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ORIGINAL ARTICLE

Antioxidant and hepatoprotective effects of *Solanum xanthocarpum* leaf extracts against CCl_4-induced liver injury in rats

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Abstract

Context: *Solanum xanthocarpum* Schard. and Wendl. (Solanaceae) has been used in traditional Indian medicines for its antioxidant, anti-inflammatory, and antiasthmatic properties.
Objective: The present study demonstrates the antioxidant and hepatoprotective effects of *S. xanthocarpum*. On the basis of *in vitro* antioxidant properties, the active fraction from column chromatography of the methanol extract of *S. xanthocarpum* leaves (SXAF) was chosen as the potent fraction and used for hepatoprotective studies in rats.
Materials and methods: The antioxidant activity was evaluated by 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and reducing power assays. Rats were pre-treated with 100 and 200 mg/kg b.w. of SXAF for 14 d with a single dose of CCl_4 in the last day. Hepatoprotective properties were determined by serum biochemical enzymes, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), antioxidant enzymes (SOD, CAT, GSH, and GST), and histopathology studies.
Results: SXAF exhibited significant antioxidant activity in scavenging free radicals with IC_{50} values of 11.72 μg (DPPH) and 17.99 μg (ABTS). Rats pre-treated with SXAF demonstrated significantly reduced levels of serum LDH (1.7-fold), ALP (1.6-fold), and AST (1.8-fold). Similarly, multiple dose SXAF administration at 200 mg/kg b.w. demonstrated significantly enhanced levels of SOD (1.78 ± 0.13), CAT (34.63 ± 1.98), GST (231.64 ± 14.28), and GSH (8.23 ± 0.48) in liver homogenates. Histopathological examination showed lowered liver damage in SXAF-treated groups.
Discussion and conclusion: These results demonstrate that SXAF possesses potent antioxidant properties as well as hepatoprotective effects against CCl_4-induced hepatotoxicity.

Keywords

DPPH, free radicals, histopathology, lipid peroxidation, oxidative stress, phenolic compounds, superoxide dismutase

History

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Introduction

It has been recognized that oxidative stress and generation of free radicals play a critical role in the development of liver disease. Therefore, some natural products with antioxidant activity have attracted great attention as potential functional ingredients to protect liver injury (Wang et al., 2009). The production of oxidative stress can be controlled by antioxidant systems in living organisms. It is commonly recognized that reactive oxygen species (ROS) are involved in a variety of physio-pathological processes, including cellular signal transduction, cell proliferation, differentiation and apoptosis, as well as ischemia-reperfusion, injuries, inflammation and many neurodegenerative disorders. ROS include free radicals such as superoxide ($^{\bullet}O_2^-$), hydroxyl radical ($^{\bullet}OH$), peroxyl radical ($RO_2^{\bullet}$) as well as non-radical species such as hydrogen peroxide (H_2O_2) (Cerutti, 1991).

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Oxidative stress is a process where the physiological balance between pro-oxidants and antioxidants is disrupted in favor of the former, ensuing in potential damage for the organism (Halliwell & Gutteridge, 1990). Antioxidants are free radical scavengers (FRS) which postpone the oxidation and block the chain initiated by high energy molecules and other consequent reactions (Cespecles et al., 2008). Hepatotoxic chemicals cause liver damage which are induced by lipid peroxidation and other oxidative damage (Appiah et al., 2009). Several compounds, such as carbon tetrachloride ($CC1_4$), acetaminophen, bromobenzene, ethanol and polycyclic aromatic hydrocarbons, have been implicated in the etiology of liver diseases (Adesanoye & Farombi, 2010). The toxicity of CCl_4 probably depends on the formation of the trichloromethyl radical ($CCl_3^{\bullet}$), which in the presence of oxygen forms the more toxic trichloromethyl peroxyl radical ($CCl_3O^{\bullet}$) (Recknagel et al., 1989). Liver cells possess a number of compensatory mechanisms to deal with ROS and their effects; among these are the induction of a number of antioxidant proteins such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHP*x*), and the tripeptide glutathione (GSH).

Plants have been used for many years as a source of traditional medicine to treat various diseased conditions. Many of these medicinal plants are also excellent sources for phytochemicals, with potent antioxidant activities. Dietary measures and traditional plant therapies are prescribed by Ayurvedic and other indigenous systems of medicine used commonly in India (Singh et al., 2005). The Solanaceae family comprises about 90 genera and 3000 species, which are widely distributed in the world. They are a rich source of active secondary metabolites (Coletto da Silva et al., 2004). Within this family, the genus *Solanum* is the largest and most complex with more than 1500 species, which yield a great variety of steroidal saponins and glycoalkaloids of interest from ecological and human health viewpoint (Roddick et al., 2001). *Solanum xanthocarpum* Schard. & Wendl., commonly known as yellow berried nightshade (synonym: Kantakari), is a pricky 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 (Hussain et al., 2012). *Solanum xanthocarpum* is an important medicinal herb in Ayurvedic medicine. Various studies have indicated that *S. xanthocarpum* possesses antiasthmatic, hypoglycemic, antibacterial, and insect-repellent properties. In addition, *S. xanthocarpum* fruit extract showed hepatoprotective effect against CCl_4-induced liver toxicity (Gupta et al., 2011). However, the leaves of this plant have not been evaluated for their bioactive potential. Hence, the present study was taken up with an aim to determine *in vitro* and *in vivo* antioxidant and hepatoprotective properties of the methanol extract of *S. xanthocarpum* leaves against CCl_4-induced toxicity.

Materials and methods

Chemicals and reagents

DPPH (1,1-diphenyl,2-picryl hydrazyl), ABTS (2,2′-azino-bis 3-ethylbenzthiazoline-6-sulfonic), 3,4,5-trihydroxybenzoic acid (gallic acid), TCA (trichloro acetic acid), and Trolox were purchased from Sigma (St. Louis, MO). Thiobarbituric acid (TBA), potassium ferricyanide ($C_6N_6FeK_3$), pyrogallol, sodium citrate, 1-chloro-2,4-dinitrobenzene (CDNB), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), hydrogen peroxide (H_2O_2), reduced glutathione (GSH), ethylene-diaminetetraacetic acid disodium salt (EDTA), CCl_4, and other chemicals were obtained from Sisco Research Laboratories (SRL), Mumbai, India. ALP and LDH kits were procured from Agappe diagnostics (Kerela, India) and AST and ALT kits were obtained from AMP diagnostics (Graz, Austria). All the other chemicals used were of analytical grade.

Plant material

The leaves of *S. xanthocarpum* were collected from Mysore, Karnataka, India, in June 2012 and were authenticated by taxonomist Dr. Sampath Kumara at the University of Mysore. Herbarium specimen has been deposited in the herbarium at the DOS in Biotechnology, University of Mysore (*S. xanthocarpum* # BJ-0021). The leaves were thoroughly washed to remove adhering dust and shade-dried. The dried leaves were pulverized in a mechanical grinder and the coarse powder was used for further studies.

Extraction procedure

Dried and ground leaves were serially extracted with hexane, ethyl acetate, and methanol using a Soxhlet apparatus (Amersham Pharmacia Biotech AB, Uppsala, Sweden). The crude extract thus obtained was transferred to a flash evaporator for complete evaporation. The dried extracts were collected and stored in a refrigerator for further studies.

Semi-purification of methanol crude extract

Fifty grams of powdered leaves of *S. xanthocarpum* were extracted in a Soxhlet apparatus with hexane, ethyl acetate, and methanol to obtain extracts with different polarities. The extracts obtained from hexane, ethyl acetate, and methanol were 0.86, 1.21, and 4.32 g, respectively. Only the methanol extract was selected for further purification as it exhibited highest antioxidant activity. Soxhlet methanol extract (2 g) (5 mg/ml) was subjected to separation by silica gel column chromatography. A glass column (55 cm × 2.2 cm dia.) was equilibrated thoroughly by passing chloroform repeatedly. The methanol extract was loaded on to the packed glass column. Flow rate was set to 10 ml/min. The column was eluted with a chloroform–methanol mobile phase with the following increasing polarity: 100:0, 80:20, 60:40, 40:60, 20:80, and 0:100. Six fractions were collected from the methanol extract to obtain active fractions. The effluents were collected in a flask and allowed to dry. The yields of six fractions collected were 0.136, 0.246, 0.312, 0.263, 0.353, and 0.472 g. *In vitro* bio assays (DPPH & ABTS) were carried out on all collected fractions to find the active fraction.

In vitro assays

Total phenolic content estimation

The total phenolic content of different extracts of *S. xanthocarpum* was determined using the Folin–Ciocalteau reagent method (Wolfe et al., 2003). To 50 µl of each extract, 2.5 ml of Folin–Ciocalteau reagent (1/10 dilution) and 2 ml of 7.5% Na_2CO_3 (w/v) were added and mixed well. The blend was incubated at 45 °C for 15 min. The absorbance of all samples was measured at 765 nm with Na_2CO_3 solution (2 ml of 7.5% Na_2CO_3 in 2.55 ml of distilled water) as a blank. The results were expressed as gallic acid equivalence (GAE) in micrograms.

Determination of antioxidant activity

DPPH-free radical scavenging activity

The DPPH assay was carried out according to the method described by Sultanova et al. (2001) with some modifications. The reaction mixture contained 5 µl of test samples (various extracts and compound dissolved in methanol) and 95 µl of DPPH in methanol. Different concentrations of test samples were prepared, while the concentration of DPPH was 300 µM in the reaction mixture. These reaction mixtures were taken in 96-well microtitre plates and incubated at 37 °C for 30 min after which the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Ascorbic acid was used as a reference standard. The level of percentage inhibition of

DPPH radical by different extracts was calculated according to the following formula:

$$\% \text{ inhibition} = \{(A_c - A)/A_c\} \times 100.$$

where A_C is the absorbance of the control and A is the absorbance of sample. Percentage scavenging was also evaluated in ascorbic acid equivalence.

ABTS radical scavenging assay

For ABTS assay, the method of Re et al. (1999) was adopted with some modifications. The stock solutions included 7 mM ABTS solution and 2.45 mM potassium persulfate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12–16 h at room temperature in the dark. The solution was then diluted by mixing 1 ml $ABTS^{\bullet+}$ solution with 60 ml methanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm using a spectrophotometer. Fresh ABTS solution was prepared for each assay. Plant extracts (10 µl) were allowed to react with 1 ml of the ABTS solution and the absorbance was noted at 734 nm after 7 min using the spectrophotometer. The $ABTS^{\bullet+}$ scavenging capacity of the extract was compared with that of Trolox and the percentage inhibition was calculated as ABTS radical scavenging activity:

$$(\%) = [(\text{Abs control} - \text{Abs sample})]/(\text{Abs control})] \times 100$$

where Abs control is the absorbance of ABTS radical + methanol; Abs sample is the absorbance of ABTS radical + sample extract/standard.

Estimation of reducing power (RP)

The reducing power of *S. xanthocarpum* extracts was evaluated according to the method of Oyaizu (1986). Different concentrations of the extracts were suspended in distilled water and mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6), and 2.5 ml of 1% $C_6N_6FeK_3$. The mixture was incubated at 50 °C for 20 min; 2.5 ml of 10% TCA was added to the mixture and centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increase in absorbance of the reaction mixture indicated reducing power. Butylated hydroxy toluene (BHT) was used as a reference compound. Reducing power was expressed as BHT equivalent (BHT/ml) and inversely proportional to BHT.

Animals and treatment

Albino Wistar rats weighing 180–200 g were used for the study. The animal care and experimental procedures performed were in compliance with the Regulations for Animal Research and Animal Ethical Committee of University of Mysore. The rats were acclimatized to the laboratory condition for 7 d before commencement of experiment.

Experimental design

Solanum xanthocarpum active fraction (SXAF) was dissolved in sterile distilled water. Two different concentrations of SXAF were orally administered. Animals were divided into five groups comprising six rats ($n = 6$) in each group as follows:

Group I: sterile distilled water, served as a positive control.
Group II: CCl_4, served as a negative control.
Group III: 100 mg/kg b.w. of SXAF orally for 14 d + single oral dose of CCl_4 on the 15th day (1 ml/kg b.w.)
Group IV: 200 mg/kg b.w. of SXAF orally for 14 d + single oral dose of CCl_4 on the 14th day (1 ml/kg b.w.)
Group V: 25 mg/kg b.w. of silymarin orally for 14 d + single oral dose of CCl_4 on the 14th day (1 ml/kg b.w.)

On the 14th day, the rats of groups II–IV were given a single oral dose of CCl_4 in olive oil (1:1) at 1 ml/kg of body weight 1 h after the last dose of SXAF. After 16–18 h of CCl_4 administration, animals were sacrificed by anesthesia. Blood was collected from heart puncture for serum separation. The liver perfused with saline was dissected out and processed immediately for biochemical assays.

In vivo hepatoprotective activity

Measurement of serum biochemical parameters

The collected blood was allowed to clot and serum was separated by centrifugation at 2500 rpm for 15 min and the serum enzymes, namely aspartate aminotransferase (AST), serum glutamate pyruvate transaminase (ALT), serum alkaline phosphatase (ALP), and lactate dehydrogenase (LDH), were assessed by commercial kit methods.

Assessment of lipid peroxidation

Lipid peroxidation (LPO) in the tissue homogenate was measured by estimating the formation of thiobarbituric acid reactive substances (TBARS) (Ohkawa et al., 1979). Tissue homogenate (10% w/v in 50 mM phosphate buffer, pH 7.4) was boiled in TCA (10%) and TBA (0.34%) for 15 min, cooled, and centrifuged. Absorbance of the supernatant was read at 535 nm. Malonyldialdehyde (MDA) is an end product of lipid peroxidation, which reacts with thiobarbituric acid to form pink chromogen thiobarbituric acid reactive substance. 1,1,3,3-Tetraethoxypropan was used as a standard for the calibration curve and was expressed as nmole/mg protein.

Assessment of reduced glutathione (GSH) activity

Hepatic GSH level was determined by the method of Ellman (1959) with slight modification. Briefly, liver tissue was homogenized in 10% TCA and 10 mM EDTA (in ratio 1:1, 10% w/v). The samples were centrifuged at 5000 rpm for 5 min at 4 °C. Supernatant (100 µl) was taken in a tube and 50 µl of Ellman's reagent (DTNB solution) was added to it. The volume was made up to 1 ml with Tris–HCl pH 8.2 and the yellow color developed was read at 412 nm.

Assessment of catalase (CAT), superoxide (SOD), and glutathione-S-transferase (GST)

The liver was perfused with cold saline to completely remove all the red blood cells. Then, it was suspended in 10% (w/v) ice-cold 50 mM phosphate buffer (pH 7.4) cut into small pieces, and the required quantity was weighed and homogenized. The homogenate was centrifuged at 10 000 *g* for 20 min at 4 °C to remove the cell debris, unbroken cells,

nuclei, erythrocytes, and mitochondria. The supernatant was used for biochemical assays. Superoxide dismutase (SOD) activity was measured using pyrogallol (2 mM) autoxidation in Tris buffer (Marklund, 1974). Decomposition of H_2O_2 in the presence of catalase (CAT) was followed at 240 nm (Aebi, 1974). One unit (U) of catalase was defined as the amount of enzyme required to decompose 1 µmol of H_2O_2 per min, at 25 °C and pH 7.0. Results are expressed as units (U) of CAT activity/mg protein. Glutathione *S*-transferase (GST) activity was assayed by the method of Warholm (1985) in phosphate buffer (0.1 M, pH 7.6) containing glutathione (0.5 mM) and CDNB (0.5 mM). The change in absorbance at 344 nm was monitored in a UV–Visible spectrophotometer.

Histopathological studies

For histological studies, the liver tissues were fixed in paraffin. Thin sections (5 µm) were cut and stained with routine hematoxylin and eosin (H & E) for photo-microscopic assessment (400×). The initial examination was qualitative, with the purpose of determining histopathological lesions in liver tissue (Amresh et al., 2007).

Statistical analysis

All results are expressed as mean ± SE. A one-way analysis of variance (ANOVA) followed by multiple comparisons with the Tukey *post hoc* test was used to compare different parameters between the groups. A p value <0.05 was considered significant.

Results

In vitro studies

Isolation of active fraction from methanol extract

The methanol extract of *S. xanthocarpum* was subjected to silica gel column chromatography. Among the eluted fractions, the fraction with a chloroform–methanol ratio of 20:80 showed highest radical scavenging antioxidant activity in DPPH and ABTS assays (Figure 1). Hence, this fraction was selected as an active fraction (SXAF) and was used for *in vivo* biological assays.

Total phenolic content

Variations in the quantity of total phenolics in the three extracts and SXAF are presented in Figure 2. All extracts showed a concentration-dependent increase in phenolic contents. Quantitative estimation proved that SXAF possessed the highest concentration of phenolics compounds among all extracts (465.12 ± 4.89 µg/ml of gallic acid equivalents/ 0.25 mg extract), while the ethyl acetate and hexane extracts contained very limited phenolic compounds.

DPPH radical scavenging activity

DPPH assay is widely used to evaluate the antioxidant activity of natural compounds. Different extracts of *S. xanthocarpum* at various concentrations, i.e., 5–25 µg/ml showed free radical scavenging activity in a dose-dependent manner (Figure 3). The SXAF showed that the IC_{50} value was 11.72 µg and it also demonstrated highest DPPH-free radical scavenging activities compared to other extracts.

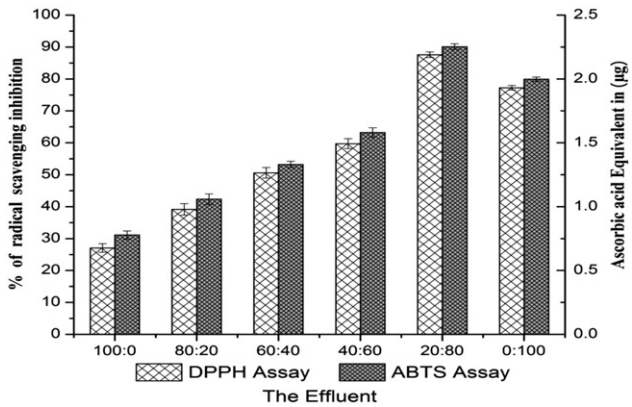

Figure 1. Comparison activities of fractions from column chromatography by DPPH and ABTS bioassays. Each value is expressed as ± SEM, $n = 3$, $p < 0.05$ compared to positive control.

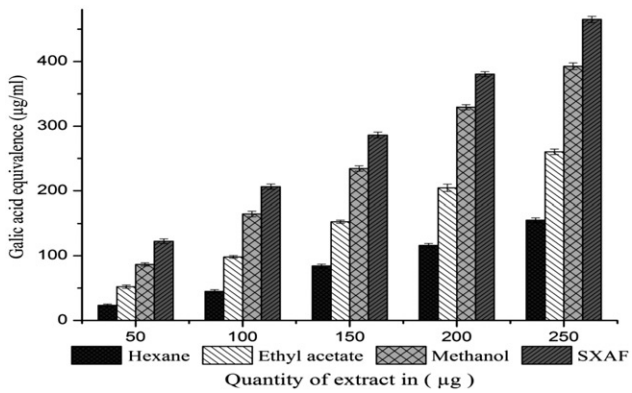

Figure 2. Total phenolic content of leaf extracts of *S. xanthocarpum* expressed as µg/ml of gallic acid equivalence (GAE). Values are expressed as ± SEM, $n = 3$, $p < 0.05$.

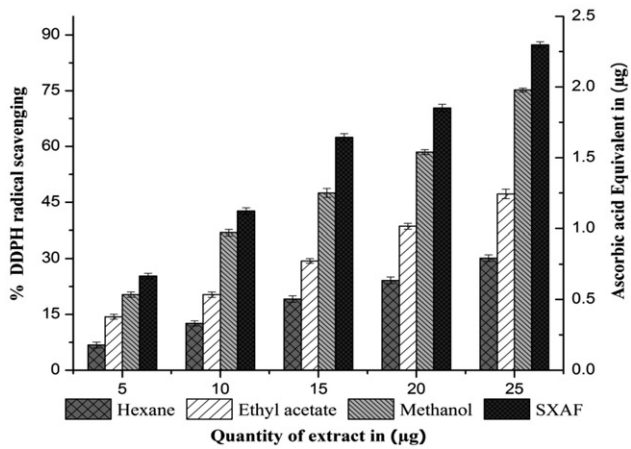

Figure 3. DPPH radical scavenging of various solvent extracts from *S. xanthocarpum*. Ascorbic acid was used as a positive control. Results are expressed as percentage of the control. Each value is expressed as ± SEM, $n = 3$, $p < 0.05$ compared to positive control.

ABTS radical scavenging activity

Figure 4 depicts the percentage scavenging of $ABTS^{\bullet+}$ radical. The relative antioxidant efficiency was compared to

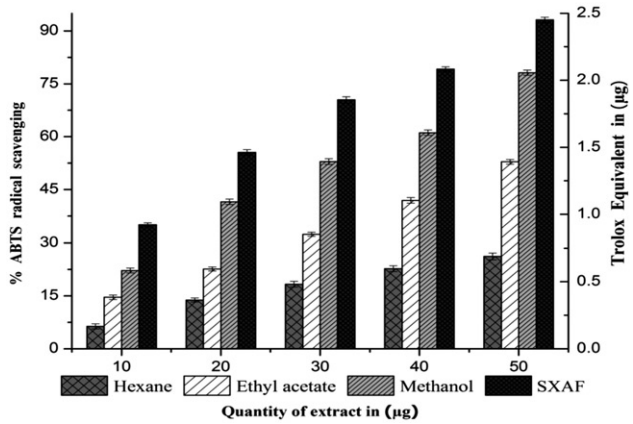

Figure 4. Scavenging of ABTS• radical by *S. xanthocarpum* leaves extract. Values are expressed as ±SEM, $n = 3$, $p < 0.05$.

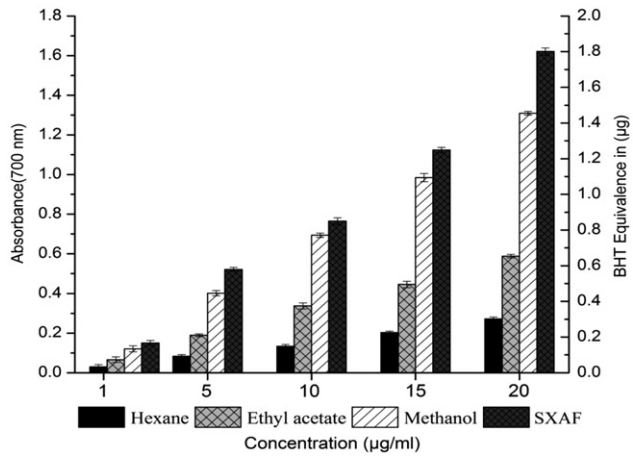

Figure 5. Reducing the power of *S. xanthocarpum* extracts based on the measurement of Fe^{+3}–Fe^{+2} transformation. Each value is expressed as ± SEM, $n = 3$, $p < 0.05$.

Trolox, and the Trolox equivalence (in µg) was plotted. Among the different solvent extracts tested, SXAF fraction showed the highest ABTS radical scavenging activity (IC_{50}, 17.99 µg). The hexane, ethyl acetate, and methanol extracts showed significantly lower ($p < 0.05$) activity.

Estimation of reducing power (RP)

The reducing power of *S. xanthocarpum* fractions is presented in Figure 5. Among all the extracts, SXAF was found to have strong and increased reducing ability at concentrations of 1–20 µg/ml. The reducing power of *S. xanthocarpum* extracts increased at higher concentrations.

In vivo studies

Based on the *in vitro* bioassays, SXAF was selected for further investigation in *in vivo* studies.

Effect of SXAF on serum enzyme

The effects of SXAF on serum enzymes in CCl_4-intoxicated rats are shown in Figure 6. Rats treated with CCl_4 (Group II) showed a significant increase in serum AST, ALT, ALP, and LDH levels compared with control animals (Group I). Treatment with SXAF at 100 and 200 mg/kg concentrations for 14 d (Groups III and IV) showed reduction in the levels of serum enzymes as AST 341.52 ($p < 0.05$), 292.16 ($p < 0.05$), ALT 74.83 ($p < 0.05$), 62.74 ($p < 0.05$), ALP 248.62 ($p < 0.05$), 210.24 ($p < 0.05$), and LDH 1624.12 ($p < 0.05$), 1237.19 ($p < 0.05$) compared with the toxic control group (Group II).

Effect of SXAF on hepatic lipid peroxidation

The results of MDA in liver are shown in Figure 7. The treatment with CCl_4 had obviously higher MDA levels than the normal control group ($p < 0.05$). MDA levels in the SXAF-treated group at 100 and 200 mg/kg were significantly lower than CCl_4-treated group 0.61 ($p < 0.05$), 0.43 ($p < 0.05$).

Effect of SXAF on hepatic antioxidant enzymes

In an attempt to obtain more information on the mechanism of protection against CCl_4 hepatotoxicity by SXAF, we monitored the natural antioxidant cell defenses including the enzymes CAT, SOD, GST, and GSH in livers of animals. The activities of their tissue antioxidant enzymes decreased significantly in animals consequent to CCl_4 (Group II)-induced hepatic damage as compared to the normal animals (Group I). Oral administration of SXAF at the dose of 200 mg/kg (Group IV) showed significant increase in CAT [34.63 ± 1.98 ($p < 0.05$)], SOD [1.78 ± 0.13 ($p < 0.05$)], GST [231.64 ± 14.28 ($p < 0.05$)], and GSH [8.23 ± 0.48 ($p < 0.05$)] when compared with CCl_4-treated rats. Treatment with SXAF at 100 mg/kg (Group III) showed only marginal increase in the level of antioxidant enzymes in liver homogenate compared with CCl_4-treated rats (Table 1). Thus, the activities of CAT, SOD, GST, and GSH were restored by SXAF treatment.

Histopathological observations

Histopathological analysis of group I (Figure 8A) animals showed normal architecture. In rats treated with CCl_4 (Group II), the normal architecture of liver was completely lost with the appearance of centrilobular necrosis. Scattered masses of necrotic tissues were detected in most of the areas in addition to the enlarged nuclei (Figure 8B). Treatment with SXAF (Groups III and IV) ameliorated the CCl_4-induced liver injury and the typical histological changes were markedly alleviated in the liver sections (Figure 8C and D). Rats treated with silymarin (Group V) showed near normal architecture with uniform sinusoids (Figure 8E).

Discussion

Polyphenols are a large and diverse class of compounds, many of which occur naturally in a wide range of food and plants. Although these compounds play an unknown role in nutrition (non-nutrients), many of them have properties including antioxidant, anti-mutagenic, anti-estrogenic, anti-carcinogenic, and anti-inflammatory effects that might potentially be beneficial in preventing disease and protecting the stability of the genome (Ferguson, 2001). The antioxidant potential of polyphenols has been correlated to the capacity of donating hydrogen radicals. The number and the configuration of H-donating hydroxyl groups are important structural features

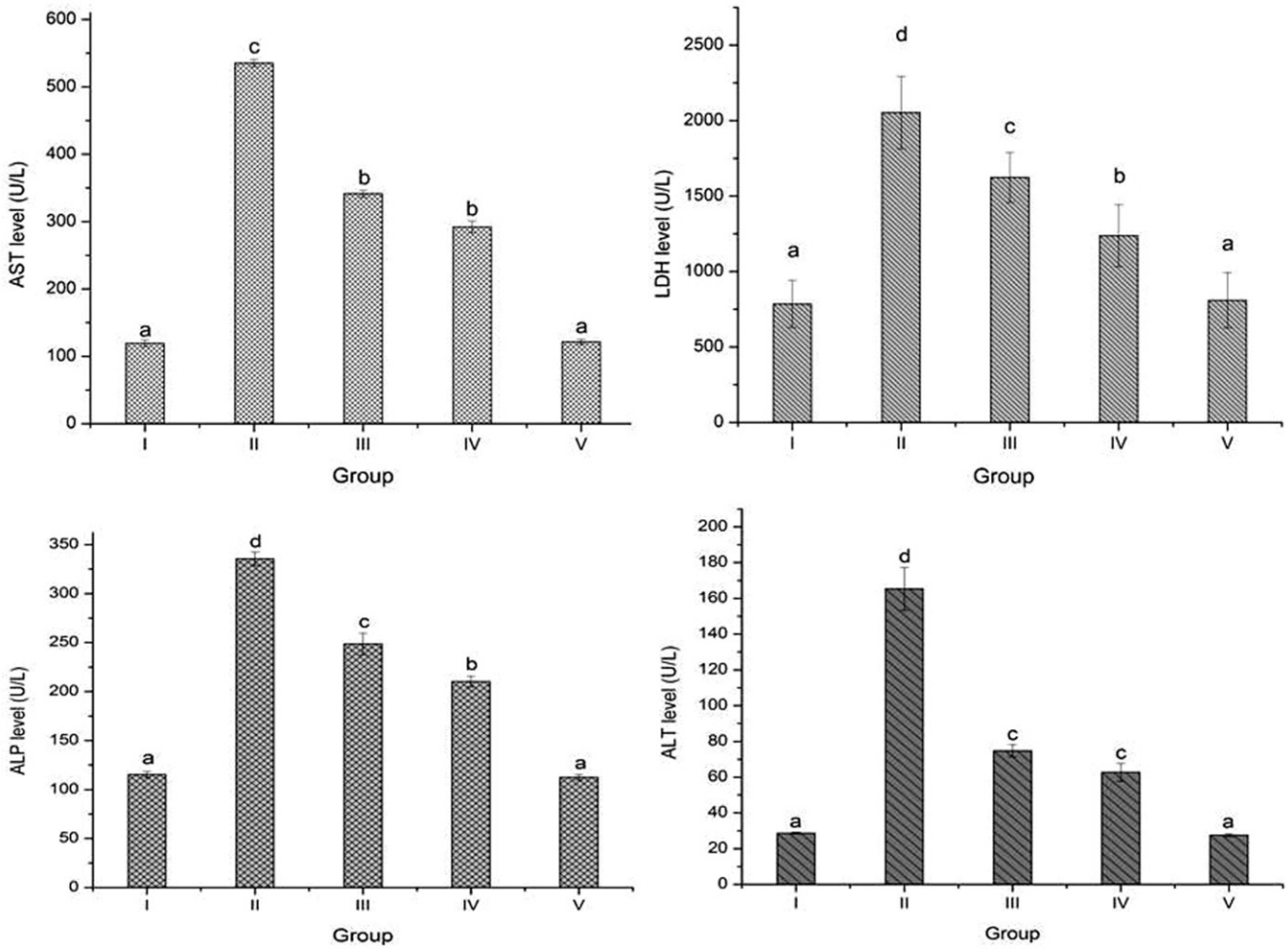

Figure 6. Effects of *S. xanthocarpum* active fraction (SXAF) on serum enzymes in CCl_4-induced hepatotoxicity in rats. Group I – control; Group II – CCl_4; Group III – SXAF 100 mg/kg b.w. + CCl_4; Group IV – SXAF 200 mg/kg b.w. + CCl_4; Group V – Silymarin 25 mg/kg b.w. + CCl_4; LDH, lactate dehydrogenase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase. Each bar represents the mean ± SE, $n = 6$; bars with different alphabets differ significantly at $p < 0.05$ level (DMRT).

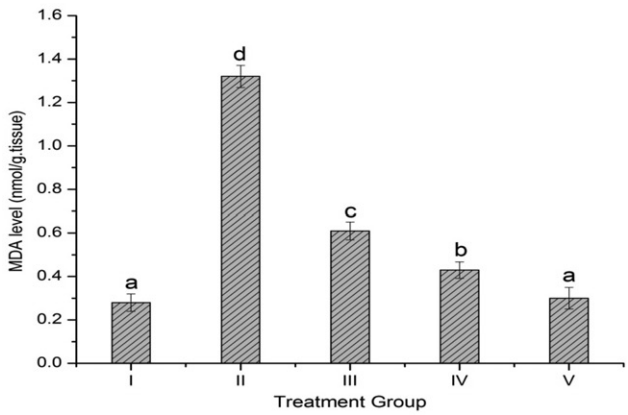

Figure 7. Effects of SXAF on the level of hepatic lipid peroxidation in CCl_4-exposed rats. The values are means ± SEM ($n = 5$), bars with different letters differ significantly at $p < 0.05$ by DMRT.

Table 1. Treatments – I: control; II: CCl_4; III: SXAF (100 mg/kg) + CCl_4 (1 ml/kg); IV: SXAF (200 mg/kg) + CCl_4 (1 ml/kg); V: Silymarin (25 mg/kg) + CCl_4 (1 ml/kg).

Group	SOD	CAT	GSH	GST
Group I	2.24 ± 0.18^{a}	33.42 ± 1.43^{b}	8.6 ± 0.52^{a}	234.52 ± 12.31^{a}
Group II	0.32 ± 0.043^{d}	29.84 ± 1.57^{c}	4.42 ± 0.47^{d}	194.36 ± 11.58^{d}
Group III	0.87 ± 0.062^{c}	30.75 ± 2.34^{c}	6.83 ± 0.37^{c}	225.64 ± 13.45^{c}
Group IV	1.78 ± 0.13^{b}	34.63 ± 1.98^{b}	8.23 ± 0.48^{b}	231.64 ± 14.28^{b}
Group V	2.42 ± 0.21^{a}	36.55 ± 2.12^{a}	8.92 ± 0.32^{a}	238.72 ± 11.34^{a}

Data are expressed as means ± SE with different suffix letters differ significantly ($p < 0.05$, $n=6$). Values are expressed as SOD, superoxide dismutase (unit/min/mg protein); CAT, catalase (μmole H_2O_2/min/mg protein); GSH, glutathione (μg/mg protein); GST, glutathione *S*-transferase (μmole CDNB conjugate/min/mg protein).

influencing the antioxidant capacity of phenolic compounds (Soobrattee et al., 2005). In the present study, the SXAF (active fraction) had the highest yield and contained the phenolic compounds.

To investigate the antioxidant activity of various extracts, we evaluated their ability to scavenge free radicals, namely DPPH and ABTS. The SXAF showed the greatest potency among all extracts and exhibited robust radical scavenging activity. This radical scavenging activity of various extracts could be related to the nature of phenolics, thus contributing to their electron transfer/hydrogen donating ability. Hexane, ethyl acetate, and methanol extracts IC_{50} values for

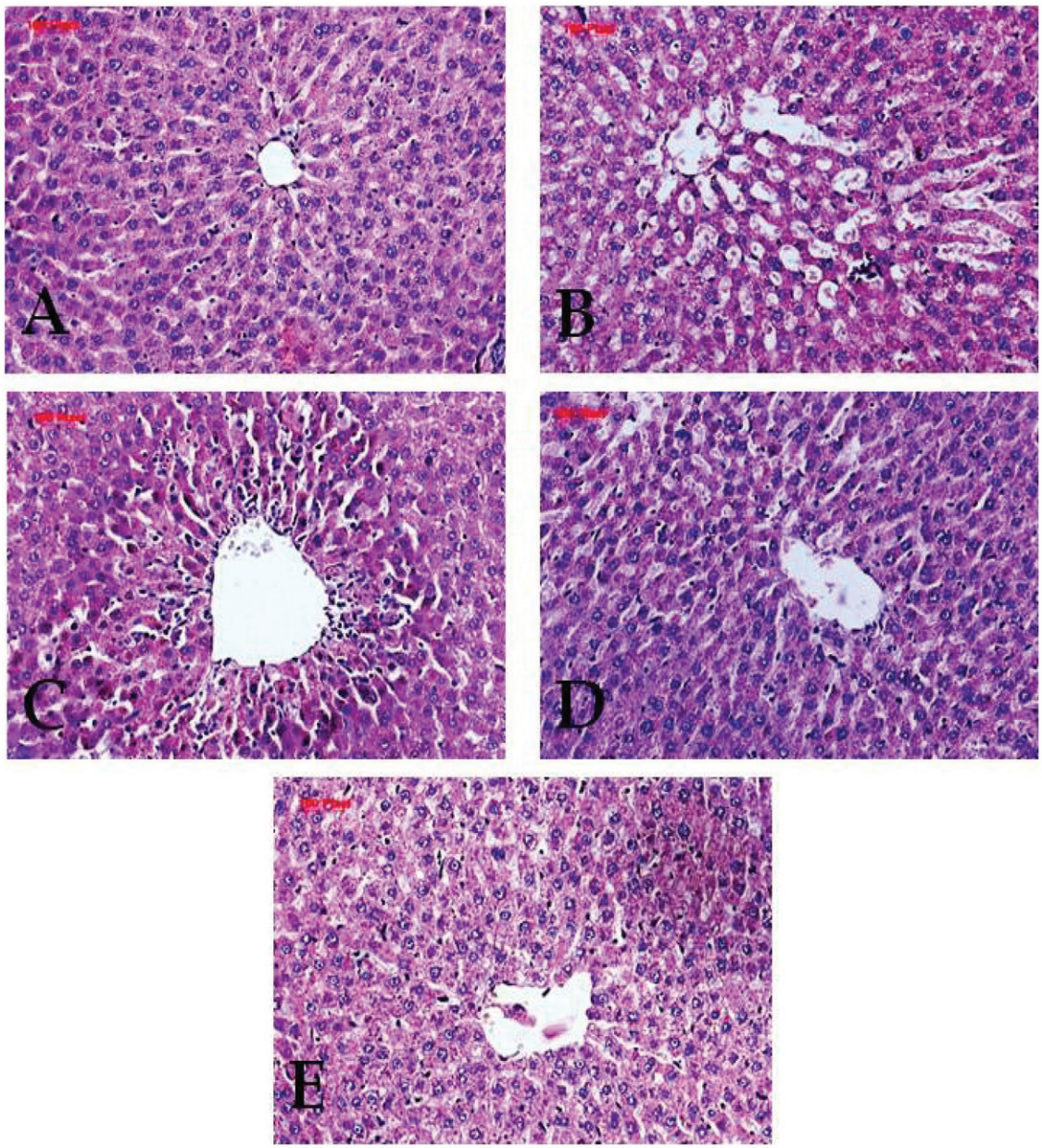

Figure 8. Effect of SXAF on the histological morphology of rat liver by hematoxylin and eosin (H & E) staining, magnification, ×400. (A) Normal control, (B) CCl_4 control, (C) SXAF 100 mg/kg b.w. + CCl_4, (D) SXAF 200 mg/kg b.w. + CCl_4, and (E) silymarin 25 mg/kg b.w. + CCl_4.

DPPH were 41.6 µg, 26.45 µg, and 11.72 µg. ABTS IC_{50} values for hexane, ethyl acetate, and methanol were 95.53 µg, 47.29 µg, and 17.99 µg. The radical scavenging activity (DPPH and ABTS) clearly showed a positive correlation (decreased IC_{50} value) with increasing total phenol content (Figures 2–4).

Reductive capacity is an important measure of the antioxidant capability of medicinal herbs. Reducing capacity was determined by using UV–Visible absorbance to monitor the transformation of Fe^{3+} to Fe^{2+} in the presence of the extracts. Increased absorbance is correlated with the reducing power of the extract (Meir et al., 1995). Based on our *in vitro* antioxidant results, SXAF was found to be the most potent extract and subsequently used to estimate the antioxidant activity of *S. xanthocarpum in vivo*. CCl_4 is being used extensively to induce hepatotoxicity in various experimental animals (Rao et al., 2003). The changes associated with CCl_4-induced liver damage are similar to that of acute viral hepatitis. The hepatotoxicity induced by CCl_4 is due to $CCl_3^{\bullet}$, 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 (Bishayee et al., 1995). The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms that have been disturbed by a hepatotoxin is the index of its protective effects (Yadav & Dixit, 2003). The increased levels of AST, ALT, ALP, and LDH are conventional indicators of liver injury (Thabrew et al., 1987). The present study revealed a significant increase in the activities of AST, ALT, ALP, and LDH levels in rat serum on exposure to CCl_4, indicating considerable hepatocellular injury. However, the treatment with SXAF (100 and 200 mg/kg b.w.) suppressed the acute hepatic damage and was consistent

with an improvement in the serum biological parameters of hepatotoxicity.

Lipid peroxidation is an important parameter of oxidative stress. This process may cause peroxidative tissue damage in inflammation, cancer and toxicity of xenobiotics, and aging (Halliwell, 1994). MDA is a cytotoxic product that is a hallmark of lipid peroxidation. Free radical scavenging is one of the major antioxidation mechanisms inhibiting the chain reaction of lipid peroxidation. In the present study, the treatment with SXAF reduced lipid peroxidation by decreasing MDA levels, indicating the free radical scavenging activity of this plant extract under *in vivo* conditions.

Both SOD and CAT are key antioxidant enzymes that protect against oxidative stress and tissue damage (Halliwell & Gutteridge, 1990). These enzymes are critical for defense mechanisms against the harmful effects of reactive oxygen species (ROS) and free radicals in biological systems. The SOD converts superoxide radicals (O_2^-) into H_2O_2 and O_2, thus participating with other antioxidant enzymes, in the enzymatic defense against oxygen toxicity. CAT is a key component of the antioxidant defense system. Inhibition of this protective mechanism results in enhanced sensitivity to free radical-induced cellular damage. The decrease of CAT may result in many deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide (Srinivasan et al., 2007).

GSH constitutes the first line of defense against free radicals. Glutathione (GSH) is a major non-protein thiol in living organisms, which plays a central role in coordinating the antioxidant defense processes in the body (Gueeri, 1995). In states of oxidative stress, GSH is converted to glutathione disulfide (GSSG) leading to lipid peroxidation. CCl_4 treatment in rats reduced the levels of tissue GSH as compared to the rats treated with CCl_4 and plant extracts. Administration of CCl_4 induced a loss of glutathione from the liver and a decrease in its hepatic content. These lowered levels may be due to the increased utilization of GSH for the detoxification process (Rajagopal et al., 2003).

GST represents one of the major cellular defense mechanisms against electrophilic xenobiotics and their metabolites. Sultana et al. (2005) have reported that CCl_4 administered chronically increases GST protein levels and that GST can utilize 4-hydroxy-2-nonenal and malondialdehyde as substrates. It has been reported that SOD, CAT, and GST constitute a mutually supportive defense against ROS (Valko et al., 2007). In the present study, we demonstrated that CCl_4 treatment induced a significant decrease in the levels of activity of the antioxidant enzymes CAT, SOD, GSH, and GST probably due to protein in activation by ROS (Tabatabaie & Floyd, 1994). These changes were reversed upon treatment with SXAF.

Conclusion

The results in the present work indicate the presence of phenolic compounds and antioxidant potential of SXAF. We demonstrated that an active fraction of methanol extract (SXAF) was able to scavenge the hydroxyl- and superoxide radical, has high reducing power, and conferred protection against biological macromolecular damage. The SXAF exhibited potent antioxidant activities both *in vitro* and *in vivo* and the phenolic compounds in the extract could be responsible for their effective antioxidant properties. It is proposed that the hydroxyl group might act as an important factor in determining the antioxidant activities of the phenolic compounds. In light of our observations, SXAF might contain several phenolic compounds and significant antioxidant activities *in vitro* and *in vivo*, SXAF should be considered as a new source of natural antioxidant for pharmaceutical studies.

Declaration of interest

The authors declare that there are no conflicts of interest. The authors acknowledge the facilities created at the University of Mysore under the Institution of Excellence Programme by the Government of India with financial support from the Ministry of Human Resource Development and University Grants Commission, New Delhi, India.

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