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ANTINOCICEPTIVE AND ANTIDIARRHEAL PROPERTIES OF THE ETHANOLIC EXTRACT OF *MANILKARA ZAPOTA* (LINN.) BARK

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ABSTRACT

Aim of the study: The present study was designed to investigate the analgesic and antidiarrhoeal activities of the ethanolic extract from the bark of *Manilkara zapota* (Linn.).

Material and methods: The analgesic activity was evaluated in acetic acid induced writhing model and the antidiarrhoeal activity was evaluated in castor oil induced diarrhoea model in white albino mice (Swiss-webstar strain).

Resuts: Phytochemical analysis of the ethanolic extract of the bark of *M. zapota* indicates the presence of reducing sugar, tannins, steroid, saponins & gum of compounds. The ethanolic extract of *M. zapota* has effect on acetic acid induced writhing in mice. At the dose of 250 mg/kg & 500 mg/kg of body weight, the extract produced 38.55% & 56.42 %writhing inhibition in test animals respectively. The results are statistically significant (P <0.001) and is comparable to the standard drug Diclofenac Na, which showed 72.07% at a dose of 25 mg/kg weight. The extract has also effect on castor oil induced diarrheal method in mice. The result show that extract inhibited the mean number of defecation which were 29.31% (P<0.01) and 41.37% (P<0.001) at the doses of 250 mg/kg and 500 mg/kg respectively. The latent period for the extract treated group (p<0.001 & P<0.01) increase respectively as compared to control group.

Conclusion: The present study tends to suggest the antinociceptive and antidiarrheal activities of the crude ethanolic extract of the bark of *M. zapota* and justify its use in folkloric remedies.

INTRODUCTION: *Manilkara zapota* (L.) (family: Sapotaceae) commonly known as Common names (Creole); sapoti, (English); chickle gum, chicle tree, common naseberry, naseberry, sapodilla, French); sapotille, sapotilleir, sapotillier commun, Sapodilla, (Hindi); chiku,, (Khmer); lomut,(Lao (Sino-Tibetan); lamud, (Malay); chiku, ciku, (Portuguese); sapota, sapoti, (Spanish); chicozapote, níspero, (Thai); has been collected from Karamjal, Sundarban, Bangladesh. *M. zapota* is a species of the lowland rainforest.

Trees grow well in a wide range of climatic conditions from wet tropics to dry cool subtropical areas. But they prefer a moist hot climate. It is an evergreen, glabrous tree, 8-15 m in height. It is cultivated throughout India, though it is native to Mexico and Central America [1]. The fruit of the *M. zapota* contains cyanogenic glycoside, phenolic compound and terpenoid [2].

The seeds are aperients, diuretic tonic and febrifuge. Bark is antibiotic, astringent and febrifuge. Chicle from bark is used in dental surgery. Fruits are edible, sweet with rich fine flavour. Bark is used as tonic and the decoction is given in diarrhoea and peludism [1]. The leaves are used to treat cough, cold, and diarrhoea [3]. Bark is used to treat diarrhoea and dysentery [2]. The leaves of the plant posses antioxidant [4] & antimicrobial activity [5]. The bark of the *M. zapota* is also traditionally used for the treatment of gastrointestinal disorder, fever and pain [6].

Pain is the most important symptom that brings the patient to physician. Analgesics relieve pain as a symptom, without affecting its cause [7]. Diarrhoeal disease is a leading cause of mortality and morbidity, especially in children in developing countries [8]. A vast majority of the people of developing countries relies on herbal drugs for the management of diarrhoea. Considering this fact the World Health Organization has constituted a diarrhoeal disease control programme, which includes studies of traditional medicinal practices, together with the elevation of health education and prevention approaches [8].

Since no literature is currently available to substantiate antinociceptive & antidiarrheal activities from ethanolic extract of *M. zapota*, therefore the present study was designed to provide scientific evidence for its use as a traditional folk remedy by investigating the antinociceptive and antidiarrheal activities that also confirm its use as pain killer and diarrhea.

MATERIALS AND METHODS:

Collection and identification of plant materials: The barks of *M. zapota* were collected from Karamjal, Sundarban, Khulna, Bangladesh. A specimen copy was deposited to Bangladesh National Herbarium for identification & the accession number was DACB-33801.

Preparation of Ethanolic Extract: The barks of *M. zapota* were freed from any of the foreign materials. Then the barks were air-dried under shed temperature followed by drying in an electric oven at 40^0 C. The dried plant materials were then ground into powder. About 400g of powdered material was taken in a clean, flat-bottomed glass container and soaked in 1300ml of 95% ethanol. The container with its contents was sealed and kept for a period of 14 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through whatman filter paper (Bibby RE200, Sterilin Ltd., UK) which was concentrated with rotary evaporator at bath temperature not exceeding 40˚C to have gummy concentrate of extract (yield approx. 9.77%).

Test Animals & Drug: Young Swiss-albino mice either sex, 3-4 weeks of age, weighing 20 -25 g, were used for *in vivo* pharmacological screening. Mice were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR,B). They were housed in standard environmental conditions at animal house of Khulna University animal lab and fed with rodent diet and water ad libitum. All experimental protocols were in compliance with University Ethics Committee on Research in Animals as well as internationally accepted principles for laboratory animal use and care.

The standard drug Diclofenac Na and Loperamide were used for this study and purchased from Square Pharmaceuticals Ltd, Bangladesh.

Phytochemical screening: The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents, by using the following reagents and chemicals, for example, alkaloids were identified by the Dragendorff's reagent, flavonoids with the use of Mg and HCl, tannins with ferric chloride and potassium dichromate solutions, and steroids with Libermann-Burchard reagent. Reducing sugars with Benedict's reagent [9-11].

Antinociceptive activity: The antinociceptive activity of the crude ethanolic extract of *M. zapota* was studied using acetic acid induced writhing model in mice [12-13]. The animals were divided into control, positive control and test groups with five mice in each group.

The animals of test groups received test substance at the dose of 250 and 500 mg/kg body weight. Positive control group was administered with Diclofenac Na (standard drug) at the dose of 25 mg/kg body weight and vehicle control group was treated with 1% Tween 80 in water at the dose of 10ml/kg body weight. Test samples, standard drug and control vehicle were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid. After an interval of 15 min, the mice were observed writhing (constriction of abdomen, turning of trunk and extension of hind legs) for 5 min.

Antidiarrheal activity: Antidiarrheal activity was tested by using Castor oil induced diarrheal method in mice [14]. Mice were fasted for 24 h and were randomly allocated to 4 groups of 5 animals each. The animals were all screened initially by giving 0.5 mL of castor oil. Only those showing diarrhoea were selected for the final experiment.

Group I received distilled water, groups III and IV received the extract orally (250 and 500 mg/kg respectively). Group II was given Loperamide (50 mg/kg, orally) in suspension. After 60 min, each animal was given 0.5 mL of castor oil and was placed in an individual cage, the floor of which was lined with blotting paper which was changed every hour, observed for 6 h and the characteristic diarrhoeal droppings were recorded. The latent period of each mouse were also counted. Normal stool was considered as numerical value 1 and watery stool as numerical value 2. Percent inhibition of defecation in mice was calculated by using the following equation:

% inhibition = {(Mo–M)/Mo}x100; where, Mo = Mean defecation of control and M = Mean defecation of test sample.

Statistical Analysis: For analgesic & Anti-diarrheal determination, data were presented as mean ± Standard deviation (S.D). Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnet's multiple comparisons. The results obtained were compared with the control group. *p* values < 0.05 were considered to be statistically significant (*p* indicates probability).

RESULTS:

Chemical group test: Results of different chemical tests on the ethanolic extract of *M. zapota* barks showed the presence of reducing sugar, tannins, steroid, saponin & gum (**Table 1**).

TABLE 1: RESULTS OF DIFFERENT GROUP TESTS OF ETHANOLIC EXTRACT OF *M. ZAPOTA* BARKS

Phytoconstituents	Ethanol extract of *M. zapota*
Alkaloid	-
Reducing sugar	+
Tannins	+
Gums	+
Flavonoids	-
Saponin	+
Steroid	+

+: Positive result; - : Negative result

Antinociceptive activity: Table 2 showed the effect of the ethanolic extract of bark of *M. zapota* on acetic acid induced writhing in mice. At the dose of 250 mg/kg & 500 mg/kg of body weight, the extract produced 38.55% & 56.42 %writhing inhibition in test animals respectively. The results were statistically significant (P <0.001) and was comparable to the standard drug Diclofenac Na, which showed 72.07% at a dose of 25 mg/kg weight.

TABLE 2: EFFECTS OF THE ETHANOLIC EXTRACT *M. ZAPOTA* ON ACETIC ACID INDUCED WRITHING OF MICE (n=5)

Group	Treatment and Dose	Number of writhes (% Writhing)	% Writhing Inhibition
Control	1% tween 80 solution 10 ml/kg, p.o.	17.9± 0.60 (100)	---
Positive Control	Diclofenac Na 25 mg/kg, p.o.	5.0 ± 0.22 * (27.93)	72.07
Test Group- 1	Et. Extract of *M. zapota* 250 mg/kg, p.o.	11.0± 0.47 * (61.45)	38.55
Test group- 2	Et. Extract of *M. zapota* 500 mg/kg, p.o.	7.80 ± 0.60 * (43.58)	56.42

*Values are expressed as mean±SEM (Standard Error Mean); Et.: Ethanolic; *indicates P < 0.001; one-way ANOVA followed by Dunnet's test as compared to control; n = Number of mice; p.o.: per oral.*

Antidiarrheal activity: Table 3 showed the effect of the ethanolic extract of bark of *M. zapota* on Castor oil induced diarrheal method in mice. The result showed that extract decreased the mean number of defecation which were 29.31% (P<0.01) and 41.37% (P<0.001) at the doses of 250mg/kg and 500mg/kg respectively. The latent period for the extract treated group was (p<0.001& p<0.01) increased as compared to control group.

TABLE 3: ANTIDIARRHEAL ACTIVITY OF THE CRUDE EXTRACT OF *M. ZAPOTA* IN CASTOR OIL INDUCED DIARRHEAL TEST METHOD ON MICE (n=5).

Sample	Dose	Mean± SEM		% inhibition
		Latent period	Defication	
Distilled water	2ml/mice, p.o.	0.65±0.06	11.60±0.25	--
Loperamide	50mg/kg, p.o.	3.29±0.10**	5.0±0.32**	56.89
Et. Extract	250 mg/kg, p.o.	2.81±0.32**	10.2±0.37*	29.31
Manilkara zapota	500 mg/kg, p.o.	1.14±0.13*	6.8±0.37**	41.37

Values are expressed as mean±SEM (Standard Error Mean); Et.: Ethanolic; *P<0.01 ; **P< 0.001; n = Number of mice; p.o.: per oral.

DISCUSSION: Antinociceptive activity of the ethanolic extract of *M. zapota* bark was tested by acetic acid induced writhing model in mice. The peripheral analgesic effect of the plant's extract may be mediated via inhibition of cyclooxygenases and/or lipoxygenases (and other inflammatory mediators), while the central analgesic action of the extract may be mediated through inhibition of central pain receptors. This hypothesis is in consonance with those of Koster *et al*. [15] and Williamson *et al.* [16] who postulated that acetic acid-induced writhing and hot-plate test methods are useful techniques for the evaluation of peripherally- and centrally-acting analgesic drugs, respectively.

With respect to the writhing test, the research group of Deraedt *et al.,* described the quantification of prostaglandins by radioimmunoassay in the peritoneal exudates of rats, obtained after intraperitoneal injection of acetic acid [17]. These authors found high levels of prostaglandins PGE_2 and $PGF_{2\alpha}$ during the first 30 min after acetic acid injection. On the basis of the result of acetic acid induced writhing test, it can be concluded that the ethanolic extract of *M. zapota* might possess an antinociceptive activity.

Castor oil or its active component ricinoleic acid induces permeability changes in mucosal fluid and electrolyte transport that results in a hypersecretory response and diarrhoea [18-19]. 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. In some diarrheas, the secretory component predominants, while other diarrhoeas are characterized by hypermotility.

The use of castor oil induced diarrhoea model in our study is logical because the autacoids and prostaglandins are involved these have been implecated in the causation of diarrhoea in human [20-21]. The liberation of ricinolic acid from castor oil results in irritation and imflammation of the intestinal mucosa, leading to release of prostaglandins, which stimulate motility and secreation [22].

These observations tend to suggest that those extracts at a dose of 500 mg/kg reduced diarrhoea by inhibiting castor oil induced intestinal accumulation of fluid. These results are recommended for previous report on ripen fruits extract of *Rhus javanica* [23].

The antidiarrhoeal activity of tannin has been reported, flavonoids, alkaloids, saponins, reducing sugars and sterols and/or terpenes [24] containing plant extracts. The phytochemical analysis of the extract showed the presence of alkaloids, saponins, flavonoids, sterols and /or terpenes and sugars. These constituents may be responsible for the antidiarrhoeal activity of *M. zapota*.

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