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In Vivo assay of Antidiarrhoeal activity of Methanolic and Petroleum ether extracts of *Manilkara Zapota* Leaves

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Abstract:

The present study was aimed to investigate the possible antidiarrhoeal action of Methanolic extract (MEMZ) and petroleum ether (PEMZ) extracts of leaves of *Manilkara zapota* (Sapotaceae). The anti-diarrheal activity of MEMZ & PEMZ extracts was investigated by castor oil and Magnesium sulfate induced diarrhea in albino mice. The parameters of this study were number of diarrheal episodes and mean weight of stool of mice. The percentage protection in extracts treated animals showing diarrhea was compared with castor oil and Magnesium sulfate treated and loperamide treated animals. In the Castor oil induced method only the PEMZ extract, showed statistically significant ($p<0.05$). In the Magnesium Sulfate induced method both the MEMZ & PEMZ extract reduced diarrhea in mice with reduction in weight of stools but the result were not significant($p>0.05$). These results indicate that the extracts possess antidiarrhoeal activity in mice.

Keywords: *Manilkara zapota*, Antidiarrhoeal activity, castor oil induced method, Magnesium Sulfate induced method.

Introduction:

The *Manilkara zapota* is a plant of Sapotaceae family, which is abundantly found in Bangladesh. It has not been studied much for significant chemical as well as biological studies; the fruits of this plant were reported to contain polyphenolic compounds that showed antioxidant activity (Islam et al.,2012) [1]. *Manilkara zapota* is a species of the lowland rainforest.It is an evergreen tree, glabrous tree, 8-15 m in height. It is cultivated throughout India, though it is native to Mexico and Central America.(Hossain et al.,2012) [2]. The leaves of this plants are used to treat cough, cold, and diarrhoea. Futhermore,the leaves of the plant posses antioxidant & antimicrobial activity (HMYB et al 1992; Chanda and Nagani,2010; Nair and Chanda,2008) [3,4,5].

No previous study was reported on the leaves of this plant in Bangladesh, thus this study was aiming to perform effect of *Manilkara zapota* leaves extracts on laboratory animals.

Materials and Method

Collection of the plant parts and identification:

For this present investigation, the *Manilkara zapota* were collected from Botanical garden, Magura (Bangladesh) on February, 2011 and were identified at the Bangladesh National Herbarium, Mirpur, Dhaka where the Accession no-35493 were deposited. The collected plant parts were

dried for one week and ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

Extraction of the plant material:

About 180 gm of powdered material was taken in a clean, flat bottomed glass container and soaked in 700 ml of 95% methanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. In the mean time 70 ml of petroleum ether was mixed with methanolic extract and kept in separating funnel. Then it was separated by filtration through Whatman filter paper (Bibby RE200, Sterilin Ltd., UK). The filtrate (pet-ether and methanol extracts) obtained was evaporated using rotary evaporator. It rendered a gummy concentrate of reddish black color. The gummy concentrate was designated as crude extracts of pet-ether and methanol. The extracts were transferred to a closed container for further use and protection. The yield of petroleum ether and methanolic extracts was 15 % and 17 % respectively.

Equipment used for experiments:

The following equipment were used:Sterile disposable syringe (1ml, 100 divisions) (CHPL, India), Tuberculin syringe with ball shaped end (Merck, Germany), Electronic and digital balance (Denver Instruments ,M-220/USA).

Antidiarrheal Effects of the Petroleum ether and Methanol Extracts of Plant *Manilkara zapota* Leaves by Castor Oil Induced Method:

The anti-diarrheal activity was performed by the method developed by (Havagiray et al., 2004) [6]**.** Animals were divided into six groups of five animals in each group. Group I received 1ml castor oil and served as control. Groups II received castor oil and standard drug, (loperamide, 3 mg/kg) and served as standard. Group III-IV received Petroleum ether and group V-VI received methanolic extracts of *Manilkara zapota* leaves (200 and 400mg /kg respectively). Diarrhea was induced in all the overnight fasted animals by administering 1 ml of castor oil orally. The test extracts and the standard drug were administered one hour prior to the treatment of castor oil. Each mouse was housed separately and observed for diarrheal episode, for a period of 3 hours. During that period, number and weight of diarrheal feces were taken and noted at every half an hour. The mean diarrheal episodes and percent protection was calculated. The anti-diarrheal activity was determined in terms of percentage protection. The data of stool weight was expressed as Mean ± SEM.

Experimental Animal

Young Swiss-albino mice of either sex aged 7-8 weeks, average weight 20-35 gm were used for the experiment. The mice were purchased from the animal Research Branch of the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR, B). They were kept in standard environmental condition (at 25±0.5°C temperature & 55-65% relative humidity and 12 hour light/12 hour dark cycle) for one week for acclimation after their purchase and fed (ICDDR, B) formulated rodent food and water *ad libitum*. The set of rules followed for animal experiment were approved by the institutional animal ethical committee **(**Zimmermann M, 1983) [7].

Experimental Design and Identification of Animals

Thirty experimental animals were randomly selected and divided into six groups denoted as group-I, group-II, group-III, group-IV, group-V and

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group-VI consisting of 5 mice in each group. Each group received a particular treatment i.e. control, standard and the dose of the extracts of the plant respectively. Prior to any treatment, each mouse was weighed properly and the dose of the test sample and control materials was adjusted accordingly. The animals were marked as M-1=Mice 1, M-2=Mice 2, M-3=Mice 3, M-4=Mice 4 and M-5=Mice 5.

Preparation of Test Materials

In order to administer the crude extract at dose of 200 and 400 mg/kg body weight of mice, required amount of extract was measured and was triturated unidirectional way by the addition of small amount of suspending agents (Tween-80). After proper mixing of extracts and suspending agent, normal saline was slowly added. The final volume of the suspension was made 5 ml. To stabilize the suspension, it was stirred well by vortex mixture. For the preparation of Loperamide at the dose of 3 mg/kg-body weight, required amount of Loperamide was taken and a suspension of 5 ml was made.

Procedure

The animals were weighed and randomly divided into six groups consisting of 5 mice in each group. At zero hour test samples *Manilkara zapota* extracts, control (1 ml castor oil) were administered orally by means of a long needle with a ball-shaped end and Loperamide was also given orally as standard. After administration of castor oil extracts and loperamide ,the number of soft and hard stools and total weight of stools of each individual mice was calculated. At one hour intervals for total 3 hours are considered for esmited experimental time to observe total diarrhoeal episode of each mice.

Statistical analysis

Data of all experiments were reported as Mean ±S.E.M (Standard error of the mean). Statistical significance testing of the values obtained were performed by one-way analysis of variance (ANOVA) and the group means were evaluated by Dunnet's multiple comparisons for antidiarrheal screening tests using SPSS program (SPSS 16.0, USA). The results were further analyzed by using Student's *t*-test to calculate significance of the results on castor oil-induced diarrhoea. In all cases the data obtained were compared with the vehicle control group. Differences were considered statistically significant when $P < 0.05$, 0.01.

Antidiarrheal Effects of the Petroleum ether and Methanol Extracts of Plant *Manilkara zapota* Leaves by Magnesium Sulfate (MgSO4) Induced Method

The anti-diarrheal activity was performed by the method developed by (Havagiray et al.,2004) [6]. Animals were divided into six groups of five animals in each group. Group I received Magnesium Sulfate (2g/kg) and served as control. Groups II received Magnesium Sulfate and standard drug, (loperamide, 3 mg/kg) and served as standard. Group III-IV received Petroleum ether and group V-VI received methanolic extracts of *Manilkara zapota* leaves (200 and 400mg /kg respectively). Diarrhea was induced in all the overnight fasted animals by administering Magnesium sulfate (2g/kg) orally. The test extracts and the standard drug were administered one hour prior to the treatment of Magnesium Sulfate. Each mouse was housed separately and observed for diarrheal episode, for a period of 3 hours. During that period, number and weight of diarrheal feces were taken and noted at every half an hour. The mean diarrheal episodes and percent protection was calculated. The anti-

diarrheal activity was determined in terms of percentage protection. The data of stool weight was expressed as Mean ± SEM.

Experimental Animal

Young Swiss-albino mice of either sex aged 7-8 weeks, average weight 25-35 gm were used for the experiment. The mice were purchased from the animal Research Branch of the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR, B). They were kept in standard environmental condition (at 25±0.5°C temperature & 55-65% relative humidity and 12 hour light/12 hour dark cycle) for one week for acclimation after their purchase and fed (ICDDR, B) formulated rodent food and water *ad libitum*. The set of rules followed for animal experiment were approved by the institutional animal ethical committee **(**Zimmermann M, 1983) [7].

Experimental Design and Identification of Animals

Thirty experimental animals were randomly selected and divided into six groups denoted as group-I, group-II, group-III, group-IV, group-V and group-VI consisting of 5 mice in each group. Each group received a particular treatment i.e. control, standard and the dose of the extracts of the plant respectively. Prior to any treatment, each mouse was weighed properly and the dose of the test sample and control materials was adjusted accordingly. The animals were marked as M-1=Mice 1, M-2=Mice 2, M-3=Mice 3, M-4=Mice 4 and M-5=Mice 5.

Preparation of Test Materials

In order to administer the crude extract at dose of 200 and 400 mg/kg body weight of mice, required amount of extract was measured and was triturated unidirectional way by the addition of small amount of suspending agents (Tween-80). After proper mixing of extracts and suspending agent, normal saline was slowly added. The final volume of the suspension was made 5 ml. To stabilize the suspension, it was stirred well by vortex mixture. For the preparation of Loperamide at the dose of 3 mg/kg-body weight, required amount of Loperamide was taken and a suspension of 5 ml was made.

Procedure

The animals were weighed and randomly divided into six groups consisting of 5 mice in each group. At zero hour test samples *Manilkara zapota* extracts, control (Magnesium Sulfate,2g/kg) were administered orally by means of a long needle with a ball-shaped end and Loperamide was also given orally as standard. After administration of magnesium Sulfate extracts and loperamide , the number of soft and hard stools and total weight of stools of each individual mice was calculated. At one hour intervals for total 3 hours are considered for esmited experimental time to observe total diarrhoeal episode of each mice.

Statistical analysis

Data of all experiments were reported as Mean ± S.E.M (Standard error of the mean). Statistical significance testing of the values obtained were performed by one-way analysis of variance (ANOVA) and the group means were evaluated by Dunnet's multiple comparisons for antidiarrhoeal screening tests using SPSS program (SPSS 16.0, USA). The results were further analyzed by using Student's *t*-test to calculate significance of the results on Magnesium sulfate induced diarrhoea. In all cases the data obtained were compared with the vehicle control group. Differences were considered statistically significant when $P < 0.05, 0.01$.

RESULT AND DISCUSSION

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Results of Antidiarrhoeal activity by castor oil induced method

The result of the castor oil induced diarrhea is given at Table-1.Only the petroleum ether extracts showed statistically significant ($p<0.05$) reduction of diarrheal episode in mice. The petroleum extracts of *Manilkara zapota* administered at the dose of 200 and 400 mg/kg showed 72.53% and 64.66 % protection respectively. The methanolic extracts of *Manilkara zapota* adminsterd at the dose of 200 and 400 mg/kg showed 43.82% and 45.73% protection respectively also. The frequency of stooling (number of wet feces and total number of feces) as well as fresh weight also decreased insignificantly and the result was found insignificant.

Treatment	Dose	Number of wet stools	Total number of stools	Total weight of stools	% Protection
Castor oil	10 ml/kg	2.250 ± 1.443	5.500 ± 1.732	0.783 ± 0.596	0
Loperamide	3 mg/kg	3.250 ± 1.518	8.750 ± 0.866	0.788 ± 0.124*	67.69
Petroleum ether extracts	200 mg/kg	0.578 ± 0.500	5.000 ± 1.943	0.403 ± 0.256*	72.53
	400 mg/kg	0.866 ± 0.750	2.500 ± 1.732	0.518 ± 0.363*	64.66
Methanolic extracts	200 mg/kg	1.750 ± 1.190	5.250 ± 1.443	0.823 ± 0.396	43.82
	400 mg/kg	1.750 ± 1.190	5.500 ± 1.667	0.795 ± 0.431	45.73

Table 1: Effects of petroleum ether and methanolic extracts of Manilkara zapota on castor oil induced diarrhea in mice.
Values are presented as Mean ± SEM (n=5). Statistical analysis was conducted by Unpaired Student's t test and the data was compared to control. The data were found statistically significant when *: $p<0.05$,

Results of Antidiarrhoeal activity by magnesium sulfate induced method

The result of the magnesium sulfate induced diarrhea is given at Table-2. Only the petroleum ether extracts showed statistically significant ($p<0.05$) reduction of diarrheal episode in mice. The petroleum extracts of *Manilkara zapota* adminsterd at the dose of 200 and 400 mg/kg showed 54.35% and 77.94 % protection respectively. The methanolic extracts of *Manilkara zapota* adminsterd at the dose of 200mg/kg showed 53.33% protection and at the dose of 400mg/kg surprisingly showed 34.87 % protection respectively also. The frequency of stooling (number of wet feces and total number of feces) as well as fresh weight also decreased insignificantly and the result was found insignificant.

Treatment	Dose	Number of wet stools	Total number of stools	Total weight of stools	% Protection
Magnesium sulfate	2 mg/kg	2.500 ± 0.333	7.000 ± 2.108	0.488± 0.100	0
Loperamide	3 mg/kg	0.000 ± 0.000	1.500 ± 0.333	0.060 ± 0.017*	87.69
Petroleum ether extracts	200 mg/kg	0.250 ± 0.288	6.250 ± 3.068	0.223 ± 0.073	54.35
	400 mg/kg	0.250 ± 0.288	2.500 ± 1.105	0.108 ± 0.040*	77.94
Methanolic extracts	200 mg/kg	0.500 ± 0.333	4.500 ± 0.745	0.228 ± 0.029	53.33
	400 mg/kg	0.250 ± 0.288	3.750 ± 1.658	0.318 ± 0.169	34.87

Table 2: Effects of petroleum ether and methanolic extracts of *Manilkara zapota* on magnesium sulfates induced diarrhea in mice.

Values are presented as Mean ± SEM (n=5). Statistical analysis was conducted by Unpaired Student's t test and the data was compared to control. The data were found statistically significant when *: p<0.05.

Discussion for Antidiarrhoeal

The use of herbal remedies in the treatment of diarrhoeal diseases is a common practice in many countries of the world including Bangladesh. A number of medicinal plants have been reported to be effective against diarrhoea and dysentery,(Gerald et al.,2007; Ghani A. (1998); Rouf et al.,2003) [8,9,10]. The use of castor oil as diarrhoea inducer is well documented. The most active component of the oil is the ricinoleic acid. Ricinoleic acid causes irritation and inflammation of the intestinal mucosa. The irritation stimulates the peristaltic activity of the small intestine, causing changes in the electrolytic permeability of the intestinal mucosa. This sequence of events leads to the release of prostaglandins which stimulates motility and secretion thereby decreasing the absorption of sodium and potassium ions(Pierce et al.,1971; Zavata et al.,1998; Rouf et al.,2003) [11,12,10].Inhibitors of prostaglandin synthesis are also known to delay diarrhoea induced by castor oil(Sunil et al.,2001) [13].Therefore, decreased frequency of stooling and fecal parameters (total number, fresh weight, and number of wet feces) observed with the extract in this study are indications of antidiarrhoeal potential. These observations also suggest that the antidiarrhoeal activity of the extract may be due to the inhibition of prostaglandin biosynthesis. In an earler studies it was demonstrated that the diarrhoeal effects of castor oil might be involved that increase the permeability of the epithelial layer to calcium ions, leading to an increase in intracellular Ca2+ and enhancement of calmodulin stimulation of NO synthase activity. NO, in turn, could stimulate intestinal secretion.It has been shown that castor-oil causes motility and secretory diarrheaThis is achieved through its dual effects on gastrointestinal motility as well as water and electrolyte transport (decreasing Na+ and K+ absorption) across the intestinal mucosa**(**Rouf et al.,2003)[10] . The inhibition of castor-oil induced intestinal fluid accumulation (enteropooling) as well as the weight of the intestinal content may be due to the ability of the extract to increase the reabsorption of electrolytes and water. This may also be due to the ability of the extract to inhibit the induced intestinal accumulation of fluid in a manner similar to loperamide(Vareinshang et al .,2004) [14] .In this study, the 200mg/kg body weight of the extracts of *Manilkara zapota* showed the best antidiarrhoeal activities. At the dose of 200mg/kg body weight, PEMZ exerted greater inhibition of 72.53% compared to MEMZ (43.83%). So PEMZ had a more prominent antidiarrheal property than MEMZ. Even PEMZ showed higher activity compared to that of the standard drug loperamide (67.69%).In magnesium sulfate, the 400mg/kg body weight of the extract of *manilkara zapota* also showed the antidiarrhoeal activities. At the dose of 400mg/kg body weight, PEMZ exerted greater inhibition of 77.95% compared to MEMZ (54.35%). So PEMZ had a more prominent antidiarrheal property than MEMZ. *The* antidiarrhoeal activities of medicinal plants have been attributed to the presence of bioactive agents such as tannins, alkaloids, saponins, flavonoids, steroids and terpenoids(Havagiray et al.,2004) [6].Therefore, the antidiarrhoeal activity of *Manilkara zapota*

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leaves observed in this study may be attributed to the presence of, flavonoids, and saponins in the methanolic and petroleum extracts. Furthermore the inhibition of castor oil and Magnesium sulfate induced enteropooling and the suppressed propulsive movement observed in this study are indications of antidiarrhoeal potential of *Manilkara zapota* leaf extract.

Conclusion

In conclusion this study shows that both the methanolic and Petroleum ether extracts of *Manilkara zapota* leaves possess moderate antidiarrheal properties due to its inhibitory effect on fluid secretion and thus presents a new perspective in the treatment of diarrhoea. So the inhibitory effect of the extracts provides a rationale for using this plant as a non-specific antidiarrheal agent in folk medicine in the treatment of dysentery and various diarrhoeal disorders. However, further Pharmacological investigations such as testing with different models of antidiarrheal activity and bioactivity guided phytochemical studies may be exploited to find out the actual active constituents which is responsible for possible antidiarrheal effects.

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References

1) M. R. Islam, M. S. Parvin, M. S. Islam,S. M. R. Hasan, and M. E. Islam ,Antioxidant Activity of the Ethanol Extract of *Manilkara zapota* Leaf, ,J. Sci. Res.2012; 4 (1), 193-202.
2) Hemayet Hossain, Md. Sariful Islam Howlader , Shubhra Kanti Dey , Arpona Hira and Arif Ahmed Antinociceptive and antidiarrheal properties of the ethanolic extract of *Manilkara zapota* (linn.) Bark , ijpsr, 2012; vol. 3(12): 4791-4795.
3) Mohiddin HMYB, Chin W, Holdsworth D. Traditional medicinal plants of Brunei, Darussalam Part III. Sengkurong. *Int J Pharmacog* 1992; 30:105-108.4.
4) S. V. Chanda and K. V. Nagani. Antioxidant Capacity of *Manilkara zapota* L. Leaves Extracts Evaluated by Four *in vitro* Methods. Nature and Science; 2010: 8(10).
5) R. Nair and Sumitra Chanda. Antimicrobial Activity of *Terminalia catappa*, *Manilkara zapota* and *Piper betel* Leaf Extract. *Indian J Pharm Sci*. 2008; 70(3): 390–393.
6) Havagiray R, Ramesh C, Sadhna K. Study of antidiarrhoeal activity of Calotropis gigantea R.B.R. in experimental animals. J Pharm Pharmaceut Sci 2004; 7: 70–75.
7) Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals pain.1983; 16: 109.
8) Gerald NT, Jules RK, Omer BN, Donatien G. Antidiarrhoeal and antimicrobial activities of Emilia coccinea (Sims) G. Don extracts. J Ethnopharmacol 2007; 112: 278– 283.
9) Ghani A. Medicinal plants of Bangladesh: Chemical constituents and uses. Asiatic society of Bangladesh, Dhaka. 1998.
10) Rouf AS, Islam MS, Rahman MT. Evaluation of antidiarrhoeal activity of Rumex maritimus roots. J Ethnopharmacol 2003; 84: 307-310.
11) Pierce NF, Carpenter CCJ, Elliot HZ, Greenough WB. Effects of prostaglandins, theophylline and Cholera exotoxin upon transmucosal water and

electrolyte movement in canine jejunum. Gastroenterol 1971; 60: 22-32.

12) Zavata MA, Perez S, Perez C, Vargos R, Perez RM. Antidiarrhoeal activity of Waltharia americana, Commelina coelestis and Althernan repens. J Ethnopharmacol 1998; 61, 41-47.

13) Sunil B, Bedi K, Singla A, Johri R. Antidiarrhoeal activity of piperine in mice. Planta Medica 2001; 67: 284–287. 53.

14) Vareinshang T, Yadav AK. Antidiarrhoeal activity of Rhus javanica ripens fruit extract in albino mice. Fitoterapia 2004; 75: 39-44.

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