

## Evaluation of central nervous system depressant activity of methanolic and petroleum ether extract of *Manilkara zapota* leaves (*in vivo*)

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### Abstract

The purpose of this study was to examine the central nervous system (CNS) -depressant activity of methanolic and petroleum ether extracts of leaves obtained from *Manilkara zapota* (Sapotaceae) *in vivo*. CNS-depressant action was evaluated by observing the effects of plant extracts on both exploratory and spontaneous locomotor activity in mice using open field and hole cross tests respectively. The extracts were found to exhibit CNS-depressant activity in a dose-dependent manner. In the open field test, both the methanolic and petroleum ether extracts reduced the exploratory behavior of mice significantly ( $p < 0.05$ ). Although the extracts reduced locomotor activity in the hole cross test, the results were found to be insignificant. Overall, these findings indicate that both types of extracts derived from *M. zapota* leaves possess CNS-depressant activity.

**Keywords** : CNS depressant, hole cross, open field, *Manilkara zapota* leaves,

### Introduction

*Manilkara zapota*, an evergreen, glabrous tree belonging to the Sapotaceae family, grows abundantly throughout Bangladesh in lowland rainforest to a maximum height of 15 metres. Common to India, the tree can also be found in Mexico and Central America [1,2]. At present, very little scientific investigation with regards to its chemical constituents or biological effects has been performed on this species. However, the fruits of *M. zapota* have been shown to contain polyphenolic compounds which elicit antioxidant activity when examined *in vivo* [3]. Medicinally, the leaves of the plant are used to treat diarrhea, coughs and colds [4]. Further, they are also reported to possess antimicrobial activity against several different species of bacteria [5, 6]. Thus, given the diverse range of pharmacological effects, this study aimed to investigate *in vivo*, the central nervous system (CNS) -depressant potential of *M. zapota* leaf extracts.

### Materials and methods

#### Collection and identification of plant parts

Fresh leaves from *M. zapota* were collected from the Magura Botanical Garden (Bangladesh). Formal identification was performed by the expert of the Bangladesh National Herbarium

where a specimen (Accession no-35493) was deposited. The collected leaves were dried for one week and ground into a coarse powder with the aid of a mortar and pestle. The powder was stored in an airtight container and kept in a cool, dark and dry place until required.

#### Extraction of plant material

Approximately 180 g of coarsely powdered material was incubated at room temperature in 700 mL of methanol (95% v/v) in a sealed container for 7 days with occasional shaking and stirring. The methanolic extract was then subjected to a coarse filtration using sterile, white cotton material. Next, 70 mL of petroleum ether was mixed with the methanolic extract and placed in a separating funnel which allowed separation by filtration through Whatman filter paper (Bibby RE200, Sterilin Ltd., UK). The filtrate (petroleum ether and methanol extracts) were dried using a vacuum pump rotary evaporator at 50°C under reduced pressure (STUART RF3022C, UK).

#### Experimental Animal

Young Swiss-albino mice of either sex weighing 25-35 g were purchased from the International Centre for Diarrheal Disease and Research Bangladesh (ICDDRDB). All mice were kept under standard environmental conditions and fed formulated rodent food

and water *ad libitum*. The guidelines for animal experimentation were strictly adhered to and were approved by the institutional animal ethical committee [7].

## Experimental Design

For each experiment, thirty animals were randomly selected and divided into six groups (indicated group I - VI) consisting of five animals in each group. Specifically, group I (control) received 1% Tween-80 only; group II acted as the standard and were given 1 mg/kg per body weight of Diazepam; groups III and IV were administered plant fractions extracted using petroleum ether at a concentration of 250 or 500 mg/kg respectively; groups V and VI received plants fractions extracted by methanol at a concentration of 250 or 500 mg/kg respectively. Before administration of any treatment, each mouse was weighed, and the dose was adjusted accordingly.

## CNS-Depressant effect of petroleum ether and ethanol extracts

### Open field test

This research followed the method as described by [8]. Briefly, the floor, measuring 0.5m<sup>2</sup> and with a wall height of 40 cm, was divided into a series of squares which were alternately colored black and white. The number of squares visited by the animals was calculated for 3 min at 0, 30, 60, 90 and 120 min time points.

### Hole cross test

The technique described by [9] was adopted for this study. Each of the six groups of mice was subject to testing. A wooden partition was fixed into the middle of a cage (30 20 14 cm) and a hole with a

diameter of 3 cm was made at a height of 7.5 cm in the center of the cage. The number of passages a mouse made through the hole from one chamber to the other was calculated for 3 min at 0, 30, 60, 90 and 120 min time point post-oral administration of the designated treatment as described in the experimental design.

## Statistical Analysis

All experimental data were reported as mean  $\pm$  standard error of the mean (SEM). Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons using SPSS program (SPSS 16.0, USA). Differences were considered statistically significant when  $P < 0.05$ .

## Results

### Open field test

The methanolic and petroleum ether extracts at various doses significantly decreased ( $p < 0.05$ ) both locomotor and exploratory activity of the mice treated with *M. zapota* fractions regardless of extraction solvent (Table 1). At 30 min, the petroleum ether extract (250 and 500 mg/kg) and methanolic extract (500 mg/kg) were shown to significantly ( $p < 0.05$ ) decrease mice movement and activity in comparison to the standard diazepam treatment group. At 60 min, both doses of petroleum ether and methanolic extract were significant ( $p < 0.05$ ) as a definitive reduction in locomotor and exploratory activity was observed. However, only the petroleum ether extract at a concentration of 250 mg/kg and both methanolic extracts (250 and 500 mg/kg) were shown to elicit a significant decrease in overall movement ( $p < 0.05$ ) and thus animal anxiety, compared to the diazepam standard at the 120 min time point.

**Table 1:** Effects of petroleum ether and methanolic extracts of *Manilkara zapota* in mice using the open field test.

Groups	Mean movements before and after treatment administration				
	0 min	30 min	60 min	90 min	120 min
I	142.50 $\pm$ 49.56	65.50 $\pm$ 23.80	41.25 $\pm$ 11.32	51.25 $\pm$ 25.92	9.75 $\pm$ 5.68
II	418.75 $\pm$ 195.13	241.25 $\pm$ 5.68*	248.50 $\pm$ 40.15*	66.75 $\pm$ 32.21	0.00 $\pm$ 0.00*
III	176.25 $\pm$ 36.76	100.50 $\pm$ 39.59*	57.75 $\pm$ 17.17*	30.75 $\pm$ 8.72	2.25 $\pm$ 0.99*
IV	120.00 $\pm$ 34.39	82.75 $\pm$ 39.47*	40.25 $\pm$ 12.82*	14.75 $\pm$ 4.38	1.75 $\pm$ 1.66
V	232.00 $\pm$ 99.81	126.75 $\pm$ 45.58	110.00 $\pm$ 9.57*	61.00 $\pm$ 9.89	1.00 $\pm$ 0.47*
VI	109.50 $\pm$ 34.79	68.25 $\pm$ 21.29*	47.25 $\pm$ 4.97*	31.75 $\pm$ 9.27	0.50 $\pm$ 0.33*

Values are presented as mean  $\pm$  SEM (n=5);  $p < 0.05^*$  calculated using ANOVA and post hoc tukey's test. (Group I = control, 1% Tween-80; group II = standard, 1 mg/kg of Diazepam; group III = petroleum extract, 250 mg/kg; group IV = petroleum extract, 500 mg/kg; group V = methanolic extract, 250 mg/kg; group VI = methanolic extract, 500 mg/kg).

## Hole cross test

To evaluate the potential reduction in locomotor activity, the hole cross test was used. Both types of extracts (methanolic and petroleum ether) educed a remarkable decrease in exploratory behavior in the trial animals from pre-treatment 0 min to 120 min

post-treatment (Table 2). At 90 min, only the methanolic extract at a concentration of 500 mg/kg reached significance ( $p < 0.05$ ), thus causing a decline in animal movement compared to the group administered the standard diazepam treatment. No other treatment decreased activity regardless of concentration or time of testing.

**Table 2:** Effects of petroleum ether and methanolic extracts of *Manilkara zapota* in mice using the hole cross test.

Groups	Mean number of hole crossings before and after treatment administration				
	0 min	30 min	60 min	90 min	120 min
I	2.75±1.59	6.00±2.62	2.50±1.73	4.00±1.41	0.75±0.55
II	2.75±0.73	3.25±2.33	1.25±0.87	0.00±0.00*	0.00±0.00
III	5.25±2.18	5.00±2.36	3.25±3.03	3.00±1.49	0.50±0.33
IV	2.00±0.82	1.50±1.00	1.25±1.44	0.75±0.55	0.33±0.33
V	5.50±2.24	2.25±0.99	3.50±1.37	1.50±1.11	0.33±0.33
VI	2.50±1.11	1.00±0.82	1.00±0.47	0.25±0.29*	0.00±0.00

Values are presented as mean ±SEM (n=5);  $p < 0.05$ \* calculated using ANOVA and post hoc tukey's test. (Group I = control, 1% Tween-80; group II = standard, 1 mg/kg of Diazepam; group III = petroleum extract, 250 mg/kg; group IV = petroleum extract, 500 mg/kg; group V = methanolic extract, 250 mg/kg; group VI = methanolic extract, 500 mg/kg).

## Discussion

A product that renders an animal quiet either has a sedative or depressive effect on the CNS [10]. In our study, the effect of methanolic and petroleum *M. zapota* derived-extracts on the CNS of young Swiss-albino mice was shown to produce a remarkable reduction in overall movement and animal anxiety. In the open field test, the extracts lessened the exploration ability of the mice significantly ( $p < 0.05$ ), thus indicating a CNS-depressive effect. A decline in exploratory behavior as a consequence of treatment with the different extracts is similar with comparable activities produced by different CNS-depressant drugs [11]. Locomotor activity is a test to measure the level of excitability of the CNS [12]. Hence, any decrease in this activity may be closely related to sedation resultant from the depression of the CNS [13]. The effects on the spontaneous motor activity demonstrated that both extracts (methanolic and petroleum ether) obtained from *M. zapota* leaves significantly decreased the rate and amplitude of movements ( $p < 0.05$ ). The CNS-depressant activity by the plant extracts may be due to an increase in the concentration of gamma-aminobutyric acid (GABA) which is the major inhibitory neurotransmitter in the vertebrate CNS [14]. Different anxiolytic, muscle relaxant and sedative-hypnotic drugs all elucidate their action through the binding of GABA to its cognate receptor (GABA<sub>A</sub>). Early investigation into phytoconstituents derived from plants showed that many flavonoids and neuroactive steroids were found to be ligands for GABA<sub>A</sub> in the CNS. As a result, this led to the well-founded assumption that such plant compounds may act as benzodiazepine-like molecules since this class of drug is able to enhance the CNS-depressive effect of GABA [15]. The sedation or

rather, the reduction in movement and activity, may be due to the interaction with benzodiazepine-like compounds contained within *M. zapota*. The leaf extracts may act by potentiating GABAergic inhibition in the CNS by membrane hyperpolarization which diminishes the firing rate of critical neurons in the brain. Conversely, the effect which was demonstrated in the mice, may in fact be due to the direct activation of GABA<sub>A</sub> by the plant extracts [16]. Moreover, phytochemical analysis into the multitude of components contained within other plants has also showed the presence of alkaloids, flavonoids, saponins and steroids [15]. Hence, any of these compounds might be responsible for *M. zapota*'s CNS-depressant activity.

## Conclusion

This study has demonstrated that both extracts (methanolic and petroleum ether) obtained from *M. zapota* leaves elicit CNS-depressive activity. Although the plant extracts did not result in significant outcomes when tested using the hole cross method, the CNS-depressive activity of *M. zapota* leaves should be further evaluated to identify the bioactive compounds which have mediated the observed effects.

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