

Development of Anti-Diarrhoeal Polyherbal Formulation “Antitrots”

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ABSTRACT

Objective: The study evaluated the antidiarrhoeal activity of polyherbal formulation “antitrots” prepared in solution form by using the hydroalcoholic fruits extract of *Phyllanthus emblica* and *Solanum nigrum* against castor oil induced diarrhoea.

Materials and Methods: Polyherbal solution was formulated using fruits extract of both the plants with excipients and evaluated for physicochemical and antidiarrhoeal activity. The standard drug used for study was loperamide.

Results: The studies revealed that at dose of 200 mg/kg polyherbal formulation showed a considerable reduction of diarrhoea but at a dose of 400 mg/kg showed significant reduction of diarrhoea compared to vehicle control group when subjected to castor oil induced diarrhoea and intestinal motility model.

Conclusion: The obtained results were nearly comparable with standard drug. Therefore the prepared formulation “antitrots” an effective antidiarrhoeal formulation.

Keywords: *Solanum nigrum*, *Phyllanthus emblica*, Polyherbal formulation, Antidiarrhoeal activity.

INTRODUCTION

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 drug reactions¹. Plants play a significant role and a valuable source of natural product obtained from them for maintaining human health for many years². The WHO suggested that medicinal plants would be the best source from which to develop a variety of medications. About 80% of population from developed countries

rely on use of medicines derived from medicinal plants. Such medicinal plants can be exploited because it has been reported that they are important sources of new chemical substances derived from them with potential therapeutic effects³. Phytomedicines are more often used in combination rather than in a single in order to get maximum benefits from their combined strength¹.

Diarrhoea is too frequent, often too precipitate passage of poorly formed stools.

In pathological term, it occurs due to passage of excess water in faeces⁴. From a mechanistic perspective, diarrhoea can be caused by an increased osmotic load within the intestine (resulting in retention of water within the lumen); Excessive secretion of electrolytes and water into the intestinal lumen; exudation of protein and fluid from the mucosa; and altered intestinal motility resulting in rapid transit (and decreased fluid absorption). In most instances, multiple processes are affected simultaneously, leading to a net increase in stool volume and weight accompanied by increase in fractional water content⁵. Diarrhea, in fact, claims the lives of 5-8 million infants and children worldwide⁶. To combat this problem, the world health organization (WHO) has initiated a diarrhoea disease control program to study traditional medicine practices and other related aspects, together with the evaluation of health education and preventive approaches^{7,8}.

Phyllanthus emblica and *Solanum nigrum* are the medicinal plants used in Ayurvedic System of Medicine for the treatment of digestive, ophthalmic, carminative, diuretics, antipyretic, diarrhoea⁹, cough, rat bite, bronchitis, fever and diarrhoea¹⁰. However no much characterization of this activity has been done on scientific basis to develop formulation from combined fruits extract of plants. Thus the present study was undertaken to explore the effects of hydroalcoholic fruits extract 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

Fruits of *Phyllanthus emblica* and *Solanum nigrum* were procured from local

market and same were authenticated by Dr. Seema Bhadhauria, Head of Department of Botany, R.B.S. College, Agra and sample specimen (Voucher No. of the Specimen: RBSC/2014/195) were deposited in the herbarium of the Department of Pharmacognosy, Anand College of Pharmacy, Agra for future reference.

Drugs and chemicals

Loperamide (Cipla Pharmaceutical Limited, Indore, India), castor oil (Triveni Aromatics and Perfumery Private Limited, Vapi, Gujarat). 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 °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*. The protocol was approved by the institutional animal ethical committee (registration number 1352/ac/10/CPCSEA) bearing no ACP/05/2014.

Preparation of hydroalcoholic extracts

The fruits were cleaned, shade dried, powder fine (400 gm.) About 200 gm each standardized powder of *Phyllanthus emblica* and *Solanum nigrum* fruits in the same concentration (1:1) were subjected to extraction by maceration with hydroalcohol (60%). After the extraction, the extract filtered and concentrated at room temperature^{11,12}. The hydroalcoholic extract was subjected to qualitative method of preliminary phytochemical analysis by adopting standard procedure^{13,14}.

Development of Polyherbal Formulation (PHF)

Oral solution containing hydroalcoholic extract and suitable excipients was prepared by dissolving all these ingredients in water^{15,16}. 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).

Standardization of polyherbal formulation (PHF)

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 studies¹⁷.

Stability studies

The stability studies were carried out to determine the quality of product during storage at different temperature (room temperature, 25°C, 45°C)¹⁸.

Toxicity studies

Toxicity studies were done as per the Organization for Economic Co-operation and Development (OECD), revised 423 guidelines¹⁹. Albino mice were fasting overnight prior to formulation administration. A total 6 animals of two groups, three in each group, received formulation from 300 mg/kg to 2000 mg/kg body weight. After each administration of dose food was withheld for further 3-4 hours. Animals were observed individually daily for a period of 14 days. Based on these studies the doses were selected for the evaluation of antidiarrhoeal activities. The LD₅₀ of the formulation falls under the class for values with no signs of acute toxicity till 2000 mg/kg body weight, so that 1/10th and 1/5th was taken as effective therapeutic

doses for formulation. The selected doses for formulation were 200 mg/kg and 400mg/kg body weight.

Administrations

Experimental animals were grouped into five, six each and were treated as given 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 and 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 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^{1,2}.

Gastrointestinal motility model

After 30 minutes, the intestinal motility was assessed by orally administering 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 transit²⁰.

Statistical analysis

Data were analysed using Graphpad Prism Software version 2.01 (GraphPad Software, La Jolla, USA). All the values were expressed as mean $\pm$ 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 of hydroalcoholic extract

The hydroalcoholic extract was subjected to qualitative method of preliminary phytochemical analysis that showed the presence of tannins, flavonoids, alkaloids, glycosides and carbohydrates.

Castor oil induced diarrhea

The wistar albino rats showed mean number of dropping recorded in the 1st, 2nd, 3rd and 4th hr. was 05 ± 1.1 , 07 ± 2.57 , 08 ± 8.91 and 08 ± 0.1 and the weight of dropping was 155 ± 6.9 , 165 ± 0.7 , 170 ± 2.1 and 160 ± 9.09 mg respectively for PHF (200mg/kg body weight). The polyherbal formulation (PHF) in solution form after administration at a dose of 200 mg/kg significantly ($F=11.2$; $P < 0.01$) reduce diarrhea as compared with negative control group. The PHF (200 mg/kg body weight) exhibited no significant effect ($F=20.0$; $P > 0.01$) on diarrhea activity when compared with vehicle control group. Whereas in comparison with positive control group (standard drug group) showed significant ($F=3.5$, $P < 0.05$; $P < 0.01$) antidiarrhoeal activity. PHF at a dose of 400 mg/kg body weight was found to be significant ($F=0.145$; $P < 0.01$; $P < 0.05$) when compared to vehicle control. PHF (400mg/kg body weight) also showed

significant ($F=0.146$; $P < 0.01$; $P < 0.05$) antidiarrhoeal activity when compared with positive control or loperamide group. It was also observed that statistical significant ($F=13.6$; $P < 0.05$) reduction in the number and weight of dropping in group receiving formulation 400 mg/kg body weight when compared with vehicle control but PHF (200 mg/kg body weight) exhibited no significant reduction ($F= 16$; $P > 0.05$). It also indicated that the PHF at the dose of 200 and 400mg/kg body weight showed significant reduction ($F= 6.3$; $P < 0.05$ and $F= 4.7$; $P < 0.05$) (Table 2).

Gastrointestinal motility model

The percent intestinal transit of PHF (200 mg/kg body weight) is reducing significantly ($F= 2.38$; $P < 0.05$) with control group and also exhibited significant ($F= 2.08$; $P < 0.05$) reduction when compared with positive control or standard drug group. The finding also indicates that the PHF (400 mg/kg body weight) showed significant reduction ($F= 2.21$; $P < 0.05$) as compared to control group and also exhibited significant reduction ($F= 2.31$; $P < 0.05$) with positive control group. The results indicated that both the PHF showed significant reduction $P < 0.05$ and were nearly comparable with standard drug group (Table 3).

DISCUSSION

The overall data in the study indicated “antitrots” as an effective antidiarrhoeal remedy. Castor oil is a bland vegetable oil obtained from the seeds of *Ricinus communis*⁴. 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 $\text{Na}^+ \text{K}^+$ ATPase activity, thus reducing normal fluid absorption²¹, activation of adenyl cyclase or mucosal Camp-mediated

active secretion, platelet activating factor²², magnesium sulfate similarly causes an increase in the electrolyte secretion by creating an osmotic imbalance. 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. 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 motility²³.

The substance which reduces the inflammation and irritation or biosynthesis of prostaglandins could effectively reduce diarrhoea induced by castor oil⁶. Literature survey revealed that *Phyllanthus emblica* and *Solanum nigrum* both plants showed anti inflammatory activity^{24,25}. The antidiarrhoeal activity of polyherbal formulation against castor oil induced diarrhoea may be due to an anti secretory 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, reducing sugar, sterols and /or terpenes. This was due to their ability to inhibit intestinal motility and hydro electrolytic secretions which are responsible to altered in this intestinal condition. It has been shown that flavonoids are able to inhibit the intestinal secretory response induced by prostaglandins E₂²². The ability of flavonoids to inhibit intestinal motility and block prostaglandin induced secretory process has been established²⁶. The fruits of

both plants are contains flavonoids as well as tannins^{27,28}. Therefore the presence of these active principles in abundance in the PHF is postulated to contributing factor responsible for its antidiarrhoeal activity. The functioning of gastrointestinal tract is largely regulated by the cholinergic and adrenergic activity and the alteration in any one of this can serve the powerful factor for induction of the diarrhea. *Phyllanthus emblica* and *Solanum nigrum* both the plants have anti cholinergic activity²⁹. Therefore this may be other contributing factors existing in polyherbal formulation responsive for its anti-diarrhoeal action and need to be explored further.

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Table 1. Formula for polyherbal formulation (PHF) in solution form

S. No.	Name of Ingredients	Quantity in grams
1	Hydroalcoholic extract	6 % w/w
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.)	100 ml

Table 2. Antidiarrhoeal activity of polyherbal formulations in castor oil induced diarrhea

Group	Mean number of dropping				Mean weight of droppings (mg)			
	1 st hour	2 nd hour	3 rd hour	4 th hour	1 st hour	2 nd hour	3 rd hour	4 th hour
Vehicle control	01 ± 1.25	03 ± 1.25	03 ± 1.05	04 ± 1.25	55 ± 1.2	100 ± 1.5	99 ± 1.25	143 ± 1.25
Negative Control	09 ± 1.25	11 ± 0.5	13 ± 2.5	10 ± 1.20	190 ± 2.5	210 ± 9.5	225 ± 6.5	215.46 ± 2.5
Positive Control	02 ± 1.1	05 ± 0.36	06 ± 0.86	06 ± 2.25	100 ± 0.3	145 ± 0.6	150 ± 1.9	145 ± 0.11
Formulation (200mg/kg b.w.)	05 ± 1.1	07 ± 2.57	08 ± 8.91	08 ± 0.1	155 ± 6.9	165 ± 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	130 ± 6.9	128 ± 8.7	120 ± 4.1	120 ± 9.09

Values are expressed in mean ± SEM, N=6; $P < 0.05$ as compared to vehicle control and positive control.

Table 3. Effect of polyherbal formulations on small intestinal transit in rats

Groups	Total length of intestine (cm)	Distance traveled by charcoal meal (cm)	% intestinal transit
Vehicle control	68 ± 1.34	60 ± 1.40	88.12 ± 1.23
Control group	65 ± 0.34	40 ± 4.90	61.53 ± 1.1
Standard group	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 Observation in each group, $P < 0.05$ compared to control groups.