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Evaluation of antidiarrhoeal potential of poly herbal formulation against castor oil induced diarrhoea in rodents

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ABSTRACT

Diarrhoea is the frequent passing of loose, watery and unformed faeces. Loss of fluids through diarrhoea can cause dehydration and electrolyte imbalance and ultimately leads to death. The aim and objective of the present study is to evaluate the antidiarrhoeal potential of the poly herbal formulation *(Anogeissus latifolia, Elephantopus scaber, Euphorbia hirta*) against castor oil induced diarrhoea. In the current study Polyherbal solution was formulated using various plant extracts listed above and mixed with excipients and evaluated for physicochemical and antidiarrhoeal activity. The standard drug used for study was loperamide. Animals was divided in to five groups of 6 animals each and diarrhea was induced by administration of castor oil at a dose of 1ml/kg b.w and the poly herbal formulation was administered at 200 & 400mg/kg dose levels orally. The antidiarrhoeal activity was evaluated by estimating the mean number of droppings mean weight of droppings and % intestinal transit. Results were analysed by oneway ANOVA followed by Dunnet's test. Studies revealed that poly herbal formulation at doses 200 & 400 mg/kg b.w p.o showed significant results in a dose dependent manner comparable with that of standard Lopramide 5mg/kg b.w.

Keywords: Diarrhoea, Poly herbal formulation, *Anogeissus latifolia, Elephantopus scaber, Euphorbia hirta,* Castor oil, *Loperamide*.

INTRODUCTION

Diarrhoea is the frequent passing of loose, watery and unformed faeces. Loss of fluids through diarrhoea can cause dehydration and electrolyte imbalance and ultimately leads to death(1). Diarrhoea has long been recognized as one of the most important health problems and leading cause of death and growth retardation in children(2). It is one of the most common clinical signs of gastrointestinal disease, but also can reflect primary disorders outside the digestive system(3). According to W.H.O. estimates for 1998, about 7.1 million deaths were caused by diarrhea and the cause of 3.3% of all deaths .Around 88% of diarrheal related deaths are caused due to inadequate sanitation and poor hygiene(4). Recently there is a greater global interest in non-synthetic, natural drugs derived from plant and herbal sources due to better tolerance and minimum potential of adverse effects(5). Although medicinal plants are used as antidiarrhoeal agents in folk medicine there have been no or few scientific studies to explain their action and usefulness as antidiarrhoeal and the adverse effects associated with the current antidiarrhoeal agents (eg. loperamide, bismuth subsalicylate, racecadotril and many more) like abdominal discomfort, dry mouth, nausea, constipation and headache etc(6) is the driving force for the researchers round the globe to look for a newer and potent non-synthetic antidiarrhoeal agent. Plants play a significant role and a valuable source of

natural products. The present study is aimed to investigate the antidiarrhoeal activity of *Anogeissus latifolia*, *Elephantopus scaber*, *Euphorbia hirta* which are used in ayurvedic system of medicine for the treatment of diabetes, hemorrhages, diarrhoea, dysentery, haemorrhoids, skin diseases, hepatopathy and general debility(7). However no much characterization of this activity has been done on scientific basis to develop formulation from combined extracts of plants. Thus the present study was undertaken to explore the effects of various extracts of combined medicinal plants in polyherbal formulation as solution form against castor oil induced diarrhea and compare these effects with loperamide as standard marketed formulation.

MATERIALS AND METHODS

PLANT COLLECTION AND IDENTIFICATION

The selected crude plant materials i.e *Anogeissus latifolia (Bark), Elephantopus scaber (leaves), Euphorbia hirta (leaves)* were collected from Erode and Madurai district of Tamilnadu. All the samples were identified and authenticated from Botanical Survey of India, Coimbatore. After authentication the materials were dried and coarsely powdered and used for further studies.

EXTRACTION

The collected plant materials were cleaned, shade dried and powdered by mechanical means. About 200 gm each standardized powder of *Anogeissus latifolia (Bark), Elephantopus scaber (leaves), Euphorbia hirta (leaves)* were subjected to extraction by Soxhlation with various solvents starting from nonpolar to polar. After the extraction, the extract was filtered and concentrated at room temperature by using Buchi rotary vacuum evaporator (8, 9). The extracts was subjected to qualitative method of preliminary phytochemical analysis by adopting standard procedure(10)

DRUGS AND CHEMICALS

Loperamide (Cipla Pharmaceutical Limited, Indore, India), castor oil. All other reagents and chemicals used for studies were of analytical or laboratory grade.

ANIMALS

Wistar albino rats (150-180 g) were obtained from animal house of Institute. They were acclimatized to animal house condition at temperature 23 ± 2 ^{0}C and room humidity 60 ± 10.4 maintained on 12:12 hours light: dark cycle, fed by standard laboratory diet (Hindustan Lever Limited, Bangalore, India) and water *ad libitum*.

DEVELOPMENT OF POLYHERBAL FORMULATION (PHF)

Oral solution containing *Anogeissus latifolia (70% etanol extract), Elephantopus scaber (Ethyl acetate extract), Euphorbia hirta (Hydroalcoholic extract)* and suitable excipients was prepared by dissolving all these ingredients in water(11, 12). The additives used were butylated hydroxyanisole (antioxidant and preservative), sodium saccharin (artificial sweetening agent), Chocolate flavor (flavoring agent) according to the quantities specified (Table 1).

Table 1: Formula for poly herbal formulation in solution form

S.No	Name of ingredient	Quantity in grams
1	Extracts	6%w/v
2	Butylated hydroxyanisol	0.2%
3	Sorbic acid	0.2%
4	Sodium saccharin	0.1%
5	Chocolate flavor	q.s
6	Purified water (q.s.)	Up to 100ml

STANDARDIZATION OF POLYHERBAL FORMULATION

Standardization of prepared formulation in solution form was done by using different organoleptic characters (color, odor and taste) as well as

physicochemical parameters like pH, visibility in light and gas evolution studies(13).

GROUPING

Experimental animals were divided into five groups of six animals each and treatment was given as below.

Group I: Vehicle control (Treated with Normal Saline, 2 ml).

Group II: Negative control (Treated with Castor oil, 1ml for castor oil induced diarrhea, 1ml 4% tragacanth for gastrointestinal motility model).)

Group III: Positive control (Treated with Loperamide, 5mg/kg body weight).

Group IV: Treatment group I (Treated with PHF, 200 mg/kg body weight).

Group V: Treatment group II (Treated with PHF, 400 mg/kg body weight).

CASTOR OIL INDUCED DIARRHOEA

After 1 hour of drug and vehicle treatment all the groups except the vehicle control group were challenged with 1ml of castor oil orally. Animals were observed for 4 h and the number of wet and dry droppings was counted every hour for a period of 4 h(14, 15).

GASTROINTESTINAL MOTILITY MODEL

After 30 minutes, the intestinal motility was assessed by orally administrating semisolid test charcoal meal consisting of 1ml of deactivated charcoal (5% deactivated charcoal in 4% aqueous tragacanth). Rats were anaesthetized using diethyl ether, the abdomen was opened and the entire small intestine starting from pyloric end to ileocaecal end was removed and placed on blotting paper. The distance traveled by charcoal meal and total length of small intestine was measured in centimeters and expressed as percentage intestinal transit(16, 17).

STATISTICAL ANALYSIS

Data were analysed using Graph pad Prism Software version 6.0 (Graph Pad Software, La Jolla, USA). All the values were expressed as mean ± standard error of mean (SEM). The significance of difference between two groups for antidiarrhoeal activity was analysed using one-way analysis of variance (ANOVA) followed by post hoc Dunnet's tests. For statistical analysis, $P<0.05$ was considered statistically significant.

RESULTS

PRELIMINARY PHYTOCHEMICAL SCREENING

Preliminary phytochemical investigations of all the plant extracts was carried out by standard protocols Presence of alkaloids in *E.hirta* is observed ,all extracts showed positive results for Tannins & phenolic compounds. Presence of terpenoids was observed for *E.scaber* and *E.hirta* and the results obtained was reported in Table2.

Table 2: Results of Preliminary Phytochemical Investigations

S.No	Tests	*Anogeissus latifolia* *(70% ethanol extract)*	*Elephantopus scaber* **(Ethyl acetate extract)**	*Euphorbia hirta* *(Hydroalcoholic extract)*
1	Alkaloids	-ve	-ve	+ve
2	Carbohydrates	+ve	+ve	+ve
3	Proteins	+ve	-ve	-ve
4	Amino acids	+ve	+ve	+ve
5	Glycosides	+ve	+ve	-ve
6	Steroids & Sterols	+ve	+ve	+ve
7	Flavonoids	+ve	-ve	+ve
8	Tannins& Phenolic compounds	+ve	+ve	+ve
9	Triterpenoids	-ve	+ve	+ve
10	Saponin	-ve	-ve	+ve
11	Fixed oil	-ve	-ve	

+ve + Present, -ve = absent

EFFECT OF POLY HERBAL FORMULATION ON CASTOR OIL INDUCED DIARRHEA

Castor oil treated animals showed significant increase in the mean number of droppings and mean weight of droppings when compared with that of vehicle control, pre-treatment with Lopramide 5mg/kg b.w and poly herbal formulation at 200 & 400 mg/kg b.w showed reduce in the mean number of droppings and mean weight of droppings significantly in a dose dependent manner when compare with negative control as shown in Table 3 and Fig 1 & 2.

Table 3: Antidiarrhoeal activity of polyherbal formulations in castor oil induced diarrhea

Group	Mean Number of Droppings				Mean weight of droppings (mg)			
	1st hr	2nd hr	3rd hr	4th hr	1st hr	2nd hr	3rd hr	4th hr
Vehicle control	01 ± 1.25	03 ± 1.25	04 ± 1.05	03 ±1.25	55 ± 1.2	100 ± 1.5	99 ± 1.25	143±1.25
Negative Control	09 ± 1.25	12 ± 0.5	12 ± 2.5	10 ± 1.20	191 ± 2.5	211 ± 9.5	226 ± 6.5	216.46 ± 2.5
Standard	02 ± 1.1	05 ± 0.36	06± 0.86	05 ± 2.25	100 ± 0.3	142 ± 0.6	151 ± 1.9	144 ± 0.11
Formulation (200mg/kg b.w.)	05 ± 1.1	07 ± 2.5	08 ± 8.91	08 ± 0.1	155 ± 6.9	65 ± 0.7	170 ± 2.1	160 ± 9.09
Formulation (400mg/kg b.w.)	02 ± 1.1	02 ± 2.57	02 ± 8.91	02± 0.1	129 ± 6.9	126 ± 8.7	120 ± 4.1	121 ± 9.09

Values are mean ± SEM N=6; $P < 0.05$ as compared to vehicle control and positive control by one way ANNOVA followed by Dunnet's test

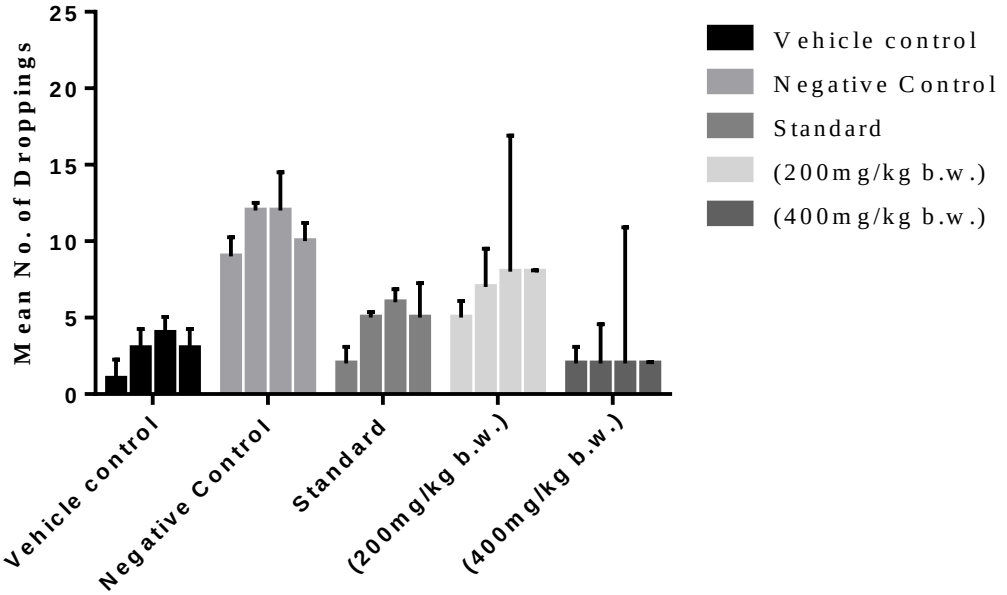

Fig-1: Effect of poly herbal formulation on Mean no. of droppings

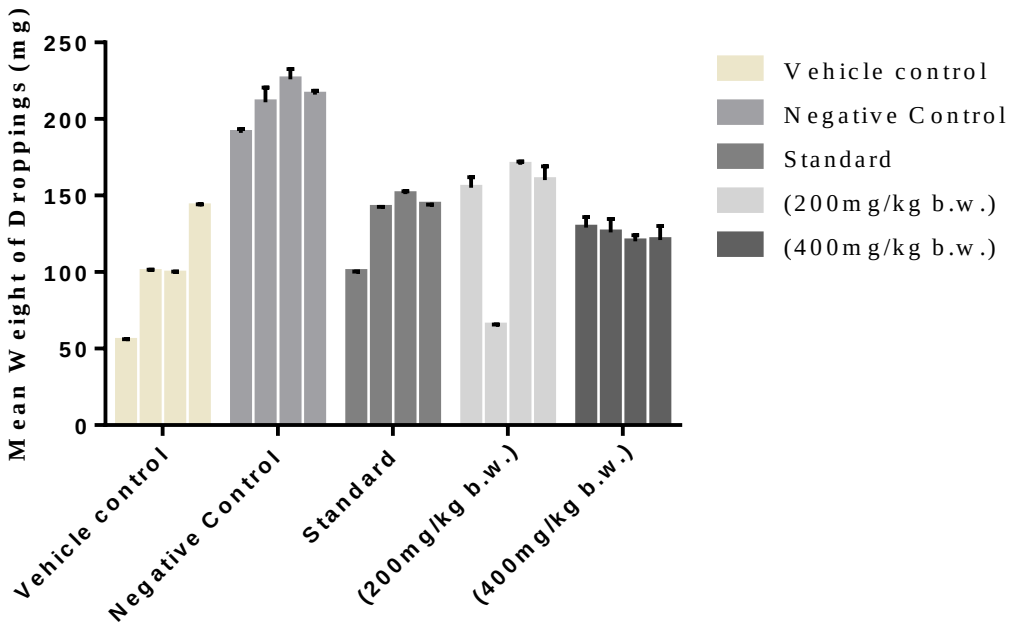

Fig-2: Effect of poly herbal formulation on Mean weight of Droppings

EFFECT OF POLY HERBAL FORMULATION ON GASTROINTESTINAL MOTILITY MODEL

Charcoal meal treated animals showed significant decrease in the % Intestinal transit when compared with that of vehicle control, pre-treatment with Lopramide 5mg/kg b.w and poly herbal formulation at 200 & 400 mg/kg b.w showed further reduce in % Intestinal transit significantly in a dose dependent manner when compare with negative control as shown in Table 4 .

Table 4: Effect of polyherbal formulations on small intestinal transit in rats

Group	Total length of intestine (cm)	Distance traveled by charcoal meal (cm)	% intestinal transit
Vehicle control	69± 1.34	60 ± 1.40	88.12 ± 1.23
Negative Control	66 ± 0.34	41 ± 4.90	61.53 ± 1.1
Standard	67 ± 1.98	19 ± 9.09	28.35 ± 0.9
Formulation (200mg/kg b.w.)	68 ± 1.56	27 ± 1.89	41.53 ± 3.0
Formulation (400mg/kg b.w.)	68 ± 0.89	23 ± 0.89	35.33 ± 3.0

Values are mean ± SEM N=6; $P < 0.05$ as compared to vehicle control and positive control by one way ANNOVA followed by Dunnet's test

DISCUSSION

The overall data in the study indicates that the prepared Poly herbal formulation is an effective antidiarrhoeal remedy. Castor oil is a bland vegetable oil obtained from the seeds of *Ricinus communis* It mainly contains triglyceride of ricinoleic acid which is a polar long chain fatty acid. The several mechanism which also explain the diarrheal property of castor are inhibition of intestinal Na^+ K^+ ATPase activity(18), thus reducing normal fluid absorption,

activation of adenyl cyclase or mucosal Camp – mediated active secretion, platelet activating factor,magnesium sulfate similarly causes an increase in the electrolyte secretion by creating an osmotic imbalance(19). Most recently nitric oxide has been claimed to contribute to the diarrhoeal effect of castor oil. And it also well reported that castor oil or its triglycerides hydrolyzed by lipase to glycerol and ricinoleic acid, which acts primarily in the small intestine to stimulate secretion of fluid and electrolytes and speed up the intestinal transit because it irritate the mucosa and stimulate intestinal contraction(20). It is also supported by the release of prostaglandins which enhance the fluid and electrolytes in small intestine due to the irritative and inflammatory action of ricinoleic acid of the intestinal mucosa. These prostaglandins then cause increase secretions into the lumen of the intestine as well as intestinal motility(21). The substance which reduces the inflammation and irritation or biosynthesis of prostaglandins could effectively reduce diarrhoea induced by castor oil Literature survey revealed that *Anogeissus latifolia* , *Elephantopus scaber* , *Euphorbia hirta* plants showed anti inflammatory activity(22). The antidiarrhoeal activity of polyherbal formulation against castor oil induced diarrhoea may be due to an antisecretary mechanism and anti-electrolyte permeability action. It is well known that antidiarrhoeal properties of medicinal plants were found to be due to tannins, flavonoids, alkaloids, saponins, sterols and /or terpenes(23). This was due to their ability to inhibit intestinal motility and hydro electrolytic secretions which are responsible to altered in this intestinal condition(24). It has been shown that flavonoids are able to inhibit the intestinal secretory response induced by prostaglandins E2 The ability of flavonoids to inhibit intestinal motility and block prostaglandin induced secretory process has been established the presence of the active principles like flavonoids and tannins in abundance in the poly herbal formulation is postulated to contributing factor responsible for its antidiarrhoeal activity.

CONCLUSION

The results of study demonstrate that Polyherbal formulation extract of *Anogeissus latifolia, Elephantopus scaber*, *Euphorbia hirta* possesses antidiarrhoeal property due to the decrease in the mean number of droppings and mean weight of droppings in castor oil induced model and decrease in % intestinal transit in charcoal meal model. Further studies are required to identify, isolate, characterize and evaluate the active principal responsible for antidiarrhoeal activity in the Poly herbal formulation.

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