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Antidiarrheal Evaluation of *Benincasa hispida* (Thunb.) Cogn. Fruit Extracts

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ABSTRACT

The methanolic extract of fruit of *Benincasa hispida* (BHFE) was evaluated for its antidiarrheal potential against several experimental models of diarrhea in rats. BHFE treated animals showed significant inhibitory activity against castor oil induced diarrhea and inhibited PGE_2 induced enter pooling in rats. It also showed significant reduction in gastro intestinal motility following charcoal meal in rats. The result obtained and establishes the efficacy of BHFE as an antidiarrheal agent.

Keywords: *Benicasa hispida, Antidiarrheal, Fruits, BHFE*

Since the diarrhoea is leading cause of mortality in developing countries, the World Health Organization (WHO) has constituted a Diarrheal Disease Control Program (CDD), which includes studies on traditional medical practices, together with the evaluation of health education and prevention approaches [1-4].

The fruit of *Benincasa hispida* (Thunb.) Cogn., commonly called as ash guard, belonging to cucurbitaceous is employed as a main ingredient in kusmanda lehyam, in Ayurvedic system of medicine. The leyham is used as rejuvenate agent and also numerous nervous disorders. Many empirical applications have been used in India centuries for various ailments such as GIT problems such as dyspepsia, burning sensation, heart disease, vermifuge, diabetes, and urinary disease [5, 6]. Though some scientific studies have been carried out reveal its anti-inflammatory activity [7], diuretic activity [8] and anti cancer [9]. The major constituents of this fruits are triterpenoids, flavanoids, glycosides, saccharides, carotenes, vitamins, β sitosterin, and uronic acid [10-12]. However there is no report on antidiarrheal activity of this plant though diarrhea is common occurrence disease. In the light of the above information the present investigation was undertaken to evaluate the antidiarrheal potential of *Benincasa hispida* fruit extract and is being reported here.

MATERIALS AND METHODS

Plant Material

The matured fruits of *Benincasa hispida* were collected from Bangalore in the month of August and September. Fruit was identified by the Botanist of Rural college of Pharmacy, Devanahalli. The voucher specimen (BCSF) kept in our laboratory for future reference.

Extract Preparation

After removing skin and the seeds, the fruit pulp was dried under shade. The coarsely powdered fruit pulp was extracted successively with petroleum ether (B.P. 60-80°C), chloroform and methanol in a Soxhlet extractor for 24-34 hrs. On evaporation of methanol from the methonolic extract *in vacuo,* a residue was obtained (yield 3.72% w/w) and was stored in desiccator. For pharmacological experiments weighed amount of the methanolic extract was suspended in 2% (w/v) aqueous tragacanth solution.

Animal Used

Albino Wistar of either sex weighing 160-180 g each were housed in standard metal cages. They were provided with food and water *ad libitum*. The rats were allowed a one-week acclimatization period before the experimental sessions.

Castor Oil Induced Diarrhea

The method followed here was the method of Awouters *et al* [13] with some modification. The original method has included only male Wister rats (220-250 g) and they were starved overnight before treatment with the selected drug in the next morning. In the present study (180-200 g) were fasted for 18 hrs. Animals were housed in five perforated steel cages containing six

Table 1. Effect of BHFE extract on castor oil-induced diarrhea in rats (Mean±SEM).

Oral Pre-treatment at 1-h	Mean defecations/group	Mean No. of wet feces/group
Tragacanth suspension (5 ml/kg)	4.08 ± 0.36	4.08 ± 0.36
Diphenoxylate (5 mg/kg)	1.31 ± 0.26**	0***
BHFE (200 mg/kg)	2.22 ± 0.16	1.28 ± 0.24*
BHFE (400 mg/kg)	1.78 ± 0.37*	0.94 ± 0.32**
BHFE (600 mg/kg)	1.38 ± 0.21**	0.62 ± 0.17**

Significance Vs control group (Tragacanth suspension group):
* $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$.
BHFE = *Benincasa hispida* fruit extract

Table 2. Inhibition of gastro-intestinal motility by BHFE.

Treatment after Charcoal Meal	Movement of Charcoal Meal as %	*p* -Valve
Saline (5 ml/kg)	84.20 ± 2.01	-
Atropine (0.1 mg/kg)	44.12 ± 2.22	< 0.001
BHFE (200 mg/kg)	71.06 ± 2.36	< 0.05
BHFE (400 mg/kg)	62.22 ± 2.46	< 0.01
BHFE (600 mg/kg)	50.13 ± 2.42	< 0.001

p-Value calculated with respect to saline control group (n=6).
BHFE = *Benincasa hispida* fruit extract

Table 3. Anti-enteropooling effect of BHFE.

Treatment	Volume of Intestinal Fluid in ml	*p*-Valve
Ethanol in saline	0.81 ± 0.12	-
PGE_2 in ethanol (100 µg/kg)	2.83 ± 0.21	< 0.001*
BHFE (200 mg/kg)	2.12 ± 0.19	< 0.05↑
BHFE (400 mg/kg)	1.83 ± 0.24	< 0.01↑
BHFE (600 mg/kg)	1.42 ± 0.11	< 0.001↑

Significance:
* with respect to ethanol in saline treatment.
↑ with respect to PGE_2 treatment (n=6).
BHFE = *Benincasa hispida* fruit extract

in each. None of the animal died even at an oral dose of 3.5 g/kg of BHFE. The doses of BHFE used were selected on a trial basis and was administered orally (200, 400, and 600 mg/kg) by gavage as suspension to three groups of animals. The fourth group received diphenoxylate (5 mg/kg) orally as suspension as standard drug comparison. Fifth group, which served as control received 2% (w/v) aqueous tragacanth solution. One hour after treatment each animal received 1ml of castor oil orally by gavage and then observed for defecation. Up to 4th hour after the castor oil challenge the presence of characteristic diarrheal dropping were noted in the transparent plastic dishes place beneath the individual rat cages.

Gastro Intestinal Motility Test [14]

Rats were fasted for 18hrs and place in 5 cages containing six in each. Each animal was administered orally with 1ml of charcoal meal (3% deactivated charcoal in 10% aqueous tragacanth). Immediately after that, the first three groups of animals were administered orally with the extract (BFHE) suspension (200, 400 and 600 mg/kg). The fourth group received atropine (0.1 mg/kg, i.p.), the standard drug for comparison. The fifth group was treated with aqueous tragacanth solution as control. Thirty minutes later, each animal was killed and the intestinal distance moved by the charcoal meal from the pylorus was cut and measured and expressed as a percentage of the distance from the pylorus to the caecum.

PGE_2-Induced Enteropooling [14]

In this method, rats of the same stock as above were deprived of food and water for 18 hrs and were placed in 5 perforated cages with 6 animals per cage. The first three groups were treated with BHFE 200, 400 and 600 mg/kg, p.o). The fourth group was then treated with aqueous tragacanth suspension as mentioned earlier, which served as control. Immediately afterwards, PGE_2 was administered orally to each rat (100 µg/kg) in 5% v/v ethanol in normal saline. After 30 minutes each rat was killed and the whole length of the intestine from the pylorus to the caecum dissected out and the contents were collected in a test tube and the volume was measured.

Statistical analysis was performed by Student's 't' test and in all the cases results are expressed as mean ± SEM.

RESULTS

Inhibition of Castor Oil-Induce Diarrhea

The extract (BHFE) like the standard antidiarrheal agent, dipheoxylate, inhibited significantly the frequency of defecation when compared to untreated rats (Table 1). Both substances also reduced greatly the wetness of fecal droppings.

Effect on Gastro-Intestinal Motility

The extract decreased propulsion of the charcoal meal through the gastrointestinal tract when compared with the control group. Atropine reduced the motility of the intestine significantly (Table 2).

Anti-Enter Pooling Activity

PGE_2 induced significant increase in the fluid volume of rat intestine when compared with control animals receiving only ethanol in normal saline and control vehicle. BHFE significantly inhibited PGE_2-induced enteropooling (Table 3).

DISCUSSION

Several studies have shown that prior administration with some plant extracts had a protective effect on the intestinal tract [15-17]. In the present study, the methanolic extract of fruit of *Benincasa hispida* (BHFE) that have not been studied so far, was evaluated for its antidiarrheal potential against castor oil induced diarrhea, gastrointestinal motility in charcoal meal test and PGE_2 induced enter pooling in Albino Wistar rats. There has been a statistically significant reduction in the incident and severity of diarrhea produced in experimental animal models.

The methanolic extract of fruit of *Benincasa hispida* (BHFE) exhibited significant antidiarrheal activity against castor oil induced diarrhea in rats. The extract had a similar activity as diphenoxylate when tested at 200, 400 and 600 mg/kg and statistically significant reduction in the frequency of defecation and the wetness of the fecal droppings when compared to untreated con-

trol rats (i.e., rats receiving neither BHFE nor diphenoxylate but castor oil (only).

It is widely known that castor oil or its active component ricinoleic acid induces permeability changes in mucosal fluid and electrolyte transport that results in a hypersecretory response and diarrhea [18, 19]. The experimental studies in rats demonstrated a significant increase in the portal venous PGE_2 concentration following oral administration of castor oil [20]. Ricinoleic acid markedly increased the PGE_2 content in the gut lumen and also caused on increase of the net secretion of the water and electrolytes into the small intestine [21]. The liberation of ricinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which stimulate motility and secretion [22]. Inhibitors of prostaglandin biosynthesis delayed castor oil induced diarrhea [13]. Based on the facts, it seems reasonable to suggest that the antidiarrheal effect of the BHFE may be due to the inhibition of prostaglandin biosynthesis.

The extract appears to act on all parts of the intestine. Thus, it reduced the intestinal propulsive movement in the charcoal meal treated model; at all doses of extract showed activity similar to that of atropine. Previous study shows that activated charcoal avidly absorbs drugs and chemicals on the surface of the charcoal particles thereby preventing absorption [23]. Thus, gastrointestinal motility test with activated charcoal was carried out to find out the effect of BHFE on peristaltic movement. The results also show that the BHFE suppressed the propulsion of charcoal meal thereby increased the absorption water and electrolytes.

The extracts also significantly inhibited the PGE_2 induced intestinal fluid accumulation (enter-pooling). It has been shown that E type of prostaglandins cause diarrhea in experimental animals as well as human beings [24]. Their mechanism has been associated with dual effects on gastrointestinal motility as well as on water and electrolyte transport [25]. PGE_2 also inhibit the absorption of glucose, a major stimulus to intestinal absorption of water and electrolytes [26]. These observations tend to suggest that the BHFE at all tested doses reduced diarrhea by inhibiting PGE_2 induced intestinal accumulation of fluid.

The above observations suggest that BHFE in graded doses reduced diarrhea by inhibiting intestinal peristalsis, gastrointestinal motility and PGE_2-induced enteropooling. These inhibitory effects of BHFE support the use of the *Benincasa hispida* in folk medicine; justify its use as non-specific antidiarrheal agent. Hence, BHFE, on preliminary studies can be claimed as a potential antidiarrheal agent, the underlying mechanism appears to be spasmolytic and anti-enteropooling property by which the fruit and/or its extract produced relief in diarrhea.

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