International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 10, Issue 5, 2018

Original Article

ANTIMOTILITY AND ANTISECRETORY RELATED ANTIDIARRHOEAL ACTIVITY OF THE *ABELMOSCHUS MOSCHATUS* MEDIK IN EXPERIMENTAL ANIMAL MODELS

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Received: 26 Dec 2017 Revised and Accepted: 11 Apr 2018

ABSTRACT

Objective: This study was intended to evaluate the anti-diarrhoeal potential of *Abelmoschus moschatus* Medik (*A. moschatus* Medik) seeds and the possible mechanism therein involved by using different experimental models in albino Wistar rats.

Methods: The hydroalcoholic seed extract of *A. moschatus* Medik. (HEAM) was orally administered at the doses of 150, 300 and 500 mg/kg respectively to the different groups in order to assess the effect of extract in castor oil induced diarrhea model in rats. In order to comprehend the mechanism involved in its anti-diarrhoeal potential, the extract was further investigated for its effect on gastrointestinal motility using charcoal meal test and antisecretory action by castor oil induced intestinal enter pooling where, atropine sulphate (5 mg/kg) and loperamide (2 mg/kg) were used as reference standards respectively.

Results: The HEAM exhibited significant ($p<0.05$, $p<0.001$) and dose-dependent anti-diarrhoeal effect by decreasing the mean number of fecal droppings produced upon castor oil administration as compared to the normal control. The effect of the extract at 500 mg/kg was near to that of loperamide (2 mg/kg). The extract (300 mg/kg) showed antimotility action by significantly ($p<0.05$, $p<0.001$) attenuating the charcoal meal transit in the intestine as compared to negative control animals. Further, the extract showed significant ($p<0.05$, $p<0.001$) inhibition in the accumulation of intestinal fluid due to castor oil.

Conclusion: The HEAM exhibited significant anti-diarrhoeal action that could be presumably related to its observed antimotility and antisecretory activities. This study justifies the usage of *A. moschatus* Medik. as an anti-diarrhoeal agent in traditional practices of medicine.

Keywords: *Abelmoschus moschatus* Medik, Anti-diarrhoeal, Loperamide, Castor oil, Charcoal

DOI: http://dx.doi.org/10.22159/ijpps.2018v10i5.24450

INTRODUCTION

Diarrhoel diseases are the most important causes of illness and mortality throughout the globe. Inadequate sanitation and poor hygiene are the causes for about 88% of diarrhoel-related deaths [1]. Diarrhoea, a critical global health problem, particularly in developing nations, accounts for over 5-8 million mortalities in children below 5 y of age, every year [2]. Plants have been vital resources for new agents. Several species of plants have been evaluated for substances with therapeutic effect [3]. According to World Health Organization, the use of traditional remedies in health care system is gradually rising. It is well known that herbal medicines are fairly safe, inexpensive and easily accessible to the layman, as compared to that of synthetic drugs. Moreover, the herbal remedies or products obtained from the natural origin are known to possess synergistic and/or side effects counteracting potentials and offer their pharmacological actions mediated normally through several pathways [1].

Abelmoschus moschatus Medik (*A. moschatus* Medik) belongs to the family malvaceae is an annual plant. The seeds and leaves of this herb are reported for their use in treating diverse clinical conditions in ethnomedical practice. The seeds have been used as an aphrodisiac, ophthalmic, cardiotonic, digestive, stomachic, antispasmodic and in the treatment of intestinal complaints and diarrhoea [4]. The literature survey revealed the reports for its antioxidant, free radical scavenging, antimicrobial, antiproliferative [5], hepatoprotective [6], antilithiatic [7], increasing insulin sensitivity [8] and plasma glucose lowering activities [9]. Despite of its traditional use in diarrhoea and intestinal complaints, the plant has not been scientifically reported. Therefore, we designed the present study to evaluate the antimotility, antisecretory and anti-diarrhoeal effects of the hydroalcoholic extract of *A. moschatus* Medik. in experimentally induced diarrheal models in rats.

MATERIALS AND METHODS

Chemicals and drugs

Atropine and castor oil (Himedia, Mumbai, India), acacia and charcoal (SD. Fine chemicals, Mumbai, India), loperamide (Cipla Pvt. Ltd., Bangalore, India) and all the other drugs and chemicals used for the experiment were of analytical grade.

Plant material

The *A. moschatus* seeds were collected from Moodigere taluk, Chikkamangalore district of Karnataka. The seeds were authenticated by Prof. D. Siddappa, Dept. of Botany, Sree Siddaganga College of Science, Tumkur, Karnataka, India and a voucher specimen (AD531) of the same was kept in the college herbarium. The seeds were washed and shade dried. The hydroalcoholic extract of *Abelmoschus moschatus* (HEAM) was prepared by dissolving the coarsely powdered seeds of *A. moschatus* using ethanol by dissolving the 1 g in 30 ml of ethanol for 3-4 h at 40-50 °C and evaporated to 4 ml. The suspensions prepared in all above cases was centrifuged for 15 min at 10,000 rpm, the resultant upper layer was collected into separate tubes and concentrated to the dry mass using vacuum evaporator stored for future use [5].

Experimental animals

Albino Wistar rats (150-200 g) of both sex were used. They were procured and maintained on a 12±h standard conditions, served with pellet diet and water was given sufficiently. The approval from the institutional animal ethical committee (IAEC) of SSCP, Tumkur, Karnataka was obtained (Ref: SSCP/IAEC-Clear/147/2013-14) prior to the experiments. The experiments were carried out in strict compliance to ethical principles and guidelines provided by the committee for the purpose of control and supervision of experiments on animals (CPCSEA).

Methodology

Castor oil induced diarrhea

The rats that are withheld from food for 18 h but freely provided with water were divided into five groups of six rats each. The animals were caged individually. The Group I served as negative control; received normal saline. The Group II was treated with loperamide (2 mg/kg), served as positive control and the other three Groups (III, IV and V) received HEAM 150, 300 and 500 mg/kg respectively. All the treatments were made orally. One hour after the pretreatment, each animal orally received 1 ml castor oil through oral feeding needles. The animals were shifted to cages containing plastic sheet at the base and then they were kept for observation up to 4 h for evaluation of different parameters [10].

Gastrointestinal motility test

The rats were allocated into four groups of six each and fasted for 18 h, however, water was freely supplied. All the treatments were made orally. The Group I (control group) received normal saline, while the Group II was served with reference standard (atropine sulphate 5 mg/kg body weight), Groups III and IV were given with the HEAM in doses of 150 and 300 mg/kg body weight respectively, thirty min thereafter, each animal received 1 ml/rat of charcoal meal (10% activated charcoal in 5% gum acacia). 30 min later, all the rats were sacrificed and the entire length of the small intestine covered by the charcoal meal was measured and expressed as a percentage of distance travelled [3].

Castor oil-induced enteropooling

Rats having free access to water but withheld from pellet food were separated into five groups. All the treatments were made orally. The Group 1 served as vehicle control which received normal saline and Group 2 as castor oil control (negative control). Group 3 and 4 were treated with 300 and 500 mg/kg of HEAM respectively, whereas Group 5 received Loperamide at 2 mg/kg (positive control). 30 min following the above treatment, rats from Groups 2 to 5 were served orally with 1 ml of castor oil and all the animals were sacrificed 1 h later. The small intestines were excised from the pylorus to caecum and volume of their content was measured. The intestinal fluid concentrations of Na^+ and K^+ were determined on a flame photometer [10].

Statistical analysis

The data were analyzed by using one way ANOVA, followed by Dunnett's t-test using Graphpad prism 5.0 software. P<0.05, P<0.001 were considered statistically significant.

RESULTS

Castor oil induced diarrhea

The HEAM exhibited significant and dose-dependent (p<0.05, p<0.001) anti-diarrhoeal activity in castor oil sourced diarrhea model. In the negative control group, there was no protection from diarrhea. The positive control group (loperamide 2 mg/kg) exhibited 83.33% protection. HEAM pretreatment (150, 300, and 500 mg/kg), significantly reduced (p<0.05, p<0.001) the mean number of fecal counts produced due to castor oil administration (17.16 at 150 mg/kg, 8.83 at 300 mg/kg and 4.66 at 500 mg/kg) as compared to castor oil control (24.16). The anti-diarrhoeal effect was found to be maximum (80.55%) with the higher dose of HEAM pretreatment (500 mg/kg) among the three doses tested. The results are elaborated in table 1.

Table 1: Effect of HEAM on castor oil induced diarrhea in rats

Group	Treatment and dose (mg/kg)	Onset diarrhoea (min)±SEM	Mean no of fecal drops±SEM	Mean weight of feces (G) ±SEM	% inhibition
1	Control (Normal Saline+Castor oil 1 ml)	38.00± 0.166	24.00± 0.703	2.506± 0.012	—
2	Standard (Loperamide 2 mg/kg)	79.166± 2.007**	3.293± 0.307**	0.578± 0.040**	86.27
3	HEAM (150 mg/kg)	40.833± 1.537 Ns	17.166± 0.542**	1.628± 0.058**	28.47
4	HEAM (300 mg/kg)	62.166± 3.060**	8.833± 0.792**	1.276± 0.012**	63.19
5	HEAM (500 mg/kg)	70.333± 2.906**	4.666± 0.477**	0.758± 0.035**	80.55

Values are expressed as mean±SEM (n = 6) *P<0.05, **P<0.001 v/s control

Gastrointestinal motility test

Both atropine sulphate and HEAM showed antimotility activities by decreasing the movement of charcoal meal in the small intestine. The pretreatment with extract exhibited significant and dose-dependent (P<0.05, P<0.001) attenuation in the forward motion of charcoal meal in the gastrointestinal tract of rats compared to control groups. The percent inhibition of charcoal meal was 58.33 % with a standard drug, 26.71 and 50.79 % with 150 mg/kg and 300 mg/kg of HEAM respectively. The results are detailed in table 2.

Table 2: Effect of HEAM on gastrointestinal motility

Group	Treatment and dose (mg/kg)	Distance traveled by charcoal meal±SEM	% inhibition
1	Control (Normal saline 5 ml/kg)	75.6± 4.567	----
2	Standard (Atropine sulphate 5 mg/kg)	31.5± 2.293**	58.33
3	HEAM (150 mg/kg)	55.4± 3.628*	26.71
4	HEAM (300 mg/kg)	37.2± 1.744**	50.79

Values are expressed as mean±SEM (n = 6) *P<0.05, **P<0.001, v/s the control

Castor oil induced enteropooling

The intestinal fluid accumulation was significantly (p<0.05 and p<0.001) inhibited in standard and extract pretreated animals. HEAM (300 mg/kg), HEAM (500 mg/kg) and Loperamide (2 mg/kg) pretreatment showed significantly reduced intestinal fluid accumulation compared to castor oil alone treated negative control group. The percentage inhibition was 29.58%, 36.16% and

39.96% respectively. The estimation of intestinal fluid concentrations of Na^+ and K^+ of all the groups showed a greater inhibitory effect on Na^+ levels than on the K^+. The results are summarized in fig. 1.

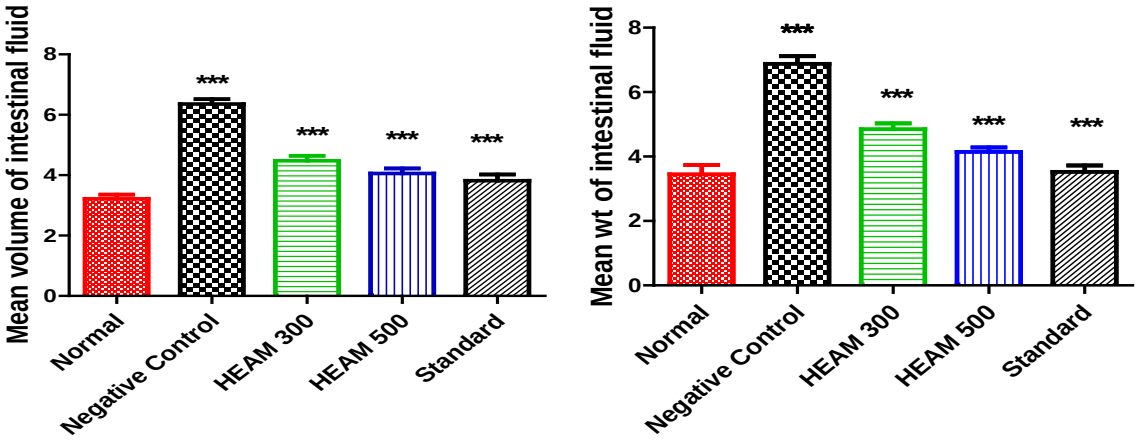

Fig. 1: Effect of the HEAM on castor oil induced enteropooling, values are expressed as mean±SEM (n = 6) *P<0.05, **P<0.001, when compared to the control (saline) and negative control (saline+castor oil)

DISCUSSION

Diarrhea is the passage of liquid stool more than three times a day or the removal of unformed contents of the bowel on a daily basis, twofold that of a person's usual rate. As an indication of underlying trouble, e. g. gastrointestinal disorder or infection, diarrhea is typified by an enhanced stool frequency, volume and fluidity, or bowel movement (gut motility) ensuing in loss of fluids, nutrients and electrolytes [11].

In the system of traditional medicine, *A. moschatus* Medik is being employed in the management of diarrhoea, antispasmodic, GI complaints by traditional healers at a lower cost. In this study, the HEAM revealed significant anti-diarrhoeal property against castor oil inflicted diarrhea model in rats.

Numerous mechanisms have been previously proposed to elucidate the basis for the castor oil caused diarrhea via; intestinal inhibition of Na^+ K^+ ATPase action, consequently decreasing usual fluid reabsorption [12], triggering of adenylate cyclase or cAMP-mediated active secretion in the mucosa [13], stimulation of endogenous prostaglandin release [14].

Yet, diarrhea caused by castor oil is well documented to its active constituent ricinoleic acid, which augments peristaltic activity in the small intestine, leading to alter in the electrolyte permeability of the intestinal mucosa through an enhanced secretory response. Thus it can be presumed that the mechanism of anti-diarrhoeal effect of the HEAM was intervened through an antisecretory action. This was as well evident from the inhibitory action of extract exhibited on castor oil inflicted intestinal fluid accumulation [3].

The HEAM appears to work on most segments of the intestine. Thus it remarkably decreased intestinal movement as evidenced by the reduction in the distance traveled by charcoal meal. These results enlighten that the HEAM attenuated the charcoal meal propulsion, thus enhancing electrolytes and water absorption. Atropine, due to its anticholinergic action reduced the propulsive movement in the charcoal meal [15].

A recent report reveals the laxative action of ricinoleic acid, the active constituent of castor oil is owing to induction of intestinal smooth muscle contraction which is intervened by stimulation of EP3 receptors [16]. Many anti-diarrhoeal agents produce their action by decreasing the intestinal secretions and/or the intestinal motility. Agents which inhibit prostaglandin biosynthesis could effectively retard castor oil inflicted diarrhoea [17].

All the doses of HEAM showed a significant defence against castor oil caused enteropooling, which might be owing to the attenuation of prostaglandins release. Enteropooling effect of the HEAM is further significant as the aversion of enteropooling facilitates in inhibiting diarrhea [18].

The preliminary phytochemical study reports on *A. moschatus* seeds indicated the presence of sterols, carbohydrates, fixed oil, proteins, tannins, lipids and considerable amounts of total polyphenols and flavonoids [19]. The seed extract has also been reported for good antimicrobial and antioxidant activity [5]. Flavonoids have been ascribed for the anti-diarrhoeal action due to their ability in inhibiting intestinal movement and water-electrolytic secretion which are changed in this intestinal state. Both *in vivo* and *in vitro* investigations have exhibited that flavonoids are competent to hinder the secretory response of intestine provoked by prostaglandins E_2 [20]. Flavonoids present as active principles in abundance in HEAM can be a contributing factor for its anti-diarrhoeal properties. Besides, flavonoids have been known for their antioxidant potential that are supposed to be responsible for exerting inhibitory effects upon numerous enzymes including those essential in the metabolism of arachidonic acid [3].

CONCLUSION

The HEAM showed a significant anti-diarrhoeal effect against castor oil caused diarrhea in rats. The antimotility and antisecretory activities of the HEAM could be the contributing mechanisms involved in the observed anti-diarrhoeal activity. This finding corroborates the use of A. moschatus Medik. as an anti-diarrhoeal agent by traditional healers. Further research is needed to validate the plant constituents that are accountable for anti-diarrhoeal action.

AUTHORS CONTRIBUTIONS

Dr. Babitha S-The present work was initiated by the author.

Akshaya Deepu HG-Author has contributed the major experiment part.

Nageena Taj-Author has helped in the manuscript preparation.

CONFLICT OF INTERESTS

The authors state that they have no conflicts of interest.

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