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Evaluation of Hepatoprotective activity of *Sphaeranthus indicus* flower heads extract

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Abstract

Objective: To investigate the protective effect of *Sphaeranthus indicus* Linn. (Asteraceae) against CCl_4-induced hepatotoxicity and the mechanism underlying these protective effects in rats. Methods: The hepatoprotective effect of *Sphaeranthus indicus* (Flower heads) extracts was studied using CCl_4 (2 ml/kg, s.c.) induced liver damage in rats. The effect of extract on bile flow was studied in anaesthetised normal rats by surgical cannulation with polyethylene tubing. The drug was given intraduodenally after one-hour bile collection. Result: The animals receiving the extracts of *Sphaeranthus indicus* has shown to possess a significant protective effect by lowering the serum aspartate and alanine aminotransferase (AST and ALT) and alkaline phosphatase (ALP). This hepatoprotective action was confirmed by hexobarbitone-induced sleeping time in mice, which was increased by CCl_4 treatment and in addition the extract-stimulated bile flow (choleretic activity) in anaesthetized normal rats. Conclusion: Methanolic extract of *Sphaeranthus indicus* flowers produces prominent hepatoprotective activity in animal models.

Keywords: Choleretic activity; Hepatoprotective; *Sphaeranthus indicus*.

1. Introduction

Sphaeranthus indicus Linn belongs to family Asteraceae is annual spreading herb, commonly known as "Gorakhmundi" in Hindi. Which grows approximately 15-30 cm in height. The plant is distributed throughout the plains and wet lands in India, Sri Lanka and Australia [1].

All parts of the plant possess medicinal uses and have been reported to have beneficial effects on several ailments. The juice of the plant is styptic and diuretic and it is said to be useful against liver and gastric disorders [2]. Roots and seeds are used as stomachic and anthelmintic [3]. They are also used as blood purifiers in skin diseases [4]. Dried and powdered leaves of *Sphaeranthus indicus* are useful in the treatment of chronic skin diseases, urethral discharges and jaundice [5]. Extract of *Sphaeranthus indicus* exhibited excellent antibacterial activity against Gram positive as well as Gram-negative bacteria [6]. The phytochemical analysis of the plant showed

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that it contains eudesmanolide type of sesquiterpene possessing immunostimulating [7] and anti-inflammatory activities [8]. However, there is paucity of scientific data on the hepato protective activity of flower heads of *Sphaeranthus indicus*. The objective of present investigation was to study the hepatoprotective activity of the methanol extract of the flower heads of *Sphaeranthus indicus* in animal models.

2. Materials and Methods

2.1 Plant material

The flower heads of *Sphaeranthus indicus* (SI) were obtained from local market of Meerut U.P, India. Authentication was conducted by Dr. H.B Singh, National Institute of Science Communication and Information Resources (NISCAIR) New Delhi, India. A voucher specimen was deposited at the herbarium of our Pharmacognosy laboratory.

2.2 Preparation of methanol extract from dry flower heads of Sphaeranthus indicus (SI)

The air-dried flower heads was powdered. The powder (100 g) was extracted with 1000 ml methanol in a soxhlet. The extract was filtered and concentrated under reduced pressure on a rotary evaporator. The concentration is expressed as mg/ml.

2.3 Animals

Male Wistar albino rats (150-200 g) and male Swiss albino mice (20-25 g) were used for the study. Animals were housed in clean metabolic cages and maintained in controlled temperature (23 ± 2°C) and they were fed with standard pellet diet and tap water *ad libitum*. Institutional ethics committee constituted for this purpose approves the protocol.

2.4 Toxicity study

Sphaeranthus indicus (SI) dried extract was dissolved in water and administered orally to different groups of mice in dose ranging from 100-1000 mg/kg for the LD_{50} study using the method of Miller and Tainter [9]. There was no lethality in any of the groups after 7 days of treatment.

2.5 CCl_4-induced hepatic damage experiments

Rats were divided into six groups (n=6). Group I, served as normal control group and received 0.5% Tween-80 (1ml/kg, p.o.) on all 5 days and received olive oil (1ml/kg, s.c.) on days 2 and 3. Group II served as CCl_4 control and were administered a single daily dose of 0.5% Tween-80 (1 ml/kg, p.o.) on all 5 days and on the 2 and 3 day, they were administered s.c., CCl_4: olive oil (1:1). Group III animals were administered SI (100 mg/kg) p.o., on all 5 days and a single dose of CCl_4 (2 ml/kg) s.c., on days 2 and 3, 30 min after SI administration. Group IV animals were administered SI (200 mg/kg p.o.) on all 5 days and a single dose of CCl_4 (2 ml/kg, s.c.) on days 2 and 3, 30 min after SI administration. Group V animals were administered Silymarin, the known hepatoprotective compound (Sigma Chemical Company, USA), at a dose of 100 mg/kg p.o., on all 5 days and a single dose of CCl_4 (2 ml/kg) s.c, on days 2 and 3, 30 min after Silymarin administration. On the fifth day, all the animals were sacrificed by mild ether anaesthesia. Blood samples were collected for evaluating the biochemical parameters.

2.6 Biochemical estimations

Serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), serum alkaline phosphatase (SAKP) and serum bilirubin (SB) were assayed according to standard methods [10,11,12].

2.7 Hexobarbitone-induced sleeping time studies

Four groups of Swiss albino mice were used for this study (six per group). Food was withdrawn on the preceding night of the

experiment. CCl_4 (50 µl/kg, p.o.) or vehicle (olive oil) was given to Groups I and II, whereas Group III and Group IV animals received CCl_4 (50 µl/kg,) p.o., and also the plant extract, SI (100 and 200 mg/kg, p.o.), 1 h after CCl_4 administration. All the four groups of animals were given hexobarbitone (60 mg/kg, i.p.) 2 h after CCl_4/vehicle treatment. The time between loss of righting reflex and its recovery was recorded and reported [13].

2.8 Choleretic activity

To study the choleretic activity of SI, an independent set of normal male rats were fasted overnight and divided into two groups of 6 rats each. These rats were anaesthetized with i.p. injection of sodium pentobarbitone. The common bile duct was surgically exposed by middle line caprotomy and cannulated with polyethylene tubing (no: 48) Body temperatures of rats were maintained by a heating lamp. Bile collected for first 10 min was discarded and then it was collected in pre weighed graduated tubes. Bile was collected for 1 h and 1 ml of 2% gum acacia was administered intraduodenally to control rats (group I). Group II animals received SI extract (100 and 200 mg/kg) intraduodenally after 1 h bile collection and then the bile was collected hourly [14].

2.9 Statistical analysis

Data were expressed as the mean standard deviation of the means (S.D.) and statistical analysis was carried out employing Student's *t*-test.

3. Results

Rats treated with CCl_4 developed significant liver damage as observed from elevated serum levels of hepatospesific enzymes as well as severe alteration in other biochemical parameters (Table 1). Activities of AST, ALT, SAKP and SB were increased in CCl_4 intoxicated rats. Treatment with SI flower head extract showed a significant protection against CCl_4 induced alteration in the serum enzyme levels & serum bilirubin as compared to normal control rats, dose dependently, almost comparable to the Silymarin treated group [14].

The time of loss of righting reflex induced by 60 mg/kg of hexobarbitone was prolonged significantly by CCl_4 administration. The administration of a single dose of SI (100mg/kg) significantly shortened the "sleeping time" compared to CCl_4 group (table 2). *Sphaeranthus indicus* treatment resulted in a significant stimulation of bile output in normal rats indicating the choleretic activity of the extract.

Table 1. Effect of *Sphaeranthus indicus* (SI) extract on rat serum enzymes after CCl_4 administration

Groups	AST (IU/l)	ALT (IU/l)	SAKP (KA/100ml)	SB (IU/l)
Normal control	87 ± 10.30	30.2 ± 4.30	45.3 ± 2.67	0.22 ± 0.01
CCl_4 control	234 ± 18.50	172.6 ± 8.60	88.3 ± 7.25	4.80 ± 2.10
CCl_4 + SI (100mg/kg)	200.3 ± 12.30	111.3 ± 7.50	58.0 ± 4.20	3.44 ± 1.30
CCl_4+ SI (200mg/kg)	91.6 ± 9.20**	41.8 ± 6.70**	54.2 ± 7.26**	1.68 ± 1.10**
CCl_4 + SI (300mg/kg)	90.0 ± 8.11**	40.8 ± 6.18**	53.9 ± 6.21**	1.56 ± 0.87**
CCl_4 + Silymarin (100 mg/kg)	88.3 ± 8.18**	40.4 ± 7.81**	52.2 ± 3.82**	1.42 ± 0.22**

Values are the mean ± S.D., n = 6, $P \leq 0.001$ significant**, compared to CCl_4 control.

Table 2. Effect of *Sphaeranthus indicus* (SI) extract on Hexobarbitone-induced sleeping time in CCl_4 intoxicated mice.

Groups	Sleeping time (min)
Normal control	16.8 ± 1.18
CCl_4 control	99.60 ± 3.18
CCl_4 + SI (100mg/kg)	49.90 ± 3.48**
CCl_4+ SI (200mg/kg)	50.9 ± 2.36**

Values are the mean ± S.D., n = 6. $P \leq 0.01$ significant**, compared to CCl4 control.

Table 3. Effect of *Sphaeranthus indicus* (SI) extract on bile flow in normal anaesthetised rats

Bile flow(ml/100 g)	Control	SI (100 mg/kg)	SI (200 mg/kg)
1 h before SI treatment (A)	0.27 ± 0.11	0.28 ± 0.12	0.28 ± 0.01
2–6 h after SI treatment (B)	0.79 ± 0.12	1.67 ± 0.01**	1.88 ± 0.01**
B/A	3.0	6.0	6.7

Values are the mean ± S.D., n = 6. $P \leq 0.01$ significant**, compared to control (0.5 % Tween-80)

4. Discussion

CCl_4 is biotransformed into cytochrome P450 in the liver endoplasmic reticulum to the highly reactive trichloromethyl free radical. This free radical in turn reacts with oxygen to form a trichloromethyl peroxyradical, which may attack lipids on the membrane of endoplasmic reticulum more readily than trichloromethyl free radical. The trichloromethylperoxy radical leads to elicit lipid peroxidation, the disruption of Ca^{2+} homeostasis, elevation of hepatic enzymes and finally results in cell death [16]. The result obtained from the present study indicate that the methanol extract of flower heads of *Sphaeranthus indicus* exhibited hepatoprotective effect against CCl_4 induced liver damage in a dose dependent manner by normalizing the elevated levels of the hepatic enzymes. This suggested the possibility that SI extract is able to condition the hepatocytes, so as to cause accelerated regeneration of parenchyma cells, thus protecting against membrane fragility and decrease of leakage of the marker enzymes into the circulation as compared to silymarin. Silymarin is a known hepatoprotective compound. It is reported to have a protective effect on the plasma membrane of hepatocytes [17]. The time of loss of righting reflex induced by 60 mg/kg of hexobarbitone was prolonged significantly by CCl_4 administered. The administration of a single dose of SI (100mg/kg) significantly shortened the "sleeping time" compared to CCl_4 groups (Table. 2) indicating its hepatoprotective potential. The amount of hypnotic compound broken down per unit time in liver damaged mice is less, thereby resulting in a prolonged sleeping time [18].

The protective effect exhibited by SI could be due to the protection of hepatic drug metabolizing enzymes. The hepatic injury caused by CCl_4 is associated with damage to the endoplasmic reticulum and any compound capable of preventing the toxicity of CCl_4 must have some direct or indirect effect on the liver [19].

Studies on the effects of bile flow (choleretic activity) revealed that SI showed marked choleretic activity in anaesthetized normal rats. This is an indication of the healthy status and strong stimulating action on the secretory activity of the liver, as reported by Shukla *et al* [20]. It is thus concluded that the methanol extract of flower heads of *Sphaeranthus* indicus has promising hepatoprotective properties.

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