EFFECT OF THE AQUEOUS EXTRACT OF *SCOPARIA DULCIS* L. ON CCL_4 INDUCED CYTOCHROME P_{450} DAMAGE IN RATS

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Summary

The cytochrome P_{450} protective activity of the aqueous extract of *scoparia dulcis* L. was evaluated against CCl_4 induced prolongation of pentobarbitone sleep time in Sprague-Dawley rats. The extract, at a dose of 0.5 g/kg, p.o., significantly prevents the CCl_4 induced prolongation of pentobarbitone sleep time ($P<0.01$), indicating the cytochrome P_{450} protection activity. However, when treated alone, the extract (0.5 g/kg, p.o.) shows a significant prolongation of pentobarbitone sleep time ($P<0.01$), indicating the intrinsic cytochrome P_{450} inhibition activity. In addition, the extract shows neither stimulant nor depressant effect on the CNS, as evident from locomotor activity test. In conclusion, the results indicate that, the aqueous extract of *scoparia dulcis* L., at an oral dose of 0.5 g/kg, p.o., shows a significant protective effect against CCl_4 induced cytochrome P_{450} damage and also show a significant intrinsic cytochrome P_{450} inhibition activity.

Key words: Cytochrome P_{450}, Carbon tetrachloride, *Scoparia dulcis* L.

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Introduction

Scoparia dulcis L. (Family: Scrophulariaceae) is a herb native to tropical America and naturalized in tropics and widely present in Karnataka, India. This plant has been traditionally used to cure many liver ailments (1). Phytochemical screening has revealed that the plant contains diterpenoids, flavonoids, tannins, alkaloids, triterpenes, hexacosonol, β-sitosterol, ketone-dulcitone and amellin, an antidiabetic compound (2-4). The different extracts of the plant has been reported to possess analgesic, anti-inflammatory, antidiabetic, antibacterial, antifungal, and insecticidal properties (5-9). The diterpenoid, scoparic acid A, isolated from the plant has been reported to be a potent β-glucuronidase inhibitor (10). The constituents, scopadulciol, scopadulcic acid-B and diacetyl scopadiol, have been shown to be responsible for the inhibitory activity of the plant on gastric H^+-K^+ ATPase enzyme (11). The diterpenoid, scopadulcic acid-B and flavone, hemenoxin, have been shown to exhibit cytotoxic and antitumor activity (12).
In the present study, both Cytochrome P_{450} (CYPs) inhibition and protection activity of the aqueous extract of *Scoparia dulcis* L. was evaluated. The intrinsic CYPs inhibition activity was evaluated by studying the ability of the plant extract to potentiate the pentobarbitone sleep time in rats. The CYPs protection activity was evaluated by studying the ability of the plant extract to prevent the CCl_4 induced prolongation of sleep time in rats.

Material and Methods

Preparation of plant extract:

Scoparia dulcis L. whole plant was collected during the month of January 2003 from the campus of Manipal College of Pharmacy, Udupi, Karnataka, and authenticated by Dr. Sreenath, Department of Botany, Bangalore University. A specimen sample of the same is preserved in the herbarium section of the department, with the voucher no. BUB12005 for future reference. The whole fresh plant was dried under shade at room temperature for seven days and then reduced to coarse powder (sieve no.10/40). This powder was used for the preparation of aqueous extract. The aqueous extract was prepared by decoction method, the extract obtained was filtered and concentrated to viscous consistency at 40-50^0C (yield 13.6%w/w).

Animals:

Male Sprague-Dawley rats, weighing 100-150 g were procured from the animal-breeding facility, NIMHANS, Bangalore. They were maintained under controlled conditions with 12 h natural light and 12 h dark light cycle, with free access to standard rodent pellet diet and water. The Institutional Animal Ethics Committee (IAEC) of Visveswarapura Institute of Pharmaceutical Sciences approved the proposal.

Chemicals:

Carbon tetrachloride was obtained from Merck Ltd., Mumbai, India. The other chemicals and reagents used were of analytical grade.

Cytochrome P_{450} protection activity:

Rats were divided into two groups of six each. Group 1 received vehicle (10 ml/kg, p.o.) and served as control, group 2 received aqueous extract (0.5 g/kg, p.o.). A week before the study, normal sleep time of all the animals was determined using pentobarbitone sodium (25 mg/kg, i.p.). The sleeping times were measured as the duration of loss of righting reflex and the rats were considered asleep unless they could right themselves 3 times in a minute. The time between loss of righting reflex and its recovery was recorded for each animal (13, 14). A week later, all the animals were given their assigned treatments 24 h before CCl_4 administration. The pentobarbitone sleep time was determined after 2 h of CCl_4 administration.

Cytochrome P_{450} inhibition activity:

Rats were divided into two groups of six each. Group 1 received vehicle (10 ml/kg, p.o.) and served as control, group 2 received aqueous extract (0.5 g/kg, p.o.). A week before the study, normal sleep time of all the animals was determined as mentioned previously. A week later, 1 h after the assigned treatments, pentobarbitone sleep time was once again determined to ascertain the cytochrome P_{450} inhibition activity.

Locomotor activity:

Rats were divided into three groups of six each. Group 1 received vehicle (10 ml/kg, p.o.) and served as control, group 2 received standard, caffeine (20 mg/kg, i.p.) and group 3 received aqueous extract (0.5 g/kg, p.o.). The basal activity scores of all the animals were recorded 2 days before the start of study using a photoactometer. On the day of the study all rats were given their assigned treatments, 30 minutes after the treatment each rat was retested for activity scores for 10 min and the difference in the activity scores were compared with the control scores.

Statistical analysis

The results were expressed in mean ± SEM, statistical significance was determined by using students ‘t’ test.

Results

Cytochrome P_{450} protection activity:

Results of the study are given in Table 1. The results reveal that the aqueous extract at an oral dose of 0.5 g/kg, significantly prevents the CCl_4 induced prolongation of pentobarbitone sleep time when compared to the control ($P<0.01$).

Table 1. Effect of the aqueous extract of *Scoparia dulcis* L. on Cytochrome P_{450} protection activity in rats.

Group	Treatment	Pentobarbitone sleep time (in minutes)	
		Before CCl_4 administration	After CCl_4 administration
01	Vehicle 10 ml/kg,p.o.	80.3 ± 1.3	166.83 ± 5.1
02	Aqueous extract 0.5 g / kg, p.o.	78.9 ± 1.5	128.0 ± 7.2*

Values are mean ± SEM, n = 6, *P< 0.01.

Cytochrome P_{450} inhibition activity:

Results of the study are given in Table 2. The results reveal that the aqueous extract at an oral dose of 0.5 g/kg, significantly increases the pentobarbitone sleep time when compared to control ($P<0.01$).

Table 2. Effect of the aqueous extract of *Scoparia dulcis* L. on Cytochrome P_{450} inhibition activity in rats.

Group	Treatment	Pentobarbitone sleep time (in minutes)	
		Before treatment	After treatment
01	Vehicle 10 ml/kg,p.o.	75.3 ± 1.9	76.8 ± .2.5
02	Aqueous extract 0.5 g / kg, p.o.	78.9 ± 1.8	132.0 ± 6.2*

Values are mean ± SEM, n = 6, *P< 0.01.

Locomotor activity:

Results of the study are given in Table 3. The results reveal that the aqueous extract at an oral dose of 0.5 g/kg, show no significant effect on the locomotor activity. Standard, caffeine, at a dose of 20 mg/kg, i.p., however, shows significant increase in the locomotor activity as compared to control ($P<0.01$).

Table 3. Effect of the aqueous extract of *Scoparia dulcis* L. on locomotor activity in rats.

Group	Treatment	Locomotor activity score	
		Before treatment	After treatment
01	Vehicle 10 ml/kg, p.o.	460 ± 21.4	468 ± 23.4
02	Caffeine 20 mg/kg, i.p.	456.4 ± 24.4	591.0 ± .36.5*
03	Aqueous extract 0.5 g / kg, p.o.	463.2 ± 18.8	436.3 ± 45.7

Values are mean ± SEM, n = 6, *P< 0.01.

Discussion

Most xenobiotics cause hepatotoxicity by getting metabolized to active metabolites through CYPs. These toxic metabolites cause damage to different cellular organelles, including CYPs. Drugs with potential ability to inhibit and protect CYPs can, therefore, prevent the formation of toxic metabolites and their damaging effects on the liver. The duration of pentobarbitone induced sleep time in intact animals is considered as an index for the activity of hepatic CYPs. Drugs with potential CYPs protection activity are likely to prevent the CCl_4 induced prolongation of pentobarbitone sleep time and drugs with inhibition activity on CYPs are likely to potentiate the pentobarbitone sleep time.Further, CCl_4 prolongs the pentobarbitone sleep time through destruction of CYPs. The destruction of CYPs is known to be mediated by free radical species generated during the metabolism of CCl_4 (15-18) Drugs having CYPs protection ability will prevent the prolongation of sleep time induced by CCl_4. In the present study animals treated with aqueous extract of *Scoparia dulcis* L. show a significant decrease in the CCl_4 induced prolongation of pentobarbitone sleep time, as compared to CCl_4 alone treated control animals (Table 1). These observations indicate the CYPs protection activity of *Scoparia dulcis* L. Further, animals pretreated with aqueous extract of *Scoparia dulcis* L. show a significant potentiation in the pentobarbitone sleep time when compared to the control (Table 2). The above findings suggest that the potentiation of pentobarbitone sleep time may be due to inhibition of CYPs by the aqueous extract of *Scoparia dulcis* L. However, variation in pentobarbitone sleep time can also be achieved by CNS drugs without altering the CYPs enzyme activity. The results obtained from the locomotor activity test (Table 3) reveal that *Scoparia dulcis* L. has neither CNS stimulant nor depressant activity, thus indicating that the potentiation of pentobarbitone sleep time is exclusively due to the inhibition of CYPs by the aqueous extract of *Scoparia dulcis* L.

In conclusion, the above results indicate that the aqueous extract of *scoparia dulcis* L. at an oral dose of 0.5 g/kg, p.o., has a significant protective effect against CCl_4 induced cytochrome P_{450} damage and also show significant intrinsic cytochrome P_{450} inhibition activity.

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