Anti-diarrhoeal activity of fruit extract of Momordica cymbalaria Hook. F.

B Vrushabendra Swamy, K Jayaveera, K Ravindra Reddy, T Bharathi

Citation

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Abstract

A study was undertaken to evaluate the effect of methanolic extract of the fruit of Momordica cymbalaria Hook F (MEMC) against several experimental models of diarrhoea in rats. MEMC treated animal's showed significant inhibitory effect against castor-oil induced diarrhea and PGE2 induced enteropooling in rats. The extract also showed a significant reduction in gastrointestinal motility in the charcoal meal test in rats. The results obtained to establish the efficacy and substantiate the folklore claim as an anti-diarrhoeal agent.

INTRODUCTION

Diarrhoea has long been recognized as one of the most important health problem in the developing countries[1]. World Wide distribution of diarrhea accounts for more than 5-8 millions deaths each year in infants and small children's less than 5 years. According to WHO estimation for the year 1998, there were about 7.1 million deaths due to diarrhea[2]. Secretory diarrhoea is most dangerous symptom of gastrointestinal problems[3] and is associated with excessive defecation and stool outputs. The stool being of abnormally loose consistency[4]. The World Health Organization has constituted a Diarrhoeal Disease Control program (CDD), which includes studies of traditional medicinal practices, together with the evaluation of health educational and prevention approaches[5].

Momordica cymabalaria Hook. F. belongs to the Cucurbitaceae family. The plant is a perennial herbaceous climber either allowed to trail on the ground or to climb on supports with the aid of tendrils. It is found in the south Indian states of Andhra Pradesh, Karnataka, Madhya Pradesh, Maharastra and Tamil Nadu as a weed. The plant is allowed to grow along bunds (boundary of fields), fences and even in the fields for the sake of fruits. However no regular cultivation is practiced. The plant has a tuberous root, which helps to maintain perennial habits, pubescent or sub glabrous. i.e., the plants dry and disappear at the end of the season. The tubers remain in the soil and emerge in the next season. The plant has a monocious stem and is very slender. The leaves are oblicular or reinform with a deeply cordate base. Flowers are unisexual. The male flower peduncle is 5–30 mm long, filiform, puberulaus, ebracteate with 2–5 flowers in racemes with a pale yellow corolla and two stamens for each flower. The female flower is solitary on a peduncle of 28 mm length. The fruits are 20–25 mm long, pyriform with 8 sharp ridges,

24× 15mm attenuated at the apex and with the base narrowed into the curved peduncle, which is fleshy, dark green and ribbed. The seeds are 4.6 mm long, ovoid shaped, smooth and shiny. Flowering occurs during October; fruits are harvested from November to January. The yield of each plant is 1.25 to 1.5kg. The tender fruits closely resemble those of a small variety of bitter gourd Athalakkai is used as a vegetable by the rural people of South Tamil Nadu and North Karnataka, India[6] The phytochemicals reported in this plants are tannins, alkaloids, phenols, proteins, amino acids[7], Vitamin C, carbohydrate and ß-Carotene[6]. The fruits of this plant reported anti diabetic and antihyperlipedimic activities (Kameswararao et al., 2003). The tubers were reported as antiovulatory activity[8].

Furthermore, literature survey of M .cymbalaria revealed that no researcher has yet reported antidiarrheal activity of this plant. Therefore, it is worth conducting an investigation on the antidiarrheal activity methanolic extract of M.cymbalaria fruits (MEMC).

MATERIALS AND METHODS

PLANT MATERIAL

The fruits of Momordica cymbalaria Hook F. was collected in November 2006 from the Bellary, Karnataka, India. The fruit material was taxonomically identified by the Regional Research Institute, Karnataka, India, and the Voucher specimen RRI/BNG/DSRU/F53/2006-07. The fruits were dried under shade with occasional shifting and then powdered with a mechanical grinder and stored in an airtight container.

PREPARATION OF EXTRACT

The powder obtained was subjected to successive soxhlet extraction with the solvents with increasing order of polarity i.e. Pet. Ether (60-80), Chloroform (59.5-61.5), Methanol (64.5-65.5) and water. Yield 3.29, 6.19, 11.70, and 15.71% respectively.

PHYTOCHEMICAL SCREENING

A preliminary phytochemical screening of all extracts carried out as described by Khandelwal K.L [9]

ANIMALS

Albino rats (Wistar) weighing 150-200g and albino mice weighing 20-25g of either sex were used in this study. They were procured from Sri Venkateshwara Enterprises, Bangalore. The animals were acclimatized for one week under laboratory conditions. They were housed in polypropylene cages and maintained at 27 C 2 C under 12 hrs dark / light cycle. They were fed with standard rat feed (Gold Mohr Lipton India Ltd.) and water ad libitum was provided. The litter in the cages was renewed thrice a week to ensure hygeinicity and maximum comfort for animals. Ethical clearance for handling the animals was obtained from the Institutional Animals Ethical Committee (RCP/IAEC/07/2006) prior to the beginning of the project work. (129/1999/CPCSEA)

ACUTE ORAL TOXICITY STUDIES

The acute oral toxicity was performed according to OPPTS following up and down procedure. Colony bred female albino rats Wistar strain (150-200gm) were maintained under controlled animal house condition with access to food and water ad libitum. The limit test carried out first at 5000mg/kg. b.w. All animals were observed for toxic symptoms and mortality for 72 h.

CASTOR OIL INDUCED DIARRHOEA

The doses of MEMC were selected on a trial basis and administered orally (200, 400 & 600mg/kg body weight) by gavage to three groups of animals. The fourth group received diphenoxylate (5mg/kg body weight) orally and the fifth group received neither drug nor extract but 2% v/v aqueous Tween 80 (1 ml) only and served as a control. After 60 min of drug treatment, each animal was administered 1ml of castor oil orally by gavage and observed for defecation up to 4hrs after castor oil administration. Characteristic diarrheal droppings were noted in the transparent plastic dishes placed beneath the individual perforated rat cages. The mean number of wet feces was calculated from the diarrhoeal droppings in the transplant plastic dishes[10, 11].

PGE INDUCED ENTEROPOOLING

For this evaluation, rats of same stock as above were deprived of food and water for 18hrs prior to the experiment. Five groups of six animals were used, which were placed in five perforated cages. The first three groups of rats were treated with MEMC (200, 400 & 600mg/kg body weight, p.o) while the fourth and fifth group received 1ml of 5% v/v ethanol in normal saline (i.p). The fourth group was then administered 1ml of normal saline and used as control. Immediately afterwards, each rat was treated with PGE_2 (100µg/kg body weight in 5% v/v ethanol in normal saline) administered orally. All the rats were sacrificed under mild anesthesia after 30min. The entire length of intestine from the pylorus to the caecum was dissected out, and its contents were collected and measured[12].

GASTROINTESTINAL MOTILITY TEST

In this method rate were tasted for 18hrs and placed in five metal cages, six in each. Each animal was given 1ml of charcoal meal (3% deactivated charcoal in normal saline). The first three groups of animals were administered MEMC orally (200, 400 & 600 mg/kg body weight) immediately after the charcoal meal treatment. The fourth group received atropine (0.1mg/kg body weight, i.p) as standard for comparison. The fifth group was treated with normal saline as control. 30min after administration of the charcoal meal, animals of each individual group were killed and the movement of charcoal from pylorus to caecum was measured. The charcoal movement in the intestine was expressed as percentage[12].

STATISTICAL ANALYSIS

For all the above experiments results were expressed as mean ±sem. statistical significance tests were performed using the students‘t' test and p-values (graph pad software) were calculated by comparison with control groups.

RESULTS

Preliminary phytochemical studies revealed the presence of tannins, alkoloids, phenols, proteins, aminoacids, flavanoids, triterpenoids, sterols, Vitamins. The MEMC found to be non toxic up to 5000 mg/kg.

INHIBITION OF CASTOR OIL INDUCED DIARRHOEA

The extract (MEMC) inhibited the frequency of defecation significantly, like standard drug (diphenoxylate) as compared to control (2% aqueous tween 80 treated). The wetness of fecal material also reduced by both the standard and extract (MEMC). The results are shown in Table – 1.

ANTI-ENTEROPOOLING ACTIVITY

PGE_2 induced a significant increase in the fluid volume of the rat as compared to control animals receiving only ethanol in normal saline. The extract (MEMC) significantly inhibited PGE_2 induced enteropooling in rats at almost all doses used (Table – 2).

EFFECT ON GASTROINTESTINAL MOTILITY

The extract (MEMC) decreased propulsion of charcoal meal through the gastrointestinal tract significantly with respect to the control group. The effect was comparable to the standard drug. The results are shown in Table – 3.

Figure 1

Table 1: Inhibition of castor oil induced diarrhea.

Oral pretreatment at 60min (mg/kg body weight)	Mean number of wet feces (mean ± SEM)
2% v/v aqueous tween 80(5)	27.2 ± 2.01
Diphenoxylate(5)	10.8 ± 1.44 [a]
MEMC (200)	21.2± 1.54 [b]
MEMC (400)	15.2 ± 1.02 [a]
MEMC (600)	11.2 ± 1.11 [a]

[a] $p<0.001$, [b] $p<0.01$ as compared to control (n=6)

MEMC: Methanol extract of *Momordica cymbalaria*

Figure 2

Table 2: Anti-enteropooling activity

Treatment	Volume of intestinal fluid ml(mean ± SEM)	P – valve
Ethanol in saline	0.76 ± 0.13	-
PGE2 in ethanol	2.93 ± 0.19	<0.001 [a]
MEMC (200mg/kg body weight)	1.87 ± 0.02	<0.001 [b]
MEMC (400mg/kg body weight)	1.16 ± 0.1	<0.001 [b]
MEMC (600mg/kg body weight)	1.02 ± 0.02	<0.001 [b]

[a] significance with respect to ethanol in saline treatment

[b] with respect to PGE_2 treatment (n=6)

MEMC: Methanol extract of *Momordica cymbalaria*

Figure 3

Table 3: Effect on gastrointestinal motility

Treatment	Dose (mg/kg body weight)	Movement of charcoal meal (%)
Control	2% aqueous tween 80	85.2 ± 2.1
Atropine	0.1	40.6 ± 2.3 [a]
MEMC	200	76.2 ± 2.2 [b]
MEMC	400	62.6 ± 2.0 [a]
MEMC	600	51.8 ± 2.1 [a]

P-valve calculated with respect to control group (n=6)

[a] $p<0.001$, [b] $p<0.02$

MEMC: Methanol extract of *Momordica cymbalaria*

DISCUSSION

In developing countries a quarter of infant and childhood mortality is related to the diarrhea[13]. The highest mortality rates have been reported to be in children less than five years of age. During the past decade oral dehydration therapy has reduced mortality from acute diarrheal disease, where as chronic diarrhea remains a life-threatening problem in those regions in which malnutrition is common co-existing and complication factors number of factors, such as infective, immunological and nutritional has been involved in the perpetuation of the diarrheal syndrome[14]. Many plants conveniently available in India are used in traditional folklore medicine for the treatment of diarrhea and dysentery of the indigenous plants used, Andrographis Peniculata, Asparagus racemosus, Butea monosperm, Cassia auriculata and other are mentioned[15]. Several studies have shown that prior administration with some plant extracts had protective effect on the intestinal tract[16, 17, 18]. In the present study, methanol extract of fruit of Momordica cymbalaria have not been studied so far was evaluated for its anti-diarrhoeal potential against castor oil induced diarrhea, gastro intestinal

motility in charcoal meal test & PGE_2 induced enteropooling in Albino Wistar rats.

The MEMC exhibited significant anti-diarrheal activity against castor oil induced diarrhea in rats. The extract had a similar activity as diphenoxylate, when tested at 200, 400 & 600mg/kg and statistically significant reduction in the frequency of defecation and the wetness of the fecal droppings when compared to untreated control rats.

It is widely known that castor oil or its active component ricinoleic acid induces permeability changes in mucosal fluid and electrolyte transport that result in hyper secretory response of diarrhea[19, 20]. The experimental studies in rat's demonstrated a significant increase in the portal venous PGE_2 concentration following oral administration of castor oil[21]. The recinoleic 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[10].

The MEMC significantly inhibited the PGE_2 induced intestinal fluid accumulation (enteropooling). It has been shown that E type of prostaglandin cause diarrhoea in experimental animals as well as human beings[23]. Their mechanism has been associated with dual effects on gastrointestinal motility as well as on water and electrolyte transport[24]. PGE_2 also inhibit the absorption of glucose a major stimulus to intestinal adsorption of water and electrolytes[25]. These observations tend to suggest that MEMC reduced diarrhea by inhibiting PGE_2 induced intestinal accumulation of fluid.

The MEMC appears to act on all parts of the intestine. Thus it reduced the intestinal propulsive movement in the charcoal meal treated model. The MEMC 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 there by preventing absorption[26]. Thus gastrointestinal motility test with activated charcoal was carried out to find out the effect of MEMC on peristalsis movement. The results shows that the MEMC suppressed the propulsion of charcoal meal thereby increased the absorption of water and electrolytes.

Previous reports have demonstrated the antidiarrhoeal activity of tannins[27], flavanoids[28], alkaloids[29], sterols and terpenes[30] containing plant extracts. The phytochemical analysis of the extract showed presence of tannins, alkaloids, sterols, terpenes and flavanoids. These constituents may responsible for the antidiarrhoeal activity.

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CORRESPONDENCE TO

Srinivasa Institute of Pharmaceutical Sciences, Sri Chowdeswari Nagar, Peddasetty Palli, Proddadur, Kadapa (Dist), Andhra Pradesh, India 516 361
vrushabendra@gmail.com, swamybm@yahoo.com Mobile: 00 91 9959661915

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Author Information

B.M. Vrushabendra Swamy
Srinivasa Institute of Pharmaceutical Sciences

K.N. Jayaveera
Department of Chemistry,, Jawaharlal Nehru Technological University College of Engineering

K. Ravindra Reddy
P. Ramireddy Memorial College of Pharmacy

T. Bharathi
Krishna Teja Pharmacy Pollege