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ORIGINAL RESEARCH ARTICLE

Analgesic and Antidiarrheal Properties of the Latex of *Calotropis Procera*

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ABSTRACT:
Calotropis procera (Ait.) R. Br. (Asclepiadaceae) is a Bangladeshi medicinal plant used traditionally to treat a various diseases. Phytochemical analyses as well as antidiarrheal and analgesic potentials of *C. procera* were evaluated. The ethanol extracts of the latex of the plant was used in this study. The analgesic activity was carried out against acetic acid-induced writhing test. The antidiarrheal potential was studied in castor oil induced diarrhea in mice. Mayer and Dragendroff's reagents, Mg and HCl, 1% gelatin-10% NaCl solution, Benedict's and Fehling's reagents were used for the analysis of alkaloids, flavonoids, tannin and reducing sugar respectively. Results showed that the intraperitoneal administration of ethanol extracts at 250 and 500 mg/kg produced a significant (p <0.05) and dose dependent analgesic effect against acetic acid induced writings. On the other hand, in castor oil induced diarrhea, oral administration at same doses was less pronounced as compared to a 4 mg/kg oral dose of loperamide. Furthermore, our phytochemical analysis also revealed the presence of alkaloids and tannins. This evidence clearly indicates that the ethanol extracts of *Calotropis Procera* possess strong analgesic potential coupled with antidiarrheal efficacy which may explain the use of the plant in traditional medicine as a popular antidiarrheal and analgesic recipe.

Keywords: Antidiarrheal activity, analgesic activity, medicinal plant.

INTRODUCTION:

Calotropis procera,(Ait.) R. Br. (Asclepiadaceae), locally known as Akand, belonging to the Asclepiadaceae family, is a soft wooded, evergreen, wild growing tropical perennial shrub, with one or few stems, few branches and relatively few leaves. It is widely distributed in Africa, South America, India and abundant in Bangladesh. It has been widely used as a traditional medicine in Ayrvedic, Unani and folker medicinal system for the treatment of many diseases like leprosy, ulcers, tumors, piles and diseases of the spleen, liver and abdomen [1].

Leaves and roots of the plant have been used to alleviate pain under different conditions. Besides the decoction of the plant has been reported to be employed in painful muscular spasm, dysentery, fever, rheumatism, asthma and as an expectorant and purgative [2, 3, 4]. Latex of the plant have been shown to confer significant anti-inflammatory activity against carageenin and formalin induced paw oedema and antipyretic effect [5,6] and it is a also a rich source of several biologically active compounds including glucosides, tannins, flavonoids and many proteins.

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Bangladesh is bestowed with many natural recourses and huge number of plants are yet to be explored. So well designed and systematic research of this plant might be helpful for the people who have been deprived of orthodox medicine. Considering the importance of this plant, we investigated antidiarrheal and analgesic activity of the ethanol extract of the *Calotropis procera* (Ait.) R. Br. (Asclepiadaceae).

In addition, phytochemical screening of the extract has also been performed to have a better view of the biologically active compounds.

MATERIALS AND METHODS:

Sample collection and extraction;

The plant was collected from the rural areas of Khulna, Bangladesh. The region was selected because of the wide availability of the plant and people of these regions use this plant as a medicine. A voucher specimen of the aerial parts of the plant was deposited in the Department of Mathematics and Natural Sciences (MNS), BRAC University, Bangladesh. Seven-day, air-dried and powdered aerial parts of the plant (150 g) was soaked for a week in 800 mL 95% ethanol with occasional shaking and stirring. The materials were filter ed through Whatman no 1 and the filtrate was completely concentrated and this dried crude extracts were used for investigation.

Animal;

For this study Swiss albino mice (20–25 g) of either sex were purchased from the Animal Research Branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDR, B) and were kept in standard stainless steel cages under environmentally controlled conditions with 12-h light/12-h dark cycle. All animals were fed with standard pellet diet (ICDDR, B) and water *ad libitum*. Animals were acclimatized to laboratory condition for one week prior to the experiment. All the experiments were carried out after the approval of the ethical committee of the institution. They were treated according to the guidelines for laboratory animals [7].

Phytochemical analysis;

Phytochemical analysis of the plant extracts were studied using the following reagents and chemicals [8,9], alkaloids with Mayer and Dragendroff's reagents, flavonoids with the use of Mg and HCl, tannin with gelatin-salt block test (1% gelatin and 10% NaCl solution), reducing sugar with Benedict's and Fehling's reagents.

Acetic acid-induced writhing test in mice;

Acetic acid induced writhing test, which measures the abdominal constriction, was carried out according to the procedures described by Koster et al. [10]. *Calotropis procera* was tested at 250 and 500 mg/kg. Ethanol extracts and the standard drug were administered intraperitoneally 30 min before the intraperitoneal administration of 0.7% acetic acid in a volume of 15 mL/kg. Control mice received 1% Tween-80 in water under same experimental condition (15 mL/kg). Each group was composed of five mice. Immediately after the injection each animal was transferred to an individual box and the writhing was counted over a period of 15 min. The number of writhing and stretching was recorded and the percentage protection was calculated using the following ratio Percentage of protection = (Control mean - Treated mean/control mean) x 100.

Tests for antidiarrheal activity;

Castor oil-induced diarrhea;

Twenty mice were allowed to fast for 18 h and divided into four groups of five animals each. Diarrhea was induced in mice with castor oil (1 mL/ mouse), administered 30 min after different doses of plant extracts (250 and 500 mg/kg), loperamide (4mg/kg) and 1% Tweeen-80 in water (0.4mL/ mouse). The animals were transferred individually in a cage lined with white paper.

RESULTS:

Phytochemical analysis;

Phytochemical analysis of the ethanol extracts revealed the presence of alkaloids, tannins but flavonoids and reducing sugars are absent in the extracts.

Acetic acid-induced writhing test in mice;

The results of the acetic acid induced writhing test in mice are given at (**Table 1**). At doses of 250 and 500 mg/kg i.p. the *C. procera* inhibited the writhing responses caused by the intraperitoneal administration of the acetic acid. The mean number of writhes was significantly lower than in the control group at these doses. The maximal inhibition of the nociceptive response was 39.09% at 500mg/kg and 28.18% at 250 mg/kg. Sodium diclophenac exerted a significant protective effect, inducing protection of 20.9% at a dose of 25 mg/kg.

Table 1: ANALGESIC EFFECT OF *C. procera* ON ACETIC ACID WRITHINGS IN MICE

Group	Dose (mg/kg)	Total no of writhings	Mean ± SD, n=5	Percentage of protection
Control	--	55.00	11 ± 1.58	---
C. procera	250	39.50	7.9 ± 1.59	28.18**
C. procera	500	33.50	6.7 ± 1.22	39.09**
Sodium dichlophenac	25	43.50	8.7 ± 1.63	20.9**

** $p < 0.05$, Dunnet test as compared to the control

Castor oil-induced diarrhea;

In castor oil induced diarrhea, the mice that did not receive the plant extract showed the typical diarrheal signs and symptoms such as watery stool and defecation. The ethanol extracts of *C. procera* produced a notable antidiarrheal effect in mice (**Table 2**). Both doses of extracts significantly increase the latent time. Latent time at 250 and 500 mg/kg was 81.8 ± 2.38 min and 106.08 ± 7.22 min, respectively compared to the 64.2 ± 3.19 min for the control group. The standard drug, loperamide at a dose of 4 mg/kg was found more effective (141.02 ± 4.23 min) than the extracts.

Table 2: **Antidiarrheal effect of *c. procera* on castor oil induced diarrhea in mice**

Group	Dose (mg/kg)	Latent time (mean ± SD, n=5)
Control	----	64.2 ± 3.19
C. procera	250	81.8 ± 2.38**
C. procera	500	106.05 ± 7.22**
Loperamide	4	141.02 ± 4.23**

** $p < 0.05$, Dunnet test as compared to the control

DISCUSSION:

The potential antidiarrheal and the analgesic activity of the ethanol extract of *Calotropis procera* were investigated.

Natural products having analgesic potentials are of great interest for many reasons. It is well established that various phlogestic agents produce inflammation through the release of several mediators of which histamine and serotonin are released in the early phase. In addition, prostaglandins (PGs) are also involved in both early and late phases of inflammation process. Cyclooxygenase (COX) that exists in two isoforms COX-1 and COX-2 are involved in the PG synthesis. COX-1 is reported to produce PGs, important for homeostasis and certain physiological functions and is expressed constitutively in most tissues and cells. COX-2 that is inducible and expressed in inflammatory cells is primarily responsible for PG production at the site of inflammation [11, 12, 13, 14]

The latex of *C. procera* is a rich source of several biologically active molecules including organic molecules, free amino acids, peptide, enzymes and some non enzymatic compounds. The analgesic test used in the present work has been chosen in order to test the chemical visceral stimuli. Acetic acid acts indirectly by inducing the release of endogenous mediators of pain sensitive to non-steroidal anti-inflammatory drugs and opioids [15]. Besides acetic acid induces an effect of writhing in mice which is an effect of acute inflammatory reaction related to the elevated level of prostaglandins E2 and $F2_{\alpha}$ in the peritoneal fluid [16, 17]. Drugs such as narcotics act both peripherally and centrally while the NSAIDs like diclophenac sodium acts only peripherally to inhibit the pain sensation [18, 19, 20]. In this study it has been found that the administration of *C. procera* produced a clear dose dependant analgesic activity. These findings suggest that central mechanism is involved in the analgesic activity of the extracts. Further investigations will be necessary about the putative narcotic like activity profile of *C. procera*. Since GABA is known to induce antinociception, an increase of GABA may also explain the antinociceptive effect of the extract [21].

Diarrhea results from an imbalance between the absorptive and secretary mechanisms in the intestinal tract accompanied by excess loss of fluid in the feces. The castor oil induced diarrhea has been widely used to analyze and evaluate the antidiarrheal potential of drugs in mice. It causes to evacuate watery stool due to its active metabolite recinolic acid within 1 hour of oral administration [22, 23, 24, 25]. Inhibition of intestinal Na^+, K^+ ATPase activity by castor oil results in the reduction of normal fluid absorption [26]. It activates adenylate cyclase or mucosal cyclic

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AMP mediated active secretion [27], stimulates the prostaglandins formation [28], and platelet activating factor. Nitric oxide has also been claimed to contribute to the diarrheal effect of castor oil [29].

In developing countries, diarrhea from infectious origin is the major cause of infant mortality. Inhibition of experimental diarrhea and reduction in fecal output by a substance are the basis of the pharmacological evaluation of a potential antidiarrheal agent. Traditional medicines are still very commonly used in Bangladesh for diarrhea and other diseases. The ability of the ethanol extracts to offer protection against castor oil induced diarrhea, and the reduction in fecal output, support the folkloric use of the plant in controlling diarrhea in animals and humans. Loperamide, the standard drug, apart from regulating the gastrointestinal tract, have also been reported to slow down transit in the small intestine, reduce colon flow rate, and consequently any effect on colonic motility [30, 31].

In this study the ethanol extract of *C. procera* exhibited a dose dependent antidiarrheal property, although loperamide displayed more efficacy than the extract. Phytochemical screening revealed the presence of tannins and alkaloids. The antidiarrheal potential of the extract could be owing to the presence of these active metabolites. Previous studies also showed that antidiarrheal and antidysenteric properties of the medicinal plant mostly due to the tannins saponins, flavonoids, sterol and triterpenes [8, 28, 32]. At present study, it is not yet shown which of the groups of phytochemicals are responsible for the observed antidiarrheal effects.

CONCLUSION:

In conclusion, we can confirm that the ethanol extracts of *C. procera* are endowed with central analgesic properties, associated with modest antidiarrheal potential. Further investigations are necessary for pharmacological and toxicological characterization.

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