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Evaluation of the Antidiarrhoeal Activity of *Swietenia mahagoni* Seed Extracts

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Abstract: *Swietenia mahagoni* belongs to Meliaceae family, native to West Indies and commonly known as mahogani. The present study was undertaken to evaluate the antidiarrhoeal activity of the ethanolic, methanolic and aqueous extracts of *S. mahagoni* seeds at the concentration (50, 100, 200 and 300 mg kg^{-1} b.wt., p.o) using different experimental model such as castor oil induced diarrhea, enteropooling and gastrointestinal motility were determined by *in vivo* experimental models. All the extracts showed significant antidiarrhoeal activity in a concentration dependent manner ($p<0.05$, $p<0.01$). Among the three solvent extracts; ethanolic extract showed most potent antidiarrhoeal activity as evidenced by the reduction in the rate of defecation and consistency of faeces. Extract produced profound decrease in intestinal transit and significantly inhibited castor oil induced enteropooling comparable to that of intraperitoneal injection of standard drug Diphenoxylate (50 mg kg^{-1} b.wt.) and atropine sulphate (2.5 mg kg^{-1} b.wt.). The prolonged onset of diarrhoea, inhibition of castor oil-induced enteropooling and the suppressed propulsive movement observed in this study support the traditional claim of *S. mahagoni* as an antidiarrhoeal drug in the Indian system of medicine without any side effects. Phytochemical analysis showed the presence of alkaloids, tannins, saponins and carotenoids which may be the active phytoconstituents.

Key words: Antidiarrhoeal, *Swietenia mahagoni,* diphenoxylate, castor oil, atropine sulphate

INTRODUCTION

According to Guerrant *et al*. (2001) diarrhoea is an alteration in the normal bowel movement, characterized by a situation in which an adult daily stool exceeds 200 g and contains 60-95% water. It leads to severe dehydration and leading cause of malnutrition and death among children in the developing countries and causes 4-5 million deaths throughout the world (Alam and Ashraf, 2003). The major causative agents in man include *Shigella flexneri*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Candida albicans* (Krause *et al*., 2001; Jouret-Mourin and Geboes, 2002). To combat the problem of diarrhea in developing countries, the World Health Organization (WHO) has constituted a diarrheal disease control programmed (DDC) (Syder and Mersom, 1982). Diphenoxylate and loperamide are the major synthetic chemicals which can be used to treatment in diarrhea but increasing development of drug resistance in human pathogens as well as the appearance of side effects, need to developed new drugs from natural sources (Hellinger, 2000). Similarly, Oral Rehydration Therapy (ORT) has been widely used as key factor in the decline of child mortality due to diarrhea (Sastry and Burgard, 2005). Many rural dwellers in the world depend largely on medicinal herbs for the treatment of diarrhoeal conditions because these herbs are readily available, affordable and are an indispensable component of traditional medicine practice. In view of this, there is the need to search for plant based antidiarrhoeal drug.

Swietenia mahagoni (L.) Jacq, is native to tropical America, Mexico, South America and widely distributed in Southern Asia (India, Sri Lanka and Bangladesh). It is an economically important timber tree, belongs to the plant family Meliaceae and commonly known as mahogany. Traditionally, different parts of this plant have been used in the treatment of fever, diabetes, malaria, hypertension and tuberculosis (Chen *et al*., 2007). Seed oil of *Swietenia mahagoni* showed strong antibacterial activity against *Salmonella typhi*, *Shigella dysenteriae* and *Staphylococcus aureus* (Majid *et al*., 2004). Ethanolic seed extract of this plant showed *in vitro* antioxidant activity (Hajra *et al*., 2011). Present investigation was undertaken to evaluate the antidiarrhoeal activity of *S. mahagoni* seed extracts using various *in vivo* experimental models.

MATERIALS AND METHODS

Plant material: Fresh, mature fruits of *Swietenia mahagoni* were collected in the month of October, 2010 from Hooghly District, West Bengal, India and were

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authenticated by botanist Prof. Pradeep Mehta, Department of Botany, Dr. H. S. Gour Central University, Sagar (M.P). A voucher specimen no. (Bot/Her/1001) has been deposited at the Departmental herbarium, Department of Botany, Dr. H.S. Gour Central University, Sagar (MP, India).

Preparation of extracts: The fruits were cut into pieces to obtain seeds then the seeds were shade dried at room temperature to prevent the loss of active constituents. The dried seeds were subjected to size reduction to a coarse powdered using a mechanical grinder. The powdered plant material (35 g) was soaked in 500 mL of each 75% ethanol, methanol and distilled water in a conical flask separately. This was covered, shaken every 30 min for 6 h, allowed to stand for about 72 h. The solution was subsequently shaken and filtered using Whatman filter paper. The filtrate was evaporated to dryness using a rotary evaporator (yield was 5.450% w/w, 4.230% w/w and 3.9% w/w). The extract was then stored below ambient temperature for further studies. The crude extract was dissolved in 5% dimethyl sulfoxide (DMSO) to prepare desired concentrations for the assessment of antidiarrhoeal activity.

Drugs and chemicals: The following drugs and chemicals were used in the study: diphenoxylate (Sigma Aldrich), atropine sulfate (Sigma Aldrich), vegetable charcoal (Carbophos®, AJC Pharma, Angoulem, France) and castor oil from a local pharmacy and other chemicals were used in analytical grade.

Phytochemical analysis: Phytochemical screening was performed to detect various compounds such as tannins, flavonoids, alkaloids, steroids etc. (Trease and Evans, 1983).

Animal used: Wistar albino rats (age 6-8 weeks, body weight 140-160 g) of either sex were used for antidiarrhoeal study. They were housed in polypropylene cages and maintained under standard environmental conditions (temperature 23±2°C, relative humidity 55±10% and 12 h light and dark place) and were fed with standard pellet diet (Hindustan lever Ltd. Kolkata, India) and water *ad libitum*. Fresh animals were used for each experiment. All the experimental procedures were carried out in strict accordance with the guidelines laid down by the Committee for the Purpose of Control and Supervision on Experimentation on Animals (CPCSEA) and experimental protocols were approved by the Institutional Animal Ethics Committee (Reg. No. -379/01/ab/CPCSEA); after performing the experiments all the waste material disposed in a safe and sanitary manner.

Castor oil-induced diarrhea in rats: Diarrhoea was induced in the rats using a modified method (Bajad *et al*., 2001; Teke *et al*., 2007). The test animals were starved for 18 h prior to the experiment but were allowed free access to water. Eighty four rats were divided into 14 groups and each group containing 6 rats. Group one served as control being received (5% DMSO), the second group (positive control) received the reference drug, Diphenoxylate (50 mg kg^{-1} b.wt., p.o). Third to fourteen groups received ethanolic, methanolic and aqueous plant extracts at the concentration of 50, 100, 200 and 300 mg kg^{-1} b.wt. separately. After 30 min of drug and extract treatment, each animal was administered 1 mL of castor oil orally and the time between oil administration and appearance of first diarrhoeal drop was noted. Observations for the severity of diarrhoea were assessed each hour for a period of 6 h by monitoring the diarrhoeal drop on a pre-weighed filter paper, placed beneath the individual rat cages. The total number of faeces, diarrhoeal faeces and the total weight of faeces excreted were expressed as average and compared with the control group. The percentage inhibition of diarrhoeal defecation in each group was also noted.

Castor oil-induced enteropooling: Intraluminal fluid accumulation was determined according to the method suggested by Chitme *et al*. (2004). The test animals were starved for 18 h prior to the experiment but were allowed free access to water. Eighty four rats were divided into fourteen groups and each group containing 6 rats. Group 1, negative control received 5% DMSO while group 2, served as positive control, received atropine sulphate at the dose of (2.5 mg kg^{-1} b.wt.). The test groups animals were orally administered with the extract at the concentration of (50, 100, 200 and 300 mg kg^{-1} b.wt.) separately. Immediately 1 mL of castor oil was administered orally to each of the rats in all the groups. After 30 min, each rat was sacrificed according to the method of (Yakubu *et al*., 2005) and the ends of the small intestine tied (at the pylorus and the caecum). The organ was dissected out and intestinal content was collected by squeezing into a measuring cylinder. The volume and the mass of the intestinal content were obtained.

Gastrointestinal motility test: Gastrointestinal motility test was performed according to the method suggested by Abdullahi *et al*. (2001). The test animals were starved for 18 h prior to the experiment but were allowed free access to water. The animals were divided in to fourteen groups and each group containing 6 rats. Group 1, negative

control received 5% DMSO while group 2, positive control received Diphenoxylate at the concentration of (50 mg kg^{-1} b.wt.). The test groups were orally administered with the extract at the concentration of (50, 100, 200 and 300 mg kg^{-1} b.wt.). After 30 min of drug and extract treatment, rats from each group were administered with 1 mL of charcoal meal (10% charcoal suspension in 5% gum acacia). After 30 min of the administration of charcoal meal, the animals from the various groups were sacrificed, small intestine was removed and the length (pylorus to caecum) as well as the distance travelled by charcoal meal through the organ was measured. This distance was expressed as a percentage of the length of the small intestine (Atta and Mouneir, 2004).

Statistical analysis: The data are expressed as Mean±SD. The difference among means has been analyzed by one-way ANOVA followed by Dunnett's test ($p<0.05$, $p<0.01$) was considered as statistically significant.

RESULTS

Castor oil-induced diarrhea: In the castor oil-induced diarrhoeal experiment, ethanolic, methanolic and aqueous extracts of *S. mahagoni* seeds significantly prolonged the time of diarrhoeal induction in a dose dependent manner. The frequency of stooling (number of wet feaces and total number of feaces), fresh weight and water content of the faeces decreased significantly (Table 1). Significant reduction in diarrhoea and percentage of defecation was observed ($p<0.05$, $p<0.01$) when compared with vehicle control group.

Castor oil induced enteropooling: In case of gastrointestinal enteropooling, all the extracts also reduced the volumes of intestinal fluid and weight of the intestinal content of the animals was observed significantly in dose dependent manner (Table 2). Among the three solvent extracts; methanolic extract showed most potent castor oil induced enteropooling by lowering significant level of volume of intestinal content (mL) and weight of intestinal content (g) (01.95±0.22 mL and 02.68±0.16 g), respectively at the concentration of 300 mg kg^{-1} b.wt. when compared with the ethanolic and aqueous extracts (02.30±0.55 mL and 02.93±0.33 g; 02.48±0.19 mL and 03.00±0.47 g), respectively at the concentration of 300 mg kg^{-1} b.wt.

Table 1: Effect of the different extracts (mg kg^{-1} b.wt.) of *S. mahagoni* seeds on castor oil- induced diarrhea

Group	Dose (mg kg^{-1} b.wt.)	Total number of faeces	Total number of diarrhoeal faeces	Inhibition (%)	Total weight of faeces (g)	Inhibition (%)
Castor oil (1 mL)+5% DMSO		25.2±1.62	19.34±1.18	0.00	9.72±0.37	0.00
Diphenoxylate+castor oil (1 mL)	50	8.6±0.42**	4.57±0.77**	76.37	1.117±0.41**	88.51
Ethanol extract+castor oil (1 mL)	50	22.4±1.32**	17.54±0.91**	9.31	7.30±0.67**	21.27
	100	19.7±0.65**	14.28±0.34**	26.16	4.82±0.29**	50.44
	200	13.83±0.42**	9.76±0.51**	49.53	2.05±0.32**	78.92
	300	10.22±0.75**	4.79±0.72**	75.23	1.01±0.19**	89.65
Methanolic extract+castor oil (1 mL)	50	21.78 ±0.29**	16.66±0.50**	13.85	6.67±0.43**	31.38
	100	18.49±0.28**	13.78±0.68**	28.75	4.22±0.26**	56.58
	200	16.34±0.32**	10.06±0.32**	47.98	2.85±0.27**	70.68
	300	12.08±0.19**	05.10±0.17**	73.63	2.10±0.15**	78.40
Aqueous extract+castor oil (1 mL)	50	23.12±0.89**	18.00±0.78*	6.93	7.784±0.38**	19.92
	100	21.09±0.65**	15.65±1.19**	19.08	5.584±0.58**	42.55
	200	16.22±0.27**	10.15±0.54**	47.52	4.167±0.41**	57.13
	300	12.78±0.71**	5.23±0.23**	72.96	2.433±0.27**	74.97

Values are expressed as Mean±SD (n = 6), *p<0.05, **p<0.01 significant when compared with vehicle-control, using one way ANOVA followed by Dunnett's test

Table 2: Effect of different extracts (mg kg^{-1} b.wt.) of *S. mahagoni* seeds on castor oil induced enteropooling

Group	Dose (mg kg^{-1} b.wt.)	Volume of intestinal content (mL)	Weight of intestinal content (g)
Castor oil (1 mL) +5% DMSO		4.55±0.33	5.22±0.34
Atropine sulphate+castor oil (1 mL)	2.5	1.52±0.15**	2.43±0.36**
Ethanol extract+castor oil (1 mL)	50	4.08±0.22^{ns}	4.85±0.28^{ns}
	100	3.75±0.55*	4.47±0.22**
	200	3.16±0.81**	3.59±0.19**
	300	2.30±0.55**	2.93±0.33**
Methanol extract+castor oil (1 mL)	50	3.68±0.46**	4.73±0.29^{ns}
	100	3.30±0.25**	3.92±0.13**
	200	2.91±0.17**	3.25±0.34**
	300	1.95±0.22**	2.68±0.16**
Aqueous extract+castor oil (1 mL)	50	4.29±0.29^{ns}	4.97±0.42^{ns}
	100	3.88±0.41^{ns}	4.55±0.26**
	200	3.42±0.61**	3.74±0.33**
	300	2.48±0.19**	3.00±0.47**

Values are expressed as Mean±SD (n = 6), *p<0.05, **p<0.01 significant, NS: Not significant when compared with vehicle-control, using one way ANOVA followed by Dunnett's test

Table 3: Effect of different extracts (mg kg^{-1} b.wt.) of *S. mahagoni* seeds on charcoal-induced gut transit changes

Group	Dose (mg kg^{-1} b.wt.)	Distance traveled by charcoal meal (%)	Inhibition (%)
5% DMSO		74.05±2.28	0.00
Diphenoxylate (standard drugs)	50	26.67±3.82**	63.98
Ethanol extract+castor oil (1 mL)	50	67.21±3.42**	9.24
	100	61.04±4.22**	17.57
	200	49.12±2.66**	33.67
	300	30.11±1.23**	59.34
Methanol extract+castor oil (1 mL)	50	69.34±02.45^{ns}	9.23
	100	61.88±2.31**	17.57
	200	52.38±1.24**	29.26
	300	33.58±0.23**	54.65
Aqueous extract+castor oil (1 mL)	50	71.20±2.10^{ns}	3.85
	100	63.73±2.25**	13.94
	200	53.31±3.92**	28.01
	300	40.71±4.44**	45.05

Values are expressed as Mean±SD (n = 6), **p<0.01 significant, NS: Not significant when compared with vehicle-control, using one way ANOVA followed by Dunnett's test

Charcoal-induced gut transit changes: In case of charcoal meal-induced gut transit changes, all the extracts reduced the distance moved by the charcoal meal, when compared with the vehicle control group in a concentration dependent manner. The extracts treated group ($p< 0.05$, $p<0.01$) produced the least transit time by reduction in the charcoal meal transit time. This activity was similar to the reference drug, Diphenoxylate Table 3. Among the three solvent extracts; ethanolic extract showed most potent activity by inhibiting distance travelled by charcoal meal (59.34%) while methanolic extract inhibiting (54.65) and aqueous extract inhibiting (45.05%) movement at the concentration of 300 mg kg^{-1} b.wt., respectively.

DISCUSSION

The use of herbal remedies in the treatment of diarrhoeal diseases is a common practice in many countries. Earlier literature review shows that the plant *S. mahagoni* has traditional claims against diarrhoea and used as astringent. Hence in the present study ethanolic, methanolic and aqueous extracts were screened for antidiarrhoeal activity by castor oil induced method, Castor oil induced enteropooling method and charcoal-induced gut transit changes model. Castor oil causes diarrhea through its active metabolites ricinoleic acid (Mujumdar, 1998). Ricinoleic acid, most active component of castor oil causes irritation and inflammation of the intestinal mucosa and responsible for diarrhoeal induction (Pierce *et al*., 1971; Zavata *et al*., 1998). The irritation stimulates the peristaltic activity of the small intestine, causing changes in the electrolytic permeability of the intestinal mucosa. This sequence of events leads to the release of prostaglandins which stimulates motility and secretion thereby decreasing the absorption of sodium and potassium ions (Rouf *et al*., 2003). Inhibitors of prostaglandin synthesis are also known to delay diarrhoea induced by castor oil (Bajad *et al*., 2001). Therefore, the prolonged time of induction of diarrhoea, decreased frequency of stooling and fecal parameters (total number, fresh weight, water content and number of wet feaces) observed with all the extracts in concentrations dependent manner in this study are indications of antidiarrhoeal potential. These observations also suggest that the antidiarrhoeal activity of the extract may be due to inhibition of prostaglandin biosynthesis. Atropine sulphate is known to produce anticholinergic effect in the evaluation of intestinal transit (Izzo *et al*., 1999) while the activated charcoal is capable of preventing the absorption of drugs and other chemicals into the body (Venkatesan *et al*., 2005). The suppressed intestinal propulsive movement of the charcoal meal by the extracts of *S. mahagoni* suggests antidiarrhoeal activity of the plant. This may be due to the ability of the extract to increase the time for absorption of water and electrolytes in the manner similar to the action of atropine sulphate. It has been shown that castor-oil causes motility and secretary diarrhea. This is achieved through its dual effects on gastrointestinal motility as well as water and electrolyte transport (decreasing Na+ and K+absorption) across the intestinal mucosa. The inhibition of castor-oil induced intestinal fluid accumulation (enteropooling) and the weight of the intestinal content may be due to the ability of the extract to increased the reabsorption of electrolytes, water and inhibit the induced intestinal accumulation of fluid in a manner similar to Diphenoxylate (Tangpu and Yadav, 2004). The antidiarrhoeal activity of medicinal plants has been attributed to the presence of bioactive agents such as tannins, alkaloids, saponins, flavonoids, steroids and terpenoids (Teke *et al*., 2007; Di Carlo *et al*., 1993). Tannins denature proteins in the intestinal mucosa by forming protein tannates which make intestinal mucosa more resistant to chemical alteration and reduce secretion. When we carried out qualitative chemical test, presence tannins, alkaloids and saponins

in the extracts may be responsible for its activity. Ethanolic, methanolic and aqueous extracts of *S. mahagoni* seed showed significant antidiarrhoeal activity, which differs in respective extract. This may be due to concentration of active phytochemicals present in particular solvent extract.

CONCLUSIONS

The prolonged onset of diarrhoea, inhibition of castor oil-induced enteropooling and the suppressed propulsive movement observed in this study support to the folkloric use of *S. mahagoni* in the local population of India as an antidiarrhoeal agent.

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