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Comparative Pharmacological Evaluation of *Ocimum sanctum* and Imipramine for Antidepressant Activity

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SUMMARY. **The present study was undertaken to evaluate the comparative antidepressant activity of *Ocimum sanctum* (OS) and imipramine using animal models of depression. Imipramine (15 mg/kg/i.p) and herbal extract of OS (500 mg/kg/p.o) were subjected for its antidepressant activity using four different animal models of depression, viz: Forced Swimming Test (FST), Reserpine Reversal Test (RRT), Haloperidol-Induced Catalepsy (HIC), and Pentobarbitone Sleeping Time (PST) in male Wistar rats. The study after single administration of OS and imipramine revealed a statistically significant reduction in immobility time in FST, RRT, and protection against HIC, compared to control respectively. However there was no significant potentiation of PST. The antidepressant activity of OS was comparable to that of standard drug imipramine. The results of the present study indicate the potential for use of OS as an adjuvant in the treatment of depression.**

INTRODUCTION

Depression is considered as an affective disorder with a prevalence of approximately 5 % in the general population. It is characterized by change in mood, lack of interest in the surroundings, psychomotor retardation and melancholia. It has been estimated that 5.8 % of men and 9.5 % of women experience a depressive episode in their lifetime and suicide is one of the most common outcomes of depression [1-3]. Depression is a common, debilitating, life threatening illness with an increasing morbidity and mortality. Furthermore, the World Health Organization (WHO) revealed that depression is the fourth leading cause of disability worldwide [4].

Despite the developments in pharmacotherapy of depression, this disorder often goes undiagnosed and untreated in many patients. Although the drugs provide some improvement in the clinical condition of patient, it is at a cost of having to bear the burden of their adverse effects [2,5,6]. This is further complicated by the difficulty in predicting the patient's response to treatment. It has been reported in earlier studies that only two out of three patients responds to any given antidepressant treatment, and of these, one would probably have responded to placebo alone [2,7]. The exact etiology of depression still remains obscure, but the most popular theory is the decrease in the neurotransmitter levels in the brain. However, recent studies have also shown the involvement of oxidative stress in the phenomenon [8,9].

Many plants have been used in the traditional systems of Medicine for the treatment of depression and associated disorders [10]. Because of the lacunae in the current treatment options, there has been an increase in the number of patients turning towards alternative and complimentary systems of medicine to obtain symptomatic relief. *Ocimum sanctum* Linn (OS), belongs to the family Lamiaceae, popularly known as Tulsi in Hindi and 'Holy Basil' in English, is one of the sacred herbs for Hindus in the Indian subcontinent. It has been in clinical use for centuries. The entire plant of OS has medicinal value although mostly the leaves, and sometimes the seeds, are used. Earlier studies with OS have indicated that the plant has hypoglycemic, hypolipidemic, adaptogenic, antidepressant, antiepileptic, hepatoprotective, anticancer, radioprotective, analgesic and anti-inflammatory properties [11]. In a preclinical study the anticataleptic property of OS in mice has already

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been reported [12]. OS is also effective against dementia, Alzheimer's disease [13] and anxiety [14]. Earlier studies with OS have demonstrated antistress activity [15,16] and found to modulate the central monoamines like noradrenaline, 5-hydroxytryptamine and dopamine. These neurotransmitters known to play an important role in pathophysiology of depression and antidepressants known to increase the level of these monoamines. Modulation of monoamines, anticataleptic activity and antistress activity of OS prompted us to study antidepressant activity in four experimental models viz. Forced Swimming Test (FST), Reserpine Reversal Test (RRT), Haloperidol-Induced Catalepsy (HIC), and Pentobarbitone Sleeping Time (PST) in male Wistar rats.

MATERIALS AND METHODS

Animals

Healthy adult male Wistar rats weighing 140-160 g, 90-120 days were used. The Institutional Animal Ethics Committee approved the study. The animals were maintained under standard conditions in the animal house approved by the Committee for the Purpose of Control, and Supervision on Experiments on Animals (CPCSEA). The animals were given pelletted food Lipton India Ltd and water *ad libitum*.

Plant Material & Chemicals

OS extract was obtained from Sami Chemicals and extracts Pvt. Ltd, Bangalore. The standard drug imipramine was procured from Torrent Pharmaceuticals, India. Plant extract was suspended in 2 % Tween 80 immediately prior to administration. The challenge drugs used in different animal models of depression were Haloperidol (Searle India Ltd., India), Reserpine (Loba-Chemie Industrial Co. India), and Pentobarbitone sodium (John Baker mc, Colorado USA).

Experimental Protocol

Healthy Male Wistar rats were randomly divided into three groups consisting of six rats each. Control group, received calculated dose of distilled water (1 ml/100 g) oral route. Standard group, imipramine 15 mg/kg/*i.p.* [17] OS group: OS 500 mg/kg/*p.o.* The dose of OS was selected based on the dose response study carried out in our laboratory.

Extracts and Drug Preparation

Weighed quantity of OS extract was suspended in distilled water using 1 % Tween 80 and administered orally, in a volume of 1 ml/ 100 gms, one hour before experimentation. Imipramine (15 mg/kg) was dissolved in distilled water and administered *i.p.* 1 h before experimentation, in a volume of 0.5 ml/100 g. Reserpine (2 mg/kg) was suspended in distilled water using 1 % Tween 80 and administered *i.p.* 24 h before experimentation in a volume of 0.5 ml/ 100 gms. Haloperidol (1 mg/kg) dissolved in distilled water and injected *i.p.* 30 min before experimentation in a volume of 0.5 ml / 100 g. Pentobarbitone sodium (30 mg/kg) dissolved in warm distilled water and administered *i.p.* in a volume of 0.5 ml/100 g.

Forced Swimming Test (FST)

In the FST [18], measurement of immobility time was carried out by observing the motor activity of the rat, which was placed in a pool of water. A glass cylinder, 25 cm in diameter, height 40 cm, was filled with water to a height of 34 cm. The temperature of water was 23 ± 1 °C. After 15 min (pretest session), the rats were towel dried and kept for 15 min in a heated enclosure (32 °C). Twenty-four h later, the animal were exposed again to the conditions outlined above and the total immobility time during 5 min period was recorded (test session). Drug/vehicle was administered to the animals 60 min before the FST. A rat was judged immobile whenever it floated passively in a slightly hunched back position with its head just above the water surface. The time spent immobile within 5 min was recorded. Immobility time is the time during which the animal floated on the surface with front paws together and made only those movements which were necessary to keep afloat. Shorter immobility time is an indicator of the stronger antidepressant effect of the tested substance.

Reserpine Reversal Test (RRT)

In the RRT [19], all groups received calculated dose of reserpine suspended in distilled water in a dose of 2 mg/kg intraperitanially 24 h before experimentation. The different experimental groups received their respective treatments and the reduction in reserpine induced immobility period was measured in individual groups one hour after the respective treatment. The time spent immobile within five minutes was recorded. Reserpine was administered to the rats (2 mg/kg/*i.p.*), after 24 hours the extracts were administered to the animals by oral route

and reduction in reserpine induced immobility period was measured one hour after drug administration. The time spent immobile within five minutes was recorded.

Haloperidol-Induced Catalepsy (HIC)

In HIC [20], the different experimental groups received the respective treatments; calculated dose of haloperidol was administered *i.p.* one hour after the administration of their respective treatments. Severity of catalepsy was measured every 30 min after haloperidol 1 mg/kg upto a total duration of 3 h. Catalepsy of an individual rat was measured in a stepwise manner by scoring method.

The method assessed the ability of an animal to respond to an externally imposed posture Step I: the rat was taken out of the home cage and placed on a table. If the rat failed to move when touched gently on the back or pushed, a score of 0.5 was assigned. Step II: The front paws of the rat were placed alternately on a 3 cm high block. If the rat failed to correct the posture within 15 s, a score of 0.5 for each paw was added to the score of the step I. Step III: the front paws of the rat placed alternately on a 9 cm high block. If the rat failed to correct the posture within 15 s, a score of 1 for each paw was added to the scores of step I and II. Thus, for an animal, the highest score was 3.5 (cut off score) and reflects total catalepsy.

Pentobarbitone Sleeping Time (PST)

In the PST test [21], the different experimental groups received the respective treatments. One hour later, calculated dose of pentobarbitone sodium (30 mg/kg / *i.p.*) was administered to the animals of all groups. The time of onset of action was noted as the animal loses it's righting reflex, that it falls asleep. The animals were placed on their backs leaving sufficient space in between two animals. The time of recovery from sleep as the animal turns to recover its normal posture was noted.

Statistical Analysis

Data are expressed as mean ± SEM. The results were subjected to one-way analysis of variance (ANOVA), followed by Dunnet's multiple comparison test to compare the treatment groups with control group.

RESULTS

Forced Swimming Test

After administration of a single oral dose, statistically significant decrease in the immobility time in forced swimming test was observed with OS 500 mg/kg, ($P < 0.001$), when compared to the control group. The extent of decrease in immobility time in case of OS was found to be very high than that of imipramine (Table 1).

Treatment	Dose (mg/kg)	Immobility (s)
Vehicle	-	223.6 ± 3.4
Imipramine	15	161.6 ± 6.1***
Ocimum sanctum	500	130.1 ± 6.8***

Table 1. Effect of acute administration of *Ocimum sanctum* and Imipramine on forced swimming induced immobility in rats.Values are expressed as mean ± SEM, n=6, *p< 0.05; **p<0.01, ***p< 0.001. Significant *versus* control Dunnet's test.

Reserpine Reversal Test

A statistically significant decrease of immobility time in reserpine induced immobility was observed after the administration of a single oral dose of OS ($P<0.001$), as compared with control group. The extent of decrease in immobility time in case of OS was found to be very high than that of imipramine (Table 2).

Treatment	Dose (mg/kg)	Immobility (s)
Vehicle	-	280.8 ± 6.8
Imipramine	15	122.1 ± 13.1***
Ocimum sanctum	500	164.1 ± 10.01***

Table 2. Effect of acute administration of *Ocimum sanctum* and Imipramine on Reserpine Induced immobility in Rats.Values are expressed as mean ± SEM, n=6, *p< 0.05; **p<0.01, ***p< 0.001. Significant *versus* control Dunnet's test.

Haloperidol Induced Catalepsy

OS ($P < 0.001$) provide highly significant protection against Haloperidol (1 mg/kg) induced catalepsy as compared to control, up to 180 min after intraperitoneal injection of Haloperidol. However, OS showed statistically significant inhibition of haloperidol induced catalepsy at 30 min after injection of HaloperidoI. The protection given by OS was more than that of imipramine (Table 3).

Pentobarbitone Sleeping Time

Compared to the control group no significant potentiation of pentobarbitone-induced loss of righting reflex was observed after pretreatment with OS extract ($P < 0.05$). However, treatment

Treatment	Dose (mg/kg)	Score of Catalepsy					
		Time (min)					
		30	60	90	120	150	180
Vehicle	-	0.5 ± 0.1	2.7 ± 0.2	3.2 ± 0.2	3.4 ± 0.1	3.5 ± 0.0	3.3 ± 0.2
Imipramine	15	± 0.1*	0.7 ± 0.3	1.0 ± 0.3	1.5 ± 0.3	1.6 ± 0.3	1.6 ± 1.4**
Ocimum sanctum	500	0.0 ± 0.0***	0.4 ± 0.1	1 ± 0.2	1.6 ± 0.2	0.6 ± 0.1	1.4 ± 0.1***

Table 3. Effect of acute administration of *Ocimum sanctum* and Imipramine on Halaoperidol Induced Catalepsy in Rats.Values are expressed as mean ± SEM, n=6, *p< 0.05; **p<0.01, ***p< 0.001. Significant *versus* control Dunnet's test.

Treatment	Dose (mg/kg)	Sleeping Time (min)
Vehicle	-	95.3 ± 7.1
Imipramine	15	137.1 ± 11.2***
Ocimum sanctum	500	97.6 ± 1.8

Table 4. Effect of acute administration of *Ocimum sanctum* and Imipramine on Pentobarbitone Induced Sleeping Time in Rats.Values are expressed as mean ± SEM, n=6, *p< 0.05; **p<0.01, ***p< 0.001. Significant *versus* control Dunnet's test.

with imipramine significantly increased (P < 0.001) the pentobarbitone induced loss of righting reflex (Table 4).

DISCUSSION AND CONCLUSION

Mood disorders, which are one of the most common mental illnesses have a lifetime risk of 10 % in general population. Of these disorders, depression alone is believed to affect around 5 % of the general population, with suicide being one of the most common outcomes [1-3]. Most of the drugs that are currently being used in the treatment of depression have adverse effects that affect the quality of life of the patient. This leads to patient's non-compliance to medication, which further complicates the problem [2,5,6]. In Ayurveda, number of single and multi drug formulations from plant origin are used in the treatment of psychiatric disorders [5,10] and are claimed to have a less side effects than conventional allopathic drugs. Individual studies using OS have also shown potent anticatalpetic activity [12] depicting interactions with the dopaminergic system in the brain. Ursolic acid, the principal constituent of OS, has been reported to have anti-inflammatory, antitumor, antioxidant and antibacterial properties [22]. The ethanolic leaf extract of OS has been found to increase the monoaminergic levels in the brain [23].

Development of immobility when rodents are placed in an inescapable cylinder of water during FST reflects the cessation of their persistent escape-directed behavior. Conventional antidepressant drugs reliably decrease the duration of immobility in animals during these tests. This decrease in duration of immobility was considered to have a good predictive value in the evaluation of potential antidepressant agents [24]. In the present study, OS caused a significant decrease in immobility induced by FST and RRT. The decrease in immobility was comparable to that produced by the standard antidepressant drug, imipramine. Neuroleptic-induced catalepsy has been linked to blockade of postsynaptic striatal dopamine D1 and D2 receptors [25]. In addition many preclinical and clinical studies have also proposed reactive oxygen species as cause of haloperidol-induced catalepsy [26]. In the present study, OS showed statistically significant inhibition of haloperidol induced catalepsy. Thus the anti-cataleptic effect of OS might be due to both its dopamine facilitatory and anti-oxidant properties. The anti-cataleptic of OS was comparable to that produced by the standard antidepressant drug, imipramine.

However, OS did not show any significant potentiation of the loss of righting reflex as induced by pentobarbitone. The barbiturates are known to induce sleep in man and animals by depressing the central nervous system by binding with high affinity to specific macromolecules within CNS. These barbiturates binding sites are closely associated with the GABAminergic receptors. GABA-chloride ionosphore, where these drugs appear to prolong rather than intensify GABA effects, it can be suggested that the antidepressant effect of the extracts observed in the present investigation could not be possibly mediated by GABAminergic receptors.

Since, OS exhibited better / equivalent effect to that of imipramine at the dose tested in FST,

RRT and HIC in rats, the drug is rated to posses potentially significant antidepressant activity.

The most prevalent theory for the pathogenesis of depression is "Monoamine hypothesis". Functional deficiency of central mono amines like noradrenaline, 5-hydroxytryptamine and dopamine are responsible for the symptoms of depression [27]. Many currently used antidepressants act by increasing the concentration of these neurotransmitters in the brain [28,29]. Evidence indicate that OS and its active principle ursolic acid is known to increase the level of noradrenaline , 5HT and dopamine level in the brain [16,23,30,31].Thus, the antidepressant like activity of OS might be due its modulatory effect on central monoamines. However, the exact mechanisms underlying the antidepressant action cannot be concluded at the moment due to the presence of large number of phytochemicals viz, euginol, aigenin, luteolin, apigenin 7-glucuronide, luteolin-7-O-glucuronide, orientin, mollusdistin and two flavonoids, orientin and vicenin [32] in the OS and further studies are being carried out to elucidate the same. In conclusion our preclinical study indicates the antidepressant like activity of OS. But, its usefulness in human beings is yet to be studied.

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