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PHARMACOLOGICAL STUDIES ON ANTI-DIARRHOEAL ACTIVITY OF *Morinda citrifolia* (L.) IN EXPERIMENTAL ANIMALS

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ABSTRACT

The purpose of the present study was to evaluate scientifically the anti-diarrhoeal effects of ethanolic (90%) extract of leaves of *Morinda citrifolia.* Linn (EEMC) was studied against castor oil-induced-diarrhoea model in rats. Antidiarrhoeal activity of 90% ethanol extract of *Morinda citrifolia* 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 reductions in fecal output and frequency of droppings whereas EEMC 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. EEMC at the doses of 200 and 400 mg/kg significantly inhibited ($P<0.001$) the castor oil induced charcoal meal transit. The EEMC 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 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 *Morinda citrifolia.* L.

Keywords: Antidiarrhoeal Activity, *Morinda citrifolia.* L, Traditional medicine, Castor Oil- induced diarrhoea, Enteropooling Method, Small intestinal transit

INTRODUCTION

Morinda citrifolia L. belonging to family Rubiaceae is Common along the coast on beaches, in beach thickets, rocky shores, roadsides, creeks, and wet areas and is widely distributed throughout the South Pacific. The chief chemical constituents [1-4] include Alizarin, morindone, morindin, rubiadin, anthraquinones and their glycosides, flavonoids, beta-sitosterol, ursolic acid, asperuloside, caproic acid, caprylic acid, hexanoic and octanoic acids. After thorough iterature survey,concluded that the plant possess Uterine muscle relaxant, analgesic, hypotensive, Antiascariasis and antibacterial activity [3-6]. It is *Traditionally used* to treat swellings, boils, ringworm, and rheumatism. Liquid pressed from young fruit is snuffed into each nostril to treat bad breath and raspy voice. It is also used in the treatment of mouth ulcers, haemorrhoids, hernia or swollen testicles, headaches, pain caused by barb of poisonous fish, removal of a splinter, childbirth, diabetes, diarrhoea and dysentery, fever, intestinal worms, filariasis, leprosy, and tuberculosis [1,7]. In Fiji, the leaves are used as a poultice for broken bones and sprains. An infusion of the root is used in treating urinary disorders and young fruits are used to treat high blood pressure. In Tonga, infusion of the bark/leaves is used to treat stomachache. The leaves are used to treat sties. In New Guinea, the root is rubbed onto centipede bites. In Micronesia, ulcerated sores on the feet are treated with remedies made from the fruit. The root is crushed and mixed with oil and is used as a smallpox salve. Polynesians apply the leaves to cuts, abscesses and inflammations. In Samoa, Tonga and Futuna, the crushed fruit is used in treating sore throat and toothache. Tahitians use the plant to treat tonsillitis,

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abdominal swellings, burns, swellings below the tongue and inflammations of fingers and toes. 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

Plant collection

The Plant material of *Morinda citrifolia* used for investigation was collected from Tirunelveli District, in the Month of August 2010. The plant was authenticated by Dr.V.Chelladurai, Research Officer Botany. C.C.R.A.S., Govt. of India. The voucher specimen (CHE-Sk-MC-09) of the plant was deposited at the college for further reference.

Preparation of extracts

The whole plant of *Morinda citrifolia 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 extract of *Morinda citrifolia L.* was found to be 16.5 % w/w.

Preliminary phytochemical screening

The phytochemical examination of ethanolic (90%) extract of whole plant of *Morinda citrifolia L* was performed by the standard methods [8].

Animals used

Albino wistar rats (150-230g) of either sex were obtained from the animal house in C.L. Baid Metha College of Pharmacy, Chennai. 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) of CPCSEA (Ref No. IAEC / XIII / 01 / CLBMCP / 2010 - 2011).

Castor oil-induced diarrhoea

Diarrhoea was induced by Nwodo and Alumanah (1991) and Nwafor *et al.*, (2005) [9,10]. 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 EEMC (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.

Castor oil-induced enteropooling

Intraluminal fluid accumulation was determined by the method of Robert *et al.,* (1976) and DiCarlo *et al.,* (1994) [11, 12]. 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 EEMC 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 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 p.o, group III received atropine (3 mg/kg, i.p.), group IV and V received EEMC 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 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 [13].

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 *Morinda citrifolia L.* revealed that presence of alkaloids, flavonoids, triterpinoids, tannins, gums and mucilage and absence of saponins and steroids.

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) (60.39%). A similar marked reduction in the number of defecations over four hours was

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achieved with *G.speciosa* at the doses of 200 or 400 mg/kg p.o. EEMC 200 and 400 significantly inhibited the defecation (42.32% and 51.86%) EEMC 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 intestinal loop. Castor oil-induced enteropooling is not influenced by diphenoxylate (5 ml/kg p.o) in rats. EEMC 200 and 400 produced a dose-dependent reduction in intestinal weight and volume. EEMC 200 and 400 mg/kg, p.o dose produced 35.93% and 54.77% 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 (89.62%), but it was reduced in both doses of extract, and much more markedly by atropine (38.25%). EEMC 200 mg/kg, p.o dose of extract produced 63.56% intestinal transit induced by castor oil respectively. Whereas, EEMC 400 mg/kg, p.o dose produced 50.05% of castor oil induced charcoal meal transit (Table 3 and Figure 3).

Table 1: Effect of *EEMC* 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)	23.13±1.47	---
II	Castor oil (2ml p.o) + diphenoxylate (5 ml/kg p.o)	9.16±0.12**	60.39
III	Castor oil (2ml p.o) + EEMC (200mg/kg p.o)	13.34±0.91*	42.32
IV	Castor oil (2ml p.o) +EEMC (400mg/kg p.o)	10.83±0.52**	53.17

Effect of EEMC on castor oil-induced diarrhoea in rats: EEMC 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 *EEMC* 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)	2.95±0.12	---
II	Castor oil (2ml p.o) +diphenoxylate (5 ml/kg p.o)	1.04±0.18**	64.74
III	Castor oil (2ml p.o) +EEMC (200mg/kg p.o)	1.89±0.05*	35.93
IV	Castor oil (2ml p.o) +EEMC (400mg/kg p.o)	1.42±0.12**	51.86

Effect of EEMC on castor oil-induced enteropooling in rats: EEMC 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 EEMC 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)	89.45 ± 1.29	47.58 ± 1.76	53.91
II	Castor oil (2ml p.o) + saline (2ml/kg i.p)	82.41 ± 2.62	74.31 ± 1.21	89.62
III	Castor oil (2ml p.o) +atropine (3mg/kg i.p)	98.24 ± 2.24	37.58 ± 1.36**	38.25
IV	Castor oil (2ml p.o) +EEMC (200mg/kg i.p)	85.78 ± 1.62	54.53 ± 1.78*	63.56
V	Castor oil (1ml p.o) +EEMC (400mg/kg i.p)	88.14 ± 1.21	44.12 ± 1.33**	50.05

Effect of EEMC on castor oil-induced small intestinal transit in rats: EEMC 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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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 *Morinda citrifolia* 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 EEMC 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 [14]. 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 [15], inhibition of intestinal Na^+,K^+-ATPase activity to reduce normal fluid absorption [16, 17], activation of adenylyl cyclase [14], stimulation of prostaglandin formation [17], platelet-activating factor and recently nitric oxide was contribute to the diarrhoeal effect of castor oil [18-19]. 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]. EEMC may act an above any one of the mechanism.

It is also noted that EEMC significantly inhibited castor oil induced intestinal fluid accumulation and the volume of intestinal content (Table 2). 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 [21]. The involvement of muscarinic receptor effect was confirmed by increased production of both gastric secretion and intraluminal fluid accumulation induced by castor oil. The EEMC 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 *Morinda citrifolia* produced relief in diarrhoea by spasmolytic activity in vivo and anti-enteropooling effects. [11].

The EEMC significantly reduced the castor oil induced intestinal transit as compared with control group (Table 3). In this study, atropine increased intestinal transit time possibly due to its anti-cholinergic effect [21]. 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 [22] by prevents the reabsorption of NaCl and water [17]. Probably EEMC increased the reabsorption of NaCl and water by decreasing intestinal motility as observed by the decrease in intestinal transit by charcoal meal.

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 [23]. The phytochemical analysis of EEMC revealed the presence of alkaloids, flavonoids, triterpinoids tannins, gums and mucilage. These constituents may mediate the anitdiarrhoeal property of the EEMC.

In conclusion, the present study has shown that *Morinda citrifolia* 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 *Morinda citrifolia* and phytochemical studies are also in progress to isolate and characterize the active constituents of *Morinda citrifolia 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.

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