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Pharmacological investigation of *Cajanus scarabaeoides* in different animal models of diarrhoea

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Abstract

The different solvent extract of *Cajanus Scarabaeoides* (Family: *Fabaceae*) was studied for its antidiarrhoeal properties in experimental diarrhoea, induced by castor oil and magnesium sulphate in rat. At the doses of 500 mg/kg per oral, the methanol extract showed significant antidiarrhoeal activity in castor oil model and magnesium sulphate-induced diarrhea. Where as the petroleum ether extract and chloroform extract did not show any such activity. The methanol extract also significantly, reduced the intestinal transit in charcoal meal test when compared to atropine sulphate (5 mg/kg; i.m.). The results showed that the methanolic extract of *Cajanus Scarabaeoides* have a significant antidiarrhoeal activity and supports its traditional uses in herbal medicine.

Keywords: *Cajanus scarabaeoides*, antidiarrhoeal, Loperamide, castor oil, magnesium sulphate.

1. Introduction

Diarrhoea is characterized by increased frequency of bowel sound and movement, wet stool and abdominal pain. It is the leading cause of malnutrition and death among children in the developing countries of the world today [1]. Several pharmaceuticals such as diphenoxylate and anti-microbial agents are available for the treatment and management of both adult and infantile diarrhoea. In recent times, emphasis has been focused on the use of oral rehydration solution (ORS) as a replacement therapy to replenish the lost fluid and electrolytes in diarrheic cases. However, there is still need for a continuing search for more effective anti-diarrhoeal agents with probably minimal side actions. Despite immense technological advancement in modern medicine, many people in the developing countries still

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rely on traditional healing practices and medicinal plants for their daily healthcare needs [2]. The World Health Organization (WHO) encouraged studies for the treatment and prevention of diarrhoeal diseases depending on traditional medical practices [3]. There is therefore an urgent need for the intensification of research into medicinal plants claimed to be effective in the management of diarrhoea. A number of medicinal plants are used traditionally in India for the management of diarrhoea. One of such medicinal plants is *C. Scarabaeoides* (Family: *Fabaceae*) commonly known as Rantur or Banna adhaki, is traditionally used in the treatment of diarrhea in cattle and sterility of women [4, 5]. The whole plant extract have also been found to exhibit antimicrobial, anthelmintic and hypoglycemic activity [6, 7, 8]. The traditional knowledge derived empirically should be supported by scientific testing when traditional diarrhoeal remedies have been tested for antidiarrhoeal activity. Plants with a traditional indication for antidiarrhoeal are more likely than randomly selected plants to show activity in standard antidiarrhoeal assays. Traditionally it has been mentioned that the *C. scarabaeoides* is used as a remedy for diarrhoea [4], so the present study is designed to evaluate the antidiarrhoeal activity of this plant.

2. Materials and Methods

2.1. Plant materials

The whole plant of *C. scarabaeoides* was collected in September 2008 from Midnapur, West Bengal, India. The whole plant material was taxonomically identified by Dr. S.C. Majumdar, Taxonomist, Botanical Survey of India, Koregaon Road, Pune 411 001. The whole plant were dried under shade with occasional shifting and then powdered with a mechanical grinder and stored in an airtight container.

2.2. Preparation of extracts

The powder obtained was subjected to successive soxhlet extraction with the solvents with increasing order of polarity i.e. petroleum ether (50°C), chloroform (50°C) and methanol (60°C).

2.3. Drugs and chemicals

The following drugs and chemicals were used with their sources: Loperamide Hcl (Veritaz Healthcare Limited, Hyderabad), Castor oil (Research Lab Fine, Mumbai), Magnesium sulphate (Research Lab Fine, Mumbai), Atropine sulphate(Research Lab Fine, Mumbai), Petroleum ether (SD Fine, Mumbai), Chloroform (SD Fine, Mumbai) and Methanol (SD Fine, Mumbai).

2.4. Preliminary phytochemical screening

Results of preliminary phytochemical tests suggest that petroleum ether extract shows the presence of alkaloids and glycosides, chloroform extract shows the presence of glycosides and steroids and methanol extract shows the presence of glycosides, flavonoids and steroids [9, 10].

2.5. Animals

Male Wister rats weighing 150-200 g were used with the approval of the Institute Animal Ethics Committee (1197/C/08/CPCSEA). Animals were fed a standard pellet (Lipton India, Ltd) and water *ad libitum* and maintained at 24-28°C temperature, 60-70% relative humidity, and 12 hours day and night cycle. Animals described as fasted were deprived of food for 18 hours but had free access to water.

2.6. Antidiarrhoeal activity

2.6.1. Castor oil induced diarrhoea

In This method Healthy albino rats of the either sex (160-190 g) were divided into 5 groups of 6 animals each. They were fasted for 18 h prior to

Table 1. Effect of *C. Scarabaeoides* on castor oil-induced diarrhoea in rats.

Treatment	Total number of faeces in 6 h	% inhibition of defecation	Total number of diarrhoeic faeces in 6 h	% inhibition of defecation	Time to start diarrhoeic faeces (hr)
castor oil+1% Tween 80	24.4 ± 0.51	0.00	17.3 ± 0.08	0.00	After 2.22 ± 0.09
castor oil+ loperamide 3 mg/kg	8.5 ± 0.63**	65.16	1.2 ± 0.06**	93.06	After 5.42 ± 0.12**
castor oil+ petroleum ether extract (500 mg/kg)	23.4 ± 0.03	4.09	16.8 ± 0.30	2.89	After 2.01 ± 0.06
castor oil+ chloroform extract (500 mg/kg)	22.6 ± 0.09	7.37	17.1 ± 0.02	1.15	After 2.52 ± 0.30
castor oil+ methanol extract (500 mg/kg)	9.3 ± 0.01**	61.88	3.1 ± 0.03**	82.08	After 5.36 ± 0.19**

Value are given as mean (±SE); n = 6;.*, *0* Values are statistically significant, compared to control Group at $p < 0.05$, $p < 0.001$ respectively

Table 2. Effect of *C. Scarabaeoides* on magnesium sulphate-induced diarrhoea in rats.

Treatment	Total number of faeces in 6 h	% inhibition of defecation	Total number of diarrhoeic faeces in 6 h	% inhibition of defecation	Time to start diarrhoeic faeces
magnesium sulphate +1% Tween 80	21.3 ± 0.33	0.00	14.5 ± 0.04	0.00	After 2.31 ± 0.07
magnesium sulphate + loperamide 3 mg/kg	7.2 ± 0.23**	66.19	1.9 ± 0.18**	86.89	After 4.58 ± 0.23**
magnesium sulphate + petroleum ether extract (500 mg/kg)	20.4 ± 0.08	4.22	13.6 ± 0.60	6.20	After 1.59 ± 0.06
magnesium sulphate + chloroform extract (500 mg/kg)	20.7 ± 0.08	2.81	14.1 ± 0.09	2.75	After 2.12 ± 0.30
magnesium sulphate + methanol extract (500 mg/kg)	9.2 ± 0.82**	56.8	3.8 ± 0.15**	73.79	After 4.43 ± 0.15**

Value are given as mean (±SE); n = 6;.*, *0* Values are statistically significant, compared to control Group at $p < 0.05$, $p < 0.001$ respectively

Table 3. Effect of *C. Scarabaeoides* on castor oil-induced small intestinal transit in rats.

Treatment	Total length of intestine(mm)	Distance travel by marker(mm)	% intestinal transit
Control	86.2 ± 2.86	83.8 ± 1.35	97.265 ± 1.6
castor oil+1% Tween 80	79.91 ± 2.08	70.63 ± 2.75	88.35 ± 1.14
castor oil+ atropine sulphate 5 mg/kg, p.o.	93.68 ± 3.92	36.98 ± 2.68**	39.47±2.34**
castor oil+ methanol extract (500 mg/kg)	86.92 ± 6.89	48.59 ± 3.72**	55.90 ± 3.12**

Value are given as mean (±SE); n = 6;.*, *0* Values are statistically significant, compared to control Group at $p < 0.05$, $p < 0.001$ respectively

the test, with free access to water. Group I received the vehicle (1% Tween 80 in water, 10 ml/kg, p.o.) and served as the control group. Groups II, III, IV and V were treated with standard drug (loperamide 3 mg/kg), petroleum ether extract (500 mg/kg), chloroform extract (500 mg/kg) and methanol extract (500 mg/kg), respectively. The doses of extract were selected by previous study [6]. All drugs/vehicle were administered orally (p.o.). Thirty minutes after the drug treatment, each rat was administered 1 ml of castor oil orally. Each animal was placed in an individual cage, the floor of which was lined with blotting paper. The floor lining was changed every hour. The diarrhoeal episodes were observed for 6 h. During an observation period of 6 h, the total number of faecal output and the number of diarrhoeic faeces excreted by the animals were recorded. Antidiarrhoeal activity was determined in terms of percentage protection [11].

2.6.2. Magnesium sulphate-induced diarrhea

A similar protocol as for castor oil-induced diarrhoea was followed. Diarrhoea was induced by oral administration of magnesium sulphate at the dose of 2 g/kg to the animals 30 min after pre-treatment with Group I received the vehicle (1% Tween 80 in water, 10 ml/kg, p.o.) and served as the control group. Groups II, III, IV and V were treated with standard drug (loperamide 3 mg/kg), petroleum ether extract (500 mg/kg), chloroform extract (500 mg/kg) and methanol extract (500 mg/kg), respectively. All drugs/vehicle were administered orally (p.o.) [12].

2.6.3. Effect on gastrointestinal motility

The test animals were starved for 24 h prior to the experiment but were allowed free access to water. Group I was treated with vehicle (1% Tween 80 in water, 10 ml/kg, p.o.), which served as control; Group II received 1 ml castor oil and vehicle (1% Tween 80 in water, 10 ml/kg, p.o.): Group III received standard drug (atropine sulphate 5 mg/kg, p.o.); Group IV with methanol extract (500 mg/kg), respectively. After 30 min, 1 ml of charcoal meal (3% deactivated charcoal in normal saline) was administered orally to all and 1 hr later, all the rats were sacrificed. The distance travelled by the charcoal meal from the pylorus to the caecum was noted [13, 14, 15].

2.7. Statistical analysis

The data obtained in the studies were subjected to one way analysis of variance (ANOVA) for determining the significant difference. The intergroup significance was analyzed using

Dunnett's *t* test. P values< 0.05 were considered to be significant. All the values were expressed as mean ± SEM.

3. Results

Different solvent extract of *C. scarabaeoides* was prepared by continuous hot extraction method using soxhlet apparatus with the solvents of increasing order of polarity i.e. petroleum ether (50°C), chloroform (50° C) and methanol (60°C) yielded 3.5, 1.19, and 8.94% respectively.

The color of the extract respectively was dark green, greenish, yellowish brown. Results of preliminary phytochemical tests suggest that petroleum ether extract shows the presence of alkaloids and glycosides, chloroform extract shows the presence of glycosides and steroids and methanol extract shows the presence of glycosides and flavonoids.

In the castor oil-induced diarrhoeal experiment in rat, the methanol extract of C. *scarabaeoides*, at the doses of 500 mg/kg and loperamide 3 mg/kg reduced significantly the total number of feces as well as the total number of diarrheic feces . The percentage inhibition of faecal count with standard drug (loperamide 3 mg/kg) and methanolic extract-treated groups were 93.06% and 82.08%, respectively (Table 1) when compared to the control group. Where as the pet ether and chloroform extract at 500 mg/kg per oral dose did not exhibit any significant antidiarrhoeal effect on rat. In the magnesium sulphate-induced diarrhoeal model in rat, the methanol extract and loperamide at the above dose levels significantly reduced the extent of diarrhoea in test animals. The percentage inhibition of faecal count with standard drug (loperamide 3 mg/kg) and methanolic extract-treated groups were 86.89% and 73.79%, respectively (Table 2) when compared to the control group. In the gastrointestinal motility test, the methanol extract, at the doses of 500 mg/kg and atropine sulphate 5 mg/kg, p.o. retarded the intestinal transit of charcoal meal in rat when compared to the control (Table 3).

4. Discussion

Consequent on oral administration of the cathartic agent, castor oil, there is immediate and efficient hydrolysis of the oil by intestinal lipase resulting in the release of free ricinoleic acid [16]. Ricinoleic acid produces an irritating action that enhances the peristaltic activity of the small intestine seen in diarrhoea [17]. Despite the fact that these numerous mechanisms have been proposed, it has not been possible to define castor oil's correct mechanism of action [18]. However, it is well documented that castor oil produces diarrhoea due to its most active component ricinoleic acid by a hypersecretory response [19, 20]. Since the methanol extract of *C. scarabaeoides* successfully inhibited the castor oil-induced diarrhoea, the extract might have exerted its antidiarrhoeal action by antisecretory mechanism. This was also evident from the reduction of total number of wet faeces in the test groups in the experiment. 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 [21, 17]. The methanol extract was found to alleviate the diarrhoeic condition in this model. The extract may have increased the absorption of water and electrolyte from the gastrointestinal tract, since it delayed the gastrointestinal transit in rat as compared to the control. The delay in the gastrointestinal transit prompted by the

extract might have contributed, at least to some extent, to their antidiarrhoeal activity by allowing a greater time for absorption. Traditionally to treat diarrhoea in cattle the whole plant is used as water decoction for overnight. Methanol is a strong polar solvent considered to extract most plant secondary constituents. Though several constituents were present in the extract, the compound responsible for the observed actions is unknown. Flavonoids possess a wide range of activities *in vitro* [22] including antidiarrhoeal activity [13, 21] may have contributed to this activity, but further studies are required. The research was able to produce scientific basis for the traditional use of *C. scarabaeoides* plant as a remedy for diarrhoea The results suggests that the methanolic extract has a potential antidiarrhoeal effect that can be explored for therapeutic advantage as an alternative treatment for diarrhoea. To improve the safety of this traditional herbal remedy, additional research is needed to define the isolation of active constituent, stability and bioactivity of this product.

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