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Antioxidant and hepatoprotective activity of *Solanum xanthocarpum* Schrad. &Wendl. fruit extract against ethanol induced liver toxicity in Experimental rodents

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ABSTRACT

The present study was aimed to evaluate antioxidant and hepatoprotective activity of the ethanolic fruit extract of *Solanum xanthocarpum* against ethanol induced hepatopathy induced liver toxicity in experimental animals. In the present study, *in- vivo* hepatoprotective effect of 50% Ethanolic fruit extract of *Solanum xanthocarpum* (SXE, 100, 200 and 400 mg/kg body weight) was evaluated using experimental models, ethanol (5 g/kg, p.o.) induced hepatotoxicity in experimental animals. The hepatoprotective activity was assessed using various biochemical parameters like AST, ALT, ALP, γ-GT and total bilirubin. Meanwhile, *in vivo* antioxidant activities as LPO, GSH, SOD and CAT were screened along with histopathological studies.Obtained results demonstrated that the treatment with SXE significantly ($P<0.05$-$P<0.001$) and dose dependently prevented ethanol induced increase in serum levels of hepatic enzymes. Furthermore, SXE significantly (up to $P<0.001$) restored the antioxidant enzymes like LPO, GSH, SOD and CAT towards normal levels. Histopathology of the liver tissue showed that SXE attenuated the hepatocellular necrosis and lead to reduction of inflammatory cells infiltration. The results of this study strongly indicate the protective effect of SXE against ethanol tempted liver injury which may be attributed to its hepatoprotective activity, and there by scientifically support its traditional use.

INTRODUCTION

Alcoholic liver disease is a leading cause of morbidity and mortality throughout the world. The three most widely recognized forms of alcoholic liver diseases are fatty liver steatosis, alcoholic hepatitis and liver cirrhosis. The aetiology of some oxidative stress-based pathological conditions in the liver has implicated excessive alcohol consumption. More than ever before, there is an upsurge in alcohol abuse and as a result, alcohol-related disorders are becoming increasingly important causes of morbidity and mortality, globally. Herbal drugs play an important role in the treatment of liver disorder with least toxicity. *Solanum xanthocarpum* Schrad and Wendl. (Solanaceae) is a prickly diffuse bright green perennial herb, commonly known as Yellow Berried Nightshade. The plant is bitter; acrid and various activities are reported in the plant vizhepatoprotective, [1]antiasthmatic, antidiabetic, [2] antioxidant, [3]immunomodulatory, [4]woundhealing, [5] diuretic, [6]antispermatogenic, antifertility, antipyretic, anticancer, anti-allergic, anthelmintic, antimicrobial and anti-inflammatory activity.[7,8] The ethanol-induced hepatotoxicity has been extensively studied, but impacts of silymarin in preventing ethanol induced hepatic injury had not received proper attention. Therefore, the present study was designed to determine the ethanol induced hepatotoxicity in male albino rats and its prevention by simultaneous administration of silymarin.

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 fruits were collected from campus garden of National Botanical Research Institute, Lucknow, India in June 2014. The plant material was identified and authenticated and the voucher specimen number NBRI-SOP-222 was deposited in the institutional herbarium. The fruits of *Solanum xanthocarpum* were dried and powdered. The powdered plant materialwas macerated with petroleum ether; the marc was exhaustively extracted with of 50% ethanol for three days. The extract was dried by rotator evaporator under reduced pressure.The extract obtained was further subjected to pharmacological investigation.

Animals

Wistar rats weighing (150-170 g) of either sex were procured from Animal house of Shri Ram MurtiSmarak College of Engineering and Technology, Bareilly. They were kept in departmental animal house in well cross ventilated room at 22 ± 2 °C with light and dark cycles of 12 h for 1 week before and during the experiments. The experiment was carried out in accordanceto the guidelines mentioned in the CPCSEA, and Institutional Animal Ethical Committee, India (Reg. No. 715/02/CPCSEA).

Acute oral toxicity studies

Swiss albino mice of either sex were divided into five groups with five animals each. SXE 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.[9]

Ethanol induced hepatotoxicity

Animals, after acclimatization (6-7 days) in the animal, were randomly divided into six groups of sixanimals each and treated in the following manner. Group I (control) received distilled water orally once daily for 28 days. Group II received ethanol (5 g/kg, 20% v/v *p.o.)* once daily for 21 days from day 8 to day 28. Groups III-V and VI received SXE (100, 200 and 400 mg/kg, *p.o.)* and silymarin (200 mg/kg, *p.o*.) respectively with ethanol. On the 29th day the animals were anesthetized by anaesthetic ether , blood was collected and liver samples were dissected. [10] On the 29th day the animals were anesthetized by anaesthetic ether the liver samples were dissected and blood was collected.

Evaluation of serum biochemical parameters

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), glutamate pyruvate transaminase (ALT, U/L),[11]alkaline phosphatase (ALP, U/L),[12]total bilirubin (mg/dL)[13] and gamma glutamyltransferase (γ-GT) using assay kits.[14]

Evaluation of antioxidant parameters

LPO content was determined by the method of Utley et al.[15] Catalase activity was measured by the method of Claiborne, [16] Superoxide dismutase activity was measured by the method of Dhindsa et al. [17], while GSH levels were determined by the method of Jollow et al.[18]

Histopathological studies

Fixed liver tissue samples were embedded after dehydration in paraffin wax, sectioned at thickness of 5M and stained with haematoxylin and Eosin (H&E) for general histopathological examination using the light microscope.

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.

RESULT

Acute oral toxicity studies

After the 2 hr. of post treatment, only reduction in locomotion and dullness were observed in some animals that treated with higher doses (2000mg/kg).*Solanumxanthocarpum,*produces no mortality at 2000 mg/kg.

Table 1. : Effect of SXE on serum AST (U/L), ALT (U/L), ALP (U/L),Total Bilirubin (TBL) level (mg/dl),γ-GT (U/L)against ethanolinduced liver toxicity in rats.

Groups	AST	ALT	ALP	TBL	γ-GT
Control	98.26 ± 2.58	92.62 ± 3.26	59.23 ± 2.8	0.71 ± 0.12	27.8 ± 2.46
Ethanol	210.05 ± 3.29†	193.05 ± 2.29†	158.23 ± 2.21†	2.3 ± 0.21†	148.21 ± 3.92†
SXE 100	197.35 ± 3.26^{a}	181.35 ± 3.25^{a}	146.32 ± 3.54^{b}	2.1 ± 0.15^{n}	136.21 ±4.26^{a}
SXE 200	141.21 ± 3.21^{c}	134.21 ± 3.11^{c}	132.24 ± 3.11^{c}	1.19 ± 0.3^{c}	98.67 ± 3.46^{c}
SXE 400	108.26 ± 4.28^{c}	118.26 ± 4.28^{c}	110.54 ± 2.94^{c}	1.01 ± 0.21^{c}	52.29 ± 2.95^{c}
Silymarin	102.68 ± 3.94^{c}	109.68 ± 3.94^{c}	87.34 ± 2.96^{c}	0.82 ± 0.1^{c}	42.28 ± 2.81^{c}

Values are mean ± S.E.M. of 6 rats in each group
n : non-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 (Ethanol)

Effect of SXE on AST, ALT, ALP, γ-GT and total bilirubin against ethanol induced liver injury in rats

The effect of various doses of SXE were studied on serum marker enzymes and total bilirubin in ethanol intoxicated animal. Hepatic injury induced by ethanol caused significant changes in marker enzyme as AST by 113.76%, ALT by 109.24%, ALP by 167.14%, γ-GT by 433.12% and total bilirubin by 223.94% compared to control group. The percentage protection in marker enzyme of treated group at 100, 200 mg/kg as AST 6 (P<0.05), 32.77 (P<0.001), ALT 6.06 (P<0.05), 30.47 (P<0.001), ALP 7.52 (P<0.01), 16.42 (P<0.001), γ-GT 8.09 (P<0.05), 33.42 (P<0.001) and total bilirubin 8.69 (ns), 48.26 (P<0.001) compared to toxic group while maximum percentage protection in marker enzyme at the dose of 400 mg/kg and silymarin (5mg/kg) as AST 48.45 (P<0.001), 51.11 (P<0.001), ALT 38.74 (P<0.001), 43.18 (P<0.001), ALP 30.13 (P<0.001), 44.8 (P<0.001), γ-GT 64.71 (P<0.001), 71.52 (P<0.001) and total bilirubin 56.04 (P<0.001), 64.34 (P<0.001) which is almost comparable to the group treated with silymarina potent hepatoprotective drug used as reference standard [Table 1].

Estimation of LPO, GSH, SOD and CAT against ethanol induced liver injury in rats

The results in [Table 2] showed clear significant percentage change in the levels of LPO in ethanol intoxicated rats as 338.21 (P<0.001) compared to control group. 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 21.77 (P<0.01), 27.45 (P<0.001) and 53.24 (P<0.001) respectively. The GSH, SOD and CAT content had significantly increased in SXE treated groups whereas ethanol intoxicated group had shown significant decrease in these parameters compared to control group. The percentage changed of GSH, SOD and CAT in ethanol intoxicated group were as 60.91 (P<0.001), 68.44 (P<0.001) and 44.95 (P<0.001) respectively. The percentage protection in GSH as 23.52 (P<0.05), 41.17 (P<0.01), 108.82 (P<0.001) and SOD 39.82 (ns), 115.21 (P <0.01), 149.10 (P<0.001) while in CAT 27.66 (P<0.05), 43.46 (P<0.01), 54.2 (P<0.001) 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 was almost comparable to those of the normal control and silymarin.

Histopathological observations

The histological observations basically support the results obtained from serum enzyme assays. Liver section in normal control rats showed normal architecture of hepatic cells, while in ethanol treated rats showed ballooning formation along with congestion in central vein, necrosis, and the loss of cellular boundaries. Whereas the SXE treated groups showed absence of cell necrosis, but with minimal inflammatory conditions. The SXE 400 mg/kg, *p.o.* treated group showed regeneration of hepatocyte around central vein with near normal liver architecture possessing higher hepatoprotective action [Figure 1].

DISCUSSION

The liver is a malleable organ in the body concerned with the regulation of the internal chemical environment. It plays a major role in detoxification and excretion of many endogenous and exogenous compounds and it is the preferred target for drug toxicity.[19]The WHO estimated that about 80 percent of the world's population still relies on plant-based medicines for their primary health care.[20] Medicinal plants and their derivatives have been used since ancient time in various forms for the treatment of liver disorders. In the present investigation, *Solanum xanthocarpum* (SXE) was evaluated for the antioxidant and hepatoprotective activity using ethanol as a hepatotoxicant. Many pathways have been proposed to play a role on how ethanol induces a state of "oxidative stress", including redox-state changes, damage to the mitochondria, acetaldehyde production, [21]and breakdown of cell membrane. [22] Oxidative stress is one major factor in aetiology of ethanol injury, mainly by Kupffer cell derived reactive oxygen. Ethanol activates Kupffer cells and Kupffer cell generates reactive oxygen species and pro-inflammatory cytokines, both of them can lead to liver damage. [23,24] Ethanol induced hepatic damage, was characterized by the raised levels of serum AST, ALT, ALP andγ-GT which are normally located in the cytoplasm and released into circulation after hepatic cellular damage. [25]γ-GT activity was considered to be one of the best indicators of liver damage. [26]Bilirubin was considered as the window parameter for hepatic function and abnormal increase in the levels of bilirubin in the serum indicate hepatobiliary disease and cause the severe disturbance of hepatocellular function. [27]

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 ethanolinduced liver toxicity in rats.

Groups	LPO	GSH	SOD	CAT
Control	1.23 ± 0.02	0.87 ± 0.03	28.33 ± 2.13	58.52 ± 3.92
Ethanol	5.39 ± 0.14†	0.34 ± 0.04†	8.94 ± 1.04*	32.21 ± 2.21*
SXE 100	4.27 ± 0.01^{b}	0.42 ± 0.01^{a}	12.5 ± 1.05^{n}	41.12 ± 2.02^{a}
SXE 200	3.91 ± 0.03^{b}	0.48 ± 0.03^{b}	19.24 ± 2.1^{b}	46.21 ±2.97^{b}
SXE 400	2.52 ± 0.02^{c}	0.71 ± 0.02^{c}	22.27 ± 2.2^{c}	49.67 ± 2.87^{c}
Silymarin	2.01 ± 0.01^{c}	0.81 ± 0.01^{c}	25.56 ± 2.7^{c}	54.28 ± 3.1^{c}

Values are mean ± S.E.M. of 6 rats in each group
n :non 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 (Ethanol)

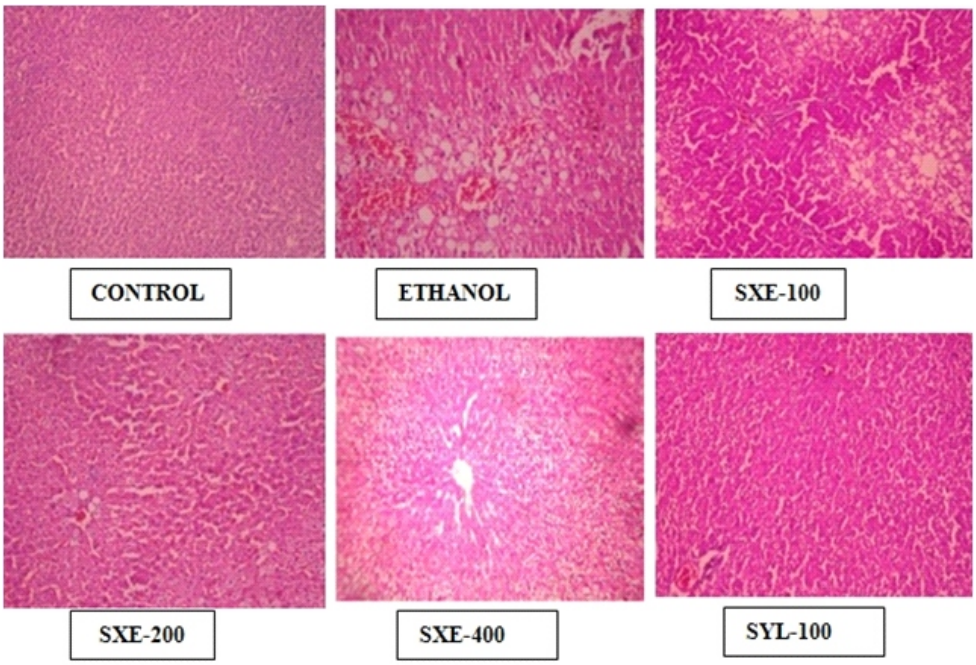

Fig 1. : C(A) Normal control rat shows normal architecture of hepatic cells. (B) Ethanol treated rats showing massive fatty changes, loss of cellular boundaries, ballooning formation along with congestion in central vein. (C) Ethanolwith 100 mg/kg of SXE showing inflammatory collections around central vein, mild ballooning with loss of cellular boundaries, (D) Ethanolwith 200 mg/kg of SXE showing less inflammatory cells around central vein. (E) Ethanolwith 400 mg/kg of SXE showingregeneration of hepatocytes.(F) Liver section of rats treated with silymarin showing normal liver architecture.

In our study, substantial increase in AST, ALT, ALP, γ-GT and TBL in the serum were observed after administration of ethanol but administration of test drug at different doses level (100, 200 and 400 mg/kg), causes subsequent recovery towards normal level as comparable to the control group I animals. SXE at different dose levels offers hepatoprotection, but 400 mg/kg is more effective than all other lower doses. Antioxidant activity was measured by determining the antioxidant parameter. SOD is an effective defence enzyme that catalyses the superoxide anions into hydrogen peroxide. Catalase catalyses' the conversion of H_2O_2 to oxygen and water and protects the tissue from oxidative damage by reactive oxygen species and hydroxyl radicals.[28]The increase in LPO levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defence mechanisms causes the formation of excessive free radicals. [29] Lipid peroxidation results from the increased oxygen radical production which may be due to the induced activity of the cytP_{450}2E1, [30]$H_2O_2$and free iron levels, as well as reduced activities of antioxidant enzymes such as SOD and CAT. [28]GSH is the major anti-oxidative tri-peptide in the cell and plays key role in the detoxification of toxicants and regulation of various pathways to maintain cellular homeostasis. [31] Ethanol intake causes liver toxicity probably by oxidative stress, increased lipid peroxidation, and by increasing GSH efflux from the liver and by inhibiting the biosynthesis, could be responsible for the depletion of hepatic GSH. [32] The rodents treated with 100, 200 and 400 mg/kg of SXE groups showed significant decrease the level of LPO while increase in the level of SOD and CAT, which indicates the antioxidant activity of the *Solanum xanthocarpum*. 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. Treatment with SXE significantly reversed all the changes. 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. Our study confirmed the protective effect of SXE against ethanol in rats. In rat, SXE hepatoprotective activity is quite similar to silymarin, a reference hepatoprotective agent.

On phytochemical screening, ethanolic fruit extract of *Solanum xanthocarpum* revealed the presence of flavonoids, steroidal alkaloids, triterpenes, flavanoids, quercitrin and apigenin glycosides are the major chemical constituents. Hence, it is possible that the mechanism of hepatoprotection of *Solanum xanthocarpum* may be due to its antioxidant property which is present in these phytochemicals and these phytochemicals reduced the oxidative stress imposed by ethanol which may prevent inflammatory hepatic damage.

CONCLUSION

The results of this study strongly indicate the protective effect of *Solanum xanthocarpum* against ethanol induced acute liver injury which may be attributed to its antioxidant and hepatoprotective activity, and there by scientifically support its traditional use.

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