International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 14, Special Issue 4, 2022

Original Article

THE ANTIDEPRESSANT ACTIVITY OF *MUNTINGIA CALABURA* L. LEAVES ETHANOL EXTRACT IN MALE SWISS-WEBSTER MICE

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Received: 17 Jul 2022, Revised and Accepted: 05 Sep 2022

ABSTRACT

Objective: The purpose of this study was to determine the activity and optimal dose of *Muntingia calabura* L. ethanol extract as an antidepressant using Forced Swimming Test (FST), Tail Suspensions Test (TST), and Open Field Test (OFT).

Methods: Twenty-five male Swiss-Webster mice were randomly divided into five groups. Group 1 as a control group, got vehicle (1% w/v CMC). As a positive control, group 2 got 0.052 mg/20 g mice BW of fluoxetine. Groups 3-5 received 0.7, 1.4, and 2.8 mg/20 g mice BW orally of *M. calabura* leaves extract. In the FST and TST, immobility time was the test parameter. In the OFT, the test parameters were the grooming duration, the duration of entries into the central square, and the duration of rearing.

Results: The results showed that all test dose groups had antidepressant activity and test dose 3 was the optimal dose marked by a decreasing in immobility time in the FST and TST with an average percentage decrease of 33.70 % and 13.95%. In the OFT method, it is characterized by increasing in the average percentage of the duration of central square and rearing by 63.46% and 76.25%, respectively and a decreasing in the average percentage of grooming duration by 27.57%.

Conclusion: From these results show ethanol extract of *M. calabura* leaves has antidepressant activity and the test dose of 3 (2.8 mg/20 g mice BW) is the optimal test dose.

Keywords: Antidepressants, *Muntingia calabura* L, Forced swimming test, Tail suspensions test, Open field test

DOI: https://dx.doi.org/10.22159/ijap.2022.v14s4.PP02 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Depression is an emotional disorder or bad mood characterized by prolonged sadness, hopelessness, feelings of guilt and meaninglessness so that all mental processes (thinking, feeling, behaving) can affect motivation to do activities in daily life and relationships interpersonal [1]. Depression and anxiety are common mental disorders with the highest prevalence. More than 200 million people worldwide (3.6% of the population) suffer from anxiety. Meanwhile, the number of people with depression is 322 million people worldwide (4.4% of the population) and almost half of them come from Southeast Asia and West Pacific regions [2]. The prevalence of emotional disorders in the population aged 15 y and over increased from 6% in 2013 to 9.8% in 2018. The prevalence of people with depression in 2018 was 6.1%. The prevalence of suicide in the population aged 15 y and over (N=722,329) was 0.8% in women and 0.6% in men [3].

Pharmacological therapy commonly used is second generation antidepressants SSRI (Selective Serotonin Reuptake Inhibitors) class, namely fluoxetine; this drug is a class of drugs that specifically inhibits serotonin uptake, causing an increase in neurotransmitters in the synaptic cleft, which ultimately results in greater postsynaptic neuron activity [4]. About 80% of people in developing countries around the world rely on traditional therapies for their primary health care, and about 85% of traditional medicine involves plant extracts [5]. *Muntingia calabura* leaves are widely used as a traditional medicine because of the content of *M. calabura* such as flavonoids, saponins and tannins [6]. Secondary metabolites present in the crude extract of *M. calabura* leaves, generally flavonoids, are known to be reliable for various bioactivities [7].

Empirically, *M. calabura* extract leaves is a medicinal plant that is often used by the public as a cough medicine, jaundice and gout [6]. In research, it was found that Muntingia calabura extract fruit can reduce uric acid levels in mice [8]. Another research on *M. calabura* leaves extract in humans was carried out by it was found that *M. calabura* leaves extract can reduce blood glucose levels [9]. Based on related research, it was stated that the *M. calabura* extract leaves with doses of 0.092 g, 0.184 g, and 0.368 g had analgesic activity [10]. *M. calabura* leaves ethanol extract has activity in reducing total cholesterol levels in the blood at a dose of 50 mg/kg BW [11]. Based on this description, researchers are interested in testing the antidepressant activity of *M. calabura* leaves ethanol extract on male white mice of Swiss Webster strain using the Forced Swimming Test (FST), Tail Suspensions Test (TST) and Open Field Test (OFT) methods.

MATERIALS AND METHODS

Plant material

The test material in this study was *M. calabura* leaves extract. *M. calabura* leaves were obtained from Sirnagalih Village, Lemahsugih District, Majalengka Regency in January 2022. The *M. calabura* leaves samples used were old leaves [12]. Plant species were determined at the Jatinangor Herbarium, Padjadaran University (Jatinangor, Indonesia) (No. 22/HB/01/2022).

Chemicals and reagents

The chemicals used are fluoxetine 20 mg (PT. Dexa medica), Na-CMC technic (PT. Dipa Prasada Husada), 96% ethanol technic (PT. Dipa Prasada Husada).

Experimental animals

The experimental animals were male Swiss-Webster mice with an average body weight of 20-30 g, and healthy and normal behavior (can be seen from the way the mice walk and habits in general). Mice were adapted for one week in cages with standard feed and water (*ad libitum*) before being treated. Induction of mild stress with a light and dark cycle every 24 h for one week. The research was carried out in accordance with the protocol standard of the Research Ethics Commission of University Bakti Tunas Husada Tasikmalaya, No.022/ec.02/kepk-bth/IV/2022.

Extraction

The dried powder of *M. calabura* leaves extract was weighed as much as 500 g and then macerated with 96% ethanol. Maceration was carried out

at room temperature for 3x24 h and occasionally stirred. The maceration filtrate is then filtered. The filtrate was then concentrated with a rotary evaporator at 60 °C. Then it was evaporated in a water bath until a concentrated extract was formed [13].

Phytochemical screening

Phytochemical screening of samples to determine the class of chemical compounds contained in *M. calabura* leaves extract includes an examination of alkaloids, flavonoids, tannins, polyphenols, saponins, steroids/terpenoids, quinones, and monoterpenes/sesquiterpenes.

Simplicia quality standards

Simplicia standardization, including determination of loss on drying, total ash content, water-soluble extract content, and ethanol-soluble extract content, was carried out.

Antidepressant activity testing

Twenty-five male Swiss-Webster mice were randomly divided into five groups. Group 1 as a control group, got a vehicle (1% w/v CMC). As a positive control, group 2 got 0.052 mg/20 g mice BW of fluoxetine. Groups 3-5 received 0.7, 1.4, and 2.8 mg/20 g mice BW orally o f *M. calabura* leaves extract. Test of FST, TST, and OFT were carried out on all groups of test animals with a one-week interval for each method. Before testing, the test animals were adapted for one week and given stress induction with a light and dark cycle every 24 h for one week. In each method, pre-test and post-test were carried out on day 8 after stress induction, the gap between the pre-test and post-test was 6 h, and the test preparation was administered 30-45 min before the post-test. The test preparation was given orally once a day [14].

Forced swimming test (FST)

Mice were placed in a cylindrical container (diameter 20 cm high 30 cm) filled with water (25 °C) with a water depth of 15 cm. The water depth was chosen so that the animal had to swim or float without the mouse's hind legs or tail touching the bottom. The test is carried out for 6 min [15]. Mice are said to be immobilized if they only move to keep their heads above the water, mice are said to be mobile if they are actively swimming and climbing [14].

Tail suspension test (TST)

This method is used to see the behavior and mood of the test animals. In this method, the mice were hung by the tail for 5 min and then the immobility duration was measured. Mice hanging by their tails are intrinsically trying to get away from this unpleasant state. However, as a result of failed attempts to escape, the mice fell into despair and became immobile [16].

Open field test (OFT)

This method is used to test motor function and behavior by measuring spontaneous activity in the open which is associated with depression. The data obtained in the form of rearing is marked by the test animal standing on both hind legs, grooming is marked by the test animal cleaning the claws, nose, face, head, body, legs, tail and genitals, and the central square is seen when the mice pass through the center of the box. Mice were put into a box without a lid and observed for 6 min then measured depression through the duration of rearing, grooming and central square activities [17].

Data analysis

The research data were analyzed using statistics which included the normality test, homogeneity test, ANOVA test, and LSD (Least Significant Differences) test; the degree of confidence used was 95%. The type of data obtained in the form of interval data with observations of immobility time on the FST and TST methods, duration of rearing, grooming and central square in the OFT method.

RESULTS

The results of the extraction of 500 g of simplicia powder obtained 114.173 g of concentrated extract, with a yield was 22.8%. Phytochemical analysis of *M. calabura* leaves extract was carried out on simplicia and extract. The results of phytochemical analysis obtained from simplicia and ethanol extract of *M. calabura* leaves can be seen in table 1. The results of the standardization of simplicia and ethanol extract of *M. calabura* leaves can be seen in table 2.

Table 1: Phytochemical analysis

Secondary metabolites	Simplicia	Extract
Flavonoids	+	+
Tannins	+	+
Saponins	+	+
Polyphenols	+	+
Quinones	+	+
Alkaloids	-	-
Steroids	-	-
Sesquiterpenes	+	+

(+) detected (-) not detected

Table 2: Quality standardization of simplicia and leaves extract

Standardization parameters	Results	(%)	Standard (%)
	Simplicia	Extract	
Loss on drying	9.6	7.8	<10
Total ash content	4.74	5.7	<6
Water soluble content	14.16	-	>12
Ethanol soluble content	22.88	-	>12.5
Water content	7.31	6	<10

Note: Standards are taken from FHI ed II, 2017, FI ed VI, 2022

The duration of immobility time in the FST method can detect psychomotor activity and hopelessness (symptoms of depression) in test animals, which play a role in psychomotor activity and hopelessness is the concentration of serotonin and glutamate (neurotransmitters); the higher the concentration of serotonin and glutamate, there will be an increase in locomotor activity and a decrease in hopelessness in test animals and vice versa [18]. FST results in each group can be seen in table 3.

Table 3: Forced swimming test result

Mice no	Immobility time (s)				
	Negative control	Positive control	Dose 1	Dose 2	Dose 3
1	99.1	63.4	90.2	72	61.18
2	97.3	57.6	95.7	68.4	60.84
3	98.5	55.3	80.1	72.1	63.45
4	83.2	62.5	89.83	70.6	64.4
5	94	69.3	86.1	70.5	63.15
mean±SD	94.42±5.88	61.62±4.88	88.38±5.15	70.8±1.42	62.6±1.37

Table 4: **Effect of *M. calabura* leaves extracts and fluoxcetine on duration of immobility by FST**

Group	Average immobility decrease (%)±SD	P value
Positive Control	34.73±4.88	p>0.05
Test Dose 1	6.41±5.15	p<0.05
Test Dose 2	25.01±1.42	p<0.05
Test Dose 3	33.70±1.37	p>0.05

Note: p>0.05 was not signicantly different, p<0.05 was signicantly different

Based on the results of statistical analysis with LSD at 95% confidence level, the positive control (fluoxetine) was significantly different from the test dose 1 (p<0.05). Test dose 2 significantly different from the positive control (p<0.05). Test dose 3 was not significantly different from the positive control (p>0.05); the average percentage decreased by 34.70% in the positive control.

The duration of immobility in the TST method can detect the state of behavior and mood (symptoms of depression) of test animals [16]. TST results in each group can be seen in table 5.

Based on the results of statistical analysis with LSD at 95% confident level, the positive control (fluoxetine) was significantly different from the test dose 1 (p<0.05). Test dose 2 and test dose 3 was not significantly different from the positive control (p>0.05). Test dose 2 and test dose 3 were able to reduce the duration of immobility with an average percentage of decrease of 13.948%; 13.95%.

The grooming response is a response that indicates fear and anxiety in test animals; the higher the duration of grooming, the more depressed, and vice versa, the smaller the duration of grooming, the less depressed [17]. *Open Field Test* (OFT) of grooming duration results in each group can be seen in table 7.

Table 5: Tail suspension test (TST) results

Mice no	Immobility time (s)				
	Negative control	Positive control	Dose 1	Dose 2	Dose 3
1	138.67	116.14	130.1	117.1	119.56
2	135.74	120.45	133.4	113.98	117.4
3	136.9	112.1	140	120.99	113.65
4	140.4	103.1	132.6	133.1	120.99
5	150.1	118.45	123.45	126.97	109.99
mean±SD	140.36±5.12	114.048±6.14	131.91±5.35	122.482±6.88	116.318±4.02

Table 6: **Effect of *M. calabura* leaves extracts and fluoxetine on duration of immobility by TST**

Group	Average percentage decreased immobility (%)±SD	P value
Positive Control	18.75±6.14	p>0.05
Test Dose 1	13.94±5.35	p<0.05
Test Dose 2	13,948±6.88	p>0.05
Test Dose 3	13.95±4.02	p>0.05

Note: p>0.05 was not signicantly different, p<0.05 was signicantly different

Table 7: Open field test (OFT) result

Mice no	*Grooming duration (s)*				
	Negative control	Positive control	Dose 1	Dose 2	Dose 3
1	24.89	16.91	19.6	19	16.77
2	25.98	17.14	18.9	18.4	18.89
3	23.9	17	19.89	19.8	17.81
4	22.99	18.19	20.9	18.45	16.89
5	25.48	18	19.29	18.99	18.9
mean±SD	24.648±1.08	17.448±0.54	19.716±0.68	18.928±0.51	17.852±0.92

Table 8: Effect of *M. calabura* leaves extracts and fluoxetine on the duration of grooming

Group	Average percentage decrease in grooming duration (%)±SD	P value
Positive Control	29.21±0.54	p>0.05
Test Dose 1	20.00±0.68	p<0.05
Test Dose 2	23.20±0.51	p<0.05
Test Dose 3	27.57±0.92	p>0.05

Note: p>0.05 was not signicantly different, p<0.05 was signicantly different

Grooming duration based on the results of statistical analysis with LSD at 95% confident level, that the positive control (fluoxetine) was significantly different from the test dose 1 and test dose 2 ($p<0.05$). Test dose 3 was not significantly different from the positive control ($p>0.05$); the average percentage decreased by 29.21% in the positive control.

Central square response shows locomotor activity, anxiety or depression in test animals; the higher the duration of the central square, the less depression, and vice versa, the smaller the duration of the central square, the more depressed [19]. OFT result on central square duration in each group can be seen in table 9.

Table 9: Open field test (OFT) result

Mice no	Central square duration (s)				
	Negative control	Positive control	Dose 1	Dose 2	Dose 3
1	8.93	16.67	9.89	11.2	14.11
2	7.85	16.98	10.1	12.4	15.34
3	8.97	12.97	11.16	15.29	13.3
4	9.98	13.4	11.67	15.96	16.6
5	7.98	15.69	12.08	13.86	12.1
mean±SD	8.742±0.77	15.142±1.66	10.98±0.86	13.742±1.77	14.29±1.57

Table 10. Effect of *M. calabura* leaves extracts and fluoxetine on the duration of entries into the central square

Group	Increased duration of central square (%)±SD	P value
Positive Control	73.20±1.66	p>0.05
Test Dose 1	25.60±0.86	p<0.05
Test Dose 2	57.19±1.77	p>0.05
Test Dose 3	63.46±1.57	p>0.05

Note: $p>0.05$ was not signicantly different, $p<0.05$ was signicantly different

On the duration of Central square based on the results of statistical analysis with LSD at 95% confident level, the positive control (fluoxetine) was significantly different from the test dose 1. Test dose 2 and test dose 3 was not significantly different with positive control ($p<0.05$). The test dose 2 and test dose 3 were able to increase the duration of Central square with an average percentage decrease of 57.19% and 63.46%.

The rearing response indicates an exploratory response related to anxiety; the higher the rearing duration, the less depressed, and vice versa, the smaller the rearing duration, the more depressed [17]. *Open Field Test* (OFT) result of Rearing duration in each group can be seen in table 11.

Rearing duration based on the results of statistical analysis with LSD at 95% confident level, that the positive control (fluoxetine) was significantly different from the test dose 1, test dose 2 and test dose 3 ($p<0.05$). Test dose 1 was not significantly different from test dose 2 ($p>0.05$). But when viewed from the percentage value, test dose 3 has the highest percentage value, which is 76,25%.

Table 11: Open field test (OFT) result

Mice no	Rearing duration (s)				
	Negative control	Positive control	Dose 1	Dose 2	Dose 3
1	20.6	37.38	26.9	28.48	35.18
2	21.4	39.21	27.1	28.85	32.4
3	19.4	39.45	27.67	27.29	35.14
4	18.56	38.16	28.10	28.86	35.88
5	19.2	37.9	28.19	29.29	36.17
mean±SD	19.832±1.02	34.42±0.79	27.592±0.52	28.554±0.68	34.954±1.34

Table 12: Effect of *M. calabura* leaves extracts and fluoxetine on duration of rearing

Group	Increase in rearing duration (%)±SD	P value
Positive Control	73.55±0.79	p<0.05
Test Dose 1	39.12±0.52	p>0.05
Test Dose 2	43.97±0.68	p>0.05
Test Dose 3	76.25±1.34	p<0.05

Note: $p>0.05$ was not signicantly different, $p<0.05$ was signicantly different

DISCUSSION

Antidepressant testing was carried out using three methods, namely FST, TST, and OFT. In the FST and TST methods, the parameters observed were immobility time in mice from each test group. Immobility time in mice can be assumed as a state of despair in humans and is one of the symptoms of depression that decreases interest and motivation [20]. In the OFT method, the parameters observed were in the form of grooming duration (indicating fear and anxiety in test animals), central square (indicates locomotor activity) and rearing (indicating an exploratory response) [19].

Antidepressant testing was carried out using three methods to obtain maximum test results. Prior to testing, the test animals were acclimatized for 1 w so that the test animals were able to adapt to the environment and weighing the body weight of mice aims to determine changes in body weight of mice during acclimatization, Pharmacological screening is also carried out to identify the health of the test animals, including observing the behavior and sensory activities of the test animals. Then the light and dark cycle stress induction was carried out, namely 24 h of light and 24 h of darkness for seven days to stress the test animals.

This antidepressant activity is influenced by the concentration of serotonin and glutamate; this may be due to antidepressant

compounds that inhibit GABA-nergic receptors so that the release of glutamate increases or because of an increase in serotonin levels which modulate the release of corticotropin hormone which refers to the release of adrenal hormones so that the energy produced is sufficient to stimulate movement (locomotor) [17].

The positive control used was fluoxetine, which belongs to the class of SSRI antidepressant drugs with the mechanism of action of inhibiting the uptake of serotonin which has been secreted in the synapse, so that serotonin levels in the brain increased. Increased levels of serotonin in the synapse are believed to be useful as antidepressants [21]. The benefits of SRRI drugs compared to other antidepressants are high tolerability, minimal side effects. Thus, SSRI antidepressants are used as the first line in the treatment of depression [4]. Average peak levels are seen in 2-8 h and have a half-life of about 16-36 h. Adult dose of fluoxetine 20-80 mg/day [22].

M. calabura leaves ethanol extract has antidepressant activity as indicated by a decrease in the duration of immobility in FST and TST methods, an increase in central square, rearing and a decrease in grooming in OFT method. Optimal antidepressant activity was shown at a test dose of 3 (2.8 mg/20 g of body weight in mice) in all test methods it can be seen in fig. 1 this was influenced by the concentration of the dose, because the higher the dose, the more activity produced [15]. The use of the FST, TST and OFT methods provide effective results for antidepressant testing.

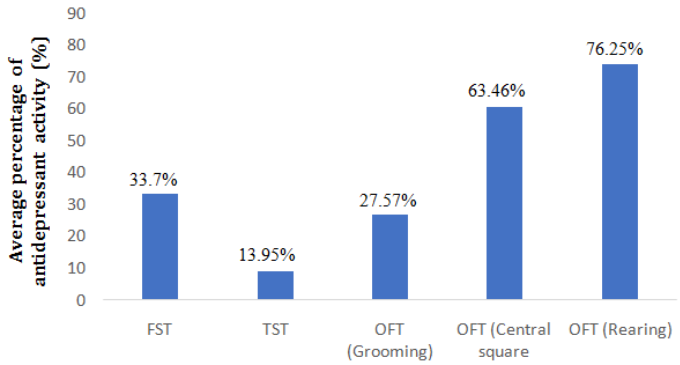

Fig. 1: Antidepressant activity test dose 3 on test method

All test dose groups of *M. calabura* extract leaf ethanol extract have antidepressant activity, presumably from the flavonoid compound, namely quarcetin [23]. In addition, it is proved by which stated that kaemferol and quarcetin compounds isolated from *Opuntia ficus-indica* var. saboten at a dose of 30 mg/kg/day can provide an antidepressant effect in mice [24]. Active compounds in plants that can be used as antidepressants include valerenic acid, methyl piperate, guinensin, piperlongumin, piperine, kaemperol, quarcetin, eugenol, curcumin and myristicin [25].

Secondary metabolite compounds in simplicia and extract of *M. calabura* leaves besides flavonoid compounds, there are other compounds, namely tannins, saponins, polyphenols, quinones and terpenoids, which have antidepressant activity. The flavonoid narigenin from grapes works by increasing serotonin (5-HT), norepinephrine (NE), and Brain-Derived Neurotrophic Factor (BDNF) levels as well as decreasing monoamine oxidase (MAO) activity. Tannins from the Terminalia chebula plant exert a neuroprotective effect and increase the availability of monoamines in the brain. Saponins from ginseng plants exhibit antidepressant effects by influencing the Brain-Derived Neurotrophic Factor (BDNF) signaling pathways, Hypothalamic Pituitary Adrenal (HPA) axis, and hippocampal neurogenesis, as well as increasing monoamine levels [26].

CONCLUSION

Ethanol extract of *Muntingia calabura* leaves has antidepressant activity and the test dose of 3 (2.8 mg/20 g mice BW) is the optimal test dose because it shows an average percentage decrease in immobility time of 33.70% in the FST method, the TST method showed an average percentage decrease in immobility time of 13.95% and the OFT method showed an increase in the duration of the central square and rearing by 63.46% and 76.25%, also in grooming showed a decrease in grooming duration by 27.57%.

ACKNOWLEDGEMENT

This article was presented at the 2022 BITP.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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