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PHARMACOLOGICAL STUDIES OF ANTI-DIARRHOEAL ACTIVITY OF BARRINGTONIA ACUTANGULA (L.) IN EXPERIMENTAL ANIMALS

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ABSTRACT

The purpose of the present study was to evaluate scientifically the anti-diarrhoeal effects of ethanol extract of roots of *Barringtonia acutangula* Linn (EBA) was studied against castor oil-induced-diarrhoea model in rats. Antidiarrhoeal activity of ethanol extract of *Barringtonia acutangula* was investigated in this study using castor oil-induced-diarrhoea model in rats. Standard drug diphenoxylate (5 ml/kg, p.o) was significant reductions in fecal output whereas EBA at the doses of 200 and 400 mg/kg p.o significantly (P<0.001) reduced the castor-oil induced frequency and consistency of diarrhoea. The EBA showed marked reduction in the number of diarrhoea stools. The results obtained establish the efficacy and substantiate the folklore claim as an anti- diarrheal agent. Further studies are needed to completely understand the mechanism of anti-diarrhoeal action of Barringtonia acutangula.

Keywords: Antidiarrhoeal Activity, Barringtonia acutangula, Traditional medicine, Castor Oil- induced diarrhoea.

INTRODUCTION

Barringtonia acutangula (L.) Gaertn. (Family: Lecythidaceae) an evergreen tree of moderate size is called as Hijja or Hijjala in Sanskrit. The fruit is spoken of as samudra-phala and various part of this plant used as a folklore medicine for curing various ailments like hemiplegia, pain in joints, eye diseases, stomach disorders, anthelmintic, diarrhoea, cough, dyspnoea, leprosy, intermittent fever, and spleenic disorders. An ethanol extract of the bark is found hypoglycemic and is reported to be used in pneumonia, diarrhea, asthma and leaf juice is given for diarrhea. Fruit is bitter, acrid, anthelmintic, emetic, expectorant and vulnerary. It is prescribed in gingivitis, as an astringent and tonic. Whole plant was reported to possess flavonols, phenolic acids, triterpenoids, tannins and steroidal compounds such as barringtogenic acid, tangulic acid and acutangulic acids. The fruit possessed saponins based on barringtogenol B, C and D. The therapeutic potential of this plant were reported to be antitumor, antibiotic, inhibit growth of Helicobacter pylori and antifungal activities [1-7].

However there are no reports on the antidiarrheal activity of the plant. Hence, the present study was designed to verify the claims of the native practitioners.

MATERIALS AND METHODS

Collection and authentication of plant material

The Plant material of *Barringtonia acutangula* (*L.*) roots was collected from Tirunelveli District, in the Month of August 2011. The plant was authenticated by Dr.V.Chelladurai, Research Officer Botany. C.C.R.A.S., Govt. of India. The voucher specimen of the plant was deposited at the college for further reference.

Preparation of plant extract

The roots of the *Barringtonia acutangula* (*L*.) are properly washed in tap water and then rinsed in distilled water. The rinsed roots are dried in an oven at 35° C for 4 days. The dried roots of *Barringtonia acutangula* was crushed to obtain powder.

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These powdered samples are then stored in airtight polythene bags protected from sunlight until use. The ethanol extract of each sample was prepared by soaking 10g of powdered sample in 200ml distilled water for 12h. The extracts are then filtered using Whatmann filter paper. Percentage yield of ethanol extract of *Barringtonia acutangula* was found to be 10.5 % w/w. The ethanol extract was administered to the animals by suspending each time in 1% CMC.

Phytochemical Screening

The phytochemical examination of ethanol extract of *Barringtonia acutangula (L.)* was performed by the standard methods [8].

Experimental animals

Adult Wistar rats of either sex weighing 180-250 gms were used in pharmacological and toxicological studies. The inbred animals were taken from the animal house and maintained in a well-ventilated room with at 12:12 hr light, dark cycle in polypropylene cages and maintained at $22\pm1^{\circ}$ C with humidity at $55\pm5\%$. They were fed balanced rodent pellet diet from Poultry Research station, Nandanam, Chennai-35 and tap water ad *libitum* throughout the experimental period. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of CPCSEA (Committee for the Purpose of Control and Supervision of Experimental Animals).

Acute toxicity study

The acute toxicity of ethanol extract of *Barringtonia acutangula* (*L.*) *roots* was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not lethal to the rats even at 2000mg/kg dose. Hence, 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for further study [9].

Castor oil-induced diarrhoea

Diarrhoea was induced by model of Nwafor *et al.*, (2005) [10, 11]. Animals were fasted for 24 h but allowed free access to water. Rats were divided into four groups of six animals each, diarrhoea was induced by administering

2 ml of castor oil orally to rats. Group I treated as control (2 ml/kg, p.o. saline), group II received diphenoxylate (5 ml/kg p.o) served as standard and group III and IV received EBA (200 and 400 mg/kg, p.o) 1 h before castor oil administration. Then observed for consistency of faecal matter and frequency of defaecation for 4 hrs.

Statistical analysis

The data were expressed as mean \pm standard error mean (S.E.M).The Significance of differences among the groups was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnet's test *P* values less than 0.05 were considered as significance.

RESULTS

Phytochemical investigation

The results of preliminary phytochemical investigation of the ethanol extract of *Barringtonia acutangula (L.)* roots (EBA) shows the presence of carbohydrates, phenols, flavanoids, glycosides, terpenes, alkaloids, tannins, and Saponins.

Acute toxicity study

Acute toxicity study in which the animals treated with the EBA at a higher dose of 2000 mg/kg did not manifest any significant abnormal signs, behavioral changes, body weight changes, or macroscopic findings at any time of observation. There was no mortality in the above-mentioned dose at the end of the 14 days of observation.

Castor oil-induced diarrhoea

After 30 min administration of castor oil the diarrhoea was clinically apparent in all the animals of control group, for the next 4 h. This was markedly reduced by diphenoxylate (5 ml/kg p.o). A similar marked reduction in the number of defecations over four hours was achieved with *Barringtonia acutangula* at the doses of 200 or 400 mg/kg p.o. EBA 200 and 400 significantly inhibited the defecation EBA 200 and 400 mg/kg, p.o. dose of extract delayed the onset of diarrhoea and only 30% of animals showed diarrhoea at first hour.

Table 1. Effect of EBA on castor oil-induced diarrhoea in rats

Group	Treatment	Mean Defecation in 4hr
Ι	Castor oil (2ml p.o) + saline (2ml/kg p.o)	26.33±1.41
Π	Castor oil (2ml p.o) + diphenoxylate (5 ml/kg p.o)	7.24±0.33**
III	Castor oil (2ml p.o) + EBA (200mg/kg p.o)	14.64±0.43*
IV	Castor oil (2ml p.o) + EBA (400mg/kg p.o)	8.42±0.15**

Effect of EBA on castor oil-induced diarrhoea in rats: EBA was administered p.o 1 h before castor oil administration. Values are expressed as mean \pm SEM from the experiments. *P<0.01, **P<0.001 when compared with **Castor oil** + saline-treated group.

DISCUSSION AND CONCLUSION

Diarrhoea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract, accompanied by hurry, resulting in an excess loss of fluid in the faeces. At doses of 200 and 400 mg/kg, the ethanol

extract of *Barringtonia acutangula* showed significant anti-diarrhoeal activity against castor oil-induced diarrhoea as compared with the control group it significantly (P<0.001) reduced the frequency of diarrhoea and consistency of defecations (Table 1). The EBA also

showed a dose related decrease in castor oil-induced diarrhoea. Several mechanisms have been supposed to be involved in the diarrhoeal effect of castor oil [12]. These include Castor oil is decreases fluid absorption, increases secretion in the small intestine and colon, and affects smooth muscle contractility in the intestine. Castor oil produces diarrhoeal effect due to its active component of recinoleic acid [13], inhibition of intestinal Na⁺, K⁺-ATPase activity to reduce normal fluid absorption [14, 15], activation of adenylyl cyclase [13], stimulation of prostaglandin formation [16], platelet-activating factor and recently nitric oxide was contribute to the diarrhoeal effect of castor oil [17-19]. Despite the fact that number of mechanisms has been involved for the diarrhoeal effect of castor oil, it has not been possible to define its correct mechanism of action [20]. EBA may act an above any one of the mechanism.

Anti-dysentric and antidiarrhoeal properties of medicinal plants were found to be due to tannins, alkaloids,

saponins, flavonoids, sterols and/or triterpenoids and reducing sugars [21]. The phytochemical analysis of EBA revealed the presence of alkaloids, flavonoids, triterpinoids carbohydrates, tannins, phenols, gums and mucilage. These constituents may mediate the anitdiarrhoeal property of the EBA.

In conclusion, the present study has shown that *Barringtonia acutangula* is a potential therapeutic option in the effective management of diarrhoea, thus justifying its widespread use by the local population for these purposes. Concerted efforts are being made to fully investigate the mechanisms involved in the pharmacological activities of *Barringtonia acutangula* and phytochemical studies are also in progress to isolate and characterize the active constituents of *Barringtonia acutangula*. The isolated compound may serve as useful prototypes of anti-diarrhoeal drugs of natural origin possessing the desired pharmacological activities while lacking certain untoward effects.

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