

PHARMACOLOGICAL INVESTIGATION OF NYCTANTHES ARBOR-TRISTIS STEM FOR ANTIDEPRESSION ACTIVITY

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Received: May 24, 2025

Accepted: Jun 23, 2025

Published: Jun 24, 2025

Abstract: Mental depression adversely affects an individual's mood, cognition, physical health, and behavior, often manifesting as a chronic condition. The biological and mental factors are also associated with symptoms of depression. The impairment of cognition, behavior, and desire are biological manifestations, while emotional markers encompass enigma, apathy, and pessimism, alongside diminished self-esteem characterized by feelings of guilt, inadequacy, and unattractiveness, as well as indecisiveness and a lack of ambition. Patients with significant depression have symptoms indicative of alterations in brain monoamine neurotransmitters, notably norepinephrine, serotonin, and dopamine. Given that all synthetic antidepressants exhibit diverse undesirable effects and hazardous interactions, our objective is to investigate the efficacy of medicinal plants in the treatment of depression. The current study proposes that the stem of *Nyctanthes arbor-tristis* exhibits greater efficacy in managing depression due to its higher concentration of phytochemical components. These phytochemical compounds exhibit antidepressant action, as demonstrated by prior scientific research. Therefore, the selected plant section possesses the most potential phytochemical constituents to substantiate the proposed research.

Keywords: *Nyctanthes Arbor-tristis*, Antidepressant activity, Depression, Mental disorder

Introduction: Depression involves many symptoms, and the person feels guilty, sad, anxious, inferior, helpless, and uneasy. People who have been diagnosed with depression are not able to carry out their tasks in a proper manner; this leads to a reduction in their appetite, poor concentration, inability to make decisions, forgetfulness, a decrease of

motor function, and withdrawal from society [1]. Depression is a neurometabolic type disorder, alteration in the levels of various hormones like serotonin, dopamine, and noradrenaline (neurotransmitters) is the hallmark of depression. The treatment approaches towards depression involves the use of psychotherapy, antidepressant drugs, stress management methods, and some complementary drugs (phytotherapy) [2]. The drug therapy of depression by allopathic drugs carries the risk of unwanted side effects, and in most cases, the drugs therapy proceeds lifelong. Medicinal herbs are used vastly in Indian subcontinent and various other countries. Flora and fauna consist of enormous number of species of plants from various parts from around the globe. Since ancient times, natural plants and their preparations have been used and approximately 80% of the population (according to senses from WHO) are dependent on medicinal plants. [3] There are about 4.25 million species of flowering plants and over 50,000 medicinal plants. Various research was performed to explore the possible sources of therapeutic activities for curing numerous diseases [4]. *Nyctanthes arbor-tristis* (Greek words, 'Nykhta'-Night and 'anthos'-flower) is a well-known herbal medicinal plant and belongs to the family Oleaceae. It is characterized as a type of small sacred ornamental tree of India and distributed from wild sub-Himalayan areas to Godavari. Commonly this medicinal plant is referred to as 'Harsinghar' or 'Night Jasmine' because flowers of this plant provide a pleasant and strong fragrance during night time [5]. This plant has been used in the Indian system of medicine and different parts of this plant are used for various pharmacological actions like antiamoebic activity, antipyretic activity, antiulcer activity, antihistaminic activity, anti-inflammatory activity immunostimulant activity, antiallergenic properties, antimalarial activity, analgesic activity, tranquilizing activity and purgative activity [6]. The active phytochemical constituents of this plant are friedelin, oleanolic acid, arborside A, arborside B and arborside C, Nyctanthic acid, β -monogentiobioside of α -crocin or crocin 3, triterpenoids lupeol, β -monogentiobioside- β -D-monoglucoside ester of α -crocin or crocin 2 and β -digentiobioside of α -crocin or crocin 1. These phytochemicals are present in different parts. The present study was undertaken for evaluation of the antidepressant activity of leaf, flower extract, and a combination of leaf and flower extract in an equal ratio of *Nyctanthes arbor-tristis* [7]. *Nyctanthes arbor-tristis* is a plant with numerous pharmacological activities and uses. It has been extensively studied for

pharmacological activities. Therefore, the study of antidepressant effects of various extracts of NT can prove to be beneficial in finding effective herbal treatment for depression. According to various studies, the safety margin of the plant was good, and there were no side effects related to plant [8]. Mental diseases encompass a range of issues, each characterized by distinct symptoms. They are typically defined by a combination of atypical thoughts, feelings, behaviors, and interpersonal relationships [9]. A mental disorder is a syndrome marked by clinically substantial disturbances in an individual's cognition, emotion, regulation, or behavior, indicating malfunction in the psychological, biological, or developmental processes that underlie mental functioning. Mental disorders are typically linked to distress or impairment in social, occupational, or other activities [10-13].

Material and Methods

Extraction & Phytochemical Screening: All chemicals and solvents were of analytical grade (AR Grade) and procured from Sigma Aldrich, Ranbaxy Fine Chemicals Ltd., LOBA Chemicals Ltd., S.D. Fine Chemicals Ltd., and Spectrochem Chemicals. Pre-coated TLC plates with a thickness of 0.2 mm containing silica gel 60 F254 were acquired from Merck. All solvents utilized for HPLC analysis were acquired from JT Baker and Fischer Scientific Ltd.

Collection and identification of plant material: The chosen plant material, *Nyctanthes Arborescens* stem, was procured from the local market in Jaunpur, Uttar Pradesh, India. *Nyctanthes arborescens*, also referred to as night jasmine or Parijat, is a sizable shrub or little tree characterized by aromatic, white blossoms that open during the night. *Nyctanthes arborescens* is a shrub or small tree reaching a height of 10 meters, characterized by its flaky grey bark.

Physicochemical Assessment: The physicochemical properties, such as ash values and extractive values, were investigated for the selected plant according to official methodologies and in accordance with WHO criteria. The total ash, acid-insoluble ash, water-soluble ash, extractive values, loss on drying, and pH were assessed for the stem of *Nyctanthes arborescens*.

Table 6.2: Physicochemical parameters of *Nyctanthes arbortristis* stem

S. No.	Physicochemical parameter values (% w/w)	<i>Nyctanthes arbortristis</i> stem
1	Total ash	12.01
2	Water soluble ash	3.21
3	Acid insoluble ash	0.26
4	Moisture content	2.14
5	Foreign organic matter determination	1.13

Table 6.3: Solvent extractive values (%w/w) of *Nyctanthes arbortrites* stem

S. No.	Name of extract	Extractive value <i>Nyctanthes arbortristis</i> stem
1	Alcohol soluble extractive value	14.12 % w/w
2	Water soluble extractive value	19.1 % w/w

Extraction Process of Drug: Extraction involves the separation of the bioactive component of plant tissues from the dormant moiety using specified solvents in standardized extraction techniques. Herbs were extracted progressively with hexane and methanol using the maceration process. The completely desiccated leaves of *Nyctanthes arbortristis* were coarsely pulverized and subsequently extracted with the non-polar solvent hexane for the defatting of the plant material. Two hundred grams of leaf powder were placed in a vessel and immersed in hexane for 24 hours; this method was repeated until full extraction was achieved. The plant material was thereafter immersed in methanol for 24 hours, and the technique was reiterated until full extraction was achieved. The methanol extract was filtered and concentrated using a rotary evaporator to achieve the final extract.

Preliminary phytochemical study of extracts: Qualitative assessment as the phytochemical analysis of any plant species is an essential process, as it provides first data regarding the existence of various chemical constituents and offers further

opportunities for future research on the specific plant species. The extracts obtained by extraction procedures were subjected to various chemical assays to identify the presence of specific classes of chemical ingredients.

In-vivo pharmacological screening (Anti-depressant activity): The animal experiments, male mice weighing about 100-125g were used. These mice had can be able to access laboratory feed and water under standard laboratory conditions. The animals used in the present study were maintained in accordance with the guidelines of National Institute of Nutrition, India and approved by Institutional Animal Ethics Committee (IAEC). Experiments performed by an observer who was unaware of the each treatment, were carried out between 1- 3p.m. For the behavioral test, different doses of the extract were separately suspended in a vehicle comprising 1% (w/v) tween 20 in distilled water and a standard drug (amitriptyline and fluoxetine) were given by gastric gavage once a day over a period of 1,3, 7, 14 and 21 days. Behavioral test was conducted 1 hour after the last treatment/administration.

A. Forced swimming test (FST):

Animal groups: The activity was performed with 42 total mice. We were randomly divided into 6 groups, each group having 6 mice. The mice of each group were treated accordingly treatment given in Table and treated as follows: Group 1 act as normal control and Group 2 have tween-20 suspensions and act as experimental control. Group 3-5 were orally administered with various doses of methanol extract of *Nyctanthes arbortristis* stem having three different doses. Group 3 treated as 75 mg extract/kg of body weight, Group 4 treated as 150 mg extract/kg of body weight, Group 5 treated as 300 mg extract/kg of body weight. The animals present in Group 6 and 7 received standard anti- depressant drug-amitriptyline and fluoxetine (10 mg/kg body weight). *Nyctanthes arbortristis* stem methanolic extract was suspended in a vehicle comprising 1% (w/v) tween 20 in distilled water (75, 150 and 300 mg extract/kg of body weight).

Table 1: Experiment animal groups (FST)

Animal groups	Treatment
Group 1	Normal control
Group 2	Tween-20 suspensions
Group 3	MNA (75mg/kg b wt.)
Group 4	MNA (150mg/kg b wt.)
Group 5	MNA (300mg/kg b wt.)
Group 6	Amitriptyline (10mg/kg b wt.)
Group 7	Fluoxetine (10mg/kg b wt.)

Experiment design: The FST conducted in mice as all the groups of mice were subjected to swimming test except group 1 in a cylindrical glass aquarium (50 x 30 cm diameter), containing 25±2°C water. Mice were allowed to swim for 6 min and the duration of immobility was measured during the final 4 min interval of the test using a video tracking system. Immobility period was regarded as the time spent by the mouse floating in the water without struggling and making only those movements necessary to keep its head above the water. Following swimming sessions, they were then towel dried. In order to determine the time-dependent effects on immobility time, oral treatments with *Nyctanthes arbortristis* stem MNA for 1, 3, 7, 14 and 21 consecutive days were investigated 39-40.

B. Tail suspension test (TST):

Animal groups: The activity was performed with 42 total mice. We were randomly divided into 6 groups, each group having 6 mice. The mice of each group were treated accordingly treatment given in Table 2 and treated as follows: Group 1 act as normal control and Group 2 have tween-20 suspensions and act as experimental control. Group 3-5 were orally administered with various doses of methanol extract of *Nyctanthes arbortristis* stem having three different doses. Group 3 treated as 75 mg extract/kg of

body weight, Group 4 treated as 150 mg extract/kg of body weight, Group 5 treated as 300 mg extract/kg of body weight. The animals present in Group 6 and 7 received standard anti- depressant drug-amitriptyline and fluoxetine (10 mg/kg body weight).

Table 2: Experiment animal groups (TST)

Animal groups	Treatment
Group 1	Normal control
Group 2	Tween-20 suspensions
Group 3	MNA (75mg/kg b wt.)
Group 4	MNA (150mg/kg b wt.)
Group 5	MNA (300mg/kg b wt.)
Group 6	Amitriptyline (10mg/kg b wt.)
Group 7	Fluoxetine (10mg/kg b wt.)

Experiment design: A box having each wall side with 35cm was used for the tail suspension test. The front surface of the apparatus was open and each mouse was suspended by fixing the tail in the center of the upper surface using a tail hanger and non-irritant adhesive tape with the head 5 cm to the bottom. The experiment was performed in darkened room with minimal background noise for duration of 5 min. The total duration of immobility (total immobility time) was observed and measured during the final 4 min interval of the test period. All test sessions were recorded by a video camera positioned directly above the box. Mice were considered immobile only when they hung passively and completely motionless.

Table 2: Effect of MNA, amitriptyline and fluoxetine pre-treatment on body weight in mice (FST groups)

Body weight (g) during different treatment period							
Days	Groups						
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
1	107.8±2.1	106.6±1.5	109.2±2.1	110.5±2.1	108.5±3.6	106.4±1.4	104.3±1.4
3	108.4±1.2	108.8±1.7	111.6±2.5	116.2±1.1	110.5±2.6	107.9±2.1	106.1±1.6
7	110.6±2.1	111.4±3.2	113.4±3.4	117.4±2.4	111.4±3.8	110.2±2.1	107.5±1.6
14	111.5±1.9	115.4±2.1	116.1±3.3	119.5±3.6	113.3±3.3	111.7±2.1	109.7±2.2
21	114.3±2.1	118.9±2.3	122.8±1.9	121.4±2.1	113.8±2.1	112.8±1.3	112.4±1.2

Values are presented as the mean ± SD (n=8). There were no significant differences at $p < 0.05$.

Table 3: Effect of MNA, amitriptyline and fluoxetine pre-treatment on immobility time in the mice (FST groups)

Group	Dose mg/ kg b.wt	Duration of immobility (s)				
		Days				
		1	3	7	14	21
Group 1	-	-	-	-	-	-
Group 2	-	110.1±10.1	100.1±9.1	105.1±8.3	101.4±8.1	102.4±6.9
Group 3	75	100.4±6.6	84.1±5.6a	87.1±5.6	71.4± 7.1a	63.2±4.6a
Group 4	150	94.2±10.3	81.5±3.3a	72.2±5.3a	57.7±5.1a	54.4±5.7a
Group 5	300	84.3±8.1a	71.1±4.1b	61.1±4.7b	39.8±6.1b	20.2±6.6b
Group 6	10	70.1±7.2b	62.3±9.1c	52.2±6.1c	27.2±8.4b	12.4±3.9c
Group 7	10	78.4±10.2a	64.6±4.1b	61.1±3.1b	34.0±5.6c	21.5±5.2b

Values are presented as the mean $\pm$ SD (n=8). Values bearing different superscripts in the same column are significantly different ($p<0.05$) (ANOVA).

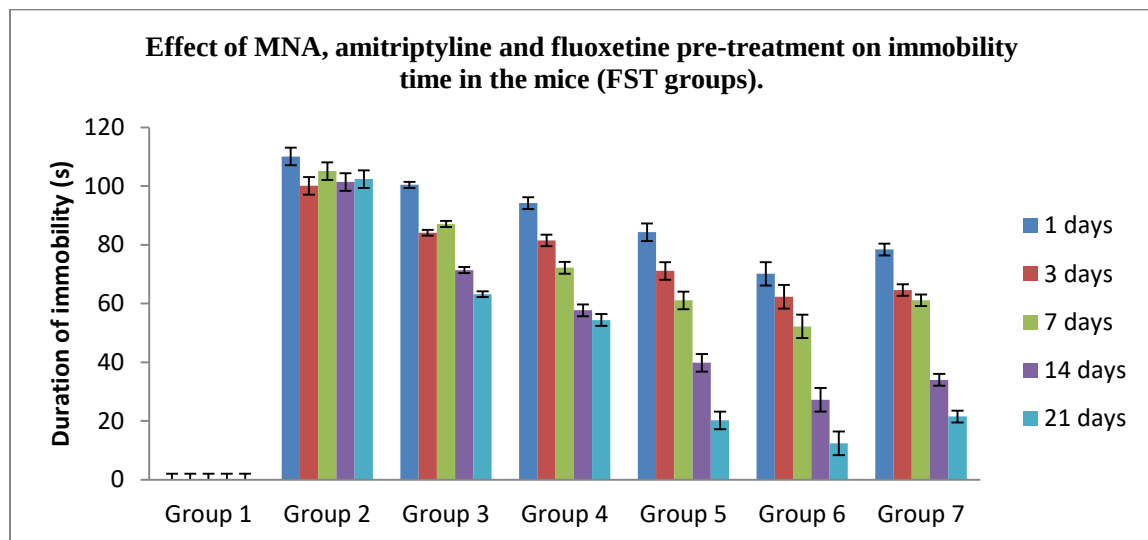


Figure 1: Effect of MNA, amitriptyline and fluoxetine pre-treatment on immobility time in the mice (FST groups)

Table 4: Effect of MNA, amitriptyline and fluoxetine pre-treatment on body weight in mice (TST groups)

Body weight (g) during different treatment period							
Days	Groups						
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
1	106.1 $\pm$ 2.2	102.1 $\pm$ 1.1	104.1 $\pm$ 1.3	108.1 $\pm$ 1.1	104.1 $\pm$ 2.6	101.1 $\pm$ 1.1	101.3 $\pm$ 2.1
3	107.7 $\pm$ 1.7	102.8 $\pm$ 3.1	106.4 $\pm$ 4.2	109.1 $\pm$ 2.1	105.2 $\pm$ 1.6	101.9 $\pm$ 1.5	102.2 $\pm$ 1.3
7	109.6 $\pm$ 1.1	104.1 $\pm$ 2.2	107.3 $\pm$ 1.4	111.1 $\pm$ 1.4	106.1 $\pm$ 1.3	104.1 $\pm$ 1.7	103.1 $\pm$ 1.1
14	110.1 $\pm$ 1.2	105.3 $\pm$ 4.1	107.9 $\pm$ 2.3	112.2 $\pm$ 2.6	108.7 $\pm$ 1.2	104.9 $\pm$ 2.2	104.2 $\pm$ 1.2
21	112.5 $\pm$ 2.3	108.2 $\pm$ 3.1	108.2 $\pm$ 2.9	114.1 $\pm$ 1.5	110.2 $\pm$ 1.8	105.8 $\pm$ 2.1	105.1 $\pm$ 1.1

Values are presented as the mean $\pm$ SD (n=8). There were no significant differences at $p<0.05$.

Table 5: Effect of MNA, amitriptyline and fluoxetine pre-treatment on immobility time in the mice (TST groups)

Group	Dose mg/ kg b.wt	Duration of immobility (s)				
		Days				
		1	3	7	14	21
Group 1						
Group 2	-	91.3±4.9	90.7±10.1	91.4±6.3	89.4±7.7	88.1±7.7
Group 3	75	85.5±3.6	87.6±8.8a	81.4±6.3	77.7±4.7a	71.2±1.7a
Group 4	150	84.1±5.1	79.8±7.6a	73.7±4.2	70.6±3.6a	68.6±2.6a
Group 5	300	83.6±4.4	69.4±8.1b	64.1±4.4a	61.8± 6.7b	58.2± 3.7b
Group 6	10	78.5±4.1	62.7±10.4 b	58.7±5.1a	52.4±4.4b	49.4±2.4b
Group 7	10	72.8±6.6	64.0±6.6b	53.4±4.6a	47.5±4.7b	43.5±5.7b

Values are presented as the mean ± SD (n=8). Values bearing different superscripts in the same column are significantly different (p<0.05) (ANOVA).

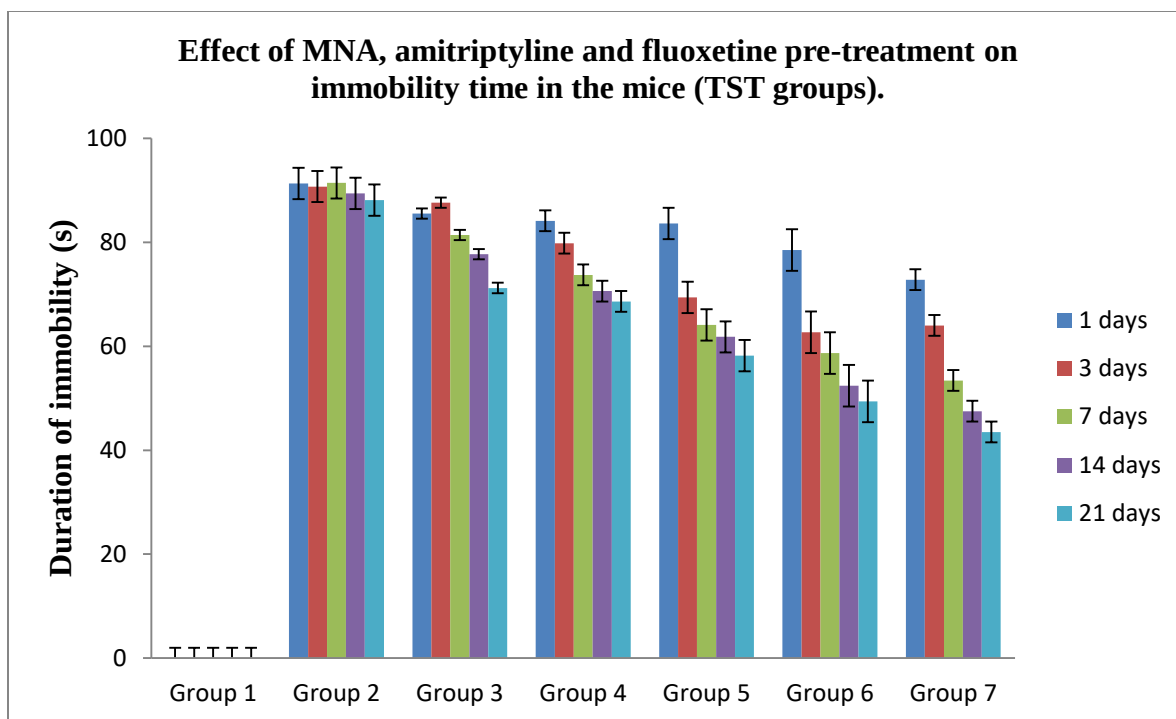


Figure 2: Effect of MNA, amitriptyline and fluoxetine pre-treatment on immobility time in the mice (TST groups)

Results and Discussion

A systematic approach is necessary in pharmacognostic study, which helps in confirmation and determination of identity, purity and quality of a crude drug. The selected crude drugs were subjected to studies organoleptic characters viz., color, odour, appearance, taste, texture etc. *Nyctanthes arbortristis* stem occur as *Nyctanthes arbortristis*, commonly known as night jasmine or Parijat, is a large shrub or small tree with fragrant, white flowers that bloom at night. *Nyctanthes arbortristis* is a shrub or a small tree growing to 10 m tall, with flaky grey bark. The extractive values are mainly useful for the determination of adulterated or exhausted drug. Alcohol soluble extractive value of *Nyctanthes arbortristis* stem were found 10.62 % w/w whereas Water soluble extractive value were found 12.4 % w/w. Methanol extract of *Nyctanthes arbortristis* stem drugs obtained by maceration method after defatting of leaves with hexane. Defatting of leaves with nonpolar solvent cause removal of chlorophyll and fatty material which can further hindered the activity of plant extract. Extract (methanol extract) of selected plant *Nyctanthes arbortristis* stem drugs obtained by maceration method was

subjected to qualitative phytochemical tests to identify the presence of secondary metabolite (viz., alkaloids, glycosides, tannins, flavonoids, sterols, fats, oils, phenols and saponins) present in them. Preliminary phytochemical screening of methanol extract of *Nyctanthes arbortristis* stem exhibited the presence of carbohydrate, tannin flavanoid, glycoside and saponin in methanol extract.

Effect of MNA, amitriptyline and fluoxetine pre-treatment on body weight in mice (FST and TST groups): The effect of extract on the body weight change is presented in Table 6.7 and 6.8. All the result was showed that there was no difference in body weight gain by the animals among all the groups subjected to 1-day, 3-days and 7-days treatment. As the treatment began from 7 days to two to three week or 14 days to 21 dias, a slight increase in weight gain was observed after oral administration. The weight gain of mice may be the normal weight gain of rats. It is confirmed that, administration of MNA did not have any effect on the weight of animals.

Effect of MNA, amitriptyline and fluoxetine pre-treatment on immobility time in the mice (FST and TST groups): In FST, mice are forced to swim in a restricted space from which they cannot escape and are induced to a characteristic behavior of immobility. This behavior, reflecting a state of despair is reduced by several agents; these are therapeutically effective in human depression. The TST also induces a state of despair in animals like that in FST. This immobility referred to as behavioral despair in animals and also recognized as a condition similar to human depression, this was investigated by possible time-dependent effects on immobility time. The effects of MNA, amitriptyline and fluoxetine on immobility in mice FST are presented. The result indicated that the slight decrease in immobility time showed after 3rd days and 7th days treatment and the decrease was non-significant at $p < 0.05$. The mice were able to swimming after MNA pre-treatment for 14th days and 21st days. There was a reduction in the duration of immobility started compared with stress control and the effect was observed with the classical anti-depressant drug fluoxetine and amitriptyline. MNA at 300 mg/kg b. wt. exhibited significant decrease in immobility duration after oral treatment for 14-days. After 21-days treatment of MNA, there was a significant treatment effect for dose in immobility time. The maximal effect was observed at 300mg/kg b.wt. allowed to reduction in immobility time with reference

as anti-depressants drug as amitriptyline and fluoxetine.

Summary And Conclusion: Mental depression encompasses biological and emotional components, with symptoms including mystery, apathy, pessimism, low self-esteem characterized by feelings of shame, inadequacy, and unattractiveness, as well as indecisiveness and a loss of ambition. The current study suggests that the stem of *Nyctanthes arbortristis* exhibits enhanced efficacy in managing depression, according to its higher concentration of phytochemical ingredients, which substantiate the proposed research. The administration of MNA at effective doses ranging from 150 mg to 300 mg produced a substantial effect on the immobility duration of all animals across various groups. The maximum effect was noted at 300 mg/kg body weight, resulting in a reduction in immobility time, comparable to the antidepressant drugs amitriptyline and fluoxetine. The methanolic extract of *Nyctanthes arbortristis* stem was determined to effectively cure depression in both rats and humans at concentrations validated in the current investigation.

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