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Screening of antidepressant activity of *marsilea minuta* in wistar albino rats

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ABSTRACT

Depression is a widely prevalent form of mental illnesses worldwide. It is commonly associated with sad mood, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, and low energy. *Marsilea minuta* has many medicinal properties, and are used in traditional medicine in the treatment of various medical conditions. This study was conducted to better understand the antidepressant activity of *Marsilea minuta*. To evaluate the *in vivo* antidepressant activity of Methanolic extract of *Marsilea minuta* leave in Swiss albino mice. Methanolic extract of *Marsilea minuta* (MEMM) leaves was prepared by a continuous method using Soxhlet apparatus. The extract was subjected to phytochemical screening followed by acute oral toxicity studies in mice. MEMM in the doses of 100mg/Kg, 200mg/Kg and 400mg/Kg mg/kg body weight was administered to test groups Group 3, 4 and 5 respectively. Imipramine hydrochloride 15mg/kg body weight was administered to Standard group by oral route. Test group 3 received 100mg/kg (p.o). Control group received Normal saline 10ml/kg body weight. Antidepressant activity was identified by using modified Forced Swimming Test (FST) and Tail Suspension Test (TST). Period of immobility was observed in both the models which was indicative of anti depressant activity. Standard statistical methods were used to evaluate the results. The results showed significant dose dependent antidepressant effect of EASL in Swiss albino mice for both the models in all the test groups (Test group I, II and III). MEMM possess significant antidepressant activity. However, further investigations are required to determine its active constituents and molecular level of target mechanism of the extract for further use in humans.

Keywords: *Marsilea minuta*, Antidepressant activity, forced swim test, Open Field Test.

INTRODUCTION

Depression: It is basically acknowledged as illness with symptoms such as anxiety and sleep disturbances. It can be a persistent, recurring illness that can cause many personal suffering for individuals and their families. At present, disability caused by depression is estimated to be the fourth most important cause of worldwide loss of life years. This has resulted into a requirement of search for effective treatments, including antidepressant drugs, herbal remedies, psychotherapy and electroconvulsive shock therapy.

THE NEUROBIOLOGY AND PHARMACOLOGY OF DEPRESSION

Neurotransmitter Systems

Within the central nervous system (CNS), the catecholamines, adrenaline, noradrenaline and dopamine forms the adrenergic systems. Out of these, few of the adrenergic neurons are radiating from the ancient limbic system and plays to role of discharging the catecholamines within the frontal cortex. Thus, the catecholaminergic pathways are claimed to be responsible for mood, alertness and stress responses. The primary neurotransmitter, which modulates the excitatory catecholamine systems of the CNS is Serotonin. The Serotonin neurons are responsible for the control of memory, mood, sex drive and appetite.[1] The systems of serotonin and noradrenaline are the important their main cell small bodies in brainstem areas that serve as headquarters for shipping axonal projections by the brains in specific pathways that mediate specific functions (See Figure No. 1 for an illustration of the serotonin projections and Figure No. 2 for an illustration of the noradrenergic projections). Multiple serotonergic and noradrenergic

pathways may be dysfunctional in depression, generating many different symptoms.

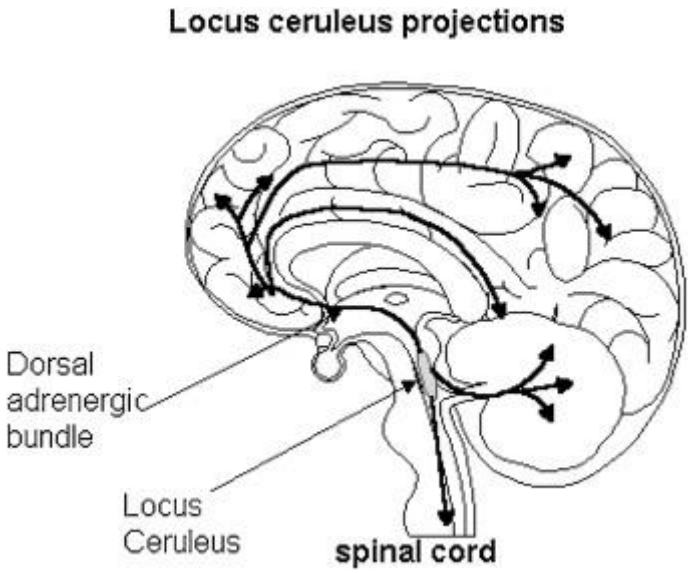

Fig 1: The projection of the noradrenaline system

The nucleus of the dorsal raphe projects the serotonin system and the raphemagnus. The serotonin receptors (5-HT) have been identified into various sub-types with the 5-HT1 and 5-HT2 sub-types being of greater interest in psychiatry. The most important of the 5-HT1 subclass is 5- HT1A which is concentrated in the hippocampus and raphe. The release of this 5 – HT from presynaptic neurons is modulated by this autoreceptor. The 5 - HT2 rceptors occur in high concentrations in the frontal cortex and nucleus accumbens.[2]

Hypotheses of Depression

Several hypotheses of the biological determinants of depression have emerged over the past century. The most important of these and the implications thereof are reviewed below. Today it is generally accepted that depression is not necessarily due to a shortage of one vital brain neurotransmitter, but rather to a disruption in the equilibrium between different regulatory systems.

The Biogenic Hypothesis of depression

The most common characteristic of depresion as claimed by monoaminergic hypothesis are a result of inadequate concentration of serotonin and noradrenaline in the synaptic clefts of the neurons in the brain.[3] This hypothesis has evolved to consider the possibility that depression may be the result of a deficiency in signal transduction from the monoamine neurotransmitter to its postsynaptic neuron, even with normal levels of neurotransmitter and receptor being present. Emerging theories that link genetic and environmental risk factors for depression suggest that stress can cause depression by downregulating certain genes, resulting in less key gene products, such as the brain-derived neurotrophic factor (BDNF), being produced. BDNF sustains the viability of neurons, so if the encoding gene is repressed the result may be atrophy or even apoptosis of neurons.

The dopamine hypothesis of depression

The original hypothesis was formulated in the late nineteen seventies by Solomon Snyder and linked schizophrenia with dopamine (DA) activity. Later, this hypothesis was extended to include depression following the observation that many antidepressants influence the metabolism of dopamine. Following chronic antidepressant treatment, the presynaptic DA receptors become subsensitised and this gets in an enhancement of DA release. A reduction in homovallinic acid (HVA), the main metabolite of dopamine, in the cerebral spinal fluid (CSF) of depressed patients who demonstrate marked motor retardation has also been reported. Therefore, a decrease in the ratio of HVA to DA is indicative of decreased turnover of DA. This hypothesis is also supported by reports of significantly reduced dopamine turnover in depressed suicide victims.

The permissive hypothesis of depression

This hypothesis emphasizes 5-HT as a neuro-modulator and its importance as a focus for antidepresant action. According to this theory, a lowered concentration in the central nervous system (CNS) of 5-HT results in an affective state regulated by NA. Decreased 5-HT and NA levels will give rise to depression. This Averages that 5-HT may act as a 'permissive' modulator of neurotransmitter function through connections between serotonergic pathways and make connections with noradrenergic and dopaminergic pathways via the associated receptors.

The glutamatergic N-methyl-D-aspartate hypothesis

As per recent researches, one of the important roles involved in the mechanism of depression is dysfunction of CNS glutamatergic pathways. Many of the researches confirm that the compounds, which induce reduction in the activities at the N – Methyl – D – Aspartate receptors produce effects similar to pharmacologically active antidepressants. Hence, it is assumed that the common pathway affected by antidepressant

drugs, whenever there are adaptive changes in NMDA receptor complex.

The kynurenine hypothesis

This hypothesis emerges from the premise that depression arises from altered levels of serotonine (5-Hydro. Trypt.) in the mind Serotonin is a metabolite of the esential A. A. tryphtophan (TRP) and all 5- Hydro. Trypt required by the neurons in the brain is synthesized in the brain because 5-Hydro. Trypt is unable to cross the BBB. Therefore, the availability of TRP is essential for the synthesis of 5-HT in CNS. There are several factors which affect the production and transport of TRP from the blood stream into the CNS, in which deficiency of Vit. B6, Stress, escalated cortisol levels and even high doses of TRP (2000m.g. of TRP).These are the factors simulating the conversion of TRP into kynurenine, which further results ito reduced TRP level. Therefore, the inhibition of liver enzyme tryptophan 2,3-dioxygenase (also known as tryptophan pyrrolase) during the first and rate - limiting step of the pathway of kynurenine would enhance circulating levels of TRP and thereby lead to increased neural production.

MATERIALS AND METHODS

The designing of methodology involves a series of steps taken in a systematic way in order to achieve the set goal (s) under the prescribed guidelines and recommendations. It includes in it all the steps from field trip to the observation including selection and collection of the medicinal plant, selection of dose value, standardization of protocol, usage of instruments, preparation of reagents, selection of specific solvents for extraction, formation of protocols and final execution of the standardized protocol. All this requires good build of mind and a good and soft technical hand to handle the materials and procedure in a true scientific manner.

Drugs and Chemicals

Drugs and Chemicals used in this study were of analytical grade and of highest purity procured from standard commercial sources in India. Materials: Imipramine, Nicholos Piramal Ltd

EXPERIMENTAL ANIMALS

Swiss albino rats, 60 in number, weighing 20-30 g, of either sex, maintained under standard conditions in the Institutional animal house were used. They were housed in clean, transparent polypropylene cages in groups of six and maintained at standard laboratory temperature and humidity (40-60%) with light/dark cycle of 12:12 hours. Animals were fed commercial pelleted chow and water. The rats were allowed to acclimatize to these conditions for a week before starting the experiments. The standard drug, Imipramine hydrochloride, was obtained from Abbot Healthcare Pvt Ltd (Depsonil 25).

Wistar rats (150-200 g) and Swiss albino mice (18-22g) of either sex selected for the study. Animals were housed in appropriate cages in uniform hygienic conditions and fed with standard pellet diet (Amrul Laboratory Animal Diet) and water ad libitum. All the animals were maintained under standard conditions, that is room temperature 26 ± 1°C, relative humidity 45 - 55% and12:12 h light – dark cycle. Animal studies had approval of IAEC.

Plant Material Collection

The fresh leaves of *Marsilea minuta* was collected from local market. The plant material was cleaned, reduced to small fragments, air dried under shade at room temperature and coarsely powdered in a mixer. The powdered material was stored or taken up for extraction process.

Preparation of plant extracts

Preparation of Methanolic Extract

The *Marsilea minuta* plants were washed, the leaves were shade dried and powdered. About 200 g of the dried leaf powder of *Marsilea minuta* was extracted with 99.9% Methanol in Soxhlet extractor for about 36 hours. The Methanol was then evaporated from the mixture by placing it in a beaker and heating it over a water bath. The extract gave a yield of brownish paste like mass weighing 6g. The yield obtained was 3% w/w with respect to dried powder.

PRELIMINARY PHYTOCHEMICAL SCREENING

Preliminary phytochemical screening of the plant extract was carried out for the analysis of Alkaloids, Carbohydrates, Tannins, Saponins, Steroids, Phenols, Flavonoids .as per the standard methods.

1. Detection of Alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide).Formation of a yellow coloured precipitate indicates the presence of alkaloids.

b).Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

c).Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

d).Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution).Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of Carbohydrates: Extracts were dissolved individually in 5ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a).Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

b).Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

c).Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A&B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of saponins

a). FrothTest: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for15 minutes. Formation of 1cm layer off a am indicates the presence of saponins.

b).FoamTest:0.5gm of extract was shaken with 2ml of water. If foam produced persists forten minutes it indicates the presence of saponins.

4. Detection of steroids.

a).Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

b).Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

5. Detection of Phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of Tannins

Gelatin Test: To the extract,1 %gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

7. Detection of Flavonoids

Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Leadacetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Selection of dose for animal study

The dose considered for the experiment on rats was obtained from conversion of human dose of *Marsilea minuta* (3-5 g/kg). The conversion factor of human dose (per 200 g body weight) is 0.018 for rats and 0.002 for mice (Ghosh 1984). Hence the calculated dose for the rats (considering human dose 3 and 5 g/kg) is 200 mg/kg and for mice is 20 mg/kg. Acute toxicity was done at dose of 2000mg/kg body weight.

PHARMACOLOGICAL EVALUATION

ACUTE ORAL TOXICITY

The acute oral toxicity of aqueous and alcoholic extracts of *Marsilea minuta* was determined by using rats and mice which were maintained under standard conditions. The animals were fasted 12 hour prior to the experiment, up and down procedure OECD guideline no. 425 were adopted for toxicity studies. Animals were administered with single dose of individual extract up to 2000mg/kg and observed for its mortality during 2days and 7days study period (short term) toxicity and observed up to 7days for their mortality, behavioral and neurological profiles.

SCREENING FOR ANTIDEPRESSANT ACTIVITY

The Methanolic extracts of *Marsilea minuta* leaves were tested for antidepressant activity using despair swim test and tail suspension test.

Treatment

The Wistar albino rats (n=60) were divided into two arms which was further divided into five groups, each group having six Wistar albino rats. Drugs were given orally after 12 hours of fasting every day, for ten days.

The drugs were prepared and administered per oral (0.1ml/10g).

Group 1 was administered normal saline (10ml/kg).

Group 2 was given standard drug Imipramine (15mg/kg). 12

Group 3, 4 and 5 received 100mg/kg, 200mg/kg, and 400mg/kg doses of the test compound Methanolic Extract of *Marsilea minuta* respectively.

For the Acute study, on day 1, one arm of 30 Wistar albino rats were subjected to Tail Suspension Test (TST), while 30 mice in the other arm were subjected to Forced Swim Test (FST), one hour after feeding the respective drugs. For Sub acute study, on day 10, the Wistar albino rats were again subjected to TST and FST, one hour after feeding respective drugs.

PROCEDURE FOR ANTIDEPRESSANT ACTIVITY

FORCED SWIM TEST (FST)

The method used was as described by Porsolt et al. The rats were individually forced to swim in a vertical plexiglass cylinder (capacity: 5L, height: 50cm diameter: 18cm) containing l5cm of water maintained at temperature: 25◦C. Rats were subjected to pre-screening, which lasted for 15 minutes. 24 hours after pre-screening, the trial was performed for 6 minutes of which the first two minutes were not recorded, and the periods of immobility for the latter four minutes was measured (in seconds) with a stopwatch. Rats were considered to be immobile when they made only the bare necessary movements to stay afloat, or when they were motionless. The Rats were taken out of the plexiglass cylinder after 6 minutes. They were dried with a dry towel, and kept under a dim lamp for drying. The water was discarded after every test, and fresh water was used for the next rats.

TAIL SUSPENSION TEST (TST)

The method used was as described by Steru et al. Antidepressants that are used in practice are able to reduce the period of immobility of rats when they try to escape when suspended by their tail. This test was a reliable screening method for antidepressants, including those involving serotonergic system. Mice ware hung on a wooden rod, 50 cm above the table, by attaching them from their tail end with the use of an adhesive tape. The first two minutes were not recorded, and the periods of immobility for the latter six minutes was recorded (in seconds) with a stopwatch. Rats were considered to be immobile only when they were motionless and not attempting to escape.

STATISTICAL ANALYSIS

Statistics The recorded data was entered in Microsoft Excel. The variables recorded followed normal distribution, hence, results have been expressed as mean (in seconds) ± standard error of mean (SEM). The data was analysed using one way ANOVA followed by post-hoc Dunnet's test. Probability 'p' value less than 0.05 was considered as statistically significant.

OPEN-FIELD TEST

For open-field test, animals were divided into four groups (n = 10 /group): control (0.9% saline), the three doses of *Marsilea minuta* (100, 200, 300 mg/kg) for one-week

treatment. To assess the effect of *Marsilea minuta* on locomotor activity, mice were evaluated in the open-field paradigm (TRU SCAN Activity Monitoring Systems, Coul bourn Instruments) previously described. Animals were individually placed in a box (40 × 60 × 50 cm). The rats were not habituated to the box before the test. The mice were placed in the center and their behavior was noted immediately and continued for 4 min. The parameters such as total movements, total distance, total ambulatory move time were recorded by video camera and registered in the computer. During the interval of the test the apparatus was cleaned.

RESULTS

Phytochemical screening test

The freshly prepared extract of the leaves of *Marsilea minuta* was subjected to phytochemical screening tests for the detection of various active constituents. The extract showed the presence of alkaloids, tannins, steroids, phenolic and flavonoids, carbohydrates, and glycosides in crude extract of *Marsilea minuta* leaves as depicted in Table 1.

Table 2: Result of chemical group tests of the Methanolic extract of *Marsilea minuta* leaves.

Carbohydrates	+
Tannins	++
Flavonoid	+++
Saponin	++
Phenols	+
Steroids	+
Alkaloids	+++
Glycosides	+

ANTIDEPRESSANT ACTIVITY OF MARSILEA MINUTA

In the Acute study, on Day 1, standard drug Imipramine (15mg/kg) and test drug EEMM (100mg/kg, 200mg/kg, 400mg/kg) showed significant reduction in immobility times when compared to control in both FST and TST (Table 1, Figure 1). In the Sub acute study, on Day 10, both Imipramine (15mg/kg) and MEMM (100mg/kg, 400mg/kg) showed significant reduction in immobility times when compared to control in both FST and TST.

Table 3: Immobility time

Day 1	Tail Suspension Test	Forced Swim Test
Normal Saline	221.3(±17.04)	136.1(±2.17)
Imipramine 15mg/kg	174.2(±1.21)*	101.15(±3.62)*
MEMM 100mg/Kg	161.5(±21.5)*	96.71(±5.16)*
MEMM 200mg/kg	181.2(±3.19)*	108.15(±2.01)*
MEMM 400mg/kg	116.1(±2.24)*	106.29(±4.39)*

*Immobility time shown in seconds as mean (± SEM),*denotes statistically significant value, # denotes statistically not significant value.*

DISCUSSION

A previous study concluded that aqueous leaf extract of *Marsilea minuta* exhibited antidepressant in forced swimming (FST) and tail suspension (TST) tests. In this study, both Imipramine and *Marsilea minuta* showed a reduction in immobility times in acute and sub acute study in both FST and TST. Lowest immobility times were recorded with Marsilea minuta at 100 mg/kg doses in most recordings, and at times, it showed comparable or even better reduction in immobility times than Imipramine in both tests in acute and sub acute study. Imipramine inhibits nor epinephrine transporter and Serotonin transporters, increasing their availability at synaptic cleft, thereby reducing depression. The antidepressant action of *Marsilea minuta* is probably similar to the mechanisms of anti-depressant agents, like Imipramine, that are effective in the above screening models. Phytochemical investigations done in a study showed the presence of alkaloids, flavonoids and tannins in the extract. It is likely that the antidepressant activity seen with *Marsilea minuta* could be because of the above mentioned phytoconstituents.

CONCLUSION

In the present study plant parts of *Marsilea minuta* have been be evaluated for antidepressant activity. As literature shows that traditionally this plant is being use in the treatment of depression. The plants materials *Marsilea minuta* used for the present studies were commercially procured from local market. Albino rats were used for the antidepressant activity. The present study provides the evidence indicating that methanolic extract of *Marsilea minuta* showed significant antidepressant activity in TST and FST models of depression. Phytochemical analysis showed the presence of Flavonoids and phenolic compounds have been reported to have multiple biological effects such as Central nervous system disorders. Similarly, the results of this study suggest that the leaf extract exhibited significant antidepressant activity with a strong psychomotor stimulation. The leaf extract was reported to contain chemical constituents such as Carbohydrates, Tannins, Flavonoid, Saponins, Phenols, Steroids, Alkaloids and Glycosides. The results obtained in this study suggest that Methanolic Extract of *Marsilea minuta* has anti-depressant activity and can be considered for use in therapy of depression after further testing.

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