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(Research article)

ANTI-DIARRHOEAL ACTIVITY OF *TODDALIA ASIATICA (L.)* IN CASTOR OIL INDUCED DIARRHOEA IN RATS

***[1]Monic Shah, [2]Pradeep Kumar Reddy P, [3]Yerram raju Behara, [4]Chandra Mouli**
Department of Pharmacology, Sri Indu Institute of Pharmacy, Hyderabad.

ABSTRACT

The purpose of the present study was to evaluate scientifically the anti-diarrhoeal effects of ethanolic (90%) extract of leaves of *Toddalia asiatica* (*L.*) (EETA) was studied against castor oil-induced-diarrhoea model in rats. Antidiarrhoeal activity of 90% ethanol extracts of *Toddalia asiatica* (*L.*) was investigated in this study using castor oil-induced-diarrhoea, enteropooling and Small intestinal transit models in rats. The weight and volume of intestinal content induced by castor oil were studied by enteropooling method. Standard drug diphenoxylate (5 ml/kg, p.o) was significant reduction in fecal output and frequency of droppings whereas EETA 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 and enteropooling. 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. EETA at the doses of 200 and 400 mg/kg significantly inhibited ($P<0.001$) the castor oil induced charcoal meal transit. The EETA 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. The results obtained to establish the efficacy and substantiate the folklore claim as an anti- diarrheal agent. Further studies are needed to complete understand the mechanism of anti-diarrhoeal action of *Toddalia asiatica* (*L.*).

Keywords: Antidiarrhoeal Activity, *Toddalia asiatica* (*L.*), Castor Oil- induced diarrhoea, Enteropooling Method, Small intestinal transit

INTRODUCTION

Diarrhoea (loose motions) is the passage of 3 or more loose or liquid stools per day, or more frequently than is normal for the individual. Diarrhoea is not itself a disease, but can be a symptom of several diseases and some times may be associated with abdominal pain, which may reduce after a stool is passed. Diarrhoea occurs due to the irritation within the lining of the small or large intestine, which leads decrease water absorption hence increase in water being passed with stools. Many factors such as food poisoning, infection (bacterial, viral, parasitic), food intolerance, malnutrition, intestinal diseases and sometimes medications can contribute to diarrhoea [1].

In developing countries, a majority of people living in rural areas almost exclusively use the traditional medicine in treating all sorts of disease including diarrhoea. Diarrhoea is a major health problem, especially for children under the age of 5 and upto 17% of children admitted in the pediatric ward die of diarrhoea. Worldwide distribution of diarrhoea accounts more than 5-8 million deaths each year in infants and children below 5 years old especially in developing countries. According to W.H.O estimates for 1998, about 7.1 million deaths were caused by

*** Corresponding author:**
Monic Shah ,
Sri Indu Institute of Pharmacy,
Hyderabad, A.P. India
E-mail address: monicvshah@gmail.com

diarrhoea. 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. 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 healers. However, the effectiveness of many of these anti-diarrhoeal traditional medicines has not been scientifically evaluated [2].

Toddalia asiatica (*L.*) Lam. (Rutaceae) are widely used in folk medicine in India to treat various ailments like cough, malaria, indigestion, influenza lung diseases and rheumatism, fever, stomach ailments, cholera and diarrhea. In our earlier communication, we have reported the antimicrobial study on the various extracts of the *root bark* and the isolation and identification of Flindersine, a quinolone alkaloid as the major active principle [3]. In the present study, we report the antidiarrhoeal activity of *Toddalia asiatica* (L.) in castor oil induced diarrhoea in rats.

Plant collection

The root bark of *Toddalia asiatica (L.)* used for investigation was collected from Tirunelveli District, in 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 in the college for further reference.

Preparation of extracts

The root bark of *Toddalia asiatica (L.)* were dried in shade, separated and made to dry powder. It was then passed through the 40 mesh sieve. A weighed quantity (100gm) 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 extracts of *Toddalia asiatica (L.)* was found to be 16.5 %

Animals used

Albino wistar rats (200-230g) of either sex were obtained from the animal house. The animals 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).

Castor oil-induced diarrhoea

Diarrhoea was induced by Nwodo and Alumanah (1991) and Nwafor *et al.,* (2005) [4,5]. Animals were fasted for 24 h but allowed free access to water. Rats were divided into four groups of six animals and 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 EETA (200 and 400 mg/kg, p.o) 1 h before castor oil administration. Then observed for consistency of faecal matter and frequency of defecation for 4 hrs.

Castor oil-induced enteropooling

Intraluminal fluid accumulation was determined by the method of Robert *et al.,* (1976) and DiCarlo *et al.,* (1994) [6, 7]. Animals were fasted for 24 h but allowed free access to water. Rats were divided four groups of six animals each. Group I received normal saline (2 ml/kg, P.o served as a control, group II received diphenoxylate (5.0 mg/kg p.o.) and groups III and IV received EETA 200 and 400 mg/kg p.o respectively 1hr 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 reweigh 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 p.o, group III received atropine (3 mg/kg, i.p.), group IV and V received EETA 200 and 400 mg/kg p.o 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 1hr, 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 the caecum [8].

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M). The Significance of differences between 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

Castor oil-induced diarrhoea

After 30-minute administration of castor oil, the diarrhoea was clinically apparent in all the animals of the control group, for the next 4 hours. This was markedly reduced by diphenoxylate (5 ml/kg p.o) (68.29%). A similar marked reduction in the number of defecations over four hours was achieved with *Toddalia asiatica* at the doses of 200 or 400 mg/kg p.o. EETA 200 and 400 significantly inhibited the defecation (50.17% and 61.26%) EETA 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 (P<0.001) (Table 1).

Castor oil-induced enteropooling

Castor oil caused accumulation of water and electrolytes in the intestinal loop. Castor oil-induced enteropooling is not influenced by diphenoxylate (5 ml/kg p.o) in rats. EETA 200 and 400 produced a dose-dependent reduction in intestinal weight and volume. EETA 200 and 400 mg/kg, p.o dose produced 37.63% and 54.01% 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.

Small intestinal transit

The percent intestinal transit was increased with castor oil (89.42%), but it was reduced in both doses of extract, and much more markedly by atropine (41.61%). EETA 200 mg/kg, p.o dose of extract produced 63.65% intestinal transit induced by castor oil respectively. Whereas, EETA 400 mg/kg, p.o dose produced 47.12% of castor oil induced charcoal meal transit (Table 3).

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 extracts of *Toddalia asiatica (L.)* 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 EETA 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 [9]. These include Castor oil decreases fluid absorption, increases secretion in the small intestine and colon, and affects smooth muscle contractility in the intestine. Castor oil produces diarrhoeal effects due to its active component of recinoleic acid [10], inhibition of intestinal Na^+,K^+-ATPase activity to reduce normal fluid absorption [11, 12], activation of adenylyl cyclase, stimulation of prostaglandin formation, platelet-activating factor and recently nitric oxide was contributed to the diarrhoeal effect of castor oil [13,14]. Despite the fact that the 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 [15]. EETA may act an above any one of the mechanisms.

It is also noted that EETA significantly inhibited castor oil induced intestinal fluid accumulation and the volume of intestinal content. 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 [16]. The involvement of muscarinic receptor effect was confirmed by increased production of both gastric secretion and intraluminal fluid accumulation induced by castor oil. The EETA may inhibit the secretion of water into the intestinal lumen, and this effect is partly mediated by both α_2-adrenoceptor and muscarinic receptor systems. The significant inhibition of the castor oil-induced enteropooling in mice suggests that the extract of *Toddalia asiatica (L.)* produced relief in diarrhoea by spasmolytic activity in vivo and anti-enteropooling effects. [11].

The EETA significantly reduced the castor oil induced intestinal transit as compared with the control group (Table 3). In this study, atropine increased intestinal transit time possibly due to its anti-cholinergic effect [17]. In castor oil induced diarrhoea, 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 [18] by prevents the reabsorption of NaCl and water [17]. Probably, EETA increased the reabsorption of NaCl and water by decreasing intestinal motility as observed by the decrease in intestinal transit by charcoal meal.

In conclusion, the present study has shown that *Toddalia asiatica (L.)* 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 *Toddalia asiatica (L.)* and phytochemical studies are also in progress to isolate and characterize the active constituents of *Toddalia asiatica (L.)* 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.

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

Group	Treatment	Mean Defecation in 4hr	% Inhibition of Defecation
I	Castor oil (2ml p.o) + saline (2ml/kg p.o)	24.16±1.24	---
II	Castor oil (2ml p.o) + diphenoxylate (5 ml/kg p.o)	7.66±0.33**	68.29
III	Castor oil (2ml p.o) + EETA (200mg/kg p.o)	12.04±0.24*	50.17
IV	Castor oil (2ml p.o) + EETA (400mg/kg p.o)	9.36±0.52**	61.26

*Effect of EETA on castor oil-induced diarrhoea in rats: EETA was administered p.o 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 EETA on castor oil induced enteropooling in rats.

Group	Treatment	Weight of Intestinal Content	% Inhibition of Weight Intestinal Content
I	Castor oil (2ml p.o) + saline (2ml/kg p.o)	2.87±0.22	---
II	Castor oil (2ml p.o) + diphenoxylate (5 ml/kg p.o)	1.14±0.32**	64.74
III	Castor oil (2ml p.o) + EETA (200mg/kg p.o)	1.79±0.15*	37.63
IV	Castor oil (2ml p.o) + EETA (400mg/kg p.o)	1.32±0.14**	54.01

*Effect of EETA on castor oil-induced enteropooling in rats: EETA was administered p.o 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 EETA 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 p.o)	92.24 ± 1.19	49.67 ± 1.16	53.85
II	Castor oil (2ml p.o) + saline (2ml/kg i.p)	85.37 ± 1.12	76.34 ± 1.22	89.42
III	Castor oil (2ml p.o) + atropine (3mg/kg i.p)	94.14 ± 1.14	39.18 ± 1.16**	41.61
IV	Castor oil (2ml p.o) + EETA (200mg/kg i.p)	88.18 ± 1.22	56.13 ± 1.18*	63.65
V	Castor oil (1ml p.o) + EETA (400mg/kg i.p)	89.44 ± 1.21	42.14 ± 1.13**	47.12

*Effect of EETA on castor oil-induced small intestinal transit in rats: EETA was administered p.o 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.*

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