

HEPATOPROTECTIVE AND ANTIOXIDANT ACTIVITIES OF *SMILAX CHINENSIS* L. ROOT

Subhash C. Mandal, Goutam Kumar Jana, Sanchita Das, Ranabir Sahu, R. Venkidesh,
Saikat Dewanjee*

Pharmacognosy and Phytotherapy Research Laboratory, Division of Pharmacognosy,
Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700032, India.
Telephone No: 0091-33-24146126 Fax: 0091-33-28371078.

* **Corresponding Author** s.dewanjee@yahoo.com

Summary

The root of the plant *Smilax Chinensis* L. (Liliaceae) is used by the tribal of South India for the treatment of liver diseases. The hepatoprotective activity of chloroform, ethyl acetate and methanol extracts at the dose of 100 mg/kg body weight orally, was evaluated against paracetamol induced liver damage in rats. Biochemical markers such as serum glutamate oxalate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase and bilirubin content of the liver were estimated. The ethyl acetate extract showed significant hepatoprotective activity and experimental data are almost comparable to that of standard drug Silymarin (25 mg/kg) with respect to all selected hepatic markers. Further, the *in vitro* antioxidant activity of the extracts was carried out with the help of some selective models namely 1, 1-diphenyl-2-picryl-hydrazil (DPPH) method, total reducing power determination and determination of total phenolic content to establish correlation between antioxidant effect and hepatoprotection. It was observed that the ethyl acetate extract showed best antioxidant effect amongst other two extracts. Thus it may be concluded that the ethyl acetate fraction produce hepatoprotective effect by antioxidant mechanism.

Key words: *Smilax chinensis*, Liliaceae, hepatoprotective, paracetamol, antioxidant

Introduction

Liver is the most important organ, which plays a crucial role in regulating various physiological processes in the body such as metabolism, secretion and storage. It has great capacity to detoxicate toxic substances and synthesize useful principles (1, 2). Therefore, damage of the liver imparts detrimental effect on the body. Aerobic organs such as the liver generate reactive oxygen species that induce oxidative tissue damage. These radicals react with cell membranes, cause inflammation and finally damage the tissue. Therefore the antioxidant mechanism is a major defense system, which converts active oxygen molecules into non-toxic compounds (3, 4) and finally revert cellular damage. Thus an antioxidant may serve as hepatoprotective agent. In spite of tremendous advances in modern medicine, there are no effective drugs available that stimulate liver function, offer protection to the liver from damage or help to regenerate hepatic cells (5).

In absence of reliable liver protective drugs in modern medicine, there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders since centuries (6). The present investigation was undertaken for the scientific exploration of hepatoprotective efficacy of a traditional plant based on its folklore claim. The *in vitro* antioxidant activity was further undertaken to perceive the mechanism of action.

Smilax chinensis L. (Liliaceae) is a deciduous climber with rounded leaves and red berries. The root tubes of which furnish the drug known as china root. It is found in the south Indian states namely Andhra Pradesh, Karnataka and Tamil Nadu (7). This plant is known to possess analgesic, antiallergic, antiasthmatic, antibiotic, antifungal, anti-inflammatory, antirheumatic, carminative, depurative, diaphoretic, diuretic, febrifuge, hepatoprotective and immunomodulatory activities (8, 9).

Material and Methods

Plant material

The roots of *Smilax chinensis* L. were collected in the month of April 2007 from Tamilnadu, India. The plant was authenticated by H J Chowdhury, Joint Director, Botanical Survey of India, Shibpur, Howrah, India. A voucher specimen JU/PPRT/DP/PT/05/07 was deposited at our laboratory for future reference.

Preparation of extracts

The roots of *Smilax chinensis* L. were dried in an incubator for two days at 40°C, crushed in a mechanical grinder to fine powder of mesh 40. The powder (500 g) was then extracted successively with petroleum ether, chloroform, ethyl acetate and methanol in a Soxhlet apparatus. Resulting extracts were filtered, concentrated, and dried *in vacuo* (yield 0.35, 0.94, 1.25, 3.46 % w/w respectively). Then the methanol, ethyl acetate and chloroform extracts were subjected for this experiment.

Phytochemical screening

The preliminary phytochemical investigation was carried out for the different extracts obtained from the crude drugs (10).

Chemicals

Silymarin was purchased from Micro labs, Tamilnadu, India. SGOT, SGPT, ALP and Bilirubin kits were procured from Span Diagnostics, Surat, India. All the reagents used were of analytical grade.

Animals

Healthy adult Wister strain albino rats of both sex between 2-3 months of age and weighing 180-240 g were used for this study. Animals were allowed to be acclimatized for a period of 2 weeks in our laboratory environment prior to the study. Rats were housed in polypropylene cages (3 animals per cage), maintained under standard laboratory conditions (*i.e.* 12:12 h light and dark sequence; at an ambient temperature of 25 ± 2°C; 35-60% humidity); the animals were fed with

standard rat pellet diet (Hindustan Liver Ltd. Mumbai, India) and water *ad libitum*. The principles of Laboratory Animals care (11) were followed and instructions given by our institutional animal ethical committee were followed throughout the experiment.

Experimental design

The rats were divided into six groups of six rats in each group.

Group I: Normal rats administered distilled water daily for 7 days.

Group II: Rats were treated with distilled water daily for 7 days.

Group III, IV and V: Rats were treated with methanol, ethyl acetate and chloroform extracts of *Smilax chinensis* L. root respectively at the dose of 100mg/kg body weight suspended in purified water for 7 days.

Group VI: Animals were treated with standard drug Silymarin solution (25 mg/kg body weight) for 7 days.

Paracetamol (750 mg/kg body weight) was administered orally in animals of group II to VI on day 7 (12).

On day 8, the animals were scarified by decapitation. Then hepatic markers were namely the SGOT (13), SGPT (13), ALP (14), bilirubin (13) were estimated in serum of rats.

***In vitro* antioxidant activity**

Determination of total phenolic compounds

The total concentration of phenolics in the Hydro alcoholic extract was determined according to the method Singleton et al. (15). Briefly, 0.1 ml of each extract solution (contains 500 µg of extract) was transferred to a 100 ml Erlenmeyer flask, and then the final volume was adjusted to 46 ml by the addition of distilled water. Afterward, 1 ml of Folin-Ciocalteu reactive was added into this mixture and after 3 min 3 ml of Na₂CO₃ (2%) was added. Subsequently, the mixture was shaken on a shaker for 2 h at room temperature, and then absorbance was measured at 760 nm. Pyrocatechol (Sigma) was used as the standard for the calibration curve. The estimation of phenolics in the fractions was carried out in triplicate, and the results were averaged. The phenolic compound content was determined as pyrocatechol equivalents using the following linear equation based on the calibration curve: $A = 0.0034C - 0.058$, $R_2 = 0.9996$. A is the absorbance, and C is pyrocatechol equivalents (µg). The test was performed in triplicate and average value was represented.

Reducing power

The reducing power of selected extracts was determined according to the method of Oyaizu, 1986 (16). 10 mg of each extract in 1ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 2.5 ml, 1% potassium ferricyanide. The mixture was incubated at 500 °C for 20 min. 2.5 ml of trichloroacetic acid (10%) was added to this mixture, which was then centrifuged at 3000 g for 10 min. The upper layer of the solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml, 0.1% FeCl₃ and the absorbance was measured at 700 nm. All the tests were performed in triplicate.

Inhibition of DPPH radical

The free radical scavenging activity of selected extracts was measured by 1, 1-diphenyl-2-picrylhydrazil (DPPH) using the method of Blois, 2000 (17). 0.1 mM solution of DPPH in ethanol was

prepared and 1ml of this solution was added to 3 ml of various concentrations of extracts. After 30 min, absorbance was measured at 517 nm. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples. All the tests were performed in triplicate.

Statistical analysis

Data were statistically calculated by utilizing one way ANOVA and expressed as mean \pm S.E.M. followed by Dunnett's *t*-test using computerized GraphPad InStat version 3.05, Graph pad software, U.S.A.

Results

Preliminary phytochemical studies revealed the presence of tannins, alkaloids and glycosides in methanol extract, flavonoids in ethyl acetate extract triterpenoids and steroids in chloroform extract.

The hepatoprotective effect of different extracts of the root of *Smilax chinensis* L. was indicated in Table 1. SGOT, SGPT, ALP and bilirubin contents of the liver were estimated. Significant elevation was absorbed SGOT, SGPT, ALP and bilirubin level in paracetamol treated animals. It has been observed that the ethyl acetate extract showed best activity in term of reduction of all hepatic markers and the results were comparable to that of standard drug Silymarin. Other two extract namely methanol and chloroform extract showed insignificant activity.

Table 1. Hepatoprotective activity of *Smilax chinensis* L. root.

Group	Treatment	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)
I	Normal control	56.23 \pm 4.88	49.57 \pm 3.58	32.72 \pm 2.72	0.55 \pm 0.03	0.089 \pm 0.01
II	Paracetamol control	108.04 \pm 3.07 ^{##}	101.49 \pm 5.12 ^{##}	89.47 \pm 5.94 ^{##}	2.43 \pm 0.12 ^{##}	0.49 \pm 0.04 ^{##}
III	Paracetamol + Methanol extract (100 mg/kg)	89.19 \pm 4.42*	93.42 \pm 5.13	82.92 \pm 5.33	1.29 \pm 0.18	0.38 \pm 0.06
IV	Paracetamol + Ethyl acetate extract (25 mg/kg)	65.05 \pm 4.45**	60.94 \pm 4.68**	46.34 \pm 4.56**	0.74 \pm 0.08**	0.102 \pm 0.06**
V	Paracetamol + chloroform extract (25 mg/kg)	97.60 \pm 7.72	97.05 \pm 3.57	77.65 \pm 6.67	1.92 \pm 0.16*	0.46 \pm 0.04
VI	Paracetamol + Silymarin (25 mg/kg)	59.67 \pm 3.29**	55.50 \pm 1.71**	35.48 \pm 1.75**	0.72 \pm 0.07**	0.093 \pm 0.07**

Values are expressed as mean \pm S.E.M. (n=6).

^{##} *p* <0.01 when compared with normal control rats.

* *p* <0.05 when compared with paracetamol control rats.

** *p* <0.01 when compared with paracetamol control rats.

Total phenolic contents compounds of methanol, ethyl acetate and chloroform extracts were expressed as mg of pyrocatechol equivalent per gram of dry weight. The level of total phenolic compounds was found 70.38 mg per gram of extracts respectively. The reducing power of extracts was determined (Fig 1). The gradual increase in absorbance with concentration is indicative of enhancement of reducing power with concentration.

The free radical scavenging activity of extracts was determined by DPPH method (Fig 2). The IC₅₀ values were found 190.14, 79.11 and 189.47, µg/ml of methanol, ethyl acetate and chloroform extract respectively whilst IC₅₀ value of standard drug α-tocopherol 147.91 µg/ml.

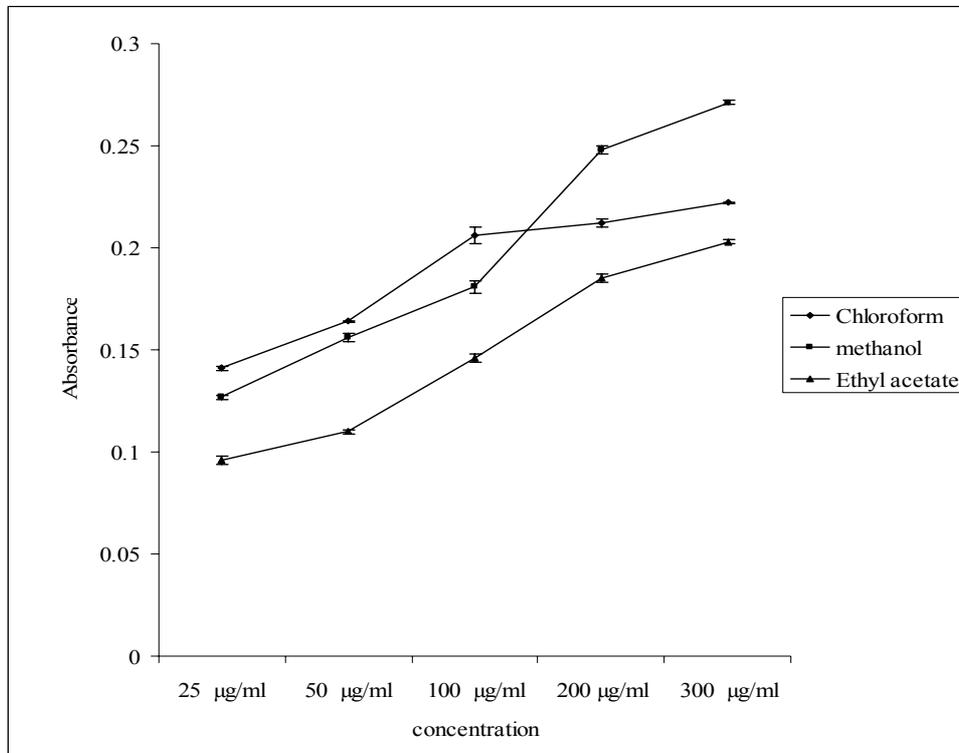


Fig.1. Reducing power determination of *Smilax chinensis* L. root extracts.

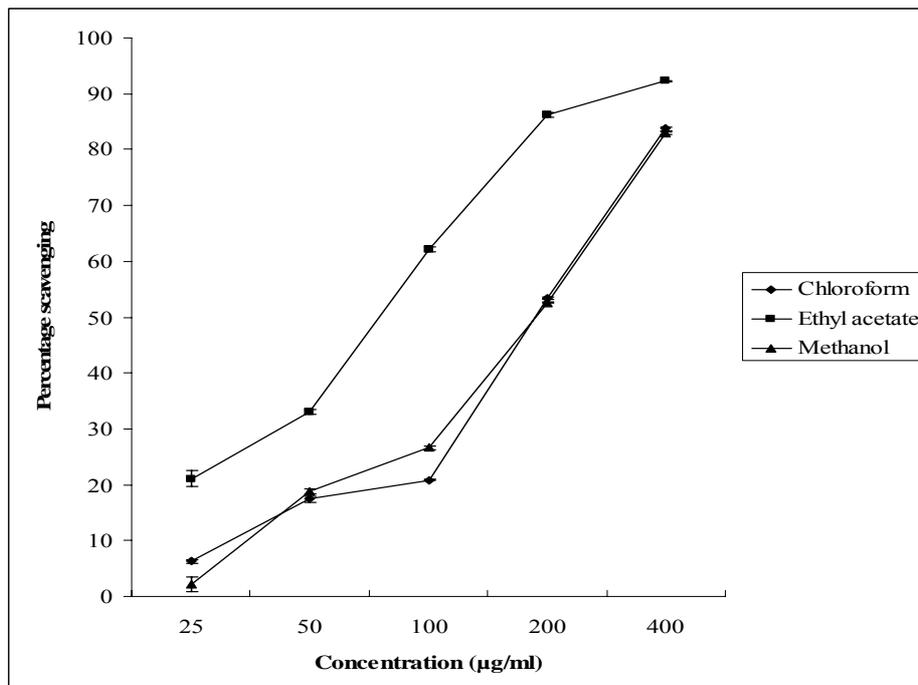


Fig. 2. DPPH (1, 1-diphenyl-2-picryl-hydrazyl) radical scavenging activity of *Smilax chinensis* L. root extracts.

Discussion

Paracetamol is a common antipyretic agent, which is safe in therapeutic doses but can produce fatal hepatic necrosis in man, rats and mice with toxic doses (18). Protection against paracetamol-induced toxicity has been used as a test for potential hepatoprotective activity by several investigations (19, 20). Hepatoprotective activity of chloroform, ethyl acetate and methanol extracts at the dose of 100 mg/kg body weight orally, was evaluated against paracetamol induced liver damage in rats in this experiment. The ethyl acetate extract exhibited promising hepatoprotective activity. The *in vitro* antioxidant activity of the extracts indicated to ethyl acetate fraction showed best activity in term of total phenolic content, reducing power and radical scavenging. Thus it may be concluded as the hepatoprotective activity of ethyl acetate extract of *Smilax chinensis* L. assumed to be due to its antioxidant effect. Further the preliminary phytochemical investigation indicated that ethyl acetate fraction is rich in flavonoid content. Flavonoids isolated from different sources have been reported to have antioxidant activity (21, 22) so the lead compound may be flavonoid. Now our intention is guided to isolate bioactive flavonoid from extract and to substantiate its effectiveness against liver diseases.

References

1. Shahani S. Evaluation of hepatoprotective efficacy of APCL-A polyherbal formulation in vivo in rats. *Indian Drugs* 1999; 36: 628–631.
2. Subramoniam A, Pushpangadan P. Development of Phytomedicine for liver diseases. *Indian J Pharmacol* 1999; 31: 166–175.
3. Halliwell B, Gutteridge JMC. Lipid peroxidation, oxygen radicals, cell damage and antioxidant therapy. *Lancet* 1984; 23: 1396-1397.
4. Hochstein P, Atallah AS. The nature of oxidants and antioxidant systems in the inhibition of mutation and cancer. *Mutat Res* 1988; 202: 363-375.
5. Chattopadhyay RR. Possible mechanism of hepatoprotective activity of *Azadirachta indica* leaf extract: Part II. *J Ethnopharmacol* 2003; 89: 217–219.
6. Chatterjee TK. Medicinal Plants with Hepatoprotective Properties, in *Herbal Opinions*, third ed. Books & Allied (P) Ltd., Calcutta 2000:135.
7. Lee H, Lin JY. Antimutagenic activity of extracts from anticancer drugs in Chinese medicine. *Mutat-Res* 1988; 204(2): 229-234.
8. Dhawan BN, Patnaik GK, Kulshrestha DK, Sarin YK. Absence of Hepatoprotective activity in *Laqotis cashmiriana*, an adulterant to *Picrohiza kurrooa*. *Ind J Pharmacy* 1991;23:121-122.
9. Handa SS, Sharma A, Chakraborty KK. Natural products and plants as liver protecting drugs. *Fitoterapia* 1986;47:307-345.
10. Kokate CK. *Practical Pharmacognosy*, Vallabh Prakashan, New Delhi, India 1994; 107-110.
11. PHS (Public Health Service), Public Health Service Policy on Humane Care and Use of Laboratory Animals, Washington, D.C., U.S., Department of Health and Human Services. Available from Office for Protection from Research Risks, Building 31, Room 4B09, NIII, Bethesda, MD 20892, 1986.
12. Bhakta T, Banerjee S, Mandal SC, Maity TK, Saha BP, Pal M. Hepatoprotective activity of *Cassia fistula* leaf extract. *Phytomedicine* 2001; Volume 8(3), 220-224.
13. Oser BL. *Hawk's Physiological Chemistry*. Tata Mc-Graw Hill Book Company Ltd., New Delhi 1965; 14 Ed, 1052-1073.

14. Bergmeyer HU, Brent E. Methods of Enzymatic Analysis, Verlag Chemie Weunheun, Academic Press, New York 1974; (2):735-760,
15. Singleton VL, Orthofer RM, Ramuela-Raventos RM. Analysis of total phenols and antioxidants and other substrates by means of Folin-Ciocalteu reagent. Methods. Enzymol 1999; 299: 152-178.
16. Oyaizu M. Studies on product of browning reaction preparation from glucose amine. Jap J Nutrition 1986; 44:307.
17. Blois MS. Antioxidant determinations by the use of stable free radical. Nature 1958;29:1199-2000.
18. Brattin WJ, Glende EA Jr, Rocknagel RO. Pathological mechanisms in carbon tetrachloride hepatotoxicity. Free Radical Biol and Med 1985;1:27.
19. Ahmed S, Rehman A, Alam, et al. Evaluation of the efficacy of *Lawsonia alba* in the alleviation of carbon tetrachloride induced oxidative stress. J Ethnopharmacol 2000;69:157.
20. Kyung Jin Lee, Eun-Rhan Woo, Chul Yung Choi, et al. Protective effect of acetoside on carbon tetrachloride induced hepatotoxicity. Life Sci 2004;74:1051.
21. Miura T, Ichhiki H, Hashimoto I, Iwamoto N, Kato M, Kubo M, Komatsu Y, Okada M, Ishida T, Tanigawa K. Antidiabetic activity of xanthone compound, mangiferin. Phytomedicine. 2001;8: 85-87.
22. Olmedilla MN. Reference values for retinal, tocopherol and main carotinoids in serum of control and insulin dependent diabetic Spanish subject. Clin. Chem. 1999;43:1066-1071.