



EVALUATION OF ANTI-DIARRHOEAL ACTIVITY OF METHANOLIC EXTRACT OF *CROTALARIA JUNCEA* LINN. IN ALBINO WISTAR RATS

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ABSTRACT

The purpose of the present study was to evaluate scientifically the anti-diarrhoeal effects of methanolic extract of leaves of *Crotalaria juncea* Linn. (MECJ) was studied against castor oil-induced-diarrhoea model and small intestine transit model in rats. Anti-diarrhoeal activity of methanolic extract of leaves of *Crotalaria juncea* Linn. was investigated in this study using castor oil induced diarrhoea, Small intestinal transit models in rats. The number of droppings and the distance traveled by charcoal in intestine were measured. Standard drug Atropine (3 mg/kg, p.o) was shown significant reductions in fecal output and frequency of droppings whereas MECJ at the doses of 200 and 400 mg/kg p.o significantly ($P < 0.001$) reduced the castor-oil induced frequency and consistency of diarrhoea. The gastrointestinal transit rate was expressed as the percentage of the longest distance travelled by the charcoal divided by the total length of the small intestine. MECJ at the doses of 200 and 400 mg/kg significantly inhibited ($P < 0.001$) the castor oil induced charcoal meal transit. The MECJ showed marked reduction in the number of diarrhoea stools as well as a modest reduction in intestinal transit. The results obtained establish the efficacy and substantiate the folklore claim as an anti- diarrhoeal agent. Further studies are needed to completely understand the mechanism of anti-diarrhoeal action of *Crotalaria juncea* Linn.

Keywords: Antidiarrhoeal Activity, *Crotalaria juncea* Linn., Traditional medicine, Castor oil induced diarrhoea, Small intestinal transit, charcoal.

INTRODUCTION

Crotalaria juncea Linn. short-day, erect herbaceous shrub, 1-3 m. tall, vegetative parts covered with short downy hairs, tap root long, strong with many well developed lateral roots and numerous branched, lobbed nodules. stems are cylindrical, ribbed and is approximately of 2 cm in diameter is a herb of annual or perennial, small shrubs of 1-3 m tall with taproots slender and vegetative parts covered with short downy hairs, stems are cylindrical, ribbed and is approximately of 2 cm in diameter erect, branched, terrete, leaves are simple with minute pointed stipules, petiole is short, opposite belonging to the family Fabaceae. *Crotalaria juncea* Linn. is generally considered to have originated in India [1]. It is distributed throughout

India, states of Bihar, Madhya Pradesh, Maharashtra, Rajasthan, Orissa and Uttar Pradesh grow this crop mainly for fibre commonly known as Sunhemp. Traditionally the plant is used in cases of amenorrhoea, blood disorders, anorexia, aphrodisiac, anti-obesity, psoriasis, anti-tumour and anti-diarrhoea. It is also used as analgesic, abortifacient, emmenagogue [2]. The native practitioners in and around Chittoor District, India, have claimed that the plant leaves are being traditionally used in diarrhoea [3]. Hence the present work is carried out to evaluate the effect of methanolic extract of leaves of *Crotalaria juncea* Linn. in an adult albino wistar rats with anti diarrhoeal activity by castor oil-induced diarrhoea and small intestine transit model.

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MATERIALS AND METHODS

Plant collection

The Plant material of *Crotalaria juncea* L used for investigation was collected from Tirumala Hills at Tirupati, Chittoor (Dist.), Andhra Pradesh, India. The plant was authenticated by Dr K. Madhava Chetty, Assistant Professor, Department of botany, S.V.University, Tirupati. The voucher specimen (PARC/2010/663) of the plant was deposited at the college for further reference.

Preparation of extract

The leaves of *Crotalaria juncea* Linn. was collected, washed, cleaned, dried and pulverized in a grinder- mixer to obtain a coarse powder and then passed through 40 mesh sieves. Weighed quantity of powdered drug was extracted successively with methanol using Soxhlet apparatus. The extraction was carried out until the extract becomes colorless. The extract was prepared. The solvent was evaporated from extract by distillation under reduced pressure. The dried extract thus obtained was kept in desiccator and was used for further experiment. Percentage yield of methanolic extract of *Crotalaria juncea* Linn. was found to be 4.9% w/w.

Preliminary phytochemical screening

The freshly prepared crude methanolic extract of *Crotalaria juncea* Linn. was qualitatively tested for the presence of phytochemical constituents by standard methods [4].

Acute toxicity studies

The acute toxicity studies of was determined as per the OECD guideline no. 423 (Acute toxic class method) [5]. It was observed that the test extract was not lethal to the rats even at 2000mg/kg dose. Hence, 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for further study.

Animals

Albino wistar rats (150-230g) of either sex were obtained from the animal house in Sree Vidyanikethan College of Pharmacy, Tirupati, Chennai. They were kept in the departmental animal house in a temperature controlled room at $25 \pm 2^\circ\text{C}$, relative humidity 44–56%, light and dark cycles of 10 and 14 h, respectively, for 1 week before and during the experiments. The animals were fed with standard pellet feed (Hindustan Lever Limited., Bangalore) and the food was withdrawn 18–24 h before the experiment, although water was allowed *ad libitum*. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA (Ref No. SVCP/IAEC/ 22-0055). All the chemicals used were of analytical grade from standard companies and water was double distilled. A

standard *Orogastric cannula* was used for oral drug administration.

Castor oil-induced diarrhoea

Animals were fasted for 24 h but allowed free access to water. Rats were divided into four groups of six animals each, diarrhoea was induced by administering 2 ml of castor oil orally to rats [6]. Group I treated as control (2 ml/kg, p.o. saline) group II received atropine (3 mg/kg p.o) served as standard and group III and IV received MECJ (200 and 400 mg/kg, p.o) 1 hr before castor oil administration. Then observed for consistency of faecal matter and frequency of defaecation for 4 hrs.

Small intestinal transit

Rats were fasted for 18 hr divided into five groups of six animals each, Group I received 2ml normal saline orally, group II received atropine (2.5 mg/kg, i.p.), group III and IV received MECJ 200 and 400 mg/kg p.o respectively, 1 hr before administration of castor oil. One ml of marker (10% charcoal suspension in 5% gum acacia) was administered orally 1 hr after castor oil treatment [7]. The rats were sacrificed after 1h and the distance travelled by charcoal meal from the pylorus was measured and expressed as percentage of the total length of the intestine from the pylorus to caecum.

RESULTS

Acute toxicity studies

Acute toxicity study in which the animals treated with the MECJ at the higher dose of 2000mg/kg did not manifest any significant abnormal signs, behavior changes, body weight changes or macroscopic findings at any time of observation. There was no mortality in the above mention dose at the end of the 14 days of observation.

Phytochemical Screening

The results of preliminary phytochemical screening of the methanolic extract of *Crotalaria juncea* Linn. revealed that presence of alkaloids, carbohydrates, tannins, gums and mucilage, aminoacids, saponins and steroids.

Castor oil-induced diarrhoea

After 30 min administration of castor oil the diarrhoea was clinically apparent in all the animals of control group, for the next 4 h. This was markedly reduced by atropine (3 mg/kg p.o) (75%). A similar marked reduction in the number of defecations over four hours was achieved with *c.juncea* at the doses of 200 or 400 mg/kg p.o. MECJ 200 and 400 significantly inhibited the defecation (25% and 50%) MECJ 200 and 400 mg/kg, p.o. dose of extract delayed the onset of diarrhoea. (Figure.1 and Table.1).

Small intestinal transit

The percent intestinal transit was increased with control (74%), but it was reduced in both doses of extract, and much more markedly by atropine (37%). MECJ 200

mg/kg, p.o dose of extract produced 20% intestinal transit induced by castor oil respectively. Whereas, MECJ 400 mg/kg, p.o dose produced 24% of castor oil induced charcoal meal transit (Figure.2 and Table.2).

Table 1. Effect of methanolic extract of *Crotalaria junea* Linn. leaves on castor oil induced diarrhoea in rats

Groups	Treatment/dose(mg/kg)	Mean defecation in 4hrs.	% Inhibition
I	Disease control (saline 2ml/kg p.o)	24.50 ± 0.42	-
II	Standard atropine(3mg/kg i.p)	12.17 ± 0.30	63.21
III	MECJ(200mg/kg p.o)	9.50 ± 0.22	56.25
IV	MECJ(400mg/kg p.o)	7.66 ± 0.33	42.38

Table 2. Effect of methanolic extract of *Crotalaria junea* Linn. leaves on castor oil induced small intestinal transit in rats

Groups	Treatment/dose(mg/kg)	Total length of the intestine	Distance travelled by marker	% of intestinal transit
I	Control (saline 2ml/kgp.o)	104 ± 0.33	74.33±0.33	73.00±0.36
II	Atropine(3mg/kgi.p)	100.6±0.37***	36.83±0.47***	37.17±0.30***
III	MECJ(200mg/kg p.o)	98.38±0.43***	19.67±0.42***	20.50±0.42***
IV	MECJ(400mg/kg p.o)	105.20±0.40***	25.33±0.16***	24.83±0.30***

Figure1. Effect of methanolic extract of *Crotalaria junea* Linn. leaves on castor oil induced diarrhoea

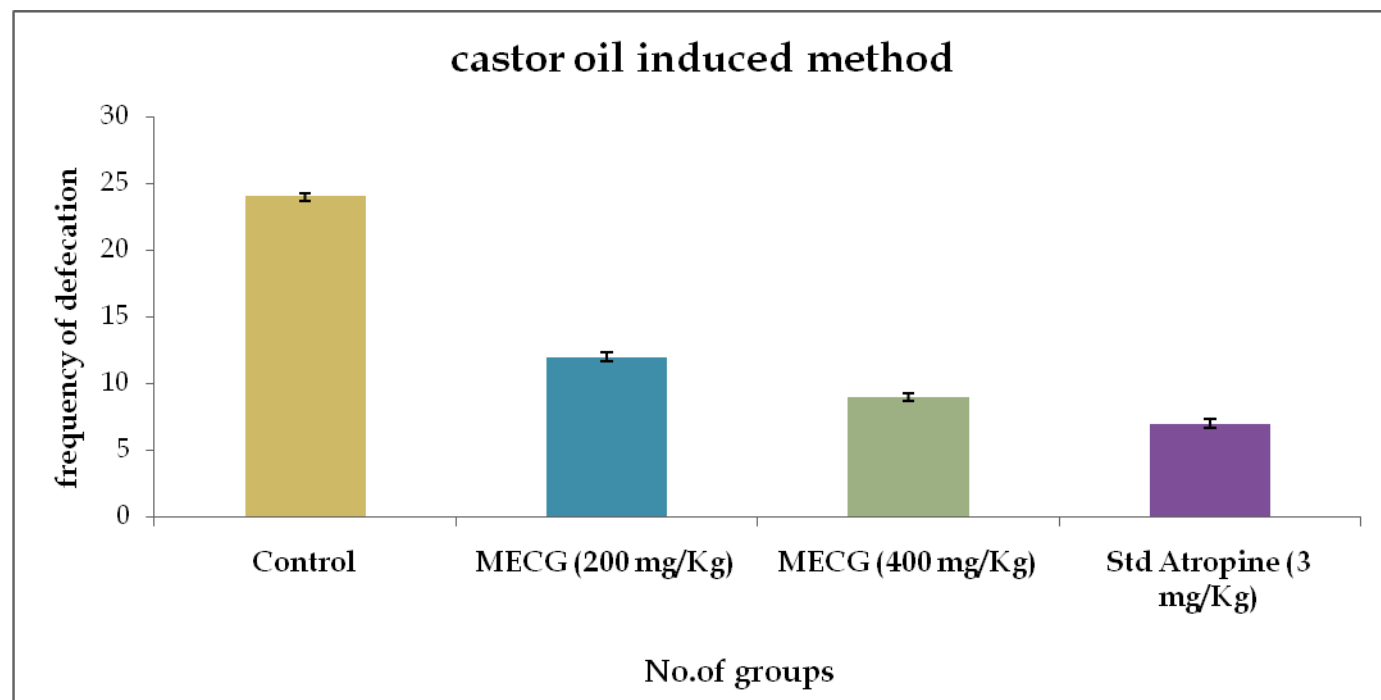
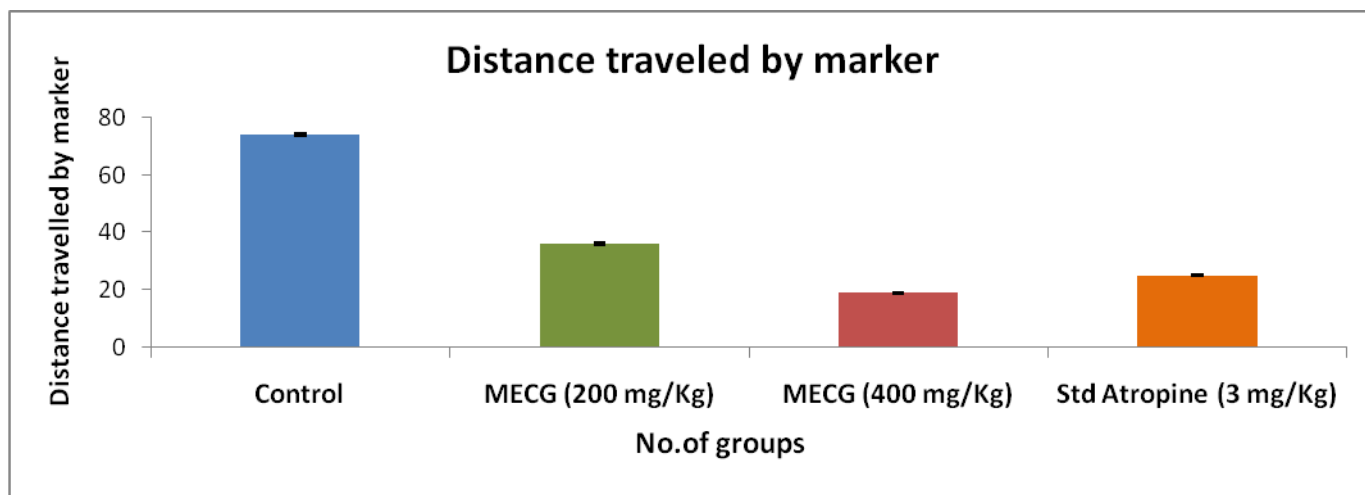


Figure 2. Effect of methanolic extract of *Crotalaria juncea* Linn. leaves on castor oil induced small intestinal transit in rats.



DISCUSSION

Diarrhoea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract, accompanied by excess loss of fluid in the faeces. At doses of 200 and 400 mg/kg, the methanol extracts of *Crotalaria juncea* Linn. showed significant antidiarrhoeal activity against castor oil-induced diarrhoea as compared with the control group. It significantly ($P < 0.01$) reduced the frequency of diarrhoea and consistency of defecations. The MECJ also showed a dose related decrease in castor oil-induced diarrhoea. Several mechanisms have been supposed to be involved in the diarrhoeal effect of castor oil. These include Castor oil decreases fluid absorption, increases secretion in the small intestine and colon, and affects smooth muscle contractility in the intestine. Castor oil produces diarrhoeal effect due to its active component of ricinoleic acid, inhibition of intestinal Na^+ , K^+ -ATPase activity to reduce normal fluid absorption, activation of adenylyl cyclase, stimulation of prostaglandin formation, platelet-activating factor and recently nitric oxide was contribute to the diarrhoeal effect of castor oil [8]. Despite the fact that number of mechanisms has been involved for the diarrhoeal effect of castor oil, it has not been possible to define its correct mechanism of action. MECJ may act against to above any one of the mechanism [9]. The MECJ significantly reduced the castor oil induced intestinal transit as compared with control group. In this study, atropine decreased intestinal transit possibly due to its anti-

cholinergic effect [10]. In castor oil induced diarrhoea, the liberation of ricinoleic acid [11]. results in irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which results in stimulation of secretion by prevents the reabsorption of NaCl and water [12]. Probably MECJ increased the reabsorption of NaCl and water by decreasing intestinal motility as observed by the decrease in intestinal transit by charcoal meal.

CONCLUSION

From the results it can be concluded that *Crotalaria juncea* Linn. is a potential therapeutic option in the effective management of diarrhoea, thus justifying its wide spread use by the local population for these purposes. Concerted efforts are being made to fully investigate the mechanisms involved in pharmacological activities of *Crotalaria juncea* Linn. and photochemical studies are also in progress to isolate and characterize the active constituents of *Crotalaria juncea* Linn. The isolated compound may serve as useful prototypes of antidiarrhoeal drugs of natural origin possessing the desired pharmacological activities while lacking certain untoward effects.

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