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Original Article

EVALUATION OF *MEDHOVIKAS*, A HERBAL FORMULATION FOR ANTIDEPRESSANT ACTIVITY

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ABSTRACT

Objective: The present study was conducted to evaluate the antidepressant activity of *Medhovikas* and its activity is compared with that of Imipramine and Mentat. The MAO-A and MAO-B levels were estimated by the Biochemical Method.

Methods: There are several methods available to evaluate the antidepressant activity. In the present study the antidepressant activity is assessed using behavioural despair method such as Forced swim test (FST) in mice. Imipramine is used as a positive control in doses of 10mg/kg and 20mg/kg, Similar activity was also observed with *Medhovikas* in doses of 200 and 400mg/kg. Biochemical method in rats to estimate the levels of MAO-A and MAO-B, the biogenic amine 5-HT is oxidized by MAO to 5-hydrixy indole acetic acid (5-HIAA) as a major metabolite and is excreted in urine. Hence the reduction in the urinary 5-HIAA level from normal after treatment with the test compound was taken as a measure of it's MAO inhibitory action.

Results : The antidepressant activity of 200 and 400mg/kg of *Medhovikas* was equivalent to that of 10 and 20mg/kg of imipramine respectively. It significantly reduced the duration of immobility indicating antidepressant activity. Significant decrease in brain MAO-A and MAO-B levels were observed which was determined by the Biochemical method.

Conclusion: Antidepressant activity was probably shown by inhibiting MAO-A and MAO-B, There are several methods to detect the monoamine oxidaise inhibitor activity of substances. These methods are based on biochemical estimation of biogenic amines and /or their pharmacological responses. This shows that *Medhovikas* is a potential antidepressant and the probable mechanism of action is in interest for further investigation.

Keywords: Antidepressant, Forced swim test, Imipramine,MAO-A & B, *Medhovika* and Mentat.

INTRODUCTION

According to world health report, about 450 million people suffer from a mental or behavioural disorder [(1)]. Depression is a heterogenous disorder that affects a person's mood, physical health and behavioural skills. Patient with major depression have symptoms that reflect changes in brain neurotransmitter, specifically norepinephrine (NE), Serotonin and Dopamine. An estimated 5.8% of men and 9.5% of women experience the depressive episodes in their life time. Suicidal tendency remains one of the common outcomes of depressive illness being responsible for 60% of the death [(2-5)]. Patients with depression have decreased social, occupational and educational functioning. An accurate diagnosis followed by an effective treatment can improve this outcome [(6)].

The main symptoms of depression are due to functional deficiency in the levels of mono aminergic transmitters: Noradrenalline, 5-hydroxy triptamine (5-HT) and Dopamine in the brain[(7)]. Drugs that increase the level of these neurotransmitters in the CNS show antidepressant activity [(8)]. The major antidepressant therapies aim for an enhancement in the transmitters levels in the neurons and thus normalize the neurotransmission[(9)]. Many of the currently available antidepressant drugs have proven to be effective but they are burdened with some disadvantages such as various adverse effects, problematic interactions and relatively low response[(10)]. In addition, it is also reported that only two out of three patients respond to any given treatment and of these, one would probably have responded to placebo alone [(11)].

Depression is a common chronic recurrent syndrome, often refractive to drug treatment affecting quality of life and overall productivity. The one-year prevalence rate is about 5%[(12)] (Paykel et al., 2005) and recurrence rates up to 85% have been reported [(13)] (Lee and Murray, 1988). Although, several classes of antidepressants are currently being used, due to clinical limitations and adverse effects there is critical interest in development of efficient and safe drugs for treatment of depression (Tran et al., 2003). Plant sources such as *Withania somnifera* (Bhattacharya et al., 2000), *Bacopa monniera* (Sairam et al., 2002) and St. John's wort extract (Kasper et al., 2006) have been reported to have antidepressant activity and can be effective therapeutic alternatives for treatment of depression. Depression is commonly accepted to be a disorder due to disturbances in neurotransmitters function, particularly serotonin, noradrenalin and dopamine [(14)] (Maes and Meltzer, 1995; Posener et al., 1994). Reduction in brain serotonin (Anguelova et al., 2003a,b; Drevets, 2001) has been reported to be one of the most important etiological factors for genesis of depression and the most widely used antidepressants namely serotonin reuptake inhibitors (SSRIs) increase extracellular availability of serotonin (Schreiber et al., 1995). Further, noradrenergic and dopaminergic systems are reported to be involved and act in tandem with the serotonergic system[(15)] (Millan et al., 2000; Koch et al., 2002). As *medhovikas* showed significant antidepressant activity in behavioural despair method in mice reported by Porsolt et al (1977)[(18)], the present study was conducted to know whether the antidepressant activity is due to the possible inhibition of monamine oxidase enzyme. The enzyme monamine oxidase enzyme (MAO) is present in several tissues. It is concerned with metabolism of biogenic amines such as 5-hydroxytryptamine (5-HT), noradrenaline etc. Inhibition of MAO activity leads to enhanced tissue levels of amines and with a reduction in the formation and excretion of their metabolites. There are several methods to detect the monoamine oxidaise inhibitor activity of substances. These methods are based on biochemical estimation of biogenic amines and /or their pharmacological responses.

MATERIALS

Animals

Swiss albino mice weighing 25-30gms and Albino rats of either sex weighing between 140-210gms were used. They were housed in

standard polypropylene cages and maintained under the standard conditions: room temperature (25±3)^0 C, humidity 45%-55%, 12/12 hr light/dark cycle. They were fed with commercially available mouse normal pellet diet and water was allowed *ad libitum.* The animals were acclimatized to the laboratory conditions one week prior to the behavioral experiments. The animal handling was performed according to the Good Laboratory Practice (GLP) guidelines. Animals used in this study were treated and cared for in accordance with the guidelines recommended (Reg.No.1217/A/08/CPCSEA/MRCP/PHD/4) by the Institutional Animal Ethics Committee of College..

Drugs: *Medhovikas* (Suveda herbals, Tirupati), *Bacopa monniera* (Suveda herbals, Tirupati), *Centella asiatica* (Suveda herbals, Tirupati), Imipramine hydrochloride (Torrent pharmaceuticals Ltd.,India), *Mentat*^{R} (Himalaya drug Co., Banglore);

Chemicals: 1-Nitroso-2-napthol (Loba-chemi, bombay); 2,4-Dinitrophenyl hydrazine GR (Loba-chemi, co, bombay);5-hydroxy indole acetic acid (Sigma chemical company, USA)

Apparatus: Apparatus consists of a glass beaker (11cm diameter, 15cm height) containing frech water upto a height of 6 cm maintained at a temperature of 22+-1^{0}C (Chaturvedi HK et al,1999)

Grouping and Treatment

For FST activity-Mice

The animals were randomly distributes into 11 groups each containing 10 animals and marked with unique identification.

Group I : Control (0.3ml of 2%gum acacia suspension).

Group II and III: Imipramine, 10mg/kg and 20mg/kg respectively.

Group IV and V : Medhovikas, 200mg/kg & 400mg/kg respectively.

Group VI and VII : Mentat, 200mg/kg and 400mg/kg respectively.

Group VIII and IX: Bacopa monniera(BM), 200mg/kg and 400mg/kg respectively.

Group X and XI : Centella asiatica(CA), 200mg/kg and 400mg/kg respectively.

For MAO activity-Rats

The animals were randomly divided into seven groups each containing five rats. they were marked with unique identifications and they were arranged in metabolic cages suitable for the collection of urine.

Rats of group A served as control and were treated orally with 2% gum acacia suspension. Rats of groups B and C were treated respectively with moclobemide 50mg/kg and 100mg/kg orally suspended in 2% gum acacia. Rats of group D, E, F, G were administered orally with *Medhovikas* (MV) 100mg/kg, *Mentat* 100mg/kg, BM 100mg/kg, CA 100mg/kg respectively in 2%gum acacia suspension. The treatment was given for 3 days in all groups. 24 hr urine samples were collected daily for 3 days before, during and after for 4 days after the treatment. the volume of urine collected during 24 hrs was noted and the aliquots of urine sample were analysed for 5-HIAA as described below.

Preparation of standard curve for estimation if 5-HIAA in urine:

1. 1-Nitroso-2-napthol: 0.1% solution in ethanol.
2. Nitrous acid reagent: to 5ml of 2N sulphuric acid, 0.2ml of 2.5% of sodium nitrate was added. It as always prepared afresh.
3. Diethyl ether (reagent grade) was washed with dilute solution of ferrous sulphate to remove peroxides and later twice with water.
4. 2,4-Dinitrophenyl hydrazine: 0.5%2,4-Dinitrophenyl hydrazine in 2N HCl
5. Phosphate buffer (0.5N) P^H>7.0

Preparation of stock solution of 5-HIAA: A stock solution was prepared by dissolving 50 mg of 5-HIAA in 100 ml of distilled water in a volumetric flask, which represents 0.5mg/ml of solution.

Preparation of standard working solutions: From the above stock solution further dilutions were made to represent 50,100,200,400,600 and 800mcg/10ml of the solution.

METHODS

Laboratory Models for Assessment of Antidepressant Activity: Forced swim test

The samples were freshly suspended in 2%gum acacia and were injected intraperitoneally on the second day before the test session. The animals were forced to swim individually, for 15min, on first day. This constitutes the 'pretest' session. Twenty four hours later, 30min after the administration of vehicle/test sample, each mouse was gently dropped in to the water in the beaker and was left for 6 minutes. The duration of immobility occurring was recorded. A mouse was judged to be immobile when it ceased struggling and remained motionless in the water making only those movements necessary to keep it's head above the water. Because little immobility is observed during first 2 minutes, the immobility observed during the last 4 minutes of 6 minutes test session was taken as response time.

1. Immobility: floating in water without swimming.
2. Swimming: active movements of extremities and circling in the container.
3. Climbing: active movements of forelimbs on the container wall.

Monamine Oxidase Inhibitory Activity Of *Medhovikas*

In the present work, the MOA inhibitory activity of the *Medhovikas* was studied by biochemical method where the urinary excretion of metabolite of 5-HT was determined in 24 hour urine samples of rats and it was compared with that of moclobemide, a known MAO inhibitor which was used as a reference substance. Simultameously MAO inhibitory activity of *Mentat,* BM, and CA was also studied as it was not reported earlier. The procedure adapted is described below.

Biochemical method in rats

The biogenic amine 5-HT is oxidized by MAO to 5-hydrixy indole acetic acid (5-HIAA) as a major metabolite and is excreted in urine (Udenfriend et al., 1955). Hence the reduction in the urinary 5-HIAA level from normal after treatment with the test compound was taken as a measure of it's MAO inhibitory action. The method reported by Udenfriend et al 1955 was followed in the present study. Principle of estimation of 5-HIAA: The 5-HIAA in solutions and urine samples was determined by measuring the optical density at 540nm on treating it with nitroso napthol reagent followed by nitrous acid reagent, after removal of the interfering keto acids by treating with 2,4dinitrophenyl hydrazine and extracting with chloroform.

Method: To prepare 10ml of standard solution in a 50 ml glass stoppered bottle, 10ml of 2,4dinitrophenyl hydrazine reagent was added. after 30min 25ml of chloroform was added, the bottles were shaken for 10min and then centrifuged. After phase separation organic layer was removed and replaced with a fresh 25ml portion of chloroform and the extraction was repeated. after centrifuging, 15ml of aqueous layer was taken into 50ml stoppered bottle. To this 8g of sodium chloride and 25ml of ether were added. These bottles were shaken for 5min on a shaker and centrifuged. After centrifugation and phase separation 20ml ether was taken into 50ml glass stoppered bottle containing 4ml of phosphate buffer at ph 7.0. It was shaken for 5min, centrifuged and ether was removed by aspiration. Later 3ml of buffer layer was taken into 15ml stoppered tubes. To this 1.5 ml of nitrosonapthol reagent and 1.5ml of nitrous acid reagent were added and mixed well and were kept at 37^{o}c for 5min. Then 5ml of ethyl acetate were added and after phase separation the ethyl acetate layer was aspirated. The above step was repeated with another 5ml of ethyl acetate. From the above aqueous solution 4ml was transferred into a cuvette and the optical density was measured at 540nm against reagent blank. The reagent blank for the blank setting of the instrument was prepared by treating 10ml of distilled water in the same manner.

STATISTICAL ANALYSIS

All the results were expressed as mean ± Standard error. A probability level of $p<0.01$ was considered as significant. The mice brain MAO-A and MAO-B levels of different groups were analysed using ANOVA, followed by Dunnett's 't' test.

RESULTS

Effect of *Medhovikas* on immobility periods in FST

The response time was recorded for each mouse and mean SEM values were calculated for each group and were given in the Table-1. All the drugs were found to reduce the response time in a dose dependent manner. The effect produced by 200 and 400mg/kg doses of *Medhovikas*,10 and 20mg/kg doses of imipramine, 200 and 400mg/kg doses of *Menat*, 200 and 400mg/kg doses of CA were statistically significant and compared to control group($P<0.001$).

Biochemical method in rats

Average values of 3 such determinations are given in Table-2. A linear relationship between the amount of 5-HIAA and the optical densities was found in the range of 50 to 800 mcg. A positive correlation between concentration of 5-HIAA and the corresponding optical densities was observed. A plot of the amount of the 5-HIAA Vs the corresponding optical densities were shown below. The amount of 5-HIAA in the unknown sample was directly read from the standard graph.
Estimation of 5-HIAA in urine: The 5-HIAA levels in urine samples were analysed in the same manner as described earlier and the amount of 5-HIAA excreted during 24hrs was calculated.

The 5-HIAA levels in 24hrs urine samples before, during and after treatment of rats with test substance are shown in the **Table-3**. and percentage changes of 5-HIAA levels calculated from average of the values obtained on the 3 days prior to drug treatment was also shown in the Table-4. The significance of *Medhovikas*, *Mentat*, Moclobemide, BM, CA treatment on urinary 5-HIAA levels compared to pre treatment levels was tested by applying unpaired student's t-test. All the drugs produced significant change. The percentage off change in 5-HIAA levels observed with *Medhovikas* (100mg/kg) was equivalent to that of moclobemide (50mg/kg) indicating that they produced similar effect. Also the percentage change of 5-HIAA levels observed with CA(100mg/kg) was almost equal to that of Moclobemide (100mg/kg) indicating that they produced similar effect.

DISCUSSION

Animal models like mother-infant separation in monkeys, amine depletion by reserpine, learned helplessness etc. are available for creating depression and for testing substances for antidepressant action. Eventhough none of animal models correspond to inherited condition of clinical depression in man, the animal models were used for screening substances for antidepressant activity as some of the underlying biochemical mechanisms were common to clinical depression and experimentally produced depression in animals. These models with their mechanisms were reviewed by Porsolt (1985).

In our studies, *Medhovikas* was tested for antidepressant activity in mice following the method reported by Porsolt(1977) since it can be used as a regular screening test in laboratory conditions. Imipramine which is used as a positive control in doses of 10 and 20mg/kg, significantly reduced the duration of immobility indicating antidepressant activity. Similar activity was also observed with *Medhovikas* in doses of 200 and 400mg/kg bodyweight.

Mentat was found to have significant antidepressant effect, following subchronic administration, which was quantitatively compared to that induced by the standard imipramine (Bhattacharya, 1994). In present study, the activity of single dose administration of *Mentat* in doses of 200 and 400mg/kg (i.p) bodyweight was found to be less effective than 200 and 400mg/kg doses of *Medhovikas* respectively. In low dose (200mg/kg), CA was found to be more effective than BM and in high dose (400mg/kg), BM was found to be more effective than CA.

The antidepressant activity of 200 and 400mg/kg of *Medhovikas* was equivalent to that of 10mg/kg and 20mg/kg of imipramine respectively. This shows that *Medhovikas* is a potential antidepressant.

The enzyme MAO is present in several tissues like liver, brain, platelets and enterochromaffin cells. It participates in the oxidative deamination of the several endogenous amines like adrenaline, noradrenaline, 5-HT etc. Several substances of diverse chemical structures are reported it inhibit this activity. compounds reported to have MAO inhibitory activity are iproniazid, tranyl cypromine and phenelezine of the two major molecular species of MAO, type A is selectively inhibited by moclobemide and prefers serotonin as a substrate. Substances that inhibit MAO activity reduce rate of formation of metabolites of monoamines or completely block their metabolism by deamination pathway with consequent reduction in the total amount of metabolite formation. This fact is made use of to provide evidence for MAO inhibitory activity of *Medhovikas*.

Table-1 :Effect Of Medhovikas on Immobility period in Forced Swim Test (FST).

Group No.	Drug treatment for 14 days P.O	Number of Animals	Dose (Kg^{-1})	Immobility Time (sec) Mean ±SEM	
				FST	% Change from Control
1	Control	10	0.3ml of 2% gum Acacia	125 ± 2.83	-----
2 and 3	Imipramine	10	10mg and 20mg	81.1 ± 3.39*** 55.1 ± 4.52***	35.4 56.1
4 and 5	Medhovikas	10	200mg and 400mg	81.7 ± 2.15*** 50.6 ± 3.25***	35.0 59.7
6 and 7	Mentat	10	200mg and 400mg	111.6 ± 5.28* 96.6 ± 2.85***	11.1 23.0
8 and 9	Bacopa monniera	10	200mg and 400mg	99.4 ± 6.43*** 45.9 ± 1.96***	20.9 63.5
10 and 11	Centella asiatice	10	200mg and 400mg	67.5 ± 3.41*** 52.9 ± 2.71***	46.3 57.9

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's t-test.*P<0.05,**P<0.01 and ***P<0.001 as compared to Control; FST-Forced Swim Test.

Table 2: Biochemical Method in rats

Concentration of 5-HIAA(µg)	Optical density
50	0.014
100	0.020
200	0.068
400	0.120
600	0.180
800	0.232

Table 3: Daily urinary 5-HIAA in rats

Days	Daily Urinary 5-HIAA ((µg) in rats treated with						
	Vehicle	**Moclobemide (50mg/kg)**	**Moclobemide (100mg/kg)**	**Medhovikas (100mg/kg)**	**Mentat (100mg/kg)**	**BM (100mg/kg)**	**CA (100mg/kg)**
Before treatment Mean ± SEM	236.23 ±7.5	466.13 ±2.26	258.23 ±3.64	470.83 ±1.11	202.2 ±3.73	318.43 ±1.59	245.1 ±2.12
During treatment Mean ± SEM	248.5 ±4.4	174.8 ±70.34*	27.87 ±13.28*	166.2 ±72.48**	71.97 ±16.79*	163.13±55.13*	38.83 ±14.06**
After treatment	235.0	461.9	86.2	463.3	191.7	298.1	119.1

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's t-test.*P<0.05,**P<0.01 and ***P<0.001 as compared to Control.

Table 4: Percentage reduction in the daily urinary 5-HIAA levels in rats.

Days	Vehicle	Moclobemide (50mg/kg)	Moclobemide (100mg/kg)	Medhovikas(100mg/kg)	Mentat (100mg/kg)	BM (100mg/kg)	CA (100mg/kg)
During treatment:	-6.0	33.4	80.4	35.2	48.2	20.2	73.4
	-8.0	70.1	89.0	72.5	69.3	46.1	86.1
1	-1.6	84.0	98.2	86.6	75.7	80.0	93.0
2	-1.2	79.2	87.0	81.2	62.4	64.7	84.2
3	-2.8	21.8	83.3	23.9	21.8	49.4	81.0
After	-1.85	3.0	74.1	5.0	9.3	20.0	68.3
treatment	0.52	0.9	66.6	1.6	5.2	6.4	51.4
1							
2							
3							
4							

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