Study of Hepatoprotective Activity on Methanol Extract of *Smilax Zeylanica* L. Leaf against Carbontetrachloride Induced Hepatic Damage in Rats

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Abstract

The present investigation was aimed at evaluating the hepatoprotective potential of the methanol extract of leaf of Smilax zeylanica L. on Carbontetrachloride induced hepatotoxicity in Wistar rats. Hepatotoxicity was induced in Wistar rats by administration of CCl4 0.5 mL/kg p.o. once a day for 7d. Methanol extract of leaf of Smilax zeylanica (MELS) was administered at doses 200, 400 and 600 mg/kg p.o. All the groups except the normal control received CCl4 0.5 mL/kg p.o. The treatments were continued once daily for 7 d. After 7 d serum levels of SGOT, SGPT, ALP, total proteins, albumin and total bilirubin were estimated. Histopathological examination of liver section was also performed. Preliminary phytochemical screening of MELS was carried out to detect the presence of various phytoconstituents. HPTLC fingerprint profiles of the detected phytoconstituents were also obtained. Administration of CCl4 0.5 mL/kg p.o. once a day for 7 d, produced profound hepatic damage as evidenced by the significant elevation in serum levels of SGOT, SGPT, ALP and total bilirubin and decrease in total proteins and albumin. The altered biochemical parameters were significantly ($p < 0.001$) restored by MELS.

Key words: S. Zeylanica Leaf, Hepatoprotective, Liver Transaminases

1. INTRODUCTION

Chopachinee is an important drug in Ayurveda and Smilax china L. is its accepted botanical source [1]. Smilax zeylanica L. is used as a substitute for Chopachinee [1,2]. S. zeylanica (Smilacaceae) is distributed in India, Myanmar and Kampuchea [1,3]. Root, rhizome and leaf of S. zeylanica are used in epilepsy, fever, venereal and skin diseases, sores, swellings and abscesses [4]. Root is also used for treating rheumatism and pain in the lower extremities [5]. The plant is also used in ritual healing techniques [6] and in bloodless dysentery [7]. S. zeylanica is used in the villages of Bangladesh for the treatment of fever, headache and wounds [8]. Phytoconstituents reported in S. zeylanica are the steroidal saponin glycosides, Dioscin, Diosgenin, Smilagenin and Sarsapogenin [9]. Antiepileptic activity studies have been reported on the roots and rhizomes of S. Zeylanica [10]. The pharmacognostical characteristics of S. zeylanica roots and rhizomes have also been investigated [11]. Antioxidant property of root, rhizome and leaf of S. zeylanica has been reported [12,13]. The hepatoprotective properties of the methanol extract of the roots and rhizomes of S. zeylanica has been reported [14]. Since no such work is reported on the leaf of S. zeylanica, this research has been undertaken.

2. METHODOLOGY

2.1 Plant Material

The leaves of S. zeylanica were collected from the forest area of Kanyakumari district, Tamil Nadu during June 2008. The plant material was identified and authenticated by Dr. S. N. Yoganarasimhan, Plant Taxonomist following local floras [15,16], and the herbarium specimen (No. 012) along with crude sample have been deposited at the herbarium and crude drug museum of PG Department of Pharmacognosy, Faculty of Pharmacy, M. S. Ramaiah University of Applied Sciences, Bangalore.

2.2 Preparation of Methanol Extract of *S. Zeylanica* Leaf

Coarsely powdered leaf material (500 g) was extracted with methanol by Soxhlation. The methanol extract (MELS) was concentrated at reduced pressure to produce a brownish semi solid mass (11.01% w/w). The phytoconstituents present in the methanol extract were identified by qualitative analysis and confirmed by HPTLC. The dried extract was suspended in 2% w/v acacia in distilled water and used for pharmacological studies.

2.3 Acute Toxicity Study

Acute toxicity studies were performed following OECD guideline 420 [18].

2.4 Hepatoprotective Activity

Hepatotoxicity was induced by administration of CCl4 (with liquid paraffin 1:1) 0.5 mL/kg, p.o. once a day for 7 d. Albino Wistar rats of either sex (170-200 g) were divided into 6 groups of 6 animals each. Group I: Normal Control treated with vehicle (2% w/v acacia, 2 mL/kg, p.o.); Group II: Positive hepatotoxic control (2% w/v acacia, 2 mL/kg, p.o + CCl4 0.5 mL/kg, p.o.); Group III:

Silymarin 100 mg/kg p.o + CCl4 0.5 mL/kg, p.o.; Group IV: MELS 200 mg/kg p.o. + CCl4 0.5 mL/kg, p.o.; Group V: MELS 400 mg/kg p.o. + CCl4 0.5 mL/ kg, p.o.; Group VI: MELS 600 mg/kg p.o. + CCl4 0.5 mL/kg, p.o.

The treatments were given once daily for 7 d. On 8th day, 18 h after the last dose of CCl4, animals were anaesthetised using anaesthetic ether. Blood was collected from retro-orbital and allowed to coagulate. Serum was separated and used for biochemical estimations. The animals were sacrificed by excess of ether anaesthesia and liver was isolated and subjected to histopathology studies [19].

2.5 Biochemical Estimations

Serum was used for the estimation of SGPT [20], SGOT [20], ALP [21], total proteins [22], albumin [23] and bilirubin [24].

2.6 Histopathological Studies

Histopathology studies were performed following standard procedures [25].

2.7 Statistical Analysis

The results were expressed as mean ± SEM and statistically analyzed by One Way Analysis of Variance (ANOVA) followed by Tukey Kramer multiple comparison test using INSTAT software.

3. RESULTS AND DISCUSSIONS

Preliminary phytochemical screening of methanol extract of *S. zeylanica* leaves revealed the presence of glycosides, saponins and flavonoids. HPTLC fingerprint profiles of the phytoconstituents were also obtained (Fig. 1, 2, 3). MELS did not show any signs or symptoms of toxicity and no mortality was recorded during the entire study.

An increase in liver weight is characteristic of CCl_4 induced hepatotoxicity. The liver weight was calculated and expressed in terms of g/100g body weight. Liver weight increased in the positive control group on daily administration of CCl_4 0.5 mL/kg. MELS 200, 400 and 600 mg/kg significantly ($p< 0.01$, $p< 0.001$) attenuated the CCl_4 induced increase in liver weight. When cell membrane of hepatocytes is damaged, a variety of cytosolic enzymes such as SGOT, SGPT and ALP are released into blood [26]. The elevated level of SGOT, SGPT and ALP in serum is an indication of cellular leakage and loss of functional integrity of liver cell membrane [27]. Estimation of these enzymes is a quantitative marker for assessing hepatic cell damage [28]. CCl_4 intoxication also produced a significant elevation in the levels of serum bilirubin. Bilirubin levels in serum of treated rats was significantly restored ($p< 0.05$, $p< 0.01$), which may be due to the inhibitory effects of the leaf extracts on cytochrome P- 450 and/or promotion of its glucuronidation [29]. SGOT, SGPT, ALP and total bilirubin levels were significantly ($p< 0.001$) increased while total proteins and albumin ($p< 0.001$) levels were lowered in the positive control group compared with the normal control animals. Treatment with the extract produced significant changes in the altered serum parameters. SGOT and SGPT levels decreased significantly ($p< 0.001$) with all the three doses of MELS. The results were independent of dose and comparable with that of the standard antioxidant silymarin. ALP levels also decreased significantly with MELS 600 mg/kg ($p< 0.05$).

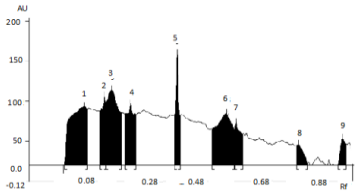

Fig. 1 HPTLC fingerprint of flavonoids in methanol extract of *S. zeylanica* leaf

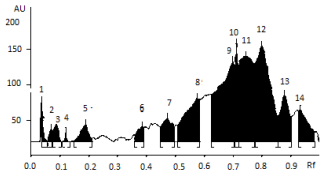

Fig. 2 HPTLC fingerprint of glycosides in methanol extract of *S. zeylanica* leaf

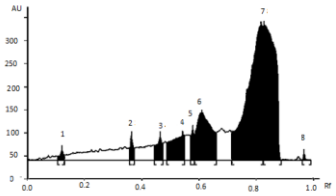

Fig. 3 HPTLC fingerprint of saponins in methanol extract of *S. zeylanica* leaf

The lowered levels of hepatic proteins in CCl_4 intoxicated rats may be attributed to the oxidative damage of some amino acids [30]. The capacity of liver to synthesize proteins especially albumin is adversely affected by hepatotoxins. Total proteins increased significantly on treatment with MELS 200 and 400 mg/kg ($p< 0.01$and $p< 0.001$respectively), however there was no significant increase with 600 mg/kg dose. Serum albumin levels in the positive control group were reduced in comparison with that of normal control. However treatment with the extract increased serum albumin levels. The effect of MELS 400 mg/kg were significant ($p<0.01$). The increased levels of total protein and albumin in the serum of extract treated animals indicate their hepatoprotective activity. The results of biochemical estimations are presented in Table 1.

Histopathology studies of liver showed ballooning degeneration, fatty degeneration, congestion, reactive changes like binucleation and loss of normal structure of hepatocytes in CCl_4 induced positive control rats, in comparison with the normal control. The extract treated groups showed regeneration of hepatocytes and normalization of fatty changes in hepatocytes. MELS 200 mg/kg treated specimens showed signs of periportal inflammation and early fibrosis. Groups treated with higher doses showed regenerative changes in hepatocytes and decreased fatty degeneration. The silymarin treated

group showed regenerative changes and fatty changes were less prominent.

Many of the phytoconstituents present in the plants under this study are reported to possess antioxidant and hepatoprotective potential. Antioxinat property of *S. zeylanica* leaf has been reported [13]. Antioxidant and hepatoprotective properties of saponins [31,32]; glycosides and flavonoids [33-36] are reported. The presence of phytoconstituents with antioxidant properties could have contributed to the hepatoprotective properties of this plant. Further work to isolate the phytoconstituent(s) responsible for the hepatoprotective effect and to elucidate the exact mechanism of action could be undertaken.

4. CONCLUSION

Leaf of *S. zeylanica* is a promising hepatoprotective agent. The hepatoprotective activity may be due to the presence of antioxidant chemicals present in it.

5. ACKNOWLEDGEMENTS

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6. CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Parameters	Normal Control	Positive control	Standard silymarin	MELS 200mg/kg	MELS 400mg/kg	MELS 600mg/kg
SGOT IU/L	140.16±6.7	910.83±110.6[a]	180.16±96.2 ***	374.66±60.13 ***	289.5±25.9 ***	267.16±38.6 ***
SGPT IU/L	41±5.7	684±79.8 [a]	37.33±6.1 ***	269.16±30.86 ***	167.16±12.6 ***	144.5±55.3 ***
ALP IU/L	214.88± 76.8	1018.16± 116.8 [a]	988±9.3	893.50±19.01	886.33±15.7	778.5±68.1*
Total proteins g/dl	6.73±0.2	4.85±1.9	6.56±0.5 *	6.28±0.2*	7.91±0.1***	3.06±0. 2
Total bilirubin mg/dl	0.433±0.2	1.43±0.1 [a]	0.91±0.2 ***	0.98±0.1*	0.92±0.0**	3.4±0.0**
Albumin g/dl	3.82±0.2	2.75±0.2[a]	3.25±0.3*	3.06±0.2	3.4±0.0**	3.25±0.1

Table 1. Effect of methanol extract of *S. zeylanica* leaf in CCl_4 induced hepatotoxicity

All values expressed as Mean ± SEM; n=6. Tukey Krammer Multiple Comparison Test [a]p<0.001 in comparison with the normal control; ***p<0.001, **p<0.01 *p<0.05, in comparison with the positive control.