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Phytochemical Screening and Hepatoprotective Potential of *Sida cordata*

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Sida cordata (Burn. f.) Borss. Waalk is highly distributed in entire part of India. The various parts of the plant are used in folklore medicine for the management of multiple diseases. So,the present study was performed to examine the hepatoprotective activity study of ethanolic extract of *S. cordata* leaves (SCLE) on alcohol mediated hepatotoxicity in a rat model. Ethanol intoxicated rats showed noteworthy elevation of liver weight and volume. Further, ethanol insulted rats also showed significant elevation in the level of biochemical markers such as AST, ALT, TG, TB, and DB) and marked variation in the histological structure of liver. Oral administration of SCLE at the dose of 200 and 400 mg/kg for 30 days) significantly reduced the liver weight, hepatic markers level and restored the histological changes annoyed by ethanol thus indicating its hepatoprotective potential. Furthermore, HPTLC analysis was performed for the identification and quality estimation of *S. cordata* leaves

Keywords: Albino rats; alcohol; hepatoprotective activity; HPTLC fingerprints; Sida cordat.

1. INTRODUCTION

The liver is a vital organ involved in the regulation of a wide range of physiological functions in the human system. It orchestrates various processes such as metabolism, synthesis, and production of various enzymes, and detoxification of toxins. Thus liver damage

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caused by various hepatotoxic agents elicits significant morbidity and mortality [1]. Mounting reports show that a high amount of alcohol ingestion elicits excessive production of free radicals, which is the chief etiological factor associated with the pathogenesis of alcoholic liver disease [2]. Chronic alcohol intake elevates the oxidizing potential of cytochrome P450 2E1 (CYP2E1) so that there is an accelerated metabolism of ethanol to form a prooxidant state the pro-oxidation burden. Thus CYP2E1 mediated ethanol oxidation generates a large number of reactive oxygen species (ROS) which is the chief culprit in the etiology of ethanol-induced liver injury [3,4]. Allopathic drugs that have been used in the management of liver diseases impose a high economic burden and serious side effects. Ayurveda, an indigenous Indian system of medicine has proven benefits for the treatment of liver disease using a wide range of plants [5].

Sida cordata (Burn. f.) Borss. Waalk. (Malvaceae) is generally referred to as Bhumibala (heartleaf fanpetal). It is a more branched hairy herb with a small main stem and extended branches and is normally distributed widely in India, specifically in dry regions [6]. The species is cited in Charak Samhita as *brmhaniya*- a mounting herb and as balya tonic and *prajasthapana*- which induces fertilization [7]. This herb is useful in the treatment of various neurological diseases like sciatic nerve damage, paralysis. Headache eye disorders, leucorrhoea, tuberculosis, hyperglycemia, pyrexia, rheumatic fever, and ovarian disease [8,9]. However, to date, no scientific studies are available to prove its folklore claim of hepatoprotective activity. Against this backdrop, the present study was undertaken to evaluate the hepatoprotective potential of *Sida cordata* leaf extract (SCLE) on alcohol-induced hepatotoxicity in a rat model. Further, HPTLC fingerprint analysis was performed for the identification and quality evaluation of *S. cordata* leaves.

2. MATERIALS AND MATHODS

2.1 Chemicals

Ethanol was procured from SD Fine Chemical, Indore. Silymarin was acquired as a gift product from Micro labs, Bangalore, India. The biochemical kits for the estimation of Alanine transaminase (ALT), Aspartate Transaminase (AST), and Bilirubin were recruited from Span Diagnostics, India. Triglycerides kit was purchased from Reckon diagnostics, India. The other chemicals used in the study were of analytical grade.

2.2 Plant Materials

The *Sida cordata* leaves were collected from rural belt of Raipur district in Chhattisgarh, India in July 2019. The identification and authentication of plant was done by Dr. M. S. Mondal, Additional Director, Central National Herbarium, Govt. of India, Howarh (India). The voucher specimen was deposited in the herbarium section of the Pharmacognosy Department, Gayatri College of Pharmacy, Sambalpur Orissa. The collected leaves was dried in shade and pulverized to coarse powder used for extraction.

2.3 Preparation of Extracts

The powdered samples was defatted using petroleum ether (60-80°C) and then subjected to hot percolation using one liter of 95 % ethanol and water. Extract was filtered and filtrate was evaporated, using a rotary evaporator under reduced pressure to the solvent free residues w/w).

2.4 Phytochemical Screening

Preliminary phytochemical analysis was performed using powdered drug and also with ethanolic extract of *Sida cordata* using standard procedures [10].

2.5 HPTLC Fingerprint Analysis

HPTLC studies were performed as per the procedure described by Harbone [11] and Wagner *et al.* [12]. 10 mg of SCLE was dissolved in 100 mL ethanol (HPTC grade) and applied on the TLC aluminium sheets pre-coated with silica gel GF_{254} (20X10cm). After trial and error methods the suitable mobile phase used in this study was toluene-ethyl acetate-formic acid (7:3:0.1). About 15 µL of extract were spotted as band with a width of around 6mm using Linomat 5 applicator in CAMAG HPTLC machine, programmed with WIN CATS software. Toluene-ethyl acetate-formic acid (7:3:0.1) was used as a mobile phase with usage of 15ml per chromography run. The chromatogram development was carried out as linear ascending in twin through glass chamber (20 x 10 cm) saturated with the mobile phase. Once the

chromatogram has been developed the plate was dried in hot air oven. Then the plates were subjected to spraying with anisaldehyde sulphuric acid reagent and dried at 100°C for 3 min. Then dried plate was observed at 366nm in photo detection chamber and the images are taken. The R_f values and finger print data were calculated using WIN CATS software.

2.6 Animals

Albino Wistar rats wistar albino rats (200-230 g) and Swiss albino mice (25-30 g) of either sex were obtained from the animal house, Gayatri College of Pharmacy, Sambalpur. The animals were assimilated to laboratory conditions and maintained at 12 h in light and dark cycle at 28 ± 2 °C. The animals were fed with standard diet pellet purchased from Anjali foods, Indore, India and free access to water.

2.7 Acute Oral Toxicity Studies

Acute toxicity study was conducted as per OECD guideline No. 423 [13]. Swiss albino mice (25-30 g) of either sex were randomly divided into six groups (n=6). SCLE was administered to mice through oral gavage at varying dose such as 250, 500, 1,000, 1,500 and 2,000 mg/kg b.wt. Animals were monitored regularly for the signs of toxicity and mortality within 24 h and then daily for 14 days. SCLE did not show any toxicity and mortality and it was safe up to the dose of 2000 mg/kg.

2.8 Alcohol Induced Hepatotoxicity

Albino rats of either sex (150-180 g) were randomly divided into five groups (n=6) and grouped as follows,

Group I: Control rats received 1 mL/kg saline p.o. for 3 days
Group II: Toxic group received 28.50% alcohol (3 mL/100 g/day p.o.) for 30 days
Group III: Standard group received Silymarin 50 mg/kg p.o.) Followed by alcohol for 30 days.
Group IV: Received SCLE (200 mg/kg p.o.) and Ethanol for 30 days.
Group V: Received SCLE (400 mg/kg; p.o.) and Ethanol for 30 days.

Alcohol (28.50%) solution was prepared using distilled water and intoxicated at the was administered in a dose of 3 mL/100 g/day p.o. for 30 days in three divided doses [14].

2.9 Assessment of Liver Function

After the experimental period the animals were anaesthetized using ether. Then blood was collected by retro orbital puncture and serum was separated by centrifugation at 3000rpm maintained at 4 °C for 15 min. The serum was stored at -80°C and used for the estimation of various biochemicals markers such as ALT, AST, Triglyceride (TG), direct bilirubin (DB), total bilirubin (TB), and total protein [15].

2.10 Histopathological Studies

The animals were anesthetized by ether anaesthesia and sacrificed by cervical decapitation and liver was removed. The isolated liver was cleared from tissue debris and rinsed in ice cold ringers solution and wet-weight and volume were determined. Further the liver tissue was subjected to histopathology to assess the severity of alcohol induced hepatic damage. The light microscopic examinations of hepatic tissues were fixed with 10% buffered formalin, embedded with paraffin. Then the paraffin embedded tissues were sectioned using microtome for a thickness of 4 µM thickness and stained with haematoxylin and eosin dye and mounted in diphenylxylene. The sections were viewed through microscopy for histological alteration in liver structure and photographs were captured.

2.11 Statistical Analysis

The data were showed as mean ± SEM. One-way analysis of variance (ANOVA) was used for the statistical analysis followed by Dunnett's procedure. P value less than 0.05 was considered as statistically significant.

3. RESULTS

3.1 Phytochemical and Chromatographic Studies

Preliminary phytochemical analysis of SCLE showed the presence of phenols, flavonoids, steroids, glycosides, saponins, tannins, terpenoids, proteins and amino acids. HPTLC fingerprint profiles for SCLE were obtained and R_f values recorded. The SCLE contained 15 phytoconstituents having R_f values of 0.16, 0.18, 0.23, 0.25, 0.30, 0.34, 0.46, 0.51, 0.58, 0.63, 0.66, 0.71, 0.79, 0.81, and 0.87 under 366 nm.

Table 1. Preliminary phytochemical screening of *Sida cordata* leaves extract

S. No.	Phytoconstituents	Petroleum ether	Ethanol	Water
1	Alkaloid	Present	Present	Present
2	Flavonoid	Absent	Present	Present
3	Terpinoid and steroids	Absent	Present	Present
4	Saponins	Absent	Absent	Absent
5	Tannin/Phenols	Absent	Present	Present
6	Carbohydrates	Absent	Present	Present
7	Reducing sugars	Absent	Absent	Absent
8	Glycosides	Absent	Present	Absent
9	Protein & amino acids	Absent	Absent	Absent

Table 2. Effects of SCLE, silymarin, and alcohol on morphological parameters of liver

Groups	Body Weight (g)	Liver Weight (g)	Liver Volume (mL)
Group I	222 ± 0.42	5 ± 0.38	6 ± 0.58
Group II	227 ± 0.54	10 ± 0.48*	10 ± 0.32*
Group III	220 ± 0.32	6 ± 0.57**	6.8 ± 0.67**
Group IV	216 ± 0.67	8 ± 0.32**	8 ± 0.42**
Group V	218 ± 0.38	7 ± 0.57*	8 ± 0.32*

*Values are mean±SEM, n=6. *Significant difference from Group I (P<0.05). **Significant difference from Group II (P<0.05)*

3.2 Acute Toxicity Studies

SCLE elicited no mortality at the dose of 2000 mg/kg. So one-tenth of the maximum dose where no mortality had observed was selected as therapeutic middle dose (200 mg/kg) and its double (400 mg/kg) was selected as high dose.

3.3 Alcohol Induced Hepatotoxicity

3.3.1 Morphological studies

There were no significant differences in body weight between the study groups. However, there was a significant ($p< 0.05$) increase in liver weight and volume in ethanol intoxicated group II animals as compared to group I control animals. Meanwhile, treatment with SCLE (200 and 400 mg/kg) significantly ($p< 0.05$) reduced the liver weight and volume as compared to the ethanol intoxicated rats [16]. The results were displayed in Table 1.

3.3.2 Biochemical estimation

The effect of SCLE and alcohol on biochemical hepatic markers level were displayed in Table 2. In this study alcohol intoxicated rats showed significant ($p<0.05$) elevation of serum AST, ALT, TG, TB, DB and decreased protein level as compared to the control animals. Meanwhile, SCLE or silymarin treatment significantly ($P<0.05$) restored the levels of hepatic markers to normal as compared to the alcohol intoxicated rats.

3.4 Histopathology

The histology appearance of control liver displayed a normal pattern with no evidence of damage (Fig. 1a). In alcohol intoxicated group fatty infiltration was observed and the hepatocytes were surrounded by large number of fat droplets (Fig. 1b). However, silymarin, SCLE (200 and 400 mg/kg) treated animals showed decreased fatty infiltration and hepatocytes architecture was f0und to be normal, the thus substantiating the hepatoprotective potential of SCLE fatty (Fig. 1c-e).

4. DISCUSSION

Chronic alcoholism is one of the global health problem causing significant morbidity and mortality Alternative medicines using herbal ingredients is one of the best regimen to reduce the alcoholic liver disease [17]. In this backdrop, the present study was undertaken to evaluate the protective efficacy of SCLE on ethanol induced hepatotoxicity.

Table 3. Effects of SCLE, silymarin, and alcohol on different biochemical parameters of liver

Groups	AST (I.U./L)	ALT (I.U./L)	T.G (I.U./L)	T.B. (I.U./L)	D.B (I.U./L)	Total protein (mg/dL)
Group I	75.81 ± 0.9	48.32 ± 1.3	184.50 ± 1.3	0.35 ± 0.06	0.15 ± 0.04	7.87 ± 0.7
Group II	170.15 ± 1.7	127.56 ± 1.8*	340.29 ± 2.7*	1.52 ± 0.12*	0.85 ± 0.06*	2.32 ± 0.5*
Group III	96.63 ± 1.2**	72.86 ± 0.5*	211.51 ± 0.8**	0.41 ± 0.07	0.30 ± 0.05*	6.45 ± 0.4**
Group IV	127.32 ± 1.5**	98.70 ± 1.3**	290.02 ± 0.9*	0.60 ± 0.05*	0.39 ± 0.03	5.13 ± 0.8**
Group V	110.67 ± 1.4*	87.23 ± 0.8*	255.18 ± 2.1*	0.52 ± 0.08**	0.31 ± 0.07*	5.87 ± 0.6*

*Values are mean±SEM, n=6. *Significant difference from Group I (P<0.05). **Significant difference from Group II (P<0.05)*

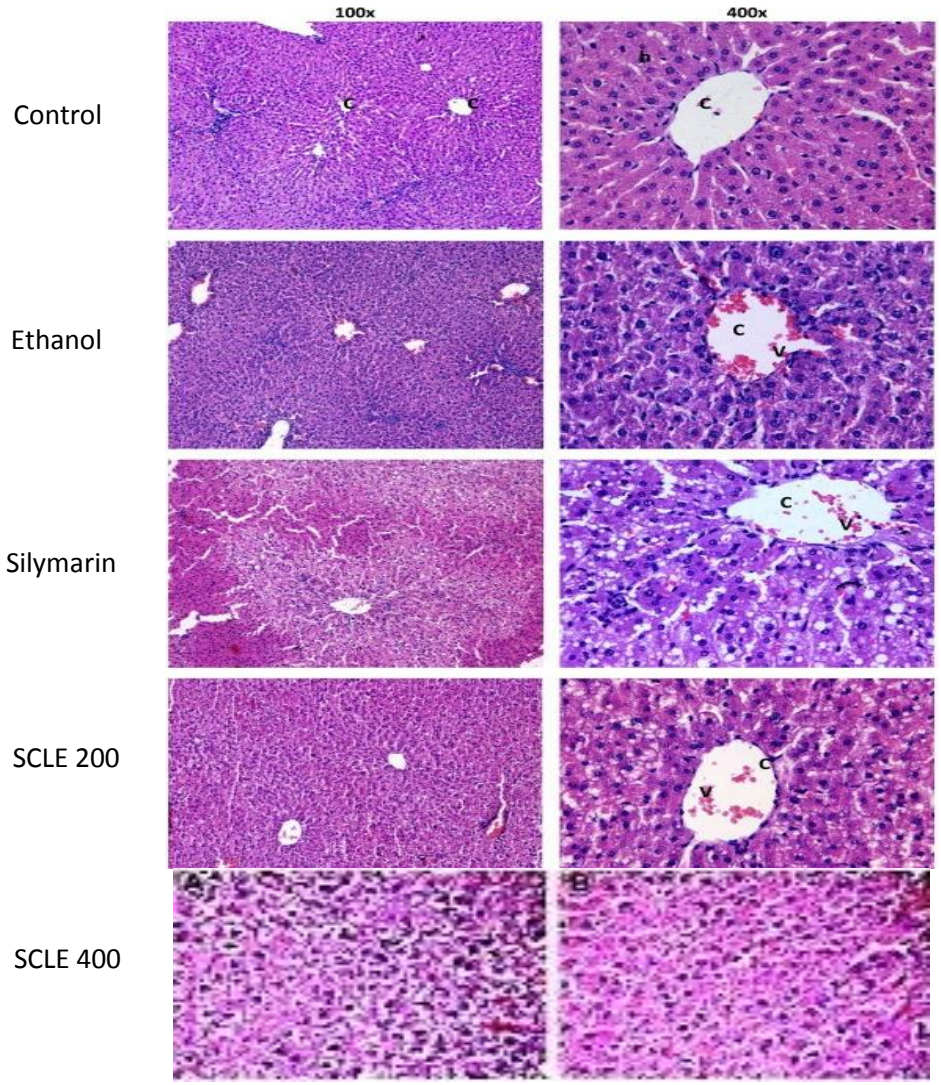

Fig. 1. Histopathology micrographs of liver tissue (a) control; (b) Ethanol control; (c) Silymarin; (d) SCLE-20 mg; and (e) SCLE-400 mg

In the event of chronic alcoholism more amount of lipids and proteins are deposited in hepatocytes which lead to hepatomegaly and reduced protein secretion in liver [18]. Water accumulation in hepatocytes leads to enlargement of liver causing increased liver weight and volume [19]. SCLE treatment effectively reduced the hepatomegaly Treatments thus substantiating its hepatoprotective activity.

During hepatotoxicity, the liver cytoplasmic membrane gets damaged and there is leakage of hepatic enzymes like AST and ALT which are intracellular in location and leaks into the systemic circulation [20]. In this study, ethanol intoxication leads to increased level of AST, ALT and decreased level of protein in serum. Further, treatment with SCLE significantly restored the increased markers enzymes and decreased protein level to normal mediated through its membrane protective activity. Estimation of serum bilirubin levels is one of vital diagnostic marker to evaluate the hepatoxicity. Thus restoration of normal bilirubin level as early as possible is a highly essential to improve the hepatocytes secretory potential [21-23]. In our study alcohol intoxicated rats showed elevated levels of TB and DB and treatment with SCLE significantly restored the increased bilirubin level to normal.

Chronic alcoholism causes hyperlipidemia as a result of activation of activation of enzyme HMG

Co-A reductase an important rate limiting enzyme in cholesterol biosynthesis. In our study increased triglycerides levels is observed in alcohol intoxicated rats which might be due to the downregulation of lipoprotein lipase, an important enzyme involved in the uptake of triglyceride-rich lipoprotein by the extrahepatic tissues. Administration of SCLE to alcohol intoxicated rats significantly reduced the TG levels to normal indicating its hepatoprotective activity. Further, in our study alcohol intoxicated rats showed marked histological alteration such as fatty infiltration and necrosis However, treatment with SCLE mitigated the histological changes and restored the hepatocytes architecture to normal.

Preliminary phytochemical screening of SCLE contains phenols, flavonoids, steroids, glycosides, saponins, tannins, and terpenoids, which may be attributed to the individual or combined effect of phytoconstituents present in it. HPTLC analysis was carried out only to resolve the number of components present in the extract. The developed chromatogram represents HPTLC finger printing of SCLE at optimized condition at 366 nm and consisting of 15 wells resolved peaks which were taken for hepatoprotective study.

Rao and Mishra [24] studied the hepatoprotective activity of *Sida cordata* powdered roots, aerial parts and their extracts against carbon tetrachloride, paracetamol, and rifampicin-induced hepatotoxic rats. It was observed that the powdered aerial and root parts showed a significant hepatoprotective activity against carbon tetrachloride followed by methanolic and aqueous extracts. Rejitha et al. [25] studied the hepatoprotective activity of 50 % ethanolic extract of the roots of *Sida cordata* L against alcohol intoxication model. Alcoholinduced toxicity is mediated through oxidative stress and it can be monitored by detecting lipid peroxidation products. Malondialdehyde, hydroperoxides, and conjugated dienes were significantly reduced in liver and protein carbonyls in the serum which was observed in the rats that were administered with ethanolic extracts of *Sida cordata*. The mRNA level of cytochrome P450 2E1, nuclear factor-κB, tumor necrosis factor alpha and transforming growth factor-β were found to be increased in the alcohol treated rats and their expressions were found to be decreased in the *Sida cordata* extracts treated rats. Silva et al. [26] also assessed the effects of the aqueous extract of S. cordifolia leaves, on liver regeneration after partial hepatectomy. The results showed that the extract stimulates liver regeneration after 67 % partial hepatectomy in rats. Qualitative HPTLC results are useful for identification and authenticating the quality of crude drug and thus substantiates the biological potential.

5. CONCLUSION

The present study unravels the hepatoprotective potential of plants so that it can be used for the management of various hepatic diseases. SCLE mitigated alcohol induced hepatotoxicity by restoration of hepatic markers and improved histological architecture. Further, phytochemistry studies concentrating on isolation is highly warranted.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All experimental studies were conducted as per the ethical protocol stated by Institutional animal ethics committee (IAEC, CPCSEA, India).

COMPETING INTERESTS

Author has declared that no competing interests exist.

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