SCREENING OF ANTI-HEPATOTOXIC POTENTIAL OF SOLANUM XANTHOCARPUM LEAF EXTRACT AGAINST CCl₄ INDUCED ACUTE HEPATOPATHY IN EXPERIMENTAL RODENTS

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ABSTRACT

The solanum xanthocarpum is claimed to possess various pharmacological activities like anti-helminthic, 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% ethanol 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 CCl₄ administration (1 ml/kg ip.). 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 (P<0.05-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 hepato cellular 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 thereby scientifically support its traditional use.

Keywords: Solanum xanthocarpum leaf extract (SXLE), Hepatoprotective, CCl₄, Antioxidant, Alkaline phosphatase.

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 established¹ 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 (CCl₄), a chemical hepatotoxin that causes free radical-mediated hepatic cell damage.² CCl₄-induced hepatotoxicity is believed to involve two phases. The initial phase involves the metabolism of CCl₄ by Cytochrome P₄₅₀ to the trichloromethyl radicals, which lead to membrane lipid peroxidation and finally to cell necrosis.² The second phase of CCl₄-induced hepatotoxicity involves the activation of Kupffer cells, which is accompanied by the production of proinflammatory mediators.² Several microarray studies have been reported describing gene expression changes caused by acute CCl₄ 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, 2–3 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.⁶ The fruits are known for several traditional medicine uses like anthelmintic, antipyretic, laxative, anti-inflammatory, urinary bladder, enlargement of the liver, anti-asthmatic 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 antiamoebic, 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 solanarpine, solanarpidine, solancarpine, solasonine, solamargine and other constituents like caffeic acid, coumarins like aesculetin and aesculin, steroids carisperter, dioxigen, campersterol, daucosterol and triterpenes like cycloartenol and cycloartenol were reported from the fruits.¹⁰ The anti spasmodic, antitumor, cardiotonic, hypotensive, antianaphylactic, arbusa tumour,¹¹ anti-urtiathiatic 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 paracetamol¹³ and anti-tubercular¹⁴ animal model is available. However, its effectiveness in protection against acute liver injury caused by carbon tetrachloride (CCl₄) 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 filtration 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. 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 bw.

Animals were observed periodically for the symptoms of toxicity and death within 24 h and then daily for 14 days.

CCL₄ 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 CCl₄ 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.

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), transfersase (ALP, U/L) and total bilirubin (mg/dl) 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-Elvienhjem 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, catalase (CAT), Superoxide dismutase (SOD) activity was estimated by the inhibition of nitroblue tetrazolium reaction system as described by Nishikimi. The concentration of GSH was determined by the method of Anderson.

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 CCl₄ intoxicated animal. Hepatic injury induced by CCl₄ caused significant change 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 7.67 (P< 0.05), ALT 6.27 (P< 0.05), ALP 4.75 (P< 0.05) and total bilirubin  11.11 (P< 0.01) compared to CCl₄ 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 CCl₄ induced liver toxicity in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALP (U/L)</th>
<th>Total bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>72.38 ± 2.51</td>
<td>62.89 ± 2.13</td>
<td>86.51 ± 2.90</td>
<td>13.61 ± 1.09</td>
</tr>
<tr>
<td>Toxic</td>
<td>221.07 ± 4.27</td>
<td>146.88 ± 4.93</td>
<td>241.08 ± 4.87</td>
<td>37.82 ± 3.02</td>
</tr>
<tr>
<td>SXLE-200</td>
<td>204.21 ± 3.86</td>
<td>136.72 ± 3.48</td>
<td>229.61 ± 3.80</td>
<td>31.81 ± 3.13</td>
</tr>
<tr>
<td>SXLE-400</td>
<td>186.32 ± 3.42</td>
<td>109.91 ± 3.24</td>
<td>182.46 ± 3.14</td>
<td>24.06 ± 2.92</td>
</tr>
<tr>
<td>SL</td>
<td>112.87 ± 2.94</td>
<td>73.38 ± 2.24</td>
<td>102.67 ± 3.03</td>
<td>17.21 ± 2.25</td>
</tr>
</tbody>
</table>

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 CCl₄ induced liver toxicity in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>LPO (nmol/min/mg)</th>
<th>GSH (nmol/mg)</th>
<th>SOD (unit/mg)</th>
<th>CAT (units/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13.61 ± 1.09</td>
<td>1.21 ± 0.02</td>
<td>27.71 ± 1.9</td>
<td>46.81 ± 2.5</td>
</tr>
<tr>
<td>Toxic</td>
<td>37.82 ± 3.02</td>
<td>0.46 ± 0.02</td>
<td>11.81 ± 1.2</td>
<td>21.04 ± 2.2</td>
</tr>
<tr>
<td>SXLE-200</td>
<td>31.18 ± 3.14</td>
<td>0.57 ± 0.01</td>
<td>16.87 ± 1.31</td>
<td>26.98 ± 2.4</td>
</tr>
<tr>
<td>SXLE-400</td>
<td>24.06 ± 2.96</td>
<td>0.72 ± 0.02</td>
<td>21.08 ± 1.41</td>
<td>34.16 ± 3.18</td>
</tr>
<tr>
<td>SL</td>
<td>17.21 ± 2.21</td>
<td>0.91 ± 0.03</td>
<td>23.04 ± 1.82</td>
<td>41.06 ± 3.68</td>
</tr>
</tbody>
</table>

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: *<0.05, *<0.01, *<0.001 compared with group II (CCIs)
susceptibility to oxidative damage and the depletion of defences may be lost, 'oxidative stress' results, chemical change of GSH, SOD and CAT in CCl₄ intoxicated hepatotoxicity, the balance between ROS production and which through a series of events deregulates the cellular functions induced hepatotoxicity, the SXLE offered hepatoprotection, effect of the SXLE was further studied on the level of these enzymes, indicating 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 CCl₄. Furthermore, a decrease in hepatic tissue GSH level was observed in the CCl₄ intoxicated 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 CCl₄ 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. 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, triterpenoids, 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.

DISCUSSION

In the present investigation, Solanum xanthocarpum (SXLE) was evaluated for the hepatoprotective activity using CCl₄ induced hepatotoxicity in rat. The hepatotoxicity induced by CCl₄ is due to its metabolite CCl₃ which is 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. Hepatocellular necrosis leads to elevation of the serum marker enzymes, which are released from the liver into blood. Hepatocellular necrosis is due to increase in the activities of AST, ALT, ALP and serum bilirubin levels on exposure to CCl₄ 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 CCl₄, and caused a subsequent recovery towards normalization comparable to the control groups animals. The hepatoprotective effect of the SXLE was further accomplished by histopathological examinations. SXLE at different dose levels offers hepatoprotection, but 400 mg/kg is more effective than all other lower doses. In CCl₄ 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 CCl₄, 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 CCl₄. Furthermore, a decrease in hepatic tissue GSH level was observed in the CCl₄ intoxicated groups.

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REFERENCES


