Research Article

Investigation of Antidepressant Potential of Methanolic Leaf Extract of *Manilkara zapota* in Experimental Animal Models

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Received: 22-10-2022; **Revised:** 20-12-2022; **Accepted:** 27-12-2022; **Published on:** 15-01-2023.

ABSTRACT

Depression is a neurological disorder leading to CNS manifestations. At present, depression is the most common issue worldwide, placed fourth on the list of disabilities affecting people of all age groups. Several conventional treatments for depression were discovered in past decades but have side effects and sometimes toxicity. Thus, an inclination to antidepressant therapy with fewer side effects and toxicity becomes more of a need for alternatives to synthetic drugs. Plant products containing active phytochemicals are used for several pharmacological activities including depression due to less toxic effects. In this present study in vivo antidepressant activity of the methanolic extract of *Manilkara zapota* leaves has been evolved by using animal experimental models. Extract of *Manilkara zapota* leaves was prepared using methanol as a solvent by a simple maceration process. The acute oral toxicity studies were performed with different doses (5,50,100,300,2000 mg/kg) of the Extract of *Manilkara zapota* and observed for behavioural changes and mortality. The antidepressant activity was studied by using the forced swimming test (FST) and tail suspension test (TST). The period of immobility was observed in both models which were indicative as a parameter for antidepressant activity. The methanolic extract of *Manilkara zapota* at the doses of 200 mg/kg ($p<0.0005$) and 400 mg/kg ($p<0.0001$) have shown significant antidepressant activity when compared to the standard marketed product Imipramine 4 mg/kg ($p<0.0001$). Thus, it was concluded that in the future plant products are becoming a vital therapy for the treatment of depression. So, further research needs to be done to determine its active constituents and molecular-level target mechanisms that are responsible for antidepressant activity in humans.

Keywords: Antidepressant activity, Manilkara zapota, forced swim test, tail suspension test, oxidative stress.

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DOI: 10.47583/ijpsrr.2023.v78i01.013

DOI link: http://dx.doi.org/10.47583/ijpsrr.2023.v78i01.013

INTRODUCTION

Depression is a multifactorial heterogeneous disorder which causes continual emotions of disappointment and reduced energy, affecting more than 350 million humans globally.[1] Major depressive disorder includes more than one aetiology including genetics, environment, physiological and biological factors.[2] It is additionally accompanied by inflammatory responses of the organism and consequent elevation of proinflammatory cytokines and increased lipid peroxidation. Another aspect of depression is oxidative stress which results due to an imbalance between prooxidants and antioxidants and sooner or later in favour of prooxidants.[3]

Depression is highly treatable. Up to 90% of people will eventually improve with medication therapy, a combination of two or more antidepressant drugs.[4] Conventional medications are more often used because they help individuals with depression or other mental illnesses get better much more quickly. But they may have many adverse effects that limit therapeutic treatment. In this era, herbal drugs are highly preferred because of their good antioxidant potential and show lesser side effects which may offer an advantage in terms of safety and tolerability, possibly by improving patient compliance.

Manilkara zapota belongs to the family Sapotaceae and is assigned various properties in the traditional system of Indian medicine.[5] Chemical composition analysis of sapota juice revealed that it is one of the prosperous sources of sugar, proteins, ascorbic acid, phenolics, carotenoids, and minerals like copper, zinc, calcium and potassium. Apart from this sapota showed potential antioxidant activity against free radicals and superoxide radicals. Methanolic extract of *Manilkara zapota* leaves has been investigated and proven for the presence of the major classes of potential phytochemicals such as triterpenes, alkaloids, polyphenols, flavonoids, anthraquinones, saponins and tannins.[7] Sapota potentially inhibit free radical-mediated lipid peroxidation. Multiple radical scavenging effects of sapota were found to be due to its nutraceutical component which is ascorbic acid, carotenoids, phenolics, etc.[5]

There have been a number of studies which have evaluated the role of antioxidants that plays a prominent role in controlling the oxidative stress which is the leading cause of a variety of health problems like "Major depressive disorder" is one of them. Methanolic leaf extract of sapota is rich in active constituents like phenolics, ascorbic acid, carotenoids, etc., which imparts good antioxidant potential to sapota. Hence, this herbal source has been selected for the present study to analyse its antidepressant potential.

This research mainly aimed at evaluating the antidepressant activity of the methanolic extract of *Manilkara zapota* leaves in experimental animal models.

MATERIALS AND METHODS

Requirements

Vertical plexiglass cylinder, thread, tape, methanol (as a solvent for extraction), normal saline, distilled water, beakers, glass rod, containers, Whatman filter paper, Imipramine (standard drug) and Oral feeder (for oral drug administration).

Animals

Albino mice irrespective of their sex were selected for the study weighing 26- 30 gm and isolated in the experimental room at temperature not exceeding 26°C, controlled humidity conditions with alternative 12 hours day and night cycles and caged in proper housing. The mice were fed with food pellets and sufficient amounts of distilled water *ad libitum*. The day prior to the experimental activity the mice were kept for overnight fasting (free access to water) for 12 hours. All the studies were carried out in accordance with the ethical standards of CPCSEA guidelines.

Collection of plant materials and Extraction process

The fresh leaves of *Manilkara zapota* (Chikko) were collected from the local areas of Mangalore and authenticated by Dr Krishma Kumar G, department of botany, Mangalore University, Konaje. The leaves were cleaned using a damp cloth to remove dust particles and kept inside a room with no sunlight to remove moisture and water content (shade drying) for 15 days. The leaves after being completely dried were powdered in a blender and the powder obtained was sieved through a 22/44 sieve, the fine powder was collected and stored in a tightly closed container at room temperature for future use.

The fine powder was weighed and set for extraction by maceration method by using methanol as a solvent for 48 hours by constant stirring every 2 hours. The mixture was filtered using Whatman's filter paper, the filtrate was collected in a beaker to evaporate by air drying for 6-7 days. The semisolid form of concentrated Methanolic extract of *Manilkara zapota* (MEMZ) leaves was collected and stored in an air-tight container and stored in a refrigerator for further use.

Acute toxicity studies

The methanolic extract of *Manilkara zapota* leaves (MEMZ) was conducted for acute oral toxicity studies. For this procedure, female mice have been chosen, and acute oral toxicity studies were carried out according to OECD guidelines 423. Different doses of extract (50, 100, 300, 1000, and 2000 mg/kg) were prepared and administered via the oral route. Animals were observed for behavioural changes and mortality for 14 days. The extract was devoid of any toxicity to the mice when given with above doses up to 2000 mg/kg. Thus, a further experiment was conducted by choosing the one by-tenth of the maximum dose and doubling doses like 200 mg/kg and 400 mg/kg doses were used for evaluation of the antidepressant activity.

Experimental design

Forced swim test and Tail suspension test these two models were chosen for the evaluation of the antidepressant activity of the methanolic leaf extract of *Manilkara zapota*. In each experimental model, the mice of either sex were taken for study and divided into 4 groups. Each group contained 5 mice (n=5). The animal groups were named as control, standard, test-1 and test-2. Firstly, each group of animals were tested for their immobility time and was noted without administration of any drug or saline, which was considered as the response of animals before administration. Then all the groups were administered orally (p. o) with their respective doses.[8] Figure 1 indicates the oral administration of drugs to the animals.

Figure 1: Oral administration of drugs to animals

GROUP 1: Control – administered with normal saline at a dose of 10 ml/kg p. o

GROUP 2: Standard - administered with standard drug (Imipramine), 4 mg/kg p. o

GROUP 3: Test 1- administered with MEMZ 200 mg/kg p. o

GROUP 4: Test 2- administered with MEMZ 400 mg/kg p. o

One hour later to the administration again animals were tested for their immobility time and responses were noted as after drug administration. The percentage reduction in immobility was calculated by using the below formula.

% Reduction in mean immobility = Mean immobility time of mice before drug administration – Mean immobility time of mice after drug administration / Mean immobility time of mice before drug administration x 100

Antidepressant models

Forced swim test model

During the test, each animal was placed inside a vertical plexiglass cylinder (40 cm in height and 18 cm in diameter), containing water up to a height of 15 cm maintained at 24-25°C. The test records the behavioural action of mice in the water with the immobility of limbs where the mice completely stop movements and remains floating without struggling which depicts the depressive action. The test duration for each mouse takes 6mins, where the initial 2 mins after placing mice in water are not recorded due to aggressive swimming and left to stabilize to assume an immobile posture. The next 4 mins in the session correspond to the antidepressant activity of mice recording the complete immobility of the limbs. After the test, the mice are removed from the water and dried for 15 mins before being placed back into the cage. The antidepressant activity of the given drug confirms positive with decreased duration of immobility.[9]

Tail suspension test model

In this test model, each mouse was suspended from the edge of the 58cm height table top with the help of an adhesive tape placed approximately 1 cm from the tip of the tail. The test records the immobility of limbs during the vertical inverted suspended position of the mice for a duration of 5mins where there is no movement shown by the mice, except for respiration. The result of the test was compared with responses before and after drug administration in each group. The decreased immobility activity confirms the test to be positive.[9]

Statistical analysis

The results obtained were analysed using Graph-prism software. The data was compiled and expressed in Mean ± SEM by one-way ANOVA for analysis of variance followed by Dunnett's multiple comparisons, a P-value of < 0.05 was considered as significant.

RESULTS

The methanolic extract of MEMZ (200 mg/kg and 400 mg/kg) was studied and observed for the changes in the duration of immobility along with the standard drug imipramine (4 mg/kg) using two models; Forced swim test model (FST) and tail suspension model (TST). In both the models, standard Imipramine and two test doses of MEMZ 200 mg/kg and 400 mg/kg were produced significant reduction ($p<0.0001$, $p<0.0005$ and $p<0.0001$) when compared with the controlled group that was treated with normal saline.

In forced swim test the standard drug Imipramine reduced the immobility time to 256.14 ± 4.938 seconds, test dose 1 (MEMZ 200 mg/kg) reduced the immobility time to 267.22 ±11.386 seconds and mice treated with test dose 2 (MEMZ 400 mg/kg) reduced the immobility time to 253.22 ±7.816 seconds when compared with control which was showing immobility time 303.01±17.178 seconds. The percentage immobility was calculated by comparing the animal behaviour before the drug administration and after the drug administration. The % reduction in immobility time was recorded for control, standard, test 1 and test 2 groups were 0.632%, 19.99%, 10.43% and 15.68% respectively. The results of the forced swim test were given in table 1. Figure 2 indicates the performance of the Forced swim test model in laboratory animals. The results are depicted in graphical representation have shown in figure 4.

Table 1: Forced Swim test model - Duration of immobility in seconds before and after administration of drug and percentage reduction in immobility

Treatment	Dose (mg/kg)	Forced swim test model, Mean duration of immobility in seconds in each group of animals (n= 5)			Statistical analysis Mean ±SEM (After administration)
		Before drug administration	After drug administration	Percentage reduction in Immobility (%)	
Control group (Normal saline)	10 ml/kg	305.010	303.08	0.632	303.008 ±17.179
Standard drug (imipramine)	4 mg/kg	320.150	256.140	19.99	256.140± 4.938****
Test dose 1 (MEMZ 200)	200 mg/kg	298.230	267.20	10.43	267.220 ±11.386***
Test dose 2 (MEMZ 400)	400 mg/kg	300.330	253.220	15.68	253.220±7.816****

Values are expressed as mean ± SEM. Comparison between control and all the other treatment groups. Statistical tests were done by using one-way ANOVA followed by Dunnett's multiple comparison test, *p<0.05, **p<0.01, ***p<0.0005 and ****p<0.0001.

In tail suspension test model, the duration of immobility time in seconds was observed in standard drug Imipramine, the test doses 200 mg/kg and 400 mg/kg treated groups were 222.46 ± 5.779, 233.96± 7.477 and 224.458 ± 4.929 respectively when compared to control response 263.402 ± 13.437. The % reduction in immobility time was recorded for control, standard, test 1 and test 2 groups were 0.605%, 18.97%, 16.10% and 23.48% respectively. The results of the tail suspension model were given in table 2. Figure 3 indicates the performance of the Tail suspension test model in laboratory animals.

Figure 2: Forced swim test model

Figure 3: Tail suspension test model

Table 2: Tail suspension test model - Duration of immobility in seconds before and after administration of drug and percentage reduction in immobility

Treatment	Dose (mg/kg)	Tail Suspension test model, Mean duration of immobility in seconds in each group of animals (n=5)			Statistical analysis Mean ± SEM (After administration)
		Before drug administration	After drug administration	Percentage reduction in immobility (%)	
Control group (Normal saline)	10 ml/kg	265.005	263.402	0.605	263.402 ± 13.438
Standard drugs (imipramine)	4 mg/kg	274.566	222.466	18.97	222.466 ± 5.779****
Test dose 1 (MEMZ 200)	200 mg/kg	278.860	233.960	16.10	233.960±7.477***
Test dose 2 (MEZ 400)	400 mg/kg	293.358	224.458	23.48	224.458±4.930****

Values are expressed as mean ± SEM. Comparison between control and all the other treatment groups. Statistical tests were done by using one-way ANOVA followed by Dunnett's multiple comparison test, *p<0.05, **p<0.01, ***p<0.0005 and ****p<0.0001.

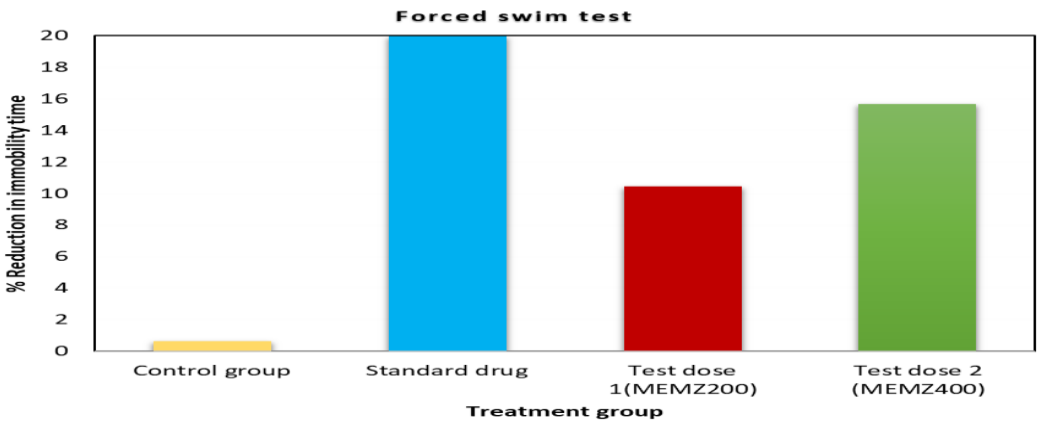

Figure 4: Graphical representation of results of forced swim test

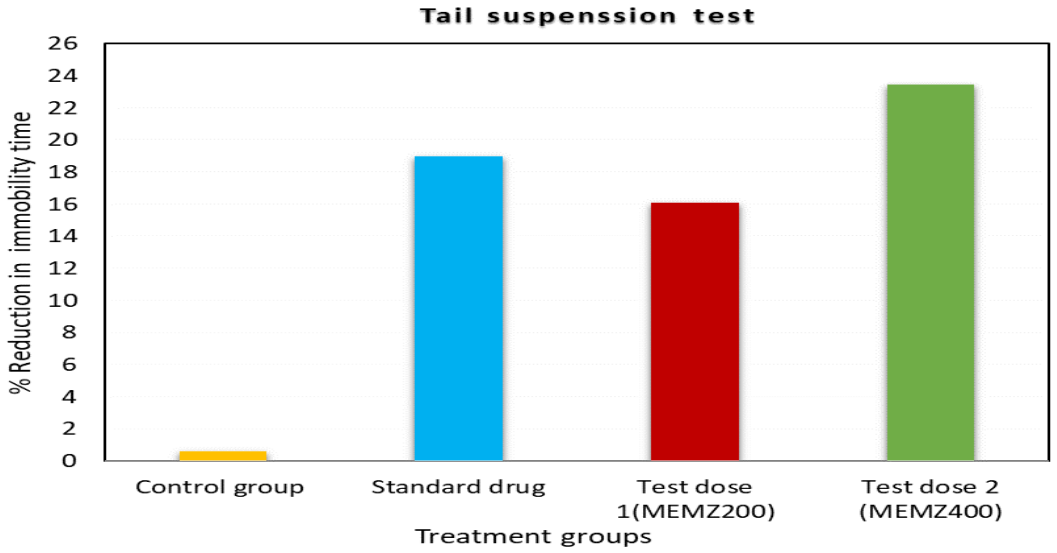

Figure 5: Graphical representation of results of tail suspension test

DISCUSSION

Mental ill health is a worldwide disorder in the present day causing various disturbances in the human race, affecting the quality of life and social interactions. Psychiatric conditions like sadness, loss of self-confidence, insomnia, confusion, and demotivation lead to issues like depression. The conventional therapy preferred for depression shows various side effects causing decreased patient compliance. The present study indicates the antidepressant activity of methanolic leaf extract of *Manilkara zapota* was carried out by using two different experimental animal models: Forced swim test (FST) and Tail suspension test (TST) models. These models are preferred as they are easy and give efficient value to immobility. Methanolic leaf extract of Manilkara zapota showed a marked decrease in immobility at the dosage of 200 mg/kg and 400 mg/kg in the Forced swim test and Tail suspension test models, the percentage reduction for standard (imipramine), test dose-1 and test dose-2 responses in Forced swim test and TST were 19.99%, 10.43%, 15.68% and 18.97%, 16.10%, 23.48% respectively. The higher dose (400 mg/kg) of MEMZ leaf extract has shown better results compared to the smaller dose (200 mg/kg) of MEMZ leaf extract. In the tail suspension test, the higher dose of MEMZ leaf has shown comparably better results than the standard Imipramine drug. The statistical analysis was conducted by one-way ANOVA was compiled and analysed to compare the data with control results. The p-values were significant for both test doses.

CONCLUSIONS

Oxidative stress can be a leading cause of depression in human beings. Natural products play a major role in controlling depression symptoms. Phytoconstituents such as ascorbic acid, phenolic compounds and zinc are rich in *Manilkara zapota* which acts as potent free radical scavengers to treat depression. Therefore, the data obtained from the study has revealed that the Methanolic extract of *Manilkara zapota* with doses of 200 mg/kg ($p < 0.0005$) and 400 mg/kg ($p < 0.0001$) have shown significant antidepressant activity similar to that of standard drug Imipramine 4 mg/kg ($p<0.0001$) when compared with control. So, it has been suggested for further molecular-level studies are needed for further evaluation of the site of the mechanism of action and the role of phytoconstituent in increasing the levels of neurotransmitters like dopamine, serotonin, norepinephrine which plays a vital role in mood stabilization.

ACKNOWLEDGEMENTS

The authors are thankful to Karavali College of Pharmacy for carrying out the project in the college laboratory, and sincerely thank our management for approval to submit the research paper to the journal.

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Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.