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Evaluation of antidepressant effects of the methanolic extract of *Achyranthes aspera* leaves in Swiss albino mice

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Abstract
Achyranthes aspera Linn. (Amaranthaceae) has been used as a treatment for piles, renal dropsy, kidney stone, snake bite, gonorrhea, and dysentery in Asian people. The recent study was designed to assess the antidepressant effects of the methanolic extract of *A. aspera* (MEAA) in different behavioral animal models on mice for 14 d treatment. The antidepressant-like activity was evaluated at the doses of 50, 100, and 200 mg/kg in the adult male mice by the forced swimming, tail suspension and open field tests. Deionized water was used as blank for control group. Imipramine hydrochloride (30 mg/kg) was employed as standard drug, respectively. Three test groups were received different doses of the MEAA for 14 d treatment. All substances were administered orally by the use of gavage. The major findings of the methanolic extract of *A. aspera* significantly reduced immobility times in both mice models for antidepressant-like activity such as FST and TST (*p<0.001). MEAA exhibited dose-dependent antidepressant activity in mice models. On the other hand, to assess the MEAA's motor stimulating activity, we performed an extra OFT. In compared to the control group, the extract also markedly boosted the effects of rearing (*p<0.001), defecation (*p<0.05), and locomotion. The results clearly showed that MEAA exerts an antidepressant-like effects in mice models. Although, need more research on the separation of active ingredients. Before using *A. aspera* to humans, it is strongly advised to investigate its mechanism of action.

Keywords: *Achyranthes aspera*, antidepressant, forced swimming, tail suspension

Introduction

Depression was recently identified as a mental condition with a significant incidence in humans, accounting for 21% of the global population [1]. The pathophysiology of depression is widely understood to include a malfunction in monoamine neurotransmitter circuits in the CNS [2]. Tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), and selective serotonin reuptake inhibitors (SSRI) are antidepressant drugs. There are also antidepressant herbal therapies like St. John's wort [4]. Furthermore, St. John's wort has been shown to disrupt drug metabolizing enzyme systems [5]. Antidepressant medicines, on the other hand, have a very limited effectiveness due to their negative effects. As a result, the demand for innovative, more tolerated, and effective treatments continues to grow. Because of their negative side effects, plant extracts may be an alternative. Due to the totality of ingredients, the active principle revalidated the therapeutic advantages of herbs [6]. As a result, herbal treatments might be called supplemental or alternative medicine. As a result, the exploration for a novel antidepressant herb with no side effects is critical. This is evident in the vast variety of herbal drugs whose potential for psychotherapy has been examined in diverse animal models.

Apang is the common name for *Achyranthes aspera* Linn. (Family: Amaranthaceae). In Bangladesh, it's recognized as "Upothlengra." In all parts of Bangladesh, a common herbaceous plant with tall spiny spikes and a woody base grows wild [8]. Many plant products are assessed in the current era of drug research and identification of novel therapeutic compounds based on their historic applications [9]. Unani physicians and local kabiraj prescribe the stems, leaves, and fruits for piles, renal dropsy, pneumonia, cough, kidney stone, skin eruptions, snake bite, gonorrhea, diarrhea, and other ailments. The extract of this plant reveals the presence of 27-Cyclohexyheptacosan-7-ol, 16-hydroxy-26 methyl heptacosan-2-one, 17-pentatriacontanol alkaloid, b-sitos-terol, spinasterol [10], fatty acids, a

number of oleonic acid, bisdesmosidic, triterpenoid based saponins, ecdysterone, n-hexacos-14-enoic, oleanolic acid, triacontanol, spinasterol, dihydroxy ketones, spathulenol, D-glucuronic, betaine, achyranthine and various amino acids [11]. It is used as antifertility agent [12], antisnake venom [13], antiobesity [14], and antiplant pathogen activity [15]. The plant is also stated to be used as antimicrobial [16], antitumor [17], antiinflammatory [18], cancer chemopreventive and antitumor property [19], immunostimulant and enhancer of the antigen clearance [20], antioxidant [21], diuretic [22], antinociceptive [23], analgesic [24], antipyretic [25], antispasmodic [26], antihepatitis [27], antiimplantation and abortifacient [28], antiarthritic [29], antileprotic [30], and an anthelmintic activity [31].

The force swimming and tail suspension tests are commonly used to determine antidepressant activity in animals [32]. These tests are simple to administer and can detect behavioral changes that are frequent in antidepressant therapies with desirable pharmacological and physiological effects. These tests can also tell you whether a medicine isn't antidepressant. As a result, several methodological changes were made in order to quantify the behavioral effects of such medications [33; 34]. Imipramine, maprotiline, and bupropion are selective noradrenergic and dopaminergic reuptake inhibitors that reduce immobility time, which is complex to tricyclic antidepressants and medications having selective effects on catecholamine transmission [34]. The development of behavioral immobility in the FST was specifically avoided by these medications [35]. In animal behavioral models, the antidepressant herbs *X. tang* and *B. monniera* considerably reduced the duration of immobility periods [36, 37]. Although, in research on rats, *A. aspera* exhibited a considerable decrease in immobility in a modified forced swimming test [38]. We have now documented antidepressant effects of the MEAA in all typical depression models in mice, where it was discovered to exhibit considerable antidepressant activity equivalent to the control group. However, significant study and long-term therapy have yet to be documented to demonstrate the MEAA's antidepressant properties in animal models of depression. This piqued our curiosity, so we decided to test the MEAA's effect in mice using animal models to look for antidepressant-like effects.

Methods

Plant material

A. aspera leaves were collected in Bogura, Dhaka, Bangladesh. Md. Abdul Mannan gathered the samples, and Principal Scientific Officer, Bangladesh National Herbarium, Mirpur-1, Dhaka, Bangladesh, identified them. For future reference, a reference sample has been dropped at the Herbarium.

Preparation of the plant extract

Fresh *A. aspera* leaves were dried at ambient temperature in an environment free of sunshine. The powder was made from the dried leaves of *A. aspera*. 250 g of ground materials were steeped in 1000 mL methanol in a beaker at 25 ± 2 ˚C for 72 hours, with a sterile glass rod stirring every 18 hours. With the aid of Whatman 102 filter paper and sterilized cotton bed, the filtrate was collected three times. Solvent was extracted using a rotary evaporator, yielding 12.23 g extract (Yield 5.09%). The acute toxicity, phytochemical screening, and antidepressant action of the extract of *A. aspera* were all investigated.

Animals

Animal Research Branch of the International Center for Diarrheal Disease and Research in Bangladesh collected 100 mature male (75 mice) and female (25 mice) Swiss albino mice 20-25 g body weight. Mice were kept in regular settings (temperature: 25˚C, humidity: 55-65%, 12-hour light/dark cycle). The mice were fed water and a pelleted mouse meal supplied by ICDDR,B. Prior to doing the studies, For 14 days, mice were acclimated to the lab setting. The night before the testing, the animals were not fed. The Ethical Principles and Guidelines for Scientific Experiments on Animals were produced by the Swiss Academy of Medical Sciences and the Swiss Academy of Sciences (1995), which were followed by all of the experimental mice. All experimental rules were authorized by Stamford University Bangladesh's Institutional Animal Ethical Committee.

Treatments

A. aspera methanolic extract was suspended in deionized water. In all of the tests, the standard medication Imipramine hydrochloride (Sandoz, Norvartis Bangladesh Ltd.) was employed. The medications were purchased from a nearby pharmacy. Methanol (Merck, Germany) and saline water were employed in the studies presented (Opsonin Pharma Ltd. Bangladesh). Mice were given a methanolic extract of *A. aspera* orally (p. o.) at dosages of 50, 100, and 200 mg/ kg, whereas the control group was given 0.1 mL/mouse deionized water via the same routes. 30 minutes before the studies, the usual medication Imipramine hydrochloride the dose of 30 mg/kg was also given in oral route. Gavage was used to provide the medication and samples to all of the groups. All groups of animals were given 14 days of treatment before the final tests. All other chemicals and reagents were of high purity and analytical grade.

Acute toxicity test

Within the weight range of 20-25 g, 25 adult healthy female mice were divided into 4 test groups and one control group (n=5). Deionized water was given to the control group (0.1 mL/mouse). The test groups were given oral at the doses of 500, 1000, 2000 and 3000 mg/kg of the extract of *A. aspera*, respectively. The animal was observed for the first four hours following medication to see whether there were any behavioral changes. They were, however, maintained under surveillance for 72 hours after receiving the medicine to see if there was any mortality [39].

Phytochemical screening

Flavonoids, alkaloids, saponins, cardiac glycosides, tannins, carbohydrates, reducing sugars, proteins, terpenoids and steroids were detected in a methanolic extract of *A. aspera* [40].

Test for alkaloids

3 mL of the extract and 3 mL of 1 percent HCl were combined on a steam bath. 1 mL of the mixture was taken in two test tubes independently. A few drops of Dragendorff's reagent were added to one test tube, and the appearance of an orange red precipitate was deemed positive. The second test tube was treated with Mayer's reagent, and the

appearance of a buff-colored precipitate was seen as a positive indicator of the presence of alkaloids.

Test for flavonoids
1 mL of the extract was mixed with 1 mL of a 10% lead acetate solution. The appearance of a yellow precipitate served as a marker for the presence of flavonoids.

Test for saponins
5 mL of the extract and 5 mL of heated deionized water were vigorously combined in a test tube. The generation of stable foam served as a proxy for the presence of saponins.

Test for tannins
Two milliliters of extract, two milliliters of deionized water, and a few drops of ferric chloride ($FeCl_3$) solution were combined. The production of a green precipitate revealed the presence of tannins.

Test for glycosides
To 2 mL of extract, 2 mL of diluted HCl, 2 mL of sodium nitropruside in pyridine, and 2 mL of sodium hydroxide solution were added. The development of a pink to blood red color is a sign that cardiac glycosides are present.

Test for carbohydrates
To each of the sections dissolved in deionized water, a few drops of Molisch's reagent were added. Next, 1 mL of concentrated H_2SO_4 was poured to the test tube's side. The mixture was given another two minutes to settle before being diluted with 5 mL deionized water. It denotes a poor outcome.
First, 1 mL of methanol extract and 1 mL of water were added to a test tube along with 20 drops of boiling Fehling's solution (A and B). The absence of precipitate red bricking at the test tube bottom indicates the absence of reducing sugars.
Second, mix 2 mL of aqueous solution with 5-8 drops of boiling Fehling's solution. The absence of a red-brick precipitate, which was present in the test tube, indicates the absence of reducing sugars.

Test for proteins
Xanthoproteic test: The extract was treated with a few drops of a strong nitric acid solution. The absence of yellow color in this test indicates a lack of proteins.
After being treated with 1 mL of a 10% sodium hydroxide solution, the extract was boiled. The mixture received a drop of 0.7% copper sulphate solution. The test's purplish violet hue indicates a lack of proteins.

Test for terpenoids
After being dissolved in 2 mL of $CHCl_3$, the methanol extract was dried. 2 mL of concentrated H_2SO_4 was then added and heated for around 2 minutes after that. A grey hue that appears when terpenoids are present is a sign.

Test for steroids
A red color was produced in the bottom chloroform layer, showing the presence of steroids, when 2 mL of methanol extract was mixed in 2 mL of chloroform and 2 mL of conc. sulphuric acid was added.
When 2 mL of the methanol extract was diluted in 2 mL of chloroform and treated with concentrated sulphuric acid and acetic acid, a greenish colour developed, indicating the presence of steroids.

Antidepressant activity tests
Forced swimming test
One of the most used pharmacological models for evaluating the effectiveness of antidepressants is the forced swimming test. Based on the observation of mice forced to swim, it was discovered that they only performed the movements required to maintain their heads above water. It was carried out using Porsolt et. al. [41].'s approach with certain changes. 25 healthy mice were randomly divided into five groups, each with five animals. Five groups of mice were given an oral treatment once a day between 1-3 p.m. for 14 days. After 14 days of treatment, mice were positioned individually in glass cylinder (Height 45 cm, Diameter 20 cm) completed to a 17 cm depth by water at 25±1 °C for 15 minutes (pre-test session). Between the pre-test session and the main session, mice were subjected to the identical circumstances for 5 minutes. Three oral drug doses were administered between the pre-test and the main session (just after the pre-test session, 5 h before the main test, and 1 h before the main test). A mouse was considered inactive unless it moved slightly to keep its head above water. Observers did it for 5 minutes between 1 and 3 p.m [42].

Tail suspension test
Antidepressants and other psychotropics may be screened using the tail suspension test, which is a simple, quick, and reliable approach [43]. This approach is founded on the observation that a mouse hanging by its tail agitates and immobilizes alternately. The tail suspension test caused immobility was decreased by a wide variety of clinically active and atypical antidepressants [44]. With minor adjustments, the test was described according to the method [45]. Twenty-five male mice were separated into five groups: control, positive control, and three test groups. There were five mice in apiece group. Two stands were set up with a 23-cm space between them, each with a clamp 22 centimeters from the floor. Mice were only considered motionless when they were passively suspended 5 cm after the end of their tail on the stand for 6 minutes. Between the hours of 1-3 p.m., the test was conducted. Observers assessed the duration of immobility [42].

Open field test
The test is a basic and straight forward method of observing mice and other rodents' behavior. The test was reported by Carlini *et al*. [46] in accordance with the procedure, with slight adjustments. Adult male mice were arbitrarily alienated into five groups, each through five animals. Over the course of 14 days, the experimental mice were given an oral treatment once a day between 1-3 p.m. Mice were placed in open field equipment after treatment, which consisted of a 40 cm diameter arena separated into 16 about equal regions. For this test, the animals were located in the middle of the box one by one and asked to keep track of the following behavioral data for 5 minutes: movement (number of squares crossing), raising (times observed upright on back legs), and number of defecations. The test was conducted between the hours of 8 and 10 a.m. [42].

Statistical analysis
The statistics was provided as a mean ± Standard Error of

the Mean. Using SPSS 18.00 software, the statistical investigation was carried out using one-way analysis of variance followed by Dunnett's post hoc test if needed. At a *p<0.001 level, differences amid groups were declared significant.

Results

The oral administration of the leave extract of *A. aspera* at dosages ranging from 500-3000 mg/kg did not result in death, but did cause behavioral abnormalities throughout 72-hours observation period. As a result, the methanolic extract of *A. aspera* has a low poisonousness profile, by an LD_{50} of more than 3000 mg/kg. Flavonoids, alkaloids, saponins, cardiac glycosides, tannins, terpenoids, and steroids were discovered in the leave extract of *A. aspera* during preliminary phytochemical screening (Table 1).

Table 1: Phytochemical screening of methanolic extract of *A. aspera* (MEAA).

Plant Constituents	Inference
Flavonoids, alkaloids, saponins, cardiac glycosides, tannins, terpenoids, and steroids	Present
Carbohydrates, reducing sugars, proteins	Absent

The mice were given the methanolic extract of *A. aspera* at dosages of 50, 100, and 200 mg/kg or the artificial antidepressant drug Imipramine hydrochloride (30 mg/kg) once day for 14 days. After 14 days, there was no differences in body weight increases across the treatment groups (Table 2).

Table 2: Effects of the extract of *A. aspera* (MEAA) in body weight (g) gain of mice.

Treatment	Dose(mg/kg)	Day 1	Day 14
Deionized water	0.1mL/mice	22.27±0.86	28.73±1.07
Imipramine hydrochloride	30	23.70±1.12	27.79±1.51
MEAA	50	22.00±1.37	25.84±2.02
MEAA	100	22.00±0.83	25.55±1.56
MEAA	200	23.20±1.06	26.33±0.76

In the forced swimming test, the MEAA at the dosages of 50, 100, and 200 mg/kg meaningfully decreased immobility time after 14 days of daily treatments (Table 3).

Table 3: Antidepressant effects of the extract of *A. aspera* (MEAA) in forced swimming test.

Treatment	Dose(mg/kg)	Immobility Time (s)
Deionized water	0.1mL/mice	117.40±2.15
Imipramine hydrochloride	30	49.20±1.85***
MEAA	50	94.40±2.13***
MEAA	100	71.00±1.51***
MEAA	200	53.40±2.13***

Values are denoted as mean ± SEM, (n= 5). *** p< 0.001 compared with the control group (Dunnett's test).

Dunnett's post hoc examination indicated that the test actions reduced immobility time considerably (***p<0.001) when likened to the control group. Similarly, the extract of *A. aspera* at the dosages of 50, 100, and 200 mg/kg considerably decreased the immobility time in the tail suspension test (Table 4).

Table 4: Antidepressant effects of the extract of *A. aspera* (MEAA) in tail suspension test.

Treatment	Dose(mg/kg)	Immobility Time (s)
Deionized water	0.1mL/mice	102.00±2.72
Imipramine hydrochloride	30	31.40±2.11***
MEAA	50	73.60±1.60***
MEAA	100	52.80±1.49***
MEAA	200	37.40±1.63***

Values are denoted as mean ± SEM, (n= 5). *** p< 0.001 compared with the control group (Dunnett's test).

However, post hoc examination revealed that the MEAA reduced immobility period considerably when compared to the control group (***p<0.001). Still, the value of lowering immobility time in the force swimming test has been demonstrated in the past with psychostimulants, which have a broad motor stimulating effect. Used to supplementary open field test to examine the motor stimulating action of the methanolic extract of *A. aspera* for this phony positive result. In the open field test, there was a behavioral shift or motor stimulating activity, demonstrating that the reduction in immobility time following oral administration of *A. aspera* methanolic extract was due to antidepressant-like effects. (Table 5).

Table 5: Antidepressant effects of the extract of *A. aspera* (MEAA) in open field test.

Treatment	Dose(mg/kg)	Locomotion (Number of squares travelled)	Rearing (Number)	Defecation (Number)
Deionized water	0.1mL/mice	96.40±2.60	31.20±1.42	1.40±0.24
Imipramine hydrochloride	30	216.20±2.67***	51.20±0.97***	2.80±0.37*
MEAA	50	115.20±2.92***	38.60±1.36**	1.60±0.24
MEAA	100	149.20±2.59***	49.60±2.24***	2.20±0.37
MEAA	200	186.40±2.78***	58.80±1.35***	3.00±0.44*

Values are denoted as mean ± SEM, (n= 5).
* p< 0.05 compared with the control group (Dunnett's test).
** p< 0.01 compared with the control group (Dunnett's test).
*** p< 0.001 compared with the control group (Dunnett's test).

At the dosages of 50, 100, and 200 mg/kg, the methanolic extract of *A. aspera* was shown to have the best locomotor effect. This test also revealed that at the dosages of 50, 100, and 200 mg/kg, the positive rearing impact was considerable. Nonetheless, the dosage of 200 mg/kg was demonstrated to have a satisfactory defecation phase impact. Furthermore, as compared to the control group, post hoc examination revealed that the extract meaningfully increased locomotion, rearing (***p<0.001), and defecation *(p<0.05) impacts.

Discussion

The goal of this research was to see that MEAA had an antidepressant effect on mice. The selection of new antidepressant medications is essential. Although, in the case of antidepressant efficacy evaluation, established

methods of a robust animal model capable of identifying distinct depressant treatments sufficiently and accurately without making errors [47]. In this scenario, forced swimming and tail suspension procedures are well established behavioral paradigms for assessing antidepressant activity. In these tests, the typical conduct is examined. Immobility time has been thought to indicate a behavioral misery comparable to the human depression. Antidepressant medicines are widely recognized for their ability to shorten animal immobility time [48]. When mice are exposed to basic stress, as a forced swimming test, their immobility duration tends to indicate a state of misery or depressed attitude, which is supposed to mimic depressive illnesses in people. Furthermore, therapy with depressing medicines has been shown to shorten immobility time [48]. Antidepressant medications' clinical effectiveness and potency were revealed to have a significant association in these animals [45; 48]. Surprisingly, all dosages of plant extract were beneficial in both experiments, according to our findings.

The forced swimming test is one of the most frequently used animal models for assessing antidepressant-like behavior. It has long been believed that the noradrenergic or serotonergic system plays a part in the pathophysiology of depression and the mechanism of antidepressant action [49; 50]. Depression is treated using substances or drugs that affect noradrenergic neurotransmission, such as monoamine oxidase inhibitors (MAOIs) and noradrenaline reuptake inhibitors (SNRIs) [51], and most antidepressants work by boosting monoamine bioavailability in the brain [52; 53]. The wide range of medicines now used to treat despair consistently raise synaptic stages of these monoamines [54]. We also discovered that imipramine hydrochloride had considerable antidepressant effects in mice based on our findings. However, originated on our outcomes, we may presume that MEAA's antidepressant effects are linked to an increase in central noradrenergic or serotonergic neurotransmissions.

The tail suspension test is a stress model that has been widely utilized as a compelling behavioral paradigm for antidepressant activity prediction. In these studies, animals are placed in an inescapable situation, and antidepressant-like activity is measured by a decrease in immobility time, which is a side effect of traditional antidepressants [55; 45]. It creates an unfavorable mental and physical environment for mice. The immobility was then documented in accordance. It's interesting to note that the 5-HT1A receptors have been linked to the therapeutic effects of antidepressants [56]. These inhibitory autoreceptors, which are found presynaptically on the soma and dendrites of 5-HT neurons in the dorsal raphe, reduce firing rate and serotonin release [57]. As a result, antidepressant drugs that block these receptors and prevent this negative feedback might be helpful [58]. However, chemicals that promote an increase in animal locomotor activity might result in a false positive TST result. The increase in locomotor activity distinguishes the effects of psychostimulant medications from those of antidepressants [35]. As a result, the open-field test [59; 60] is used to rule out the possibility that the reduction in immobility time caused by a specific drug in both tests is related to an increase in locomotor activity in animals. The first hypothesis of depression was proposed around 40 years ago, claiming that the principal symptoms of depression are caused by a functional shortage of cerebral monoaminergic transmitters such as (NE), 5HT, and dopamine (DA) at synapses [61]. Another study [62] that looked at the antidepressant effects of natural estrogen 17 estradiol, ethynyl estradiol and diethyl-stilbesterol likewise confirmed Imipramine hydrochloride's antidepressant-like effects. The flavonoid, which binds to the central benzodiazepines receptor with high affinity, has significant anxiolytic [63; 64] and depressive properties [65]. It is suggested that flavonoid glycosides, which reach brain tissues through the metabolizing process, protect brain function from CNS disturbance and, as a result, display antidepressant-like effects, are a component of one of the MEAA's antidepressant processes.

Acute toxicity is defined as "any consequence that produces functional impairment or biochemical lesions that may compromise the whole animal's performance or reduce the organ's ability to react to subsequent challenges" [66]. As a result, a substance that enters the body through the mouth for a short period of time and generates any harmful consequence with slight latency is considered orally and extremely poisonous. Acute toxicity test, when correctly done and attentively monitored, has been said to provide more information about a chemical compound's biologic features than any other single test [67]. Adult twenty-five Swiss albino female mice were utilized in this investigation of acute oral toxicity to examine the toxicity effects of the MEAA leaves. Mice have been demonstrated to be a superior predictor of human acute lethal dosage in studies. At the dosage of 3000 mg/kg, the MEAA extract appears to be harmless, and the LD_{50} is estimated to be >3000 mg/kg [68]. The crude extract was given to the mice orally, so they had to fast before taking the dosage to avoid food and other substances in their digestive systems interfering with the extract components' responses. When evaluating acute toxicity, the oral route of administration is often the most convenient and widely employed. It is less expensive and causes no harm to the animal [68]. As a result, no anesthetic is required during the injection of the extract. The test animals, on the other hand, were treated in the same way as traditional healers treated their patients. This would allow any results obtained in mice to be easily translated to what may be expected in humans.

Furthermore, the presence of monoterpenoid chemicals such as carvone and thujone in *Aloysia polystachya* essential oil has been linked to anxiolytic and antidepressant-like effects [69]. Similarly, MEAA's antidepressant-like effects might be attributed to terpenoids, which are one of its ingredients. Nonetheless, the current investigation found that MEAA has an antidepressant-like effect when it interacts with the noradrenergic or serotonergic neurotransmitter systems. Though, further research is required to fully comprehend the mechanism of its effect.

Conclusions

MEAA exhibits an antidepressant effect in mice, according to the force swimming, tail suspension, and open field tests. The pattern of effects seen in these studies implies that the noradrenergic or serotonergic neurotransmitter system may be involved in the antidepressant effects. Before using the leave extract of *A. aspera* in humans, more research on the extraction and fractionation of the active constituents, as well as the mechanism of action, is strongly advised.

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Competing interests
No conflicts of interest were reported by the authors.

References
1. Schechter LE, Ring RH, Beyer CE, Hughes ZA, Khawaja X, Malberg JE, Rosenzweig-Lipson S. Innovative approaches for the development of antidepressant drugs: current and future strategies. NeuroRx. 2005;(2):590–611.
2. Nemeroff CB, Owens MJ. Treatment of mood disorders. Nature Neuroscience. 2002;5:1068-1070.
3. Belmaker RH, Agam G. Major depressive disorder. New England Journal of Medicine. 2008;358:55-68.
4. Chambers CD, Hernandez-Diaz S, Van Marter LJ, Werler MM, Louik C, Jones KL, *et al*. Selective serotonin-reuptake inhibitors and risk of persistent pulmonary hypertension of the newborn. N Engl J Med. 2006;354:579-587.
5. Markowitz JS, Donovan JL, DeVane CL, Taylor RM, Ruan Y, Wang JS, *et al*. Effect of St John's wort on drug metabolism by induction of cytochrome P450 3A4 enzyme. JAMA. 2003;290:1500-1504.
6. Zhang ZJ. Therapeutic effects of herbal extracts and constituents in animal models of psychiatric disorders. Life Sci. 2004;75:1659-1699.
7. Anonymous. The Wealth of India - Raw Materials, Council of Scientific and Industrial Research (CSIR). New Delhi, 2007, 55-57.
8. Ghani A. Medicinal Plants of Bangladesh: Chemical Constituents and Uses. Asiatic Society of Bangladesh. Dhaka, 1998, 63.
9. Jain JB, Kumane SC, Bhattacharya S. Medicinal flora of Madhya Pradesh and Chattisgarh: A Review. Ind J of Trad Know. 2006;5:237-242.
10. Prain D. Bengal plants, Botanical survey of India. Calcutta, 1963, 646-655.
11. Srivastava PK. *Achyranthes aspera*: a potent immunostimulating plant for traditional medicine. International journal of pharmaceutical sciences and research. 2014;5:1601-1611.
12. Pakrashi A, Mookerji N, Basak B. Effect of chromatographic fractions of the plant *Achyranthes aspera* Linn on fertility in female albino mice. J. Reprod. Fertil. 1975;43:127-128.
13. Samy RP, Thwin MM, Gopalakrishnakone P, Ignacimuthu S. Ethnobotanical survey of folk plants for the treatment of snakebites in southern part of Tamilnadu, India. J Ethnopharmacol. 2008;115:302-312.
14. Mangal A, Sharma MC. Evaluation of certain medicinal plants for antiobesity properties. Indian Journal of Traditional Knowledge. 2009;8:602-605.
15. Singh HNP, Prasad MM, Sinha KK. Efficacy of leaf extracts of some medicinal plants against disease development in banana. Letters in Applied Microbiology. 1963;17:269–271.
16. Thilagavathi G, Kannaian T. Application of Pricklychaff (*Achyranthes aspera* Linn.) leaves as herbal antimicrobial finish for cotton fabric used in healthcare textiles. Natural Pro Rad. 2008;7:330-334.
17. Chakraborty A, Brantner A, Mukainaka T, Nobukuni Y, Kuchide M, Konoshima T, *et al*. Cancer chemopreventive activity of *Achyranthes aspera* leaves on Epstein - Barr virus activation and two stage mouse skin carcinogenesis. Cancer letters. 2002;177:1-5.
18. Vetrichelvan T, Jegadeesan M. Effect of alcohol extract of *Achyranthes aspera* Linn. on acute and subacute inflammation. Phytother Res. 2003;17:77-79.
19. Chakraborty A, Brantner A, Mukuinaka T, Nobukuni Y, Kuchido M, Konoshima T. Cancer chemo preventive activity of *Achyranthes aspera* leaves on Epstein-Barr virus activation and two stage mouse skin carcinogenesis. Cancer Letters. 2002;177:1-5.
20. Chakrabarti R, Vasudeva Rao Y. *Achyranthes aspera* stimulates the immunity and enhances the antigen clearance in *Catla catla*. Int. Immuno pharmacol. 2006;6:782-790.
21. Suresh Kumar P, Sucheta S, Deepa VS, Selvamani P, Latha S. Antioxidant activity in some selected Indian medicinal plants. African Journal of Biotechnology. 2008;7:1826-1828.
22. Subramaniam R. Cystone -a vegetable diuretic. The Antiseptic. 1961;2:103-106.
23. Barua CC, Talukdar A, Begum SA, Lahon LC, Sarma DK, Pathak DC, *et al*. Antinociceptive activity of methanolic extract of leaves of *Achyranthes aspera* Linn. (Amaranthaceae) in animal models of nociception. Indian J. Exp. Biol. 2010;48:817-821.
24. Kumar PS, Sucheta S, Deepa VS, Selvamani P, Latha S. “Antioxidant activity in some selected Indian medicinal plants, African Journal of Biotechnology.0 2008;7:1826-1828.
25. Sutar NG, Sutar UN, Sharma YP, Shaikh IK, Kshirsagar SS. Phytochemical investigation and pharmacological screening of leaves of *Achyranthes aspera* Linn. as analgesic and antipyretic. Biosciences Biotechnology Research Asia. 2008;5:841-844.
26. Aswal BS, Goel AK, Kulshrestha DK, Mehrotra BN, Patnaik GK. Screening of Indian plants for biological activity. Part XV., Ind. J. Exp. Biol. 1996;34:444-467.
27. Dange SV, SA Phadke. Comparative efficacy of five indigenous compound formulations in patients of acute viral hepatitis. Maharashtra Medical Journal. 1989;36:75.
28. Vasudeva N, Sharma SK. Post-coital antifertility activity of *Achyranthes aspera* Linn root. J Ethnopharmacol. 2006;107:179-181.
29. Aggarwal D, Singh H. Kshara basti in amavata (rheumatoid arthritis), Sachitra Ayurved. 2006;59:223-224.
30. Tripathi SN, Chaturvedi GN, Dube GP. Effect of *Achyranthes aspera* in the treatment of leprosy. J. Med. Sci. 1963;4:103-112.
31. Sujitha K, Phani Sri A, Mohan Rao PM, Lal Mahammed, Srinivasarao K, Karuna Sree V. Preliminary screening of Syzygium cumini and *Achyranthes aspera* for their anthelmintic activity. Research Journal of Pharmacognosy and Phytochemistry. 2010;2:441-445.
32. Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. Nature. 1977a;266:730-732.
33. Cryan JF, Mombereau C, Vassout A. The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. Neuroscience and Biobehavioral Reviews. 2005;29:571-625.

34. Lucki I. The forced swimming test as a model for core and component behavioral effects of antidepressant drugs. Behav Pharmacol. 1997;8:523-532.
35. Borsini F, Meli A. Is the forced swimming test a suitable model for revealing antidepressant activity? Psychopharmacol. 1988;94:147-160.
36. You-Zhi Z, Yun-feng LI, Neng-jiang YU. Antidepressant- like effects of the ethanolic extracts of Xiaobuxin-Tang, a traditional Chinese herbal prescription in animal models of depression. Chinese Med J. 2007;120:1792-1796.
37. Mannan MA, Abir AB, Rahman MR. Antidepresssant-like effect of the methanolic extract of *Bacopa monniera* in mice. BMC Complementary and Alternative Medicine. 2015;15:337.
38. Barua CC, Begum SA, Talukdar A, Pathak DC, Barua AG, Borah P, *et al*. Effect of *Achyranthes aspera* Linn on modified forced swimming in rats. Pharmacologyonline. 2010;1:183-191.
39. Seth UK, Dadkar NK, Kamat UG. Selected Topics in Experimental Pharmacology. Kothari Book Depot, Bombay, 1st edition, 1972, 126.
40. Ghani A. Medicinal Plants of Bangladesh with Chemical Constituents and Uses. 2nd Ed. The Asiatic Society of Bangladesh, Dhaka, 2003, 331-332.
41. Porsolt RD, Bertin A, Jalfre M. Behavioural despair in mice: a primary screening test for antidepressants. Acrh.Inter. Pharmacodyn. Ther. 1977b;229:327-336.
42. Sakakibara H, Ishida K, Grundmann O, Nakajima JI, Seo S, Butterweck V, *et al*. Antidepressant effect of extracts from *Ginkgo biloba* leaves in behavioral models. Biol Pharm Bull. 2006;29:1767-1770.
43. Wesołowska A, Nikiforuk A, Stachowicz K, Tatarczyńska E. Effect of `the selective 5-HT7 receptor antagonist SB 269970 in animal models of anxiety and depression. Neuropharmacology. 2006;51:578-586.
44. Nanjappa K, Shalam M, Harish M, Prabhu, Rao S, Kutty B. Pharmacological and neurobiochemical evidence for antidepressant-like effect of Sumind, A herbal product in animals. The Internet Journal of Nutrition and Wellness, 2006, 4.
45. Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacol. 1985;85:367-370.
46. Carlini EA, Contar J, De DP, Silva-Filho AR, Da Silveira-Filho NG, Frochtengarten ML, *et al*. Pharmacology of lemongrass (*Cymbopogon citratus* Stapf). I. effects of teas prepared from the leaves on laboratory animals. J Ethnopharmacol. 1986;17:37-64.
47. Willner P. The validity of animal models of depression. Psychopharmacology (Berl). 1984;83:1–16.
48. Porsolt RD. Behavioral despair, Antidepressants: neurochemical, behavioral and clinical perspectives. Edited by Enna SJ, Malick JB, Richelson E. New York: Raven Press, 1981, 121-139.
49. Frazer A. Norepinephrine involvement in antidepressant action. Journal of Clinical Psychiatry. 2000;61:25-30.
50. Nutt DJ. The role of dopamine and norepinephrine in depression and antidepressant treatment. Journal of Clinical Psychiatry. 2006;67:3-8.
51. Pa´ ez-Pereda M. New drug targets in the signaling pathways activated by antidepressants. Progress in Neuropsychopharmacology and Biological Psychiatry. 2005;29:1010-1016.
52. Blier P. Possible neurobiological mechanisms underlying faster onset of antidepressant action. Journal of Clinical Psychiatry. 2001;62:7-11.
53. Brunello N, Mendlewicz J, Kasper S, Leonard B, Montgomery S, Nelson J, Paykel E, *et al*. The role of noradrenaline and selective noradrenaline reuptake inhibition in depression. European College of Neuropsychopharmacology. 2002;12:461-475.
54. Risch SG, Nemeroff CB. Neurochemical alterations of serotonergic neuronal systems in depression. Journal of Clinical Psychiatry. 1992;53:3-7.
55. Cryan FJ, Valentino RJ, Lucki I. Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. Neurosci Biobehav Rev. 2005;29:547-569.
56. Celada P, Puig M, Amargo´s-Bosch M, Adell A, Artigas F. The therapeutic role of 5-HT1A and 5-HT2A receptors in depression. Journal of Psychiatry and Neuroscience. 2004;29:252-265.
57. Shrestha S, Hirvonen J, Hines CS, Henter I, Svenningsson P, Pike VW, *et al*. Serotonin-1A receptors in major depression quantified using PET: controversies, confounds, and recommendations. Neuroimage. 2012;59:3243–3251.
58. Blier P, Ward NM. Is there a role for 5-HT1A agonists in the treatment of depression? Biological Psychiatry. 2003;53:193-203.
59. Rodrigues ALS, Rocha JB, Mello CF, Souza DO. Effect of perinatal lead exposure on rat behavior in open-field and two-way avoidance tasks. Pharmacology and Toxicology. 1996;79:150–156.
60. Rodrigues ALS, da Silva GL, Mateussi AS, Fernandes ES, Miguel OG, Yunes RA, *et al*. Involvement of monoaminergic system in the antidepressant-like effect of the hydroalcoholic extract of Siphocampylus verticillatus. Life Sciences. 2002;70:1347-1358.
61. Schildkraut JJ. The catecholamine hypothesis of affective disorders. A review of supporting evidence. Am J Psychiat. 1965;122:509.
62. Estrada-Camarena E, Fernandez-Guasti A, Lopez-Rubalcava C. Antidepressant like effect of Different estrogenic Compounds in the Forced Swimming Test. Neuropsychopharmacol. 2003;28:830-838.
63. Svenningsen AB, Madsen KD, Liljefors T, Stafford GI, Van SJ, Jager AK. Biflavones from Rhus species with affinity for the GABA (A) /benzodiazepine receptor. J Ethnopharmacol. 2006;103:276-280.
64. Rang H, Dale M, Ritter J, Moore P. Pharmacology. 6th ed. Edinburgh: Churchill Livingstone, 2007.
65. Jager AK, Saaby L. Flavonoids and the CNS. Molecules. 2011;6:1471-1485.
66. Barahona MV, Sanchez-Fortu'n S. Toxicity of carbamates to the brine shrimp Artemia salina and the effect of atropine, BW284c51, iso-OMPA and 2-PAM on carbaryl toxicity. Environ Pollut, 104, 469–476.
67. Walum E. (1998). Acute oral toxicity. Enviro Health Perspect. 1999;106:497-503.
68. Syahmi AR, Vijayarathna S, Sasidharan S, Latha LY, Kwan YP, Lau YL. Acute oral toxicity and brine shrimp lethality of Elaeis guineensis Jacq. (oil palm leaf) methanol extract. Molecules. 2010;15:8111-8121.
69. Mora S, Diaz-veliz G, Millan R. Anxiolytic and antidepressant like effect of the hydroehanolic extract from *Aloysia polystachya* in rats. Pharmacol Biochem Behav. 2005;82:373-378.