ANTIDIARRHEAL ACTIVITY OF THE HYDROETHANOLIC EXTRACT OF *CALOTROPIS GIGANTEA* R. BR. (ASCLEPIADACEAE)

Gaurav Lodhi[1], H. K. Singh[3], K. K. Pant[1], Ch. V. Rao[2], Zeashan Hussain[2,]*

[1]*Department of Pharmacology and Therapeutics, CSM Medical University, Lucknow 226003, India.*
[2]*Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute, (Council of Scientific and Industrial Research) Rana Pratap Marg, Post Box No. 436, Lucknow-226 001, Uttar Pradesh, India*
[3]*Azad Institute of Pharmacy and Research, Azad Puram, Lucknow, India-226002.*
* Corresponding author. Tel: +91 9935844704
E-mail address: zeashanhussain01@gmail.com

Summary

The antidiarrheal activity of the hydroethanolic extract of *Calotropis gigantea* R. Br. (Asclepiadaceae) stems was assessed on experimental animals. The hydroethanolic extract (250 and 500 mg/kg, po) exhibited a dose dependent decrease in Mean defecation in 4h. Further, *C. gigantea* at 500 mg/kg dose produced a significant and dose dependent reduction in intestinal fluids accumulation.
The extract at 500 mg/kg reduced the small intestinal transit from 86.3 ± 1.6 % for castor oil treated rats to 76.1 ± 4.1% . The extract of C. *gigantea* had no effect on normal defecation at 250 mg/kg in mice. However at 500 mg/kg, the extract inhibited defecation by 100% in the initial 2 h and the activity was reduced to 68.0% in the third hour.

***Keywords*:** *Calotropis gigantea* (*Asclepiadaceae*); antidiarrhoeal; castor oil; small intestine transit.

Introduction

Diarrheal disease is a leading cause of mortality and morbidity, especially among children in developing countries resulting in a major health care problem. Medicinal herbs constitute an indispensable component of the traditional medicine practiced worldwide due to the economical viability, accessibility and ancestral experience. Despite the availability of a vast spectrum of approaches for diarrheal management, a vast majority of the people of the developing countries rely on herbal drugs for the management of diarrhea. WHO has encouraged studies for treatment and prevention of diarrheal diseases depending on traditional medical practices[1].

Calotropis gigantea R. Br. (*Asclepiadaceae*), a wildly growing plant, has been reported to possess a number of medicinal properties and is used in toothache and earache, sprain, anxiety, pain, epilepsy and in mental disorders[2]. The aerial parts of the plant have been reported to possess anti-diarrheal properties[3] and the flowers have been reported for their analgesic activity[4]. The roots of the plant have shown CNS activity[2] as well as pregnancy interceptive properties[5].

The stem bark of *C. gigantea* yields resin and wax. The wax contains β- amyrin and its isovalerate, α and β- calotropeols, mixture of tetracyclic triterpene, traces of sterols, C_{31} and C_{33} hydrocarbons, fatty acids and giganteol. The stem of *C. gigantea* yields latex. The latex contains the cardiac glycosides, calotropin, uscharin, calotoxin, uscharidin and gigantin. Calotropin, gigantin and uscharin show digitalis like action on the heart. A proteolytic enzyme, calotropain has been isolated from the latex. Calotropain has marked anti-blood coagulating activity. The latex consists of calotropin D_I and D_{II} and calotropain F_I and F_{II} and an enzyme with invertase activity. It is a promising anti-inflammatory agent[6].

The present study was designed to evaluate the effect of aqueous ethanolic extract of *C. gigantea* on castor oil induced diarrhea.

Experimental

Plant material: The stems of *Calotropis gigantea* were procured locally from Lucknow district of Uttar Pradesh in India and were identified at Hygia Institute of Pharmaceutical Education and Research, Lucknow (India). Voucher specimens are being maintained in the herbarium (HIPER /07/12) of the Institute for further references.

Extract Preparation: Stems of *Calotropis gigantea* were washed with tap water, chopped in to pieces and dried in shade. Dried pieces of stem were ground to coarse powder and stored in an airtight container. The dried stems were extracted (250 g) with ethyl alcohol (50%, V/V) in a Soxhlet extractor for 18–20 h. The extract was concentrated to dryness under reduced pressure and controlled temperature (40–50 ° C). The extract thus obtained was preserved in a desiccator to prevent degradation by moisture. For the pharmacological studies the *C. gigantea* extract was suspended in double distilled water containing carboxymethyl cellulose (1%, W/V, CMC).

Experimental animals: Wistar albino rats (180–220 g) and albino mice (18-24 g) of either sex were used. The animals were placed individually in specially designed cages and maintained under standard laboratory conditions (temperature 25 ± 2 °C) with light and dark cycles of 12 and 12 h respectively. Animals were provided with the standard rodent pellet diet (Amrut, India) and the food was withdrawn 24 h before the experiment but water was allowed *ad libitum*. All procedures described were reviewed and approved by the Institutional Animal Ethical Committee

Evaluation of the effect on normal defecation: Four groups of six mice each were placed individually in separate cages with filter papers at the bottom. The doses of extracts (250 and 500 mg/kg) were administered orally to different groups. The nonspecific antidiarrheal reference drug diphenoxylate HCl (5.0 mg/kg, po) and 1% CMC (10 ml/kg, po) were administered to two groups and they later served as controls[7]. The total number of faecal droppings in each group was assessed for the next 4 h. Percent reduction in the total number of faeces in the treated groups was obtained by comparison with control animals.

Castor oil induced diarrhea: Rats were divided into five groups of six animals each. Diarrhea was induced by administering 1 ml of castor oil orally to rats. Group I served as control, group II, III, IV and V received atropine (0.1 mg/kg, ip), phenylbutazone (PBZ; 100 mg/kg orally) and *C. gigantea* extract at doses of 250 and 500 mg/ kg respectively 1 h before castor oil administration. The numbers of both dry and wet diarrhoeal droppings were counted every hour for a period of 4 h. Mean number of the stools passed by the treated groups was compared with that of control. The number of animals protected from diarrhea was also analyzed in each group[8].

Castor oil induced enteropooling and electrolyte secretion: Intraluminal fluid accumulation was determined by the method of Robert et al. (1976)[9]. Rats were divided into four groups of six animals each. Group I received 2 ml of 1%, W/V, CMC, group II received 2 ml of castor oil, group III and IV received *C. gigantea* extract at doses of 250 and 500 mg/kg respectively 1 h before the oral administration of castor oil. 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. The Na^+ and K^+ concentrations were analyzed in the intraluminal fluid by flame photometry.

Small intestinal transit: Rats were divided into four groups of six animals each. Group I received 2 ml of 1%, W/V, CMC, group II received 2 ml of castor oil, group III and IV received *C. gigantea* extract at doses of 250 and 500 mg/kg respectively 1 h before oral administration of castor oil. One ml of a marker (10% charcoal suspension in 5% gum acacia) was administered orally 1 h after castor oil treatment. The rats were sacrificed after 1 h and the distance travelled by charcoal from the pylorus was measured and expressed as a percentage of the total length of the intestine from the pylorus to caecum[10].

Result and Discussion

The aqueous ethanolic extract of *C. gigantea* stems at 250 mg/kg dose had no effect, 500 mg/kg dose inhibited defecation by 100% in the initial 2 h compared to normal defecation in mice. The activity was reduced to 68% in the third hour.

Castor oil is an effective laxative. The action of castor oil as diarrhea inductor has been largely studied and it is known that its most active component is the ricinoleic acid, which produces an irritating activity in the small intestine[8,11]. *C. gigantea* extract at 250 mg/kg mildly inhibited diarrhea induced by castor oil but at 500 mg/kg dose it produced a significant ($P < 0.01$) reduction in number of stools passed (3.1 ± 0.7) as compared to the castor oil treated control group (7.1 ± 0.7). Phenylbutazone (1.6 ± 0.8) and atropine (1.1 ± 0.8) also produced a significant ($P < 0.001$) reduction in the number of stools passed as compared to the castor oil treated control group (Table 1).

Administration of castor oil significantly ($P < 0.001$) decreased the propulsion of charcoal meal through gastrointestinal tract when compared to the normal rats.

C. gigantea extract at 500 mg/kg dose significantly ($P < 0.05$) reduced the propulsion of charcoal meal through small intestinal tract when compared to the castor oil treated rats. The percentage of intestinal length traversed by charcoal meal in *C. gigantea* 250 and 500 mg/kg pretreated, normal and castor oil treated rats was 81.2 ± 3.7, 76.1 ± 4.1, 99.1 ± 0.7 and 86.3 ± 1.6 respectively (Table 2).

Aqueous ethanolic extract of *C. gigantea* stems was also found to possess anti-enteropooling activity. Oral administration of castor oil produced a significant ($P < 0.01$) increase in the intestinal fluid (2.4 ± 0.3 ml) as compared to normal rats (1.1 ± 0.2 ml). *C. gigantea* stems extract, when given orally at a dose of 500 mg/kg , 1h before castor oil, significantly inhibited the enteropooling (1.5 ± 0.2 ml; $P < 0.05$) and the volume of intestinal fluid was comparable to that obtained in normal group (Figure 1). The weight of the intestinal content was also significantly ($P < 0.01$) increased in rats treated with castor oil (3.0 ± 0.4 gm) in comparison to normal rats (1.3 ± 0.03 gm) however *C .gigantea* extract administration at both the doses produced a very marginal change in the weight of the intestinal content when compared to castor oil treated rats, it was 2.7 ± 0.4 and 2.3 ± 0.6 gm for 250 and 500 mg/kg doses respectively.

Castor oil administration significantly increased the Na^+ concentration in rats to 12.0 ± 0.5 meq/l as compared to the control group (6.9 ± 0.4 meq/l). *C .gigantea* extract pretreatment however did not alter the Na^+ concentration in intestinal fluid as compared to the castor oil treated group. It was 12.1 ± 1.3 and 11.6 ± 1.7 meq/l for 250 and 500 mg/kg doses respectively.

None of the treatments produced a significant change in the K^+ concentration (Figure 2). The K^+ concentration for *C. gigantea* 250 and 500 mg/kg pretreated, normal and castor oil treated rats was 33.8 ± 4.7, 34.2 ± 6.3, 35.9 ± 3.8 and 34.0 ± 4.2 meq/l respectively.

Table 1: Effect of *C. gigantea* extract on castor oil induced diarrhea

Treatment	Mean defecation in 4 h
Castor oil (1 ml p.o.)	7.1 ± 0.7
Castor oil + Atropine (0.1 mg/kg ip)	1.1 ± 0.8[c]
Castor oil + Phenylbutazone (0.1 mg/kg po)	1.6 ± 0.8[c]
Castor oil + *C. gigantea* extract (250 mg/kg po)	4.3 ± 1.1
Castor oil + *C. gigantea* extract (500 mg/kg po)	3.1 ± 0.7[b]

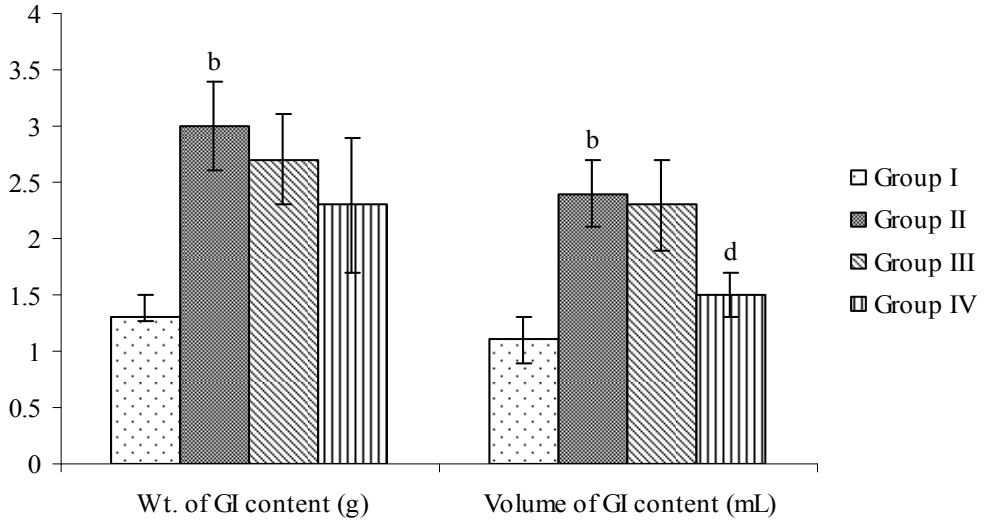
Values represent mean ± SEM of six animals in each group
([a] $P < 0.05$ vs. Castor oil, [b] $P < 0.01$ vs. Castor oil, [c] $P < 0.001$ vs. Castor oil)

Table 2: Effect of *C. gigantea* extract on small intestine transit

Treatment	Intestinal transit (%)
Control (2 ml of 1%, W/V, CMC po)	99.1 ± 0.7
Castor oil (2 ml po)	86.3 ± 1.6[c]
Castor oil + *C. gigantea* extract (250 mg/kg po)	81.2 ± 3.7
Castor oil + *C. gigantea* extract (500 mg/kg po)	76.1 ± 4.1[d]

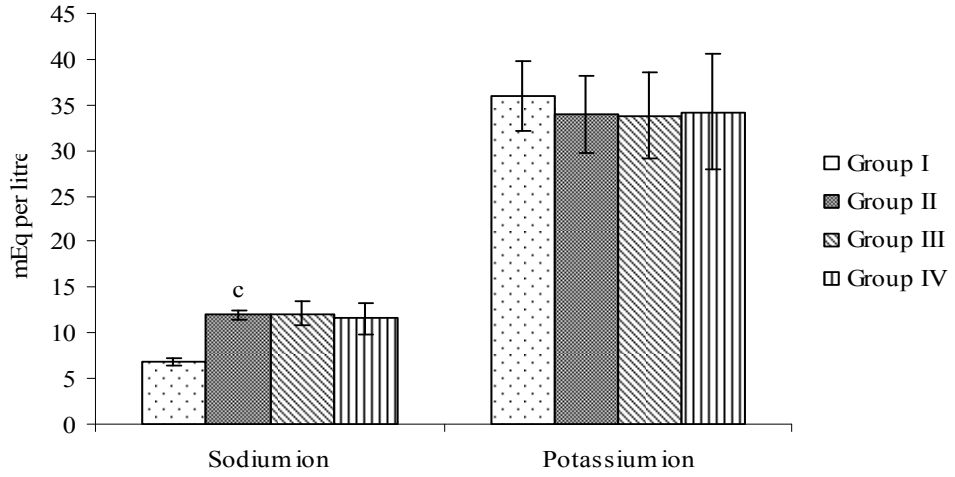
Values represent mean ± SEM of six animals in each group
([c] $P < 0.001$ vs. Control, [d] $P < 0.05$ vs. castor oil)

Fig. 1: Effect of *C. gigantea extract* on castor oil induced enteropooling



Values represent mean ± SEM of six animals in each group; Group I: Normal Control (saline 2 ml po), Group II: Castor oil (2 ml po), Group III: *C. gigantea* extract (250 mg/kg) + Castor oil (2 ml po), Group IV: *C. gigantea* extract (500 mg/kg) + Castor oil (2 ml po) (^{b} P < 0.01, ^{c} P < 0.001 vs. control, ^{d} P < 0.05 vs. castor oil)

Fig. 2: Effect of *C. gigantea extract* on castor oil induced change in electrolyte concentration in intestinal fluid



Values represent mean ± SEM of six animals in each group; Group I: Normal Control (saline 2 ml po), Group II: Castor oil (2 ml po), Group III: *C. gigantea* extract (250 mg/kg) + Castor oil (2 ml po), Group IV: *C. gigantea* extract (500 mg/kg) + Castor oil (2 ml po) (^{c} P < 0.001 vs. control)

References

1. Atta AH, Mouneir SM. Antidiarrhoeal activity of some Egyptian medicinal plant extracts. J Ethnopharmacol 2004; 92: 303-309.
2. Pathak AK, Argal A. CNS activity of *Calotropis gigantea* roots. J Ethnopharmacol 2006; 106: 142-145
3. Chitme HR, Chandra R, Kaushik S. Studies on anti-diarrhoeal activity of *Calotropis gigantea* r.br. in experimental animals. J Pharm Pharma Sci 2004; 7: 70-75.
4. Pathak AK, Argal A. Analgesic activity of *Calotropis gigantea* flower. Fitoterapia 2007; 78: 40–42.
5. Srivastava SR, Keshri G, Bhargavan B, Singh C, Singh MM. Pregnancy interceptive activity of the roots of *Calotropis gigantea* Linn. in rats. Contraception 2007; 75: 318-322.
6. Anonymous. The wealth of India, a dictionary of Indian raw materials and industrial products. CSIR, New Delhi: Indian
7. Melo L, Thomas G, Mukherjee R. Antidiarrhoeal activity of bisnordihydrotoxiferine isolated from root bark of *Strychonus trinervis* (Vell.) Mart. (Longaniaceae). J Pharm Pharmacol 1988; 40: 79–82.
8. Awouters F, Niemegeers CJE, Lenaerts FM, Janssen PAJ. Delay of castor oil diarrhoea in rats: a new way to evaluate inhibitors of prostaglandin biosynthesis. J Pharm Pharmacol 1978; 30: 41–45.
9. Robert A, Nezamis JE, Lancaster C, Hanchar AJ, Klepper MS. Enteropooling assay: a test for diarrhoea produced by prostaglandins. Prostaglandins 1976; 11**:** 809–828.
10. Mascolo N, Izzo AA, Autore G, Barbato F, Capasso F. Nitric oxide and Castor-oil induced diarrhoea. J Pharmacol Exp Ther 1994; 268: 291–295.
11. Nwodo OFC, Alumanah EO. Studies on *Abrus precatorious* seed. II. Antidiarrhoeal activity. J Ethnopharmacol 1991; 31: 395–398.