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TO STUDY THE EFFECT OF INDIAN MEDICINAL PLANTS AS ANTI-DEPRESSIVE AGENTS

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Abstract-

Depression is a mental state of low mood and aversion to activity, which affects more than 280 million people of all ages (about 3.5% of the global population). Classified medically as a mental and behavioral disorder, the experience of depression affects a person's thoughts, behavior, motivation, feelings, and sense of well-being. In this study Screening and isolation of extract from natural products to protect the depressive behaviour. Effect of natural products in FST & TST induced depression models in order to identify its anti-depressive efficacy and Biochemical parameters investigation in the collected blood serum after the treatment with plant extract.

Key words- Depression, Anti-Depressive Agents, Melissa officinalis, mental disorders

1. INTRODUCTION

Depression is a common mental disorder that presents with depressed mood, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, low energy, and poor concentration. These problems can become chronic or recurrent and lead to substantial impairments in an individual's ability to take care of his or her everyday responsibilities. At its worst, depression can lead to suicide, a tragic fatality associated with the loss of about 850 000 lives every year. (1-3) Depression is the leading cause of disability as measured by YLDs and the 4th leading contributor to the global burden of disease (DALYs) in 2000. By the year 2020, depression is projected to reach 2nd place of the ranking of DALYs calculated for all ages, both sexes. Today, depression is already the 2nd cause of DALYs in the age category 15-44 years for both sexes combined. Depression occurs in persons of all genders, ages, and backgrounds. Depression is a disorder of the brain. There are a variety of causes, including genetic, environmental, psychological, and biochemical factors. Depression usually starts between the ages of 15 and 30 and is much more common in women. Women can also get postpartum depression after the birth of a baby. Some people get seasonal affective disorder in the winter. Depression is one part of disorder. There are effective treatments for depression, including antidepressants and talk therapy. Most people do best by using both. Depression is common, affecting about 121 million people worldwide. Depression is among the leading causes of disability worldwide. Depression can be reliably diagnosed and treated in primary care. Fewer than 25 % of those affected have access to effective treatments. Depression can be reliably diagnosed in primary care. Antidepressant medications and brief, structured forms of psychotherapy are effective for 60-80 % of those affected and can be delivered in primary care. However, fewer than 25 % of those affected (in some countries fewer than 10 %) receive such treatments. Barriers to effective

care include the lack of resources, lack of trained providers, and the social stigma associated with mental disorders including depression. Research by 20 different researchers, says depression affects nearly 121 million people worldwide. It is the second contributor to shorter lifespan for individuals in the 15-44 age group(4).

1.1 DEPRESSION SYMPTOMS

Feelings of sadness or unhappiness, Irritability or frustration, even over small matters, Loss of interest or pleasure in normal activities , Reduced sex drive, Insomnia or excessive sleeping, Changes in appetite depression often causes decreased appetite and weight loss, but in some people it causes increased cravings for food and weight gain, Agitation or restlessness for example, pacing, handwringing or an inability to sit still, Irritability or angry outbursts, Slowed thinking, speaking or body movements, Indecisiveness, distractibility and decreased concentration, Fatigue, tiredness and loss of energy even small tasks may seem to require a lot of effort, Feelings of worthlessness or guilt, fixating on past failures or blaming yourself when things aren't going right, Trouble thinking, concentrating, making decisions and remembering things, Frequent thoughts of death, dying or suicide, Crying spells for no apparent reason, Unexplained physical problems, such as back pain or headaches.

A number of recent studies have shown that depression predicts the onset of number of clinical conditions including coronary heart disease, cancer, hyper-tension, hypothyroidism, neurological disorders, and diabetes mellitus (4) and constitutes the most potent risk factors for suicide, a leading cause of death worldwide. So, drugs having properties to combat with depression, with few side effects, might be useful for such clinical conditions. To overcome these adverse effects, extensive investigations have searched for novel and better-tolerated molecules from plant sources. Herbal medicines play an important role in healthcare programs worldwide. The search for novel pharmaco-therapy from medicinal plants for psychiatric illnesses has progressed significantly in the past decade and their therapeutic potential has been assessed in a variety of animal models (1). Depression is not a normal part of growing older, and most seniors feel satisfied with their lives. However, depression can and does occur in older adults. Unfortunately, it often goes undiagnosed and untreated. Many adults with depression feel reluctant to seek help when they are feeling down. Therefore, the present study will design to investigate the antidepressant effects of these extracts by using various experimental depression paradigms in rodents (5)

2. MATERIAL AND METHOD

2.1 Materials:

2.1.1 Chemicals: Chemicals used in the present study have been listed below along with the name of the manufacturers:

Chemical	Manufacturer
Boric acid	SD Fine Chemicals Ltd., India
Bovine serum albumin	Sigma Chemicals, USA
Bradford's reagent	Sigma Chemicals, USA
Bromophenol blue	Sigma Chemicals, USA
Copper sulphate	Sisco Research Laboratories, India
Dimethyl sulphoxide (DMSO)	Qualigens, India
Ethanol	Merck, Germany
Folin's Reagent	Sisco Research Laboratories, India
Formaldehyde	Sisco Research Laboratories, India

Formamide	Sisco Research Laboratories, India
Glacial Acetic acid	SD Fine Chemicals Ltd., India
Glucose	Sigma Chemicals, USA
Hydrogen peroxide	M.P. Biomedicals, India
Indomethacin	Fluka, USA
Magnesium chloride ($MgCl_2$)	Sisco Research Laboratories, India
Methanol	Merck, Germany
Perchloric acid	Sigma Chemicals, USA
Phenophthalein	Sisco Research Laboratories, India
Potassium dihydrogen phosphate	Sisco Research Laboratories, India
Sodium acetate	Sisco Research Laboratories, India
Sodium Chloride (NaCl)	Sisco Research Laboratories, India
Sodium hydroxide (NaOH)	Sisco Research Laboratories, India
Streptozotocin (STZ)	Sisco Research Laboratories, India
Triton X-100	Sisco Research Laboratories, India

2.1.2 Instruments used in the study:

- Autoclave, Vertical SMI-102 (Jindal, SM Scientific Pvt. Ltd, Delhi, India).
- Digital pH meter (Model- CL-54, Toshniwal Instruments Manufacturing Pvt. Ltd. Chennai, India).
- Electrophoresis chambers (Miniphor UVT System, Maxiphor UVT System Banglore Genei, Banglore, India.
- Freezers (-20ºC Refrigerated, RQFV-265(D), Remi Instruments (-85ºC Ultrafreezer, Model-U41085, New Brunswick, Canada), 4ºC Refrigerators, Samsung India Electronics Pvt. Ltd., New Delhi, India).
- Fluorescent Spectrophotometer (Cary Eclipse Fluorescent Spectrophotometer, PCB 150 Water Peltier System, Varian Inc., the Netherlands).
- Homogenizer with 1/8 Hp motor and auto-transference.
- Ice machine (Ice Boy, Model-ZX-120 ELW, New Delhi, India).
- Incubator (Jindal, SM Scientific Pvt Ltd, Delhi, India).
- Magnetic Stirrer (Remi laboratory Instruments, Mumbai, India).
- Microplate reader (Powerwave ^{TM}-XSL, Biotek India, Mumbai, India).
- Microscopes (Trinocular Stereozoom Microscope SZ-CTV Olympus Optical Co., Tokoyo, Japan).
- Microwave oven (LG Electronics India Pvt. Ltd. Greater Noida, India).
- Spectrophotometer (Shimadzu UV1201, Shimadzu Scientific Instruments, Columbia, USA).
- Shaking Water Bath (LabTech, Diahan Labtech Co. India Pvt. Ltd. New Delhi, India).
- Vortex Mixer (Lead Instrument Pvt. Ltd., Banglore, India).
- Weighing Balances (Micro Electronic Balance, JS 110, Chyo, Japan)

2.1.3 Plants material:

All the plants were collected and the voucher specimen numbers were assigned and preserved in the Herbarium of the Institute. All the extraction procedures of plant extracts were performed. In the present study, the active constituents of plant were used to evaluate their anti-depressive potential.

2.1.4 Melissa officinalis:

The **Melissa officinalis** were purchased from the local herbal market and the authentification was done by the Institute. The dried leaves of **Melissa officinalis** (200 g) were cut into small pieces and were placed in glass percolator with 1.2 lit of ethanol: distilled water (1:1) and allowed to stand at room temperature for 24h. The percolate was collected and this process was repeated for four times. The combined percolate was concentrated under vacuum using rotary evaporator at 40^0C. The weight of extract obtained was found to be 10 gm.

2.2 Methods:

2.2.1 Experimental Animals:

Experimental protocols were approved by the Institutional Ethical and Usage Committee following the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals Reg. No.- 2005/PO/RcBT/S/18/CPCSEA) which complies with international norms of INSA (Indian National Science Academy). Adult male Albino male Swiss mice, weighing 20–25 g were used in this study. Animals were housed in raised bottom mesh cages to prevent coprophagy and were kept in environmentally controlled rooms with temperature regulated at 22 ± 2◦C, 12/12 h light and dark cycle. Each animal was used once in the behavior tests.

2.2.2 Treatment schedule:

Pilot investigation with the graded doses of plant extract were tested against depressant model to identify the effective dose and selected for further studies. Various plant extract; reference drug Imipramine (IMP) (60 mg/kg) were freshly prepared in 1% carboxymethyl cellulose (CMC) as suspension and administered orally to the animals. The rats were randomly divided into various groups, each consisting of 6 animals.

2.2.2.1Treatment groups for depressant model:

Mice were divided into three groups.

Group I (Control group): Control group of animals were treated with 1% CMC, for 21 d after induction of depression in mice.

Group II (Treatment groups): Mice were treated with herbal extract M. exotica (400 mg/kg body weight) in depressant models.

Group III (Reference drug treated): Mice were treated with marketed drug Imipramine (IMP) (60mg/kg) 1 hr prior of any depressive models.

2.2.3 Anti-Depression models in swiss albino mice

2.2.3.1 Tail suspension test

We followed the Steru et al., 1985 for tail suspension test. In brief, the mice were individually suspended 60 cm above the surface of table with an adhesive tape placed 1 cm away from the tip of the tail. After 1 min acclimatization, immobility duration was recorded for 5 min. Mice were considered immobile only when they hung passively and were completely motionless

2.2.3.2 Forced swim test

In these test swiss mice is a behavioral despair model (6). The mice were placed individually in glass cylinders (20 cm height, 10 cm diameter) containing 10 cm depth of water at 25°C. After 5 min, the animals were removed from water, dried, and returned back to their home cages. They were again placed in the cylinder 24 h later and after the initial 1 min acclimatization period, the total duration of immobility was measured for 5 min. Mice were considered to be immobile when they were floating motionless. The duration of swimming was measured by digital counter.

2.2.3.3 Rotarod test in mice

Rota rod test is commonly used for evaluation of neuromuscular coordination in mice and the protocol was used as described by Dunham and Miya (1957) (7) and studied in the Rotamex 4/8 apparatus (Columbus Instruments). Basically, the rotarod consists of a rod that is coated with polypropylene foam to provide friction and to prevent animals from slipping off the rod. The distance between the rod and floor is kept 15 cm to avoid intentional jumping of mice. The rod is driven by a motor and the rotational speed can be regulated, which is maintained at 8 rpm in our study. Animals were trained on the rotarod for duration of 2 min per trial, with three trials per day for 2 days. On the third day, mice were given trials before and after treatment of extract. The ME extract was used at the highest dose of 400 mg/kg, p.o. in present study to evaluate any defects in motor coordination.

2.2.4 Protein assay:

The Protein content of the samples was estimated by the method of Lowry et al. (1951) (7) using Folin Phenol reagent. Bovine serum albumin (BSA) (1 mg/ml) was used as standard.

2.2.4.1 Protein estimation of lowery method

This method is reasonably sensitive, detecting 10µg/cm^{-3} of protein. This method is based on the reaction when the folin's reagent together with copper sulphate solution is mixed with protein solution, blue purple colour is formed which can be quantified at 660nm range.

Requirements BSA Solution (Standard Protein solution), 1mg/ml.**,** Solution A: Mix solution (i) and (ii)**,**2% Sodium carbonate**,** 0.1N NaOH**,** Solution B: Mix solution (i) and (ii)**,** 1.56% $CuSO_4$**,** 2.37% Sodium Potassium Tartarate**,** Solution C: Mix 2 ml solution B in 100 ml solution A. **,**Solution D: Dilute Folin Ciocalteau reagent (1:1), at the time of use.

Procedure Prepare standard solution of stach in increasing concentration and add distilled water to make up volume 1ml in each tube, as shown in table **.**Took 2 ml of solution C in each test tube and added 200 µl of protein solution of each concentration. Incubated for 10 minutes at RT**..**After incubation added 200 µl of 1X Folin reagent, further incubated for 30 minutes dark**..**Took the O.D at 660 nm.

2.2.5 Statistical analysis:

Data were expressed as mean ± S.E.M. Analysis will be performed with Prism version 5.0 software using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. $P<0.05$ was considered to be statistically significant.

3. **RESULTS AND DISCUSSION**

3.1 Melissa officinalis:

Melissa officinalis belonging to the Lamiaceae family and commonly known as lemon balm. M. officinalis is an evergreen, tropical plant with tiny, white, scented flowers, which is cultivated as an ornamental tree. Various parts of this plant have been used in traditional medicine. **Melissa officinalis** have anti-inflammatory, antioxidant (8), anti-diabetic effects. (9) and anti-anxiety effects (10).are traditionally used in China and its common name is Chinese box.

Figure 1: Representative photographs of Melissa officinalis

3.2 Evaluation of anti-depressive effect of Melissa officinalis against depressant mice model:

Extract of Melissa officinalis (MO) at graded doses (200, 400 and 600 mg/kg, p.o.) were used and 400 mg/kg showed maximum reversal of depression in comparison of others dose. In tail suspension experiment plant extract MO at 200 mg/kg had no significant effect on the immobility duration when compared with the 1% CMC-treated control groups. Further, doses of MO at 400 and 600 mg/ kg produced a significant reduction in the immobility time by 57% and 55%, respectively [$P < 0.001$]. Whereas the antidepressant reference drug IMP (60 mg/kg, p.o.) exhibited potential reduction in the immobility time ($P < 0.001$) (90%). Furthermore, 400 mg/kg, p.o. was selected as the efficacious dose and the activity was confirmed in other models of depression.

Figure Image depicts Tail Suspension Test

3.4 Effect of Melissa officinalis (MO) in Force Swim Test

The dose of 400 mg/kg of MO was used to review the degree of immobility in mice performed Forced Swim Test (Figure 2). We found very interesting results and the immobility time in plant extract MO at dose of 400 mg/kg and reference drug IMP at 60 mg/kg treated mice was reduced by 40 and 91%, respectively [$P< 0.001$].

Figure. Image depicts Forced Swim Test

Whereas Table 1 depicts the treatment schedule and effect of MO plant extract on forced swim test depressant models. This experiment was divided into three groups in first group control; it was treated with 1% CMC only, in 2nd group plant extract MO only. Furthermore in 3rd group reference drug Imiparimine was used at 60 mg/kg.

Table 1: Effect of **Melissa officinalis** (MO) extract and reference drug Imipramine (IMP) on forced swim depressant models. (n= 6 in each group).*Statistically significant at P<0.05 and **P< 0.01, in comparison to control. n = 6 in each group.

Treatment groups	Forced Swim test Mobilization time (sec)
Control 1% CMC	72.7±6.02
MO (400 mg/kg)	122.0±30.89*
Imipramine (60 mg/kg)	273.0±19.28

3.5 Evaluation of motor coordination of Melissa officinalis against rotarod model:

MO at the highest dose, 600 mg/kg, p.o., studied in the experiments rotarod was tested for motor coordination. Both the control group delivered them only 1% CMC and plant extract MO treated group. Both groups were allowed to spend 120 sec on the rotating rod, without falling down even once. This result depicts our finding that the plant extract MO had no motor impairments.

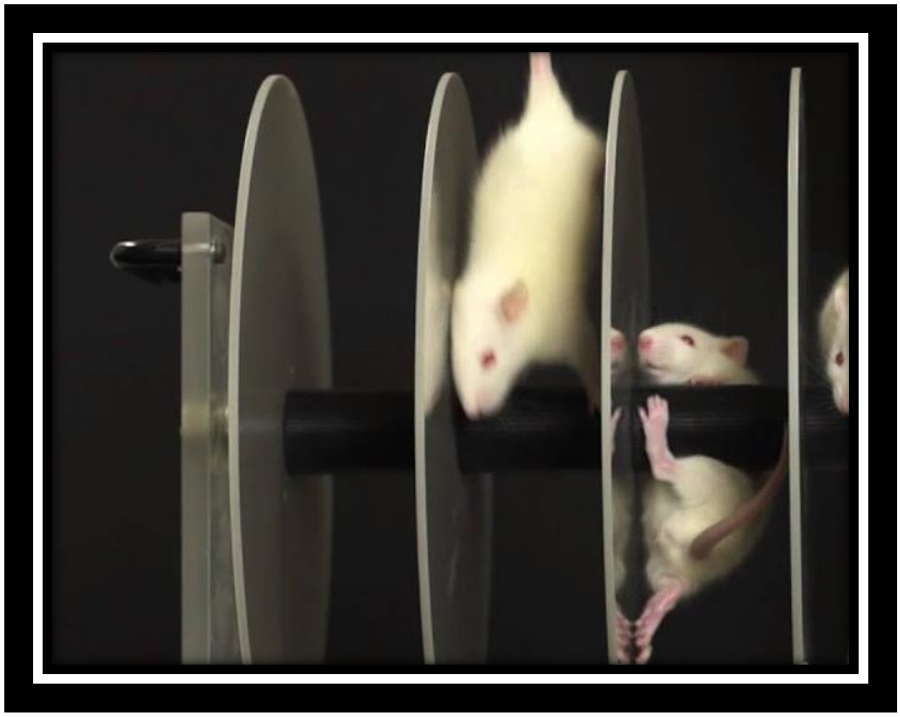

Figure This image depicts how rotarod works

Depression associated most of the clinical disorders including thyroid, cardiovascular, neuronal and postpartum condition. So it is very important to treat depression. A lot of marketed drugs are available in the market claiming effective against depression. Herbal products brought substantial contributions to drug innovation by providing novel chemical structures and/or mechanisms of action (Rates, 2001)(11). In India; a large number of herbal extracts are used in folk medicine to treat various types of disorders. We have also studied the antidepressant effects of the MO extract in tail suspension and forced swim models of depression, which provides a rapid and reliable behavior screening test for antidepressants. The present study has been conducted to evaluate the anti-depressant activity of Melissa officinalis extract against various depressant models of experimentally induced depression and to evaluate its mechanism of action.

A preliminary study for the dose fixation of Melissa officinalis crude extract was conducted and 400mg/kg was found to be the optimum dose that can give the highest protection. The screening results were summarized in the table 1. Tail suspension induced depression is a well-accepted model for the induction of depression. TST related animal experiments appear to be a very good mimic of the human condition and have allowed studies into pathogenic mechanisms as well as useful therapeutic intervention. Thus, the significant protection imparted by MO in this model reflected the possibility of its involvement in the regulatory mechanism of depression. The immobility has been expected to reflect a state of "behavioral despair and variants" or "failure to adapt to stress" (Willner & Muscat, 1991). This interesting finding in TST model intrigued to further explore its effects on other models of depression in mice. It was observed that plant extract MO produces its huge outcome at the dose of 400 mg/kg, p.o. and produces a reduction of immobility time in mice exposed to both the models, comparable with that of reference drug impramine (IMP).

The effects of the crude extract MO on forced swim induced depressant model and was examined and compared with the reference drug Imipramine (Table 4). MO shows antidepressant activity at the dose 400 mg/kg. However, plant extract MO did not cause alteration in motor coordination on the rotarod test in the protocol studied, suggesting that the decreased locomotor action observed may not be exerted through peripheral neuromuscular blockage or centrally mediated impairment of motor function (12).

The wide variety of neuropharmacological actions of this plant extract opens up interesting avenues for further research (13). The activity of plant extract as an potentantidepressant needs further assessment. This offers new perspectives in the treatment of these diseases, as there is compelling evidence that symptoms of anxiety and depression overlap with one another (13). Many antidepressants have been reported to be of use in depression . There are several reports pertaining to antidepressant effects of serotonergic drugs (14). These reports show that depressant may share some common etiological factors and drugs showing both anxiolytic and antidepressant activities are to be extensively studied for their therapeutic beneficial uses. Based on our studies, we conclude that the MO demonstrates significant antidepressant potential and also rules out any side effect of the extract on motor coordination. Thus, our results fortify the ethnopharmacological importance of MO in psychiatric disorders like depression however; more experimentation on pharmacological mechanisms and neurotransmitter analysis is required for a definitive conclusion.

4.Conclusion

This study explains evidence based-information regarding the anti-depressant activity of ethanol extract of MO in different depressant models. It was obvious from the result that, the ethanol extract of MO played a beneficial role in reduction of depression. In conclusion, MO inhibit the formation of depression in mice. MO also exhibit anti-oxidative properties by scavenging the free radicals and reactive oxygen species. MO might be a potent therapeutic agent in treating depressant incidences since it possesses anti- depressant activity.

MO results shown in this study indicated that the **MO** extract are most protective and able to reduce the depression. Further potent anti-oxidant activity of MO exhibiting their strong anti-depressants and could therefore be used as a remedy for the treatment of depression.

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