Anti-Diarrhoeal Activity of Root Extracts of *Elephantopus scaber* L

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Summary

A study was undertaken to evaluate the effect of methanolic extract of the root of *Elephantopus scaber* L (MEES) against several experimental models of diarrhea in rats. MEES treated animal's showed significant inhibitory effect against castor-oil induced diarrhea and PGE_2 induced enteropooling in rats. The extract also showed a significant reduction in gastrointestinal motility in the charcoal meal test in rats. The results obtained to establish the efficacy and substantiate the folklore claim as an anti-diarrhoeal agent.
Key words: *Elephantopus scaber*, MEES, diarrhea, methanol extract.

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Introduction

Diarrhoea has long been recognized as one of the most important health problem in the developing countries (1). World Wide distribution of diarrhoea accounts for more than 5-8 millions deaths each year in infants and small children's less than 5 years. According to WHO estimation for the year 1998, there were about 7.1 million deaths due to diarrhea (2). Secretory diarrhoea is most dangerous symptom of gastrointestinal problems (3) and is associated with excessive defecation and stool outputs. The stool being of abnormally loose consistency(4). The World Health Organization has constituted a Diarrhoeal Disease Control program (CDD), which includes studies of traditional medicinal practices, together with the evaluation of health educational and prevention approaches (5).

Elephantopus scaber L. (Asteraceae) is a small herb, which grows in the wild throughout the hotter parts of India. In the Indian system of medicine, the medicinal attribution of this species has been known for a long time. As per the traditional claims, the roots were used as an antipyretic, cardiotonic, and diuretic. Decoction of the roots and leaves is used as emollient and given in dysuria, diarrhea, dysentery, and in stomach pain. The aqueous extract of leaves is applied externally to treat eczema and ulcers. Phytochemically the plant has been reported to contain sesquiterpene lactones deoxyelephantopin, isodeoxy-elephantopin, and scabertopin. It also contains epifriedelinol, lupeol, and stigmasterol (6).

Materials and Methods

Plant Material:

The roots of *Elephantopus scaber* L. were collected from Warangal, Andhrapradesh, India in February 2007. The plant was identified by routine pharmacognostical studies including organoleptic tests, microscopic and macroscopic observation. The vouches specimen has been retain in our laboratory for future reference. The collected roots were air dried and pulverized using mechanical grinder.

Preperation of Extract

The powdered roots (500g) were extracted using 85% methanol. The solvent was completely removed from the extract by distillation *in vocuo* and a brown coloured semisolid mass obtained. (Yield 6.2% w/w, with respect to the dry powder). The extract was stored in dessicator and used for further experiment after suspend in aqueous tween 80 solution (2%). The chemical constituents of the extracts were identified by qualitative tests and further confirmed by TLC study for the presence of alkaloids, tannins, sterols, and carbohydrates.

Animals

Wistar Albino rats (either sex) weighing between 150-200gm were used. The animals were divided into five groups (n=6) and were housed in polyacrylic cages with standard diet and water *ad libitum* except in the castor oil model, in which the animals were placed individually in separate cages. The animals were maintained under standard laboratory conditions for acclimatization period of 7 days prior to perform the experiment. The animals were fasted for 18hours prior to the experiment.

Experimental Design

Toxicity Study

An acute toxicity study relating to the determination of LD_{50} value was performed using different doses of the extract according to the method described by Ghosh et al., 1984. from the toxicity study; it was observed that the root extract is non-toxic up to dose of 3.2g/kg body weight. It is safe and was used in different doses for further studies.

Castor Oil Induced Diarrhoea (8).

The doses of MEES were selected on a trial basis and administered orally (100, 200 & 400mg/kg body weight) by gavage to three groups of animals. The fourth group received diphenoxylate (5mg/kg body weight) orally and the fifth group received neither drug nor extract but 2% v/v aqueous Tween 80 (1 ml) only and served as a control. After 60 min of drug treatment, each animal was administered 1ml of castor oil orally by gavage and observed for defecation up to 4hrs after castor oil administration. Characteristic diarrhoeal droppings were noted in the transparent plastic dishes placed beneath the individual perforated rat cages. The mean number of wet feces was calculated from the diarrhoeal droppings in the transplant plastic dishes (9).

Pge_2 – Induced Enteropooling (10).

For this evaluation, rats of same stock as above were deprived of food and water for 18hrs prior to the experiment. Five groups of six animals were used, which were placed in five perforated cages. The first three groups of rats were treated with MEES (100, 200 & 400mg/kg body weight, p.o) while the fourth and fifth group received 1ml of 5% v/v ethanol in normal saline (i.p). The fourth group was then administered 1ml of normal saline and used as control. Immediately afterwards, each rat was treated with PGE_2 (100µg/kg body weight in 5% v/v ethanol in normal saline) administered orally. All the rats were sacrificed under mild anesthesia after 30min. The entire length of intestine from the pylorus to the caecum was dissected out, and its contents were collected and measured.

Gastrointestinal Motility Test (10).

In this method rats were fasted for 18hrs and placed in five metal cages, six in each. Each animal was given 1ml of charcoal meal (3% deactivated charcoal in normal saline). The first three groups of animals were administered MEES orally (100, 200 & 400 mg/kg body weight) immediately after the charcoal meal treatment. The fourth group received atropine (0.1mg/kg body weight, i.p) as standard for comparison. The fifth group was treated with normal saline as control. 30min after administration of the charcoal meal, animals of each individual group were killed and the movement of charcoal from pylorus to caecum was measured. The charcoal movement in the intestine was expressed as percentage.

Statistical Analysis

For all the above experiments results were expressed as mean ±SEM. Statistical significance tests were performed using the students't' test and p-values (Graph Pad software) were calculated by comparison with control groups.

Results

Chemical Analysis

The results of the preliminary photochemical screening of methanolic extract of *Elephantopus scaber* corresponding root have been presented in Table – 1.

Table-1: Phytochemical screening of *Elephantopus scaber* L

Test	Methanol extract
Alkaloids	+
Tannins	+
sterols	+
Carbohydrates	+

L, -, absence; +, presence.

Toxicity Study

From the toxicity study it was observed that the root extract is non-toxic and caused no death up to a dose of 3.2g/kg orally. It is safe and was used in doses for further studies. Results are shown in Table-2.

Inhibition of Castor-Oil Induced Diarrohea

The extract (MEES) inhibited the frequency of defecation significantly, like standard drug (diphenoxylate) as compared to control (2% aqueous tween 80 treated). The wetness of fecal material also reduced by both the standard and extract (MEES). The results are shown in Table – 3.

Table-2: Toxicity Study:

Treatment	Dose (mg/kg body weight)	No. of animals	No. of survives	No. of deaths	LD_{50} valve
Control	2% tween 80 solution	20	20	0	-
MEES	100	20	20	0	-
	200	20	20	0	-
	400	20	20	0	-
	800	20	20	0	-
	1600	20	20	0	-
	3200	20	20	0	>3.2g/kg body weight

MEES: Methonolic extract of *Elephantopus scaber* L.

Table-3: Inhibition of castor oil induced diarrhoea.

Oral pretreatment at 60min (mg/kg body weight)	Mean number of wet feces (mean ± SEM)
2% v/v aqueous tween 80(5)	27.2 ± 2.01
Diphenoxylate(5)	10.8 ± 1.44 [a]
MEES(100)	20.3± 1.64 [b]
MEES(200)	14.6 ± 1.12 [a]
MEES(400)	11.8 ± 1.19 [a]

[a] $p<0.001$, [b] $p<0.01$ as compared to control (n=6); MEES: Methanol extract of *Elephantopus scaber* L

Anti-Enteropooling Activity

PGE_2 induced a significant increase in the fluid volume of the rat as compared to control animals receiving only ethanol in normal saline. The extract (MEES) significantly inhibited PGE_2 induced enteropooling in rats at almost all doses used. Results are shown in Table – 4.

Effect on Gastrointestinal Motility

The extract (MEES) decreased propulsion of charcoal meal through the gastrointestinal tract significantly with respect to the control group. The effect was comparable to the standard drug. The results are shown in Table – 5.

Table-4: Anti-enteropooling activity

Treatment	Volume of intestinal fluid ml(mean ± SEM)	P – valve
Ethanol in saline	0.76 ± 0.13	-
PGE2 in ethanol	2.93 ± 0.19	<0.001 [a]
MEES(100mg/kg body weight)	1.82 ± 0.10	<0.001 [b]
MEES(200mg/kg body weight)	1.18 ± 0.11	<0.001 [b]
MEES(400mg/kg body weight)	1.04 ± 0.05	<0.001 [b]

[a] significance with respect to ethanol in saline treatment. [b] with respect to PGE_2 treatment (n=6); MEES: methanol extract of *Elephantopus scaber* L.

Table-5: Effect on gastrointestinal motility

Treatment	Dose (mg/kg body weight)	Movement of charcoal meal (%)
Control	2% aqueous tween 80	85.2 ± 2.1
Atropine	0.1	40.6 ± 2.3 [a]
MEES	100	75.2 ± 2.2 [b]
MEES	200	64.6 ± 2.1 [a]
MEES	400	53.8 ± 2.2 [a]

P-valve calculated with respect to control group (n=6); [a] $p<0.001$, [b] $p<0.02$; MEES: Methanol extract of *Elephantopus scaber* L.

Discussion

In developing countries a quarter of infant and childhood mortality is related to the diarrhoea (11).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, where as chronic diarrhea remains a life-threatening problem in those regions in which malnutrition is common co-existing and complication factors , such as infective, immunological and nutritional has been involved in the perpetuation of the diarrhoeal syndrome(12). Many plants conveniently available in India are used in traditional folklore medicine for the treatment of diarrhea and dysentery of the indigenous plants used, *Andrographis Peniculata*, *Asparagus racemosus*, *Butea monosperm*, *Cassia auriculata* and other are mentioned (13). Several studies have shown that prior administration with some plant extracts had protective effect on the intestinal tract (14)/(15)/(16).

In the present study, methanol extract of root of *Elephantopus scaber* have not been studied so far was evaluated for its anti-diarrhoeal potential against castor oil induced diarrhea, gastro intestinal motility in charcoal meal test & PGE_2 induced enteropooling in Albino wistar rats.

The MEES exhibited significant anti-diarrhoeal activity against castor oil induced diarrhea in rats. The extract had a similar activity as diphenoxylate, when tested at 100, 200 & 400mg/kg and statistically significant reduction in the frequency of defecation and the wetness of the fecal droppings when compared to untreated control rats.

It is widely known that castor oil or its active component ricinoleic acid induces permeability changes in mucosal fluid and electrolyte transport that result in hyper secretory response of diarrhoea (17)/(18). The experimental studies in rat's demonstrated a significant increase in the portal venous PGE_2 concentration following oral administration of castor oil (19). The recinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which stimulate motility and secretion (20). Inhibitors of prostaglandin biosynthesis delayed castor oil induced diarrhea.

The MEES significantly inhibited the PGE_2 induced intestinal fluid accumulation (enteropooling). It has been shown that E type of prostaglandin cause diarrhoea in experimental animals as well as human beings (21). Their mechanism has been associated with dual effects on gastrointestinal motility as well as on water and electrolyte transport (22). PGE_2 also inhibit the absorption of glucose a major stimulus to intestinal adsorption of water and electrolytes (23). These observations tend to suggest that MEES reduced diarrhea by inhibiting PGE_2 induced intestinal accumulation of fluid.

The MEES appears to act on all parts of the intestine. Thus it reduced the intestinal propulsive movement in the charcoal meal treated model. The MEES 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 there by preventing absorption (24). Thus gastrointestinal motility test with activated charcoal was carried out to find out the effect of MEES on peristalsis movement. The results shows that the MEES suppressed the propulsion of charcoal meal thereby increased the absorption of water and electrolytes.

Previous reports have demonstrated the antidiarrhoeal activity of tannins (25), alkaloids (26), sterols and reducing sugars (27), containing plant extracts. The phytochemical analysis of the extract showed presence of tannins, alkaloids sterols and reducing sugar. These constituents may responsible for the antidiarrhoeal activity.

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