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ANTI-DIARRHOEAL ACTIVITY OF *ALOCASSIA MACRORHIZA* (L.) AGAINST CASTOR OIL INDUCED DIARRHOEA MODEL IN RATS

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ABSTRACT

The present study was to scientifically evaluate anti-diarrhoeal effects of ethanolic (90%) extract of bark of *Alocassia macrorhiza (L.)* (EEAM) was studied against castor oil-induced-diarrhoea model in rats. The weight and volume of intestinal content induced by castor oil were studied by enteropooling method. Standard drug atropine (3mg/kg, i.p) showed significant reductions in fecal output and frequency of droppings whereas EEAM at the doses of 100 and 200 mg/kg i.p significantly retarded the castor-oil induced enteropooling and intestinal transit. The gastrointestinal transit rate was expressed as the percentage of the longest distance travelled by the charcoal divided by the total length of the small intestine. EEAM at the doses of 100 and 200 mg/kg significantly inhibited ($P<0.001$) weight and volume of intestinal content. The results obtained establish the efficacy and substantiate the folklore claim as an anti- diarrheal agent.

Keywords: Anti-diarrhoeal activity, *Alocassia macrorhiza (L.)*, Traditional medicine, Castor oil- induced diarrhoea, Enteropooling method.

INTRODUCTION

Worldwide distribution of diarrhoea accounts for more than 5-8 million deaths each year in infants and children below 5 years old especially in developing countries (Fauci *et al*., 1998). According to W.H.O. estimates for 1998, about 7.1 million deaths were caused by diarrhoea (Park *et al.*, 2000). The incidence of diarrhoeal diseases still remains high despite the efforts of many governments and international organizations to curb it. It is therefore important to identify and evaluate available natural drugs as alternatives to currently used anti-diarrhoeal drugs, which are not always free from adverse effects (Hardman *et al*., 1992). A range of medicinal plants with anti-diarrhoeal properties is widely used by traditional healers. However, the effectiveness of many of these anti-diarrhoeal traditional medicines has not been scientifically evaluated.

Alocassia macrorhiza (L.) (Family: Araceae) is probably native to Indo-malesia but widely distributed by aboriginal peoples throughout South-East Asia into the tropical Pacific. According to literature review its constituents, oxalic acid, calcium oxalate, flavonoids, cyanogenic glycosides, alocasin, cholesterol, beta-sitosterol, stigmatosterol, camposterol, fucosterol, amino acids, citric acid, malic acid, ascorbic acid, succinic acid, glucose, fructose and sucrose. Arabino-galactan proteins and betalectins (Cambie RC, and Ash J, 1994; Yeoh *et al*., 1986). This plant traditionally using, In Fiji, the sap of the

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Therefore, the present study was performed to verify the folklore claim of anti-diarrhoeal activity of *Alocassia macrorhiza (L.)* against castor oil-induced-diarrhoea model in rats.

MATERIALS AND METHODS

Plant collection

The bark of *Alocassia macrorhiza (L.)* used for investigation was purchased from Tamil Nadu Medical Plant Farms & Herbal Medicine Corporation Limited (TAMPCOL). The plant was authenticated by Dr.V.Chelladurai, Research Officer Botany. C.C.R.A.S., Govt. of India. The voucher specimen (CHE- AM-0525) of the plant was deposited at the college for further reference.

Preparation of extracts

The bark of *Alocassia macrorhiza (L.)* are dried in shade, separated and made to dry powder. It was then passed through the 40 mesh sieve. A weighed quantity (90gm) of the powder was subjected to continuous hot extraction in Soxhlet Apparatus. The extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample. Percentage yield of ethanolic extract of bark of *Alocassia macrorhiza (L.)* (EEAM) was found to be 10.5 % w/w.

Preliminary phytochemical screening

The phytochemical examination of ethanolic (90%) extract of bark of *Alocassia macrorhiza (L.)* was performed by the standard methods (Harbone JP, 1973).

Animals used

Albino wistar rats (150-230g) were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. The animals were fed with standard pellet feed (Hindustan Lever Limited., Bangalore) and water was given *ad libitum*. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA.

Castor oil-induced diarrhoea

Rats were divided into four groups of six animals each, diarrhea was induced by administering 1 ml of castor oil orally to rats. Group I treated as control (2 ml/kg, i.p. saline), group II received atropine (3mg/kg, i.p.) served as standard and group III and IV received EEAM (100 and 200 mg/kg, i.p.) 1 h before castor oil administration. The number of both wet and dry diarrheal droppings were counted every hour for a period of 4 h mean of the stools passed by the treated groups were compared with that of the positive control group consisted of animals given an intraperitoneal injection of saline (2ml/kg, i.p) (Awouters *et al*., 1978).

Castor oil-induced enteropooling

Intraluminal fluid accumulation was determined by the method of Robert *et al* (Robert *et al*., 1976). Overnight fasted rats were divided four groups of six animals each. Group I received normal saline (2 ml/kg, i.p.) served as a control, group II received atropine (3mg/kg, i.p.) and groups III and IV received EEAM 100 and 200 mg/kg intraperitoneally respectively. 1h 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.

Small intestinal transit

Rats were fasted for 18 h divided into five groups of six animals each, Group I received 2 ml normal saline orally, group II received 2 ml of castor oil orally with saline 2 ml/kg intraperitoneally, group III received atropine (3 mg/kg, i.p.), group IV and V received EEAM 100 and 200 mg/kg intraperitoneally respectively, 1 h before administration of castor oil. One ml of marker (10% charcoal suspension in 5% gum acacia) was administered orally 1 h after castor oil treatment. The rats were sacrificed after 1h and the distance traveled by charcoal meal from the pylorus was measured and expressed as percentage of the total length of the intestine from the pylorus to caecum (Izzo *et al*., 1999).

Statistical analysis

The data were expressed as mean ± 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 screening

The results of preliminary phytochemical screening of the ethanolic extract of inner bark of *Alocassia macrorhiza (L.)* revealed that presence of alkaloids, flavonoids, tannins, phenols, gums and mucilage and absence of saponins and steroids.

Castor oil-induced diarrhoea

30 min after 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 the intraperitoneal injection of atropine, 3 mg/kg (72.33%). A similar marked reduction in the number of defecations over four hours was achieved with *Alocassia macrorhiza (L.)* at the doses of 100 or 200 mg/kg i.p. EEAM 100 and 200 significantly inhibited the defecation (46.28% and 62.59%) EEAM 100 and 200 mg/kg, i.p. dose of extract delayed the onset of diarrhoea and only 30% of animals showed diarrhoea at first hour (P<0.001) (Table 1).

Castor oil-induced enteropooling

Castor oil caused accumulation of water and electrolytes in intestinal loop. Castor oil-induced enteropooling is not influenced by atropine in rats at the dose of 3 mg/kg, i.p. EEAM 100 and 200 produced a dose-dependent reduction in intestinal weight and volume. EEAM 100 and 200 mg/kg, i.p. dose produced 35.54% and 56.25% inhibition of volume of intestinal content respectively with significance (P<0.001). The weight of intestinal content was also reduced significantly at both the doses. **(**Table 2**).**

Small intestinal transit

The percent intestinal transit was increased with castor oil (86.81%), but it was reduced in both doses of extract, and much more markedly by atropine (40.57%). EEAM 100 mg/kg, i.p. dose of extract produced 72.21% intestinal transit induced by castor oil respectively. Whereas, EEAM 200 mg/kg, i.p. dose produced 56.60% of castor oil induced charcoal meal transit (Table 3).

Table 1: Effect of *EEAM* on castor oil-induced diarrhoea in rats.

Group	Treatment	Mean Defecation in 4hr	% Inhibition of Defecation
I	Castor oil (1ml p.o) + saline (2ml/kg i.p)	25.45±1.22	---
II	Castor oil (1ml p.o) + atropine (3mg/kg i.p)	7.04±0.24**	72.33
III	Castor oil (1ml p.o) + EEAM (100mg/kg i.p)	13.67±0.84*	46.28
IV	Castor oil (1ml p.o) + EEAM (200mg/kg i.p)	9.52±0.19**	62.59

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

Table 2: Effect of EEAM on castor oil induced enteropooling in rats.

Group	Treatment	Weight of Intestinal Content (g)	% Inhibition of Weight Intestinal Content
I	Castor oil (1ml p.o) + saline (2ml/kg i.p)	2.56±0.22	---
II	Castor oil (1ml p.o) + atropine (3mg/kg i.p)	1.58±0.14**	38.28
III	Castor oil (1ml p.o) + EEAM (100mg/kg i.p)	1.65±0.17*	35.54
IV	Castor oil (1ml p.o) + EEAM (200mg/kg i.p)	1.12±0.15**	56.25

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

Table 3: Effect EEAM on castor oil-induced small intestinal transit in rats.

Group	Treatment	Total Length of Intestine	Distance Travelled By Marker	%Intestinal Transit
I	saline (2ml/kg i.p)	98.24 ± 1.32	46.57 ± 1.16	47.40
II	Castor oil (1ml p.o) + saline (2ml/kg i.p)	85.46 ± 2.16	74.19 ± 1.42	86.81
III	Castor oil (1ml p.o) + atropine (3mg/kg i.p)	92.58 ± 2.52	37.56 ± 1.22**	40.57
IV	Castor oil (1ml p.o) + EEAM (100mg/kg i.p)	86.44 ± 1.42	62.42 ± 1.29*	72.21
V	Castor oil (1ml p.o) + EEAM (200mg/kg i.p)	89.72 ± 1.15	50.79 ± 1.33**	56.60

*Effect of EEAM on castor oil-induced small intestinal transit in rats: EEAM was administered i.p 1 h before castor oil administration. Values are expressed as mean ± SEM from the experiments. *P<0.01, **P<0.001 when compared with* Castor oil + *saline-treated group.*

DISCUSSION

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. 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 (Horton *et al*., 1968; Greenbargena *et al*., 1978). The liberation of ricinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which stimulate motility and secretion (Pierce *et al*., 1971). The results of the present study show that EEAM produced a statistically significant reduction in the severity and frequency of diarrhoea produced by castor oil. It is also noted that EEAM significantly inhibited castor oil induced intestinal fluid accumulation and the volume of intestinal content, dose dependently more than atropine. The EEAM significantly reduced the castor oil induced intestinal transit. In this study, atropine produced a significant reduction in the number of stools and increased intestinal transit time possibly due to its anti-cholinergic effect (Brown JA and Taylor P, 2000).

However, it did not inhibit castor oil induced enteropooling and gain in weight of intestinal content suggesting thereby that mediators other than acetylcholine are involved in castor oil induced enteropooling. An increase in intestinal transit time with atropine could also result due to reduction in gastric emptying (Mascolo *et al*., 1994). Castor oil is also reported to induce diarrhoea by increasing the volume of intestinal content by prevention of the reabsorption of water. The liberation of ricinoleic acid results in irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which results in stimulation of secretion (Pierce *et al*., 1971). Thereby prevents the reabsorption of NaCl and H_2O (Ammon HV and Soergel KH, 1985). Probably EEAM increased the reabsorption of NaCl and water by decreasing intestinal motility as observed by the decrease in intestinal transit by charcoal meal.

The secretory diarrhoea is associated with an activation of Cl- channels, causing Cl- efflux from the cell, the efflux of Cl- results in massive secretion of water into the intestinal lumen and profuse watery diarrhoea (Longanga_Otshudi *et al*., 2000). The EEAM may inhibit the secretion of water into the lumen by reverting this mechanism. Anti-dysentric and antidiarrhoeal properties of medicinal plants were found to be due to tannins, alkaloids, saponins, flavonoids, sterols and/or triterpenes and reducing sugars (Vimala *et al*., 1997). The phytochemical analysis of EEAM revealed the presence of alkaloids, flavonoids, triterpinoids carbohydrates, tannins, phenols, gums and mucilage. These constituents may mediate the anitdiarrhoeal property of the EEAM.

Although the antidiarrhoeal properties of the reported active terpenoids are well established, aspects of their mechanism of action remain poorly understood. 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 (Veiga *et al*., 2001; Milanova *et al*., 1995; Nikiema *et al*., 2001).

CONCLUSION

The EEAM showed marked reduction in the number of diarrhoea stools and the reduction in the weight and volume of the intestinal contents, as well as a modest reduction in intestinal transit. This study did not go further, to demonstration as to whether the extract altered the activity of Na/K ATPase or activation of chloride channels. Whatever, may be the mechanism of action, the ethanolic extract of *Alocassia macrorhiza (L.)* may be useful in a wide range of diarrhoeal states, due to both disorders of transit e.g. functional diarrhoeas, radiation diarrhoea or due to abnormal secretory mechanisms like in cholera or E.coli enterotoxin induced diarrhoea. Further studies are needed to completely understand the mechanism of anti-diarrhoeal action of *Alocassia macrorhiza (L.).*

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