Anti-Depressant Effect of Myristica Malabarica in Depressed Animal Model

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ABSTRACT

The Myristica malabarica was estimated in the present study for antidepressant activity. The extraction was carried out by petroleum ether, chloroform, and ethanol. Pet-ether extract showed the presence of alkaloids, flavonoids, and triterpenoids. Chloroform extract showed the presence of alkaloids, glycoside, tannins& phenolics, saponins, flavonoids, carbohydrates, proteins & amino acids, phytosterols, and terpenoids. Ethanol extract showed the presence of alkaloids, glycosides, tannins& phenolics, saponins, flavonoids, carbohydrates, proteins & amino acids, and phytosterols. This activity was evaluated using the mice behavioral model i.e. forced swimming and tail suspension model for 14 days, with estimation of neurotransmitters in the animal's brain. In depression, the brain reflected low monoamine levels like release of noradrenaline, dopamine, and serotonin but 14 days after successive administration of the ethanolic extract (100 and 200 mg/kg). Still, at a dose of 200mg/kg, it shows more potent antidepressant activity, and their levels were significantly increased. In conclusion, Myristica malabarica showed significant anti-depressant activity.

I. INTRODUCTION

Nature constantly stands as a golden mark to demonstrate the outstanding phenomenon of symbiosis. The abiotic and biotic elements of nature are all interdependent. Plants are vital to man in his life. The three important necessities of life are food, cloth, and shelter, and a host of other useful products are supplied to him by the plant origin[1]. Nature has not only provided important necessities but also provided a complete storehouse of medicine to cure all ailments. The knowledge of drugs has built up over thousands of years as a result of man's curious nature so that today we possess many effective means of ensuring health care.

The World Health Organization (WHO) estimates that about 80% of developing countries people almost rely exclusively on plant-based medicine for their primary health care. The medicinal plants play a main role in the development of the backbone of the traditional medicine (TM). Indian Materia medica includes about 2000 drugs of natural origin, almost all being used in different traditional medicinal systems and folklore practices. Indian Materia medica included 400 drugs derived from mineral and animal origin while the rest from vegetable origin (plant)[2]. India has a rich heritage of traditional health care and traditional medicinal systems have been flourishing for many centuries. It mainly consists of three major systems viz.., Ayurveda, Siddha and Unani. For the development of the traditional medicinal system, a lot of efforts have been taken by the government and private sectors[3].

Therapeutic potentials of crude drugs range from plant parts, simple extracts and isolated active constituents. There has been increasing interest in plants as a source of lead molecules in medicinal system in the last few decades. An herbal product has occupied the most part in the market for curing different human diseases and disorders. These drugs have been known and are being used by man for many centuries in the form of plant part, but isolation and evaluation of phytoconstituents from many plants has not still taken place[4]. Dealing with natural products, the most vital and potent therapeutic important is the medicinal plants. The medicinal plants have huge commercial potential throughout the world. In concurrence with the increased interest in herbals, there has been an explosion on quality control of these medicinal plants are of most essential with respect of safety and efficacy. In the herbal boom worldwide, it is estimated that high-quality phytomedicines will provide safe and effective medication and scientists engaged therein will provide safe and effective medication[5,6].

Materia Medica of India provides lot of information on the folklore practices and therapeutically traditional aspects of important natural substances. Indian TM is based on many systems mainly, Ayurveda, Siddha, and Unani. The quality control of these drugs is mostly based on physiochemical, phytochemical, pharmacological, toxicological, and related

approaches which mainly include various instrumental techniques like microscopy, chromatography, hyphenated techniques, and others. The evaluation of the rich heritage of the Indian TM is essential. The government as well as private sectors is trying their best to develop all potentials for the evaluation of these systems to bring out therapeutic approaches existing in the original system of medicine and assist in generating data to put these medicinal products on national health care programs.
Herbal drugs are of great importance to the health of individuals and communities. Furthermore, commercial Ayurvedic products can make a dent in the international market, which are walking towards alternative medicine for ailments to which even modern system has no answer. The ailments include metabolic or degenerative disorders like arthritis, lifestyle induced problems of heart and diabetes, cancer, dementia, age-related disorders, immunological disorders and gynecological disorders. There is a big market for these products.Chemical diversity in natural products is a highly rich source of lead pharmaceuticals, cosmetics, agrochemicals and other economically important chemicals. Presently numerous pure compounds are isolated from the plants for this purpose. Moreover, many plants are used in treating a broad variety of diseases. In Western medicine, several plants are successfully used, and particularly in the US a rapid increase in the sales of medicinal plants is observed. In non-western medicine, numerous plants are being used as well. Moreover, the food industry is working on proving the benefits of various foods for our health and nutraceuticals (food supplements) which is another rapidly growing branch of pharmaceutical field.

II.MATERIAL AND METHODS

Collection of Plant Materials:

Dried *Myristica malabarica* was purchased from an herbal Market of Hyderabad, Telangana, India and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, S.V University, and Tirupati. A specimen voucher was deposited at the Department of Pharmacology, of our institution.

Extraction Methodology

The stems *Myristica malabarica* of were pulverized in grinder. The pulverized material (# 60-80) was utilized for extraction as mentioned below.

Technique: Continuous Hot extraction (Soxhlet apparatus)

Solvent:

1. Petroleum ether (60-80°C)
2. Chloroform
3. Methanol

The extraction was carried out in several batches. Total quantity of dried pulverized stems processed = 1000g.

Petroleum ether extraction: The extraction was carried out by continuous hot extraction method using Soxhlet extractor till all constituents are removed. The end of completion of extraction was indicated by no colour with iodine fumes. After completion of extraction, solvent was distilled out and dried extract was obtained. This extract was kept in desiccators for storage. Petroleum ether extract usually contains colouring matter like chlorophyll, carotenoids, lipids, free sterol and triterpenes etc.

Chloroform extraction: The marc after exhaustive petroleum ether extraction (defatted material) was air-dried and subjected to Soxhlet extraction with chloroform (middle polar solvent). Completion of extraction was determined by reaction with iodine fumes. The chloroform extract usually contains glycosides, alkaloids, steroids, triterpenoids, colouring matter etc.

Methanol extraction: The marc after exhaustive chloroform extraction was air-dried and subjected to Soxhlet extraction with methanol (highly polar solvent). Completion of extraction was determined by reaction with iodine fumes. The methanol extract usually contains glycosides, flavonoids, tannins, resinous substances, acids like cinnamic acid, benzoic acids, carbohydrates, proteins etc.

Pharmacological investigation

Oral toxicity study:

Rationale- Acute oral toxicity of the extracts of *Myristica malabarica* was carried out by the up and down procedure (UDP). The principal value of UDP is to minimize the number of animals required to estimate the acute oral toxicity of chemical and estimating the median lethal dose. The LD50 can be estimated using 6-10 animals of 1 sex. Animals are dosed, one at a time, at 24 H intervals.

Depending on outcome the dose for the next animals is adjusted up or down. The dose is increased if the animal survives and the dose is decreased if animal dies. After reaching the reversal on initial outcome, i.e., point where an increasing or decreasing dose pattern is reversed by giving a smaller or higher dose. Four additional animals are dosed the same UDP. In absence of any information about the substance, the starting dose may be 200 or 500 mg/kg body weight. For further doses, a dose progression factor of 1:3 is used. The next dose was administered according to mortality of the animal.

Procedure

An individual animal was administered with 250mg/kg body weight dose one at a time orally. The animal was observed for 24 hours. The animal survived; the 750mg/kg body weight dose one at a time orally was given to the next animal. Again, theanimal was observed for 24 hours. The animal survived; the 2000mg/kg body weight dose one at a time orally was given to the next animal. Again, theanimal was observed for 24 hours. The animal survived, andfour additional animals were dosed using the same dose. No deaths occurred, 3 animals of the other sex were tested at the same dose level. If mortality was not registered again, the test was terminated.

III.RESULT AND DISCUSSION

Extraction:

The extraction was carried out by continuous hot extraction method using Soxhlet extractor till all constituents removed. The end of completion of extraction was indicated by no color with iodine fumes when spot on TLC plate. Percentage and color of extract are given in Table 4.1.

Table no-1: Extractive values after continuous extraction of *Myristica malabarica*

Extract	Colour of the extract	% (w/w) of Extract obtained
Petroleum Ether	Yellowish green	1.13 ± 0.13
Chloroform	Dark brown	2.67 ±0.28
Ethanol	Dark brown	4.26 ± 0.43

Extraction:

The extraction was carried out by continuous hot extraction method using Soxhlet extractor till all constituents removed. The end of completion of extraction was indicated by no color with iodine fumes when spot on TLC plate. Percentage and color of extract are given in Table 4.1.

Table no-2: Extractive values after continuous extraction of *Myristica malabarica*

Extract	Colour of the extract	% (w/w) of Extract obtained
Petroleum Ether	Yellowish green	1.13 ± 0.13
Chloroform	Dark brown	2.67 ±0.28
Ethanol	Dark brown	4.26 ± 0.43

Acute toxicity study

Acute toxicity studies revealed that nontoxic nature of extracts *Myristica malabarica*. There were no lethality or toxic reactions found at 2000mg/ kg body weight of the study period. All the animals were alive, healthy and active during the observation for the given dose so the doses were fixed for pharmacological study.

Table no-3: Effect of extracts of *Myristica malabarica* in acute toxicity

Day	Dose (mg Per kg body weight)	No. of animal(s)	Observation
Day 1	250	3	Survived
Day 2	750	3	Survived
Day 3	2000	3	Survived
Day 4	2000	1	Survived

Antidepressant Activity:

A) Effects of Myristica malabaricaextracts on the immobility time in the Force swim test

The chloroform extract of *Myristica malabarica* induced a significant antidepressant effect in the FST because it significantly reduced the immobility time compared with the vehicle-treated group (185.17±6.20s) (Figure 6.49). On the contrary, the ethanol extract of *Myristica malabarica* did not give this behavior. The immobility time of chloroform extract of *Myristica malabarica* was found to be 152.67±11.04, 138.33±5.731sec and for ethanol extract of *Myristica malabarica* 185.33±4.349, 186.50±4.262sec. for the doses of 100 and 200mg/kg/ day on the 7th day respectively. The standard group treated with fluoxetine (10mg/kg) exhibited powerful activity (111.83±4.826s). No significant difference was observed in the immobility time of *Myristica malabaricas* extracts on 4th day and 7th day in FST.

Table no-4: Effect of extracts of *Myristica malabarica* and fluoxetine on immobility time in force swim test in albino mice

Sample	**Immobility time (sec.)** (Mean±SEM)	
	Day 4	Day 7
Control	187.00±3.99	185.17±6.20

CEMM	146.50±8.03**	152.67±4.66**
CEMM	140.67±6.61***	138.33±5.73***
EEMM	185.33±4.54	187.00±4.35
EEMM	186.50±4.52***	185.83±4.26***
Fluoxetine	116.00±5.87	111.83±4.83

Values are expressed as mean±S.E.M. (n=6). *P≤0.05 **P≤0.01 ***P≤0.001 compared with the vehicle treated control group (two-way ANOVA followed by Dunnett's test)

Figure no-1: Effect of extracts of Myristica malabaricaand fluoxetine on immobility time in force swim test in albino mice

B) Effect of Myristica malabaricaextracts on the immobility time in the tail suspension test

In the TST, the chloroform extract of *Myristica malabarica*showed a significant effect on decreasing the immobility time, compared with the vehicle-treated control group (184.20±5.375 sec.) (Figure6.50). The mean immobility time of the chloroform extract of *Myristica malabarica* treated group for 100 and 200 mg/kg dose was 137.50± 9.86 and 117.00± 3.83sec., respectively. While methanol extract of *Myristica malabarica*did not show any effect on immobility time (192.20±8.98, 176.83±6.07sec). Chloroform extract of *Myristica malabarica* 200 mg/kg showed a significant effect when compared with control. The standard group treated with imipramine (10 mg/kg), also significantly diminished the immobility time (107.67±5.10sec.). No significant difference was observed in the immobility time of *Myristica malabarica* extracts on the 4th days and 7th days in TST.

Table no-5: Effect of extracts of Myristica malabaricaand imipramine on immobility time in the tail suspension test in albino mice

Sample	**Immobility time (sec.)** (Mean±SEM)	
	Day 4	Day 7
Control	185.50±7.56	182.50±10.33
CEMM 100	138.00±8.32***	137.50± 9.86***
CEMM 200	123.00±11.16***	117.00± 3.83***
EEMM 100	178.00±15.01	192.17±8.98
EEMM 200	180.33±7.68	176.83±6.07
IMIPRAMINE	121.00± 6.59***	107.67±5.10***

Values are expressed as mean±S.E.M. (n=6). *P≤0.05 **P≤0.01 ***P≤0.001 compared with the vehicle treated control group (two-way ANOVA followed by Dunnett's test)

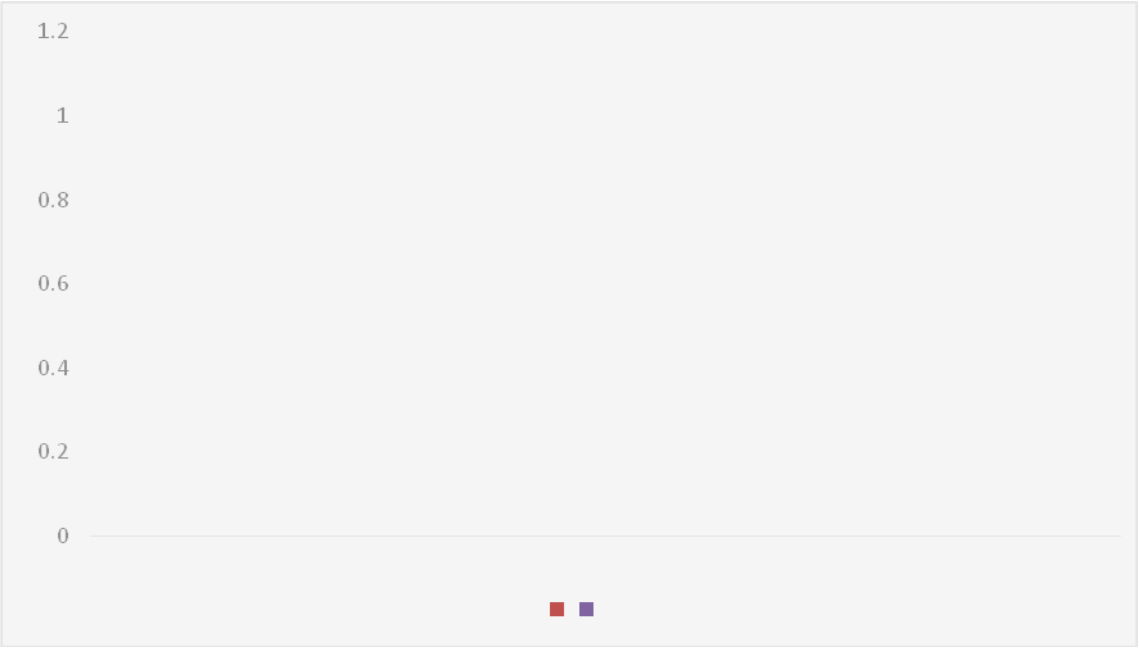


Figure no-2: Effect of extracts of *Myristica malabarica* and imipramine on immobility time in the tail suspension test in albino mice.

C) Effects of *Myristica malabarica* extracts on the open field test

Table no-6: Effects of *Myristica malabarica*extracts and fluoxetine for 7 days administration on the number of crossings, rearing and grooming in open field test.

Sample	No. of crossing	No. of grooming	No. of rearing
Control	73.17±2.651	7.50±1.147	17.80±2.709
CEMM 100	78.83±4.895	7.50±1.088	19.60±1.030
CEMM 200	76.83.±3.260	7.83±1.515	17.40±2.676
EEMM 100	73.17±5.089	8.33±0.49	17.60±3.076
EEMM 200	77.33±5.414	8.33±1.38	18.00±2.530
Fluoxetine	77.00±3.933	8.00±1.43	19.60±1.568

Values are expressed as mean±S.E.M. (n=6). *P≤0.05 **P≤0.01 ***P≤0.001 compared with the vehicle treated control group (two-way ANOVA followed by Dunnett's test)

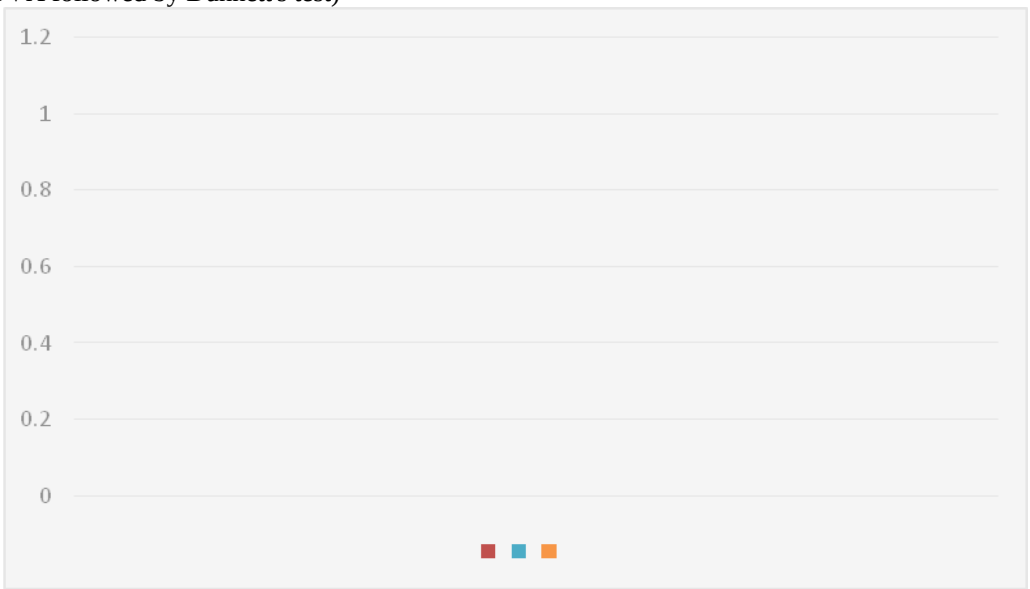


Figure no-3: Effects of *Myristica malabarica*extracts and fluoxetine for 7 days administration on the number of crossings, rearing, and grooming in open field test.

No significant differences were observed in the number of squares crossed, rearing, and grooming between vehicle treated group and *Myristica malabarica* extracts as well as standard treated groups ($P < 0.05$) (Figure 6.51). Although *Myristica malabarica* has been used to treat nervous shock in traditional medicine, its specific neuropharmacological activities have not been demonstrated yet. Principal value of UDP is to minimizing the number of animals required to estimate the acute oral toxicity of *Myristica malabarica* extract and estimating the median lethal dose. *Myristica malabarica* extract not showed toxicity. The forced swim test and tail suspension test are the most common animal models of depression used for antidepressant screening. In both tests, animals are placed in an inescapable situation and the antidepressant like activity is expressed by the decrease in immobility time[90]. In the FST, mice are forced to swim in a restricted space from which they cannot escape and are induced to assume a characteristic behavior of immobility. This behavior reflects a state of despair or lowered mood, which can be reduced by several agents that are therapeutically effective in human depression. The TST also induces a state of immobility in animals like that in the FST. The chloroform extract of HPS decreases immobility time while methanol extract not showed any effect in TST as well as FST. Chloroform extract of *Myristica malabarica* showed dose-dependent activity. This immobility in TST and FST referred to as behavioral despair in animals is believed to reproduce a condition similar to human depression.

The compounds which able to increases locomotor activity in OFT including psych stimulants, convulsant and anticholinergic give a false positive result in TST and FST. In general, hyperkinesis also produces false positive effect in TST and FST by shortening the immobility time. Therefore, OFT was used to exclude these false effects that could be associated with psycho stimulants, convulsants and anticholinergics or hyperkinesis activity[91]. The main difference between antidepressants and psychostimulants is that antidepressants would not increase locomotor activity. In addition, the finding suggested that reduction of immobility time elicited by CEMM in FST as well as in TST was specifically arises via its antidepressant mechanism. In TST and FST chloroform extract of MM decreases immobility time which is not due to any psychostimulant, anticholinergic, convulsant effect, or hyperkinesis activity.

Estimation of neurotransmitters

Effect of ethanolic extract of *Myristica malabarica* on dopamine levels

Treatment with piracetam and ethanolic extract of ***Myristica malabarica***at a dose level of 100 and 200 mg/ kg, p.o. for 14 days significantly ($p < 0.01$) decreased brain dopamine level in a dose-dependent manner when compared to their corresponding control treated groups respectively.

Effect of ethanolic extract of *Myristica malabarica* on serotonin levels

Ethanolic extract of ***Myristica malabarica***at a dose level of 100 and 200mg/ kg, p.otreatment for 14 days significantly ($p < 0.01$) increased brain serotonin level in dose dose-dependent manner in comparison to their corresponding control-treated groups. **Fluoxetine** also significantly ($p < 0.01$) increased brain serotonin levels when compared to their corresponding control treated groups respectively.

Effect of ethanolic extract of *Myristica malabarica* on noradrenaline levels

The ethanolic extract 100 and 200 mg/kg of ***Myristica malabarica*** to mice significantly increased the brain noradrenaline (*** $p<0.001$) levels when compared with the control group.

Table no-7: Effect of*Myristica malabarica* extracts on dopamine, serotonin, and noradrenaline levels on mice brain

SL. No.	Treatment	Dopamine (ng/mg tissue)	Serotonin (ng/mg tissue)	Noradrenaline (ng/g tissue)
1	Control	381.68±9.93	121.66±3.29	240 ± 0.02
2	EEMM (100mg/kg)	112.82±1.23*	160.27±5.33*	490 ± 0.01**
3	EEMM (200mg/kg)	98.75±1.62*	168.3±3.89*	570 ± 0.03**
4	**Fluoxetine**	133.24±2.83*	174.5±6.55*	660 ± 0.01***

Statistical significance test was done by ANOVA followed by Dunnet's test (n=6) Values are mean ± SEM of 6 animals per groups. *P< 0.01vs control, *** p<0.001 vs control.

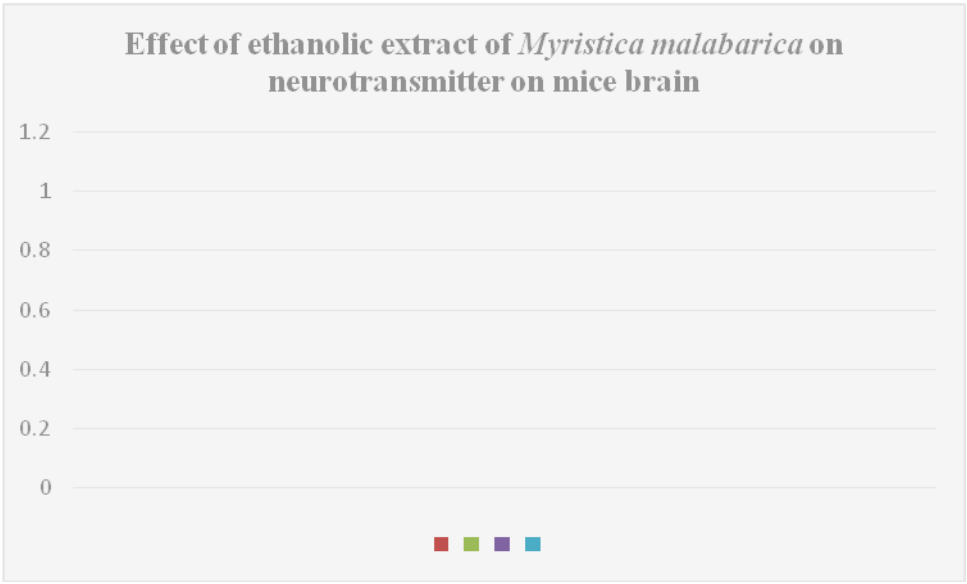


Figure no-4: Effect of*Myristica malabarica* extracts on dopamine, serotonin, and noradrenaline levels on mice brain

The spontaneous and experimentally induced deficiencies in monoamines (serotonin, noradrenaline, and dopamine) are well documented and implicated in the onset of depression. A much experimental procedure designed to increase monoaminergic activity proved antidepressant properties [98]. In the present study, ***Myristica malabarica*** (200 mg/kg) showed a significant increase in the level of monoamines such as (serotonin, noradrenaline, and dopamine) in brain tissue while at a lower dose, 100 mg/kg showed a less significant increase in monoamines.

Effect of *Myristica malabarica* ethanolic extraction MAO A and B activities in mice whole brain

The effects of *Myristica malabarica* ethanolic extract and fluoxetine for 14 days on the MAO A and B activities in rat whole brain was shown in Table 3. The MAO-A and B activities in the normal group were 31.6 ±/1.9 nmol/mg protein h and 25.8 ±1.5 nmol/mg protein h, respectively. Oral administration of the extract at doses of 100 and 200mg/kg significantly inhibited MAO-A activity in a dose-dependent manner, providing 33.4 and 44.7 % inhibition. However, only *Myristica malabarica* ethanolic extract at a dose of 200 mg/kg significantly inhibited MAO B activity, producing 37.3 % inhibition. Fluoxetine at the dose of 20 mg/kg also reduced the MAO A and B activity significantly ($p < 0.01$).

Table no-8: Effect of *Myristica malabarica* ethanolic extraction MAO A and B activities in mice whole brain

Treatment	Dose (mg/kg)	MAO activity (nmol/mg protein h)		MAO inhibition (%)	
		A	B	A	B
Control	---	31.6±1.9	25.8±1.5	----	---
Fluoxetine	20	19.5±1.8**	17.3±0.9**	16.2	13.4
EEMM	100	13.6±1.7*	15.9±1.7*	33.4	17.6
EEMM	200	17.2±1.5**	18.6±1.7*	44.7	37.3

EEMM: Ethanolic extract of *Myristica malabarica*. Results were expressed Mean ± SEM *p < 0.05, **p < 0.01 when compared with control groups, * p < 0.05, **p < 0.01 when compared with model groups.

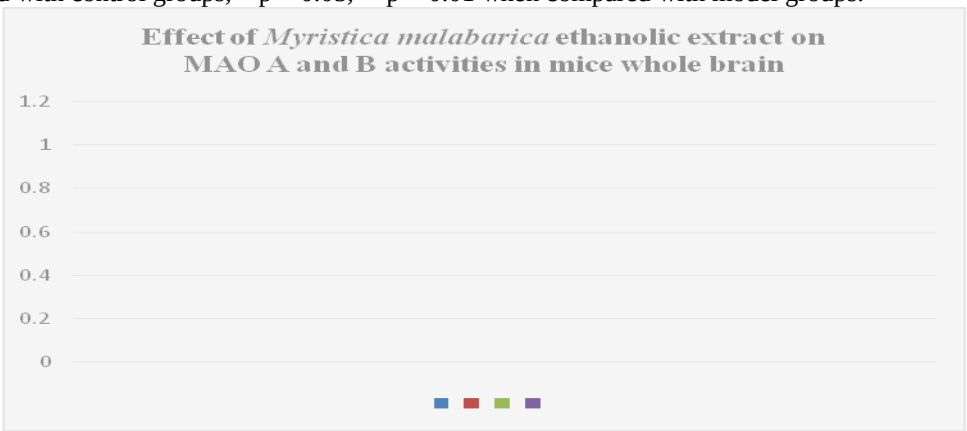


Figure no-5: Effect of *Myristica malabarica* ethanolic extraction MAO A and B activities in mice whole brain

MAO is an important enzyme in the metabolism of a wide range of monoamine neurotransmitters, including noradrenaline, dopamine, and 5- hydroxytryptamine[99-101]. MAO exists in two forms, A and B. MAO-A is more important than MAO B in the metabolism of the major neurotransmitter monoamines. MAO-A inhibitors have been regarded as drugs for

treating depression in clinical[102-104]. In the present investigation, we have demonstrated that the EEMM significantly inhibited in vivo MAO-A activity in rat whole brain in a dose-dependent manner. However, only EEMM at a dose up to 400 mg/kg exhibited to have the MAO B inhibitory activity. These findings suggest that anti-depressant effects of EEMM in mice models of immobility tests may be related to the inhibitory activity of MAO.

Effect of *Myristica malabarica* ethanolic extract on Gamma Amino Butyric Acid (GABA)

The effect of ***Myristica malabarica* ethanolic extract** (100 and 200 mg/kg, orally) and fluoxetine (30 mg/kg, orally) after treatments in the **Gamma Amino Butyric Acid (GABA)** levels in the mice shown in Tables 4 and 5. Significant decrease gamma amino butyric (GABA) levels in mice were observed (5-HT; $F = 34.56$, $P < 0.01$). The maximum effect was observed with mice was treated 200mg /kg dose of EEMM.

The level of GABA was significantly increased when it's treated with ***Myristica malabarica* ethanolic extract**. As a conclusion, the data showed the efficacy of ethanolic extract of ***Myristica malabarica***against depression in mice. Overall, results justify and support the use of ***Myristica malabarica***as antidepressant medicine.

Table no-9: Effect of *Myristica malabarica* ethanolic extract on Gamma Amino Butyric Acid (GABA)

Sl. No.	Treatment	Dose (mg/kg)	GABA (g/ml)
1	Control	-----	0.2 ± 0.01
2	Fluoxetine	30	0.5 ± 0.04
3	EEMM	100	0.4 ± 0.01
4	EEMM	200	0..3 ± 0.03

Values are expressed as mean ± S.E.M (n = 6)

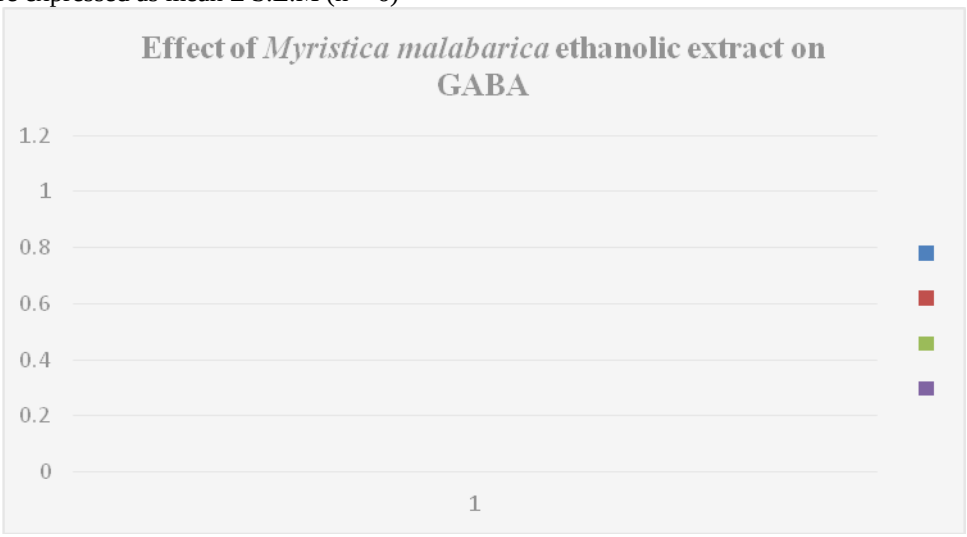
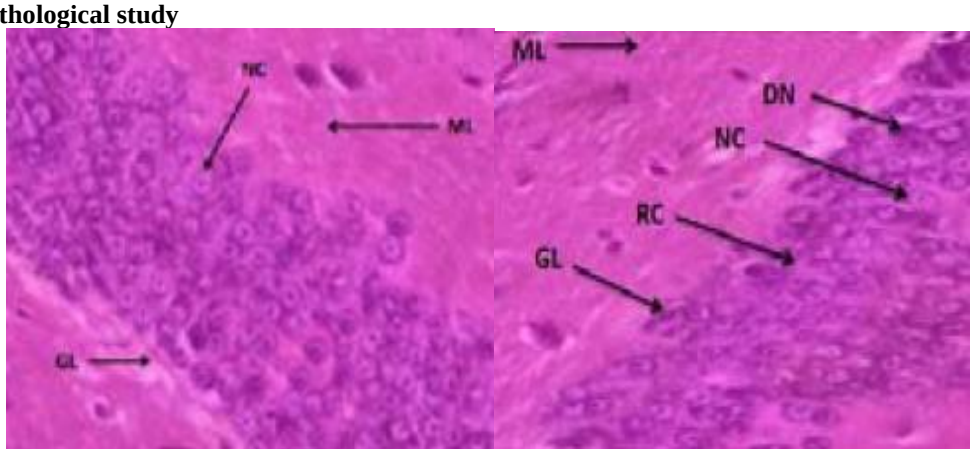
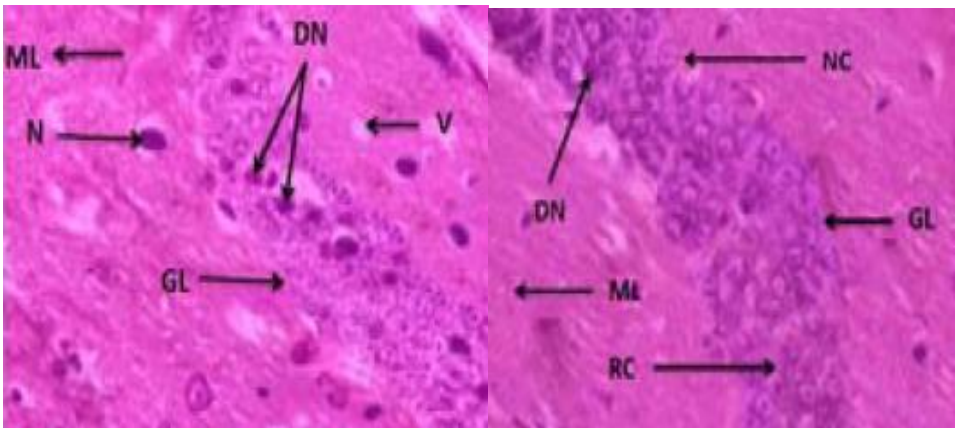


Figure no-6: Effect of *Myristica malabarica* ethanolic extract on Gamma Amino Butyric Acid (GABA)

Histopathological study



(A) Normal control (B) Fluoxetine



(C) EEMM 100mg/kg (D) EEMM 200mg/kg

Figure no-7: Effect of EEMM on Hippocampus region of brain in HIC performed animals.

(A) Normal: Transverse section of hippocampus showing normal histo-architecture (H&E 40x). **(B)Fluoxetine:** Transverse section of hippocampus showing almost normal histo-architecture, slight degenerated neurons, and regenerative changes (H&E 40x). **(C) EEMM 100 mg/kg:** Transverse section of hippocampus showing degenerated neurons, vacuolization, and neuronal necrosis (H&E 40x). **(D)EEMM 200mg/kg:** Transverse section of hippocampus showing almost normal neuronal cytoarchitecture, but rare degenerative changes and degenerative neurons (H&E 40x).

(GL granular layer, NC Normal cell, DC degenerative changes, V vacuolization, DN degenerated neuron ML molecular layer, N necrosis RC regenerative changes SD structural damage).

CONCLUSION

Herbal remedy for human mental illness is much preferred over synthetic pharmaceuticals because of various side effects. Herbal treatment not only advances persevering compliance but furthermore, there are possibilities of enhancing the bioavailability of numerous pharmaceuticals. Active constituents extracted from parts of plant origin is beneficial in treatment of mental illness sources have proved to be beneficial.[96]

It is concluded that ***Myristica malabarica*** at a dose of (100 and 200 mg/kg), showed antidepressant activity similar to fluoxetine (100 mg/kg) in mice.

The present findings indicate antidepressant property of ***Myristica malabarica*** extract, thereby validating its claim as a nervine tonic in the Indian system of medicine. In traditional medicine, it is conventional to utilize herbs for the achievement of treatment purposes and simultaneously to enhance beneficial effects without causing severe side effects.Further study is warranted for its potential use in humans.

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