

Evaluation of Antidiarrhoeal Activity of *Thunbergia fragrans* Roxb.

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Acetonic and ethanolic extracts of *Thunbergia fragrans* (Acanthaceae) leaves on gastrointestinal motility was investigated by using Swiss albino mice. Acetonic and ethanolic extracts at dose of 500 mg/kg body weight were orally administered to mice. The antidiarrhoeal activity was evaluated using charcoal meal model and castor oil induced diarrhoea model in albino mice. Both extracts demonstrated a significant antidiarrhoeal activity ($p < 0.001$). These observations prove the antidiarrhoeal activity of *T. fragrans*. The acute toxicity studies of these two extracts upto a dose level of 2000 mg/kg body weight of the mice showed no mortality.

Key Words: *Thunbergia fragrans*, Antidiarrhoeal activity, Charcoal meal, Loperamide.

INTRODUCTION

Diarrhoea is one of the leading cause of mortality in developing countries. In view of this, the World Health Organization (WHO) has initiated diarrhoea disease control program to study traditional medical practices and other related aspects¹. During ethnobotanical survey in Nilgiris district of Tamil Nadu it was observed that tribal population *viz.*, Irulas are using *Thunbergia fragrans* to relive stomach disorders as well as to control diarrhoea.

Thunbergia fragrans Roxb. belongs to the family Acanthaceae, is a large woody climber grown in India, Burma and China. A decoction made of leaves of this plant used in stomach complaints and fungal infection². Entire plant contains "Trigonelline" a protein³, flowers contain apogenin-7-O β glucuronide and daucosterol⁴ were reported. In this study the antidiarrhoeal activity of the acetonic and ethanolic extract of *T. fragrans* in animals was studied.

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EXPERIMENTAL

The leaves of *T. fragrans* were collected from various places of Nilgiris, Tamilnadu in the month of August. The plant sample was authenticated by Dr. Rajan, Botanist, Department of Botany, Government Arts College, Ootacamundj, where voucher specimens were deposited. The plant material was thereafter shade-dried and reduced to a powder form.

Extraction of plant material: Weighed amount of the powdered sample was extracted exhaustively in acetone and ethanol. The resulting extract was then concentrated over a water bath until a solid extract sample was obtained. These extracts were dried and preliminary phytochemical investigation was carried out.

Animals: Swiss albino mice weighing about 120-150 g of either sex were obtained from the animal house, J.S.S. College of Pharmacy, Ootacamund, Tamilnadu (India). The animals were maintained under standard conditions and fed standard pellet diet (Kamadenu Enterprises, Bangalore) and water *ad libitum*.

Acute Toxicity Studies⁵: Swiss albino mice of either sex divided into groups of 6 animals each. Group I received normal saline (10 mL/kg, orally) and served as control, while other groups received acetone and ethanol extract of *T. fragrans* of at different dose levels of 5, 50, 300 and 2000 mg/kg body weight respectively. Immediately the animals were observed for 6 h for continuously for their behaviour and thereafter daily for 14 d for mortality.

Gastrointestinal motility in mice: The method described by Akah *et al.*⁶ with slight modifications was used to test the effect of the extract on gastrointestinal motility. Swiss albino mice were divided into 4 groups, 6 animals each. Group I received vehicle (normal saline 10 mL/kg), group II received Loperamide (2 mg/kg) as standard, group III and IV received acetone and ethanol extract of *T. fragrans* (500 mg/kg body weight p.o.). After 20 min, 1 mL of 10% charcoal suspension in 5% acacia solution was administered to each mouse orally. The animals were sacrificed after 20 min the abdomen was opened. The small intestines were dissected out and placed on a clean surface. The distance traveled by the charcoal meal from the pylorus was measured. The entire length of the small intestine was also measured for percentage distance traveled by the charcoal plug along small intestine was then estimated for both the extracts.

Castor oil-induced diarrhoea in mice: The method was described by Awouters *et al.*⁷, using mice. The animals were fasted for 12 h prior to the commencement of the experiment and were randomly divided into 4 groups of 6 mice each. The mice in the first group received 10 mL/kg normal saline, while mice in the second group received loperamide 2 mg/

kg orally. The third and fourth groups received acetone and ethanol extracts of *T. fragrans* 500 mg/kg orally. After 30 min of administration of the extract, castor oil (0.2 mL/mouse) was given orally. The animals were placed on individual cages over clean filter paper. The cages were inspected for the presence of the number of defecation per animal were recorded up to 4 h.

Statistical analysis: The observations are reported as mean \pm SEM. The statistical analysis is carried out using one way analysis of variance (ANOVA) followed by Tukey's test. P values less than 0.001 and 0.01 were considered as significant.

RESULT AND DISCUSSION

Acute toxicity studies: The acute toxicity studies of acetone and ethanol extract of *T. fragrans*, at 2000 mg/kg administered orally caused neither any behavioral change nor mortality. The LD₅₀ of *T. fragrans*, was thus found to be more than 2000 mg/kg body weight.

Effect on gastrointestinal motility (Charcoal meal test): The result indicates that the acetone and ethanol extract showed significant ($p < 0.01$) reduction in propulsion of charcoal meal through gastrointestinal tract as compared to control group (Table-1). This study suggested that both extracts possess significant antidiarrhoeal activity compared to Loperamide.

TABLE-1
EFFECT OF ACETONE AND ETHANOL EXTRACT OF *Thunbergia fragrans* ON CASTOR OIL-INDUCED IN MICE GASTROINTESTINAL MOTILITY BY CHARCOAL MEAL METHOD

Treatment	Dose (mg/kg, p.o.)	% Distance traveled	Percentage protection
Control	–	80.67 \pm 1.48	–
Loperamide	2	41.20 \pm 1.62**	48.92
Acetone extract	500	35.89 \pm 1.47**	55.51
Ethanol extract	500	32.80 \pm 1.45**	59.34

Values are mean \pm SEM, (n = 6);

**p < 0.01 vs. Control (Normal saline- 10 mL/kg)

Inhibition of Castor oil induced diarrhoea: The acetone and ethanol extracts of *T. fragrans* (500 mg/kg) significantly ($p < 0.001$) protected the mice against castor oil-induced diarrhoea when compared with the control (Table-2). Both extracts of *T. fragrans* showed potent inhibitory effect when compared to loperamide.

T. fragrans exhibited antidiarrhoeal activity was found to be comparable to loperamide, a drug widely employed against diarrhoeal disorders

which effectively antagonizes diarrhea induced by prostaglandins and cholera toxin^{8,9}. The pharmacological effect of loperamide was found to be due to its antimotility and antisecretory properties¹⁰. The observations of the charcoal meal test demonstrate the significant reduction in the propulsive movement in the small intestine after treating with the plant extracts. Both the extracts showed potent inhibition of peristaltic movements than standard drug loperamide. Castor oil causes diarrhoea through the active metabolite ricinolic acid¹¹. Ricinolic acid increases peristaltic activity. The mechanism of action of castor oil induced diarrhoea was found to be through elevated prostaglandin biosynthesis^{7,12,13}. Prostaglandins contribute to the pathophysiological function in the gastrointestinal tract¹⁴. From our investigations, it is likely that the extracts mediate their effects through similar mechanism. The preliminary phytochemical screening done by us reveals that extracts of plant contain flavonoids, tannins and saponins. Flavonoids are known to modify the production of cyclo-oxygenase 1 and 2 (COX-1 & 2) and Lipo-oxygenase (LOX)^{15,16} thereby inhibition of prostaglandin production. Flavonoids and other constituents are responsible for the observed effects.

TABLE-2
EFFECT OF ACETONE AND ETHANOL EXTRACT OF *Thunbergia fragrans* ON CASTOR OIL-INDUCED DIARRHOEA IN MICE

Treatment	Dose in mg/kg (orally)	Number of mice with diarrhoea	Percentage of protection
Control	0.2 mL/mouse	6	0
Loperamide	2	0	100***
Acetone extract	500	0	100***
Ethanol extract	500	0	100***

Values are mean \pm SEM, (n = 6);

***p < 0.001 vs. Control (Normal saline- 10 mL/kg)

Conclusion

This study demonstrates the efficacy and safety of both acetonic and ethanolic extracts of *T. fragrans* as antidiarrhoeal agent scientifically justifying the use of this plant as an antidiarrhoeal agent by the tribal people of Kunjapanai of Nilgiris, Tamilnadu (India).

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