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Anti-ulcer activity of *Smithia conferta* in various animal

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Abstract *Smithia conferta* Sm. (Leguminosae), is a commonly used plant in Indian traditional medicine. In the current study anti-ulcer activity of its petroleum ether, alcohol and aqueous extracts of leaves were investigated using different animal models. All extracts were also subjected to phytochemical analysis and their toxic potential. Petroleum ether extract was found to contain steroids; alcohol extract revealed the presence of isoflavonoids, alkaloids and carbohydrates; while aqueous extract was found to contain amino acids, carbohydrates and flavonoids. *S. conferta* aqueous and alcoholic extracts were found to be non-toxic up to 5000 mg/kg dose level while petroleum ether extract was safe only up to a dose of 2000 mg/kg after single dose administration of the extracts.

During confirmation of the claimed anti-ulcer activity, treatment with aqueous and alcoholic extracts showed significant reduction in ulcer index, free acidity as well as total acidity in pylorus ligated rats. However, petroleum ether extract showed relatively less profound reduction in all these indices. The anti-ulcer activity observed in aqueous extract treatment group was nearly equivalent to the standard group.

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1. Introduction

Smithia conferta Sm. (Leguminosae) is commonly known as Duthi in Hindi, Lakshman booti in Sanskrit, and Bhaji in Marathi (Chopra et al., 1956; Yadaya, 1956; Geissman and Venkatraman, 1962). Bhat community use this plant for regulating fertility, and as Bhaji in Marathi (Lal and Lata, 1980). It is found basically throughout the world but abundantly in Bundelkhand region of Uttar Pradesh (India). It is widely used in the traditional medical practice of peoples living in India, Indonesia and China to treatment of biliousness, ulcers (peptic) rheumatism, uterine trouble along with sterility problem

in women (Harvone and Mobry, 1982), laxative and used as tonic (Mobr et al., 1970), the powdered form of leaves mixed with honey was prescribed for cholera (Inuma et al., 1991) and its ointment is used to cure elephantiasis. Earlier some worker (Sahelian, 2003; Prakash and Gupta, 2005; Begum et al., 2005) have reported the presence of various biologically active constituents in this plant (Yadaya, 1956; Yadava, 2005).

In Bundelkhand region this plant is traditionally used to treat stomach ache and ulcers (Vogel, 2002). Since its recognition to peptic ulcer as an important chemical entity, various efforts have been made to find a suitable remedial measure, as the drugs used to reduce acid secretion have dominated the pharmacological basis of ulcer therapy (Freston, 1990). Peptic ulcer disease can be prevented by strengthening as cytoprotection (Robert, 1981), the defensive mechanism of gastric and mucosa rather than attenuating factor of aggression causing ulceration (Freston, 1990). Keeping in view the frequent folklore use of *S. conferta*, the present study was carried out to determine the anti-ulcer activity of *S. conferta* leaves using animal models.

2. Materials and methods

2.1. Plant material and preparation of its extracts

S. conferta leaves were collected locally from the region of Bundelkhand (U.P.), sun dried and grinded into powder form. The powdered leaves (200 g) were successively extracted in Soxhlet extractor using petroleum ether and absolute alcohol for 24 h. The aqueous extract was prepared by maceration of leaves with water for 48 h. The yield for all the three extracts were: 4.94, 9.16 and 13.12 g, respectively (percentage yield: was 2.47, 4.58, and 6.56 g%, respectively).

2.2. Preliminary phytochemical screening

All the three extracts were subjected to preliminary phytochemical investigation for the presence of various phytochemical constituents (Khandelwal, 2000).

2.3. Acute oral toxicity studies (LD_{50})

The acute toxicity of petroleum ether, alcoholic and aqueous extracts of *S. conferta* leaves were determined in albino mice fasted for 3 h (which examined that/DEL). The highest oral dose administered was 4 g/kg body weight (which was equivalent to powder crude drug 28.95 g/kg of body weight). Up to 4 g/kg dose levels no signs of toxicity appeared. The LD_{50} of the test extracts were calculated using AOT 425 software (Sen et al., 1992).

Oral toxicity: not considered as toxic (DL_{50} oral/rats > 40 g/kg body weight).

2.4. Preparation of drugs and chemicals

Standard ranitidine injection (Aciloc Injection, Cadila, Health Care Ltd., Mumbai, India), Tween 80 (laboratory grade) were used. Other chemicals used were: petroleum ether, absolute alcohol, anesthetic ether, formalin and EDTA ethylene di-amino tetra acetic acid (EDTA).

2.5. Experimental animals

Adult Swiss albino mice (20–25 g) and Wister rats (150–180 g) were used to study antistress and anti-ulcer activity. All these animals were maintained under standard husbandry protocol and conditions (light/dark period of 12 h light/dark and temperature $25 \pm 3^\circ\text{C}$) with free access to food and water ad libitum and all experiments were carried out between 10 am to 5 pm daily (Khandelwal, 2000).

2.6. Anti-ulcer activity

2.6.1. Pylorus ligation induced gastric ulcers in rats

Starved rats (48 h), weighing 150–180 g having free access to drinking water were placed in separate single–single cages with raised bottom in order to avoid cannibalism and coprophagy (Vogel, 2002). The rats were randomly allotted to five groups containing six animals each (Khandelwal, 2000) as follows.

DEL/each group having six animals/DEL. {Group 1: Group 1st received petroleum ether extract (500 mg/kg body weight), Group 2nd received alcoholic extract (500 mg/kg), Group 3rd received aqueous extract (500 mg/kg), Group 4th received standard (ranitidine 20 mg/kg) and Group 5th received (Tween 80) all the drugs are given by oral route. DEL.} (Table 1).

Under ether anesthesia a midline abdominal incision was made. The pylorus was ligated representing that neither blood supply was damaged nor traction occurred on the pylorus. The test compounds were given orally by gavage. The animal was kept for 6 h under experimental conditions (Sen et al., 1992) (after completing/DEL). At the end of treatment, the mucosa of animals in each group were examined under microscope. The number of ulcers (was noted/DEL) and their severity (Khandelwal, 2000), were recorded using arbitrary scale as follows.

0 = no ulcer, 0.5 = spot ulcer, 1.0 = superficial ulcers, 2.0 = deep ulcers and 3.0 = perforation.

Mean of ulcer score for each animal was expressed by the formula given below:

Percentage protection

$$= 100 - \frac{\text{Ulcer index of treated group}}{\text{Ulcer index of controlled group}} \times 100$$

The volume of the gastric content was measured after centrifugation, while acidity was determined by titration with 0.01 N NaOH using Toppfer's reagent and phenolphthalein as indicators (Kulkarni, 1999).

2.6.2. Water immersion stress induced ulcer

Stress ulcers were induced by forced swimming in the glass cylinder (Alder, 1984) with a height 15 cm; diameter 25 cm containing water to the height of 35 cm maintaining at 25°C

Table 1 Experimental groups and treatment given.

Groups	Treatment (dose/kg, p.o.)
Group 1	Petroleum ether extract 500 mg/kg
Group 2	Alcoholic extract 500 mg/kg
Group 3	Aqueous extract 500 mg/kg
Group 4	Ranitidine 20 mg/kg [standard]
Group 5	Tween 80

for 1 h. The animals were fasted for 24 h before performing the experiment. The animals were assigned to five groups with six animals in each group. After treatment, the animals were allowed to swim according to the standard protocol and ulcers were recorded as described earlier (Khandelwal, 2000).

The drugs treatment similar to previous method animals were subjected to swim in water for 1 h/DEL. Then they are removed, dried and injected (i/v) 20 mg/kg. Evans blue after 10 min they were sacrificed in ether anesthesia and performing complete experiments. The number of ulcers was noted when examined under a dissection microscope and the severity recorded as mentioned above the ulcer index was calculated/DEL.

2.7. Statistical analysis

The results are expressed as mean \pm SEM. Statistical difference between means were determined by one-way ANOVA followed by Dunnett's post hoc test (Del/was) were used to analyze and compared data with $P > 0.05$ as the limit of significance (SEM = standard of error of mean).

3. Results and discussion

3.1. Preliminary phytochemical investigation

The preliminary phytochemical screening with leave extracts of *S. conferta* revealed the presence of carbohydrates, amino acids and flavonoids in aqueous extract, alkaloids, flavonoids and carbohydrates in alcoholic extract and only steroids in petroleum ether extract.

3.2. Acute toxicity study

Aqueous and alcoholic extracts up to a dose of 5000 mg/kg body weight and petroleum ether extracts up to a dose of 2000 mg/kg were found to be safe.

3.3. Effect of *S. conferta* extracts in pylorus ligation induced gastric ulcers

Pretreatment with all extracts significantly decreased ulcer index $P < 0.001$, with aqueous and alcoholic extracts while $P < 0.01$ with petroleum ether extract. There was significantly rise in pH with reduction in volume of gastric contents, free acidity, total acidity with/in extract treated group as compared to extract untreated rats. Significant with aqueous and alcoholic extract and percentage protection was comparable with that of standard (ranitidine) as shown in Table 2.

3.4. Water immersion stress induced ulcer

Pretreatment with all the extracts has significantly reduced ulcers index $P < 0.001$ and percentage protection of ulcerogenic effect with aqueous extract (86.12) was more than the standard (71.38) and extract like alcoholic and petroleum ether extracts did not show much protection in Table 3.

All the extracts of *S. conferta* in pylorus ligation induced ulcer model reduced ulcer index, gastric volume, free acidity and total acidity. Thus, they exhibit the antiseecretory mechanism involved in the extracts for their antiulcerogenic activity. Ulcer index parameter was used for the evaluation of anti-ulcer activity since factors such as gastric volume, free acidity and total acidity is directly related to ulcer formation.

Pylorus ligation increased the acid secretion that is why the gastric volume is increased. Thus low pH increases free acidity and total acidity as a result ulcer index is increases.

Water immersion test is one of the best model of stress in rats to induced ulcer. The model provides both physiological stress as well as emotional stress to the animals. In case of water immersion induced stress in rats, all extracts represented significant ($P < 0.001$) ulcer inhibition.

The results indicated that *S. conferta* extracts produced antiulcerogenic effects possessing antiseecretory, cytoprotective and H_2 blocking/proton pump inhibition mechanism.

Table 2 Effect of *S. conferta* extract on ulcer index, pH, volume of gastric juice, free acidity, total acidity and percentage protection in pylorus ligated rats.

Treatment groups	Petroleum ether extract	Alcoholic extract	Aqueous extract	Standard (ranitidine)	Control
Dose mg/kg	500	500	500	20	
Ulcer index	1.18 \pm 0.26**	0.84 \pm 0.11***	0.74 \pm 0.42***	0.50 \pm 0.24***	4.26 \pm 1.03
pH	3.64 \pm 0.94	4.64 \pm 0.62**	5.17 \pm 0.46*	4.81 \pm 0.77**	1.34 \pm 0.32
Volume of gastric juice	3.64 \pm 0.48	5.08 \pm 0.47	2.68 \pm 0.16**	2.26 \pm 0.43**	4.85 \pm 0.29
Free acidity	51.63 \pm 14.61	47.3 \pm 13.97	32.86 \pm 10.63*	36.0 \pm 15.32*	88.98 \pm 8.80
Total acidity	110.8 \pm 14.38	76.87 \pm 21.19*	67.83 \pm 6.44**	45.30 \pm 21.03*	151.12 \pm 11.20
% protection	72.43	80.42	83.48	89.17	—

Results are expressed as mean \pm SEM, significance at * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ as compared to control.

Table 3 Effect of *S. conferta* extract on ulcer index and percentage protection in stress induced ulcers in rats.

Group	Treatment on group	Ulcer index	Percentage protection in %
1	Petroleum extract	4.6 \pm 0.396	3.68
2	Alcoholic extract	3.5 \pm 0.242	25.08
3	Aqueous extract	0.69 \pm 0.19***	86.12
4	Ranitidine (standard)	1.34 \pm 0.312**	71.38
5	Control	4.68 \pm 0.422	—

Results are expressed as mean \pm SEM, significance at * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ as compared to stress.

The present study demonstrated the potential of *S. conferta* leaves to exert anti-ulcer activity especially the aqueous extract.

4. Conclusion

In conclusion, the present study demonstrated the potential of *S. conferta* leaves extracts to exert anti-ulcer activity especially the aqueous extract and thus justify the uses of the plant in treating ulcer related ailments.

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