# Pharmacological And Neurobiochemical Evidence For Antidepressant-Like Effect Of Sumind, A Herbal Product In Animals

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**Citation**

K Nanjappa, M Shalam, M Harish, Prabhu, S Rao, B Kutty. *Pharmacological And Neurobiochemical Evidence For Antidepressant-Like Effect Of Sumind, A Herbal Product In Animals*. The Internet Journal of Nutrition and Wellness. 2006 Volume 4 Number 1.

**Abstract**

In this study the sumind, a polyherbal formulation was explored for its antidepressant properties using Forced swim test (FST) paradigm. Many of its individual constituents have been for central nervous system (CNS) activities but no systematic work was carried on the formulation. In this study its effect on depression was explored in rats. For this purpose different doses of sumind (92 mg/kg, 184 g/kg, 274 mg/kg and 367 mg/kg, PO) were selected for both acute and chronic studies in rats. Sumind exhibited significant antidepressant activity, as indicated by its ability to decrease swim stress induced immobility time in rats as well as restoring the biogenic amines to normal level that was altered by the swim induced stress in whole rat brain assay by HPLC Method. These results indicate that sumind can be a potential candidate for managing depression. However further studies are required to substantiate the same.

## INTRODUCTION

Depression is considered as an affective disorder characterized primarily by change of mood. It is associated with significant morbidity and mortality. The prevalence of major depression in the general population is estimated at 5 %. In medical patients, the prevalence ranges from 9% in ambulatory setting to as high as 30% in hospitalized patients[1]. According to the World Health report (WHO, 2001), approximately 450 million people suffer from a mental or behavioral disorder, yet only a small minority of them receives even the most basic treatment. This amounts to 12.3% of the global burden of disease, and will rise to 15% by 2020[2]. In the search for new therapeutic products for the treatment of neurological disorders, medicinal plant research, worldwide, has progressed constantly, demonstrating the pharmacological. A number of single and compound drug formulations of plant origin are mentioned in Ayurveda, the ancient traditional system of medicine for the treatment of psychiatric disorders[3]. In Ayurveda, compound formulations are generally used in the therapy based on the concept that such a combination provide synergistic therapeutic effect and also include ingredients, which help to minimize the adverse effects of the major drugs[4]. Sumind is such a herbal formulation with the following composition (Ayurvedic nomenclature and the quantity of each ingredient are given in parentheses), Nardostachys atamans Linn (Jatamansi, 100 mg), Acorus calamus linn (Vacha, 80 mg), Celastarus paniculata Linn (Jotishmati, 50 mg), Convolvulus microphyllus Linn (Shankapushpi, 80 mg), Bacopa monnieri Linn (Brahmi, 80 mg), Withania somnifera Linn (Ashvagadha, 50 mg), Valerian wallichii Linn (Tagara,10 mg), Eclipta alba Linn (Bhringaraja , 100 mg).

So far there are no pharmacological evidences to demonstrate the antidepressant effect. However, there are reports to show that some of the ingredients of this formulation, Withania somnifera possessing anti stress activity, anti inflammatory activity, anti tumour activity, anticonvulsant activity and CNS depressant action [5], [6] also there are studies to show the antagonism of strychnine induced convulsion, sedative and tranquilizing effect of Acorus calamus [7]. Animal study has shown that the chronic treatment of Nardostachys jatamasi significantly increase the biogenic amines like NA, DA, 5HT and the inhibitory amino acids in rat brain [8] . Bacopa manniera and Convolvulus microphyllus has been used since time immemorial as nerve tonic for improvement of memory [9], [10]. Animal studies has shown that Celastrus paniculata effective in tranquilizing,

hypolipidemic, antiatherosclerotic, sedative and anticonvulsant activities [11]. We have used the behavioral changes in FST model of depression and level of biogenic amines of DA, NA and 5HT as parameters for evaluating antidepressant activity of the sumind in rats.

## MATERIAL AND METHODS

### ANIMALS

The experiments were performed on female wistar rats (180-250gm) and swiss albino mice (25-35g) of either sex procured from Central animal research facility NIMHANS or Drug testing laboratory, Bangalore, India. The animals were group housed in colony cages at an ambient temperature of 25 ± 1°C and 45 to 55 % relative humidity with a 12 hr/12 hr light dark cycle and had free access of food and water ad libitum. The experiments were carried out between 0900 to 1400 hrs and animals were acclimatized for one week before the start of experimentation. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Government College of Pharmacy, Bangalore, India, and were in accordance with the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

### DRUGS AND CHEMICALS

The investigational drug, sumind, was a gift sample from Anglo-French drugs and Industries Ltd, India. Alprazolam and Diazepam were gifted by Bal Pharmaceutical Ltd. India and Venlafaxine, Imipramine and Fluoxetine by Torrent Pharmaceuticals, India. Noradrenaline, Dopamine, 5-Hydroxy tryptamine and Corticosterone were purchased from Sigma Chemicals, USA. All the other chemicals used in the study were of analytical grade.

The experimental drug was suspended in distilled water and administered orally. Alprazolam, Diazepam, Venlafaxine, Imipramine and Fluoxetine were all dissolved in dw for administration and served as positive controls.

### EXPERIMENTAL DESIGN

### ACUTE TOXICITY TEST

Acute toxicity of the preparation was determined using female albino mice. The animals were fasted 3 hrs prior to the experiment according to the procedure (OECD guide line no. 425). Animals were observed for 48 hrs for any mortfollowing administration of the different doses of the preparation orally as per the guidelines.

### LOCO MOTOR ACTIVITY

Naive pretreated drugs or vehicle were placed in loco motor activity chamber to which photoelectric cells were attached (Digital photoactometer-Hicon instrument) allowing loco motor activity to be recorded over a period of 10 minutes [12].

### TAIL SUSPENSION TEST (TST) IN MICE

TST is a simple, rapid and reliable method to screen antidepressants and other class of psychotropics [13]. This method is based on the observation that a mouse suspended by the tail shows alternate agitation and immobility which is indicative of a state of depression. TST induced immobility is reduced by a large no of clinically active and atypical antidepressants. Device used for this consists of metallic gallows which were connected to a nylon catheter (d=1.5 mm, l=150 mm) with a hook attached to its extremity. The distance between the floor of the device and the hook was 350 mm. The mouse was hung in the hook by an adhesive tape placed 20 mm from the extremity of its tail, the mouse was 150 mm away from the nearest object. The articulated stylus of the gallows was connected to kymograph drum, marking on a cylinder covered with black smoke paper which is rotated at 2 cm/min. Mice were divided into different groups each containing 10 animals, sumind (92,184, 367 mg/kg b.w, p.o. single dose), Venlafaxine (32 mg/kg), Imipramine (32 mg/kg), Fluoxetine (16 mg/kg b.w) or distilled water (10ml/kg, p.o) and were administered 1 h prior to the beginning of TST in case of sumind and 0.5 h prior in case of standard drugs. Mice were suspended individually by the tail by securing the tail to the shelf by adhesive tape placed apprx. 2 cm away from the tip of the tail. And the total immobility time (flat recording in the graph).

### FORCED SWIMMING TEST (FST)

The FST is the most widely used pharmacological model for assessing antidepressant activity. The development of immobility when the rodents are placed in an inescapable cylinder of water reflects the cessation of persistent escape-directed behavior. The test was performed according to a modification suggested [14] of the traditional method described by Porsolt et al. [15]. The apparatus consisted of a transparent Plexiglas cylinder (50 cmhigh×20 cm wide) filled to a 30 cm depth with water at room temperature. Animals were divided into following groups each consisting 10 animals, sumind (92,184 and 367 mg/kg b.w, p.o. single dose), sumind 92, 184, 275 and 367 mg/kg b.w, p.o once daily for 15 days), Venlafaxine (8 and 32 mg/kg b.w, i.p single dose),

Imipramine (32 mg/kg), Fluoxetine (16 mg/kg b.w, i.p single dose) or distilled water ( 2ml/kg, p.o) and were administered 1 h prior to the beginning of swimming session in case of sumind and 0.5 h prior in case of standard drugs. The study was initiated 24 hrs after the pretest session which consisted of allowing the animals to swim for 15 min. At the end of this session animals were returned to their respective home cages after drying them in a heated enclosure. The rats were judged to be immobile when it is floated in an upright position, and made only small movements to keep its head above water. The duration of immobility was recorded during the last 5 min of the 6 min testing session.

## ESTIMATION OF BIOGENIC AMINES

There are various studies showing the alterations in biogenic amines following varying degrees of stress. Forced swimming was used to induce stress in rats. The method for stress was similar to FST above. Animals were divided into following groups each consisting of 8 rats, Normal control (vehicle, 2ml/kg, p.o), Stress control (Stress + vehicle 2 ml/kg. p.o), Venlafaxine HCL (32 mg/kg b.w i.p) single dose, sumind (275 and 367 mg/kg b.w. p.o) once daily for 5 days, sumind (275 and 367 mg/kg b.w p.o) once daily for 15 days. All rats were subjected to forced swimming for 25 min, 90 min after the administration of last dose. However in case of standard, Venlafaxine HCL which was administered 30 min prior to the FST. All the animals were decapitated after swimming session and the whole brain was dissected out, weighed and separated on an ice packing and homogenized in ice-cold buffer solution (pH 3.96). Then the homogenate was centrifuged at 4500 rpm for 30mins, supernatant separated and ultra filtered through a 0.22um filter paper. The clear ultrafilterate was used for quantification of DA, NA and 5HT by HPLC method with electrochemical detection [16].

## ESTIMATION OF CORTICOSTERONE

The excessive adrenocortical hormones have been implicated in the development of depression. Elevated Corticosterone and ACTH have been found in stress (Huda et al., 1995)[22]. The role of stress is well known in the pathogenesis of depression. Hence, forced swimming was used to induce stress in rats and the effect of only chronic treatment with sumind was observed. The method for stress was similar to FST above. Animals were divided into following groups each consisting of 8 rats, Normal control (vehicle, 2ml/kg, p.o), Stress control (Stress + vehicle 2 ml/kg. p.o), Venlafaxine HCL (32 mg/kg b.w i.p) single dose, sumind (275 and 367 mg/kg b.w p.o) once daily for 15 days. All rats were subjected to forced swimming for 25 min, 60 min after the administration of last dose of the respective treatment. However in case of standard, Venlafaxine HCL which was administered 30 min prior to the FST. At the end of swimming session blood was collected from carotid bleeding under mild ether anesthesia. Plasma was separated by centrifugation, the supernatant 1 ml of plasma was collected in separating funnel to which 25 ml of chloroform is added and shaken for 5 min and organic layer was collected in a beaker and this is repeated for 3 times. The pooled organic layers are evaporated on boiling water bath. The residue collected is dissolved in absolute methanol and the solution is filtered through 0.22 mm membrane filter and the filtrates were injected into the HPLC system for analysis of corticosterone. (Singh et al 2000)[23].

## STATISTICAL ANALYSIS

Results are represented as mean SEM. Data was analyzed using a statistical package (Graph pad prism version 3.00 to Windows, Graph pad software, San Diego, California, USA). Comparisons between various groups were made using a one-way analysis of variance (ANOVA) a post-hoc comparisons were performed using Tukey-multiple comparison, Kruskal-wallis and dunnett's multiple comparison tests. $P<0.05$ is considered as statistically significant.

## RESULTS

### EFFECT OF SUMIND ON ACUTE TOXICITY TEST IN MICE

Animals were observed for mortality for 48 hrs and the preparation was found to be safe upto a dose of 3000 mg/kg b.w.

### LOCO MOTOR ACTIVITY IN MICE

Sumind 180 mg/kg and 367 mg/kg and venlafaxine 32 mg/kg significantly increased the loco motor activity ($p<0.01$ and $p <0.05$ respectively) this suggests that sumind and Venlafaxine were psycho stimulant, no sedative effects were observed at any of the doses tested. Whereas in case of Imipramine HCl (32 mg/kg) significantly decrease the locomotor activity suggesting a sedative effect (table 1).

### Figure 1

Table 1: Effect on immobility time in FST in rats and TST in mice, locomotor activity in mice using photoactometer following treatment with SUMIND

| Treatment groups | Immobility time in FST ( sec) | | Immobility time in TST (sec) | Total number of counts in photoactometer |
|---|---|---|---|---|
| | Acute | Chronic | | |
| Control | 198.2±12.73 | 169.5 ± 10.06 | 230.7 ± 11.06 | 193.6 ± 13.27 |
| Venlafaxine HCl 8 mg | 158.2±10.48$^{ns}$ | ------- | 213.5±11.2$^{ns}$ | 288.1±29.66$^{ns}$ |
| Venlafaxine HCl 32 mg | 98.2±11.24*** | 34.21±5.76*** | 80.40±18.8*** | 288±14.92* |
| Imipramine 32 mg | 125.5±11.55*** | 91.88±11.43*** | 155.7±23.46* | 96.25±19.30* |
| sumind 92 mg/kg | 157.3±7.89 $^{ns}$ | 109.8 ± 13.58*** | 210.9 ± 15726 $^{ns}$ | 272.3 ± 32.82 $^{ns}$ |
| sumind 184mg/kg | 128.8±10.46*** | 67.88 ± 12.94*** | 159.6 ±12.5* | 357.1 ± 25.05*** |
| sumind 275mg/kg | 120.32±5.76*** | 90.75 ± 14.59*** | 160.4±7.3* | 320.25± 27.32* |
| sumind 367mg/kg | 98.8±14.46*** | 54.88 ± 8.21*** | 151.8 ± 18.40* | 289.8 ± 13.45* |

Results are expressed as mean ± SEM obtained from 10 animals.
ns- statistically non significant, *P<0.05, ***P<0.001 vs. normal control.

## EFFECT OF SUMIND ON IMMOBILITY TIME IN TAIL SUSPENSION TEST IN MICE

The behavioral score of immobility in control, standard drugs and sumind treated groups are shown in. Single dose administration of sumind with different dose range in mice showed dose dependent decrease in immobility time (sumind 184 mg/kg and 367 mg/kg, p<0.001) and the effect was qualitatively comparable to standard antidepressant drugs. However sumind 94 mg/kg did not show any significant effect. (table 1).

## EFFECT OF SUMIND ON IMMOBILITY TIME IN FORCED SWIMMING TEST IN RATS

The behavioral score of immobility in control, standard drgus and sumind treated groups are shown in (table1). Single dose administration of sumind with different dose range in rats showed dose dependent decrease in immobility time (sumind 184 mg/kg, p<0.001 and 367 mg/kg, p<0.001). In chronic administration, all the doses of sumind decreased total period of immobility (p<0.001) as compared to compared to vehicle treated control group. The effect was qualitatively comparable to standard antidepressant drugs.

## EFFECT OF SUMIND ON BIOGENIC AMINES IN RAT WHOLE BRAIN

The effect of swim stress, Venlafaxine HCl, sumind treatment on the levels of DA, NA and 5 HT in rat whole brain are shown in (table2). Sumind 275 mg/kg and 367 mg/kg shown that there is a significant increase of NA (p<0.05) DA (p<0.01) and incase of 5 HT (p<0.5 and p<0.001) level. Venlafaxine 32 mg/kg increases the all the neurotransmitter levels NA (p<0.05), DA (p<0.01) and 5HT (p<0.001) showing high statistical significant after single IP administration as compared with vehicle treated swim stress group.

### Figure 2

Table 2: Effect on swimming induced alterations in biogenic amines of rat brain following acute of 5 days and chronic treatment of 15 days with SUMIND

| Treatment period | Treatment groups | Biogenic amine in rat whole brain (ng/g of wet tissue) | | |
|---|---|---|---|---|
| | | Noradrenaline | Dopamine | 5-HT |
| Acute treatment | Normal control | 2419±281.1 | 2971±298.8 | 3957±427.0 |
| | Stress control | 2070 ± 270.4 | 2514±174.4 | 3419±362.6 |
| | Venlafaxine HCl | 2935±153.3* | 4051±166.9*** | 5937±436.5*** |
| | Sumind 275 mg/kg | 2842 ± 147* | 3579 ± 225.1** | 4980 ± 347.7* |
| | Sumind 367 mg/kg | 3033 ± 84.44** | 4224 ± 153.2*** | 5943 ± 279.5*** |
| Chronic treatment | Normal Control | 2419 ± 281.1 | 2971±298.8 | 3957±427.0 |
| | Stress control | 2070±270.4 | 2514±174.4 | 3419±362.6 |
| | Venlafaxine HCl | 2935±153.3* | 4051±166/9*** | 5937±436.5*** |
| | Sumind 275 mg/kg | 2842 ± 143* | 4039 ± 595* | 5071 ± 485.2* |
| | Sumind 367 mg/kg | 3139 ± 152.9* | 3824 ± 189.4* | 5832 ± 5832.6** |

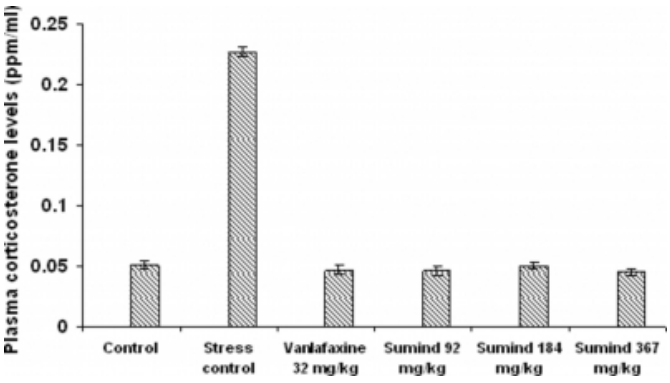
Results are expressed as mean ±SEM obtained from 8 animals.
ns- Statistically non significant, *P<0.05, **P<0.01, ***

## EFFECT OF SUMIND ON PLASMA CORTICOSTERONE IN RATS

The effect of swim stress, Venlafaxine HCl, sumind treatment on the plasma levels of Corticosterone rats is shown in (Fig.1). The stress increased significantly the Corticosterone levels in rats when compared to normal control. All the doses of sumind have decreased the stress induced Corticosterone levels significantly (P<0.001) which was comparable to standard.

### Figure 3

Figure 1: Effect on swimming induced alterations in plasma corticosterone levels following treatment with SUMIND




Results are expressed as mean SEM obtained from 8 animals. ns- Statistically non significant, ###P<0.001 vs. Normal control, ***P<0.001 vs. Stress control

## DISCUSSION

Major mood disorders are the most common mental illnesses with a life time morbid risk of perhaps 10% in the general population. As many as 10% to 15% of individuals with this disorder and upto 25% of those with bipolar disorder display suicidal behavior during their lifetime.

In the present study, the formulated polyherbal ayurvedic preparation SUMIND, consisting of eight Indian Medicinal plants, was evaluated for antidepressant activity.

Sumind was found to be safe in as no mortality was observed following treatment upto a dose of 3000 mg/kg. The increase in ambulatory behavior indicates a stimulant effect and the sumind has shown stimulant activity in a dose dependent manner [15]. This prompted us to study it further using other paradigms of depression like TST and FST. The immobility exhibited by test animals in these models is a indicative of a behavioral despair ness which reflects a state depressive state. Sumind has significantly reduced the immobility time in these paradigms following acute and chronic treatment. Thus it was proven to be a potential antidepressant. However depression is a complex disorder resulting from changes in central noradrenergic, serotonergic and dopaminergic systems or adrenomedullary system. Hence it was thought to be worthwhile to estimate all the three neurotransmitters in brain of rats depressed by forced swimming. Untreated controls have shown decreased levels of NA, 5-Ht and DA indicating a state of depression. On the other hand pretreated animals exhibited increased levels of these biogenic amines. In that sumind 275 mg/kg and 367 mg/kg produced a significant increase in NA, DA and 5 HT levels following both acute and chronic treatments. This effect was comparable to the standards used.

The various types of stressors induce hormonal alterations in experimental animals which are reminiscent of those observed in depressed patients. Forced swimming in rats is used as a swim stressor to study the action of antidepressant drugs on endocrine hormone systems. Our study showed that the swim stress for 20 minutes after chronic vehicle treatment causes increase in adrenocortical steroid hormone corticosterone in blood plasma when compared to the unstressed rats. The results of this study showed that on chronic treatment of SUNIND, significantly reduced the level of corticosterone in plasma ($P<0.001$) when compared to control group.

## ACKNOWLEDGEMENT

We are grateful to Prof. M.Laxman, Ex- Principal, Government College of Pharmacy and heartily thanks to Dr.T.R.raju Professor and H.O.D. of Neurophysiology Department Nimhans for the facilities provided estimation of Biogenic amines, for giving a highly valuable suggestion, moral support and advise for the research work. We express thanks to Anglo-French Drugs and Industries Limited for the research grant towards my project and supplying sumind for the study.

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