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Anti-diarrhoeal activity of ethanolic extract of heartwood of *Pterocarpus marsupium* roxb.

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ABSTRACT

Diarrhoea is a common cause of death in developing countries and 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. They were found to contain tannins, alkaloids, saponins, sterols, triterpenes and reducing sugars. This study evaluated the antidiarrhoeal activity of ethanolic extract of heartwood of Pterocarpus marsupium induced by castor oil and magnesium sulphate in rat at 200 and 400 mg/kg b.w. The doses were given orally and showed significant antidiarrhoeal activity comparable with that of the standard drug loperamide. The statistical analyses of results were carried out using one-way analysis (ANOVA) followed by Student t-test. On the basis of these findings, it can be assumed that Pterocarpus marsupium could be a potential source for novel discovery for antidiarrhoeal. These results may support the fact that this plant is used traditionally to cure diarrhoea.

Keywords: Pterocarpus marsupium, Anti-Diarrhoeal, Castor Oil, Magnesium Sulphate, Loperamide

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INTRODUCTION

Diarrhoea has long been recognized as one of the most important health problems in the developing countries and a major cause of infant mortality and morbidity [1-2]. Worldwide distribution of diarrhoea accounts for more than 5-8 million deaths each year in infants and small children less than 5 year. According to WHO estimation for the year 1998, there were about 7.1 million deaths due to diarrhoea[3]. Secretory diarrhoea is the most dangerous symptom of gastrointestinal problems [4] and is associated with excessive defecation and stool outputs, the stools being of abnormally loose consistency[5]. So the present study was aimed to evaluate the traditional claim of antidiarrhoeal activity of ethanolic extract of heartwood of *Pterocarpus marsupium* in various experimental models.

MATERIAL AND METHODS

Collection and Authentication of Plant Material:

The heartwood of *Pterocarpus marsupium* Roxb. were obtained from Haridwar city and authenticated by Department of Botany, IFTM University, Moradabad, U.P. A voucher specimen was preserved in the Department of Pharmacy, IFTM University for future reference.

Preparation of Extract:

Heartwood of *Pterocarpus marsupium* washed with distilled water to remove dirt and soil and shade dried in a ventilated place at room temperature. The dried plant materials were reduced to coarse powder by mechanical grinder, extracted with 95% ethanol as solvent in soxhlet extractor for 18 hours. The ethanolic extract of heartwood of *Pterocarpus marsupium* was filtered and concentrated under reduce pressure using rotavapor (Buchi, USA), then freeze-dried (Freezone® 4.5, Labconco, USA) and stored in deep freezer for further use. Solutions of the extracts were prepared freshly for each study.

Preliminary Phytochemical Screening:

The Ethanolic Extract of *Pterocarpus marsupium* (EEPM) was tested for the presence of various chemical constituents such as saponins, flavonoids, glycosides, alkaloids, tannins and reducing sugar.

Pharmacological Studies :

Animals:

Wistar albino rats of either sex weighing 200 ±25g were kept in Departmental animal house at a temperature (25 ± 2)°C and 12 hours light/dark cycle respectively for one week before and during the experiments and fed with standard

diet and water ad libitum. Animal studies were conducted according to the Institute Animal Ethics Committee. All the experiments were performed in the morning according to the current guidelines for the care of laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals.

Drugs and Chemicals:

Magnesium sulphate, Loperamide (Ranbaxy (I) Ltd, Castor oil (Galaxo) and all other chemicals were of analytical grade.

Acute Oral Toxicity Study (LD50):

Acute oral toxicity studies were performed as per OECD-423 guidelines to determine the safety doses. Acute Oral Toxicity studies of the extract were carried out on female wistar strain albino rats. Rats were fasted over night and weight of each animal was recorded just before study. Animals were divided into ten groups. They were fed orally with the ethanolic extract of heartwood in increasing dose levels of 100, 200, 400, 500, 1000, 1500, 2000, 3000, 4000 and 5000 mg/kg b.w. through oral feeding needle. The animals were observed continuously for changes in signs and symptoms and mortality. The LD_{50} was 4 g/kg body weight therefore the ED_{50} was found to be 400 mg/kg body weight. The doses for the study have been selected from these results and the experiments were carried out.

Antidiarrhoeal Experiment Models:

Castor Oil-induced Diarrhoea:

Wistar albino rats of either sex (200 ±25g) were fasted for 24 h before starting the experiment. The animals were randomly housed in individual cages and divided into four groups (n=6). The group I received 1% CMC (10 ml/kg p.o.) served as the control and group II received loperamide (3 mg/kg) acting as the standard. The last two groups received different doses (200 and 400 mg/kg p.o.) of the plant extract. One hour after the treatment, each animal received castor oil (10 ml/kg, p.o.) through a feeding needle. At 4 hours after dosing the castor oil, the individual rats cages were inspected for the presence of unformed water faecal pellets; their absence was recorded as a positive result, indicating protection from diarrhoea at that time[6-7].

Magnesium Sulphate-induced Diarrhoea:

Animal divided in four group (n=6) and diarrhoea were induced by oral administration of magnesium sulphate at the dose of 2 mg/kg b.w. to the animals, one hour after pre treatment with 1% CMC (10 ml/kg p.o.) to the control group, loperamide (3mg/kg p.o.) to the standard group, and the plant extract at the doses of 200 mg/kg and 400 mg/kg to the remaining groups. During an observation period of 4 hours, the total number of faecal output and the number of diarrhoeic faeces excreted by the animals were recorded[8].

Statistical Analysis:

The experimental results were expressed as the mean ± standard error of the mean (S.E.M.). Data were evaluated by student's t-test and the means were compared using Graph pad prism 5 software t- test at $p< 0.001$.

RESULTS

Phytochemical Analysis of Plant Extracts:

The phytochemical analysis of heartwood of *Pterocarpus marsupium* (95% ethanol extract) were revealed the presence of various chemical constituents such as alkaloids, saponins, glycosides, tannins, flavonoids, reducing sugar etc. (Table 1)

Table 1: Qualitative Analysis of Ethanolic Extract of *Pterocarpus marsupium*:

S. No	Phytochemical Constituent	Ethanolic Extract	S. No	Phytochemical Constituent	Ethanolic Extract
1.	Carbohydrates	+	**8.**	Proteins & amino acids	**-**
2.	Alkaloids	+	**9.**	Phenolic compounds	**+**
3.	Phytosterols	+	**10.**	Tannins	+
4.	Fats & oils	**-**	**11.**	Flavonoids	+
5.	Gums & mucilages	**+**	**12.**	Triterpenes	**-**
6.	Saponins	+	**13.**	Volatile oils	**-**
7.	Glycosides	+	**14.**	Steroids & triterpenoids	**-**

Effect on Castor Oil-induced Diarrhoea:

In the castor oil-induced diarrhoeal model in rat, the 95% ethanolic extract of *Pterocarpus marsupium* at the 200 & 400 mg/kg dose levels were found to reduce the severity of diarrhoea in test animals. There was less significant effect with the dose of 200 mg/kg (p.o.) of the extract compared with control. The reduction on castor oil-induced diarrhoea at 400 mg/kg (p.o.) of plant extract treatment was found to be almost comparable with that of treatment by 3 mg/kg of loperamide (Table 2).

Table 2: Effect of *Pterocarpus marsupium* on castor oil induced diarrhoea in rats:

Treatment	Dose (mg/kg)	Total no of faeces in 4 hr	Total no of wet faeces in 4 hr	Reduction (%)
Control (1%, 10ml/kg CMC)	.....	13.66 ± 0.01135	10.63 ± 0.003594	.....
Loperamide	3	2.466 ± 0.1464***	1.431 ± 0.0119***	86.53***
EEPM	200	5.229 ± 0.006436*	3.640 ± 0.01072*	65.75*
EEPM	400	2.764 ± 0.2202**	1.927 ± 0.04667**	81.87**

Values are mean ± SEM (n=6) *** $p< 0.001$ compared to control group.

Effect on magnesium sulphate-induced diarrhoea:

In the magnesium sulphate-induced diarrhoeal model in rat, the 95% ethanolic extract of *Pterocarpus marsupium* at the 200 & 400 mg/kg dose levels were found to reduce the severity of diarrhoea in test animals. There was less significant effect with the dose of 200 mg/kg (p.o.) of the extract compared with control. The reduction on magnesium sulphate -induced diarrhoea at 400 mg/kg (p.o.) of plant extract treatment was found to be almost comparable with that of treatment by 3 mg/kg of loperamide (Table 3).

Table 3: Effect of *Pterocarpus marsupium* on Magnesium Sulphate induced diarrhoea in rats:

Treatment	Dose (mg/kg)	Total no of faeces in 4 hr	Total no of wet faeces in 4 hr	Reduction (%)
Control (1%, 10ml/kg CMC)	.....	11.15 ± 0.1017	10.65 ± 0.009204	…….
Loperamide	3	3.155 ± 0.01206***	2.741 ± 0.01177***	74.26***
EEPM	200	6.278 ± 0.1711*	4.943 ± 0.01866*	53.58*
EEPM	400	3.954 ± 0.2115**	3.035 ± 0.03847**	71.50**

Values are mean ± SEM (n=6)*** p<0.001 compared to control group.

DISCUSSION

It is well know that the traditional uses of plants and their effects are due to the presence of secondary metabolites. These metabolites are alkaloids, glycosides, flavonoides, tannins, triterpenes etc. The medicinal value of plants depends on the presence of these metabolites qualitatively & quantitatively. Number of factors, such as infective, immunological and nutritional has been involved in the perpetuation of the diarrhoeal syndrome[2]. Many plants conveniently available in India are used in traditional folklore medicine for the treatment of diarrhoea and dysentery[9] of the indigenous plants used *Andrographis paniculata, Asparagus racemosus, Butea monosperma, Cassia auriculata*, and others are mentioned[10]. Several studies have shown that prior administration with some plant extracts had a protective effect on the intestinal tract[11-13]. In the present study, the newer plant have used by tribens and rural have not been studied so far, was evaluated for its anti-diarrhoeal potential against castor oil induced diarrhoea in Wistar albino rats. 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 diarrhoea. Ricinoleic acid markedly increased the PGE-2 content in the gut lumen and also caused on increases of the net secretion of the water and electrolytes into the small intestine. 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[14-15].The mechanism involved has been associated with dual effects on gastrointestinal motility as well as on water and electrolyte transport (decreasing Na+ and K+ absorption) across the intestinal mucosa[15]. These conditions tend to suggest that the extracts of *Pterocarpus marsupium* reduces diarrhoea by increasing reabsorption of electrolytes and water or by inhibiting induced intestinal accumulation of fluid just as loperamide. Loperamide acts by decreasing the transit velocity and increasing the capacity of the intestines to retain their fluids[16]. So the dose of 400 mg/kg reduced diarrhoea by inhibiting castor oil induced intestinal accumulation of fluid. On the other hand, magnesium sulphate has been reported to induce diarrhoea by increasing the volume of intestinal content through prevention of reabsorption of water. It has also been demonstrated that it promotes the liberation of cholecystokinin from the duodenal mucosa, which increases the secretion and motility of small intestine and thereby prevents the reabsorption of sodium chloride and water [17-18].The ethanolic extract was found to alleviate the diarrhoeic condition in this model. The *Pterocarpus marsupium* extract have increased the absorption of water and electrolyte from the gastrointestinal tract in rat as compared to the control. Previous reports have been demonstrated that the antidiarrhoeal activity of flavanoids[17], alkaloids[19], tannin[20], saponins, reducing sugars and sterols and/or terpenes[21] containing plant extracts. The phytochemical analysis of the extract showed the presence of alkaloids, saponins, sterols/or terpenes, tannins, mucilage and sugars. Therefore, these constituents might be responsible for the antidiarrhoeal activity of ethanolic extract of *Pterocarpus marsupium*. The results of the present study justify the traditional claims of *Pterocarpus marsupium* extract being an antidiarrhoeal drug. Moreover, the active constituents are responsible for the antidiarrhoeal activity remain to be identified.

CONCLUSION

The 95% ethanolic extract of selected plant materials showed antidiarrhoeal activity in primarily evaluation of diarrhoeic conditions in test animals. The obtained results thus give the experimental basis to understand the use of selected traditional medicine, as an antidiarrhoeal agent. However, further bioassay guided phytochemical and pharmacological studies are required to identify the active principle(s) and exact mechanism(s) of action.

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