

Original Article

ANTIDIARRHOEAL EVALUATION OF AQUEOUS EXTRACTS OF GARCINIA INDICA & CUMINUM CYMINUM AND A POLYHERBAL FORMULATION

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ABSTRACT

Objective: The objective of the study was to assess and verify the traditional claims of use of *Garcinia indica* and *Cuminum cyminum* in diarrhoea. One of the marketed herbal tablet was also assessed for its antidiarrhoeal activity.

Methods: In the present study, the aqueous extracts of *G. indica*, *C. cyminum* and Bi-Quinol tablets were evaluated *in vivo* in mice by castor oil-induced diarrhoea method.

Results: Both *G. indica* and *C. cyminum* at doses of 200 mg/kg and 400 mg/kg significantly reduced the total number of faeces ($P < 0.001$) as well as diarrhoeic faeces ($P < 0.05$ and $P < 0.01$) in a dose-dependent manner when compared with the control. Bi-Quinol tablets at the dose of 100 mg/kg reduced the total number of faeces ($P < 0.001$) as well as diarrhoeic faeces ($P < 0.01$) when compared with the control. The reduction in total number of faeces was greater in *C. cyminum* as compared to *G. indica* at both the doses tested. The percent protection with respect to total number of wet faeces against diarrhoea was found to be 87.5 for Loperamide, 66.67 and 62.5 for *G. indica* (400 mg/kg) and *C. cyminum* (400 mg/kg) respectively and 70.83 for Bi-Quinol (100 mg/kg). At lower doses of 200 mg/kg, *G. indica* and *C. cyminum* showed 54.17% and 45.83% percent protection respectively. The plants and the Bi-Quinol tablets extracts showed the presence of alkaloids, carbohydrates, flavonoids, phenols, tannins, phytosterols and saponins. The results were found to be statistically significant with P value set at 95% confidence interval.

Conclusion: The results obtained showed that *G. indica*, *C. cyminum* and the Bi-Quinol tablets decreased the number of wet faeces as well as the total number of faeces. It was concluded that the extracts might have exerted antidiarrhoeal activity through the anti-hypersecretory mechanism. A decrease in contractility of the ileum (smooth muscle) may be another mechanism responsible for the protective action of the extracts against diarrhoea.

Keywords: *Garcinia indica*, *Cuminum cyminum*, Antidiarrhoeal activity.

INTRODUCTION

The World Health Organisation (WHO) statistics states that diarrhoea is the cause of 10% of all child deaths globally, under the age five [1]. Diarrhoea affects people of all ages, due to several causes- dehydration, malnutrition, infection (bacterial, viral and parasitic organisms), zinc deficiency, functional bowel disorders, food intolerances & sensitivities and reaction to medicines. Severe diarrhoea becomes life-threatening, particularly in young children and people who are malnourished or have impaired immunity. Other symptoms that accompany diarrhoea are cramping, abdominal pain, nausea and loss of bowel control [2]. Modern medication is still a challenge for a vast majority of the population in the third world countries. Traditional herbal remedies are an integral component of people's cultural beliefs and also represent a part of struggle of the people to meet essential drug needs. The dependence on plants as source of medicine is still relied upon in many parts of the world. The knowledge of traditional medicine and its practice has been passed down through generations. The antidiarrhoeal activity of medicinal plants has been attributed to presence of various constituents like alkaloids, flavonoids, saponins and tannins [3, 4, 5, 6]. *Cuminum cyminum* Linn, Apiaceae (seeds) & *Garcinia indica* Choisy, Clusiaceae (rinds) are the some plants of natural origin used in folklore medicine to treat various gastrointestinal disorders like diarrhoea, abdominal pain, flatulence and as stomachics [7, 8]. These plants are commonly used household spices having medicinal and commercial importance. To date, the folklore claim of antidiarrhoeal activity of these plants has not been validated scientifically/pharmacologically. Based on market survey, the herbal formulation Bi-Quinol was selected for evaluation of antidiarrhoeal activity. The antidiarrhoeal activity for the components of the tablet namely *Aegle marmelos* (fruit), *Holarrhena antidysenterica* (bark and seed), *Berberine aristata* has been reported [9, 10, 11, 12]. Therefore; the objective of this present study was to assess and verify the traditional claim of antidiarrhoeal activity of above plant extracts and the tablet Bi-Quinol.

MATERIALS AND METHODS

Preparation of extract

The dried rinds of *G. indica* fruit and dried *C. cyminum* seeds were procured from the local market. Both the plant parts were authenticated by Dr. Ganesh N. Iyer at Ramnarain Ruia College, Mumbai, Maharashtra. The dried rinds of kokum fruit and dried cumin seeds were coarsely powdered. A quantity of 100 gms of each of the above mentioned powders were separately refluxed in a round bottom flask for 3 hours using 400 ml distilled water. The extracts obtained were filtered. The extracts were concentrated in a rotary evaporator. The extracts were then subject to qualitative phytochemical tests for identification of phytoconstituents [13]. Samples of a marketed herbal antidiarrhoeal tablet Bi-Quinol were purchased from the local pharmacy.

Drugs

Castor oil (Ashwin Fine Chemicals and Pharmaceuticals), Loperamide hydrochloride – Cipla Pharmaceuticals Ltd, Bi-Quinol (Bhairavi Pharmaceuticals), Tween 80 (S.D. Fine Chem Ltd.)

Animals

Mus musculus "Swiss albino" mice of either sex, weighing 25–45 gm; obtained from Haffkine Institute, Mumbai, were used for the experiments. The animals were acclimatized to standard environmental conditions at $22 \pm 2^\circ\text{C}$ on a 12 hour light–dark cycle with free access to pellet food and water *ad libitum* for five days prior to the experiments. All experiments were performed after an overnight fast of 12 hours.

Ethics

The study was approved by Institutional Animal Ethical Committee of SPP School of Pharmacy & Technology Management, SVKMS's NMIMS, Vile Parle (w), Mumbai (CPCSEA/ IAEC/SPTM/P-07/2014).

Dose selection

The LD₅₀ cut off value reported for *Garcinia indica* in Wistar rats is 2000 mg/kg [14]. *Cuminum cyminum* at a dose of 2000 mg/kg did not cause any morbidity or mortality in rats during the 14 days of observation period [15]. Therefore, for the evaluation of anti-diarrhoeal activity, two doses were selected; first was one-tenth of the LD₅₀ cut off value and second was twice that of one-tenth dose (200 mg/kg & 400 mg/kg P.O. single dose for both the extracts). A dose of 100 mg/kg was selected for the tablet Bi-Quinol.

Antidiarrhoeal Activity

Castor oil-induced diarrhoea method

The method described by Shobha and Thomas (2001) was followed with minor modification [9, 16]. The animals were divided into control and test groups of five each. Each animal was placed in an individual cage, the floor of which was lined with blotting paper. The floor lining was changed every hour. The control group received vehicle 1% Tween 80 in water at the dose of 10 ml/kg. Thirty minutes following the administration of loperamide and test extracts, each animal was administered 0.3 ml castor oil orally. The parameters observed for a period of 4 hours were: onset time of diarrhoea, the total number of faeces as well as the number of diarrhoeic faeces excreted by the animals in 4 hours and the total weight of diarrhoeal stools in that period of time. A numerical score based on stool consistency were assigned as follows: normal stool=1, semi-solid stool=2 and watery stool=3. The onset was measured as the time interval in minutes between the administration of castor oil and the appearance of the first diarrhoeal stool (wet faeces that leave a halo on the filter paper). The percent protection against diarrhoea was calculated with respect to number of wet faeces.

Grouping of animals

Group I (Control): Vehicle Control (1% tween 80 in water, 10 ml/kg, p.o.)

Group II (GI AE 200): *Garcinia indica*, aqueous extract (dose: 200 mg/kg, p.o.)

Group III (GI AE 400): *Garcinia indica*, aqueous extract (dose: 400 mg/kg, p.o.)

Group IV (CC AE 200): *Cuminum cyminum*, aqueous extract (dose 200 mg/kg, p.o.)

Group V (CC AE 400): *Cuminum cyminum*, aqueous extract (dose: 400 mg/kg, p.o.)

Group VI (Bi-Quinol 100): Bi-Quinol, aqueous extract (dose: 100 mg/kg, p.o.)

Group VII (Loperamide 3): Loperamide (dose: 3 mg/kg, p.o.)

Statistical analysis

The data obtained was subjected to one way of analysis of variance (ANOVA) followed by Dunnett's-Comparison Test using Graph Pad Prism software. A 'P' value less than 0.05 was considered to be statistically significant. All the values are expressed as Mean ± SEM.

RESULTS

Phytochemical screening

Preliminary phytochemical tests of the aqueous extracts of *G. indica* and *C. cyminum* and Bi-Quinol revealed the presence of alkaloids, carbohydrates, flavonoids, phenols and tannins and absence of proteins & amino acid. The presence of phytosterols & saponins were noted for *C. cyminum* & Bi-Quinol extracts but were absent in *G. indica*. The presence of glycosides was observed only for the extract of Bi-Quinol tablets (Table 1).

Castor oil-induced diarrhoea

In the experimental animals subject to castor oil-induced diarrhoea, both the aqueous extracts of *Garcinia indica* and *Cuminum cyminum* at doses of 200 mg/kg and 400 mg/kg significantly reduced the total number of faeces (P<0.001) as well as diarrhoeic faeces (P<0.05 and P<0.01) in a dose-dependent manner when compared with the control. Bi-Quinol tablets at the dose of 100 mg/kg reduced the total number of faeces (P<0.001) as well as diarrhoeic faeces (P<0.01) when compared with the control. The reduction in total number of faeces was greater in *C. cyminum* as compared to *G. indica* at both the doses tested. The percent protection with respect to total number of wet faeces against diarrhoea was found to be 87.5 for Loperamide, 66.67 and 62.5 for *G. indica* (400 mg/kg) and *C. cyminum* (400 mg/kg) respectively and 70.83 for Bi-Quinol (100 mg/kg). At lower doses of 200 mg/kg, *G. indica* and *C. cyminum* showed 54.17% and 45.83% percent protection respectively. The results were found to be statistically significant with P value set at 95% confidence interval.

Table 1: Results of phytochemical screening of the aqueous extracts of *Garcinia indica* and *Cuminum cyminum* and Bi-Quinol tablets

Sr. No.	Tests	<i>G. indica</i>	<i>C. cyminum</i>	Bi-Quinol tablets
1	Alkaloids	+	+	+
2	Carbohydrates	+	+	+
3	Flavonoids	+	+	+
4	Glycoside	-	-	+
5	Phenols	+	+	+
6	Proteins & amino acids	-	-	-
7	Phytosterols	-	+	+
8	Saponins	-	+	+
9	Tannins	+	+	+

(+) = Present, (-) = Absent

Table 2: Evaluation of anti-diarrhoeal activity of *Garcinia indica* and *Cuminum cyminum* and Bi-Quinol tablets against castor oil-induced diarrhoea

Treatment	Dose (mg/kg)	Onset time of diarrhoea (mins)	Total number of faeces	Number of wet faeces	Total weight of wet faeces (mg)
Control	10 ml/kg	48.4 ± 5.25	11.8 ± 0.37	4.8 ± 0.37	722.4 ± 14.79
<i>Garcinia indica</i>	200	65.4 ± 5.88 ^{ns}	6.6 ± 0.40 ^{***}	2.2 ± 0.86*	568.6 ± 16.28 ^{***}
<i>Garcinia indica</i>	400	109.8 ± 4.08 ^{***}	6.4 ± 0.24 ^{***}	1.6 ± 0.60 ^{**}	438 ± 12.85 ^{***}
<i>Cuminumcyminum</i>	200	75 ± 9.75 ^{ns}	8.2 ± 0.86 ^{***}	2.6 ± 0.24*	584.2 ± 20.68 ^{***}
<i>Cuminumcyminum</i>	400	88 ± 10.56 ^{**}	6 ± 0.32 ^{***}	1.8 ± 1.06 ^{**}	526.6 ± 20.34 ^{***}
Bi-Quinol	100	57 ± 4.64 ^{ns}	8 ± 0.89 ^{***}	1.4 ± 0.40 ^{**}	506.4 ± 9.22 ^{***}
Loperamide	3	220.8 ± 8.07 ^{***}	1.6 ± 0.24 ^{***}	0.6 ± 0.60 ^{***}	348.6 ± 10.23 ^{***}

Values are expressed as Mean ± SEM, n=5, *P < 0.05, **P < 0.01, ***P < 0.001, ns=not significant when P > 0.05.

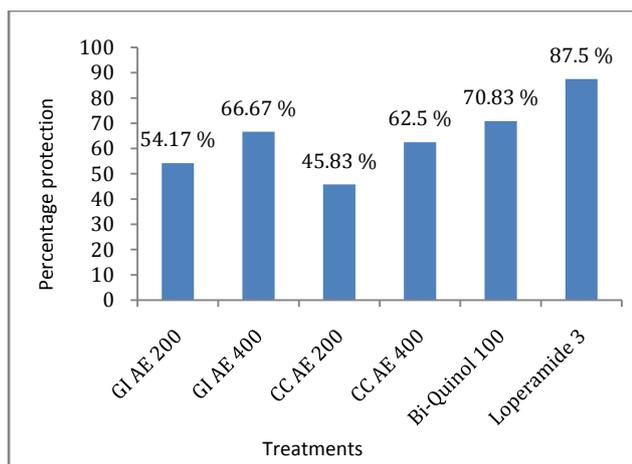


Fig. 1: Percentage protection offered by the aqueous extracts of *Garcinia indica* and *Cuminum cyminum* and Bi-Quinol tablets against castor oil-induced diarrhoea

DISCUSSION

Castor oil-induced diarrhoea method is considered as a suitable model of the complex hypersecretion process. Ricinoleic acid formed by hydrolysis in the upper small intestine, causes irritation and inflammation of the intestinal mucosa leading to prostaglandin release. This induces change in the net secretion of water and electrolyte transport, resulting in a hypersecretory response in the small intestine [17, 18]. The total number of wet faeces was significantly lower ($P < 0.05$ and $P < 0.01$) in the treatment groups as compared to the control. The Bi-Quinol treated group showed a 70.83% decrease, whereas *G. indica* and *C. cyminum* at a dose of 400 mg/kg showed a 66.66 and 62.5% decrease in the total number of wet faeces respectively. There was observable difference in the total number of faeces between the two treatment groups of *C. cyminum* (2.2 at $P < 0.001$). This difference was relatively larger than that obtained in the groups treated with *G. indica* (0.2 at $P < 0.001$). These findings suggest the anti-hypersecretory mechanism of the aqueous extracts of *G. indica*, *C. cyminum* and the Bi-Quinol tablets against castor oil-induced diarrhoea method. In the *in vitro* antispasmodic studies conducted, *G. indica*, *C. cyminum* and the Bi-Quinol tablets were also found to inhibit Acetylcholine and Histamine induced contraction in the ileum. Thus the extracts may be exerting an effect by blocking the muscarinic receptors which are located on glandular/smooth muscle thus inhibiting the inositol trisphosphate (IP_3)/diacylglycerol (DAG) transducer mechanism, thereby decreasing the contractility [19]. The difference in the onset of diarrhoea was significant only in the groups treated with *G. indica* and *C. cyminum* at a dose of 400 mg/kg ($P < 0.001$ and $P < 0.01$ respectively) as compared to the control.

CONCLUSION

The extracts of the *G. indica* and *C. cyminum* and the Bi-Quinol tablets showed the presence of alkaloids, carbohydrates, flavonoids, phenols, tannins, phytosterols and saponins. The extracts of *G. indica* and *C. cyminum* as well as Bi-Quinol were found to inhibit both Acetylcholine as well as Histamine induced contractions in the isolated ileum experiments suggesting that the extracts may possess antispasmodic activity. The results obtained from the anti-diarrhoeal study indicate that *G. indica* and *C. cyminum* and the Bi-Quinol tablets decreased the total number of wet faeces as well as the total number of faeces. These findings indicate that the extracts may exert anti-diarrhoeal activity through a decrease in the contractility and antihypersecretory mechanism. Further evaluation to study the effect of the extracts on the other mechanisms that might be responsible for anti-diarrhoeal effect like the release of prostaglandin E_2 and nitrous oxide pathway can be taken up based on these results [18, 20]. Further isolation of the active principles of the plants could ascertain the true anti-diarrhoeal potential, with specific mechanism of action.

CONFLICT OF INTEREST: Nil

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