Vol. 9/2 (2009) 197 - 201

JOURNAL OF NATURAL REMEDIES

Antidiarrheal activity of methanolic extracts of seeds of *Lepidium sativum*

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Abstract

The objective of the present study is to investigate the antidiarrheal activity of the methanolic extract of *Lepidium sativum* (MELS). The preliminary phytochemical investigation was carried out to identify the various chemical constituents present in the extract. It was found that the MELS contain alkaloids, carbohydrates, glycosides, saponins, proteins, steroids, flavonoids, tannins and phenolic compounds. Acute toxicity studies revealed that the MELS was safe upto 2000mg/kg. The antidiarrheal activity was observed in three experimentally induced diarrhea models i.e. Castor oil induced diarrhea; Prostaglandin E2 (PG-E2) induced enteropooling in rats and charcoal meal test in mice. In castor oil induced model MELS (50, 100 and 200mg/kg.p.o.) showed significant dose dependent reduction of cumulative wet faecal mass. In PG-E2 induced enteropooling model, MELS (50, 100 and 200mg/kg.p.o.) inhibited PG-E2 induced secretions. Similarly in charcoal meal test MELS (50, 100 and 200mg/kg.p.o.) decreased the movement of charcoal indicating its antimotility activity. It was observed that MELS possess significant anti-diarrheal activity.

Key words: *Lepidium sativum*, anti-diarrheal activity, Castor oil, Prostaglandin E2, Charcoal meal test.

1. Introduction

Diarrhea, an important health problem worldwide, especially in developing countries, accounts for more than 5-8 million deaths in infants and children under 5 years, each year [1]. In recent years, there has been a great interest in herbal remedies for the treatment of a number of ailments. Medicinal plants are promising source of antidiarrheal drugs [2]. Indigenous plants such as *Andrographalis paniculata, Asparagus racemosus, Butea monosperma, Cassia auriculata, Ficus hispida, Hemidesmus indicus, Guiera senegalensis* etc are widely used for treatment of diarrhea [3].

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Lepidium sativum is an erect herbaceous plant belongs to the family Cruciferae. The roots are useful in secondary syphilis and tenesmus. The leaves are stimulant, diuretic and antibacterial. The seeds are bitter, thermogenic, depurative, rubefacient, galactogogue, emmenagogue, tonic, aphrodisiac, ophthalmic and diuretic [4].

2. Materials and methods

2.1 Plant material and extraction

The seeds of *Lipedium sativum* were collected from the local market from Bangalore. The plant was authenticated by Dr. K.P. Sreenath, Taxonomist, department of Botany, Bangalore University, Bangalore. A sample specimen (LS.No.1) was deposited in department of Pharmacognosy, PES College of Pharmacy, Bangalore. The dried seeds were powdered and subjected to solvent extraction in soxhlet apparatus using methanol at 60°C. After that, the extract was concentrated at reduced temperature and pressure, until a concentrated residue was obtained.

2.2 Drugs and Chemicals

Castor oil (Medinova chemicals. Bangalore), Deactivated charcoal (New India chemical enterprises. Kochi), PG-E2 (Zidus Alidac. Ahmedabad.), Atropine (S.D.Fine chemicals. Mumbai.) and Loperamide (Torrent Pharmaceuticals. Ahmedabad, India) were used for the study.

2.3 Experimental animals

Swiss albino mice (20-25g) and Wistar albino rats (150-200 g) of either sex were acclimatized for 7 days under standard husbandry conditions i.e. room temperature 26 ± 1°C. relative humidity 45-55% and light : dark cycle 12:12 h. the experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of PES College of Pharmacy, Bangalore and conducted according to the guidelines of the Committee for the Purpose of the Control and Supervision on Experiments on Animals (CPCSEA).

2.4 Antidiarrheal activity

2.4.1 Castor oil induced Diarrhea

The method described by Awouters *et al* [5] as followed. Albino rats of either sex weighing 150-200 g were used. They were divided into 5 groups each group containing six animals. Rats were fasted 24 hrs before the test with free access to water. Rats were treated orally with vehicle or methanolic extract or standard. One hour after drug treatment, each rat received castor oil (2ml/100g, p.o). Each rat was then housed separately in cage over clean filter paper. Then diarrhea episodes were observed for a period of 4 hours. During this period, first defecation time, frequency of defecation and cumulative wet faecal mass were recorded. Antidiarrheal activity was determined in terms of percentage reduction in cumulative faecal mass with respect to vehicle treated group [6].

2.4.2 Prostaglandin-E2 induced Diarrhea

Five groups of rats (150-200 g) consisting of 6 animals in each group were deprived of food and water for 18 hours prior to the experiment. Rats were treated orally with vehicle or MELS or loperamide one hour prior to prostaglandin-E2 administration. All the rats were administered with PG-E2 (100 µg/kg in 2% v/v Tween 80 orally) except normal control group. Thirty minutes after PG-E2, all the rats were sacrificed; the whole length of the intestine from the pylorus to the caecum was dissected out and its contents were collected and measured [6].Percentage reduction of intestinal secretion (volume) was calculated.

2.5 Charcoal meal test

Albino mice of either sex weighing 20-25 g were used. Mice were fasted for 4 hours before

Table 1. Effect of methonolic extract of *Lepidium sativum* seeds on Castor oil induced diarrhea.

Sl.No	Drug	Dose mg/kg	Cumulative fecal mass	% Inhibition
1	Control	-	7.533 ± 0.219	-
2	Loperamide	1	1.408 ± 0.172***	81.30
3	MELS	50	4.417 ± 0.31***	41.36
4	MELS	100	2.967 ± 0.25***	60.61
5	MELS	200	1.850 ± 0.303***	75.44

Values are Mean ± S.E.M. (n=6); Significance vs. Control group: ***P < 0.001

Table 2. Effect of methonolic extract of *Lepidium sativum* seeds on PG-E2 induced diarrhea.

Sl.No	Drug	Dose mg/kg	Volume of secretion	% Inhibition in secretion
1	Control	-	2.62 ± 0.25	-
2	Loperamide	3	0.96 ± 0.17***	63.35
3	MELS	50	1.42 ± 0.18**	45.80
4	MELS	100	1.008 ± 0.20***	61.52
5	MELS	200	0.95 ± 0.12***	63.74

Values are Mean ± S.E.M. (n=6); Significance vs. Control group: ***P < 0.001, **P< 0.01

Table 3. Effect of methonolic extract of *Lepidium sativum* seeds on Charcoal meal test.

Sl.No	Drug	Dose mg/kg	% Movement	% Inhibition
1	Control	-	84.16±1.88	-
2	Atropine	1	28.27±1.84***	67.2
3	MELS	50	58.82±1.56***	31.7
4	MELS	100	50.31±1.80***	41.6
5	MELS	200	44.89±1.58***	47.9

Values are Mean ± S.E.M. (n=6); Significance vs. Control group: ***P < 0.001

commencing the experiment with free access to water. After 1 hour of vehicle or MELS or standard treatment, 1ml of charcoal meal [3% deactivated charcoal in 2% aqueous tween 80 orally] was administered by oral route to all the animals in each group. After 50 minutes of charcoal treatment each mouse was sacrificed and distance moved by the charcoal meal from the pylorus to caecum was measured to express as a percentage of distance travelled by the charcoal meal in ratio to the intestinal length. Percentage inhibition produced by extracts was calculated [6].

2.6 Statistical analysis

Values are expressed as mean ± SEM from 6 animals. Statistical differences in mean were analyzed using one way ANOVA (analysis of variance) followed by Dunnett's test $p<0.05$ was considered significant.

3. Results

3.1 Castor oil induced diarrhea

The standard drug Loperamide (1mg/kg), MELS (50, 100 & 200 mg/kg) significantly reduced the mean weight of the faeces when compared to vehicle treated control rats. The MELS has showed significant reduction in the mean weight of faeces in dose dependent manner. The results are shown in Table 1.

3.2 Prostaglandin E2 induced diarrhea

The standard drug Loperamide (3mg/kg) and MELS (50, 100 & 200 mg/kg) has showed significant inhibition of PG-E2 induced intestinal secretions when compare to vehicle treated group. The results are shown in Table 2.

3.3 Charcoal meal test

The standard drug atropine (1mg/kg) and MELS (50, 100 & 200 mg/kg) has showed significant decrease in propulsion of charcoal meal when compared to vehicle treated group. The results are shown in Table 3.

4. Discussion

Methanolic extract of seeds of *Lepidium sativum* (MELS) which has not been studied so far for its antidiarrheal activity was evaluated for the antidiarrheal potential against experimentally induced diarrhea models namely, castor oil induced diarrhea in rats, PG-E2 induced enteropooling in rats and Charcoal meal test in mice.

MELS exhibited significant antidiarheal activity against castor oil induced diarrhea in rats. The MELS (50, 100 and 200 mg/kg) showed significant antidiarrheal activity at all the dose levels, at higher dose level MELS (200 mg/kg) has showed equally significant activity as that of loperamide (1 mg/kg). 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 hyper secretory response and diarrhea [7, 8].

Ricinoleic acid markedly increases the PG-E2 in portal vein and gut lumen and also causes an increase in secretion of water and electrolytes in to the small intestine [9, 10].

Ricinoleic acid also produces irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which stimulate motility and secretion [11]. Inhibition of prostaglandin biosynthesis delayed castor oil induced diarrhea [5]. Based on these observations, it seems that the antidiarrheal effect of MELS may be due to the inhibition of prostaglandin biosynthesis or by decreasing the peristaltic movement.

To ensure that MELS modify the action of prostaglandin, effect of MELS on PG-E2 induced diarrhea was studied in rats. MELS significantly inhibited the PG-E2 induced intestinal fluid accumulation (enteropooling). It has been shown that E type of prostaglandins cause diarrhea in experimental animals as well as human beings [12]. Their mechanism has been associated with dual effects on gastrointestinal motility as well as on water and electrolyte transport [13]. These observations tend to suggest that MELS (50, 100 & 200 mg/kg) reduced diarrhea by inhbiting PG-E2 induced intestinal accumulation of fluid.

Studies showed that activated charcoal readily adsorbs drugs and chemical on the surface of the charcoal particles and their by preventing absorption [14]. Hence gastrointestinal motility test with deactivated charcoal was carried out to find out the effect of MELS on peristaltic movement. The MELS appear to act on all parts of intestine. Thus, it reduced the intestinal propulsive movement in the charcoal meal treated model; at the doses 50, 100 and 200

mg/kg. The results also showed that MELS suppressed the propulsion of charcoal meal there by increased the absorption of water and electrolytes. The inhibition of peristaltic movement with MELS may be due to the anti histaminic and anticholinergic actions. From these models we can suggest that MELS non-specifically inhibit diarrhea either by decreasing intestinal motility or by decreasing the prostaglandin biosynthesis.

The result indicates that MELS possess significant antidiarrheal activity due to their inhibitory effect both on gastrointestinal propulsion and fluid secretion.

The data obtained is consistent with literature reports [4] on antidiarrheal activity of *Lepidium sativum* seeds using gastrointestinal motility test in mice and castor oil induced diarrhea in rats and PGE_2 induced intraluminal accumulation of fluids in rats. The inhibitory effect of the seed extract justified the use of the seed as a non-specific antidiarrheal agent in folklore medicine. Further detailed investigations are needed to determine the phytoconstituents which are responsible for antidiarrheal activity.

5. Acknowledgement

The authors are thankful to Prof.Dr.S.Mohan, Principal and management members of P.E.S.College of Pharmacy for providing all necessary facilities to carry out the research work. Special thanks to Dr.K.P.Sreenath, Reader in Botany, Bangalore University for authentifying the plant material and also to Dr.Anitha, Asst.Prof. Siddaganga Ayurvedic College, Bangalore for technical support.

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