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EVALUATION OF ANTIDEPRESSANT ACTIVITY OF METHANOLIC EXTRACT OF *HOPPEA DICHOTOMA* ROOTS IN MICE

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ABSTRACT

The present study was undertaken to evaluate the antidepressant effect of methanolic extract of roots of Hoppea dichotoma (MEHD) in mice using Forced Swim Test (FST) and Tail Suspension Test (TST). Fluoxetine (20 mg/kg) was used as a standard drug. Duration of immobility was noted in FST and TST models. In this study, MEHD (200mg/kg,300 mg/kg,400mg/kg) administered orally for 14 successive days significantly reduced (p<0.01) the duration of immobility in FST and TST as comparable to control group of animals. Further the effect of MEHD on monoamine oxidase (MAO) activity was evaluated using spectrophotometer. Significant decrease(P<0.01) in brain MAO-A and MAO-B levels were observed upon methanolic extract administration to mice as compared to control. These findings demonstrate that methanolic extract of Hoppea dichotoma root showed significant antidepressant like activity probably by inhibiting MAO and subsequent increase in the brain monoamines. Therefore, it can be a potent candidate for the management of depression.

KEY WORDS

Antidepressant, Forced Swim Test, Hoppea dichotoma, Tail Suspension Test

INTRODUCTION

Depression is a disorder that affects person's behavior, mood, thought and physical health. Patients with depression have symptoms include retardation of thought, apathy, pessimism, indecisiveness and loss of motivation that reflects the changes in brain neurotransmitters, especially norepinephrine (NE), serotonin (5-HT) and dopamine (DA)[1]. According to world health organization estimation 121 million people worldwide suffer from depression [2]. The major drugs used an antidepressant include monoamine oxidase-A(MAO-A) inhibitors, selective serotonin reuptake inhibitors (SSRI'S), selective norepinephrine reuptake inhibitors (SNRI'S). However, the side effects and drug interactions limit the use of these drugs [3]. Several plants have been explored to possess antidepressant activity like Asparagus *racemosus,Bacopa monia, Catharanthus tinctorius,,Glycyrhiza glabra*[4].Therefore, the search for herbal antidepressant drugs with least side effects and therapeutic efficacy is important.

Hoppea dichotoma belongs to the family Gentianaceae. It is a small tufted annual herb grows up to 12 cm height. In India the roots of this plant used to treat epilepsy, paralysis and whole plant is used to treat piles and snake bites [5]. The plant is widely used for various medicinal purposes but its antidepressant activity has not been reported scientifically. Therefore, our study was focused on the evaluation of antidepressant potential of methanolic extract of roots of *Hoppea dichotoma* in laboratory animals.

MATERIALS AND METHODS

Collection and Preparation of Plant extract

The roots of the *Hoppea dichotoma* were collected from Tirumala Hills, Tirupati, India. The plant was authenticated by Dr. Madhava Chetty, Professor of Botany, Sri Venkateshwara University, Tirupati and

voucher specimen of the plant were preserved at institute herbarium library. The roots are washed, shade dried and powdered. The powder was subjected to soxhlet extractor using methanol. The extract was filtered and then solvent was evaporated under reduced pressure to a solvent free concentrated mass, which was then stored in air-tight container in a cool and dry condition.

Preliminary phytochemical screening.

The methanol extract of *Hoppea dichotoma* was screened for the presence of various phytoconstituents like steroids, alkaloids, glycosides, flavonoids, carbohydrates, proteins and phenolic compounds [6].

Animals

Swiss albino mice of either sex weighing 25 to 30 g were used in the present study. All the animals were maintained under controlled conditions of temperature (23 ± 2 C), humidity (50 ± 5%) and 12 h light-dark cycles. All the animals were acclimatized for seven days before the study. The animals were randomized into experimental and control groups and housed individually in sanitized polypropylene cages containing sterile husk as bedding. They had free assessed to standard pellets as basal diet and water ad libitum. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) of Malla Reddy Institute of Pharmaceutical sciences (Reg. No: 1662/PO/Re/S/12/CPCSEA).

Acute toxicity study

Acute toxicity test was performed according to OECD guideline 423. Swiss albino mice were divided into test group comprising of six animals in each group.The animals were subjected for acute toxicity study using both plant extracts at a dose of 2000 mg/kg. The mice were observed continuously for 1 h and then half hourly for 4 h for any gross behavioral change and general motor activities like writhing, convulsion, response to tail pinching, gnawing, pupil size, fecal output, feeding behavior, etc., and further up to 72 h for any mortality [7].

Experimental protocol

For this experiment total 30 Swiss albino mice were divided into five groups and the treatment was given as follows;

Group 1: Normal control, given distilled water, p.o; for 14 days.

Group 2: Standard group received fluoxetine in dose of 20 mg/kg i.p.; for 14 days.

Group 3: Administered methanolic extract of *Hoppea dichotoma* (200mg/kg) orally for 14 days.

Group 4: Administered methanolic extract of *Hoppea dichotoma* (300mg/kg) orally for 14 days.

Group 5: Administered methanolic extract of *Hoppea dichotoma* (400mg/kg) orally for 14 days.

The antidepressant activity was carried out using two different models.

Forced swim test (FST)

Forced swim test was proposed as a model to test antidepressant activity by Porsolt et.al [8]**.** Mice were individually forced to swim in open glass chamber (25 × 15 × 25cm) containing fresh water to a height of 15 cm and maintained at 26°±1°C. At this height of water, animals were not able to support themselves by touching the bottom or the side walls of the chamber with their hind-paws or tail. Each animal showed vigorous movement during initial 2 min period of the test. The duration of immobility was manually recorded during the next 4 min of the total 6 min testing period. Mice were considered to be immobile when they ceased struggling and remained floating motionless in water, making only those movements necessary to keep their head above water. Following swimming session, mice were towel dried and returned to their housing conditions [9-11].

Tail suspension test (TST)

The total duration of immobility induced by tail suspension was measured according to the method described as a means of evaluating potential antidepressants [12]. Each mouse was individually suspended to the edge of a table, 50 cm above the floor, by adhesive tape placed approximately 1 cm from the tip of the tail. Each animal under test was both acoustically and visually isolated from other animals during the test. The total period of immobility was recorded manually for 6 min. Animal was considered to be immobile when it didn't show any body movement, hung passively and completely motionless. The test was conducted in a dim lighted room and each mouse was used only once in the test. The observer, recording the immobility of animals, was blind to the drug treatments given to the animals under study [13].

Biochemical Estimation

On 14th day, rats were sacrificed after 6 min exposure to FST and TST, the brain samples were collected immediately on an ice plate. The collected brain samples were washed with cold 0.25M sucrose, 0.1 M

Tris, 0.02 M EDTA buffer (pH 7.4) and weighed. The whole procedure of brain isolation was completed within five minutes [14,15]. The collected brain samples were analyzed for MAO-A and MAO-B.

Estimation of MAO-A and MAO-B

The brain mitochondrial fraction was prepared following the procedure of Schurr and Livne, 1976[15]. The MAO activity was accessed using spectrophotometer. Briefly, the buffer washed brain sample was homogenized in 9 volumes of cold 0.25 M sucrose, 0.1 M Tris, 0.02 M EDTA buffer (pH7.4) and centrifuged twice at 800 g for 10 min at 4°C in cooling centrifuge. The pellets were discarded and the supernatant was then centrifuged at 12000 g for 20 min. The precipitates were washed twice with about 100 ml of sucrose-Tris-EDTA buffer and suspended in 9 volumes of cold sodium phosphate buffer (10 mM, pH 7.4, containing 320 mM sucrose) and mingled well at 4°C for 20 min. The mixture was then centrifuged at 15000g for 30 min at 4°C and the pellets were re-suspended in cold sodium phosphate buffer [16,17]. The protein concentration was estimated by Lowry method using bovine serum albumin [18].

For estimating MAO-A activity, 2.75 ml sodium phosphate buffer (100 mM, pH 7.4) and 100 µl of 4 mM 5-hydroxytryptamine were mixed in a quartz cuvette which was then placed in double beam spectrophotometer. This was followed by the addition of 150 µl solution of mitochondrial fraction to initiate the enzymatic reaction and the change in absorbance was recorded at wavelength of 280 nm for 5 min against the blank containing sodium phosphate buffer and 5-hydroxytryptamine. For estimating MAO-B activity, 2.75 ml sodium phosphate buffer (100 mM, pH 7.4) and 100 µl of 0.1 M benzyl amine were mixed in a quartz cuvette which was then placed in double beam spectrophotometer. This was followed by the addition of 150 µl solution of mitochondrial fraction to initiate the enzymatic reaction and the change in absorbance was recorded at wavelength of 249 nm for 5 min against the blank containing sodium phosphate buffer and benzylamine.

Both MAO-A and MAO-B values were expressed as Unit/g protein. Specific activity was expressed as the number of units of activity per gram of protein.

Statistical analysis:

All the values are expressed as the mean ± SEM. The data were analyzed by using one-way ANOVA followed by Dunnet's, $P<0.01$ was considered as statistically significant.

RESULTS

Preliminary Phytochemical Screening:

The preliminary phytochemical screening revealed the presence of alkaloids, glycosides, flavonoids, tannins, steroids, saponins, proteins.

Acute Toxicity Studies

Methanolic extract of *Hoppea dichotoma* root showed no behavioral changes nor mortality at a dose of 2000 mg/kg.

Effect of *Hoppea dichotoma* root extract on immobility period in Forced Swim Test and Tail Suspension Test

Methanolic extract of *Hoppea dichotoma* root of three doses 200mg/kg, 300mg/kg, 400mg/kg administered for 14 successive days to mice decreased the immobility period significantly in a dose dependent manner in FST (Figure 1) and TST (Figure 2), indicating significant antidepressant like activity. A dose of 400mg/kg p.o of methanolic extract showed most potent antidepressant like activity in both FST and TST indicated by more decrease in the immobility time (Tables 1 and 2).

Effect of *Hoppea dichotoma* root extract on brain Mono amine oxidase (MAO) activity

Methanolic extract of *Hoppea dichotoma* administered for 14 successive days significantly reduces the brain MAO-A (Figure 3) and MAO-B (Figure 4) in a dose dependent manner when compared to the control (Tables 3 and 4).

Table -1: Effect of methanolic extract of *Hoppea dichotoma* root extract on immobility period of mice in Forced Swim Test

S.No	Treatment	Immovable time in sec
1	Vehicle(saline)	112.74±5.134
2.	Fluoxetine (20mg/kg)	52.77±3.79[a]
3.	MEHD (200mg/kg)	81.07±4.330[b]
4.	MEHD (300mg/kg)	75.17±3.043[b]
5	MEHD (400mg/kg)	71.33±3.480[b]

Values are expressed as mean ± S.E.M(n=6). [a]P<0.01 as compared to vehicle treated group. [b]P<0.01 as compared to vehicle treated group. MEHD: methanolic extract of *Hoppea dichotoma*

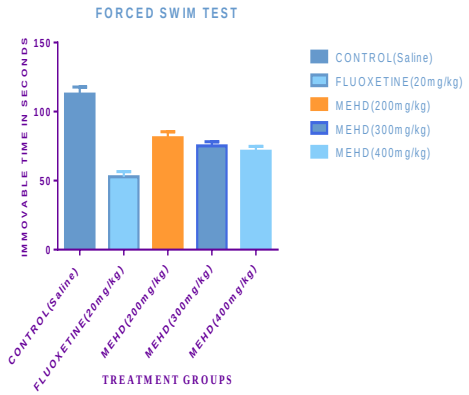

Figure 1: Effect of methanolic extract of *Hoppea dichotoma* root extract on immobility period of mice in forced swim test

Table -2: Effect of methanolic extract of *Hoppea dichotoma* root on immobility period of mice in tail suspension test

S.No	Treatment	Immovable time in sec
1	Vehicle(saline)	138.66±3.904
2.	Fluoxetine (20mg/kg)	81.83±3.30[a]
3.	MEHD 200mg/kg	113.16±4.73[b]
4.	MEHD 300mg/kg	96.76±4.21[b]
5	MEHD 400mg/kg	92.33±3.80[b]

Values are expressed as mean ± S.E.M(n=6). [a]P<0.01 as compared to vehicle treated group. [b]P<0.01 as compared to vehicle treated group. MEHD: methanolic extract of *Hoppea dichotoma*

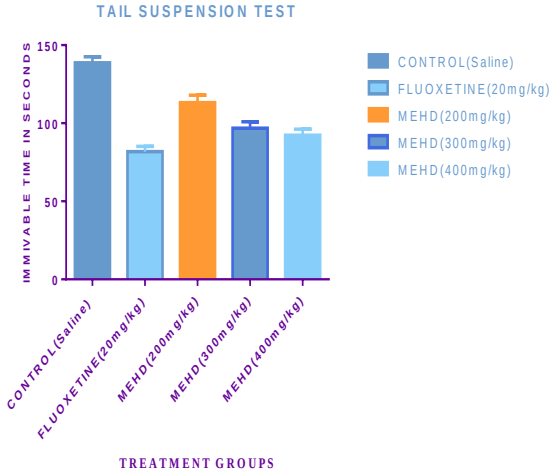

Figure 2: Effect of methanolic extract of *Hoppea dichotoma* root on immobility period of mice in tail suspension test

Table-3: Effect of methanolic extract of *Hoppea dichotoma* on mice brain monoamine oxidase-A (MAO-A) activity

S.No	Treatment	MAO-A activity µ/g protein
1	Vehicle(saline)	35.16±1.55
2.	Fluoxetine (20mg/kg)	19.16±1.40[a]
3.	MEHD (200mg/kg)	27±1.52[b]
4.	MEHD (300mg/kg)	25.5±1.91[b]
5	MEHD (400mg/kg)	23.3±1.11[b]

Values are expressed as mean ± S.E.M(n=6). [a]P<0.01 as compared to vehicle treated group. [b]P<0.01 as compared to vehicle treated group. MEHD: Methanolic Extract of *Hoppea dichotoma*

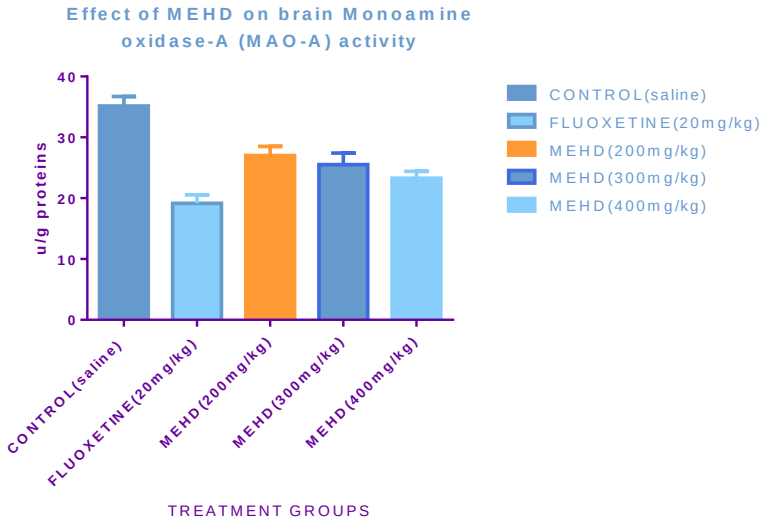

Figure 3: Effect of *Hoppea dichotoma* root extract on brain Mono amine oxidase-A (MAO-A) activity

Table -4: Effect of methanolic extract of *Hoppea dichotoma* on mice brain monoamine oxidase-B (MAO-B) activity

S.No	Treatment	MAO-B activity µ/g protein
1	Vehicle(saline)	46.16±2.72
2.	Fluoxetine (20mg/kg)	28.66±2.10[a]
3.	MEHD (200mg/kg)	36.5±1.43[b]
4.	MEHD (300mg/kg)	34.5±1.78[b]
5	MEHD (400mg/kg)	32.83±1.24[b]

Values are expressed as mean ± S.E.M(n=6). [a]P<0.01 as compared to vehicle treated group. [b]P<0.01 as compared to vehicle treated group. MEHD: Methanolic Extract of *Hoppea dichotoma*

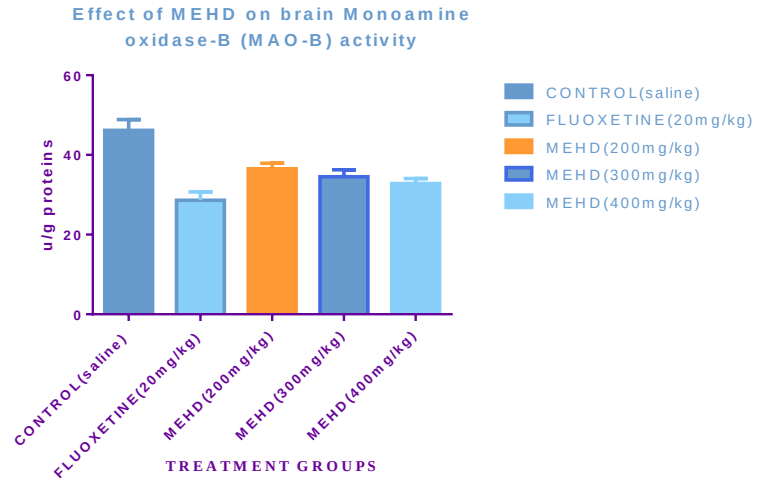

Figure 4: Effect of *Hoppea dichotoma* root extract on brain monoamine oxidase-B (MAO-B) activity

DISCUSSIONS

Depression is complex neuropsychiatric disorder, affects the life style and daily life with social relations of a person and that has been classified and treated in a variety of ways. Now a day, number of synthetic antidepressant drugs is available, these drugs have limited effect or restrictions in their clinical applications and associated with more side effect as well as the chronic toxicity that affect almost every organ system [19].

In this regard the antidepressant effect methanolic extract of *Hoppea dichotoma* root extract was studied by using Forced Swim Test and Tail Suspension Test. The extract administered for 14 successive days at doses (200mg/kg, 300mg/kg, 400mg/kg) produced significant antidepressant activity in mice employing both FST and TST in dose dependent manner. The efficacies of the extracts were comparable to the standard drug Fluoxetine. The methanolic extracts (200 mg/kg, 300 mg/kg, and 400 mg/kg p.o) administered for 14 successive days to mice significantly decreased brain MAO-A and MAO-B activity as compared to control. Hence, methanolic extract showed antidepressant activity probably by inhibiting MAO enzyme, thus increased brain levels of monoamines.

It has been previously suggested by Reneric and Lucki [20] that an increase in both swimming and climbing behaviors in the FST occurs when the animal is treated by a drug which increases serotonin, norepinephrine, and dopamine levels in the nerve terminals. Immobility in the FST represent a state of hopelessness in the animal which correlate to negative mood when place in an inescapable place. The immobility time is decreased by various types of antidepressants. An increase in all the three neurotransmitters could be by inhibition of monoamine oxidase activity in the brain. A growing body of research indicates that besides depletion of serotonin and catecholamine neurotransmitters, depression could result from various other pathophysiological mechanisms as well. Researchers suggest that depression may inhibit neurogenesis in the hippocampus [21]. This idea is supported by the finding that antidepressants can promote neurogenesis.

Phytochemical screening of MEHD revealed presence of flavonoids, saponins, sterols, proteins, tannins and carbohydrates. Moreover triterpepenoids (steroidal compounds) are present in the plant, those are able to cross blood brain barrier (BBB) due to their lipophilic nature and so it can be assumed that such steroidal compounds might also be responsible to elicit antidepressant and other neuropharmacological activities at molecular level in CNS (brain)[22].

CONCLUSION

The present study provides the first evidence indicating that methanolic extract of *Hoppea dichotoma* root showed significant antidepressant activity in TST and FST models of depression. Further research is required to know the mechanism of its action.

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Conflict of interest

We declare that we have no conflict of interest.

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