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Antidepressant activity of ethanolic extract of leaves of *Ocimum sanctum* in mice

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ABSTRACT

Depression is a widespread psychiatric disorder affecting around 5% of the population. Furthermore, it is difficult to predict which patient will respond to any given treatment. In the traditional systems of medicine, many plants and formulations have been used to treat depression for thousands of years. *Ocimum sanctum*(OS), 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 a versatile role in traditional medicine. Therefore, the present study was undertaken to evaluate the antidepressant potential of acute and chronic administration of OS in forced swim test (FST) and tail suspension test (TST). Inbred adult male Swiss Albino mice weighing 25-30g were used in the study. Standard drug (imipramine) and test drug (OS) were suspended in 1% gum acacia. The vehicle (10ml/kg, p.o), imipramine (10mg/kg, p.o) and OS (1.75mg/kg, 4.25mg/kg, 8.5mg/kg, p.o. respectively) were administered 1hour prior to acute study. In chronic study, all drugs were given once a day for 10 days and the last dose was given 1hour before the experiment. Duration of immobility was noted in both the models. In our study, both imipramine and OS significantly reduced the duration of immobility in both experimental models as compared to the animals in the control group. 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.

Keywords: Ocimum sanctum, Forced swim test, Tail suspension test, 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]

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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*(OS) Linn, 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 antiinflammatory properties[11], From our laboratory we have already reported the anticataleptic property of OS in mice[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-Hydoroxytryptamine 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 two experimental models viz. forced swim test(FST) and tail suspension test(TST) in mice.

MATERIALS AND METHODS

Animals

Swiss albino mice weighing 25-30 gm from our breeding stock

were used in this study. The animals were housed at 24±2°C with 12:12 h light and dark cycle. They had free access to food and water. The animals were acclimatized for a period of seven days before behavioral studies. All experiments were carried out during day time from 0900 to 1400 hours. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Kasturba Medical College, Manipal University, Mangalore and care of animals was taken as per guidelines of CPCSEA, Department of Animal Welfare, Government of India.

Drugs and Chemicals

Imipramine (Ranbaxy Pvt Ltd. Mumbai) was used as the standard drug. The test drug,OS leaf ethanolic (70% v/v) extract was standardized and supplied by Quality Control Laboratory, M/s. Natural Remedies, Bangalore. It was found to contain =2.5% w/w ursolic acid by HPLC (Lab ref no.0408817 dt 30-8-2004). All drugs were dissolved/ suspended in 1% gum acacia[17], which served as the vehicle.

Experimental design

On the day of the experiment, the animals were divided into five groups (n=6). Group I received the vehicle (10ml/kg) and served as the control, group II received the standard drug imipramine (10mg/ kg), groups III, IV and V were received the test drug OS in doses of 1.75, 4.25 and 8.50mg/kg respectively by the oral route.

Drug/vehicle was administered to the animals 60 minutes before the behavioural evaluation in acute study. For chronic study, a new set of animals were used. They were grouped as in acute study and were administered the drugs/vehicle for a period of ten days. Behavioural evaluation was carried out 60 minutes post drug/vehicle administration on tenth day. The antidepressant activity of the test drug was evaluated using the following experimental models of depression:

Tail suspension test (TST):

The method described by Steru, et. al. was used in our study[18]. The animals were hung by the tail on a thin horizontal steel rod,75 cm above the surface with the help of an adhesive tape placed approximately 1 cm from the tip of the tail. Immobility was recorded during the six minutes of observations.. Mice were considered to be immobile only when they hung passively and were completely motionless.

Forced Swim Test (FST):

The method described by *Porsolt, et. al.* was used in our study[19]. Each animal was placed individually in a five liters glass cylinder, filled with water upto a height of 15 cm and were observed for duration of six minutes. The duration of immobility was recorded during the last four minutes of the observation period. The mouse was considered immobile when it floated motionlessly or made only those moments necessary to keep its head above the water surface. They were removed and dried with a towel. The water was changed after each test.

Statistical analysis

The data is expressed as mean±SEM and was analysed by one-way ANOVA followed by Dunnet's 't' test . $P< 0.05$ was considered to be statistically significant.

RESULTS

Forced swim test (FST):

Forced swim test (FST): A significant decrease in the duration of immobility was seen with the standard drug imipramine in both, acute and chronic study. In the OS treated groups, in acute study, a significant reduction in duration of immobility against control was seen only in the groups treated with 4.25 and 8.5mg/kg. However on chronic administration, OS at all the doses tested (1.75, 4.25 and 8.50mg/kg) produced significant reduction in immobility compared to vehicle treated group. Imipramine significantly reduced the immobility both on acute and chronic administration (Table 1).

Table 1. Duration of immobility (in seconds) of mice in forced swim test

Groups(n = 6)	Treatments Dose per kg	Duration of immobility (in seconds)	
		Acute study	Chronic study
Group - I	Control- 1% gumacacia 10ml	120.00 ± 3.94	128.00 ± 3.30
Group - II	Imipramine 10mg	62.33 ± 12.00	88.16 ± 2.37**
Group -III	*Ocimum sanctum* 1.75mg	104.50 ± 1.50	99.10 ± 2.46**
Group - IV	*Ocimum sanctum* 4.25mg	95.00 ± 2.51*	95.00 ± 2.97**
Group - V	*Ocimum sanctum* 8.5mg	62.33 ± 3.41*	91.83 ± 4.97**

*(Values are expressed as mean ±SEM. *P<0.05, **P<0.01 compared with control)*

Table 2. Duration of immobility (in seconds) of mice in Tail suspension test

Groups (n = 6)	Treatments Dose per kg	Duration of immobility (in seconds)	
		Acute study	Chronic study
Group - I	Control- 1% gumacacia 10ml	247.17 ± 2.91	254.00 ± 4.48
Group - II	Imipramine 10mg	187.00 ± 16.68**	205.50 ± 4.83**
Group -III	*Ocimum sanctum* 1.75mg	239.50 ± 4.90	224.00 ± 4.01**
Group - IV	*Ocimum sanctum* 4.25mg	237.17 ± 5.91	221.00 ± 3.36**
Group - V	*Ocimum sanctum* 8.5mg	225.00 ± 2.76	215.00 ± 3.19**

*(Values are expressed as mean ± SEM. **P<0.01 compared with control)*

Tail suspension test (TST):

Tail suspension test (TST): A significant decrease in the duration of immobility was seen with the standard drug imipramine both in acute and chronic study. OS at all the doses tested (1.75, 4.25 and 8.50mg/kg) failed to alter the duration of immobility to a significant level in acute study. However, on chronic administration, OS significantly reduced the duration of immobility at all the tested (1.75, 4.25 and 8.50mg/kg). (Table 2).

DISCUSSION

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. From our laboratory we have already reported the central actions of a polyhrebal formulation NR-ANX-C[20,21], which contains OS as one of the main constituents. 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 suspended by their tail during TST and when they were 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 ethanolic extract of OS reduced the duration of immobility in both models of depression viz. FST (only in chronic study) on acute and chronic administration in FST model and on chronic administration in TST. However, the reduction was more pronounced in chronic administration than after single dose administration The reduction was comparable to that produced by the standard antidepressant drug, imipramine (10mg/kg). Reduction in duration of immobility is suggestive of antidepressant like activity of the extract.

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[25]. Many currently used antidepressants act by increasing the concentration of these neurotransmitters in the brain[26,27]. Evidence indicate that OS and its active principle ursolic acid known increase the level of noradrenaline , 5HT and dopamine level in the brain[16,23,28,29].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[30] 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 yet to be studied.

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