www.gbtrp.com
NPT (2011) 1(1):5-10.
ISSN: 2231-0436

Nature of Pharmaceutical Technology

Original Article

Anti diarrhoeal activity of rare orchid *Eulophia epidendraea* (Retz.)Fisher

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Received: 11.10.2010; Revised: 29.11.2010; Accepted: 01.01.2010; Published: 15.01.2011.

Abstract

The anti-diarrhoeal activity of ethanolic-tuber extract of *Eulophia epidendraea* was investigated on the castor oil - induced rat models. The anti-diarrhoeal activity measurement of faecal output was reduced to 75.60% and 82.33% respectively at the higher dose 500mg extract of *E. epidendraea* tuber in active against 4h. The observed the mechanism of its anti-diarrhoeal activity, its effect was further evaluated on intestinal transit, castor oil induced intestinal fluid accumulation and electrolyte concentration in the small intestinal fluid. The highest inhibition of gut motility was obtained by 500mg ethanolic extract of *E. epidendrae*a tuber. These observations demonstrated the inhibitory effect of *E. epidendraea* tuber extract on the castor oil- induced diarrhoea, peristaltic activity in small intestine. The result of most effective anti-diarrhoeal dose of 500mg/kg identified as tuber extract of *E. epidendraea*

Keywords: Orchidaceae, *Eulophia epidendraea*, tuber, leaf, phytochemicals,flavonoids

Introduction

Diarrhoea has long been recognized as one of the most important health problems in the developing countries (Snyder and Merson, 1982). In developing countries, diarrhoea is almost universally infectious in origin. According to WHO estimation for the year 1998, there were about 7.1 million deaths due to diarrhoea (Park, 2000). Secretory diarrhoea is the most dangerous symptom of gastrointestinal problems (Fontaine, 1988), and is associated with excessive defecation and stool outputs, the stools being of abnormally loose consistency (Aranda-Michel *et al*., 1999). Appropriate clinical management of diarrhoea includes oral rehydrate therapy and chemotherapy (Irfan, *et al*., 2001).

Medicinal plants play a key role in the development and advancement of modern studies on diarrhoeal acitivities of substances. According to World Health Organization in its Diarrhoeal Disease Control Program has given a special emphasis on the use of traditional folklore medicine in the control and management of diarrhoea (Anonymous, 1979). Medicinal plants of orchid *Eulophia epidendraea* (Retz.) Fischer belongs to the Orchidaceae family, which is found in South Africa and India. The orchid *Eulophia epidendraea* has been traditionally used by the local people of Yadav community for the treatment of tumour, abscess and healing of wound (Maridass *et al*.,2008). However, there is no scientific proof justifying the traditional use of *Eulophia epidendraea* tuber in the treatment of diarrhoea. Hence, the present study was undertaken to evaluate its potential antidiarrhoeal efficacy in different experimental models of diarrhoea in inbred Wistar rats.

Materials and Methods

Plant material

Fresh tubers of *Eulophia epidendraea* (Retz.) Fisher (Orchidaceae) was collected from Kambli Malaikovil Forest, Tirunelveli District, Tamil Nadu, India. The tuber of *E. epidendraea* was air- dried and powdered. About 250g of this powder was extracted with Absolute Alcohol in a Soxhlet apparatus. The extraction process was continued for 8h. The solvent was evaporated under reduced pressure. After determining the yields, extracts were stored at 4^{o}C until further use.

Qualitative phytochemical tests

The tests were done to find the presence of the active constituents such as alkaloids, glycosides, terpenoids and steroids, flavonoids, reducing sugar and tannin by the following procedure:

Alkaloid

Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. Alkaloid solution produces white yellowish precipitate when a few drops of Mayer's reagents are added. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent. The alcoholic extract was evaporated to dryness and the residue was heated on a boiling water bath with 2%

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hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. The samples were then observed for the presence of turbidity or yellow precipitation.

Glycoside

Glycosides are compounds which upon hydrolysis give rise to one or more sugars (glycones) and a compound which is not a sugar (aglycone or genine). To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer.

Terpenoids and steroid

4mg of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpenoid and green bluish color for steroids.

Flavonoid

4 ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and orange color for flavones.

Tannins

To 0.5 ml of extract solution 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue color was observed for gallic tannins and green black for catecholic tannins.

Reducing Sugar

To 0.5 ml of extract solution, 1 ml of water and 5-8 drops of Fehling's solution was added at hot and observed for brick red precipitate.

Anti diarrhoeal activity

Test Animals

Inbred Wistar rats of either sex weighing 150-180g were used for castor oil - induced anti-diarrhoeal activity. All animals were fed standard animal feed and tap water *ad libitum* before the experiments. Each experimental group consisted of six animals housed in separate cages.

Castor oil - Induced Diarrhoea

Inbred Wistar rats were divided into five groups of six animals each, diarrhea was induced by administering 2ml of castor oil orally to rats. Group 1 served as control (2ml/kg, i.p. saline), group 2 received loperamide (3mg/kg, i.p.) served as standard and group 3, 4 and 5 received extract (100, 200 and 500 mg/kg, i.p) 1h before castor oil administration. The number of both wet and dry diarrhoeal droppings were counted every hour for a period of 4h mean of the stools passed by the treated groups were compared with that of the positive control group consisted of animals given an intraperitonial injection of saline (2ml/kg, ip) (Awouters *et al*.,1978).

Castor oil - Induced Enteropooling

Intraluminal fluid accumulation was determined by the method of Robert *et al.*, (1976). Overnight fasted rats were divided five groups of six animals each. Group 1 received normal saline intraperitoneal (2ml/kg i.p.) served as a control, group 2 received atropine (3mg/kg, i.p.) and groups 3, 4 and 5 received the extract of 100, 200 and 500mg/kg intraperitoneally respectively 1h before the oral administration of castor oil. Two hours later the rats were sacrificed, the small intestine was removed after tying the ends with thread and weighed. The intestinal contents were collected by milking into a graduated tube and their volume was measured. The intestine was reweighed and the difference between full and empty intestines was calculated (Robert *et al.*,1976) .

Small Intestinal Transit

Wistar rats were fasted for 18h divided into six groups of six animals each, Group 1 received 2ml normal saline orally, group 2 received 2ml of castor oil orally with saline 2ml/kg intraperitoneally, group 3 received atropine (3mg/kg, i.p.), group 4, 5 and 6 received 100, 200 and 500mg/kg intraperitoneally of the plant extract respectively, 1h before administration of castor oil. 1ml of marker (10% charcoal suspension in 5% gum acacia) was administered orally 1h after castor oil treatment. The rats were sacrificed after 1h and the distance traveled by charcoal meal from the pylorus was measured and expressed as percentage of the total length of the intestine from the pylorus to caecum (Mascolo,1994).

Statistical Analysis

The experimental results are represented as mean ± S.E. (Standard error of the mean). Student's *t*-test was sued for the evaluation of data and $P<0.05$ accepted as significant.

Results

Measurement of Faecal Output**:**

The extract of *E. epidendraea* tuber of 100 mg had no effect, 200 and 500 mg doses inhibited defecation by 100% in the initial 1, 2, 3 and 4 hrs compared to normal defecation in Inbred Wistar rats. The activity of was reduced to 75.60% and 82.33% respectively at the higher doses in the 4 hrs. When compared to the control group (Table-1).

Table-1: Effect of *E. epidendraea* extract on castor oil - induced diarrhoea in inbred wistar rats

Treatment	Total No. of faecal matter passed (hr)				% Reduction
	1	2	3	4	
Control (Saline)	5.0 ± 0.88	16.0 ± 2.16	23.0 ± 2.16	35.66 ± 1.70	-
100mg	0.0 ± 0.00	5.0 ± 0.82	12.3 ± 1.25	20.33 ± 2.05	42.99
200mg	0.0 ± 0.00	0.0 ± 0.00	2.7 ± 2.5	8.7 ± 3.3	75.60
500mg	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	6.3 ± 2.05	82.33
Loperamide	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	6.3 ± 2.05	82.33

Values are expressed as Mean ± SE from the experiments. **P*<0.05 when compared with control and treated group.

Table -2: Effect of *E. epidendraea* tuber extract on castor oil induced enteropooling in inbred wistar rats

Treatment (Extract)	Weight of intestinal Content (gm)	% inhibition weight of intestinal content
Castor oil + Saline	1.859 ± 0.021	-
Castor oil + 100mg	1.661 ± 0.014	27.79 ± 0.080
Castor oil + 200mg	1.278 ± 0.033	42.01 ± 0.053
Castor oil + 500mg	0.975 ± 0.008	63.73 ± 0.096
Atropine	1.796 ± 0.02	14.34 ± 0.011

Table- 3: Effect of *E. epidendraea* tuber extract on charcoal meal-stimulated gastrointestinal transit in inbred wistar rats

Treatment	Dose (mg/kg^{-1})	Total length of Intestine (cm)	Mean distance of traveled by charcoal (cm)	Reduction (%)
Control	-	80.43 ± 0.41	58.63 ± 1.48	27.11
Extract + charcoal	100	87.37 ± 1.49	44.50 ± 2.78	49.07
Extract + charcoal	200	87.47 ± 1.99	25.43 ± 0.58	58.38
Extract + charcoal	500	84.33 ± 0.38	26.06 ± 0.43	69.10
Atropine + charcoal	0.1	85.33 ± 1.03	28.90 ± 1.27	66.11

Statistical significance test with control was done by independent student t test. (*P*< 0.5) w hen compared to control.

Castor oil-Induced Enteropooling

Castor oil caused accumulation of water and electrolytes in intestinal loop. Castor oil - induced enteropooling is not influenced by atropine in rats (3mg/kg, i.p.). Each dose of the extract produced a dose-dependent reduction in intestinal weight and volume. 100mg/kg, i.p. dose of extract produced 23.82 % inhibition of volume of intestinal content (*P*<0.01). However, 200 and 500mg/kg, i.p. dose produced 39.86 and 61.35 % inhibition of volume of intestinal content respectively with significance (*P*<0.001). The weight of intestinal content was also reduced significantly at all the doses. 100, 200 and 500mg/kg, i.p. dose dependently inhibited weight of intestinal content of 27.79, 42.01 and 63.73 % respectively (Table-2).

Effect of *Eulophia epidendraea* tuber extract on castor oil induced enteropooling in rats. Values are expressed as Mean ± SE from the experiments. *P*<0.01, *P*<0.001, when compared with castor oil + saline group.

Small Intestinal Transit

The effect of *Eulophia epidendrae* on castor oil stimulated gastro intestinal transit was also significant and dose dependent. The reduction in the gastrointestinal transit of 49.07 to 69.10 was comparable to the standard drug atropine (66.11) seen in Table-3. The highest inhibition of gut motility was however obtained with the 500 mg ethanolic extract of *E. epidendrae*a tuber. These observations demonstrate the inhibitory effect of the *E. epidendraea* tuber extract on the castor oil-induced diarrhoea, peristaltic activity in small intestine.

The anti diarrhoeal active constituents of tuber extract of *E. epidendraea* results seen in Table-4.

Table-4: Active principles of *E. epidendraea*

Test	Ethanol extract
Alkaloids	+
Flavonoids	+++
Glycosides	++
Tannins	+
Steroids& Terpenes	+

+, Presence; -, Absence

Discussion

In developing countries, a quarter of infant and childhood mortality is related to the diarrhoea (Jousilahti *et al.,* 1997). The highest mortality rates have been reported to be in children less than five years of age. During the past decade oral dehydration therapy has reduced mortality from acute diarrhoeal disease, whereas chronic diarrhoea remains a life-threatening problem in those regions, in which malnutrition is a common co-existing and complication factor. Number of factors, such as infective, immunological and nutritional has been involved in the perpetuation of the diarrhoeal syndrome (Galvez, *et al*., 1995). Many plants conveniently available in India are used in traditional folklore medicine for the treatment of diarrhoea. Of the indigenous plants used, *Andrographis paniculata, Asparagus racemosus, Butea monosperma, Cassia auriculata,* and others are mentioned (Chopra *et al*., 1956). Several studies have shown that prior administration with some plant extracts had a protective effect on the intestinal tract (Rani *et al*., 1999; Majumdar, *et al.,* 2000; Kumar *et al*., 2001). In the present study, ethanol extracts of *Eulophia epidendraea* that have not been studied so far, was evaluated for its anti-diarrhoeal potential against castor oil induced diarrhoea, gastrointestinal motility in charcoal meal test and induced enteropooling in inbred Wistar rats.

In traditional system, many plants are claimed to have anti-diarrhoeal activity without any scientific basis. The aim of the present study was to evaluate the putative anti-diarrhoeal effects on the tuber of *Eulophia epidendraea,* which are used in indigenous medicines of abscess, healing of wound, and diarrhoea. In establishing the pharmacological evaluation of a potential anti-diarrhoeal agent, the inhibition of experimentally induced diarrhoea, reduction in the faecal output and gastrointestinal motility tests have remained the most common parameter in several reporter (Majumdar *et al*.,2000;Tangpu *et al*.,2004; Venkatesan *et al*.,2005).

The ethanol extract of *Eulophia epidendraea* exhibited significant anti-diarrhoeal activity against castor oil induced diarrhoea in rats. The extracts had a similar activity as loperamide, when tested at 200 and 500 mg/kg and statistically significant reduction in the frequency of defecation and the wetness of the faecal droppings when compared to untreated control rats.

The extracts also significantly inhibited the castor oil induced intestinal fluid accumulation (enteropooling). It is widely known that castor oil or its active component ricinoleic acid induces permeability changes in mucosal fluid and electrolyte transport that results in a hypersecretory response and diarrhoea (Ammon *et al*., 1974; Gaginella *et al*., 1975). Diarrhoea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract, accompanied by hurry, resulting in an excess loss of fluid in the faeces. In some diarrheas, the secretory component predominants, while other diarrhoeas are characterized by hypermotility. The use of castor oil induced diarrhoea model in our study is logical because the autacoids and prostaglandins are involved these have been implecated in the causation of diarrhoea in man (Horton *et al*., 1968; Greenbargena, 1978). The liberation of ricinolic acid from castor oil results in irritation and imflammation of the intestinal mucosa, leading to release of protaglandins, which stimulate motility and secreation (Pierce, 1971). These observations tend to suggest that those extracts at a dose of 500 mg/kg reduced diarrhoea by inhibiting castor oil induced intestinal accumulation of fluid. These results are recommended for previous report on ripen fruits extract of *Rhus javanica* (Tangpu, 2004).

The extract appears to act on all parts of the intestine. Thus, it reduced the intestinal propulsive movement in the charcoal meal treated model; at 500 mg/kg *Eulophia epidendraea* tuber extract showed activity similar to that of atropine. Previous study shows that activated charcoal avidly absorbs drugs and chemicals on the surface of the charcoal particles thereby preventing absorption (Levy, 1982). Thus, gastrointestinal motility test with activates charcoal was carried out to find out the effect of ethanol and aqueous extracts on peristaltic movement. The results also show that the ethanol and aqueous extracts suppressed the propulsion of charcoal meal thereby increased the absorption water and electrolytes.

The results indicate that the ethanol extract of *Eulophia epidendraea* possesses significant anti-diarrhoeal activity due to its inhibitory effect both on gastro intestinal propulsion and fluid secretion. The data obtained are consistent with antidiarrhoeal activity of wild plants *Asparagus pubescens,* and *A. racemosus* using gastrointestinal motility test and castor oil induced diarrhoea and intraluminal accumulation of fluid in rats (Nawfor, *et al.,* 2000; Venkatesan *et al*., 2005). The inhibitory effect of the extract justified the use of the plant as a non -specific anti-diarrhoeal agent in folk medicine.

Previous reports have demonstrated the antidiarrhoeal activity of tannin (Mukherjee *et al*.,1998), flavonoids (Galvez *et al*.,1993), alkaloids (Gricilda Shoba *et al*.,2001), saponins, reducing sugars and sterols and/or terpenes (Otshudi *et al*.,2000) containing plant extracts. The phytochemical analysis of the extract showed the presence of alkaloids, saponins, flavonoids, sterols and /or terpenes and sugars. These constituents may responsible for the antidiarrhoeal activity of *Eulophia epidendraea* tuber extract.

Conclusion

The results of this study seem to provide a support for the use of orchids *Eulophia epidendraea* tuber as antidiarrhoeal agent in the traditional medicine system of Yadav community. Further study, however, is necessary to isolate and identify the active phytochemical of tubers and their precise mechanism of action.

Acknowledgement

The authors are grateful to Principal, St. Xavier's College (Autonomous), for providing all laboratory facilities to carry out this research work.

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