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ANTIDIARRHEAL ACTIVITY OF RHODODENDRON ARBORIUM LEAVES IN WISTAR RATS

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Abstract: Diarrhea is a world wide spread disease which is a major public health problem mainly in developing countries. It is responsible for high degree of mortality and morbidity for all age groups. *Rhododandron arborium* (family: Ericaceae) is being traditionally used in the Indian system of medicine for the treatment of various disorder such as dysentery, analgesics, hepatoprotective and antipyretics etc. In our present work we investigated the antidiarrheal activity of *R. arborium* on wistar rats. It was evaluated by PGE2 induced enteropooling, castor oil induced diarrhea and the gastric motility was evaluated by administrating charcoal meal to the rats. Loperamide 3mg/kg and atropine 0.1mg/kg were used as standard drugs for comparison. *R. arborium* ethanolic extract was used at doses of 40, 60 and 100mg/kg. Comparison with standard drugs showed a significant ($p<0.01$) dose dependent reduction in diarrhea produced by different agents in all the three models in rats. The antidiarrheal property of *R. arborium* may be due to the presence of quercetin related flavonoids saponins and phenolic compounds present in the leaves of *R. arborium*. The result clearly indicated that the ethanolic extract of leaves of *R. arborium* is effective against diarrheal diseases.

Keywords: *R. arborium*, Castor oil induced diarrhea, Entropooling method, Small Intestine Transit

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INTRODUCTION

A healthy adult drinks 2 L of fluid per day, to which 1 L of saliva, 2 L of gastric juice, 1 L of bile, 2 L of pancreatic juice and 1 L of intestinal secretion is added. Out of these 9 L of fluid presented to the intestine, less than 200 g of stool is excreted per day, of which 65% to 85% is water [1]. Diarrhea is one of the major health problems in most of the developing countries and is responsible for the deaths of millions every year [2]. The precise definition of diarrhea is elusive and gives the considerable variation in normal bowel habits. An increase in stool mass, stool frequency and/ or stool fluidity are perceived as diarrhea by most patients. For many individuals, this consists of daily stool production in excess of 250 g containing 70% to 90% water. However, over 14 L of fluid may be lost per day in severe cases of diarrhea. Diarrhea is often accompanied by pain, urgency, perianal discomfort and incontinence [3].

A research made by the World Health Organization (WHO), have shown that nearly 3-5 billion cases of diarrhea occur every year (1 billion in children less than 5 years of age), and approximately 5 million deaths are due to diarrhea annually (2.5 million in children less than 5 years of age)[4]. The treatment is mainly based on the symptomatic relief of diarrhea and the very first approach is the use of Oral Rehydration Solution (ORS) in order to balance the electrolyte disturbance which is one of the major complications of diarrhea. Plants continue to serve as possible sources for new drugs and chemicals but they have been much less explored. These can be extremely useful as lead structure for synthetic modification and optimization of bioactivity [5]. Widespread use of botanicals as medicinal products in developing countries and herbal products are also becoming a part of the integrated health care systems of industrialized nations. Nowadays a scientific evaluation of herbs, according to their traditional and folk uses, in the treatment of various diseases can be included in “complementary alternatives system of medicines” (CAM). Safety and efficacy of natural herbal product is therefore a cause of concern to promote and rationalize their use**.**

One such herbal plant, *R. arborium* (family: Ericaceae) is being traditionally used in the treatment of diarrhea and blood dysentery [6]. Its name means “tending to be woody or growing in a tree like form It is an evergreen shrub or small tree with a showy display of bright red flowers [7]. The young leaves are astringent and poultice, paste of leaves is used in relief of headaches, the bark juice is used in treatment of cough. The juice of the flowers is used in the treatment of menstrual disorders. The fresh and dried corolla that is acid-sweet in nature is given when fish bones get struck in the gullet [8]. So the aim of the present study was to evaluate the anti-diarrheal activity of *R. arborium.*

MATERIAL AND METHODS:

Plant material

After proper identification by Taxonomist of the Botanical Survey of India, Dehradun, India, the leaves of *R. arborium* were collected from the Himalaya, Garhwal region. The leaves were dried under shade at room temperature and coarsely powdered by using a mechanical grinder. The powdered drug was then extracted successively with petroleum ether (60-70° C), chloroform (50-60°C) and ethanol (80-90°C) each for 24 hrs. The extracts were concentrated under reduced pressure. The dried extracts were stored in airtight containers.

Animals

Wistar albino rats of either sex (150-250 g) were housed in a spacious cage for 10 days after obtaining approval from 'Institute Ethics Committee'. During the experiment, rats were fed standard chow diet. After randomization into various groups, the rats were acclimatized for 2 to 3 days in the new environment before initiation of experiment. Animals had free access to food and drinking water till before 30 minutes of sampling.

Chemicals

Castor oil was used to induce diarrhea and was bought from local market. Another chemical PGE_2 which was used to induce diarrhea was purchased from Rolex Chemical Ltd. Atropine and Loperamide were used as standard drugs and were procured from Total health care Pvt. Ltd, Baddhi, Himachal Pradesh.

Experiments

1. Castor oil induced diarrhea

Wistar rats of either sex (150-250 g) were fasted for 18 h before the experiment. They were then divided into five groups. The first group was administered with aqueous 1% tragacanth suspension and served as control group. The second group received standard drug Loperamide (3mg/kg) orally as suspension and served as standard group. The ethanolic extract was administered orally at 40 mg/kg to third group, 60 mg/kg to fourth group and 100 mg/kg to fifth group as suspension and served as test groups. After 60 min of treatment, animals of each group received 1 ml of castor oil orally and the watery fecal material and number of defecation were noted up to 4 h in the transparent metabolic cages with filter paper at the base. Weight of paper before and after defecation was noted [9][10].

2. PGE2 induced enteropooling

Wistar rats of either sex (150-250 g) were fasted for 18 h. They were then divided into five groups, each containing six rats. A solution of PGE_2 was made in 5% v/v ethanol in normal saline. The first group which served as PGE_2 control was administered with PGE_2 (100 µg/ kg p.o.) only. The second group which served as vehicle control was administered with aqueous 1% tragacanth suspension by oral route. Then the third, fourth and fifth groups were given a varied dose of 40 mg/kg, 60 mg/kg and 100 mg/kg respectively of ethanolic extract of *R. arborium*. Immediately, PGE_2 was administered and after 30 min each rats was sacrificed and whole length of the intestine from pylorus to caecum was dissected out and its content were collected in measuring cylinder and volume was measured[11][12].

3. Gastrointestinal motility test by charcoal meal:

Rats of either sex (150-250 g) were fasted for 18 h. Five groups (n=6) were made. The first group was denoted as control and was administered with aqueous 1% tragacanth suspension. The second group was given standard drug atropine (0.1 mg/kg) subcutaneously. The ethanolic extract of *R. arborium* was administered orally at 40 mg/kg to third group, 60 mg/kg to fourth group and the fifth group received 100 mg/kg in suspension form. After 30 minutes of the treatment, the animals were given 1ml of 10% activated charcoal suspended in 10% aqueous tragacanth powder p.o. Animals were sacrificed 30 min after charcoal meal administration by inhalation of ether. The abdomen was cut off and the small intestine was carefully removed. The distance travelled by charcoal plug from pylorus to caecum was measured and expressed as percentage of the distance travelled by charcoal plug for each of the animal[13][14].

Statistical Analysis

All results are expressed as the mean ± SEM. The results were analyzed for statistical significance using one-way analysis of variance (ANOVA); comparison was made by using Dunnett's test. P values <0.05 were considered as significant and P values <0.01 were regarded as very significant.

RESULTS:

Phytochemical analysis

The phytochemical screening of different extracts of *R. arborium* showed that the petroleum ether extract contains phytosterol, saponins and fixed oils. The chloroform extract contains proteins and the ethanolic extract contains carbohydrates, saponins, flavonoids, phytosterols, tannins and phenolic compounds [6].

Castor oil induced diarrhea:

Castor oil produced watery diarrhea, which lasted up to 24 h in the vehicle treated control group. The ethanolic extract of *R*. *arborium* exhibited pronounced antidiarrheal effect in a dose dependent manner following oral pretreatment on castor oil-induced diarrhea compared with the control. The extract delayed the time of defecation by 70.83 min, 132.33 min and 154.55 min at 40 mg/kg, 60 mg/kg and 100 mg/kg doses respectively. Although there was a delay in defecation but comparatively it was less than that caused by Loperamide (200.00 min). The ethanolic extract significantly ($p < 0.01$) inhibited both the frequency of defecation as well as the wetness of the fecal droppings of rats (Table 1).

Table - 1: Castor oil induced diarrohea

Sr. No.	Treatment N= 6	Mean wet defecation	Mean increase in weight of paper	Delay in defecation time (min)
1.	Control	9.167 ± 0.87	3.023 ± 0.49	32.167±7.305
2.	Loperamide (3 mg/kg)	1.833 ± 1.05 **	0.533 ± 0.29 **	200.00± 22.509**
3.	*R. arborium* ethanolic extract (40 mg/kg)	4.667 ± 0.99 **	2.140 ± 0.25^{ns}	70.833±2.845^{ns}
4.	*R. arborium* ethanolic extract (60 mg/kg)	3.667 ± 0.80 **	1.407 ± 0.51 *	132.33±29.261**
5.	*R. arborium* ethanolic extract (100 mg/kg)	2.30±0.56**	1.25±0.11*	154.55±25.11**

Number of animals (N) =6
Values are expressed as mean ± SEM
**** = P < 0.01 = very significant, * = P < 0.05 = significant, Not significant (ns) = P > 0.05**

PGE2 induced diarrhea:
As shown in Table 2, PGE_2 induced a significant increase in the fluid volume of rat's intestine in the control group. The extract inhibited PGE_2 induced enteropooling significantly ($p<0.01$) in rats by the three doses. The volume of intestinal fluid for 40 mg/kg, 60 mg/kg and 100 mg/kg doses of *R. arborium* was found to be 1.93 ml, 1.30 ml and 1.24 ml respectively.

Table - 2: PGE_2 induced enteropooling

Sr. No.	Treatment N= 6	Volume of intestinal fluid (ml)
1.	PGE_2 Control	3.240 ± 0.09798
2.	Vehicle Control	3.080 ± 0.1020^{ns}
3.	Ethanolic extract of *R. arborium* (40 mg/kg)	1.933 ± 0.1978**
4.	Ethanolic extract of *R. arborium* (60 mg/kg)	1.300 ± 0.1125**
5.	Ethanolic extract of *R. arborium* (100mg/kg)	1.24±0.1025**

Number of animals (N) =6
Values are expressed as mean ± SEM
**** = P < 0.01 = very significant, Not significant (ns) = P > 0.05**

Gastrointestinal motility by charcoal meal:

Ethanolic extract of *R. arborium* (40 mg/kg, 60 mg/kg and 100 mg/kg) and atropine sulphate (0.1 mg/kg) decreased the propulsion of the charcoal meal through the gastrointestinal tract when compared with the control (Table 3). Distance travelled by the charcoal meal was reduced to 74.80%, 63.91% and 51.61% in the ethanolic extract of *R. arborium* treated groups with the dose of 40, 60 and 100 mg/kg respectively, compared to control group. Atropine on the other hand, produced a marked decrease in the propulsive movements and the intestinal length traversed by charcoal meal was 49.87%. These observations were significantly ($p< 0.01$) different from what was seen in the control group. The dose 100 mg/kg produced significant decrease in the movement of charcoal meal and was almost equal to that produced by atropine sulphate (0.1mg/kg).

Table-3: Charcoal meal induced gastrointestinal transit

Sr. No.	Treatment N= 6	% Movement of charcoal meal
1.	Control	98.440 ± 1.560
2.	Atropine sulphate (0.1 mg/kg)	49.878 ± 8.043**
3.	*R. arborium* ethanolic extract (40 mg/kg)	74.802 ± 2.946**
4.	*R. arborium* ethanolic extract (60 mg/kg)	63.913 ± 5.188**
5.	*R. arborium* ethanolic extract (100 mg/kg)	63.913 ± 5.188**

Number of animals (N) =6
Values are expressed as mean ± SEM
****=P<0.01= very significant**

DISSCUSSION:

Diarrhea is the frequent passage of liquid feces which is generally accompanied by abdominal cramps and sometimes nausea and vomiting. It may be viewed as physiological mechanism for rapidly ridding the gut of poisonous or irritating substances. There are numerous causes, including underlying disease, infection, toxins and even anxiety. Repercussions ranges from mild discomfort and inconvenience to medical emergencies: requiring hospitalization, parentral fluid and electrolyte replacement therapy. Globally acute diarrheal disease is one of the principal causes of death in malnourished infants, especially in developing countries where medical care is less accessible. The three main approaches for treatment of diarrhea are maintenance of fluid and electrolyte balance, use of anti- infective and lastly use of spasmolytic or other antidiarrheal agents.

Castor plant contains two well known noxious ingredients, which are extremely toxic, ricin and oil composed chiefly of triglycerides of ricinoleic acid. The triglyceride is hydrolyzed in the small bowel by the action of lipases into glycerol and the active agent, ricinoleic acid, which acts primarily in the small intestine to stimulate secretion of fluid and electrolyte and speeds up intestinal transit [15]. The liberation of ricinoleic acid from castor oil produces an irritating reaction and inflammation on the wall of intestinal mucosa and thus enhances the release of prostaglandin, which stimulate the peristaltic activity, and secretion in the small intestine.

Ricinoleic acid also acts as an anionic surfactants which reduce the net absorption of water and electrolyte and produce diarrhea [16].

Loperamide is a well known antidiarrheal agent [17], an analog of meperidine, has opioid like actions on the gut, activating presynaptic opioid receptors in the enteric nervous system to inhibit acetylcholine release and decrease peristalsis [18].

Fluidity of stool is one of the basic signs of diarrhea. The extract also significantly inhibited the PGE_2 induced intestinal fluid accumulation. PGE_2 is a pro-secretary inflammatory mediator and within the mucous it can not only reduce the intestinal absorption but also initiate a pro-secretory state in intestines [19]. Prostaglandins have complex pharmacological and biological activities in the modulation of gastrointestinal functions, including contraction or relaxation of smooth muscles and inhibition or enhancement of neurotransmitter release [20]. The extract also has said to have anti-muscarinic agent like atropine in reducing the gastric motility as well as inhibiting the contraction of ileum [21].

The result showed that the ethanolic extract of the leaves of *R. arborium* was found to be effective against diarrhea induced by different models. The antidiarrheal effects of the leaves of *R. arborium* may be due to the reduction of gastrointestinal motility, inhibition of the synthesis of PGE_2 and NO or presence of more than one antidiarrheal principle and their synergistic properties.

CONCLUSION:

The antidiarrheal activity of the ethanolic extract of leaves of *R. arborium* showed the significant results. Hence, it is considered that it can be used in the treatment of diarrhea along with the rehydration therapy.

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