Pharmacological Screening of *Mangifera indica* Seeds for Antidepressant-like Action Along with a Mechanistic Study

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ABSTRACT: Depression is emerging as a major global issue. There are several antidepressants available in the market, but their efficacy is usually unpredictable. Therefore, there is a need to find an alternative therapeutic agent with better therapeutic efficacy and availability. In the current investigation, the antidepressant-like action of the aqueous methanolic extract of *Mangifera indica* seeds (25, 50, and 100 mg/kg) was evaluated by two predictive models like the tail suspension test and forced swimming test along with the determination of the mechanism of action working behind this action. The results of the acute treatment with the extract show a dose-dependent reduction in the duration of immobility in both models. The antidepressant-like action of the extract (100 mg/kg) was blocked by the administration of *p*-chlorophenyl alanine, *α*-methyl-*p*-tyrosine, prazosin, and sulpiride while remaining unaffected with propranolol. In contrast, the administration of d-serine along with the extract (a full agonist of glycine/N-methyl-D-aspartate, NMDA, receptors) diminished the anti-immobility action. The administration of the extract along with nitro-L-arginine-methyl ester synergizes into the anti-immobility action of the extract, and intake of l-arginine remained unable to effect this action, whereas sildenafil blocks the effect. The antidepressant-like action of the extract is probably due to the involvement of serotonergic and adrenergic (mainly *α* receptors are involved) systems, an NMDA receptor complex, and the nitric oxide pathway.

1. INTRODUCTION

Sadness and unhappiness are common human reactions. Everyone experiences such feelings occasionally, but it will diminish within a few days, whereas in cases of depression, the duration and severity differ from the normal period. It affects over 300 million individuals globally. A report conducted by the WHO show that cases of depression are rising globally and consuming a big share of the budget for its treatment. The symptoms of depression can be improved with the help of antidepressant pharmacotherapy along with psychotherapy sessions. The previous studies and pathophysiology of depression suggested the involvement of certain receptors such as serotonergic and noradrenergic behind the mechanism of action for various antidepressant classes (tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), and monoamine oxidase inhibitors (MAOIs)). According to the monoamine hypothesis, the reduction in levels of serotonin and noradrenaline in the central nervous system is responsible for the development of depression. Therefore, all effective therapeutic regimens are designed to elevate levels of serotonin or noradrenaline in the brain. With the presently prescribed standard antidepressant drugs, an encounter with certain disadvantages like the delayed onset of therapeutic efficiency, high occurrences of insensitive patients, weight gain, and sexual problems occurs. Special scientific interest has been provoked by seeds discarded as a wasted part of *Mangifera indica* (*M. indica*) because of the existence of a high content of bio-active compounds, which are helpful in improving the human health. The seeds are used traditionally for treatment of urinary disorders as they contain diuretic properties, increasing the strength of the nervous system and circulatory system of blood, eliminating body contaminants, and treatment of anemia, diarrhea, rheumatism, diabetes, asthma, syphilis, gastric and hepatic disorders, astringent conditions, emetic conditions, toothache, and cough as the seeds are a good resource of carbohydrates (58–80%), protein (6–13%), along with the content of amino acids, and lipids (6–16%). There are a number of natural antioxidant products present in *M. indica* seeds (MISs) such as phenolic compounds, carotenoids, vitamin C, phenolic acid, and minerals (calcium, magnesium, potassium, sodium, phosphorus, iron, manganese, and zinc).

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HPLC analysis quantified the presence of some compounds such as tannin (20.7%), gallic acid (6%), caffeeic acid (7.7%), vanillin (20.2%), mangiferin (4.2%), ferulic acid (10.4%), and cinnamic acid (11.2%).

Its chemical constituents stated in the literature review reveals its antidepressant potential. The seed extracts (MISEs) have been examined for various pharmacological properties like antioxidant, anti-inflammatory, antidiabetic, and immune-modulatory activities. The chemical structures of these bioactive moieties are not similar with existing antidepressant drugs. The literature review revealed that there is no previous scientific evaluation conducted to explore the antidepressant pharmacological activity of M. indica seeds. The current study was planned to determine a new antidepressant and also to illustrate the possible modes of action that may provide new therapeutic options for the treatment of depression.

2. RESULTS

2.1. Acute Antidepressant Activity. 2.1.1. Behavioral Studies. 2.1.1.1. Forced Swimming Test (FST). The result obtained after the administration of MISE 1 h before the test period session showed a significant dose-dependent reduction in immobility time. The swimming time and climbing activity were also increased in MISE-treated groups that indicate their antidepressant-like action. Similarly, the standard drugs fluoxetine and reboxetine produce a significant reduction in immobility time in mice when compared to the negative control group. Fluoxetine significantly increased swimming time but not the climbing activity. Reboxetine significantly increased the swimming time along with augmentation of climbing activity (Figure 1). The result of the MISE at 100 mg/kg was comparable to that of standard (fluoxetine of 10 mg/kg and reboxetine of 20 mg/kg) drugs.

2.1.1.2. Tail Suspension Test (TST). The result obtained after the administration of MISE 1 h before the test period session showed a significant dose-dependent reduction in immobility time. The swinging time and curling activity were also augmented in a dose-dependent manner in MISE-treated groups that indicate their antidepressant-like action. Similarly, the standard drugs fluoxetine and reboxetine produce a significant reduction in immobility time in mice when compared to the negative control group. Fluoxetine significantly increased swimming time but not the curling activity. Reboxetine also significantly augmented the swimming but not the curling activity (Figure 2). The result of the MISE at 100 mg/kg was comparable to that of standard (fluoxetine of 10 mg/kg and reboxetine of 20 mg/kg) drugs.

Figure 1. Effects of MISE (25; 50 and 100 mg/kg) administration on immobility time and swimming and climbing time in the FST. Values are given as means ± SEM (n = 6), a: ***p < 0.001, b: **p < 0.01, and c: *p < 0.05 when compared with the disease control.

Figure 2. Effects of MISE (25, 50, and 100 mg/kg) administration on immobility time, swimming time, and climbing time in the TST. Values are given as means ± SEM (n = 6), a: ***p < 0.001, b: **p < 0.01, c: *p < 0.05, and ns: nonsignificant (p > 0.05) when compared with the disease control.
2.1.2. Mechanistic Study. 2.1.2.1. Influence of Serotonergic Depletion. The results showed that pretreatment of pCPA alone (300 mg/kg) for up to three days remains unable to change the immobility time when compared to a negative control group in the TST, whereas the pretreatment produced a significant inhibition in the anti-immobility effect of MISE (100 mg/kg) when compared to the negative control group receiving normal saline (Figure 3). A similar result was obtained when fluoxetine (SSRI) was administered in pCPA-pretreated mice. The pretreatment of pCPA remained unable to alter the antidepressant-like action of reboxetine (NRI).

2.1.2.2. Influence of Catecholamine Depletion. The results showed that pretreatment of α-methyl-p-tyrosine (AMPT, 100 mg/kg i.p.) alone remains unable to change the immobility time when compared to the negative control group. Pretreatment of AMPT (100 mg/kg i.p.) 4 h before administration of MISE (100 mg/kg) and reboxetine (20 mg/kg) significantly inhibited the anti-immobility effect in the TST when compared to a negative control group receiving normal saline. The TST was performed 60 min later after the administration of MISE and reference drug treatments. Meanwhile, AMPT was unable to produce any alteration in the anti-immobility effect of fluoxetine when compared to the negative control group (Figure 4).

2.1.2.3. Influence of Some Antagonists on the MISE Activity. The antagonist such as prazosin, propranolol, and sulpiride was administered 30 min before the administration of MISE and reference drugs. The pretreatment of prazosin (3 mg/kg p.o.) produced a significant decrease in the anti-immobility action of MISE (100 mg/kg), whereas propranolol (3 mg/kg p.o.) remained ineffective in reversing the antidepressant-like action of the extract when compared to the negative control group in the TST (Figure 5), while pretreatment of sulpiride (50 mg/kg i.p.) remains effective in reversing the anti-immobility effect of the extract when compared to the negative control.

2.1.2.4. Evaluation of N-Methyl-D-aspartate (NMDA) Participation. Administration of D-serine (600 mg/kg i.p.) along with MISE and standard drugs (fluoxetine and reboxetine) resulted in a significant inhibition in the anti-immobility effect in the TST when compared to a negative control. Although the administration of D-serine alone did not produce any change in the mobility time when compared to the negative control in the TST.

2.1.2.5. Contribution of the L-Arginine-NO-cGMP Pathway. The pretreatment of L-arginine (750 mg/kg i.p.) and sildenafil caused a blockage in the anti-immobility effect of MISE in the TST when compared to the negative control. The pretreatment of L-NAME (30 mg/kg i.p.) enhances the anti-immobility activity of the extract in the TST. Administration of L-arginine, L-NAME, and sildenafil alone does not produce any effect on the mobility pattern when compared to the negative control group receiving normal saline (Figure 6).

3. DISCUSSION

Depression is a frequent and usual psychiatric ailment. Initially, the therapeutic ability of MISE as a natural source of an antidepressant drug was examined. For evaluation of the antidepressant-like action of MISE, the FST and TST were used. The FST comprises an observation of two different phases such as the passive phase (immobile behavior) and the active phase (swimming and climbing) of stressed mice. The TST is a reputable test having some advantages over the FST such as better pharmacological sensitivity, a reduced risk of hypothermia, and a fast recovery toward normal voluntary behavior after the termination of the trial. The TST is a reputable test having some advantages over the FST such as better pharmacological sensitivity, a reduced risk of hypothermia, and a fast recovery toward normal voluntary behavior after the termination of the trial. It is used for the assessment of particular behavioral aspects for the evaluation of the antidepressant potential of the test substance as well as for differentiating between standard drugs and other agents acting as an antidepressant-like agent as the traditional antidepressants work by modulation of the serotonergic pathway decreasing the immobility time and increasing the swinging behavior, whereas opioids also decrease the immobility time and increase the curling behavior without producing any influence on the swinging time.
The antidepressant-like action of the MISE was assessed by the most commonly used animal tests like the FST and TST as done by Can Ö et al. In the FST, the mice were enforced to swim in a constricted area from which emission is impossible. This test accounts for both the behaviors of animals such as those of passive (immobility) and active (swimming and climbing). The TST is also a reputable method for assessment of antidepressant-like action that shares the same focal point of behavioral despair of the FST. It differs in the aspect that the animal was suspended by the tail for the induction of immobility.

In the current investigation, the result obtained from the FST showed that the MISE at a dose of 100 mg/kg significantly reduced the immobility time in mice when compared to the negative control group. Standard drugs fluoxetine (10 mg/kg) and reboxetine (20 mg/kg) also reduce the immobility time as expected. Moreover, the administration of MISE also augmented the active behavior such as swimming and climbing. The decrease in passive behavior and augmentation of the active behavioral phase suggests the antidepressant-like action of MISE.

Similarly, the results of TST showed that the MISE administered at a dose of 100 mg/kg significantly reduced the immobility time along with the escalation of curling and climbing activities in mice when compared to the negative control group that exhibited the antidepressant-like action of MISE (Figure 2). The standard drugs fluoxetine (10 mg/kg) and reboxetine (20 mg/kg) also reduce the immobility time as compared to the negative control group. Standard drugs fluoxetine (10 mg/kg) and reboxetine (20 mg/kg) also reduce the immobility time as expected. Moreover, the administration of MISE also augmented the active behavior such as swimming and climbing. The decrease in passive behavior and augmentation of the active behavioral phase suggests the antidepressant-like action of MISE.

![Figure 5](image-url)
The reuptake of 5HT, along with an advantage of rapid onset of action of D-serine (600 mg/kg, i.p., agonist of the NMDA glutamate receptor) pretreatment on the behavioral response of NS (10 mg/kg, p.o.), MISE (100 mg/kg, p.o.), standard fluoxetine (10 mg/kg, p.o.), and standard reboxetine (20 mg/kg, i.p.) in the tail suspension test. All values are presented as means ± SEM (n = 6). Significant difference existing against animals administered with a vehicle, ***p < 0.01 and **p < 0.001; significant difference against treatment group b (p < 0.001).

The down regulation of serotonin receptors or inhibition of the reuptake of certain neurotransmitters influences the mood; for example, the response of norepinephrine (NE) is mediated by the family of G protein-coupled receptors known as the adrenergic receptors, and levels of extracellular NE are regulated by synaptic clearance via the NE transporter and modulation of its metabolism. Almost all of these noradrenergic system components serve as direct molecular targets for antidepressant drugs, including the newer SHT/NE reuptake inhibitors (SNRIs) such as duloxetine, atypical antidepressants like mirtazepine, and the older TCAs and MAOIs. The down regulation of bioactivity at β1 and α2 adrenergic receptors is required for treatment of depression by decreasing the number of binding sites or by reduction of functional response to agonists. Some drugs such as SSRIs work by inhibition of SHT reuptake into the nerve terminal eventually increasing their levels in the synaptic cleft. Some nonselective β adrenergic receptor blockers preferentially affect the SHT receptors and inhibit the reuptake of SHT along with an advantage of rapid onset of action. Previous reports suggest that the antidepressant-like effect of SSRIs, serotonin–norepinephrine reuptake inhibitors, and tricyclic antidepressants is abolished with D-serine because of activation of the glycine site of the NMDA receptor. Various brain activities are controlled by the interaction between serotonin and excitatory amino acids.

After the illustration of the antidepressant-like action of MISE, the mechanistic studies were conducted for clarification of possible receptors and pathways involved in the development of antidepressant-like effects of MISE. To perform the first portion of mechanistic studies, the involvement of the serotonergic system was evaluated behind the antidepressant-like action of MISE. For the execution of this, pretreatment of pCPA (inhibitor of serotonin synthesis) was given to mice and the influence was assessed through the TST. Although behavioral depression is not always related to the depletion of 5-HT, in the current study, the depletion of serotonin caused by pCPA blocks the antidepressant effect of fluoxetine with no influence on antidepressants that work as a norepinephrine reuptake inhibitor. According to earlier investigations, the continuous three-day administration of pCPA (100 mg/kg) can successfully delete the endogenous stores of serotonin without producing any influence on noradrenergic or dopaminergic levels. The results of the current study showed that the antidepressant-like activity of fluoxetine was blocked and, in the case of the MISE-administered group, it was diminished and remained unaffected in the case of reboxetine that was administered along with pCPA in the TST. The results showed the participation of the 5-HT receptor (serotonergic system) behind the acute antidepressant-like activity of MISE.

To evaluate the involvement of the noradrenergic and/or dopaminergic neurotransmission pathway behind the antidepressant-like activity of MISE, a pretreatment with a selective inhibitor of tyrosine hydroxylase (AMPT) was given to mice. Tyrosine hydroxylase acts as rate-limiting enzyme giving information on noradrenaline (NA) and dopamine (DA). Previous reports revealed that AMPT produces a reduction in the synthesis of NA (53%) and DA (57%) that resulted in prolongation of immobility time during the evaluation test (TST) in mice with no influence on the serotonin level. In the current study, the antidepressant-like activity of reboxetine was blocked and the MISE level was diminished and remains unaffected in the case of fluoxetine with AMPT administration when evaluated by the TST. The present results follow those of O’Leary et al. who worked on the evaluation of the behavioral response of different antidepressants in a state of deficiency of serotonin and the catecholamine neurotransmitter. This effect revealed the possible involvement of the catecholaminergic system in the development of the antidepressant-like response of MISE.

The catecholaminergic system encountered both receptors of adrenergic and dopaminergic that are involved in the development of antidepressant-like action of agents. Therefore, to evaluate the contribution of these receptors, various particular receptor antagonist agents were used.

For evaluation of the possible contribution of α and β adrenergic receptors in the development of antidepressant-like action of MISE, prazosin and propranolol were used.
respectively. The anti-immobility effect of MISE was blocked by pretreatment with prazosin, whereas pretreatment with propranolol resulted in an ineffective blockage of MISE antidepressant-like action in the TST. This result suggests the possible involvement of the α-adrenergic receptor in the antidepressant-like outcome of MISE, whereas the contribution of the dopaminergic receptor was evaluated by pretreatment of mice with sulpiride 60 min prior to administration of MISE. The anti-immobility action of MISE was blocked by pretreatment with sulpiride. The data collected from these mechanistic evaluations showed that an elevated level of catecholamine is essential for the development of the antidepressant-like action of MISE along with the contribution of agonistic action at α-adrenergic and dopaminergic receptors.

Various studies of the past decade showed the influence of the NMDA class of glutamatergic receptors behind the mechanism of antidepressant treatment and also the evaluation of pathophysiological pathways behind the development of depression.25 Previous evaluations also exhibit the participation of NMDA receptor antagonists in the development of antidepressant-like action in mice.26 A full agonist of the glycine site on the NMDA receptor (d-serine) was used for evaluating the affinity of the MISE for the NMDA receptor. The results of the TST showed that d-serine alone does not influence the immobility time, whereas simultaneous administration of d-serine with MISE, reboxetine, and fluoxetine produces an inhibition of the anti-immobility effect.

Previous reports suggest that the antidepressant-like activity of SSRI, serotonin–norepinephrine reuptake inhibitors, and tricyclic antidepressants is abolished with d-serine because of activation of the glycine site of the NMDA receptor.17−19 Various brain activities are controlled by the interaction between serotonin and excitatory amino acids. The affinity of the serotonergic pathway for the NMDA receptor is more evident as compared to that of the noradrenergic pathway. The antagonist of the NMDA receptor causes a release or increase in the concentration of serotonin in the brain (Szewczyk et al.), so it can be assumed that the antidepressant-like activity of MISE may be due to its affinity for the glycine site of the NMDA receptor.25

In the brain, nitric oxide (NO) acts as a signaling molecule and shows an association to synaptic plasticity, neurotransmission, behavioral aspects (learning, perception of pain, and aggression), and establishment of CNS disorders (anxiety and depression).25,26 The rise in metabolites of plasma nitric oxide was encountered in patients having suicidal affinity as compared to normal individuals or non-suicidal psychiatric patients.27 Similarly, the stressed state also causes an increase in nitric oxide levels due to the rise in the production of NO.28 It has been reported that suicidal patients exhibited significantly increased levels of plasma nitric oxide metabolites as compared to non-suicidal psychiatric patients or healthy subjects.29,30 Various previous studies revealed the significant participation of the l-arginine-NO-cGMP pathway in the pathophysiology of depression. In the current study, the probable involvement of the l-arginine-NO-cGMP pathway was evaluated for the antidepressant-like action of MISE. Results of the current study revealed that pretreatment with l-arginine (nitric oxide synthase (NOS) substrate) remains unable to block the anti-immobility property of MISE in animals during the TST. The simultaneous administration of the MISE with nitro-l-arginine-methyl ester (l-NNAME), a nonselective NOS inhibitor, produces a synergistic effect in the production of the antidepressant-like action of MISE. Previous investigations revealed that the compound having an NOS inhibition property is a good candidate for antidepressant-like effects.31 Various reports showed that inhibition of NOS produces an improvement in behavioral properties of certain antidepressant classes such as the TCAs and SSRIs during the FST.32,33 Therefore, it can be assumed that the antidepressant-like activity of MISE may be due to inhibition of nitric oxide synthesis that eventually enhances its SSRI-like action. The possible mechanism of action behind the antidepressant-like action of MISE is presented in Figure 7.

A study conducted on the evaluation of the influence produced by cGMP levels show that the rise in levels causes the onset of depression and the vice-versa result is produced when the levels decrease.35 The inhibition of soluble guanylate cyclase with the administration of methylene blue or a reduction in nitric oxide function (by L-NAME) showed a reduction in cGMP levels. The degradation product of cGMP is a phosphodiesterase enzyme (PDE) that causes the degradation of cGMP into guanosine monophosphate (GMP). Therefore, inhibition of the phosphodiesterase enzyme causes a depression-like state due to an increase in cGMP levels.36 Sildenafil acts as a selective PDE5 inhibitor that subsequently produces a rise in the level of cGMP in the target tissue.34,35 In the current study, pretreatment of sildenafil blocked the anti-immobility action of MISE in the TST of mice. Results presented the participation of the cGMP pathway...
in the development of the antidepressant-like activity of MISE. The outcome of the current study is in line with previous investigations conducted for evaluation of the influence produced by the PDE5 inhibitor on experimental animals conducted for evaluation of antidepressant compounds.36−38

4. MATERIALS AND METHOD

MISs were procured from a local market in Faisalabad from May to August in 2018 for collection and preparation of extracts. The seeds were identified and authenticated by a taxonomist, Dr. Mansoor Hameed of the Department of Botany, University of Agriculture Faisalabad (UAF). The dried sample was deposited into the UAF herbarium via voucher no. 79-1-2019.

4.1. Extraction. Seeds were manually removed from their endocarp, shade-dried, and ground to a coarse powder. The extract was prepared by soaking 500 g of the powder in aqueous methanol (20:80) solvent (2.5 L) for 72 h with intermittent stirring. The extract was filtered through muslin cloth initially then by Whatman filter paper no.1. The extra solvent was removed from the filtrate by a rotary evaporator at 40 °C, and the semisolid form of the extract was stored in an amber-colored bottle for analysis.

4.2. Animals. Swiss albino male mice (22–30 g, 180 days old) were bred in the animal house of Government College University, Faisalabad. They were kept in a controlled environment in groups of eight, each in propylene cages at a temperature of 23 ± 2 °C in a 12 h light/dark cycle with a humidity of 35–65% and fed at a normal pellet diet and water ad libitum. The mice were acclimatized for 3 weeks before the commencement of behavioral studies that were performed between the period of 9:00 am to 2:00 pm and fasted for 2 h before and 2 h after receiving the drug.

4.3. Acute Antidepressant Activity. 4.3.1. Behavioral Studies. 4.3.1.1. Forced Swimming Test (FST). The animals were forced to swim in a glass chamber (25 × 15 × 25 cm3) filled with fresh water maintained at 26 °C at a height of 15 cm. After the completion of the test, the session water was changed because unchanged water affects the behavior of the remaining animals. Initially, mice showed a highly energetic behavior, for instance, forceful swimming in a circular motion, wall climbing, or diving toward the bottom. This behavior was diminished at the end of second minute and changed into a phase of immobility consisting of cessation of struggling, floating for an increased interval, and restricted movements just to retain the head above the water. This immobile phase was recorded for the next four minutes manually from the total 6 min test duration. After this swimming session, mice were dried with a towel and put back to their cages.

4.3.1.2. Tail Suspension Test (TST). The tail suspension test is centered on the principle of hanging upside down, which leads to an inactive behavior that has a similarity with human depression. Tests were performed by individually suspending the animals for 5 min by the tail with the help of adhesive tape applied 1 cm apart from the tip on a horizontal ring bar stand that was 30 cm above from the floor. The duration of immobility was noted. Immobility is characterized as inactive or immobile hanging phases of the animal. The test was not scheduled for conduction immediately on the next day after the execution of force swimming tests. The reduction in the immobility duration reflects the antidepressant potential of giving the treatment.39

4.3.2. Mechanistic Studies. For determination of the mechanism of action behind the antidepressant-like action of MISE, the TST was used because it has certain advantages over the FST such that it provides an objective assessment of the immobility phase and it prevents the animal from the risk of hypothermia that may occur due to water immersion.11,15

4.3.2.1. Influence of Serotonergic Depletion. For determination of the possible participation of the serotonergic system behind the effect of the MISE, a pretreatment of para-chlorophenylalanine (pCPA) or normal saline was given to mice. pCPA is attributed to a fall in the concentration of serotonin in the brain due to inhibition of its biosynthesis.18,20 Mice were administrated with normal saline (negative control) or pCPA (i.p.). For depletion of serotonin synthesis, pCPA was given at a dose of 300 mg/kg once a day for three days consecutively. On the fourth day, after 24 h since the previous pCPA administration, the mice were administered with MISE (100 mg/kg), fluoxetine (10 mg/kg), reboxetine (20 mg/kg), and normal saline (10 mL/kg) 60 min before the commencement of behavioral assessment in the TST.

4.3.2.2. Influence of Catecholamine Depletion. For evaluation of the possible contribution of catecholamines behind the antidepressant effect of MISE, pretreatment of AMPT (400 mg/kg i.p.) or a vehicle was given 4 h before administration of MISE (100 mg/kg), reboxetine (20 mg/kg), fluoxetine (10 mg/kg), and normal saline (10 mL/kg). Then, the TST was conducted over 60 min to assess the behavioral aspects. The immobility time was noted and compared with the negative control group. AMPT is associated with inhibition of the tyrosine hydroxylase enzyme and depletion of recently synthesized pools of catecholamines.40

4.3.2.3. Influence of Some Antagonists on MISE Activity. For assessment of the influence produced by the antagonists of α, β, and dopaminergic receptors, the different antagonists were given in an appropriate dose that was selected from the literature. Pretreatment with an α1 adrenergic blocker (prazosin of 3 mg/kg p.o.), β-adrenergic blocker (propranolol of 3 mg/kg p.o.), and dopamine antagonist (sulpiride 50 mg/kg i.p.) or normal saline was given to mice 30 min before the administration of MISE (100 mg/kg) and normal saline (10 mL/kg). The behavioral assessment was done 45 min after the administration of MISE and normal saline by the TST.

4.3.2.4. Evaluation of N-Methyl-D-aspartate (NMDA) Participation. To assess the possible contribution of the NMDA receptor behind the activity of MISE, sub-effective doses of d-serine (600 mg/kg i.p.) selected from literature were given 15 min before the administration of MISE (100 mg/kg), reboxetine (20 mg/kg), fluoxetine (10 mg/kg), and normal saline (10 mL/kg). After 45 min, the immobility time was noted and compared with a negative control in the TST.17,42

4.3.2.5. Contribution of the l-Arginine-NO-cGMP Pathway. To explore the possible participation of the l-arginine-nitric oxide pathway behind the antidepressant activity of MISE, a sub-effective dose of the nitric oxide precursor (l-arginine 750 mg/kg i.p.) or vehicle was given 20 min before the administration of MISE (100 mg/kg) and normal saline (10 mL/kg). The behavioral assessment test (TST) was performed 45 min after the administration of MISE and normal saline, and the immobility time was compared with that of the negative control.

In other experiments, a sub-effective dose of the non-selective nitric oxide synthase (NOS) inhibitor (L-NAME 30
mg/kg i.p.) or normal saline was given 20 min before the administration of MISE (100 mg/kg p.o.) and normal saline (10 mL/kg p.o.). For the behavioral assessment, the TST was performed 45 min after the administration of MISE and normal saline, and the immobility time was compared with that of the negative control.43

For assessment of the possible participation of cyclic guanosine monophosphate (cGMP) behind the antidepressant-like action of MISE, a phosphodiesterase 5 inhibitor (sildenafil, 5 mg/kg i.p.) or vehicle was administered 20 min prior to the administration of MISE (100 mg/kg) and normal saline (10 mL/kg). The TST was performed 45 min after the administration of MISE and normal saline.42

5. STATISTICAL ANALYSIS

Results were expressed as means ± S.E.M. (n = 6). Two-way analysis of variance (ANOVA) followed by “Bonferroni posttests” was applied using Graph Pad Prism version 5. P < 0.05 was set as statistically significant.

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Author Contributions
S.I. performed the experimental work. U.S. supervised the project and drafted the manuscript. B.A. also helped in writing the original draft.

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