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Research Article

In Vivo Evaluation of Antidiarrhoeal Activity of the Seed of Swietenia macrophylla King (Meliaceae)

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Abstract

Purpose: *The seeds of Swietenia macrophylla are used in traditional medicine for the treatment of diarrhoea. Thus the petroleum ether extract of Swietenia macrophylla (Meliaceae) seeds was investigated for its anti-diarrhoeal property in Wister albino rats to substantiate folklore claim.*

Methods: *Petroleum ether extract of the seeds of this plant, at graded doses (25, 50 & 100mg/kg body weight) was investigated for anti-diarrhoeal activity in term of reduction in the rate of defecation and consistency of faeces in castor oil induced diarrhoea. To understand the mechanism of its anti-diarrhoeal activity, its effect was further evaluated on intestinal transit and castor oil induced intestinal fluid accumulation (enteropooling).*

Results*: At various doses (25, 50 & 100mg/kg body weight) the extract showed a remarkable anti-diarrhoeal activity evidenced by the reduction in the rate of defecation and consistency of faeces. Results are comparable to that of standard drug diphenoxylate (50mg/kg body weight). A single oral dose of Swietenia macrophylla extract of 100mg/kg body weight produced a significant decrease in the severity of diarrhoea. Extract produced profound decrease in intestinal transit (4.45 - 34.60%) also significantly inhibited castor oil induced enteropooling comparable to that of intraperitoneal injection of standard drug atropine sulphate at doses of 0.1 mg/kg body weight and 3 mg/kg body weight respectively.*

Conclusions: *Experimental findings showed that petroleum ether extract of seeds of Swietenia macrophylla possess significant anti-diarrhoeal activity and may be a potent source of anti-diarrhoeal drug in future.*

Key words: *Swietenia macrophylla, anti-diarrhoeal activity, castor oil, atropine sulphate.*

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INTRODUCTION

Diarrhoea is characterized by increased frequency of bowel movement, wet stool and abdominal pain [1]. It is a leading cause of malnutrition and death among children in the developing countries of the world today [2]. Many governments and international organizations are trying to control this disease but the rate of incidence is still high, about 7.1 million per year [3]. Many synthetic chemicals like diphenoxylate, loperamide and antibiotics are available for the treatment of diarrhoea but they have some side effects. The natural drugs are used as anti-diarrhoeal drugs, which are not always free from adverse effects [4]. Therefore, the search for safe and more effective agents has continued to be an important area of active research. Since ancient times, diarrhoea has been treated orally with several medicinal plants or their extracts based on folklore medicine.

The plant *Swietenia macrophylla* (Family: Meliaceae) is a beautiful, lofty, evergreen large tree native to tropical America, Mexico and South America usually 30-40m in height and 3-4m in girth [5].The seed of *Swietenia macrophylla* has been reported for its anti-inflammatory, antimutagenecity and antitumor activity [6]. Swietenine, swietenolide [7], swietemahonin, khayasin, andirobin, augustineolide, 7-deacetoxy-7-oxogedunin, 6-deoxy swietenine, proceranolide, 6-O-acetyl swietonolide, 2-hydroxy swietenine have been isolated from the seeds of this plant [8]. Seed of *Swietenia macrophylla* is traditionally used by the local healers of East Midnapore, West-Bengal, India for curing diarrhoea. The present study was undertaken to evaluate the antidiarrhoeal potential of petroleum ether extract of seeds of *Swietenia macrophylla* in normal and castor oil induced diarrhoeal rats.

MATERIALS AND METHODS

Plant material

Seeds of *Swietenia macrophylla* King. (Family: Meliaceae) were collected in the months of December and January, from the villages of Midnapore (E), West Bengal, India. The plant was authenticated by the Botanical Survey of India. A voucher specimen number entitled CNH/1-1(64) was deposited at our institute for future reference.

Preparation of extract

The powdered seeds of *Swietenia macrophylla* (600 g) were extracted with petroleum ether using Soxhlet apparatus. The resulting extract was evaporated in vacuum (Yield = 12.42%) and stored in desiccators for future use. The crude extract was dissolved 2% Tween 80 prior to the experiment and used.

Animals

Swiss albino rats (150 – 180 g) of either sex were selected for the experiments. Animals were allowed to be acclimatise for a period of 2 weeks in our laboratory environment prior to the study. Animals were housed in polypropylene cages (4 animals per cage), maintained under standard laboratory conditions (*i.e.* 12:12 hour light and dark sequence; at an ambient temperature of 25±2ºC; 35-60% humidity); the animals were fed with standard rat pellet diet (Hindustan Liver Ltd. Mumbai) and water *ad libitum*. The principles of Laboratory Animal Care (NIH, 1985) were followed and instructions given by our institutional animal ethical committee were maintained throughout the experiment.

Chemicals and Reagents

Atropine sulphate and diphenoxylate (standard reference antidiarrhoeal drugs), castor oil (laxative agent), normal saline solution (0.9% NaCl), charcoal meal (10% activated charcoal in 5% gum acacia) and vehicle (2%v/v Tween 80 in distilled water) were used. Atropine sulphate was procured from Samarth Pharma. Pvt. Ltd., Mumbai, India and diphenoxylate was purchased from Maiden Pharma. Pvt. Ltd., Delhi, India.

Castor oil-induced diarrhoea

Rats were fasted for 18 h and divided into five groups of six animals per group. Castor oil at a dose of 1 ml/animal orally, was given to all groups of animals for the induction of diarrhoea [9]. Thirty minutes after castor oil administration, the first group (control group) received vehicle (0.5% v/v Tween 80 in distilled water), while the second, third and fourth groups were given petroleum ether extract at doses of 25, 50 and 100 mg/kg body weight respectively by oral route. The fifth group received the reference drug, diphenoxylate (50 mg/kg body weight) [10].

Animals of all groups were placed separately in individual cages lined with filter paper. The filter papers were changed every hour and the severity of diarrhoea was assessed hourly for six hours. The total number of faeces excreted and the total weight of faeces were recorded within a period of six hour and compared with the control group. The total number of diarrhoeal faeces of the control group was considered 100%. The results were expressed as percentage of inhibition of diarrhoea [11].

Gastrointestinal motility test

This experiment was done by using charcoal meal as a diet marker [12]. The rats were divided into five groups of six animals each and fasted for eighteen hours before the experiment. The first group (the control group) was orally administered the vehicle (0.5% Tween 80 in distilled water). The second, third and fourth groups orally received petroleum ether extract at doses of 25, 50 and 100 mg/kg body weight respectively. The fifth group received the standard drug, atropine sulphate (0.1 mg/kg body weight intraperitoneal). Thirty minutes later each animal was given 1 ml of charcoal meal (10% activated charcoal in 5% gum acacia) orally. Each animal was sacrificed thirty minutes after administration of charcoal meal. The distance covered by the charcoal meal in the intestine was expressed as a percentage of the total distance traveled from the pylorus to the caecum[13].

Castor oil-induced enteropooling

Intraluminal fluid accumulation was determined by the method of Boominathan *et al*. 2005. Over night fasted rats were divided into five groups of six animals each. Group 1 which received normal saline (2 ml/kg intraperitoneal) served as the control group. Group 2 received atropine (3 mg/kg intraperitoneal) and groups 3, 4 and 5 received extract of 25, 50 and 100mg/kg intraperitonealy, respectively, one hour before the oral administration of castor oil (1 ml). Two hours later, the rats were sacrificed; the small intestine was removed after tying the ends with threads and weighed. The intestinal content was collected by milking into a graduated cylinder and their volume was measured. The intestine was reweighed and the difference between the full and empty was calculated.

Statistical analysis

Data were analyzed by one-way ANOVA followed by Dunnett's *t*-test using computerized GraphPad InStat version 3.05 (Graph Pad software, U.S.A.).

RESULTS

The petroleum ether extract was found to be effective against castor oil induced diarrhoea on experimental rats at various doses of 25, 50 & 100 mg/kg body weight has been shown in Table 1. A single oral dose of *Swietenia macrophylla* extract of 100 mg/kg body weight produced a significant decrease in the severity of diarrhoea in terms of reduction in the rate of defecation and consistency of faeces in Wistar albino rats. The percentage inhibition for the number of wet faeces as well as wet mass indicates the presence of antidiarrhoeal activity in extract as compared with that of control group. Experimental results reflect the activity is more pronounced at the dose of 100mg/kg body weight. The percentage of inhibition of number of wet faeces as well as wet mass found 67.23% and 69.46% respectively at the dose of 100 mg/kg body weight very much comparable to that of standard drug diphenoxylate (50mg/kg body weight). The petroleum ether extract produced profound decrease in intestinal transit of 4.45-34.60% at the dose range of 25-100 mg/kg body weight and significantly inhibited castor oil induced enteropooling in terms of volume and weight of intestinal content comparable to that of intraperitoneal injection of standard drug atropine sulphate at doses of 0.1 mg/kg body weight and 3mg/kg body weight respectively as indicated in Tables 2 and 3.

DISCUSSION

Diarrhoea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract accompanied by hurry resulting in an excess loss of fluid in the faeces. In some diarrhoea the secretory component predominates while other diarrhoea is characterized by hypermotility [14]**.** Castor oil causes diarrhoea due to its active metabolite, ricinoleic acid [15, 16], which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal

Table 1: Effect of the petroleum ether extract of *S. macrophylla* seeds at different dose levels on castor oil- v induced diarrhoea.

Group	Total number of faeces (g)	Total number of diarrhoeal faeces	Inhibition (%)	Total weight of faeces (g)	Inhibition (%)
Castor oil (1ml) + vehicle (0.5% Tween 80)	24.2 ± 1.49	18.0 ± 0.71	0.00	9.76 ± 0.34	0.00
Diphenoxylate (50 mg/kg) + castor oil (1ml)	7.8 ± 0.37 ***	5.2 ± 1.30 ***	71.12	1.76 ± 0.57***	81.97
Petroleum ether extract (25 mg/kg) + castor oil (1 ml)	23.8 ± 1.59	17.8 ± 0.84	1.20	6.98 ± 1.87	28.48
Petroleum ether extract (50 mg/kg) + castor oil (1 ml)	18.68 ±0.87 *	6.3 ± 0.84 ***	65.00	4.76 ± 1.47*	51.23
Petroleum ether extract (100 mg/kg) + castor oil (1 ml)	18.0 ± 0.71 **	5.9 ± 1.30 ***	67.23	2.98 ± 0.58**	69.46

*Values are expressed as mean ± S.E.M. (n=6). *p < 0.05, **p <0.01, ***p <0.001when compared with vehicle-control.*

Table 2: Effect of the petroleum ether extract of *S. macrophylla* seeds at different dose levels on charcoal-induced gut transit changes.

Group	Distance traveled by charcoal meal (%)	Inhibition (%)
Vehicle (0.5% Tween 80) (control)	73.23 ± 8.75	0.00%
Atropine sulphate (0.1 mg/kg)	41.24 ± 5.32*	43.68%
Petroleum ether extract (25 mg/kg)	69.97 ± 7.89	4.45%
Petroleum ether extract (50 mg/kg)	63.78 ± 6.86	12.90%
Petroleum ether extract (100 mg/kg)	47.89 ± 5.53	34.60%

*Values are expressed as mean ± S.E.M. (n=6). *p< 0.05 when compared with vehicle-control.*

mucosa. Its action also stimulates the release of endogenous prostaglandin [17]. Castor oil reported to induce diarrhoea by increasing the volume of intestinal contents by preventing the re-absorption of water. The liberation of ricinoleic acid results in irritation and inflammation of intestinal mucosa leading to release of prostaglandin [18]. In this study, the petroleum

Table 3: Effect of the petroleum ether extract of *S. macrophylla* seeds at different dose levels on castor oil enteropooling.

Group	Volume of intestinal content (ml)	Weight of intestinal content (g)
Normal saline + castor oil (1ml)	4.23 ± 0.16	4.67 ± 0.58
Atropine sulphate + castor oil (1ml)	1.54 ± 0.45 **	2.69 ± 0.57*
Petroleum ether extract (25mg/kg) + castor oil (1ml)	3.97 ± 0.24	3.97 ± 0.45
Petroleum ether extract (50mg/kg) + castor oil (1ml)	1.55 ± 0.67 **	2.37 ± 0.28*
Petroleum ether extract (100mg/kg) + castor oil (1ml)	1.62 ± 0.73**	2.43 ± 0.42*

*Values are expressed as mean ± S.E.M. (n=6). *p < 0.05, **p <0.01, when compared with control.*

ether extract of *Swietenia macrophylla* seeds exhibited a significant dose-dependant anti-diarrhoeal activity. The results were comparable to that of the standard drug diphenoxylate (50 mg/kg) with regard to the severity of diarrhoea. Petroleum ether extract also significantly reduced intestinal transit as observed by the decrease in transit motility of charcoal meal. This may be due to the fact that the extract may increase the reabsorption of water by decreasing intestinal motility as observed in the decrease of intestinal transit by charcoal meal. The extract also led to a marked reduction in the weight and the volume of the intestinal contents on castor oil-induced enteropooling.

Above observations suggest that the extract in graded doses reduce diarrhoea by inhibiting peristalsis, gastrointestinal motility and castor oil induced enteropooling. It is equally effective in prevention and curing of diarrhoea. Phytochemical screening revealed the presence of steroids (Libermann-Burchard test and Salkowski test both showed positive results) and triterpenes (Libermann-Burchard test and Noller test both showed positive results) in petroleum ether extract. Steroids are useful for the treatment of diarrhoea and also may enhance intestinal absorption of Na^+ and water [19, 20]. Hence, steroid may be responsible for the anti-diarrhoeal activity.

CONCLUSION

In conclusion, the results of this investigation revealed that petroleum ether extract contains pharmacologically active substance(s) with anti-diarrhoeal properties. This provides the rationale for the use of the seed extract of *Swietenia macrophylla* as an anti-diarrhoeal drug by traditional healers. Further research is to be carried out to fractionate and purify the extract, in order to find out the molecule responsible for the anti-diarrhoeal activity observed.

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