

Research Article

EVALUATION OF ANTIDIARRHOEAL ACTIVITY OF THE ROOT OF *CLITORIA TERNATEA* LINN

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ABSTRACT

The roots of *Clitoria ternatea* Linn are used in traditional medicine for the treatment of diarrhoea. Thus the alcoholic extract of *Clitoria ternatea* Linn (Fabaceae) root was investigated for its anti-diarrhoeal property in Wistar albino rats to substantiate Ayurvedic claim. Alcoholic extract of the root of this plant, at graded doses (100, 200 & 400 mg/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). At various doses (100, 200 & 400 mg/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 loperamide (3 mg/kg body weight). 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 dose 5 mg/kg body weight. Experimental findings showed that alcoholic extract of *Clitoria ternatea* Linn (Fabaceae) root possess significant anti-diarrhoeal activity and may be a potent source of anti-diarrhoeal drug in future.

Keywords: *Clitoria ternatea* Linn, Anti-diarrhoeal activity, castor oil and atropine sulphate.

INTRODUCTION

Diarrhoea is characterized by increased frequency of bowel movement, wet stool and abdominal pain¹. It is a leading cause of malnutrition and death among children in the developing countries of the world today². Many governments and international organizations are trying to control this disease but the rate of incidence is still high, about 7.1 million per year³. 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 antidiarrhoeal drugs, which are not always free from adverse effects⁴. 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. *Clitoria ternatea* Linn. (Family: Fabaceae) a perennial twing herb, stems are terete, more or less pubescent. There are two varieties of *Clitoria ternatea* white-flower and blue flower varieties⁵ found in Indo-China, Philippines and Madagascar, since the flowers of the plant resemble a conch shell; it is commonly called "Shankpushpi"⁶. *Clitoria ternatea* is reported to be a good "Medhya" (toning the brain) drug mainly used in the treatment of "Masasika" roga (mental illness), but it is also said to be useful in hectic fever, severe bronchitis, asthma and remedy for snakebite and scorpion sting⁷. The root with a few tortuous branches, cylindrical, 1-5 mm in thickness, a few places show cracks due to presence of lenticels, colour light brown, fracture fibrous; taste bitter. It has a sharp bitter taste and cooling, laxative, diuretic, anthelmintic, anti-inflammatory

properties; they are useful in severe bronchitis, asthma and hectic fever^{8,9}. Phytochemical screening of methanol extract of *Clitoria ternatea* Linn. root show the presence of tannins, resins, starch, taraxerol, and ternatins¹⁰. In Ayurvedic formulation root of *Clitoria ternatea* Linn. is used for curing diarrhoea¹¹. The present study was undertaken to evaluate the antidiarrhoeal potential of alcoholic extract of root of *Clitoria ternatea* Linn. in normal and castor oil induced diarrhoeal rats.

MATERIALS AND METHODS

Plant material

Roots of *Clitoria ternatea* Linn were collected from Amreli localities, Amreli district (Gujarat), the plant was identified and authenticated by Dept. of Botany, Smt. U. B. Bhagat Science Mahila college, Amreli, Gujarat.

Preparation of extract

Roots of *Clitoria ternatea* Linn (600 g) were extracted with methanol using Soxhlet apparatus. The resulting extract was evaporated in vacuum (Yield = 4.72%) and stored in desiccators for future use. The crude extract was dissolved 2% Tween 80 prior to the experiment and used.

Animals

Wistar 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 $25 \pm 2^\circ\text{C}$; 35-60% humidity); the animals were fed with standard rat pellet diet (Hindustan Liver Ltd. Mumbai) and water *ad libitum*. The experiments on animal were conducted in accordance with the international accepted principles for laboratory animal use and the experimental protocols duly approved by the institutional Ethical Committee (Reg. No. IAEC/365/01/ab/CPCSEA).

Chemicals and Reagents

Atropine sulphate and loperamide (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 (0.5 %v/v Tween 80 in distilled water) were used.

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 diarrhoea¹². 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 100, 200 and 400 mg/kg body weight respectively by oral route. The fifth group received the reference drug, loperamide (3 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.¹³

Gastrointestinal Motility Test

This experiment was done by using charcoal meal as a diet marker¹⁴. 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 100, 200 and 400 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.¹⁵

Castor oil-induced enteropooling

Intraluminal fluid accumulation was determined by the method of overnight 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 (5 mg/kg intraperitoneal) and groups 3, 4 and 5 received extract of 100, 200 and 400mg/kg intraperitoneally, 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.¹⁴

Statistical analysis

The values were expressed as mean \pm standard deviation. The statistical analysis of data was by Analysis of Variance (ANOVA) using 5% level of significance. The statistical package used was SPSS 9.0. A One-way ANOVA enabled the significant differences between the values to be observed. The Duncan test was used to identify these differences.

RESULTS

The alcoholic extract was found to be effective against castor oil induced diarrhoea on experimental rats at various doses of 100, 200 & 400 mg/kg body weight has been shown in Table 1.

Table 1: Effect of the alcoholic extract of *Clitoria ternatea* Linn root at different dose levels on castor oil induced diarrhoea.

Group	Total number of faeces (g)	Total number diarrhoeal faeces (g)	Inhibition (%)	Total weight of faeces (g)	Inhibition (%)
Castor oil (1ml) + vehicle (0.5% Tween80)	24.76 \pm 5.34 ^d	21.76 \pm 4.34 ^b	0	9.76 \pm 1.38 ^c	0
Loperamide (3 mg/kg) + castor oil (1ml)	9.76 \pm 0.94 ^a	7.46 \pm 1.60 ^a	65.72	2.45 \pm 0.37 ^a	74.90
Alcoholic extract (100 mg/kg) + castor oil (1 ml)	20.82 \pm 2.34 ^{cd}	6.58 \pm 2.75 ^a	69.76	6.81 \pm 1.98 ^b	30.23
Alcoholic extract (200 mg/kg) + castor oil (1 ml)	18.76 \pm 1.67 ^c	5.76 \pm 1.30 ^a	73.53	4.21 \pm 1.54 ^b	56.87
Alcoholic extract (400 mg/kg) + castor oil (1 ml)	14.45 \pm 1.56 ^c	4.24 \pm 0.84 ^a	80.52	2.96 \pm 1.47 ^b	69.68

Values in the same column with different letter superscripts are significantly different ($p < 0.05$)



Table 2: Effect of the alcoholic extract of *Clitoria ternatea* Linn root at different dose levels on charcoal-induced gut transit changes

Group	Distance traveled by charcoal meal (%)	Inhibition (%)
Vehicle (0.5% Tween 80) (control)	74.52 ± 8.42 ^d	0
Atropine sulphate (0.1 mg/kg)	42.65 ± 4.48 ^a	42.76
Alcoholic extract (100 mg/kg)	66.28 ± 7.25 ^{cd}	11.06
Alcoholic extract (200 mg/kg)	59.35 ± 5.52 ^b	20.35
Alcoholic extract (400 mg/kg)	49.97 ± 4.34 ^b	32.94

Values in the same column with different letter superscripts are significantly different (p<0.05).

Table 3: Effect of the alcoholic extract of *Clitoria ternatea* Linn root at different dose levels on castor oil enteropooling

Group	Volume of intestinal content (ml)	Weight of intestinal content (g)
Normal saline + castor oil (1ml)	4.16 ± 0.26 ^d	4.64 ± 1.45 ^c
Atropine sulphate (5mg/kg) + castor oil (1ml)	1.92 ± 0.34 ^c	2.56 ± 0.25 ^b
Alcoholic extract (100mg/kg) + castor oil (1ml)	1.50 ± 0.44 ^{abc}	2.51 ± 0.37 ^b
Alcoholic extract (200mg/kg) + castor oil (1ml)	1.42 ± 0.15 ^{bc}	2.54 ± 0.42 ^b
Alcoholic extract (400mg/kg) + castor oil (1ml)	1.34 ± 0.27 ^{bc}	2.71 ± 0.31 ^b

Values in the same column with different letter superscripts are significantly different (p<0.05)

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 400mg/kg body weight. The percentage of inhibition of number of wet faeces as well as wet mass found 80.52% and 69.68% respectively at the dose of 400 mg/kg body weight very much comparable to that of standard drug loperamide (3mg/kg body weight). The alcoholic extract produced profound decrease in intestinal transit of 11.06-32.94% at the dose range of 100-400 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 5mg/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.¹⁶ Castor oil causes diarrhoea due to its active metabolite, ricinoleic acid^{17,18} which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. Its action also stimulates the release of endogenous prostaglandin.¹⁹ Castor oil reported to induce diarrhoea by increasing the volume of intestinal contents by preventing the reabsorption of water. The liberation of ricinoleic acid results in irritation and inflammation of intestinal mucosa leading to release of prostaglandin.²⁰ In this study, the alcoholic extract of *Clitoria ternatea* Linn root exhibited a significant dose-dependent antidiarrhoeal activity. The results were

comparable to that of the standard drug loperamide (3 mg/kg) with regard to the severity of diarrhoea. Alcoholic 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.

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