidative damage and the depletion of hepatic GSH has been shown to be associated with an enhanced toxicity to chemicals, including CCl_4[24]. Furthermore, a decrease in hepatic tissue GSH level was observed in the CCl_4 treated groups. The increase in hepatic GSH level in the rats treated with 100, 200 and 400 mg/kg of SXE may be due to de novo GSH

synthesis or GSH regeneration. The level of lipid peroxide is a measure of membrane damage and alterations in structure and function of cellular membranes. In the present study, elevation of lipid peroxidation in the liver of rats treated with CCl_4 was observed. The increase in LPO levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defence mechanisms to prevent the formation of excessive free radicals[25]. Treatment with SXE significantly reversed all the changes. Hence, it is possible that the mechanism of hepatoprotection of *S. xanthocarpum* may be due to its antioxidant activity.

On preliminary qualitative phytochemical screening, SXE revealed the presence of flavonoids, steroidal alkaloids, triterpenes, flavanoids, quercitrin and apigenin glycosides are the major chemical constituents. These antioxidant phytochemicals might contribute to the hepatoprotective and antioxidant activities of the fruit of *S. xanthocarpum*. In conclusion, this study showed that the ethanolic fruit extract of *S. xanthocarpum* has hepatoprotective effects that were proven by biochemical and histopathological analysis. The SXE has shown dose dependent activity among which at the dose level of 400 mg/kg,
p.o. shows greater activity which is comparable with the control and standard groups. However, further investigation is in process on the fruit extract to identify the active constituents responsible for hepatoprotection.

Conflict of interest statement

We declare that we have no conflict of interest.

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