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Antidiarrhoeal Activity of *Terminalia catappa* Linn. Leaf Extracts in Rats

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The methanol, water and petroleum ether extracts of *Terminalia catappa* Linn were screened for their antidiarrhoeal potential at dose level of 1/5th of their lethal doses screened against several experimental models of diarrhoea in rats. The methanol and aqueous extracts showed significant inhibitory activity against castor oil induced diarrhea and inhibited PGE_2 induced enteropooling in rats. The methanol and aqueous extracts also showed significant reduction in gastrointestinal motility following charcoal meal in rats.

Key Words: Antidiarrhoeal Activity, *Terminalia catappa*, Methanol, Aqueous extracts, PGE_2, Castor oil.

INTRODUCTION

Diarrhoea has long been recognized as one of the most important health problems in the developing countries[1]. Worldwide distribution of diarrhoea accounts for more than 5–8 million deaths each year in infants and small children less than 5 years. According to WHO estimation for the year 1998, there were about 7.1 million deaths due to diarrhoea[2]. Secretory diarrhoea is the most dangerous symptom of gastrointestinal problems[3] and is associated with excessive defecation and stool outputs, the stools being of abnormally loose consistency[4].

Terminalia catappa linn (Combretacae) is found throughout the warmer parts of India and called as Indian almond, Malabar almond and Tropical almond. It is a medium sized tree with leaves clustered towards the ends of the branches. The various extracts of leaves and bark of the plant have been reported to be anticancer

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and antioxidant[5], anti-HIV reverse transcriptase[6], hepatoprotective and anti-inflammatory[7], anti-hepatitis[8] and aphrodisiac[9]. The phytochemicals of this plant include tannins (punicalagin, punicalin, terflavins A and B, tergallagin, tercatain, chebulagic acid, geranin, granatin B, corilagin)[10], flavanoids (isovitexin, vitexin, isoorientin, rutin)[11] and triterpinoids (ursolic acid, 2α,3β,23-trihydroxyurs-12-en-28-oic acid)[12].

However, there is no report is available on the traditional use of *Terminalia catappa* Linn extracts in the treatment of diarrhoea. Hence, the present study was undertaken to evaluate its potential anti-diarrhoeal efficacy in different experimental models of diarrhoea in albino rats.

EXPERIMENTAL

Fresh tender leaves of *Terminalia catappa* were collected in the month of October from the herbal garden of Rural College of Pharmacy. The plant was identified by the botanist of Rural College of Pharmacy, Devanahalli. The voucher specimen (TCL-2) was kept in our laboratory for future reference.

Preparation of extracts

The leaves were shade-dried at room temperature. The dried leaves were subjected to size reduction to coarse powder by using dry grinder and passed through sieve No. 40. The powder was packed into soxhlet apparatus and extracted successively with petroleum ether (60–80°), methanol and distilled water (yield 2.1 ± 0.2, 8.1 ± 0.56 and 8.8 ± 0.7) respectively. All the extracts were dried at 45°C in a hot air oven till solid to semisolid mass was obtained and were stored in air-tight containers in a refrigerator below 10°C. The suspensions of methanol and petroleum ether extracts were prepared by using 0.5% Tween-80 (SD Fine Chemicals, Mumbai, India) in normal saline and the solution of aqueous extract was prepared by using normal saline as solvent for the experiment.

Animals used

Wister albino rats (150–200 g) and Wistar albino mice (20–25 g) were procured from Indian Institute of Sciences, Bangalore, India. Before and during the experiment the rats were fed with standard diet (Gold Mohr, Lipton India Ltd.). After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity and dark/light cycle. Animals described as fasting were deprived of food and water for 16 h *ad libitum*. All the experiments were carried out under the guidance of Ethical Committee of Rural College of Pharmacy (Registration No. 129/99/CPCSEA).

Toxicological studies

Preliminary oral LD_{50} doses of petroleum ether, methanol and aqueous extracts of *Terminalia catappa* in mice were found to be 345, 205 and 220 mg/kg respectively.

Castor oil induced diarrhoea

Rats were divided into five groups ($n = 6$) and fasted for 18 h and water provided *ad libitum*. Group A received 10 mg/kg 0.5% v/v aqueous Tween-80 and served as negative control. Group B received standard lopermide (3 mg/kg p.o.) as positive control. Groups C, D and E were treated with leaf extracts of *Terminalia catappa* at 1/5th of LD_{50} doses of the petroleum ether extract (69 mg/kg, p.o.), methanol extract (41 mg/kg, p.o.) and aqueous extract (44 mg/kg, p.o.), respectively. After 1 h of treatment, all the animals were challenged with 1 mL of castor oil orally and observed for consistency of fecal material[13]. The frequency of defecation was noted in transparent plastic dishes placed beneath the individual rat cages up to 4 h[14].

Gastrointestinal motility

Rats were divided into five groups ($n = 6$) and fasted for 18 h before the experiment. Each rat was orally administered with 1 mL of charcoal meal (5% deactivated charcoal in 10% aqueous tragacanth). Group A treated with 0.5% v/v aqueous Tween-80 (10 mL/kg p.o.) and served as negative control. Group B received atropine (0.1 mg/kg, i.p.) served as positive control. Groups C, D and E were treated with petroleum ether extract (69 mg/kg p.o.), methanol extract (41 mg/kg, p.o.) and aqueous extract (44 mg/kg, p.o.), respectively. Thirty minutes later, each animal was killed and the intestinal distance moved by the charcoal meal from the pylorus to caecum was measured and expressed as percentage of distance moved[15].

PGE_2 induced enteropooling

In this method the rats were deprived of food and water for 18 h and placed in five cages with six animals per cage. The first three groups (A, B and C) were treated with petroleum ether extract (69 mg/kg, p.o.), methanol extract (41 mg/kg, p.o.) and AE (44 mg/kg, p.o.), respectively. The fourth group D was then treated with 1 mL of 5% v/v ethanol in normal saline (i.p.) and then it was treated with 0.5% Tween-80 suspension, which served as negative control. Immediately after extract administration, PGE_2 (Astra Zeneca, India) was administered orally to each rat (100 µg/kg) in the first four groups. The fifth group E was treated with PGE_2 (100 µg/kg) as well as 0.5% Tween-80 suspension and served as the PGE_2 control group. After 30 min following administration of PGE_2, each rat was sacrificed and the whole length of intestine from the pylorus to caecum was dissected out, its content collected in a test tube and volume measured[15].

Statistical Analysis

All the data were analyzed statistically using one-way analysis of variance followed by Dunnett's t-test. The data are expressed as mean ± S.E.M. P-values less than 0.05 imply significance.

RESULTS AND DISCUSSION

Castor oil induced diarrhoea

Administration of castor oil produced characteristic semi-solid diarrhoea dropping in 18 h starved rats of the control group during 4 h observation period

(Table-1). The methanol and aqueous extract showed significant ($P < 0.01$) reduction in the number of defecations over 4 h when compared to that of untreated castor oil rats, the activity similar to that of lopermide (3 mg/kg), standard antidiarrhoeal agent. Both aqueous extract and methanol extract delayed the onset of diarrhoea and 100 and 80% of rats were protected against castor oil induced diarrhoea at 4 h, respectively. The petroleum ether extract did not show any significant activity.

TABLE-1
EFFECT OF PETROLEUM ETHER (PE), METHANOL (ME) AND AQUEOUS (AE) LEAF EXTRACTS OF *TERMINALIA CATAPPA* LINN ON CASTOR OIL-INDUCED DIARRHOEA IN RATS

Oral pretreatment at 0 h* castor oil 1 mL p.o., at 1 h	Mean number of wet faecus in 4 h
Control (10 mL/kg)	4.7 ± 0.75
Standard (Lopermide 3 mg/kg)	0.00†
PE (69 mg/kg)	3.21 ± 0.16
ME (41 mg/kg)	1.14 ± 0.54†
AE (44 mg/kg)	1.15 ± 0.56†

The test drug, lopermide and vehicle were given p.o.
Results are expressed in mean ± S.E.M., n = 6.
*P < 0.05, †P < 0.01 when compared to control.

Gastrointestinal motility

The methanol and aqueous extract significantly decreased ($P < 0.001$) propulsion of the charcoal meal through the gastrointestinal tract and not by the petroleum ether extract as compared with the control group (0.5% Tween-80). A similar reduction of gastrointestinal transit of charcoal meal in rat was achieved with intraperitoneal injection of atropine sulfate (0.1 mg/kg) (Table-2).

TABLE-2
EFFECT OF PETROLEUM ETHER (PE), METHANOL (ME) AND AQUEOUS (AE) LEAF EXTRACTS OF *TERMINALIA CATAPPA* LINN ON GASTROINTESTINAL TRANSIT IN RATS

Charcoal meal followed by the test drug p.o.*	% Movement of charcoal meal
Control (10 mL/kg)	85.32 ± 1.21
Standard drug (Atropine 0.1 mg/kg)	33.12 ± 0.67*
PE (69 mg/kg)	79.16 ± 1.89
ME (41 mg/kg)	41.16 ± 1.25*
AE (44 mg/kg)	51.23 ± 1.23*

The test drug and vehicle were given p.o. and Atropine was given i.p.
Results are expressed as mean ± S.E.M., n = 6.
*P < 0.001 when compared to control.

PGE_2-Induced enteropooling

The methanol and aqueous extract were inhibited PGE_2 enteropooling in rats (Table-3). This effect did not show in petroleum ether extract. PGE_2 induced a significant increase in the fluid volume of the rat's intestine when compared with animals who received ethanol in normal saline.

TABLE-3
ANTI-ENTEROPOOLING EFFECT OF PETROLEUM ETHER (PE), METHANOL (ME) AND AQUEOUS (AE) LEAF EXTRACTS OF *TERMINALIA CATAPPA* LINN IN RATS

The test drug * followed by PGE_2, p.o.	Volume of intestinal fluid (mL)	P-values
Ethanol in saline	0.83 ± 0.06	—
PGE_2 in ethanol	3.07 ± 0.21	0.001[a]
PE (69 mg/kg)	2.98 ± 0.12	0.001[a]
ME (41 mg/kg)	1.82 ± 0.13	0.01[b]
AE (44 mg/kg)	2.01 ± 0.16	0.01[b]

The test drug and vehicle were given p.o.
Results are expressed as mean ± S.E.M., n = 6.
[a]With respect to ethanol in saline treatment.
[b]With respect to PGE_2 treatment.

Conclusion

In developing countries, a quarter of infants and childhood mortality is related to diarrhoea[16]. The highest mortality rates have been reported to be in children less than five years of age. During the past decade oral dehydration therapy has reduced mortality from acute diarrhoeal disease, whereas chronic diarrhoea remains a life-threatening problem in those regions in which malnutrion is a common coexisting and complication factor. A number of factors such as infective, immunological and nutritional have been involved in the perpetuation of the diarrhoeal syndrome[17]. Many plants available in India are used in traditional folk medicine for the treatment of diarrhoea and dysentery. Of the indigenous plants used, *Andrographis paniculata*, *Asparagus racemosus*, *Butea monosperma*, *Cassia auriculata* and others are mentioned[18]. Several studies have shown that prior administration of plant extracts has a protective effect on the intestinal tract[19-21]. In the present study, the petroleum ether, methanol and aqueous extract of *Terminalia catappa* that have not been studied so far, were evaluated for their antidiarrhoeal potential against castor oil induced diarrhoea, gastrointestinal motility in charcoal meal test and PGE_2 induced enteropooling in albino rats.

The methanol and aqueous extract of *Terminalia catappa* Linn exhibited significant antidiarrhoeal activity against castor oil induced diarrhoea in rats. The extracts had a similar activity as lopermide.

It is widely known that castor oil or its active component recinoleic acid induced permeability changes in mucosal fluid and electrolyte transport that results in a hypersecretory response and diarrhoea[22, 23]. The experimental studies

in rats demonstrated a significant increase in the portal venous PGE_2 concentration following oral administration of castor oil[24]. Recinoleic acid markedly increased the PGE_2 content in the gut lumen and also caused on increase of the net secretion of water and electrolytes in the small intestine[25]. The liberation of recinoleic acid from castor oil results in irritatian and inflammation of the intestinal mucosa, leading to release of prostaglandins biosynthesis delayed castor oil induced diarrhoea.

The methanol and aqueous extract appear to act on all parts of intestine. Thus, it reduces the intestinal propulsive movement of charcoal meal treated model. The AE and ME showed similar to that of atropine. The previous study shows that activated charcoal avidly absorbs drugs and chemicals on the surface of the charcoal particles thereby preventing absorption[26]. Thus, gastrointestinal motility test with activated charcoal was carried out to find out the effect of extracts of *Terminalia catappa* on peristalsis movement. The results also show that the methanol extract and aqueous extract suppressed propulsion of charcoal meal thereby increased the absorption of water and electrolytes.

The methanol and aqueous extract also significantly inhibited the PGE_2 induced intestinal fluid accumulation (enteropooling). It has been shown that E type of prostaglandins causes diarrhoea in experimental models as well as human beings[27]. Their mechanism has been associated with dual effects on gastrointestinal motility as well as on water and electrolyte transport[28]. PGE_2 also inhibits absorption of glucose, a major stimulus to intestinal absorption of water and electrolytes[29]. These observations tend to suggest that methanol and aqueous extract reduced diarrhoea by inhibiting PGE_2 induced intestinal accumulation of fluid.

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