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Evaluation of antidiarrhoeal property of the hydroalcoholic extract of roots of *Calotropis gigantea* R.Br. on Caster-induced diarrhoea in rats

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Abstract

Diarrhoea (Greek and Latin: dia, through and rheein: to flow or run) is characterized by increased frequency of bowel movement, wet stool and abdominal pain. Diarrhoea is a major health problem especially for children under the age of 5 years. Worldwide distribution of diarrhoea accounts for more than 5-8 million deaths each year in infants. According to W.H.O. estimates for 1998, about 7.1 million deaths were caused by diarrhoea. The incidence of diarrhoeal diseases still remains high despite the efforts of many governments and international organisations to curb it. Calotropis gigantea Linn. (Asclepiadaceae) is a glabrous or hoary, laticiferous shrubs or small trees, commonly known as Swallow-Wort or Milkweed or wasteland weed or Ak or Boro Akanda. In this study, we examined the Antidiarrhoeal property of the hydroalcoholic extract of root of C. gigantea on Caster-induced diarrhoea in rats. From this study, the use of the root of C. gigantea in traditional medicine as a non-specific antidiarrhoeal agent has been justified. Further studies are, however, needed to establish the safety of the extract and to possibly isolate the active principle responsible for the observed effects.

INTRODUCTION

Calotropis gigantea Linn. (Asclepiadaceae) is a glabrous or hoary, laticiferous shrubs or small trees, commonly known as Swallow-Wort or Milkweed or wasteland weed or Ak or Boro Akanda.[1,2] It grows in topical region and most abundant in Bangladesh, India, Burma, Pakistan and in the sub Himalayan tract.[3]

The chemical constituents of *C. gigantea* have been extensively investigated, leading to the isolation of many cardenolides, cardiac glycosides, flavonoids, terpenes, pregnanes and

giganticine (a nonprotein amino acid). [4, 5] Cardenolide glycosides, calotropin, frugoside and 4′-O-beta-D-glucopyranosyl frugoside were isolated from the root of *Calotropis gigantea* which are toxic to cell lines of human origin. Some bioactive compounds such as gigantean (a novel insect antifeedant nonprotein amino acid), isorhamnetin-3-O-rutinoside, isorhamnetin-3-O-glucopyranoside, taraxasterylacetate, calotroposides (A and B) etc. were also isolated and purified from Calotropis gigantean. Recently two new cardenolides, 19-Nor and 18,20-epoxy-cardenolides having inhibitory effect against KB,BC and NCI-H187 cancer cell lines, are *isola*ted from the leaves of *Calotropis gigantean*.[3**]** In 1980, Pal and Sinha had isolated, crystallized and studied the properties of calotropins D_1 and D_2 from *C.gigantea*.[5**]** A new pregnanone, named calotropone (1), was isolated from the EtOH extract of the roots of *Calotropis gigantea* L. together with a known cardiac glycoside.[5]

Traditionally it is used to treat common diseases such as fever, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting and diarrhoea, either alone or with other medicines. The whole plant, root bark, roots, leaves and flowers are to treat many diseases and abnormalities in humans. [2]

In literature, it was also reported that extract of *Calotropis gigantea* showed Antimicrobial Activity [4], Wound Healing Activity [1], Insecticidal Activity [4], Free radical scavenging activity [7], anti-inflammatory[8], hepatoprotective[9] and antiinflammatory[10].

Diarrhoea (Greek and Latin: dia, through and rheein: to flow or run) is characterized by increased frequency of bowel movement, wet stool and abdominal pain. Many synthetic chemicals like diphenoxylate, loperamide and antibiotics are available for the treatment of diarrhoea but they have some side effects and increasing resistance of pathogen to the common antibiotics. In recent years, special attention is being given on alternative safe natural bio-remedies to cure diseases because of their less or no side effects and resistance in microbes against them. Hence, the objective of present study was to evaluate the anti-diarrhoeal activity of hydroalcoholic extract of root of *Calotropis gigantea* against castor oil-induced diarrhoea model to scientifically justify the traditional claims. [11].

MATERIALS AND METHODS

Plant material

Collection of plant material

The root of *Calotropis gigantean* R.Br**.** were collected from wild sources surrounding Ahimamau, Lucknow U.P. and authenticated by Dr. Tariq hussain the scientist Head The of department herbarium, N.B.R.I., Lucknow, U.P., India. (Reference No. 243/93. 16/02/2009)

Preparation of plant material

250 gm of air dried powder was extracted with water: ethanol (50:50) by cold maceration in a specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand for eighteen hours. The extract was filtered, concentrated under reduced temperature using rotary evaporator and dried extracts was subjected for phytochemical and biological activity on experimental animal model.

Animal selection: Healthy adult wister albino rats were used for all the experimental study of various parameters of antidiorrhoea activity. The body weight of these animals varied from 160 to 190gm and the age from 4-6 months. Male or female rats were selected to antidiorrhoeal activity.
Animals were housed individually with free access to food and water under standard condition and the basal food intake, body weights to the nearest gram were noted. The animals were starved 18 hr prior to starting biological activity.

Experimental procedures
Phytochemical screening
The hydro-alcoholic extract of the root of *Calotropis gigantea* R.Br. was evaluated for the presence of flavonoids, tannins, alkaloids, saponins, glycosides and sterols/triterpenes [12]

Drugs and chemicals
Loperamide (standard reference antidiarrhoeal drug), castor oil (laxative agent), normal saline solution (9% NaCl) and vehicle (0.5% v/v Tweens 80 in distilled water) were used.

Screening methods for antidiarrhoeal activity:
Castor oil-induced diarrhoea
The rats were grouped into five groups of five animals each according to their weights and housed in separate locally fabricated metabolic cages. Two control groups were employed. The first control group received only normal saline (1ml/kg body wt.) whereas the second control animals were treated with the standard anti-diarrhoeal drug (Loperamide). The three test groups of animals were given graded doses of the extract (200, 300 & 400mg/kg body wt. respectively).The extract and Loperamide were administered p.o. 1 hour before the oral administration of the cathartic agent castor oil (2 ml per rat). Oral administration of castor oil was facilitated by the use of a stomach tube. The animals were monitored for 12 hours for consistency of stool and the frequency of defecation. At the end of this period, the total number of the fecal matter for each group and the number of diarrhoeic (wet) faeces were recorded and the mean value for each group calculated.

Schedule for screening of hydro-alcoholic extracts for anti diarrhoeal activity

Groups	**Dose (p.o)**
Control	2% Tween 80 Saline.
Standard	3 mg/kg. b.w. of Loperamide in 2%Tween 80 Saline.
Test extract-1	100mg/kg.b.w.in 2%Tween 80 Saline
Test extract-2	150mg/kg.b.w.in 2%Tweenb 80 Saline.
Test extract-3	250mg/kg.b.w.in 2%Tween 80 Saline.

Statistical analysis
The experimental results are represented as mean ± S.E. (Standard error of the mean). Student's t-test was used for the evaluation of data and $P<0.05$ accepted as significant.

RESULT

Physical constants:
Various physical constants for roots were determined which includes extractive values, moisture content, ash values and reported in table no:02.

Table no: 2

S. No.	Parameter	Result in %w/w
1.	Total ash value	**3.5**
2.	Acid soluble ash	**1.5**
3.	Water soluble ash	**2.3**
4.	Loss on drying	**11.0**

Castor oil-induced diarrhoea
The animals were monitored for 12 hours for consistency of stool and the frequency of defecation. At the end of this period, the total number of the fecal matter for each group and the number of diarrhoeic (wet) faeces were recorded and the mean value for each group calculated. Values for the treated groups were compared with that of control rats that received normal saline. The mean number of diarrhoeic feaces –pooled by the group that received normal saline (1ml/kg body wt.) and Castor oil was considered as 100%. Percentage inhibition of wetness of feaces and frequency of stooling caused by the extract and Loperamide were obtained by comparing them with castor oil.

Table no: 9 Screening result of *Calotropis gigantea* hydroalcoholic extracts on Caster oil-induced diarrhea in experimental animal

Group	Dose (p.o)	Mean defection in 4hrs.	% Inhibition of defecation
Saline	10ml/kg b.w.	2.99±0.335	--
Standard	**3mg/kg b.w.**	**1.09±0.205**	**66%**
Extract-1	**100mg/kg b.w.**	**1.96±0.225**	**51%**
Extract-2	**150mg/kg b.w.**	**1.92±0.303**	**53%**
Extract-3	**250mg/kg b.w.**	**1.46±0.367**	**63%**

Values were considered significant when $P<0.05$ compared with normal saline group, n=6

SUMMARY AND CONCLUSION

In most cases of diarrhoea result from disorders of intestinal water and electrolyte transport.[13] There are numerous causes of diarrhoea including infectious agents, toxins, anxiety, drugs, and

so forth.[14] Diarrhoea is a major health problem especially for children under the age of 5 years. Worldwide distribution of diarrhoea accounts for more than 5-8 million deaths each year in infants. According to W.H.O. estimates for 1998, about 7.1 million deaths were caused by diarrhoea. The incidence of diarrhoeal diseases still remains high despite the efforts of many governments and international organisations to curb it. [15] In recent times, emphasis has been focused on the use of oral rehydration solution (ORS) as a replacement therapy to replenish the lost fluid and electrolytes in diarrhoeic cases.[16] Generally, the treatment of diarrhoea is non-specific, and is usually aimed at reducing the discomfort and inconvenience of frequent bowel movements. [12, 17]

The castor oil test has been used extensively to screen and evaluate antidiarrhoeal properties of drugs in mice. Within 1 h of oral administration of the oil, the animals begin to evacuate watery stools [18, 19]. The use of castor oil induced diarrhoea model in our study is logical because the autocoids and prostaglandins are involved these have been implicated in the causation of diarrhoeas in man [20, 21]. Castor oil causes diarrhoea due to its active metabolite, ricinolic acid [22], which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. Its action also stimulates the release of endogenous prostaglandin which results in stimulation of secretion [23, 24]. Thereby prevents the reabsorption of NaCl and H_2O [23] Loperamide, apart from regulating the gastrointestinal tract, is also reported to slow down transit in the small intestine, reduce colon flow rate, and consequently any effect on colonic motility. [25]

In this study, the hydroethanolic extract of root of *C.gigantea* displayed a signifi cant and dose-dependent antidiarrhoeal property.Previous studies showed that antidysenteric and antidiarrhoeal properties of medicinal plants were mostly due to tannins, alkaloids, saponins, flavonoids, sterol and triterpenes.[25] Sesquiterpenes, diterpenes, terpenes, flavonoids and terpenoid derivatives are known for inhibiting release of autocoids and prostaglandins, thereby inhibit the motility and secretion induced by castor oil. [26, 27, 28, 29]

The antidiarrhoeal property of the hydroalcoholic extract of *C.gigantea* found in the present study could be owing to the presence of glycosides, alkaloids, flavonoids, carbohydrates, proteins & amino acids and sterols in this plant. The anti-diarrhoeal activity of the extract may also be due to the presence of denature proteins forming protein tannates, protein tannates make the intestinal mucosa more resistant and reduce secretion (30)

From this study, the use of the root of *C. gigantea* in traditional medicine as a non-specific antidiarrhoeal agent has been justified. Further studies are, however, needed to establish the safety of the extract and to possibly isolate the active principle responsible for the observed effects.

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