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Evaluation of antidiarrheal activity of *Anjanavaerathi chooranam*

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Abstract
Diarrhea is a serious problem affecting 3.5 billion people per year around the world. Indian literature from siddha texts and other books claim the potency of several plants in the treatment of diarrhea. Researches are doing experiment to establish the relation between the claimed action and observed pharmacological activities. The present study was undertaken to evaluate theanti diarrheal activity of *Anjanavaerathi chooranam* using different experimental model such as castor oil induced diarrhea GIT motility and enteropooling. It is hoped the article would stimulate future clinical studies because of the paucity of knowledge in this area.

Keywords: siddha system, *Anjanavaerathi chooranam*, antidiarrheal activity

1. Introduction
Due to unhygienic livelihood condition, peoples of the third world counties are very prone to several common diseases including diarrhea. According to the World Health Organization (WHO), diarrhea is the second leading reason of death of children less than five years of age [1]. During diarrhea, the normal bowel movement becomes changed, which results in an increase in water content, volume, or frequency of the stools [2]. The common reason for causing diarrhea is gastrointestinal infection by various types of bacteria, virus, and parasites. This infection can be spread out through food, drinking water, and unhygienic environment. Besides other pathological conditions, usually four major mechanisms are responsible for pathophysiology in electrolyte and water transportation, such as increasing of luminal osmolarity and electrolyte secretion, decreasing of electrolyte absorption, and acceleration of intestinal motility ultimately decreasing of transition time [3].
Despite the efforts of international organizations to control this disease, still the incidence of diarrhoea is very high [4]. Some antibiotics are used as antidiarrheal drug, but these drugs sometimes show some adverse effects and microorganisms are tend to develop resistance towards them [5]. Therefore the search for safe and more effective agents from plant origin has continued to be an important area of active research. However, plants have long been a very important source of new drugs. Many plant species have been screened for substances with therapeutic activity. For the treatment of diarrhea, medicinal plants are a potential source of antidiarrheal drugs [6]. Moreover, many international organizations including WHO have encouraged studies pertaining to the treatment and prevention of diarrheal diseases using traditional medical practices [7–9]. At present, around 25% of drugs are isolated from plants and there are numerous evidences available about the use of medicinal plants including their pharmacological and biochemical properties [10].
However, there are no available medicinal claims about antidiarrheal activity of siddha formulation *Anjanavaerathi chooranam* That is why we are interested in examining the antidiarrheal activity of siddha formulation *Anjanavaerathi chooranam.*

Drugs and Chemicals
Castor oil *(WELL's Heath Care, Spain*), 0.9% sodium chloride solution (normal saline) (Orion Infusions Ltd., Bangladesh), charcoal meal (10% activated charcoal in 5% gum acacia), and loperamide (*Square Pharmaceuticals Ltd., Bangladesh*) were used for antidiarrheal activity test, and dimethyl sulfoxide (DMSO*) (Sigma-Aldrich, USA).*

Experimental animals
Albino Wistar rats (180–220 g) were collected from central animal house, K. M. College of pharmacy, Madurai, which were used as the experimental model for investigation of the antidiarrheal activity. All the animals housed under standard laboratory condition at °C and 12 h light: dark cycle, acclimatized for 10 days before experiment. Standard diet and water were provided constantly.

Castor oil-induced diarrhea in rats
Rats of both sexes (180–220 g) were fasted for 18 hours. The selected rats for castor oil-induced diarrheal test were divided into four groups. Group I was given normal saline (2 mL/kg) orally as control group and Group II received loperamide (5 mg/kg) as standard group. Groups III-IV received *Anjanavaerathi chooranam* (200 and 400 mg/kg b. wt. i.p., resp.). After 1 h, all groups received castor oil 1 mL each orally. Then they were placed in cages lined with adsorbent papers and observed for 4 h for the presence of characteristic diarrheal droppings. 100% was considered as the total number of feces of control group [11]. The activity was expressed as % inhibition of diarrhea. The percent (%) inhibition of defecation was measured using the following formula: where is mean

number of defecation time caused by castor oil and is mean number of defecation time caused by drug.

Castor oil-induced enteropooling

Castor oil-induced enteropooling test helps to determine the prevention of fluid accumulation ability of drug. Here also rats of both sexes (180-220 g) were fasted for 18 hours. The selected rats for this test were divided into four groups . Group I (controlled group) was given normal saline (2 mL/kg) orally while Group II (standard group) received loperamide (5 mg/kg). The rest of the groups (Groups III-IV) received *Anjanavaerathi chooranam* (200 and 400 mg/kg b. wt. i.p. resp.). After 1 h, all groups received castor oil, 1 mL orally per animal. Two hours later, all rats were sacrificed and the small intestine from the pylorus to the caecum was isolated. The intestinal contents were collected by milking into a graduated tube and their volume was measured [12].

Gastrointestinal motility test

This test was done according to the method of Mascolo *et al*. and Rahman *et al*. For this test, selected rats were divided into four groups of five rats in each. At first, 1 mL castor oil was given orally in every rat of each group to produce diarrhea. After 1 h, Group I (control group) received saline (2 mL/kg) orally. Group II received standard drug (loperamide 5 mg/kg b. wt. i.p) and Groups III-IV (the rest of the two groups) received *Anjanavaerathi chooranam* (200 and 400 mg/kg b. wt. i.p. resp.). After 1 h, all animals received 1 mL of charcoal meal (10% charcoal suspension in 5% gum acacia) orally. One hour after following the charcoal meal administration, all animals were sacrificed and the distance covered by the charcoal meal in the intestine, from the pylorus to the caecum, was measured and expressed as percentage of distance moved [13, 14].

Statistical analysis

The results are presented as mean ± standard error of mean (SEM). The one-way ANOVA test with Newmannkeuls multiple range tests was used to analyze and compare the data using graphpadsoftware, while0.01 were considered as statistically significant.

Results

Castor Oil-Induced Diarrhea

In case of castor oil-induced diarrheal test, the *Anjanavaerathi chooranam* showed a marked antidiarrheal effect in the rats (Table 1). In both doses, 200 mg/kg and 400 mg/kg, *Anjanavaerathi chooranam* produced significant defecation.

The *Anjanavaerathi chooranam* doses of 200 mg/kg and 400 mg/kg decrease the total amount of wet feces produced upon administration of castor oil (and g) at doses 200 mg/kg and 400 mg/kg compared to the control group (g) at the dose of 5 mg/kg.

Castor Oil-Induced Enteropooling

In this test, *Anjanavaerathi chooranam* at both of the 200 and 400 mg/kg doses produced significant and dose dependent reduction in intestinal weight and volume (Table 2).The *Anjanavaerathi chooranam* decreased intestinal volume by 30.33% and 40.16% at doses 200 and 400 mg/kg, respectively. The standard drug loperamide (5 mg/kg) also significantly inhibited () the intestinal fluid accumulation (42.58%).

Gastrointestinal Motility Test

The *Anjanavaerathi chooranam* lessened gastrointestinal distance (cm to cm) traveled by the charcoal meal in the rats noticeably compared with the control group. Loperamide (5 mg/kg) produced a marked (46.53%) decrease in the propulsion of charcoal meal through gastrointestinal tract (Table 3).

Table 1: Effect of *Anjanavaerathi chooranam* on castor oil-induced diarrhea in rats.

Groups	Treatment	Total number of feces	% Inhibition of defecation	Total number of diarrheal feces	% Inhibition of diarrhea
I	Castor oil + Saline (2 mL/kg p.o)	18.26 ± 1.88	------	11.15 ± 1.18	—
II	Castor oil + Loperamide (5 mg/kg i.p)	7.85 ± 0.75	57.32	5.18 ± 0.44	54.90
III	Castor oil + *Anjanavaerathi chooranam* (200 mg/kg i.p)	10.3 ± 0.80	45.12	6.40 ± 0.92	42.84
IV	Castor oil + *Anjanavaerathi chooranam* (400 mg/kg i.p)	8.4 ± 1.60	56.05	5.84 ± 0.56	57.88

Values were expressed as mean ± SEM. ()., when compared with control group (ANOVA followed by newmann keuls multiple range tests).

Table 2: Effect of *Anjanavaerathi chooranam* on castor oil induced enterpooling in rats.

Group	Treatment	Weight of intestinal content (g)	Volume of intestinal content (mL)	Inhibition (%)
1	Castor oil + Saline (2 mL/kg p.o)	3.30 ± 0.12	2.84 ± 0.36	—
II	Castor oil + Loperamide (5 mg/kg i.p)	1.93 ± 0.48	1.62 ± 0.15	42.85
III	Castor oil +*Anjanavaerathi chooranam*(200 mg/kg i.p)	2.44 ± 0.10	2.20 ± 0.16	30.35
IV	Castor oil + *Anjanavaerathi chooranam* (400 mg/kg i.p)	1.94 ± 0.10	1.65 ± 0.19	40.22

Values were expressed as mean ± SEM. ()., when compared with control group (ANOVA followed by newmann keuls multiple range tests).

Table 3: Effect of ***Anjanavaerathi chooranam*** leaves on small intestinal transition in rats.

Group	Treatment	Total length of intestine (cm)	Distance traveled by marker (cm)	Inhibition (%)
I	Castor oil + Saline (2 mL/kg p.o)	109.5 ± 2.40	102.0 ± 2.80	--------
II	Castor oil + Loperamide (5 mg/kg i.p)	103.35 ± 1.82	44.0 ± 0.90	46.30
III	Castor oil +*Anjanavaerathi chooranam* (200 mg/kg i.p)	102.25 ± 3.15	67.5 ± 2.20	33.20
IV	Castor oil + *Anjanavaerathi chooranam* (400 mg/kg i.p)	93.0 ± 2.65	57.3 ± 1.42	43.52

Values were expressed as mean ± SEM. ()., when compared with control group (ANOVA followed by newmann keuls multiple range tests).

Discussion

Traditionally, people use plant(s) or plant-derived preparations considering them to be efficacious against diarrheal disorders without any scientific basis [15]. These experimental models were therefore employed to validate antidiarrheal efficacy of siddha formulation *Anjanavaerathi chooranam* in the current study.

Diarrhea can be described as the abnormally frequent defecation of feces of low consistency which may be due to a disturbance in the transport of water and electrolytes in the intestines. Instead of the multiplicity of etiologies, (i) increased electrolytes secretion (secretory diarrhea), (ii) increased luminal osmolarity (osmotic diarrhea), (iii) deranged intestinal motility causing a decreased transit time, and (iv) decreased electrolytes absorption may be responsible for pathophysiology [16,17]. Recent study claims that nitric oxide in castor oil is responsible for the diarrheal effect, although it is evidenced that ricinoleic acid produces diarrhea through a hypersecretory response which is the most active component of castor oil [18, 19]. There are several mechanisms proposed to explain the diarrheal effect of castor oil including inhibition of intestinal Na+ K+ ATPase activity, consequently reducing normal fluid absorption [20, 21], activation of adenylatecyclase or mucosal cAMP-mediated active secretion [22], and stimulation of prostaglandin formation and platelet activating factor [15]. Usually castor oil is metabolized into ricinoleic acid in the gut, which causes irritation and inflammation in the intestinal mucosa, resulting in the release of inflammatory mediators (e.g., prostaglandins and histamine). The released prostaglandins initiate vasodilatation, smooth muscle contraction, and mucus secretion in the small intestines. In experimental animals as well as in human beings, prostaglandins of the E series are considered to be good diarrheagenic agents.

Our study showed that the overall antidiarrheal study reveals the dose dependent activity. In our study, *Anjanavaerathi chooranam* showed significantly reduced amount of feces in castor oil-induced rat by 44.99% and 55.99% at the doses of 200 and 400 mg/kg, respectively, and % inhibition of diarrhea was 42.67 and 57.57 at 200 and 400 mg/kg, respectively. Moreover, our results directly demonstrate an inhibition of castor oil-induced enteropooling with reduction of the weight and volume of intraluminal contents by 30.33% and 40.16% at 200 and 400 mg/mL, respectively. These results suggest that *Anjanavaerathi chooranam* contain antidiarrheal components. Also, from these results, it can be predicted that reduction of water and electrolytes secretion into the small intestine may enhance electrolyte absorption from the intestinal lumen consistent with inhibition of hypersecretion [23]. Besides different pathophysiological conditions of diarrhea, hypermotility characterizes diarrhea where the secretory component is not the causative factor [24]. Castor oil produces ricinoleic acid leading to irritation, inflammation of intestinal mucosa, and ultimately diarrhea. At this condition, prostaglandins stimulate gastrointestinal motility and secrete water and electrolytes. It is also well established that loperamide inhibits diarrhea induced by castor oil and charcoal passage test is used to determine the effect of test substance on gut motility [25]. In gastrointestinal motility, *Anjanavaerathi chooranam* suppressed the propulsive movement or transit of charcoal meal through the gastrointestinal tract which demonstrates that the leaves extract may be able to reduce the frequency of stool in diarrheal conditions.

It was reported that flavonoids and polyphenols were responsible for the antidiarrheal activity properties [26]. However, previous studies also have shown that flavonoids have ability to inhibit intestinal motility and water and electrolytes secretion [27]. Moreover, in vivo and in vitro tests have also shown that flavonoids are able to inhibit prostaglandin E2 induced intestinal secretion and spasmogens induced contraction and also inhibit release of prostaglandins and autocoids [28]. Thereby, flavonoids as the inhibitors of prostaglandins biosynthesis are considered to delay castor oil-induced diarrhea [29]. Polyphenols also can show antidiarrheal property by interacting and inhibiting cytochrome P450 systems [30]. So, the antidiarrheal activity of the *Anjanavaerathi chooranam* could therefore be due to the presence of flavonoids and phenols.

Conclusion

The findings of the present study provide convincing evidence that *Anjanavaerathi chooranam* possesses remarkable antidiarrheal activity.. Antidiarrheal effect is rapid, long lasting, and statistically significant at both 200 and 400 mg/kg doses. Determination of antidiarrheal effect in other models as well as the effect on gut motility may give a clear idea about the mechanism(s) of antidiarrheal activity. However, further chemical and pharmacological studies are required to isolate the bioactive compounds and elucidate the precise mechanisms responsible for the observed pharmacological activities of this *Anjanavaerathi chooranam.*

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