

Evaluation of Anti-diarrhea activity of *Rhizophora mucronata* bark extracts

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Abstract

Rhizophora mucronata is seen along the tropical coastal line is associated with many medicinal activities. The purpose of present study is to investigate the anti-diarrhea activity of *Rhizophora mucronata* bark extracts as traditional used by people of India. The chloroform, ethyl acetate methanol and water extracts were subjected for antidiarrhea activity in castor oil induced and gastrointestinal motility test in albino mice and effect on spasmogen induced contractility in isolated guinea pig ileum. The phytochemical analysis was carried. The bark extracts showed inhibition of castor oil induced diarrhea and very significant percentage inhibition of charcoal meal in mice. The bark extracts did not inhibit the acetylcholine and histamine induced contraction of isolated guinea pig ileum up to 10 mg/ml concentrations. The study supports the anti diarrhea activity of *Rhizophora mucronata* bark, as evident from the castor oil induced and gastrointestinal motility and indicating a different mechanism of action.

INTRODUCTION

Diarrhea is characterized by frequent, watery bowel movements, often accompanied by stomach cramps, abdominal pain and gas. Diarrhea may be of various colors and contain mucous, blood or pus. According to World Health Organization, Diarrhea occurs worldwide and causes 4 % of all deaths and 5 % of health loss due to disability. It is most commonly caused by gastrointestinal infections, which kill around 2.2 million people globally each year. It is most commonly caused by gastrointestinal infections, which kill around 2.2 million people globally each year, mostly children in developing countries [1]. Enteric pathogens in Thailand have developed resistance to virtually all antibiotics routinely used in the treatment of diarrhea, as well as the newer fluoroquinolone and macrolide classes of drugs [2]. An urge to develop new and better drugs is always emerging. Nature has provided infinite folk remedies for various ailments. In both China and India herbs like *Chebula*, *Swertia chiraita* and Black pepper have found place in ayurvedic and Chinese practice to treat diarrhea [3]. Various traditional medicinal herbs have scientifically evaluated for antidiarrhea activity [4,5,6]. *Rhizophora mucronata* commonly known as Asiatic mangrove, widely distributed along the coastal tropical and subtropical region has been reported to possess several medicinal properties. In

countries like Burma, India and China bark of *Rhizophora mucronata* has been used as traditional medicine in the treatment of diarrhea, dysentery, blood in urine and, fever [7]. The present investigation is to scientifically evaluate the anti diarrhea activity of the various extracts of *Rhizophora mucronata* bark in experimental animal model.

EXPERIMENTAL PLANT MATERIAL

Rhizophora mucronata bark was collected in the month of November from the coastal area of Kundupura, Karnataka, India. Bark was authenticated from the Department of Botany, Bangalore University, India. A voucher specimen is deposited for further reference.

CHEMICALS

Atropine used as standard antidiarrhoeal drug, Castor oil (laxative agent), activated charcoal, Gum Acacia, acetylcholine and histamine were of pharmacological grade. All other reagents and solvents were of analytical grade and obtained from Merck.

ANIMALS

The protocol for screening the antidiarrhea activity in animals was cleared by the ethical committee of Krupanidhi college of Pharmacy, Bangalore, India. Albino mice 24-28 g

and Wistar rats 180-240 g and guinea pigs were obtained from animal house of Krupanidhi College of Pharmacy. The animals were housed under standard laboratory environmental condition for acclimatization for a period of 14 days prior to perform the experiments.

PREPARATION OF PLANT MATERIAL AND PHYTOCHEMICAL ANALYSIS

Rhizophora mucronata bark was dried under shade and was pulverized to a fine coarse powder. A weighed quantity of the powder bark 500 g was subjected for successive solvent extraction using petroleum ether (40-60°C), followed by chloroform (RMCE), ethyl acetate (RMEE), methanol (RMME), by using soxhlet apparatus and aqueous extract (RMWE) by boiling marc with water. Each extract was concentrated under vacuum and dried to a constant weight. The successive solvent extracts were tested for their chemical constituents by their color reaction.

CASTOR OIL INDUCED DIARRHEA

Wistar rats were divided into 10 groups (n = 6) and, fasted for 18 h and water was provided ad libitum prior to the experiment. A dose of 250 mg/kg and 500 mg/kg was selected for all the solvent extract. Group I served as control, to which tween 80 (5 %) 3 ml/kg was administered orally; group II received atropine 3 mg/kg p. o, which was taken as positive control, animals of groups III- X, received orally 250 and 500 mg/kg of chloroform, ethyl acetate, methanol extract and aqueous extract respectively. After 1 h, all the animals were orally administered castor oil one ml/kg and were observed for onset of defecation, number of wet stools and number of solid stools and total number of stools for period of 4 h [8].

GASTROINTESTINAL MOTILITY TEST

Albino mice of either sex were divided into 10 groups (n=6) and were fasted for 18 h and water was given ad libitum prior to the experiment. Group I animals served as control and was treated orally with tween 80 (5%) one ml/kg and group II were treated with atropine 3 mg/kg, a positive control. The other groups III-X of animals received orally 250 mg/kg and 500 mg/kg of chloroform, ethyl acetate, methanol extract and aqueous extract respectively. After 1 h, each animal was administered orally with charcoal meal 0.25 ml (10% charcoal in 5% gum acacia). Twenty minutes later, the animals were sacrificed. Total small intestine from pylorus to caecum was isolated and the total length and the length traveled by the charcoal meal were measured [9].

EFFECT ON SPASMOGEN INDUCED CONTRACTILITY IN ISOLATED GUINEA PIG ILEUM

The effect of the extract on histamine- and acetylcholine-induced muscle contraction of guinea pig ileum was investigated. About 10-20 mm long strips were taken from a portion 10-30 cm proximal to the ileocecal junction of the ileum. The contents of the intestine were washed off using Tyrode solution and the mesenteric residue eliminated. 20 ml jacketed organ bath containing Tyrode solution at 37 °C continuously bubbled with air was used to record muscle contraction. After the initial equilibration period of about 30-60 min, concentration curves were recorded for acetylcholine (Ach), histamine (Hist) and all the solvents extracts. Closing response that gave 75% response to the agonists was then used as the dose for interaction with the extract. Different preparation was used for each agonist and each experiment was repeated at least four times [10].

RESULTS

CHEMICAL ANALYSIS

The percentage yields of successive solvent extracts of *Rhizophora mucronata* bark are Petroleum ether -0.25 %, chloroform -0.5 %, ethyl acetate 1%, methanol- 2.8 % and aqueous 1%. The Phytochemical constituents present in the successive solvent extracts, pet ether (40-60°C), chloroform, ethyl acetate, methanol, and aqueous extracts *Rhizophora mucronata* are presented in Table 1. The yield of pet ether extract was negligible hence; the extract was not subjected for anti-diarrhea study.

Figure 1

Table 1: Phytochemical analysis of *Rhizophora* bark extract Present +, absent

Chemical constituents	Pet ether (40-60 °C)	Chloroform	Ethyl acetate	Methanol	Aqueous
Alkaloids	-	-	-	-	-
Carbohydrates	-	-	-	-	+
Glycosides	-	+	+	+	+
Flavonoids	-	-	+	-	-
Sterols	-	+	+	+	+
Triterpenoid	+	+	+	+	-
Saponins	-	-	-	+	-
Phenol and	-	-	+	+	+
Fats and oils	+	-	-	-	-
Proteins	-	-	-	-	+

STATISTICAL ANALYSIS

The data was 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

CASTOR OIL INDUCED DIARRHEA

A significant delay was seen in the onset of semi- solid defecation after administration of chloroform and methanol extracts (500 mg/kg) in comparison to control (P<0.05). A dose dependent significant inhibition in the frequency of defecations was observed with chloroform and methanol extract (P<0.01) and a statistically significant inhibition in frequency of defecation were noted in the ethyl acetate extract treated animals (P<0.05) with respect to control. The aqueous extract did not show any anti-diarrhea effect (Table 2).

Figure 2

Table 2: Effects of *Rhizophora* bark extracts in castor oil induced diarrhea in rats n=6

Treatment n=6	Mean Onset of time	Mean No of wet feces	Mean No defecation
Control tween 80 (5%) 3ml/kg	36.00 ± 12.360	4.00 ± 0.8306	3.20 ± 0.9326
Atropine 3 mg/kg + co	152.00 ± 42.180*	0.0000**	0.52 ± 0.2836**
RMCE 250 mg/kg +co	56.33 ± 15.75	1.5 ± 0.178*	1.33 ± 0.42*
RMCE 500mg/kg +co	147.50 ± 41.508*	0.50 ± 0.2236**	0.66 ± 0.1637**
RMEE 250 mg/kg +co	15.5 ± 6.94	2.33 ± 0.84	5.16 ± 0.22
RMEE 500 mg/kg + co	130.00 ± 36.492	1.33 ± 0.494**	1.05 ± 3.1031*
RMME 250 mg/kg +co	31.33 ± 3.70	1.33 ± 0.33**	1.5 ± 0.22*
RMME 500 mg/kg + 500 mg/kg + co	144.16 ± 31.129*	0.83 ± 0.3073**	0.56 ± 0.120**
RMWE 250 mg/kg + co	26.83 ± 0.79	5.83 ± 0.40	4.83 ± 0.307
RMWE 500 mg/kg+ co	42.00 ± 4.233	3.00 ± 0.7.303	2.10 ± 0.4282

Values are expressed as Mean ± SEM, *P<0.05 significant, **P<0.01 very significance Castor oil=co

GASTROINTESTINAL MOTILITY

A very significant decrease (P<0.01) in the propulsion of charcoal meal through the gastro intestinal tract was exhibited in experimental animals, which was dose dependent at 250 and 500 mg/kg of the RMCE, and RMME with respect to control, whereas the RMEE showed activity

at 500 mg/kg, but the aqueous extract (RMWE) showed no such activity. Table 3 depicts the results of gastrointestinal motility from *Rhizophora mucronata* bark extracts.

Figure 3

Table 3: Effect of *Rhizophora* bark extracts in inhibition of transit of charcoal meal.

Treatment (n=6)	Total length of intestine	Distance traveled (Cms)	% Transit of charcoal meal
Control tween 80 (5 %) 1 ml/kg)	36.53 ± 1.179	22.312 ± 1.146	65.44 ± 0.6411
Atropine 3 mg/kg	35.34 ± 0.167	11.230 ± 0.122**	33.49 ± 1.0090**
RMCE 250 mg/kg	37.32 ± 0.02	17.621 ± 0.23*	38.41 ± 0.1021*
RMCE 500 mg/kg	38.58 ± 0.874	13.833 ± 0.2108**	36.49 ± 3.9140**
RMEE 250 mg/kg	34.29 ± 0.67	21.013 ± 0.23	63.47 ± 0.80
RMEE 500 mg/kg	34.83 ± 1.195	13.033 ± 1.470**	36.93 ± 3.9140**
RMME 250 mg/kg	36.25 ± 0.99	16.210 ± 0.302*	37.75 ± 0.22*
RMME 500 mg/kg	33.83 ± 1.108	11.233 ± 0.4333**	33.098 ± 0.7274**
RMWE 250 mg/kg	34.20 ± .011	19.85 ± 0.59	57.96 ± 0.36
RMWE 500 mg/kg	35.83 ± 1.166	21.33 ± 0.1851	43.065 ± 2.774

Values expressed as Mean ± SEM, **P<0.01 very significant, *P<0.05 significant

EFFECT ON SPASMOGEN INDUCED CONTRACTILITY-ISOLATED GUINEA PIG ILEUM:

All the extracts, chloroform, ethyl acetate methanol and aqueous of *Rhizophora mucronata* did not inhibit acetylcholine or the histamine receptor of the isolated guinea pig ileum up to 10 mg/ml concentrations.

DISCUSSION

The cause for diarrhea being, infection, inflammation, immunological and nutritional and is characterized by excessive secretion of electrolytes and water into the intestinal lumen, exudation of protein and fluid from the mucosa, and altered intestinal motility, resulting in rapid transit time and an increase in wet faeces [11]. In most instances, multiple processes are simultaneously affected involving several factors, a particular factor becoming a dominant player in a given environment; however, motility and /or secretory disturbances usually remain a common dominant in most cases [12].

Castor oil-induced induces diarrhea by increasing peristaltic activity and alters the permeability of the intestinal mucosa to water and electrolytes [13]. The liberation of ricinoleic acid

from castor oil results in irritation and inflammation of prostaglandin's, which stimulates motility and secretion [14,15]. The involvement of nitric oxide from neurons in the diarrhea induced by the castor oil has also been proposed [16]. Autocoids and prostaglandin's are involved and implicated in the induction of diarrhea [17,18]; Castor oil increases the induction of prostaglandins [19], causes changes in the permeability and mucosal injuries and stimulates PAF biosynthesis which may result in inflammation of intestinal mucosa [20]. Therefore the use of castor oil induced diarrhea model in the present study is logical, since the chloroform, ethyl acetate, methanol and aqueous extract of *Rhizophora mucronata* bark demonstrated the dose dependent manner inhibition of castor oil –induced diarrhea. It can be assumed that anti diarrhea action was mediated by decrease in peristaltic effect and increase in water absorption and electrolytes. *Rhizophora mucronata* bark might possess some compounds with antisecretory properties which may account for its efficacy against diarrhea induced by castor oil in mice. The inhibition of autocoids and prostaglandin which are responsible for increase in stimulation of motility and secretion can also be accounted in the present study; by the fact that the extracts have shown significant anti inflammation activity in dose dependent manner in various anti inflammatory animal model.

The effect on gut motility is determined by measuring the ability of an active drug to block the contraction evoked by agonist (acetyl choline, histamine and nicotine) [13]. The inhibition in percentage of transit of the marker, charcoal meal through the gastrointestinal tract of the albino mice was significantly seen in dose dependent manner by the chloroform, ethyl acetate, methanol and aqueous extract of *Rhizophora mucronata*. However, the extract failed to inhibit the contraction produced by the acetylcholine and histamine agonist in the isolated spasmogen induced contractility guinea pig ileum model experiment.

The anti diarrhea activity of the extract may also be due to the presence of denature of proteins forming protein tannates, which makes the intestinal mucosa more resistant and reduce secretion [21]. The tannins present in the ethyl acetate and methanol extract may responsible for the observed effects in our study.

CONCLUSION

The anti diarrhea effect of *Rhizophora mucronata* bark extract is evident from the experimental model like- castor oil induced diarrhea, significant decrease in the number of

wet faeces; gastrointestinal motility- the remarkably decrease in the propulsive movement of the gastrointestinal contents. On the isolated guinea pig ileum, the extract did not appreciably affect acetylcholine and histamine induced contractions.

The investigation concludes that folk fore use of *Rhizophora mucronata* for treating diarrhea. The study suggest the anti diarrhea activity is probably due to inhibition of prostaglandin's and/ or decrease in the peristaltic activity and alteration of intestinal permeability. Reports of plant extract containing saponins, reducing sugars and sterols and/or terpenes [22], tannin [23], flavonoids [24], have shown anti diarrhea activity. The possibility that number of phytochemicals including triterpenoids, flavonoids, sterols, tannins and phenols present in the chloroform, ethyl acetate, methanol extract of *Rhizophora mucronata* bark acts synergistically to produce the therapeutic effect. Further studies with purified constituents are needed to completely understand the mechanism of anti-diarrheal action of *Rhizophora mucronata* brack extracts.

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