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Research Article

SCREENING OF ANTI-HEPATOTOXIC POTENTIAL OF *SOLANUM XANTHOCARPUM* LEAF EXTRACT AGAINST CCL_4 INDUCED ACUTE HEPATOPATHY IN EXPERIMENTAL RODENTS

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ABSTRACT

The *solanum xanthocarpum* is claimed to possess various pharmacological activities like anti-helmintic, antipyretic laxative, anti-inflammatory, enlargement of the liver, anti-asthmatic, aphrodisiac activities, anti-asthmatic, anti-nociceptive, anti-fungal and molluscicide activities. Aim of the present study to investigate the hepatoprotective potential of *solanum xanthocarpum* (Solanaceae) leaf extract in experimental rats to validate its traditional claim. 50% ethanolic leaf extract of *solanum xanthocarpum* (SXLE, 200 and 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. Obtained results demonstrated that the treatment with SXLE significantly ($P<0.05$-$P<0.001$) and dose-dependently prevented chemically induced increase in serum levels of hepatic enzymes. Furthermore, SXLE 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 SXLE attenuated the hepatocellular necrosis and led to reduction of inflammatory cells infiltration. The results of this study strongly indicate the protective effect of SXLE against acute liver injury which may be attributed to its hepatoprotective activity, and there by scientifically support its traditional use.

Keywords: *Solanum xanthocarpum* leaf extract (SXLE), Hepatoprotective, CCl_4, Antioxidant, Alkaline phosphatise.

INTRODUCTION

Liver diseases are one of the most serious health problems in the world today but, despite tremendous advances in modern medicine, their prevention and treatment options still remain limited. However, the pathogenesis of hepatic diseases as well as the role of oxidative stress and inflammation therein is well established[1] and accordingly, blocking or retarding the chain reactions of oxidation and inflammation process could be a promising therapeutic strategy for prevention and treatment of liver injury. Recently, the most common *in-vivo* model used in the investigation of new hepatoprotective agents has been a well-characterized rodent model of liver injury induced by carbon tetrachloride (CCl_4), a chemical hepatotoxin that causes free radical-mediated hepatocellular damage.[2] CCl_4-induced hepatotoxicity is believed to involve two phases. The initial phase involves the metabolism of CCl_4 by Cytochrome P_{450} to the trichloromethyl radicals, which lead to membrane lipid peroxidation and finally to cell necrosis.[3] The second phase of CCl_4-induced hepatotoxicity involves the activation of Kupffer cells, which is accompanied by the production of proinflammatory mediators.[4] Several microarray studies have been reported describing gene expression changes caused by acute CCl_4 toxicity.[5]

Solanum xanthocarpum Schrad. & Wendl. (family: Solanaceae) commonly known as Yellow Berried Nightshade (syn: Kantakari), is a prickly diffuse bright green perennial herb, woody at the base, 2–3m 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.[6] The fruits are known for several traditional medicine uses like anthelmintic, antipyretic, laxative, antiinflammatory, urinary bladder, enlargement of the liver, antiasthmatic and aphrodisiac activities.[7] The stem, flowers and fruits are prescribed for relief in burning sensation in the feet accompanied by vesicular eruptions.[8] *S. xanthocarpum* has shown antiasthmatic, anti-nociceptive, anti-fungal and molluscicide activities.[9] 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.[10] The antispasmodic, antitumor, cardiotonic, hypotensive, antianaphylactic, arbuda tumour,[11] anti-urolithiatic and natriuretic activities were also reported.[12] 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 few research report on hepatoprotective effect against paracetamol[13] and anti-tubercular[14] animal model is available. However, its effectiveness in protection against acute liver injury caused by carbon tetrachloride (CCl_4) of leaf extract had not been previously established. Therefore, present study was designed to evaluate the effect of *solanum xanthocarpum* leaf extract (SXLE) against carbon tetrachloride induced acute liver injury in experimental animals.

MATERIALS AND METHODS

Chemicals

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

Preparation of plant extract

Fresh and matured leaf was 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-79024 was deposited in the institutional herbarium. The freshly collected leaf (2.5 kg) of *solanum xanthocarpum* were dried and powdered. The powdered plant a material (750 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 rota vapour (Buchi, USA) and then dried in lyophilizer (Labconco, USA) under reduced pressure. The yield obtained was 154.86 g of solid residue (yield 20.64% w/w).). Preliminary qualitative phytochemical screening of SXLE has given the positive testes for, flavonoids, steroidal alkaloids, triterpenes, quercitrin and apigenin glycosides.[15]

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 °C, 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).

Acute oral toxicity studies

Acute toxicity study was performed according to Organisation for Economic Co-operation and Development guidelines No. 423.[16] Swiss albino mice of either sex were divided into six groups with six animals each. SXLE was administered orally as a single dose to mice at different dose levels of 250, 500, 1000, 1500 and 2000 mg/kg b.w. Animals were observed periodically for the symptoms of toxicity and death within 24 h and then daily for 14 days.

CCl_4 induced hepatotoxicity

The animals were divided into five 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 and IV received orally 200 and 400 mg/kg body weight of SXLE in (1 %, w/v, CMC) respectively along with carbon tetrachloride as in group II. Group V received silymarin, the known hepatoprotective compound (Sigma Chemicals Company, USA), at a dose of 100 mg/kg, *p.o.*, along with carbon tetrachloride. The SXLE 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.[17]

Assessment of hepatoprotective activity

The collected blood was allowed to clot and serum was separated at 2500 rpm for 15 min and the biochemical parameters like serum enzymes: aspartate aminotransferase (AST, U/L), alanine aminotransferase (ALT, U/L),[18] serum alkaline phosphatase (ALP, U/L)[19] and total bilirubin (mg/dL)[20] were assayed using assay kits.

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, [21] catalase (CAT).[22] Superoxide dismutase (SOD) activity was estimated by the inhibition of nicotinamide adenine dinucleotide (reduced)-phenazine methosulphate–nitrobluetetrazolium reaction system as described by Nishikimi.[23] The concentration of GSH was determined by the method of Anderson.[24]

Histopathological studies

For histological studies, the liver tissues were fixed with 10% phosphate buffered neutral formalin, dehydrated in graded (50-100%) alcohol and embedded in paraffin. Thin sections (5µm) were cut and stained with routine hematoxylin and eosin stain for photo microscopic assessment. The initial examination was qualitative, with the purpose of determining histopathological lesions in liver tissue.

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.

RESULTS

Acute toxicity studies

Solanum xanthocarpum produces no mortality at 2000 mg/kg. Therefore, one-tenth of the maximum no mortality dose of extract was selected as therapeutic middle dose (200 mg/kg) and just double dose of it as highest (400 mg/kg) respectively, in this study.

Effect of SXLE on AST, ALT, ALP and total bilirubin

The effect various doses of SXLE were studied on serum marker enzymes and total bilirubin in CCl_4 intoxicated animal. Hepatic injury induced by CCl_4 caused significant changed in marker enzyme as AST by 205.42%, ALT by 133.43%, ALP by 178.67% and total bilirubin by 75% compared to control group. The percentage protection in marker enzyme of treated group at 200 mg/kg as AST 7.6 ($P< 0.01$), ALT 6.27 ($P< 0.05$), ALP 4.75 ($P< 0.05$) and total bilirubin 11.11 ($P< 0.01$) 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 15.76 ($P< 0.001$), 48.94 ($P< 0.001$), ALT 25.13 ($P< 0.001$), 50.01 ($P< 0.001$), ALP 20.53 ($P< 0.001$), 57.41 ($P< 0.001$) and total bilirubin 23.87 ($P< 0.001$), 29.36 ($P< 0.001$) which is almost comparable to the group treated with silymarin, a potent hepatoprotective drug used as reference standard (Table 1).

Table 1: Effect of SXLE 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.

Group	AST	ALT	ALP	TBL
Control	72.38 ± 2.51	62.89 ± 2.13	86.51 ± 2.90	13.61 ± 1.09
Toxic	221.07±4.2†	146.81 ± 4.3†	241.08 ± 4.8†	37.82 ± 3.02†
SXLE-200	204.21 ±3.8^{b}	136.72 ± 3.4^{a}	229.61 ± 3.8^{a}	31.81 ± 3.1^{b}
SXLE-400	186.32 ± 3.4^{c}	109.91 ± 3.2^{c}	182.46 ± 3.1^{c}	24.06 ± 2.9^{c}
SL	112.87 ± 2.9^{c}	73.38 ± 2.24^{c}	102.67 ±3.03^{c}	17.21 ± 2.2^{c}

Table 2:Effect of SXLE on liver LPO (MDA nmol/min/mg of protein), GSH (nmol/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	13.61 ±1.09	1.21 ± 0.02	27.71 ± 1.9	46.81 ± 2.5
Toxic	37.82±3.02†	0.46 ± 0.02†	11.81 ± 1.2 †	21.04 ± 2.2†
SXLE-200	31.18 ± 3.1^{n}	0.57 ± 0.01^{b}	16.87 ± 1.31^{a}	26.98 ± 2.4^{n}
SXLE-400	24.06 ± 2.9^{b}	0.72 ± 0.02^{c}	21.08 ± 1.41^{c}	34.16 ± 3.1^{b}
SL	17.21 ± 2.2^{c}	0.91 ± 0.03^{c}	23.04 ± 1.82^{c}	41.06 ± 3.8^{c}

Values are mean ± S.E.M. of 6 rats in each group
n: no significant
P values: †<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)

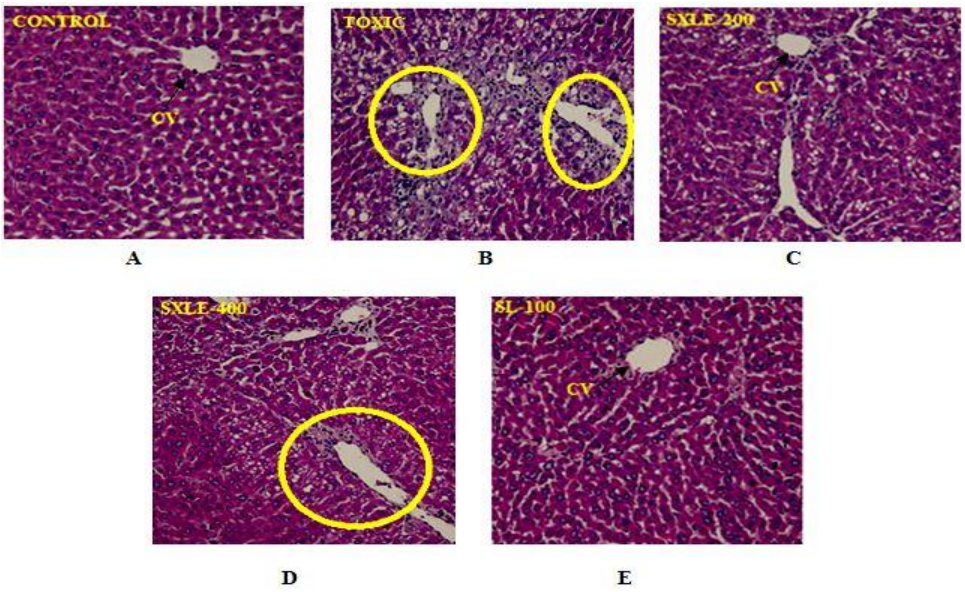

Figure Legends 1:Histopathology of liver tissues: A- Liver section of normal control rat shows central vein surrounded by hepatic cord of cells i.e. normal architecture. B- Liver section of CCl_4 treated rats showing massive fatty changes along with congestion in central vein, necrosis, sinusoidal dilation, ballooning degeneration and the loss of cellular boundaries. C- Liver section of rats treated CCl_4 and 200 mg/kg of SXLE showing inflammatory collections around central vein, less ballooning and focal necrosis with sinusoidal dilatation. D- Liver section of rats treated CCl_4 and 400 mg/kg of SXLE showing regeneration of hepatocyte's around central vein toward near normal liver architecture, absence of ballooning but showing sinusoidal dilation. E- Liver section of rats treated CCl_4 and 100 mg/kg of silymarin showing normal liver architecture.

Estimation of LPO, GSH, SOD and CAT

The results in Table 2 ligand showed clear significant percentage change in the levels of LPO in CCl_4 intoxicated rats as 177.88 ($P< 0.001$) compared to control group. Treatment with SXLE at the doses of 200 and 400 mg/kg significantly prevented this heave in levels and the percentage protection in LPO were 17.55 (ns) and 36.38 ($P< 0.01$) respectively. The GSH, SOD and CAT content had significantly increased in SXLE 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 61.98 ($P< 0.001$), 57.3 ($P< 0.001$) and 55.05 ($P< 0.001$) respectively. The percentage protection in GSH as 23.91 ($p<0.01$), 56.52 ($P< 0.001$) and SOD 42.84 ($p<0.05$), 78.49 ($P < 0.001$) while in CAT 28.23 (ns), 62.35 ($P < 0.001$) at the doses levels 200 and 400 mg/kg, respectively. In different doses level of SXLE, 400 mg/kg has shown maximum protection which was almost comparable to those of the normal control and silymarin.

DISCUSSION

In the present investigation, *solanum xanthocarpum* (SXLE) 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.[25] Hepatocellular necrosis leads to elevation of the serum marker enzymes, which are released from the liver into blood.[26] 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 SXLE at different doses level (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 SXLE was further accomplished by the histopathological examinations. SXLE 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 200 and 400 mg/kg of SXLE groups showed significant increase in the level of these enzymes, which indicates the antioxidant activity of the SXLE. 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.[27] 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 200 and 400 mg/kg of SXLE 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.[28] Treatment with SXLE significantly reversed all the changes. Hence, it is possible that the mechanism of hepatoprotection of SXLE may be due to its antioxidant activity. On preliminary qualitative Phytochemical screening, SXLE revealed the presence of flavonoids, steroidal alkaloids, triter penes, flavonoids, quercitrin and apigenin glycosides are the major chemical constituents. These antioxidant phytochemicals might contribute to the hepatoprotective and antioxidant activities of the leaf of SXLE.

CONCLUSION

In conclusion, this study showed that the ethanolic leaf extract of *solanum xanthocarpum* has hepatoprotective effects that were proven by biochemical and histopathological analysis. The SXLE 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 leaf extract to identify the active constituents responsible for hepatoprotection.

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