ANTI-DIARRHOEAL ACTIVITY OF ETHANOLIC HEARTWOOD EXTRACT OF PTEROCARPUS MARSUPIUM

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Summary

Diarrhoea is a common cause of death in developing countries and the second most common cause of infant's death worldwide. *Pterocarpus marsupium* is a medicinal herb belonging to the family *Fabaceae* has been traditionally used in the treatment of diarrhoea. Therefore, the present study was designed to investigate antidiarrheal activity of ethanolic heartwood extract of *Pterocarpus marsupium* (EEPM). The extract was evaluated for an antidiarrheal activity using castor oil and charcoal induced gastrointestinal motility test in rats. EEPM at a dose of 250 and 500 mg/kg, significantly reduced the frequency and severity of diarrhoea. At the same doses, the extract significantly delayed the intestinal transit of charcoal meal in the test animals as compared to the control. The results of the present study confirm antidiarrheal potential of the heartwood of *Pterocarpus marsupium*, thus may provide the scientific basis for the traditional use of this plant as the modality for diarrhoea.

Keywords: *Pterocarpus marsupium,* anti-diarrheal, castor oil, loperamide.

Introduction

Diarrhoea is a common cause of death in developing countries and the second most common cause of infant's death worldwide (1). The loss of fluids through diarrhoea can cause severe dehydration, which is one of the major causes of death in diarrhoea sufferers. Green plants synthesize and preserve a variety of biochemical products, many of which are extractable and used as chemical feed stocks or as raw material for various scientific investigations. Many secondary metabolites of plant are commercially important and find use in a number of pharmaceutical compounds (2).

Pterocarpus marsupium (Fabaceae) commonly known as Vijaysar is a medium to large, deciduous tree that can grow up to 30 meters tall. In different language the plant is known as Bijasal (Hindi), Biyo (Gujrati), Pitasara (Sanskrit) and Chandanam (Telgu) (3). As per the traditional claim heartwood of *Pterocarpus marsupium* are the potential source of drugs used as an astringent, anti-inflammatory, anthelmintic, leprosy, skin diseases, diabetes, diarrhoea, asthma, bronchitis and grayness of hairs (4). It has been scientifically reported for hypolipidemic (5), hepatoprotective (6), anti-ulcer (7), anti-inflammatory (8), and anti-diabetic activity (9, 10). A variety of flavonoids and their derivatives have been isolated from various parts of the plant. It is also a rich source of polyphenolic compounds (11). However, till date the ethanolic extract of heartwood of *Pterocarpus marsupium* (EEPM) has not been scientifically investigated for anthelmintic activity.

Despite immense technological advancement in modern medicine, many people in the developing countries still rely on the healing practices and medicinal plants for their daily health care needs. However, EEPM has not been yet scientifically investigated for its anti-diarrheal activity. Therefore, present study was designed to investigate the role of EEPM as an anti-diarrheal and to find out it's possible mechanism of action.

Methods

Collection of plant material and preparation of extracts

The heartwood of *Pterocarpus marsupium* was purchased from the local market. The plant material was authenticated at Agharkar Research Institute; Pune and voucher specimen (09130) was deposited. The powdered material was extracted with ethanol (95%) by continuous Soxhlet extraction method. It was then filtered and concentrated in vacuum under reduced pressure using rotary evaporator. Suspension of extract was prepared by using 2 % gum acacia.

Preliminary phytochemical screening (12)

The extract was screened for preliminary phytochemical tests for the presence of alkaloids, glycosides, flavonoids, volatile oils, tannins and phenolic compounds.

Animals

Wistar albino rats (150-200 g) of either sex were used. The animals were maintained under standard laboratory conditions at temperature 23 ± 2^0C with relative humidity 55 ± 10 % and 12 h light and dark cycle throughout all the experiments. Animals had free access to food and water *ad libitum*. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethics Committee.

Experimental Design

Evaluation of EEPM in castor oil induced diarrhoea (13)

The 18 h fasted wistar rats weighing 150-250g were divided into four groups (n=5) as follows,

Group-I: Received 2% gum acacia (1 ml/kg, *p.o.*) and 1 h later castor oil (2 ml/rat; *p.o.*)

Group-II: Received EEPM (250 mg/kg, *p.o.*) and 1 h later castor oil (2 ml/rat; *p.o.*)

Group-III: Received EEPM (500 mg/kg, *p.o.*) and 1 h later castor oil (2 ml/rat; *p.o.*)

Group-IV: Received Loperamide (5 mg/kg, *p.o.*) and 1 h later castor oil (2 ml/rat; *p.o.*)

Experimental diarrhoea was induced in rats by oral administration of castor oil. The number of both hard and soft pellet was counted at every hour over six hour period for each rat. Diarrhoea was defined as the presence in the stool with fluid material that stained the paper placed beneath the cages.

Percent inhibition (PI) of diarrhoea was calculated as follows:

PI= Mean defecation (Control group - Treated group) × 100/ Mean defecation of control group

Evaluation of EEAR on gastrointestinal motility test (14)

The 18 h fasted wistar rats weighing 150-250g were divided into four groups (n=5) as follows,

Group-I: 2% gum acacia (1ml/kg, *p.o.*) and 30 min later 5 % charcoal meal (1 ml/rat; *p.o.*)

Group-II: EEPM (250 mg/kg, *p.o.*) and 30 min later 5 % charcoal meal (1 ml/rat; *p.o.*)

Group-III: EEPM (500 mg/kg, *p.o.*) and 30 min later 5 % charcoal meal (1 ml/rat; *p.o.*)

Group-IV: Atropine (5 mg/kg, p.o.) and 30 min later 5 % charcoal meal (1 ml/rat; p.o.)

After half an hour all the rats were sacrificed and the distance travelled by the charcoal meal from pylorus to ileuo-cecum junction was measured.

Statistical analysis

Results were expressed as mean ± SEM. The data obtained were analyzed by one- way ANOVA followed by Tukey test[18]. The level of significance was set at $p<0.05$.

Results

Effect of EEPM on castor-oil induced diarrhoea in rat

Diarrhoea induced with castor oil showed significant increase in the total number of feces. EEPM at a dose of 250 and 500 mg/kg were significantly reduced the total number of feces (6.2 ± 0.96; $p<0.01$ and 3.4 ± 0.82; $p<0.001$; respectively). This is comparable with the reference drug loperamide (2.4 ± 0.57; $p< 0.001$).

Effect of EEPM on gastrointestinal motility in rat

Treatment with EEPM at a dose of 250 and 500 mg/kg were significantly (75.80 ± 1.06 $p<0.01$ and 68.80 ± 8.89; $p<0.001$; respectively) reduced the gastrointestinal motility in rats. This is comparable with the reference drug atropine (67.60 ± 2.11; $p< 0.001$).

Table 1: Effect of EEPM on castor-oil induced diarrhoea in rat.

Group	Total number of feces	Inhibition of diarrhoea (%)
Group-I	13.60 ± 1.88	--
Group-II	6.2 ± 0.96 *	↓ 54.41
Group-III	3.4 ± 0.82 **	↓ 75
Group-IV	2.4 ± 0.57 **	↓ 82

Values are expressed as mean ± S.E.M (n = 5), where* p<0.05 and **p < 0.01, when compared to the control.

Fig 1: Effect of EEPM on castor-oil induced diarrhoea in rat

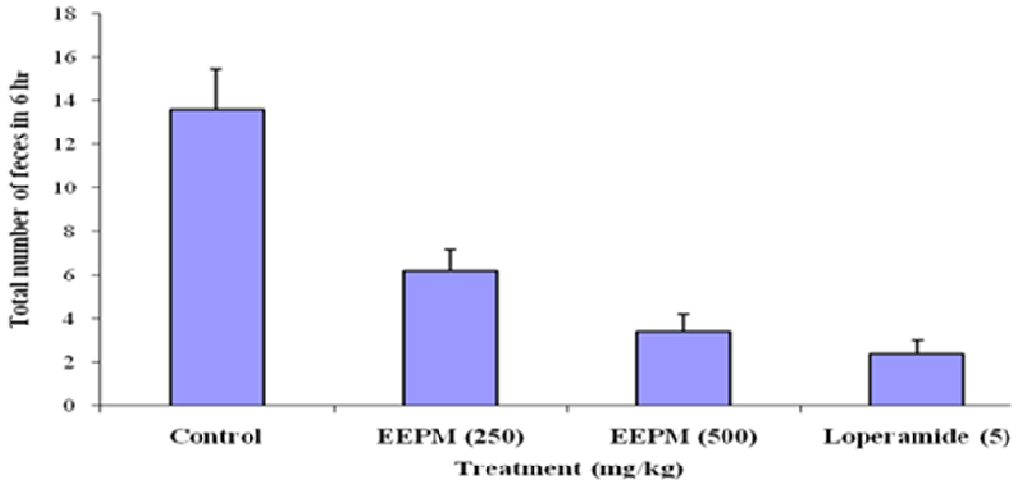
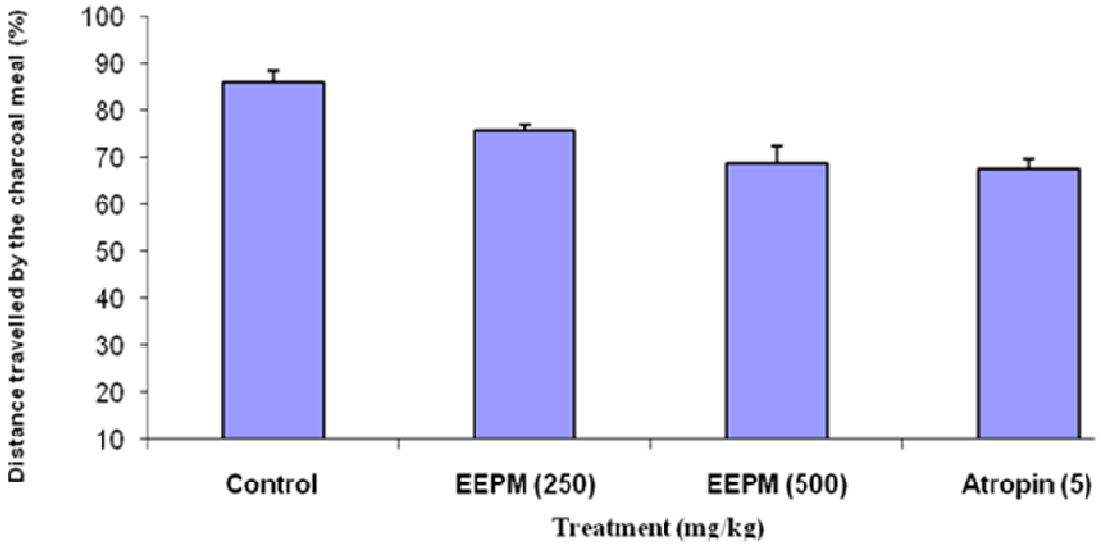


Table 2: Effect of EEPM on gastrointestinal motility in rat.

Group	**Distance travelled by Charcoal meal (cm)**	**Inhibition of diarrhoea (%)**
Group-I	86.20± 2.28	--
Group-II	75.80± 3.06	↓ 12.00
Group-III	68.80± 1.89 **	↓20.18
Group-IV	67.60± 2.11 **	↓22.27

Values are expressed as mean ± S.E.M (n = 5), where **p < 0.0 1, when compared to the control.

Fig 2: Effect of EEPM on gastrointestinal motility in rat



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Discussion

In the traditional system of medicine heartwood of *Pterocarpus marsupium* is used in the management of diarrhoea. Present investigation aims to study the effect of ethanolic extract of *Pterocarpus marsupium* heartwood on castor oil- induced diarrhoea in experimental animals. Phytochemical studies of EEPM showed the presence of glycoside, carbohydrates, flavonoids and tannins.

EEPM showed a dose dependent significant activity against castor oil-induced diarrhoea. The result is almost comparable to the effect of widely used antidiarrheal drug loperamide when tested at a dose level of 5 mg/kg. Castor oil is made up of 90% ricinoleate (15) which is metabolized to ricinoleic acid, causes the irritation and inflammation in the intestinal mucosa, leading to release of prostaglandins, and stimulate the net secretion of water and electrolytes into the small intestine (16). Therefore the antidiarrheal effects of EEPM might be due to the inhibition of prostaglandin biosynthesis.

The extract significantly reduced intestinal transit as an evidenced by the decrease in the distance traveled by charcoal meal. These results also show that the extract suppressed the propulsion of charcoal meal thereby increasing the absorption of water and electrolytes.

Antidiarrheal properties of medicinal plants were found to be due to tannins, flavonoids, alkaloids, saponins, reducing sugar, sterols and/or terpenes (17, 18). The antidiarrheal activity of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydro-electrolytic secretions (19) which are altered in this intestinal condition. In vitro and in vivo experiments have shown that flavonoids are able to inhibit the intestinal secretory response induced by prostaglandins E2 (20). In addition, flavonoids present antioxidant properties which are presumed to be responsible for the inhibitory effects exerted upon several enzymes including those involved in the arachidonic acid metabolism (21). These constituents may be responsible for the antidiarrheal activity of the ethanolic extract of *P. marsupium*.

Thus, results of this study reveals that the heartwood extract of *P. marsupium* contains pharmacologically active substances with antidiarrheal properties. These properties could be a potential source of modern pharmaceutical products. Further investigation is necessary for isolation, identification and characterization of different active compounds from the extract and for elucidating their mode of action.

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