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EVALUATION OF ANTIDIARRHOEAL EFFICACY OF POLYHERBAL FORMULATION

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ABSTRACT

Present work was designed to investigate the antidiarrhoeal potential of the polyherbal formulation prepared using extract of *Aegle marmelos*, *Punica granatum* and *Piper nigrum* with excipients. Antidiarrhoeal effect was evaluated against castor oil and magnesium sulphate induced diarrhoea model in mice. Antimotility effect was evaluated in charcoal meal test in mice while antisecretory effect was evaluated in castor oil induced intestinal secretions in mice. The studies revealed that at a dose of 8 ml/kg polyherbal formulation showed significant reduction of diarrhoea compared to vehicle control group when subjected to castor oil and magnesium sulphate induced diarrhoea, intestinal motility and castor oil induced intestinal secretions in mice. Polyherbal formulation was found to be the effective antidiarrhoeal remedy as the antidiarrhoeal efficacy of the polyherbal formulation was commensurable to standard drug Loperamide.

Keywords: Polyherbal formulation, antidiarrhoeal, antimotility and antisecretory

INTRODUCTION

Diarrhoea is characterized by the passage of loose, watery and unformed faeces leading to excess loss of fluid, salt and nutrients. If it is untreated can cause dehydration and electrolyte imbalance and ultimately leads to death **[1]**. Worldwide diarrhoea is a frequent medical problem accounts for more than 5-8 million deaths annually, majority of whom are children and infants below 5 years old especially in tropical and subtropical poor

countries. Even in the wealthy industrialized countries acute diarrhoea in children leads to significant morbidity and mortality. Chronic diarrhoea is also a major problem in some other clinical situations **[2, 3]**.

Synthetic drugs are available in the market for the treatment of diarrhoea have many undesirable effects like nausea, dryness of the mouth, drowsiness, diziness, restlessness, allergy, pyrexia, constipation, and abdominal cramps **[4]**. Since the herbal products possess less side effects and better tolerance when compared to the synthetic drugs, there is a greater global interest in natural drugs derived from plant and herbal sources **[5]**. In order to counter the problems of diarrhoea globally, the World Health Organization in Diarrhoeal Disease Control Program has given a special importance on the use of traditional medicines in the management and control of diarrhoea as medicinal herbs constitute an indispensable component of the traditional medicine practiced worldwide because of economical viability, accessibility and ancestral experience. Thus, there is a call for the development of a new, effective, safe, and low-cost antidiarrhoeal agent **[6]**.

Aegle marmelos, *Punica granatum*, and *Piper nigrum* are the medicinal plants used in Ayurvedic System of Medicine as digestive, appetizer, antipyretic, memory improving, astringent, aromatic, stimulant, stomachic, carminative bio-availability enhancer, antidiarrhoeal and antidysenteric **[7, 8, 9]**. In spite of that not much characterization of this activity has been done on scientific basis to develop formulation from combined extracts of these plants. Hence the present study was undertaken to explore the effects of extracts of medicinal plants together as polyherbal formulation in solution form against castor oil and magnesium sulphate induced diarrhea and compared these effects with Loperamide as standard marketed formulation.

MATERIALS AND METHODS

Drugs and chemicals

Loperamide (Cipla Pharmaceutical Limited), Castor oil (Paras Chemical Industries) and Magnesium sulphate (Merck). All other chemicals and reagents used in the studies were of analytical or laboratory grade.

Plant collection and identification

The selected crude plant materials *Aegle marmelos* (fruit), *Punica granatum* (seeds) and *Piper nigrum* (fruit) were procured from local market and authenticated by Herbarium incharge, Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad (M.S.), India, where a voucher specimen has been deposited.

Preparation of extracts

The plant materials were cleaned, shade dried and powdered by mechanical means. 100 gm standardized powder of each Aegle *marmelos* (fruit), *Punica granatum* (seeds*)* and *Piper nigrum* (fruit), were subjected to extraction by maceration with hydroalcohol (60%). Thereafter the extract was filtered and concentrated at room temperature.

Development of Polyherbal formulation

Oral solution containing hydroalcoholic extract (6% w/w) with suitable excipients like butylated hydroxyanisole (0.2 %), Sorbic acid (0.2 %), sodium saccharin (0.1 %), Chocolate flavor (q.s.) was prepared by dissolving all these ingredients in water up to 100 ml. Polyherbal Formulation in solution form was standardize by using different organoleptic characters such as color, odor and taste as well as physicochemical parameters like pH, visibility in light and gas evolution studies.

Animals

Swiss albino mice of either sex, weighing between 25-35 gm obtained from VIPER, Pune, were used for the experiments. Mice were kept in standard environmental condition, fed standard laboratory diet and water ad libitum. All experiments were performed after an overnight fast. The protocol was approved by the institutional animal ethical committee (GCPA/IAEC/2011/235).

Acute toxicity study

Polyherbal Formulation studied for acute oral toxicity as per revised OECD guidelines number 423. Polyherbal Formulation was devoid of any toxicity up to 20 ml/kg in albino mice by oral route.

Experimental procedures for antidiarrhoeal activity

Effect of Polyherbal Formulation on castor oil induced diarrhoea

Six mice of each group were treated as outlined below:

Group 1 (Vehicle control group): Distilled water 10 ml/kg, p.o.,

Group 2 (Standard group): Loperamide 2 mg/kg, p.o.,

Group 3 (Test group): Polyherbal Formulation 2, 4 and 8 ml/kg p.o.,

Castor oil (0.2 ml) was administered to each mouse after 30 minutes of above treatment. The animals were then placed under separate glass funnels, with the floor lined with blotting paper, for observation for 4 h. The parameters observed were: onset of diarrhoea, total number of faecal output, and number of wet faeces **[10]**.

Effect of Polyherbal Formulation on magnesium sulfate induced diarrhoea

Protocol similar to castor oil induced diarrhoea was followed. Magnesium sulfate was given in the dose of 2 g/kg to the animals 30 minutes after pre-treatment with **[11]**.

Group 1 (Vehicle control group): Distilled water 10 ml/kg, p.o.,

Group 2 (Standard group): Loperamide 2 mg/kg, p.o.,

Group 3 (Test group): Polyherbal Formulation 2, 4 and 8 ml/kg p.o.,

Effect of Polyherbal Formulation on castor oil induced gastrointestinal motility

Six mice were allotted to different groups. Treatment was then carried out as outlined below:

Group 1 (Vehicle control group): Distilled water 10 ml/kg, p.o.,

Group 2 (Standard group): Loperamide 2 mg/kg, p.o.,

Group 3 (Test group): Polyherbal Formulation 2, 4 and 8 ml/kg p.o.,

After 30 minutes of above treatment, each animal was given 0.2 ml castor oil orally. All animal were given 0.2 ml of charcoal meal (3% charcoal in 5 % gum acacia) orally, 30 minutes after castor oil administration. Animals were sacrificed 30 minutes after administration of charcoal meal and the small intestine was immediately isolated. Peristaltic index was expressed as percentage of the distance travelled by the charcoal meal relative to the total length of the small intestine **[12]**.

Effect of Polyherbal Formulation on castor oil induced small intestinal secretions

Effect of Polyherbal Formulation on intestinal secretion was indirectly studied by enteropooling assay.

Group 1 (Vehicle control group): Distilled water 10 ml/kg, p.o.,

Group 2 (Standard group): Loperamide 2 mg/kg, p.o.,

Group 3 (Test group): Polyherbal Formulation 2, 4 and 8ml/kg p.o.,

Castor oil (0.2 ml, p.o.) was administered to each mouse after 30 minutes of above treatment. The mice were sacrificed 30 minutes after castor oil administration and the entire small intestine from each animal was weighed and their group average was calculated. The difference in the weight of intestine in castor oil treated group and control was considered as the castor oil induced accumulation of intestinal fluid **[13]**.

Statistics

The results of all experiments were reported as mean ± S.E.M. Statistical analysis was carried out using Student's 't'-test. A level of

significance of $P < 0.05$ was regarded as statistically significant.

RESULTS

Antidiarrhoeal effect of Polyherbal Formulation on castor oil induced diarrhoea

Castor oil produced copious diarrhoea in all the mice in control group. Pretreatment of mice with the different doses of Polyherbal Formulation caused a significant dose dependent delay in the onset of copious diarrhea and decrease in the number of wet stools as shown in **Table 1**. Polyherbal Formulation showed 52.95%, 73.52%, 86.76% inhibition of diarrhoea at doses of 2 ml/kg and 4 ml/kg and 8 ml/kg respectively. Loperamide showed 89.76%, inhibition of diarrhoea at dose of 2 mg/kg.

Antidiarrhoeal effect of Polyherbal Formulation on magnesium sulphate induced diarrhoea

Administration of magnesium sulphate to the mice in control group produced diarrhoea. Polyherbal Formulation showed a significant dose dependent delay in the onset of diarrhea and reduction of number of wet stools as shown in **Table 2**. Polyherbal Formulation produced 59.05%, 75.39%, 88.58% inhibition of diarrhoea at doses of 2 ml/kg and 4 ml/kg and 8 ml/kg respectively. Loperamide produced 90.15%, inhibition of diarrhoea at dose of 2 mg/kg.

Effect of Polyherbal Formulation on castor oil induced gastrointestinal motility

The results revealed that Polyherbal Formulation significantly inhibited the castor oil induced gastrointestinal transit of charcoal in mice as shown in **Table 3**. Polyherbal Formulation inhibited the castor oil induced gastrointestinal transit of charcoal in mice by 16.52%, 30.64% and 38.01% at doses of 2 ml/kg and 4 ml/kg and 8 ml/kg, respectively. Loperamide (2 mg/kg) showed 40.98% inhibition of gastrointestinal transit.

Effect of Polyherbal Formulation on castor oil induced small intestinal secretion

Polyherbal Formulation showed the inhibition of castor oil induced intraluminal accumulation of fluid as shown in **Table 4**. Polyherbal Formulation inhibited the castor oil induced intraluminal accumulation of fluid by 44.03%, 62.62% and 74.55% at doses of 2 ml/kg and 4 ml/kg and 8 ml/kg, respectively. Loperamide (2 mg/kg) showed 79.25% inhibition of castor oil induced intraluminal accumulation of fluid.

Table 1: Effect of Polyherbal Formulation on castor oil induced diarrhoea in mice.

Group	Dose (/kg)	Onset of diarrhoea (min)	Number of wet stools	% Inhibition
Control		49 ± 2.06	11.33 ± 0.21	
Formulation	2 ml	79 ± 2.47	5.33 ±0.28	52.95
Formulation	4 ml	107 ± 3.72	3.00 ± 0.30	73.52
Formulation	8 ml	174 ± 4.81	1.5 ± 0.21	86.76
Loperamide	2 mg	195 ± 5.36	1.16 ± 0.16	89.76

Values are mean ± standard error of mean. $P < 0.05$ vs. control, student's '*t*' test

Table 2: Effect of Polyherbal Formulation on magnesium sulphate induced diarrhoea in mice

Group	Dose (/kg)	Onset of diarrhoea (min.)	Number of wet stools	% Inhibition
Control		54 ± 2.73	10.16 ± 0.33	
Formulation	2 ml	81 ± 3.71	4.16 ±0.28	59.05
Formulation	4 ml	132 ± 4.18	2.5 ± 0.21	75.39
Formulation	8 ml	210 ± 5.92	1.16 ± 0.22	88.58
Loperamide	2 mg	218 ± 5.25	1.00 ± 0.16	90.15

Values are mean ± standard error of mean. $P < 0.05$ vs. control, student's '*t*' test

Table 3: Effect of Polyherbal Formulation on castor oil induced intestinal transit in mice.

Group	Dose (mg/kg)	Percent intestinal transit	% Inhibition
Control		78.42 ± 2.38	
Formulation	2 ml	65.46 ± 1.72	16.52
Formulation	4 ml	54.39 ± 2.95	30.64
Formulation	8 ml	48.61± 1.54	38.01
Loperamide	2 mg	46.28 ± 2.72	40.98

Values are mean ± standard error of mean. $P < 0.05$ vs. control, student's '*t*' test

Table 4: Effect of Polyherbal Formulation on castor oil induced intraluminal fluid accumulation in mice.

Experimental Group	Dose (mg/kg)	Weight of small intestine (mg)	Castor oil induced intraluminal fluid (mg)	% Inhibition
Normal		1078 ± 22		
Control		1589 ± 39	511 ± 26	
Formulation	2 ml	1364 ± 34	286 ± 19	44.03
Formulation	4 ml	1269 ± 27	191 ± 13	62.62
Formulation	8 ml	1208 ± 19	130 ± 11	74.55
Loperamide	2 mg	1184 ± 22	106 ± 09	79.25

Values are mean ± standard error of mean. $P < 0.05$ vs. control, student's '*t*' test

DISCUSSION

Polyherbal formulation or herb-herb combination has been used all throughout the world due to its medicinal and therapeutic application **[14]**. The results of this study indicate that the prepared Polyherbal formulation is an effective antidiarrhoeal remedy. Antidiarrhoeal activity of Polyherbal formulation was analysed by using castor oil and magnesium sulphate induced diarrhoea, charcoal meal test and castor oil induced enteropooling models.

Ricinoleic acid, the active component of castor oil is mainly responsible for the induction of diarrhoea. After the ingestion of castor oil, ricinoleic acid is liberated in the small intestine by pancreatic lipases. Prostaglandins are released due to irritating and inflammatory actions of ricinoleic acid on the intestinal mucosa. It causes changes in

electrolyte transport and increase in the permeability of the mucosal cells results in to hypersecretory response along with increase in peristaltic activity results in diarrhoea **[15]**. Thus the diarrhoea induced by castor oil is secretory diarrhea because of hypersecretory action of recinolic acid. Polyherbal formulation successfully inhibited the castor oil induced diarrhoea by reducing the total number of wet faeces indicates its antisecretory mechanism.

An osmotic property of the magnesium sulphate produces the diarrhoea by preventing reabsorption of water ions which leads to increase in the volume of the intestinal content. It enhances the liberation of cholecystokinin from the duodenal mucosa results in to increase in the secretion and motility of small intestine which prevents the reabsorption of sodium chloride and water **[16]**. Polyherbal formulation reduced the diarrhoea in this model may be due to increase in the absorption of water and electrolyte from the gastrointestinal tract.

Effect of Polyherbal formulation on the motility of intestine is evaluated by charcoal meal test in mice. Polyherbal formulation was found to be the inhibitor of intestinal motility. Castor oil effectuate permeability changes in the intestinal mucosa membranes to water and electrolytes which results in to the increase in the fluid and watery luminal content that flow rapidly through intestines **[17]**. Polyherbal formulation inhibited the castor oil induced intestinal fluid accumulation. These results indicate that Polyherbal formulation produced antidiarrhoeal effect through its antimotility and antisecretory effect. The delay in the gastrointestinal transit prompted by the Polyherbal formulation might have imparted to their antidiarrhoeal activity by enabling a greater time for absorption.

CONCLUSION

In present study Polyherbal formulation has shown analogous antidiarrhoeal activity to the standard drug Loperamide by inhibiting the castor oil and magnesium sulphate induced diarrhoea, intestinal motility and castor oil induced intestinal secretions in mice.

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