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Antidiarrhoeal effect of an isolated fraction (JC) of *Jatropha curcas* roots in mice

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Abstract

Objective : To study antidiarrhoeal activity of JC fraction isolated from *Jatropha curcas* L. roots in albino mice. Materials and methods : The roots of *Jatropha curcas* were collected from Awasari ghat near Pune, Maharashtra. The methanol extract of defatted roots was fractionated by solvent wash and preparative chromatography to yield JC fraction. This fraction at 50 and 100 mg/kg was tested for faecal output in normal and castor oil or magnesium sulphate induced diarrhoea in albino mice. The effect of JC fraction 100mg/kg was also studied on fluid and electrolyte secretion after castor oil administration and charcoal meal adsorption. Results : JC fraction showed activity against castor oil and magnesium sulphate induced diarrhoea . It also reduced intestinal motility as well as castor oil induced increased intraluminal fluid and sodium ion secretion in small intestine. Conclusion : JC fraction shows antidiarrhoeal activity through inhibition of prostaglandin biosynthesis and reduction of osmotic pressure. It also decreases peristaltic activity and castor oil induced permeability changes in intestinal mucosal membrane to water and electrolyte.

Key words : *Jatropha curcas* roots, Antidiarrhoeal activity, Castor oil, Magnesium sulphate, Charcoal meal.

1. Introduction

Diarrhoea is a leading cause of mortality in developing countries. In view of this, the World Health Organization has given due importance to studies on traditional medicinal practices. During ethnobotanical survey of Konkan area, a part of West coast of India , use of *Jatropha curcas* L. roots was recorded to control dysentery and diarrhoea. In order to control these symptoms, two tablespoons of root suspension is recommended once or twice depending on severity along with butter milk. It is interesting to note that this use is exactly opposite to well-known purgative action of seeds of this plant. Review of literature for ethnobotanical information discloses the use of roots after triturating with buttermilk in dyspepsia and diarrhoea [1-3].

Jatropha curcas L. (Euphorbiaceae) is a native of tropical America. It is soft-wooded shrub or small tree 5 to 50 ft. high commonly grown as

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a fence near villages. Seed oil has commercial value and is mainly used for manufacture of candles, soaps, varnishes and lubricants.

This oil is also used externally for various local diseases. Blue dye obtained from bark and leaves is used in industry [4-6]. Recently, based on the ethnobotanical lead, the methanolic extract of roots of this plant was evaluated for antidiarrhoeal activity in albino mice [7].

In the present communication results of JC fraction obtained after purification of methanol extract for antidiarrhoeal activity are reported.

2 . Materials and methods

2.1 Plant material & Extraction procedure

Roots of *Jatropha curcas* were collected in bulk quantities from Awasari ghat, a part of Western ghat of India in winter season of 1997-1998. The specimen of collected material was deposited in Agharkar Herbarium of Maharashtra Association at Agharkar Research Institute, Pune, India (voucher specimen number AHMA : 17567).

The collected material was washed with distilled water to remove dirt and soil. The root pieces were further shade dried and then coarsely powdered. Based on our earlier results the roots were defatted and extracted with methanol.

This extract was dried at low temperature to remove solvent (yield 5.51%) and was processed further monitoring antidiarrhoeal activity in albino mice. The dried extract was washed with petroleum ether (60-80°C) and residue (94.78% of methanol extract) was subjected to preparative TLC on Silica Gel G Layer using Peroleum ether : Diethyl Ether : Ethyl acetate (50: 50 :1.5) solvent system. This fraction was designated as JC fraction.

The HPTLC study of this fraction was carried out on instrument comprising of Linomat IV for spotting, using Densitometer - TLC Scanner III with "CATS" software (Camag, Switzerland). These studies were carried out on pre-coated aluminum non-fluorescent plates (E. Merck) with Benzene : Diethyl ether 70:30 system at 254 nm wavelength, using D2 lamp, which showed major spot at 0.88 Rf.

2.2 Experimental animals

Swiss albino mice of either sex weighing between 20-25 g were used for all experiments. They were originally obtained from the National Institute of Virology, Pune, India and have been inbred in the animal facilities at the Agharkar Research Institute, Pune, (ARI) for several generations for the last 16 years. They were housed in polypropylene cages in an air conditioned area at 25 ± 2°C with 10:14 hours light and dark cycle.

They were given Amrut brand balanced animal feed and water *ad-libitum.* For each treatment at least six animals were used. The protocols used for animal experiments were approved by the Institutional Animal Ethics Committee of ARI.

2.3 Drugs used

i) The extracts / fractions of *Jatropha curcas* roots were suspended in 1% Carboxy Methyl Cellulose (CMC) in water for administration.

ii) Castor oil (refined pure) - Paras Chemical Industries.

iii) Diphenoxylate Hydrochloride - Searle, India.

iv) Chlorpromazine hydrochloride - Rhone Poulene (India) Limited.

v) Activated charcoal - E. Merck (India) Limited.

vi) Atropine sulphate - Loba Chemicals.

vii) Solvents- SQ grade of Qualigens fine chemicals.

Table 1 .
Antidiarrhoeal activity of JC fraction in albino mice

Sl.No.	Group	Mean number of defections ± SEM -- in four hours
1.	Normal	9.00 ± 0.68
2.	JC fraction 50mg/kg	8.50 ± 0.96
3.	JC fraction 100 mg / kg	10.67 ± 1.29
4.	Castor oil 4 ml / kg	26.23 ± 2.66 *
5.	Castor oil 4 ml / kg + JC fraction 50 mg / kg	16.00 ± 2.28 **
6.	Castor oil 4 ml / kg + JC fraction 100 mg / kg	15.00 ± 0.62 **
7.	Castor oil 4 ml / kg + Diphenoxylate HCl 5mg/kg	4.30 ± 0.74 **
8.	$MgSO_4$ 2g / kg	32.17 ± 2.05@
9.	$MgSO_4$ 2g / kg +JC fraction 50 mg / kg	17.83 ± 2.51@@
10.	$MgSO_4$ 2g / kg+JC fraction 100 mg / kg	13.00 ± 1.00@@
11.	$MgSO_4$ 2g / kg + Diphenoxylate HCI 5mg / kg	7.67 ± 0.73@@

n=6; * Significant as compared to normal P<0.001;
** Significant as compared to Castrol oil treatment only P<0.001;
@ Significant as compared to normal P<0.001 ; @@ Significant as compared to $MgSO_4$ treatment only P<0.001

2.4 Effect of JC fraction on fecal output

The JC fraction was tested at 50 and 100 mg/kg in normal and castor oil (4ml / kg, p.o.) or magnesium sulphate (2g / kg, p. o.) induced fecal output in mice using diphenoxylate HCl (5mg/ kg, p.o.) as a positive control. Treatment to various groups of mice was given 30 min prior to castor oil or magnesium sulphate as shown in Table-1. The total number of defecations per animal were recorded up to 4 hours.

2.5 Effect of JC fraction on Castor oil induced small intestinal secretions

The effect of JC fraction on castor oil (8 ml/ kg; p.o.) induced secretion of fluid and electrolyte in small intestine was studied in mice. It was studied by enteropooling assay and its sodium ion estimation was carried out using atomic absorption spectrophotometer (UNICAM 929). The chlorpromazine 30 mg/ kg I.P. was used as a standard drug as shown in Table 2. The castor oil was administered 30 min after the treatment of JC fraction or chlorpromazine or vehicle [8].

2.6 Small intestinal transit

The effect of JC fraction on small intestinal transit was studied in group of overnight fasted mice. Thirty minutes after treatment of JC fraction or atropine sulphate or vehicle, mice were administered with 0.2 ml of charcoal meal (3% charcoal in 5% gum acacia) by oral route. All animals were sacrificed after 20 min and distance traveled by charcoal with reference to total length of small intestine was calculated for each mouse to express percentage of distance traveled as shown in Table -3 [8].

2.7 Statistical analysis

The results of all experiments were reported as mean ± SEM. These results were further analyzed by using Student's *t* -test to calculate significance of the results. P values less than 0.05 were considered as statistically significant.

3. Results

3.1 Effect of JC fraction on fecal output

There was no effect on fecal output by JC fraction up to 100 mg/kg in normal mice,

Table2.
Effect of JC fraction on castor oil stimulated intraluminal fluid and Na^+ secretion in small intestine of albino mice .

Sl. No.	Group	Weight of intestine mg / 20g ± S.E.M	Castor oil induced intraluminal fluid in mg	Na^+ m eq /L ± SEM
1.	Control	1062 ± 80	-	6.31 ± 0.55
2.	Castor oil 8ml/kg	1606 ± 80*	544	10.75 ± 0.55*
3.	Castor oil 8ml/kg+CMC	1690 ± 72	628	9.66 ± 0.86
4.	Castor oil 8ml/kg +JC fraction 100mg/kg	1295 ± 44##	233	7.34 ± 0.52 #
5.	Castor oil 8ml/kg + Chlorpromazine 30mg/kg	1083 ± 90##	21	3.64 ± 0.35##

n=6; #Significant as compared to castor oil P < 0.01; ##Significant as compared to castor oil treatment P < 0.001; *Significant as compared with control P < 0.001

however , it showed dose dependant inhibition of castor oil and magnesium sulphate induced diarrhoea in mice. This effect is significant as compared to control, however this activity was less as compared to diphenoxylate HCl as shown in Table- 1.

3.2 Effect of JC fraction on Castor oil induced small intestinal secretions

The castor oil induced intraluminal secretion of fluid was inhibited by JC fraction as well as chlorpromazine as shown in Table -2. This reduction was highly significant as compared to castor oil treatment only. So also there was significant reduction in sodium ion secretion in small intestine by JC fraction and chlorpromazine treatment.

3.3 Small intestinal transit

The results of present study revealed that JC fraction, atropine sulphate 5 mg/kg significantly inhibited the gastrointestinal transit of charcoal in mice by 52.68 % and 45.61%,respectively as compared to control as shown in Table-3 .

4. Discussion

The use of medicinal plants for the treatment of diarrhoea in folk medicine is a common practice in many countries. In most of the cases these practices are followed empirically without knowing probable mechanism and limitations of the treatment.

Castor oil increases peristaltic activity and produces permeability changes in the intestinal mucosal membrane to electrolytes and water. The precise mechanism of action of castor oil is through elevated prostaglandin biosynthesis [9-10]. Prostaglandin contributes to the patho-physiological functions in gastrointestinal tract [11]. The magnesium sulphate induced diarrhoea is presumed to be by osmotic properties and cholecystokinin production [12].

Thus, dose dependant antidiarrhoeal activity of JC fraction against castor oil and magnesium sulphate induced diarrhoea in mice is complex in nature. The effect of JC fraction on enteropooling assay and sodium ion concentration in intestinal fluid, after castor oil challenge showed significant activity against permeability changes leading to water and electrolyte secretion.

The experimental observations carried out after charcoal meal administration are significantly contributing to antidiarrhoeal activity of JC fraction by reduction in peristaltic activity . In light of these multifarious observations, ethnobotanical lead of antidiarrhoeal activity of *Jatropha curcas* roots can be explained.

Table-3 Effect of JC fraction on gastrointestinal transit of charcoal in albino mice

Group	Mean distance traveled by charcoal (%of total length of small intestine) ± SEM	% inhibition
Control	72.36 ± 1.65	_
JC fraction 100mg/kg	34.24 ± 4.31#	52.68
Atropine sulphate 5mg/kg	39.35 ± 6.86#	45.61

n = 6; #Significant as compared to control, P < 0.001

Diarrhoea is considered as a consequence of altered motility and fluid accumulation. Since a number of flavonoids have been reported to inhibit intestinal motility and secretion [13], they may presumably exert an antidiarrhoeal action [8]. Similar observations were also noted with Bisnor (bisnordihydrodrotoxiferine) a dimmer tertiary indole alkaloid compound isolated from *Strychnos trinervis* roots [12, 14]. In order to understand the complex mechanism of action, JC fraction is being purified to assess antidiarrhoeal activity using precise animal models.

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