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Research Article

ANTI-DIARRHEAL ACTIVITY OF ALCOHOLIC AND AQUEOUS EXTRACT OF *CALOTROPIS PROCERA* R.Br. LEAVES IN RATS

G. ABHINAYANI, N. SRAVYA AND R. NAGA KISHORE*

Department of pharmacology, Geethanjali College of pharmacy, Hyderabbad, AP. India. Email: rnkishore.sm24@gmail.com

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ABSTRACT

Objective: The purpose of the present study was to evaluate scientifically the anti-diarrheal effect of *Calotropis procera* R.Br. used traditionally in Indian system of medicine using castor oil-induced diarrhoea model.

Method: The anti-diarrheal effect of aqueous and alcoholic extract of leaves of *Calotropis procera* R.Br. was studied against castor oil-induced-diarrhea model in rats. The gastrointestinal transit rate was expressed as the percentage of the longest distance traversed by the charcoal divided by the total length of the small intestine. The weight and volume of intestinal content induced by castor oil were studied by enteropooling method.

Results: Like atropine (3mg/kg, i.p.) there were significant reductions in fecal output and frequency of droppings when the plant extracts of aqueous 100 and 200 mg/kg doses were administered intraperitoneally compared with castor oil treated rats. All doses of the plant extract significantly retarded the castor-oil induced enteropooling and intestinal transit.

Conclusion: The remarkable anti-diarrheal effect of *Calotropis procera* R.Br extract against castor oil-induced diarrhea model attests to its utility in a wide range of diarrheal states.

Keywords: *Calotropis procera* R.Br., Anti-Diarroheal, Castor oil, Atropine.

INTRODUCTION

Diarrhea is a condition that involves the frequently passing of liquid faeces with or without blood or mucus; it is one of the leading causes of mortality in developing countries and major cause of this disease is malnutrition[1,2]. WHO has encouraged studies for treatment and prevention of diarrheal diseases depending on traditional medicinal practices[3].

Calotropis procera R.Br. (Asclepiadaceae) commonly known as small crown plant, found in most parts of the world in dry, sandy soils and in warm climates[4]. These are used against dysentery, toothache, earache, sprain, anxiety, pain and serpent bites. It is most commonly grown in Asian countries that include India, Indonesia, Malaysia, Philippines, Thailand, Srilanka and China. *Calotropis procera* R.Br. is scientifically reported for its antielemintic, anti-Candida, cytotoxic, antipyretic and wound healing activity[5,6]. Current study was focused to investigate the antidiarrheal effect of crude leaves extract of *Calotropis procera* R.Br. has been explored to gain its possible mechanism by using castor oil-induced diarrhea model[7].

MATERIAL AND METHODS

Plant Material

The leaves of *Calotropis procera* R.Br. were collected around the local area of Hyderabad city. The leaves were dried under shade, dehydrated leaves were powered to a fine texture and 100g of the dried powdered leaves were repeatedly extracted with alcohol and water separate. The extracts were concentrated under vacuum and the residue was used in the experiments[8]. The dried leaves extracts were freshly re-dissolved in normal saline and given to adult albino Swiss rats.

Animals

Albino Swiss rats of either sex weighing 150-200g were used for castor oil-induced anti-diarrheal and intestinal transit activity. All animals were fed standard animal feed and tap water *ad libitum* before the experiments. Each experimental group consisted of six animals housed in separate cages.

Castor oil-induced diarrhea

Albino Swiss rats of either sex (150-180g) were divided into six groups of six animals each. The animals were kept in fasting for 24 hours before the test, with free access to water. Diarrhoea was induced by administering 1ml of castor oil orally. Group 1 was treated with 2ml/kg of normal saline, which served as control; Group 2 received standard drug (Atropine 3mg/kg). Groups 3 and 4 received aqueous extract (100 and 200 /kg) and group 5and 6 received alcoholic extract (100 and 200mg/kg, i.p.) respectively 1 h before castor oil administration. Each animal was placed in an individual cage, the floor of which was lined by blotting paper[9]. The floor lining was changed every hour. The consistency of number of both the wet and the dry diarrheal droppings were counted every hour for a period of 4 hours stools passed by the treated groups were compared with that of the positive control group consisted of animals given an intra peritoneal injection of saline(2ml/kg,i.p.)

Castor oil-induced enteropooling

Albino Swiss rats of either sex (150-200g) were divided into six groups of six animals each. They were fasted overnight prior to the experiment, but allowed free access to water. Group 1 was treated with 2ml/kg of normal saline, which served as control; Group 2 received standard drug (Atropine 3mg/kg). Groups 3 and 4 received aqueous extract (100 and 200 /kg) and group 5and 6 received alcoholic extract (100 and 200mg/kg, i.p.) Respectively 1 h before castor oil administration. Two hours later the rats were sacrificed, the small intestine was removed after tying the ends with thread and weighed. The intestinal contents were collected by milking into a graduated tube and their volume was measured. The intestine was reweighed and the difference between full and empty intestines was calculated[10].

Small Intestinal Transit

Albino Swiss rats of either sex (150-180g) were randomly divided into seven groups of six rats each. The animals were kept in fasting for 18 hours before the test, with free access to water. This was done according to the method previously described using charcoal mean as a diet marker. Diarrhea was induced by administering 1ml of castor oil orally. Group 1 was treated with 2ml/kg of normal saline, which served as control; Group 2 received 2ml of castor oil orally with saline 2ml/kg intraperitoneally, Groups 3 and 4 received aqueous extract (100 and 200 /kg) and group 5and 6 received alcoholic extract (100 and 200mg/kg, i.p.) Respectively 1 h before castor oil administration. One ml of marker (10% charcoal suspension in 5% gum acacia) was administered orally 1h after castor oil treatment[11]. The rats were sacrificed after 1h and the distance travelled by charcoal meal from pylorus was measured and

expressed as percentage of the total length of the intestine from the pylorus to ceacum.

Statistical analysis

The experimental results are represented as mean ± S.E.M (standard error of the mean). The data obtained in the studies were subjected to using one-way analysis of variance (ANOVA), followed by student t-test. P<0.01 were considered statistically significant.

RESULTS & DISCUSSION

Castor oil-induced diarrhea

In the rats with castor oil induced diarrhea, the aqueous extracts of leaves of *Calotropis procera* R.Br. showed percentage of inhibition of diarrhea at 100 mg/kg (12.3%) and 200 mg/kg (54.86%), similarly the alcoholic extracts showed percentage of inhibition of diarrhea at 100 mg/kg (9.73%) and 200 mg/kg (38.40%), which are significant with that of atropine (50%) (Table 1).

Castor oil-induced enteropooling

Enteropooling due to Castor oil is by the mechanism of accumulation of water and electrolytes in intestins. Extracts showed dose dependant reduction in the intestinal weight and volume and much more markedly by Aqueous extracts at 100 mg/kg (17.24%) and 200 mg/kg (37.93%), similarly the alcoholic extracts showed percentage of inhibition at 100 mg/kg (10.34%) and 200 mg/kg (34.48%). Extracts showed significant with that of atropine 3mg/kg (6.89%) (Table 2).

Small Intestinal Transit

The percent intestinal transit was increased with castor oil (88.22%), but was reduced in Aqueous extracts at 100 mg/kg (98.78%) and 200 mg/kg (72.27%), similarly the alcoholic extracts showed percentage of inhibition at 100 mg/kg (88.70%) and 200 mg/kg (73.33%). The percent intestinal transit was reduced with atropine 3mg/kg (46.96%) (Table 3).

Table 1: Effect of leaves of *Calotropis procera* R.Br. extract on castor oil induced diarrhea in rats

S. No.	Treatment	Mean Defection	% Inhibition Of Defection
1.	Castor Oil (1ml p.o.) + Saline (2mg/kg, i.p.)	22.6 ± 3.2	-
2.	Castor Oil + Atropine (3mg/kg, i.p.)	11.3 ± 1.0	50%
3.	Castor Oil+Aqueous Extract (100mg/kg, i.p.)	19.8 ± 2.6	12.3%
4.	Castor Oil+Aqueous Extract (200mg/kg, i.p.)	10.2 ± 1.8	54.86%
5.	Castor Oil+Alcohol Extract (100mg/kg, i.p.)	20.4 ± 3.3	9.73%
6.	Castor Oil+Alcohol Extract (200mg/kg, i.p.)	13.9 ± 1.5	38.4%

Values are expressed as mean ± SEM. p<0.05, when compared with atropine-treated group.

Table 2: Effect of leaves of *Calotropis procera* R.Br. extract on castor oil induced enteropooling in rats

S. No.	Treatment	Wt. Intestinal Content	% inhibition wt. intestinal content
1.	Castor Oil (1ml P.O.) + Saline (2ml/kg, i.p.)	2.9 ± 0.2	-
2.	Castor Oil + Atropine(3mg/kg, i.p)	2.7 ± 0.1	6.89%
3.	Castor Oil+Aqueous Extract (100mg/kg i.p.)	2.4 ± 0.1	17.24%
4.	Castor Oil+Aqueous Extract (200mg/kg i.p.)	1.8 ± 0.2	37.93%
5.	Castor Oil+Alcohol Extract (100mg/kg i.p.)	2.6 ± 0.1	10.34%
6.	Castor Oil+Alcohol Extract (200mg/kg i.p.)	1.9 ± 0.3	34.48%

Values are expressed as mean ± SEM. p<0.05, when compared with atropine-treated group

Table 3: Effect of leaves of *Calotropis procera* R.Br. extract on castor oil induced small intestinal transit in rats

S. No.	Treatment	Total length of intestine	Distance travelled by marker	% Intestinal transit
1.	Saline (2ml p.o.)	90.1 ± 2.4	85.4 ± 1.5	94.78%
2.	Castor Oil (2ml p.o) + Saline (2mg/kg, i.p.)	88.3 ± 3.0	77.9 ± 2.8	88.22%
3.	Castor Oil + Atropine (3mg/kg i.p.)	92.4 ± 1.2	43.4 ± 2.2	46.96%
4.	Castor Oil+Aqueous Extract (100mg/kg i.p.)	90.6 ± 2.9	89.2 ± 3.6	98.78%
5.	Castor Oil+Aqueous Extract (200mg/kg i.p.)	77.2 ± 3.5	55.8 ± 2.5	72.27%
6.	Castor Oil+Alcohol Extract (100mg/kg i.p.)	85.9 ± 1.4	76.2 ± 1.4	88.70%
7.	Castor Oil+Alcohol Extract (200mg/kg i.p.)	81.0 ± 2.6	59.4 ± 3.8	73.33%

Values are expressed as mean ± SEM. p<0.05, when compared with atropine-treated group

CONCLUSION

Castor oil is a suitable model of diarrhea in rats, since it induce diarrhea by increasing the volume of intestinal content and allows the observation of measurable changes in the number of stools, enteropooling and intestinal transit. From this current study, it can be concluded that extracts of leaves of *Calotropis procera* R.Br. possesses marked anti diarrheal activity in dose dependant manner. Hence we conclude that *Calotropis procera* R.Br. could be a potential source for novel lead discovery for anti diarrheal drug development and number of preclinical trials. Futher studies are necessary to evaluate the active principle[12] and to understand mechanism of anti diarrheal action of *Calotropis procera* R.Br.

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