Original Article

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EVALUATION OF *CALOTROPIS GIGANTEA* ROOT IN EXPERIMENTAL DIARRHEA

*Rumana Rahman, Fazlu.Rehman, Shashank,B, Rajashekar S, Gangadhar B, Chandrasekhar S, Sandeep V L V S
Global College of Pharmacy, Moinabad, RR District,
Andhra Pradesh, India – 501 504.

Abstract

Calotropis gigantea is a wasteland weed and considered a medicinal plant in India. Invivo antidiarrheal activity of an ethanolic extract of 'Akondmul' (*Calotropis gigantea* root extract - CGE) was investigated in wistar rats by Castor oil induced diarrhea, enteropooling assay and small intestinal transit models. Preliminary phytochemical screening revealed the presence of carbohydrates, alkaloids, glycosides, flavonoids and tannins. CGE showed dose dependent decrease in diarrhea at three doses 100mg/kg, 200mg/kg and 400mg/kg in all the three experimental models. CGE reduced the mean fecal output in castor oil induced diarrhea which indicated reduction in diarrheal symptoms. It also reduced the intestinal fluid accumulation resulting in a decrease in the weight and volume of intestinal fluids indicating some prostaglandin inhibitory action. CGE could also reduce the small intestinal transit suggesting an anti motility effect. The anti diarrheal effects were found to be significant ($p<0.05$) at 200mg/kg and 400mg/kg in all the models. The study shows anti diarrheal potential of *Calotropis gigantea* roots which can be exploited as a cheap and effective remedy in non specific and infectious diarrheas.

Key words: Antidiarrheal activity, Akondmul, *Calotropis gigantea root*, Castor oil induced diarrhea, Enteropooling assay, Small intestinal transit.

Introduction

Plants have long been one a very important source of antidiarrheal drugs[1]. Since medicinal plants used by traditional healers have a history of usage, it can become the shortest route for scientists to discover plant-derived drugs. The average success rate of obtaining new medicines from botanical sources is one in 125, whereas the comparative rate of success of obtaining useful medicines from synthetic chemicals is about one in 10,000[2].

Author for Correspondence:
Rumana Rahman,
Global College of Pharmacy,
Moinabad, RR District,
Andhra Pradesh,
India – 501 504.
Email: syedrrahman17@gmail.com

Diarrhea is characterized by increased frequency of bowel movement, wet stool and abdominal pain. It is a leading cause of malnutrition and death among children in the developing countries of the world today. Many governments and international organizations are trying to control this disease but the rate of incidence is still high, about 7.1 million per year[3].Secretory diarrhea is the most dangerous symptom of GIT problems and is associated with excessive defaecation and stool output, the stools being of abnormally loose consistency[4].Therefore the World Health Organization encouraged studies for the treatment and prevention of diarrheal diseases depending on traditional medical practices[5].

Calotropis gigantea (Asclepiadaceae) is considered a traditional medicinal plant of India[6].The roots of *Calotropis gigantea* are traditionally called "Akondmul"[7] and are reported to possess cytotoxic

principles[8,9,10,11]. It has been screened for a number of medicinal properties such as Hepatoprotective[12,13,14,15], antioxidant[16,17], pregnancy interceptive activity[18], CNS activity[19], insecticidal[20], anticonvulsant[21], immune modulatory[22], analgesic anti inflammatory[23], fibrino -lytic[24], antidiabetic[25] and wound healing activity[26,27]. Literature survey revealed the presence of some unique phytoconstituents in Akondmul which have not yet been screened for their potential against diarrhea. The present work was therefore undertaken to screen the effectiveness of *Calotropis gigantea* roots in diarrhea for validating its traditional use.

Materials and Methods

Animals

Wistar albino rats (150 – 200 g) of either sex were housed in polypropylene cages, maintained under standard laboratory conditions and provided with standard diet and water *ad libitum*. They were acclimatized for one week to the laboratory environment before initiating the study. Handling of animals and experimentation were carried out according to the guidelines laid by Committee for the Protection and Control of Scientific Experimentation on Animals and with prior approval from the Institutional Animal Ethical Committee. Animals were fasted for 18hrs prior to experimentation during which food but not water was withheld.

Chemicals and drugs

Atropine sulphate and Acetyl Salicylic acid (standard antidiarrheal drugs), castor oil (laxative), ethanol (solvent),carboxy methyl cellulose(suspending agent) and deactivated charcoal(marker) were procured from SD Fine chemicals, Mumbai, India.

Plant material

Roots of *Calotropis gigantea* were collected in the month of March from our campus and authenticated in the Pharmacognosy department. A voucher specimen (R11-01) was deposited for the same for future reference. The roots were cleaned, washed and dried under the shade for 3 weeks. The powdered material (1.5kg) was macerated with ethanol (x4) for one week and the solvent was evaporated under vacuum to obtain the crude ethanolic extract of *Calotropis gigantea* root (CGE).

Experimentation

Phytochemical Screening

Preliminary photochemical screening of CGE was carried according to the standard procedures[28].

Acute Oral Toxicity[29]

Acute oral toxicity was conducted according to OECD guideline 425. Five animals were randomly selected and fasted overnight. CGE was administered at a single oral dose of 2000mg/kg in distilled water. Animals were housed singly in cages and observed for 48 hrs for signs of toxicity. One-twentieth, one-tenth, one-fifth of the maximum tolerable dose were selected for the study.

Castor oil induced diarrhea [30]

Animals were divided into five groups each having six animals and were dosed as follows:

Group I (diarrhea control) received 1ml of 0.6% CMC, p.o

Group II (standard control) received Acetyl salicylic acid (100mg/kg in 0.6% CMC, p.o)

Group III-V(CGE treated) received CGE at three selected doses i.e 100mg/kg, 200mg/kg and 400mg/kg in 0.6%CMC, p.o

Thirty minutes later, Castor oil (10ml/kg p.o) was administered to all the animals. One hour after castor oil administration and every hour, upto four hours, the fecal output was determined by placing each animal in an individual cage lined with absorbent paper. The fecal stained papers in the cages were replaced with new papers every hour. Total number of diarrheal feces of control group was considered 100% and % protection for the CGE treated groups was calculated.

Enteropooling Assay [30]

Animals were divided into five groups of six animals each .The grouping and dosing was done as follows:

GroupI (diarrhea control) received 1ml of 0.6% CMC, p.o

GroupII (standard control) received Acetyl salicylic acid (100mg/kg in 0.6% CMC, p.o)

Groups III-V (CGE treated) received extract at the three selected doses 100mg/kg, 200mg/kg and 400mg/kg in CMC, p.o.

Thirty minutes after administration of the doses, all animals received Castor oil (10ml/kg p.o). Thirty minutes later all the animals were sacrificed by cervical dislocation and their small intestines were removed after tying the pyloric and ileocaecal ends separately with pieces of thread. The weight of full intestines was noted and the intestinal contents were carefully collected in a graduated cylinder by milking. The empty intestines were reweighed and the weight and volume of intestinal contents was determined.

Small Intestinal Transit (SIT)[31]

For the determination of the effectiveness of the extract on small intestinal transit animals were divided into five groups, each having six animals and dosed in the following manner:

Group I *(diarrhea control) received 1ml distilled water, p.o*

Group II (standard control) received Atropine sulphate (0.1mg/kg in distilled water, ip)

Groups III-V (CGE treated) received CGE at three selected doses, 100mg/kg, 200mg/kg and 400mg/kg respectively in distilled water p.o

Thirty minutes later all the animals received Castor oil (10ml/kg p.o), thirty minutes after which they were given 1ml of 10% deactivated charcoal in 0.6% CMC, as a marker. Thirty minutes after charcoal administration all the animals were sacrificed by cervical dislocation and the small intestine was removed by tying threads and then cutting at the pyloric and ileo caecal junctions. Care was taken not to stretch the intestine and the distance covered by the marker in the intestine was expressed as a percentge of the total distance travelled from the pylorus to the caecum.

Statistical Analysis

Data was analysed by one-way ANOVA followed by Dunnett's test using Graph Pad Prism 5.04 software. P value less than 0.05 was considered statistically significant.

Results and Discussion

The yield of the CGE was found to be 7.18%. No deaths were reported at the end of 48hrs indicating an LD_{50} greater than 2000mg/kg. Phytochemical investigation revealed the presence of sugars, tannins, flavonoids, alkaloids, steroids and glycosides.

Table 01: Phytochemical Investigation

S.No	Constituent	Result
1.	Carbohydrates	+
2.	Alkaloids	+
3.	Glycosides	+
4.	Tannins	+
5.	Steroids	+
6.	Flavonoids	+

CGE exhibited a dose dependent antidiarrheal effect in the models tested. Eventhough it was not as effective as the standard drugs Acetyl Salicylic acid and Atropine, the higher doses of 200mg/kg and 400mg/kg exhibited appreciable antidiarrheal activity in all the models tested.

CGE at the two higher doses tested, significantly reduced the fecal output (Table 2) as well as the intraluminal fluid accumulation. Castor oil or its active component ricinoleic acid induces permeability changes in mucosal fluid and electrolyte transport which result in a hypersecretory response and diarrhea. Ricinoleic acid increases PGE_2 in portal venous blood and gut lumen, increasing water and electrolyte secretion and produces irritation and inflammation of the intestinal mucosa which stimulates motility. Inhibition of PG biosynthesis delays castor oil induced diarrhea. Based on these observation it is proposed that the CGE might be acting by decreasing the synthesis of prostaglandins[32].The delay of castor oil induced diarrhea and inhibition of intestinal fluid secretion have been shown to characterize nonsteroidal anti-inflammatory drugs.

Moreover it has been reported that indomethacin reduces intestinal fluid secretion and inhibits the luminal release of prostaglandin E in patients with acute diarrhea. aspirin like drugs were tested and a positive correlation was observed in their ability to prevent diarrhea induced by castor oil and inhibition of inflammation caused by carrageenan[30].

Table 02: Castor oil induced diarrhea

Group	No.of diarrheal animals	Number of feces in 4hrs	% protection
Control	6/6	10.00±0.54	-
Standard (acetyl salicylic acid 100mg/kg)	1/6	0.99±0.75***	90.1
CGE(100mg/kg)	4/6	6.03±0.42***	39.7
CGE(200mg/kg)	3/6	4.33±0.75***	56.7
CGE(400mg/kg)	2/6	2.27±0.51***	77.3

n=6, values are expressed as Mean ± SEM analysed by ANOVA followed by Dunnett's post-test,***p<0.001 considered very significant, compared to control.

To strengthen this theory further intra luminal fluid accumulation was studied by enteropooling assay. The CGE effectively reduced the volume and weight of intestinal fluid eventhough it was not as effective as the standard drug Acetyl Salicylic acid (Table 3). The decrease in volume of intestinal contents indicated an alteration in the electrolyte transport.Since absorption of water and electrolytes is affected by the motility of the intestine the influence of CGE on the intestinal motility was studied. Activated charcoal readily adsorbs drugs and chemicals preventing absorption[32]. Hence the small intestinal motility was studied using deactivated charcoal as a diet marker. The CGE might have reduced the intestinal motility by an antimuscarinic or an antihistaminic action which suppressed the propulsion of deactivated charcoal (Table 4).

Table 03: Enteropooling Assay

Group	Volume of intestinal contents(ml)	Weight of intestinal contents(g)
Control(vehicle)	1.9±0.14	2.2±0.08
Standard (acetyl salicylic acid 100mg/kg)	0.43±0.12***	0.98±0.05***
CGE(100mg/kg)	1.45±0.13***	1.57±0.03***
CGE(200mg/kg)	1.03±0.12***	1.36±0.02***
CGE(400mg/kg)	0.58±0.17***	1.15±0.03***

n=6,Values are expressed as mean±SEM, analysed by ANOVA followed by Dunnett's post-test,*** p<0.001 considered extremely significant, compared to control.

The decrease in the number of feces , intestinal fluid accumulation and the intestinal transit after the administration of CGE was not as much as the standard drugs Acetyl Salicylic acid or Atropine respectively. This indicates that even though the extract has anti diarrheal activity it has lesser potential to cause constipation. This finding can be of importance since the modern day anti diarrheals suffer from this drawback.

Table 04: Study of Small Intestinal Transit

Group	%SIT=distance travelled by marker/total length of intestine x 100	% protection
Control	90.33±1.28	-
Standard (atropine 0.1mg/kg)	44.0±1.36***	56.0
CGE(100mg/kg)	83.0±1.15**	17.0
CGE(200mg/kg)	71.83±1.24***	28.17
CGE(400mg/kg)	56.83±1.30***	43.17

n=6, Values are expressed as Mean ± SEM analysed by ANOVA followed by Dunnett's post-test,**p<0.01 considered very significant,*** p<0.001 compared to control

The CGE may have exerted its antidiarrheal effect through an antimuscarinic or an antihistaminic effect. It may also be acting as a non specific smooth muscle relaxant like papaverine.The plant can serve as a profitable alternative in treating not just non specific diarrheas but also infectious diarrheas due to bacterial and helminth invasions as antibacterial [33] and antihelminthic[34] properties of the plant have already been established. Cardenoglycosides such as calotropin frugoside, 4-o-beta-d-glucopyranosyl frugoside, calotropins A and B and flavonoids reported in Akondmul may be responsible for the antidiarrheal activity. Flavonoids have been known to enhance intestinal absorption and possess spasmolytic action [35].Tannins, on the other hand have the ability to cause protein denaturation by forming protein tannates[36]. Eventhough our phytochemical investigation could establish the presence of flavonoids and tannins a specific constituent could not be assigned to the antidiarrheal effect. Further detailed studies need to be performed for elucidation of the exact mechanism of action and to understand the involvement of various other peptides and hormones and to assign a specific constituent(s) for the antidiarrheal activity.

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