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Pharmacological evaluation of *Jatropha curcas* L. extract for Anti-diarrhoeal Activity

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The present study provides the pharmacological evaluation of stem bark extract of *Jatropha curcas* L. for anti-diarrhoeal activity in rats. We made an attempt to study the effect of stem bark extract of *Jatropha curcas* L. on diarrhoeal disease. The different activities studied were castor oil-induced diarrhoea, magnesium sulphate induced diarrhoea and charcoal meal transit test. The result of the study reflected that methanol extract of the stem bark (100, 300 mg/kg) decreased total no. of faeces, wet faeces and distance travelled by charcoal plug and showed the anti-diarrhoeal activity. *Jatropha curcas* L. extract demonstrates the anti-diarrhoeal activity in rats.

Keywords : Castor oil-induced diarrhoea, charcoal meal transit test, diarrhoeal, *Jatropha curcas,* magnesium sulphate induced diarrhoea.

Diarrhoea is a common gastrointestinal disorder characterized by an increase in stool frequency and a change in stool consistency.[1] It is one of the leading causes of mortality in developing countries. In view of this, the World Health Organization has initiated Diarrhoea Disease Control Program to study traditional medical practices and other related aspects.[2]

Jatropha curcas L. or Physic nut is a bush or small tree (up to 5 m height) and belongs to the Euphorbiaceae family and contains approximately 170 known species.[3] *Jatropha*, a drought-resistant shrub or tree, which is widely distributed in the wild or semi-cultivated areas in Central and South America, Africa, India and South East Asia.[4] It is a multipurpose, drought resistant, perennial plant gaining lot of importance for the production of biodiesel. It has thick glorious branch lets. The tree has a straight trunk and grey or reddish bark masked by large white patches. It has green leaves with a length and width of 6 to 15 cm, with 5 to 7 shallow lobes. The branches contain whitish latex, which causes brown stains. Inflorescences are formed terminally on branches. The plant is monoecious and flowers are unisexual.[5-6] After pollination, a trilocular ellipsoidal fruit is formed. The seeds are black and in the average 18 mm long and 10 mm wide ripe *Jatropha* fruits.[7] It is a multipurpose species with many attributes and considerable potential. The wood and fruit of *Jatropha* can be used for numerous purposes including fuel. It is used against dermatomucosal diseases, arthritis, gout, jaundice, toothache, gum inflammation, gum bleeding, diarrhoea and pyorrhea.[8] Plant extract used to treat allergies, burns, cuts and wounds, inflammation, leprosy, leucoderma, scabies and small pox. Water extract of branches used in HIV, tumor and wound healing. The plant contains organic acids, cyclic triterpenes stigmasterol,[9] curcacycline A, curcin,[10] a

lectin phorbolesters esterases, sitosterol and its d-glucoside.[11] The leaf and bark have been shown to contain glycosides, tannins, phytosterols, flavanoids and steroidal sapogenins.[8]

In order to search for newer remedy for diarrhoea and dysentery, this study aimed at the investigation of the antidiarrhoeal activity of the extract of the barks of *Jatropha curcas* L. in castor oil-induced diarrhoea, magnesium sulphate-induced diarrhoea and charcoal meal transit models in rats.

Materials and methods

Plant materials and preparation of extract

Fresh stem bark of *Jatropha curcas* L. collected from a local area of Jaipur was identified in the department of botany, Rajasthan University, Jaipur. A voucher specimen number RUBL20844 was deposited in the department of botany, Rajasthan University, Jaipur. The fresh stem bark was air-dried to constant weight, pulverized and stored in an air-tight container for further use. 200 g powder of dried stem bark was subjected to soxhelet extraction with methanol. The extract was then filtered and the filtrate was concentrated to dryness. The extract was subjected to phytochemical tests for tannins, steroids, alkaloids and glycosides, flavanoids, carbohydrates, proteins and amino acid using reported methods.[12-13]

Animals

Albino rats of either sex (150-200 g) were used for experimental purpose. The animals were housed in hygienic cages (6 rats / cage) under standard conditions of temperature $(25\pm2)^0$C, relative humidity (45±20) % and (light) 12h: (dark) 12h cycle. The rats were fed with standard pellet diet (Amrut feeds, Chakan) and water *ad libitum*. The animals were allowed to acclimatize to experimental conditions by housing them for 8-10 days prior to the experiments. The experimental design and research plan along with animals handling and disposal procedure were approved by Institutional Animal Ethical Committee of Jaipur National University (1054/ac/07/CPCSEA) and IAEC approval number was JNU/IAEC/2010/02.

Acute toxicity study

The acute toxicity study (LD_{50}) was performed according to **OECD guidelines no. 423 (Organization for Economic Corporation and Development)**. Adult wistar rats (approx.200-300g.) of either sex were used. The selected albino rats were used to determine the dose. The animals were divided into four groups of six in each. The animals were fasted overnight prior to the acute experimental procedure. Distilled water was used as vehicle to suspend the extracts and administered orally as following doses – 100, 300, 1000 and 2000 mg/kg body wt. immediately after dosing, the animals were observed continuously for first 4 hours for behavioral changes and for mortality at the end of 24 hrs and daily for 14 days respectively.[14]

Anti-diarrhoeal activity

Castor oil-induced diarrhoea

Rats were divided into five groups of six animals each. Diarrhoea was induced by administering 1 ml of castor oil orally to rats. Group 1 served as control, group 2 received loperamide 3 mg/kg, group 3 received *Jatropha curcas* methanol extract (JCME) 100 mg/kg, group 4 received JCME 300 mg/kg body weight 1 h before castor oil administration. The total number of defecations and wet diarrhoeal defecations were counted for a period of 4 h. Mean number of the stools passed by the treated groups was compared with that of control. The total score of diarrhoeic feaces of the control group was considered to be 100%

diarrhoea. The results were expressed as a percentage of inhibition. The number of animals protected from diarrhoea was also analyzed in each group.[15-16]

Magnesium sulphate induced diarrhoea

Diarrhoea in rats was induced by administering $MgSO_4$ at dose of 2g/ kg, *p.o.* Rats were divided into four groups of six animals each. Group 1 served as control, group 2 received loperamide 3 mg/kg, group 3 received JCME 100 mg/kg, group 4 received JCME 300 mg/kg body weight 1 h before magnesium sulphate administration. The total number of defecations and wet diarrhoeal defecations were counted for a period of 4 h. Mean number of the stools passed by the treated groups was compared with that of control. The total score of diarrhoeic feaces of the control group was considered to be 100% diarrhoea. The results were expressed as a percentage of inhibition.[15, 17]

Charcoal meal transit test

The animals were grouped into 4 (n=6) and treated as follows: group 1 served as a control, group 2 received 0.1 mg/kg of atropine (*s.c.*), while groups 3 and 4 received the JCME (100 and 300 mg/kg *p.o.*) respectively. A suspension of charcoal meal containing 5% charcoal in 10% aqueous tragacanth powder was administered *intragastrically* to rats 30 min after treatment. 30 min after administration of the charcoal meal, animals of each individual group were sacrificed and the movement of charcoal from pylorus to caecum was measured. The charcoal movement in the intestine was expressed as a percentage.[16]

Statistical analysis

Results are expressed as mean± S.E.M. Statistical significance was determined by using the one way analysis of variance (ANOVA) and repeated measures (ANOVA) followed by Dunnett's multiple comparison tests. $P < 0.05$ was considered statistically significant.[18]

Results and discussion

Phytochemical screening

The Preliminary phytochemical investigation revealed the presence of phytoconstituents and their results are given in (Table 1).

Table 1: Preliminary qualitative tests of *Jatropha curcas* Stem bark extracts

S.NO.	TESTS	JCME
1.	Alkaloids	++
2.	Glycosides	++
3.	Carbohydrates	-
4.	Flavanoids	++
5.	Triterpenoids	++
6.	Saponin	-
7.	Tannin and Phenolic compound	++
8.	Protein and Amino acid	-
9.	Steroid	++
10.	Fixed Oil & Fat	-

[+] – Present, [-] – Absent

Acute toxicity study The extracts of *Jatropha curcas* Stem Bark did not cause any mortality up to 2000 mg/kg and hence dose of (100 and 300 mg/kg, *p.o.*) were selected for the present study. Their result are shown in (Table 2).

Table 2: Acute toxicity study (OECD guidelines 423)

Treatment	Dose mg / kg, *p.o.*	Number of animals	Number of deaths	% Death	Toxicity profile
JCME	2000	6	0	0	Safe

Castor oil-induced diarrhoea

In the castor oil-induced diarrhoeal rats, the methanolic extract of the barks of *Jatropha curcas* L., at the doses of 100 and 300 mg/kg, reduced the total number of faeces as well as of diarrhoeic faeces and the results were statistically significant (Table 3). As compared to control methanolic extract of the barks of *Jatropha curcas* L. at the dose of 100 and 300 mg/kg, exhibited prominent antidiarrhoeal activity.

Table 3: Effect of *Jatropha curcas* methanolic extract on castor oil induced diarrhea

Treatment	Dose (mg/kg)	Total number of feaces in 4 hr. Mean± SEM	Total number of wet feaces in 4 hr. Mean± SEM
Control	-	12.83 ± 0.6	9.33 ± 0.66
Standard	3	1.67 ± 0.33***	1.33 ± 0.21***
JCMSE	100	6.33 ± 0.76***	5.17 ± 0.6***
JCMSE	300	3.83 ± 0.47***	3.00 ± 0.36***

Values are expressed as mean ± S.E.M. (n= 6). *** P<0.001 as compared to control. One way Anova followed by Dunnett's multiple comparison test

Magnesium sulphate induced diarrhoea

In the Magnesium sulphate induced diarrhoeal rats, the methanolic extract of the barks of *Jatropha curcas* L., at the doses of 100 and 300 mg/kg, reduced the total number of faeces as well as of diarrhoeic faeces and the results were statistically significant (Table 4). As compared to control methanolic extract of the barks of *Jatropha curcas* L. at the dose of 100 and 300 mg/kg, exhibited prominent antidiarrhoeal activity.

Table 4: Effect of JCME on magnesium sulphate induced diarrhoea in rats

Treatment	Dose (mg/kg)	Total number of faeces in 4 h Mean ± SEM	Total number of wet faeces in 4 h Mean ± SEM
Control	-	10.33 ± 0.66	9.5 ± 0.42
Standard	3	1.16 ± 0.30***	0.83 ± 0.30***
JCMSE	100	7.83 ± 0.48**	6.83 ± 0.48**
JCMSE	300	5.16 ± 0.6***	4.67 ± 0.61***

Values are expressed as mean ± S.E.M. (n= 6).
** P< 0.01, *** P<0.001 as compared to control.
One way Anova followed by Dunnett's multiple comparison test

Charcoal meal transit test

In the gastrointestinal motility test, (Table 5) show the effect of methanolic extract on charcoal meal transit test in albino rats. The methanolic extract of the barks of *Jatropha curcas* L., at the doses of 100 and 300 mg/kg, significantly decrease distance travelled by charcoal plug when compared to control and exhibited good anti diarrhoeal activity.

Table 5: Effect of JCME on charcoal meal transit test in rats

Treatment	Dose (mg/kg,)	Distance travelled (cm)
Control	10 ml/kg (*p. o.)*	42.9±2.11
Atropine	0.1 (*s. c.)*	13.9±1.23***
Extract	100 (*p. o.)*	32.37±1.41**
Extract	300 (*p. o.)*	26.63±1.22***

Values are expressed as mean ± S.E.M. (n= 6).
** P< 0.01, *** P<0.001 as compared to control.
One way Anova followed by Dunnett's multiple comparison test

Diarrhoea is a common and major public health problem among people with poor standard of hygiene especially in developing countries and it remains the leading cause of morbidity and mortality in all age groups, with as many as four million cases occurring each year, [1, 19] based on the results from their investigation, concluded that herbal treatments remain important as home remedy for diarrhoea. It have been also reported that despite the availability of simple and cheap treatments for diarrhoea (ORT), healers and patients in many communities still rely on locally available phytomedicines.[20]

Like many other mangrove plant species, *Jatropha curcas* L. contains high amounts of polar compounds mainly phenolics, which could be extracted easily by maceration using methanol. To avoid any solvent effect on the experimental animals, the solvent was evaporated completely to dryness to yield a non-sticky solid mass.

The castor oil-induced diarrhoea demonstrates secretory diarrhoea, since recinolic acid, the active ingredient of castor oil induces diarrhoea by a hypersecretory response.[21- 22] In this study, the extract caused a delay in the onset of copious diarrhoea, decreased the frequency of purging, weight of wet stools and severity of diarrhoea. The methanolic extract of *Jatropha curcas* L. significantly reduced total no. of faeces and the wet faeces in 4 h induced experimentally in rats by castor oil. 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 cholecytokinin from the duodenal mucosa, which increases the secretion and motility of small intestine and thereby prevents the reabsorption of sodium chloride and water.[23] The methanol extract was also found to alleviate the diarrhoeic condition in this model. The extract offered an increased absorption of water and electrolyte from the gastrointestinal tract. Since the extract (ME) delayed the gastrointestinal transit in rats as compared to the control, they might have antimotility property. 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. Properties such as these may underlie the observed antidiarrhoeal effects of plant. On the basis of these findings, it can be assumed that *Jatropha curcas* L. could be a potential source for novel 'lead' discovery for antidiarrhoeal drug development.

Conclusion

The study on anti-diarrhoeal activity showed that treatment with JCE (100 and 300 and mg/kg p. o.) showed significant $P < 0.01^{**}$ and 0.001^{***} as compared to the control. The extract caused a delay in the onset of copious diarrhoea, decreased the frequency of purging, weight of wet stools and severity of diarrhoea.

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