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In Vivo Assay of Analgesic Activity of Methanolic and Petroleum Ether Extracts of *Manilkara zapota* leaves

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Authors' contributions

This work was carried out in collaboration between all authors. Author MIUH designed the study and wrote the protocol. Author FS wrote the first draft of the manuscript. Author M conducted the experimental works. Author MARC performed the statistical analysis. Author MTH managed the literature searches and analyses of data. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: The main objective of this work was to observe the analgesic activity of *Manilkara zapota* (leaves) on mice.
Study Design: The Present study was designed to observe pharmacological activities of the crude extracts of the plant *Manilkara zapota* (leaves). The study protocol consisted of Cold extraction at room temperature of the whole plant with distilled methanol. Afterwards, Filtration of the crude Methanolic and Petroleum ether extracts by using the Markin cotton cloth and subsequently through the filter paper and solvent evaporation. Finally, screening of analgesic activity of crude extracts on Swiss Albino mice.
Place and Duration of Study: School of Science and Engineering, Department of Pharmacy, Southeast University, Bangladesh, January 2011 to August 2012.
Methodology: The analgesic activity was investigated for its peripheral pharmacological actions by using acetic acid-induced writhing test in mice.
Results: The Methanolic and Petroleum Ether extracts, at the dose of 200 mg/kg body weight, displayed 96.82% & 94.27% pain inhibition which was significant ($p<0.001$)

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compared to control. These results indicate that the extracts possess significant analgesic activity.
Conclusion: This study suggests that the Methanolic and Petroleum Ether extract of *Manilkara zapota* leaves have analgesic activity in a dose dependent manner which supports it's as an analgesic drug in folk medicine.

Keywords: Manilkara zapota leaves; analgesic activity; writhing test.

1. 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 [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 [2]. The leaves of this plant are used to treat cough, cold, and diarrhea. Furthermore, the leaves of the plant posses antioxidant & antimicrobial activity [3,4,5].

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

2. MATERIALS AND METHODS

2.1 Preparation of Plant Extract

2.1.1 Collection of the plant parts and identification

For this present investigation, the *Manilkara zapota* leaves were collected from Botanical garden, Magura (Bangladesh) on, January 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.

2.1.2 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 (petroleum-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 petroleum-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.

2.2 Analgesic Activity by Acetic Acid Induced Writhing Method

2.2.1 Principle

In this method Koster et al. [6], acetic acid (0.7% v/v) was administered intra-peritoneal to the experimental animals to create pain sensation. As a result, the animals squirms their body at regular interval out of pain. This squirm or contraction of the body was termed as "writhing". As long as the animals feel pain, they continue to give writhing. Each writhing was counted and taken as an indication of pain sensation. Any substance that has got analgesic activity was supposed to lessen the number of writhing of animals within in a given time frame and with respect to the control group. The writhing inhibition of positive control was taken as standard and compared with test samples and control. As positive control, any standard NSAID drug can be used. In the present study, Indomethacin was used to serve the purpose.

2.2.2 Drugs and chemicals

Acetic acid, DMSO (Merck, Germany), Indomethacin (Square Pharmaceuticals ltd, Bangladesh), Tween-80 (BDH Chemicals Ltd, UK), Normal Saline Solution (Beximco Infusion Ltd, Bangladesh) was purchased.

2.2.3 Experimental animal

Male young Swiss-albino mice of 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 ± 0.5℃ 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 [7].

2.2.4 Experimental design

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.

2.2.5 Preparation of test materials

In order to administer the crude extract at dose of 100 mg/kg and 200mg/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 Indomethacin at the dose of 10 mg/kg-body weight, required amount of Indomethacin was taken and a suspension of 5 ml was made.

2.2.6 Procedure

At zero hour, test samples and Indomethacin were administered orally by means of a force-feeding tip. For control group, acetic acid was administered by means of a syringe at that time. After 40 minutes, acetic acid (0.7%) was administered intraperitoneally to each of the animals of all the groups. After, the forty minutes interval between the oral administration of test materials and intra-peritoneal administration of acetic acid was given to assure proper absorption of the administered samples. Finally, acetic acid was administered after five minutes, number of squirms or writhing was counted for each mouse for fifteen minutes.

2.2.7 Counting of writhing

Each mouse of all groups were observed individually for counting the number of writhing they made in 15 minutes commencing just 5 minutes after the intraperitoneal administration of acetic acid solution. Full writhing was not always accomplished by the animal, because sometimes the animals started to give writhing but they did not complete it. This incomplete writhing was considered as half-writhing. Accordingly two half writhing were taken as one full writhing [8].

2.3 Statistical Analysis

Data of all experiments were reported as Mean ± SEM (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 analgesic 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 acetic acid induced analgesic activity. In all cases the data obtained were compared with the vehicle control group. Differences were considered statistically significant when $P < 0.05$, 0.01.

3. RESULTS AND DISCUSSION

3.1 Results of Analgesic Activity

The effects of analgesic activity of both methanolic and petroleum ether extracts of *Manilkara zapota* leaves (Table 1), at the dose 200 mg/kg showed statistically significant ($p<0.001$) and give maximum 94.27 % and 96.82 % inhibition respectively on acetic acid induced writhing in mice. Besides at the dose of 100mg/kg body weight both Methanolic and Petroleum Ether Extracts of *Manilkara zapota* displayed 35.67% and 59.87% inhibition respectively.

Table 1. Effect of methanolic and petroleum ether extracts of *Manilkara zapota* on acetic acid induced writhing in mice

Treatment	Dose	Route	No. of writhing (Mean ± SEM)	% Inhibition
Control	0.5 ml/mouse	p.o	39.250 ± 8.019	0%
Indomethacin	10 mg/kg	p.o	18.500 ± 7.638*	52.87%
Methanol extract	100 mg/kg	p.o	25.250 ± 9.109	35.67%
	200 mg/kg	p.o	1.250 ± 1.093**	96.82%
Petroleum-ether extract	100 mg/kg	p.o	15.750 ± 9.981	59.87%
	200 mg/kg	p.o	2.250 ± 0.866**	94.27%

*Values are presented as Mean ± SEM (n=5). Statistical analysis: *: P<0.05, **: P<0.001, Dunnett test as compared to control.*

3.2 Discussion for Analgesic

The methanolic and petroleum ether extracts was evaluated in acetic acid-induced writhing test for its analgesic activity. Acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from tissue phospholipids [9]. The acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesics. The response is thought to be mediated by peritoneal mast cells [10] acid sensing ion channels [11] and the prostaglandin pathways [12]. In our experiment, both Methanolic & Petroleum Ether Extracts of *Manilkara zapota* showed 96.82% & 94.27% pain inhibition respectively at the dose of 200 mg/kg body weight which was significant compared to control. So the results found in the present study demonstrated that the plant extracts has Strong analgesic activity.

4. CONCLUSION

In conclusion this study shows that both the methanolic and Petroleum ether extracts of *Manilkara zapota* leaves possess significant analgesic activity in acetic acid induced writhing method. Further investigations are required to find the active component of the extract in order to confirm the mechanism of action in the development of a potent analgesic agent.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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