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Research Article

ANTIDIARRHOEAL ACTIVITY OF ETHANOLIC EXTRACTS OF *RUTA GRAVEOLENS* LEAVES AND STEM

PINKEE PANDEY*, ARCHANA MEHTA AND SUBHADIP HAJRA

Lab of Plant Biotechnology, Dept. of Botany, School of Biological and Chemical Sciences, Dr. H. S. Gour Central University, Sagar 470003, Madhya Pradesh, India , Email: pinkee.9d@gmail.com

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ABSTRACT

Ruta graveolens is a peritoneal herb commonly known as Rue belongs to the Rutaceae family. The present study was undertaken to evaluate the antidiarrhoeal activity of the ethanolic extracts of *Ruta graveolens* leaves and stem at the concentration (50, 100, 200 and 300 mg / kg bw, p.o) using different experimental animal models such as castor oil induced diarrhea, enteropooling and gastrointestinal motility test. Both the extracts showed concentration dependent antidiarrhoeal activity (*p<0.05, **p<0.01). Leaves extract showed most potent antidiarrhoeal activity as compared to stem extract. Extract produced profound decreased in intestinal transit and significantly inhibited castor oil induced enteropooling comparable to that of standard drug Diphenoxylate (50 mg/kg bw) and atropine sulphate (2.5 mg/kg bw). The prolonged onset of diarrhoea, inhibition of castor oil-induced enteropooling and the suppressed propulsive movement observed in this study support the antidiarrhoeal activity of *R. graveolens* leaves and stem extracts.

Keywords: Antidiarrhoeal, *Ruta graveolens,* leaves and stem, diphenoxylate, castor oil, atropine sulphate.

INTRODUCTION

Diarrhoea is an alteration in the normal bowel movement, characterized by increased in the water content in the intestine and frequency of stools.[1] It is caused by *Shigella flexneri, Staphylococcus aureus, Escherichia coli, Salmonella typhi* and *Candida albicans* and one of the primary causes of infant mortality especially in developing countries.[2, 3] It is the world's third highest killer disease (about 7.1 million per year), despite the efforts of international organizations to control this disease. Some synthetic chemicals like diphenoxylate, loperamide are used for the treatment of diarrhoea but their used are restricted due to their some adverse effects. Therefore; the search for safe and more effective agents for treatment of diarrhoeal condition from plant origin has continued to be an important area of active research. Now days several naturally occurring compounds are enormously used for the treatment of diarrhoea because these herbs are readily available, affordable and are an indispensable component of traditional medicine practice. For this reason, international organizations including the World Health Organization (WHO) have encouraged studies pertaining to the treatment and prevention of diarrheal diseases using traditional medical practices.[4] On the basis of above background here *R. graveolens* leaves and stem were selected for evaluation of antidiarrhoeal activity.

Ruta graveolens is a hardy ever green plant belongs to Rutaceae family, commonly known as rue. The leaves are small, oblong, deeply divided, pinnate (bipinnate or tripinnate), alternative (3-5 cm) long, feathery appearance, green to strongly glaucous blue-green in colour, glandular dotted, 2-3 pinnatisect, covered with ashy bloom when fresh. Flowers are small (13 mm), yellow in colour, clusters during spring and summer, held well above the blue-green foliage and often covering most of the plant. In the traditional system of medicine the whole plant is used as a drug. Hydro alcoholic leaves extract showed potent anthelmentic, antioxidant and α-amylase inhibitory activity while its stem extract showed significant antimicrobial activity.[5, 6, 7] Ruta in combination with $Ca_3(PO_4)_2$ is found to be effective in treatment of brain cancers particularly glioma.[8] Leaves extract also reported to possess anti-inflammatory activity.[9]

MATERIALS AND METHODS

Plant material

Ruta graveolens leaves and stem were collected in the month of August, 2010 from the Botanical garden and authenticated. A voucher specimen no. Bot/Her/1514 has been deposited at the Departmental herbarium, Department of Botany, Dr. H. S. Gour Central University, Sagar, (M.P) India.

Preparation of extracts

The plant materials were washed in running tap water to remove dust material as much as possible and air dried for 6-7 days at room temperature to prevent the loss of active phytoconstituents. The air-dried leaves and stem (35g) were powdered using a mechanical grinder and soaked separately in 500 ml of 75% ethanol and kept in a shaker for 72 h. The crude extract was collected by filtration and evaporated under reduced pressure to give a blackish green amorphous mass for leaves and reddish mass for stem (yield was 2.120w/w; 2.55w/w) respectively. Extractives were dissolved in distilled water to get desired concentrations.

Drugs and Chemicals

The following drugs and chemicals were used in the study: diphenoxylate (Sigma Aldrich), atropine sulfate (Sigma Aldrich), vegetable charcoal (Carbopho, AJC Pharma, Angoulem, France) and castor oil from a local pharmacy and other chemicals were used in analytical grade.

Phytochemical analysis

Phytochemical screening was performed to detect various compounds such as tannins, flavonoids, alkaloids, steroids etc., [10]

Animal used

Wister albino rats (age 6-8 weeks, body weight 140–160 g) of either sex were used for antidiarrhoeal study. They were housed in polypropylene cages and maintained under standard environmental conditions (temperature 23 ± 2°C, relative humidity 55±10% and 12 h light and dark place) and were fed with standard pellet diet (Hindustan lever Ltd. Kolkata, India) and water *ad libitum*. Fresh animals were used for each experiment. All the experimental procedures were carried out in strict accordance with the guidelines laid down by the Committee for the Purpose of Control and Supervision on Experimentation on Animals (CPCSEA) and experimental protocols were approved by the Institutional Animal Ethics Committee (Reg. No. -379/01/ab/CPCSEA); after performing the experiments all the waste material disposed in a safe and sanitary manner.

Castor oil-induced diarrhoea in rats

Diarrhoea was induced in the rats using a modified method. [11, 12] The test animals were starved for 18 h prior to the experiment but were allowed free access to water. Sixty rats were divided into 10 groups and each group containing 6 rats. Group one served as vehicle control being received distilled water, the second group

(positive control) received the reference drug, Diphenoxylate (50 mg/kg bw, p.o). Third to six groups received leaves extract while seven to ten group received stem extract at the concentration of 50, 100, 200 and 300 mg/kg bw separately. After 30 min of drug and extracts treatment, each animal was administered 1 ml of castor oil orally and the time between oil administration and appearance of first diarrhoeal drop was noted. Observations for the severity of diarrhoea were assessed each hour for a period of 6 h by monitoring the diarrhoeal drop on a pre-weighed filter paper, placed beneath the individual rat cages. The total number of faeces, diarrhoeal faeces and the total weight of faeces excreted were expressed as average and compared with the control group. The percentage inhibition of diarrhoeal defecation in each group was also noted.

Castor oil-induced enteropooling

Intraluminal fluid accumulation was determined according to the method.[13] The test animals were starved for 18 h prior to the experiment but were allowed free access to water. Sixty rats were divided into ten groups and each group containing 6 rats. Group 1, served as vehicle control, received distilled water while group 2, served as positive control, received atropine sulphate at the concentration of (2.5 mg/kg bw). The test groups animals were orally administered with the extract at the concentration of (50, 100, 200 and 300 mg/kg bw) separately. Immediately 1 ml of castor oil was administered orally to each of the rats in all the groups. After 30 min, each rat was sacrificed according to the method of [14] and the ends of the small intestine tied (at the pylorus and the caecum). The organ was dissected out and intestinal content was collected by squeezing into a measuring cylinder. The volume and the mass of the intestinal content were obtained.

Gastrointestinal motility test

Gastrointestinal motility test was performed according to the method suggested by.[15] The test animals were starved for 18 h prior to the experiment but were allowed free access to water. The animals were divided in to ten groups and each group containing 6 rats. Group 1, vehicle control, received distilled water while group 2, positive control received Diphenoxylate at the concentration of (50 mg/kg bw). The test groups were orally administered with the extracts at the concentration (50, 100, 250 and 300 mg/kg bw). After 30 min of drug and extracts treatment, rats from each group were administered with 1 ml of charcoal meal (10% charcoal suspension in 5% gum acacia). After 30 min of the administration of charcoal meal, the animals from the various groups were sacrificed, small intestine was removed and the length (pylorus to caecum) as well as the distance travelled by charcoal meal through the organ was measured. This distance was expressed as a percentage of the length of the small intestine.[16]

STATISTICAL ANALYSIS

The data are expressed as mean ±S.D. The difference among means has been analyzed by one-way ANOVA followed by Dunnett's test (*p<0.05, **p<0.01) was considered as statistically significant.

RESULTS

Castor oil- induced diarrhea

In the castor oil-induced diarrhoea experiment, ethanolic extracts of *R. graveolens* leaves and stem significantly prolonged the time of diarrhoeal induction in a dose dependent manner. The frequency of stooling (number of wet feaces and total number of feaces), fresh weight and water content of the faeces decreased significantly (Table 1). Significant reduction in diarrhoea and percentage of defecation was observed (*p< 0.05, **p<0.01) when compared with vehicle control group.

Castor oil induced enteropooling

In case of gastrointestinal enteropooling, both extracts reduced the volumes of intestinal fluid and weight of the intestinal content of the animals significantly in dose dependent manner (Table 2). Among the two extracts; leaves extract showed most potent castor oil induced enteropooling by lowering significant level of volume of intestinal content (ml) and weight of intestinal content (g) (03.19±0.32**ml and 03.81±0.23**g) when compared with the stem extract (03.21±0.24**ml and 03.73±0.16**g) at the concentration of 300 mg/kg bw respectively.

Table 1: Effect of the ethanolic extracts (mg/kg bw) of *R. graveolens* leaves and stem on castor oil- induced diarrhea

Group	Dose (mg/kg bw)	Total number of faeces	Total number of diarrhoeal faeces	Inhibition (%)	Total weight of faeces (g)	Inhibition (%)
Castor oil (1ml) + Distilled water		35.5±0.72	19.34±1.18	00	9.72±0.37	00
Diphenoxylate + castor oil (1ml)	50	08.6±0.42**	04.57±0.77**	76.37	1.117±0.41**	88.51
Ethanolic leaves extract + castor oil (1ml)	50	31.4±0.13**	18.03±0.21**	06.77	08.19±0.56**	15.74
	100	26.34±0.23**	15.10±0.38**	21.92	06.51±0.46**	33.02
	200	19.25±0.38**	11.51±0.17**	40.48	04.87±0.25**	49.89
	300	13.70±0.04**	07.13±0.31**	63.13	02.56±0.34**	73.66
Ethanolic stem extract + castor oil (1ml)	50	33.64±0.34**	18.42±0.34*	04.75	08.89±0.22**	08.53
	100	29.40±0.21**	16.65±0.19**	13.90	07.49±0.17**	22.94
	200	23.28±0.16**	12.31±0.25**	36.34	05.81±0.20**	40.22
	300	16.31±0.48**	09.15±0.31**	52.68	03.47±0.16**	64.30

Values are expressed as mean ± S.D., (n=6), One way ANOVA followed by Dunnett's test *p<0.05, **p<0.01 significant when compared with vehicle-control.

Table 2: Effect of ethanolic extracts (mg/kg bw) of *R. graveolens* leaves and stem on castor oil induced enteropooling

Group	Dose (mg/kg bw)	Volume of intestinal content (ml)	Weight of intestinal content (g)
Castor oil (1ml) + Distilled water		04.78±0.23	05.44±0.18
Atropine sulphate + castor oil (1ml)	2.5	01.52 ± 0.15**	02.43±0.36**
Ethanolic leaves extract + castor oil (1ml)	50	04.65±0.12^{ns}	05.15±0.23^{ns}
	100	04.29±0.24**	04.89±0.26^{ns}
	200	03.84±0.34**	04.47±0.10*
	300	03.19±0.32**	03.81±0.23**
Ethanolic stem extract + castor oil (1ml)	50	04.72±0.18^{ns}	05.27±0.11^{ns}
	100	04.45±0.17^{ns}	05.02±0.11^{ns}
	200	03.93±0.31**	04.62±0.18*
	300	03.21±0.24**	03.73±0.16**

Values are expressed as mean ± S.D., (n=6) One way ANOVA followed by Dunnett's test *p<0.05, **p<0.01 significant, ns= not significant when compared with vehicle-control.

Charcoal-induced gut transit changes

In case of charcoal meal-induced gut transit changes, both the extracts reduced the distance moved by the charcoal meal, when compared with the vehicle control group in a concentration dependent manner. The extracts treated group (*p< 0.05, **p<0.01) produced the least transit time by reduction in the charcoal meal transit time. This activity was similar to the reference drug, Diphenoxylate (Table 3). Ethanolic leaves extract showed most potent activity by inhibiting distance travelled by charcoal meal (51.84%) while stem extract inhibiting (48.88 %) movement at the concentration of 300 mg/kg bw respectively.

Table 3: Effect of ethanolic extracts (mg/kg bw) of *R. graveolens* leaves and stem on charcoal-induced gut transit changes.

Group	Dose (mg/kg bw)	Distance traveled by charcoal meal (%)	Inhibition (%)
Distilled water	-	73.37±1.57	00.00
Diphenoxylate (standard drugs)	50	26.67±3.82**	63.65
Ethanolic leaves extract + castor oil (1ml)	50	70.55±2.18 ns	03.84
	100	63.04±2.18**	14.08
	200	52.12±2.15**	28.96
	300	35.33±2.15**	51.84
Ethanolic stem extract + castor oil (1ml)	50	72.11±0.54 ns	01.70
	100	64.04±1.21**	12.71
	200	52.88±2.37**	27.92
	300	37.50±1.23**	48.88

Values are expressed as mean ± S.D., (n=6). One way ANOVA followed by Dunnett's test **p<0.01 significant, ns= not significant when compared with vehicle-control.

DISCUSSION

Phytochemical screening of the ethanolic leaves and stem extracts of *Ruta graveolens* reveals the presence of alkaloids, flavonoids, tannins and steroids. Ricinoleic acid the active component of castor oil mainly responsible for antidiarrhoeal activity by inhibition of intestinal Na^+ K^+ ATPase activity, thus reducing normal fluid absorption, activation of adenylate cyclase or mucosal cAMP-mediated active secretion, stimulation of prostaglandin formation, and platelet activating factor. The second physiological effect of released ricinoleic acid is to cause inflammatory swelling of the intestinal mucosa. [17, 18] Atropine is an antimuscorinic drug with the capacity to reduce gastrointestinal motility and secretions. The antimotility effect of the extract in this study may also be via the muscorinic receptors. It is significantly reduced intestinal transit time due to its anticholinergic effect. Diphenoxylate is used for the treatment of diarrhea that acts by slowing intestinal contractions and peristalsis allowing the body to consolidate intestinal contents and prolong transit time, thus allowing the intestines to draw moisture out of them at a normal or higher rate and therefore stop the formation of loose and liquid stools. Therefore it can be assumed that the antidiarrhoeal action of the extract was mediated by an antisecretory mechanism. This was also evident from the inhibition of castor oil-induced fluid accumulation by the extract. The results were comparable to those of the standard drug, atropine sulphate.

The inhibition of castor-oil induced intestinal fluid accumulation (enteropooling) and the weight of the intestinal content may be due to the ability of the extract to increased the reabsorption of electrolytes, water and inhibit the induced intestinal accumulation of fluid in a manner similar to Diphenoxylate. [19] The antidiarrhoeal activity of medicinal plants has been attributed to the presence of bioactive agents such as tannins, alkaloids, saponins, flavonoids, steroids and terpenoids. [20, 21] Tannins denature proteins in the intestinal mucosa by forming protein tannates which make intestinal mucosa more resistant to chemical alteration and reduce secretion. When we carried out qualitative chemical test, presence tannins, alkaloids and saponins in the extracts may be responsible for its activity. Ethanolic extracts of *R. graveolens* showed significant antidiarrhoeal activity, but leaves extract showed more significant activity as compared to stem extract. This may be due to concentration of active phytochemicals present in particular solvent extract.

CONCLUSIONS

The prolonged onset of diarrhoea, inhibition of castor oil-induced enteropooling and the suppressed propulsive movement observed in this study strongly claim the antidiarrhoeal activity of *R. graveolens* leaves and stem in a concentration dependent manner. The results of this study showed that there has been a statistically significant reduction in the incident and severity of diarrhoea with the extracts in experimental animal models. Leaves extract at the concentration of 100, 200 and 300 mg/kg body weight significantly lowered several typical parameters of diarrhoea. Further studies are required to confirm the underlying mechanism of the observed activity of the plant.

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