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Antidiarrheal Potential of *Adenanthera pavonina* Linn Seed Aqueous Extract in Experimental Animals

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Abstract: The objective of present investigation is to investigate antidiarrheal potential of *Adenanthera pavonina* seed aqueous extract (APSAE) in experimental animals. The APSAE was administered orally to three groups of animals (six per group) in order to investigate activity against castor oil and magnesium sulphate-induced diarrhoea in rats. The effect of extract on gastrointestinal transit using charcoal and castor oil induced enteropooling was assessed using Loperamide 3mg/kg was used as reference standard. Oral administration of APSAE at doses 50,100 and 200 mg/kg exhibited dose-dependent significant ($P<0.05$) antidiarrheal potential against castor oil and magnesium sulphate-induced diarrhoea in rats. APSAE also produced significant ($P<0.05$) reduction in propulsive movement in castor oil-induced gastrointestinal transit using charcoal meal in rats when compared with reference standard Loperamide. These findings demonstrate that *Adenanthera pavonina* seed aqueous extract shows significant antidiarrhoeal potential, thus justifying its traditional use in diarrhoea.

Keywords: *Adenanthera pavonina*, Diarrhoea, Castor Oil, $MgSO_4$, Loperamide

1. Introduction

Diarrheal diseases are one of the leading causes of childhood morbidity and mortality in developing countries. An estimated 1000 million episodes occur each year in children under 5 years of age. Diarrhoea causes an estimated 5 million deaths in children less than 4 years of age per year [1]. Incidence of diarrheal diseases still remains high despite intervention of government agencies and international organization to halt the trend. Many synthetic drugs like diphenoxylate, loperamide and antibiotics are available for the treatment of diarrhoea but they have some side effects [2]. The World Health Organization (WHO) encourages studies for the treatment and prevention of diarrheal diseases based on traditional medical practices [3].

Adenanthera pavonina Linn. (Family: Leguminosae-Mimosaceae), is a deciduous tree, 18-24 m tall, bole erect and 60 cm in diameter [4]. Many species of adenanthera, including Adenanthera pavonina, have been used as traditional herbal medicine against a variety of diseases including diabetes, lipid disorders, diarrhoea, haemorrhage from the stomach, haematuria and as anti-inflammatory agent in gout. The seed contains an anti-inflammatory active principle O-acetylethanolamine. The leaves contain octacosanol, dulcitol, glucosides of betasitosterol and stigmasterol. The bark contains stigmasterol glucoside [5]. Phytochemical studies shows the presence of the glycosides, saponins and steroids in seed and pod [6], [7]. Pavonin, a new five-membered lactone ring compound isolated from *Adenanthera pavonina* [8]. Oil from seeds reported membrane-stabilizing activity by reducing lytic effect on erythrocytes [9]. The methanol seed extract has been reported to demonstrate anti-inflammatory and analgesic activities [10]. Neuroprotective effect of seeds in neuropathic pain in streptozotocin-induced diabetic rats [11].

On the basis of reported activities and chemical

constituents the aqueous extract of seed was chosen for the present study. Therefore, taking into consideration the reported pharmacological activities of *Adenanthera pavonina* Linn., the present study is planned to investigate antidiarrheal potential of *Adenanthera pavonina* Linn. seed aqueous extract in rats.

2. Materials and Methods

2.1. Plant Materials

The seeds of Adenanthera pavonina were collected in the month of April, 2009, from Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India and were authenticated by Dr. P. G. Diwakar, Joint Director, Botanical Survey of India, Pune. A voucher specimen (BSI/WRC/Tech/2010/463) kept in herbarium, BSI for future reference.

2.2. Chemicals

Loperamide, the standard drug (Micro labs, Bangalore). All other chemicals including Castor oil, magnesium sulphate, atropine sulphate, charcoal meal (SD Fine chemicals, Mumbai) were of analytical grade.

2.3. Preparation of Extract

The seeds of plant were washed with distilled water, shed dried and latter powdered. This powder was then defatted with petroleum ether which was further macerated with distilled water for 72 hrs with occasional shaking. It was then filtered and evaporated. The yield of APSAE was 2.6% w/w.

2.4. Preliminary Phytochemical Screening

The preliminary phytochemical screening of APSAE was carried out for qualitative identification of type of phytoconstituents present [12].

2.5. Animals

Healthy wistar albino rats weighing 150-200g of either sex were obtained from in house breed at the animal house of M. E. S's College of Pharmacy, Sonai and were housed in polypropylene cages lined with husk in standard environmental conditions (Temperature 25±2°C; relative humidity 55 ± 10%; and 12:12 light: dark cycle). The animals were fed on a standard pellet diet (Amrut rat and mice feed, Sangli, India) and water ad libitum.

Animals were acclimatized to the laboratory condition for at least 1 week prior to the experiment and were maintained in a well-ventilated animal house. The experimental protocol was approved by the Institutional animal Ethical Committee (MES/COP/IAEC/2009-10/03) and the care of the laboratory animals was taken as per the current CPCSEA regulations.

2.6. Experimental Design

2.6.1. Acute Toxicity Study (OECD 420, 2001)

The present study was conducted according to the organization for economic cooperation and development (OECD) revised fixed dose procedure for acute toxicity testing (OECD guideline 420, 2001). Two groups of five healthy albino wistar rats of either sex (3-month old, 150–200 gm b.wt.) were administered a limit dose of 2000 and 5000 mg/kg of the APSAE and animals were observed for mortality and clinical signs for the first hour, then hourly for 3 h and finally periodically until 48 h. All of the experimental animals were maintained under close observation for 14 days and the number of rats that died within the study period was noted. The LD_{50} was predicted to be above 2000 or 5000 mg/kg if three or more rats survived [13].

2.6.2. Castor Oil Induced Diarrhoea

Wistar albino rats were divided into five groups of six animals (n=6) each. All rats were fasted for 18 hrs and received castor oil at a dose of 1 ml/animal orally (p.o.) using orogastric tubes for induction of diarrhoea [14]. Thirty minutes after castor oil administration, rats of group I (control) received 1.0 ml/100 g of 0.9% NaCl in distilled water (normal saline), group II (Reference) received standard drug, loperamide (3 mg/kg p.o.) and rats of groups III, IV and V received 50,100 and 200 mg/kg APSAE, p.o. respectively. The animals were placed separately in metabolic cages over white clean Whatmann filter paper, which was changed every hour. The severity of diarrhoea was assessed each hour for 4 hrs. The total number of diarrheal faeces of the control group was considered 100%.

2.6.3. Magnesium Sulphate Induced Diarrhoea

Wistar albino rats were divided into five groups of six animals (n=6) each. All rats were fasted for 18 hrs and received magnesium sulphate at a dose of 2 g/kg orally (p.o.) using orogastric tubes for induction of diarrhoea [14]. Thirty minutes after magnesium sulphate administration, rats of group I (control) received 1.0 ml/100 g of 0.9% NaCl in distilled water (normal saline), group II (Reference) received standard drug, loperamide (3 mg/kg p.o.) and rats of groups III, IV and V received 50,100 and 200 mg/kg APSAE, p.o. respectively. The animals were placed separately in metabolic cages over white clean Whatmann filter paper, which was changed every hour. The severity of diarrhoea was assessed each hour for 4 hrs. The total number of diarrheal faeces of the control group was considered 100%.

$$\% \text{ inhibition} = (\text{Control} - \text{Test}) \times 100/\text{Control} \quad (1)$$

2.6.4. Measurement of Gastrointestinal Transit Time Using Charcoal

Wistar albino rats were fasted for 18 hrs and divided into five groups of six animals each. Castor oil (1 ml) was administered orally to the animals. One hour later, Group I (control) was administered 1.0 ml/100 g of 0.9% NaCl in distilled water (normal saline), group II (Reference) received standard drug, atropine sulphate (5mg/kg p.o.) and rats of groups III, IV and V received 50,100 and 200 mg/kg APSAE, p.o. respectively. After 30 min of the administration, 1 ml of charcoal meal (10% suspension in 5% gum acacia) as a marker diet was given orally to rats in all groups. The rats

were sacrificed by ether (20% v/v) anaesthesia and small intestine was carefully separated from mesentery avoiding being stretched. For each animal, gastrointestinal transit was calculated as percentage distance travelled by charcoal meal to the total length of intestine. The inhibitory effect of APSAE on gastrointestinal transit was calculated relative to atropine sulphate [15].

2.6.5. Statistical Analysis

The results were expressed as Mean ± SEM, statistical difference was done by using one-way analysis of variance (ANOVA) followed by Dunnette's multiple comparison test. A difference in the mean P value <0.05 was considered as statistically significant.

3. Results

3.1. Preliminary Phytochemical Screening

The study showed the presence of steroids, terpenoids, alkaloids, tannins, phenolic compounds, flavonoids, sugars and amino acids.

3.2. Acute Toxicity Study

Oral administration of APSAE was found safe at dose of 2000 mg/kg, p.o. and produced no signs of toxicity. However, from 5gm/kg APSAE caused slow movement of animal, decreased aggressiveness, altered touch and pain sensibility but did not cause any negative behavioural changes such as excitement, respiratory distress, convulsions or coma. No mortality was observed up to 14 days. Hence, the median lethal dose (LD_{50}) of the APSAE was then greater than 2000 mg/kg body weight. Therefore doses 50,100 and 200 mg/ kg b.wt. were selected for all in vivo experiments.

3.3. Castor Oil Induced Diarrhoea

The APSAE was found to be effective against castor oil induced diarrhoea in rats at various doses of 50, 100 and 200 mg/kg body weight as compared to control. However, at the end, there was a significant 81.36%, 19.16%, 40.49% and 66.03% reduction of diarrheal faeces with the loperamide and APSAE (50,100 and 200 mg/kg) respectively in a dose dependent manner when compared with control group "Table 1".

Table 1. *Effect of APSAE on castor oil induced diarrhoea in rats.*

Groups	Treatment	Total no. of diarrheal	Total wt. of	%
	Dose mg/kg	faeces	faeces	Inhibition
Control	-	20.16±0.60	7.83±0.30	-
Loperamide	3	4.66±0.33**	1.46±0.16**	81.36
APSAE	50	16.16±0.60*	6.33±0.33*	19.16
APSAE	100	11.00±0.36*	4.66±0.42*	40.49
APSAE	200	6.50±0.42**	2.66±0.33**	66.03

* P< 0.05, **P<0.01 Values are Mean ± SEM, n=6, when compared with control by using one way ANOVA followed by Dunnette's multiple comparison test.

3.4. Magnesium Sulphate Induced Diarrhoea

The APSAE was found to be effective against magnesium sulphate induced diarrhoea in rats at various doses of 50, 100 and 200 mg/kg body weight as compared to control. However, at the end, there was a significant 81.40%, 21.97%, 45.98% and 69.99% reduction of diarrheal faeces with the loperamide and APSAE (50,100 and 200 mg/kg) respectively in a dose dependent manner when compared with control group "Table 2".

Table 2. *Effect of APSAE on magnesium sulphate induced diarrhoea in rats.*

Groups	Treatment	Total no. of diarrheal	Total wt. of	%
	Dose mg/kg	faeces	faeces	Inhibition
Control	-	16.16±0.47	8.33±0.21	-
Loperamide	3	3.66±0.33**	1.55±0.13**	81.40
APSAE	50	12.33±0.42*	6.50±0.34*	21.97
APSAE	100	6.83±0.30**	4.50±0.42*	45.98
APSAE	200	4.33±1.03**	2.50±0.22**	69.99

* P< 0.05, **P<0.01 Values are Mean ± SEM, n=6, when compared with control by using one way ANOVA followed by Dunnette's multiple comparison test.

3.5. Measurement of Gastrointestinal Transit Using Charcoal Meal

Administration of APSAE shows significant reduction of gastrointestinal transit against castor oil induced diarrhoea in rats. There was a significant 56.12%, 84.51%, 73.26% and 60.86% reduction of gastrointestinal transit with atropine and APSAE 50,100 and 200 mg/kg respectively in a dose dependent manner when compared with control "Table 3".

Table 3. *Effect of APSAE on gastrointestinal transit using charcoal meal in castor oil induced diarrheal rats.*

Groups	Treatment	Total length of	Distance travelled by	%
	Dose mg/kg	intestine (cm)	charcoal meal (cm)	Inhibition
Control	-	75.66±0.21	73.33±0.49	96.92
Atropine sulphate	3	73.33±0.61	41.66±0.74*	56.12
APSAE	50	77.50±0.56	65.50±0.42*	84.51
APSAE	100	81.66±0.49	59.83±0.47*	73.26
APSAE	200	84.33±1.03	51.33±0.75*	60.86

* P< 0.05, **P<0.01 Values are Mean ± SEM, n=6, when compared with respective group by using one way ANOVA followed by Dunnette's multiple comparison test.

4. Discussion

Diarrhoea is the frequent passage of liquid faeces and it involves both an increase in the motility of the gastrointestinal tract along with increased secretion and decreased absorption of fluid and thus a loss of electrolytes (particularly sodium) and water [16]. Therefore to restore personal comfort and convenience many patients require antidiarrheal therapy and treatment is carried out to achieve

other objectives such as increased resistance to flow (segmental contraction, decreased propulsion and peristalsis) and increased mucosal absorption or decreased secretion [17], [18]. The present investigation involves evaluation of the antidiarrheal potential of *Adenanthera pavonina* against castor oil and magnesium sulphate induced diarrhoea. APSAE effect also investigated on gastrointestinal transit using charcoal meal in castor oil induced diarrheal rats in comparison with reference to actions of drugs like atropine sulphate in reducing gastrointestinal transit.

Castor oil, a very effective laxative is hydrolysed in the upper small intestine to ricinoleic acid [19], which can stimulate fluid secretion, inhibit water and electrolyte absorption, reduce active Na^+ and K^+ absorption and decrease Na^+, K^+-ATPase in the small intestine and colon [20], [21]. Castor oil also increases the peristaltic activity and produces permeability changes in the intestinal mucosal membrane to electrolytes and water. Furthermore, ricinoleic acid can also lead to the release of endogenous prostaglandins [22], which play an important role in the modulation of GIT, stimulate motility and secretion leading to diarrhoea [23]. In this study, the results showed that APSAE reduced castor oil induced diarrhoea as well as the number of diarrheal faeces and total weight of faeces in a dose dependent manner, which could be taken as antidiarrheal potential. Loperamide is widely employed antidiarrheal drug which effectively antagonized the diarrhoea induced by castor oil – prostaglandin [25] or cholera toxin [26]. The therapeutic effect of loperamide is believed to be due to its antimotility and antisecretory activity [27].

Magnesium sulphate has been reported to induce diarrhoea by increasing the volume of intestinal content through prevention of reabsorption of water. It has been demonstrated that it promotes the release 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 [12]. The APSAE was also found to reduce magnesium sulphate induced diarrhoea significantly which could be due to increased absorption of water and electrolytes.

The APSAE exhibited significant antidiarrheal effect on gastrointestinal transit using charcoal meal in rats. Hyper motility characterizes forms of diarrhoea where the secretary component is not the causative factor [13]. The APSAE suppressed the propulsive movement or gastrointestinal transit of charcoal meal which clearly indicates that extract may be capable of reducing the frequency of stools in diarrheal conditions. The extract inhibits gastrointestinal motility in diarrhoea through anticholinergic effect. Anticholinergic agents are known to inhibit gastrointestinal hyper motility. Castor oil induced gastrointestinal hyper motility has been suggested to be indirectly mediated by cholinergic system since it is inhibited by atropine sulphate, a known anticholinergic agent [28].

Phytochemical screening revealed the presence of numerous constituents such as steroids, terpenoids, alkaloids, tannins, phenolic compounds, flavonoids, Sugars and amino acids. Antidiarrheal properties of medicinal plants were found to be due to tannins, flavonoids, alkaloids, saponins, reducing sugar, sterol and terpenes [29], [30]. Hence tannins, reducing sugars and sterols may be responsible for antidiarrheal potential of APSAE. Hence, these findings demonstrate that *Adenanthera pavonina* has the potential to treat GI disorders such as diarrhoea owing to its antidiarrheal effect. Further studies are necessary to substantiate above claim and to work out exact mechanism of action involved in antidiarrheal activity of this plant.

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