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BENEFICIAL EFFECT OF PLANT EXTRACT FROM *SESBANIA BISPINOSA* ON ENZYMATIC AND NONENZYMATIC ANTIOXIDANTS IN DEPRESSION INDUCED ANIMAL MODELS

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Abstract

Depression is one of the most common psychiatric illnesses that is a major cause of disability and public health problems. Oxidative stress plays a part in the pathogenesis of depression.Plant treated groups has been shown to decrease the stress and ameliorates depressive symptoms. The objective of the current study was to determine the activity of enzymatic (SOD, CAT, GPx) and nonenzymatic (Vitamin E) antioxidants in depression induced rats.Female Wistar rats weighing about 120-150 g were separated into 4 groups of 6 animals in each: Control group, Chronic Unpredictable Mild Stress (CUMS) induced depression group, Imipramine treated group and *Sesbania bispinosa*leaf extract treated group. The animals of CUMS groups were exposed to stress protocol for 2 weeks and the control group was maintained under normal conditions. Depression behaviour was confirmed by Forced Swim Test (FST). Depressed rats were made to swim for 15 minutes on the first day until 60 minutes on the last day for 28 days. Plant extract treatment given for 28 days showed marked improvement in the levels of enzymatic antioxidant *viz*., SOD, Catalase and GPx as well as in the levels of non-enzymatic antioxidants Vitamin E.

Keywords: Antioxidants, Depression, Imipramine, *Sesbania bispinosa*.

1. Introduction

Depression, a common incapacitating psychiatric condition, puts a major health burden on society [1]. According to the most accepted hypothesis of depression, the monoamine theory, patients with major depression have symptoms that are reflected with changes in brain monoamine neurotransmitter levels, specifically in nor-epenephrine and serotonin levels [2]. Depression is a leading cause of disability and death around the world. Despite substantial advancements in treatment, major depression remains a prevalent condition that causes severe morbidity and mortality [3].

Mood conditions are the world's second leading cause of disability-adjusted life years and the leading cause of years spent disabled in all age groups. Drugs used to treat this condition have a 60% success rate. In addition, several treatments need many weeks of therapy until signs and symptoms improve, and antidepressants have several side effects [4]. Medical plant therapies may be effective alternatives in the treatment of depression, and have progressed significantly in the past decade [5]. encouraging evidence has shown that exercise and physical activity have beneficial effects on depression symptoms that are often comparable to those of antidepressant treatments [6].

In the present study, the oxidative stress induced by immobilization stress was assessed using free radical scavenging enzymes such as Superoxide dismutase, Catalase, and Glutathione S Transferase, as well as non-enzymatic antioxidants (Vitamin E). The antioxidant potential of *S. bispinosa* leaves and its active constituents were also investigated on pre and post immobilization stress induced rats.

2. Materials and Method

2.1. Plant collection and authentication: The whole plant of *Sesbania bispinosa*were collected from the local areas (folklore shops) of Coimbatore district, Tamil Nadu, India. The plant was then dried at room temperature in the shade. The dried whole plant was submitted and authenticated (No.BSI/SRC/5/23/2014-15/Tech-1641) at Botanical Survey of India, Southern Regional Centre, Coimbatore, India.

2.2. Procurement of animals: Young female Wistar Albino rats of 120-150 g procured from Chettikulam, Nagercoil, India were used for the study. The Ethical authorization for handling of experimental animals were obtained from the Institutional Animal Ethics Committee (IAEC) constituted for the purpose and care of laboratory animals as per the supervision of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social justice and empowerment, Government of India (CPCSEA/No: 264/2015/IAEC).

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2.3. Grouping of animal

2.3.1. Experimental set up: The animals were acclimatized for 3 days and maintained under standard laboratory conditions with regulated temperature (29 ± 5°C), humidity (55 ± 5%) and 12 hours light/dark cycles throughout the experimental period given in Table 1.

Table 1: Experimental design for each screening method

Group I	Normal control
Group II	Depression induced by CUMS protocol
Group III	Depression induced+12.5mg/kg Imipramine treated
Group IV	Depression induced + 500 mg/kg of leaf extract treated

2.4. Induction of depression [7]**:** Depression was induced in albino rats using the CUMS protocol. A Forced Swim Test (FST) was carried out on the seventh day of induction to determine the status of depression.
Each stress regimen was carried out for two periods with the following stressors:

- Food deprivation for 24 hours
- Day-night reversal
- Soiled bedding (150ml water per cage) for 22hours
- Cage tilting (45 degree inclined) for 22hours
- Crowded housing (10 animals per cage)
- Exposure to novel odour (household air freshener)

2.5. Screening of antidepressant activity

2.5.1.Forced swim test (FST): The experimental group rats were placed in a cylindrical container of diameter 10 cm and height 25 cm with water level of 20 cm depth at 25°C for a total of 6 minutes. The first 2 minutes were ignored, but the time span during which the animals attempted to flee, which happened to be the final 4 minutes, was used to measure the animals' immobility inside the water container [7].

2.6.Collection of serum and tissues: After 30 days, the animals were sacrificed by cervical dislocation under mild chloroform anaesthesia. By cardiac puncture, blood was extracted and the serum was separated by centrifugation for 10 minutes at 5000 rpm. The brain was excised immediately and thoroughly washed in saline before use [8].

2.7.Preparation of tissue homogenate: A 0.1 M cold Tris-HCl buffer (pH 7.4) in a potter homogenizer fitted with a Teflon plunger operating at 600 rpm for 3 minutes, a 10% homogenate of the washed animal tissue was prepared. For different biochemical assays, prepared homogenate was then used [9].

2.8.Biochemical analysis

2.8.1. Determination of superoxide dismutase (SOD) activity: SOD activity was assayed by Beauchamp and Fridovich (1971). The mixture of reactions was 0.5 ml brain PMS, 1 ml 50 mM sodium carbonate, 0.4 ml 25 µM NBT and 0.2 ml 0.1 mM EDTA. The response was initiated by adding 1 mM of hydroxylamine-hydrochloride to 0.4 ml. At 560 nm, the absorbance change was reported. Simultaneously, the control was run without brain homogenate. SOD activity units were expressed as the amount of enzyme required to inhibit a 50 % reduction in NBT [10]**.**

2.8.2. Determination of catalase (CAT) activity: By the method of Claiborne (1985), CAT behaviour was assayed. 1.95 ml of phosphate buffer (0.05 M, pH 7.0), 1.0 ml of H_2O_2 (0.019 M), 0.05 ml of hepatic PMS (10 %, w / v) consisted of the assay mixture. Absorbance changes were registered at 240 nm. The CAT behaviour was measured in terms of nmol H_2O_2 / min / mg protein intake [11]**.**

2.8.3. Determination of glutathione peroxidase (GPX) activity: According to Mohandas et al.'s (1984), GPX activity was assessed. The reaction mixture consisted of 1.44 ml of 0.05 M phosphate buffer, pH 7.0, 0.1 ml of 1mM EDTA, 0.1mM of sodium azide, 0.05 ml of 1 U / ml of glutathione reductase, 0.10 ml of 1mM GSH, 0.1 ml of 2mM NADPH, 0.01 ml of 0.25mM H_2O_2 and 0.1 ml of 10% PMS for a total volume of 2 ml. At 25^{0}C the disappearance of NADPH at 340 nm was reported [12]**.**

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2.8.4. Determination of Ascorbic acid activity: Ascorbic acid (vitamin E) activity was estimated by the method of Sadasivam and Manickam, (1996). Ascorbic acid is dehydrogenated by bromination first. The dehydroascorbic acid is then reacted to form osazone with 2.4-dinitrophenyl hydrazine and dissolved in sulfuric acid to provide an orange-red solution estimated at 540 nm [13].

2.9. Statistical analysis: All values were expressed as Mean ± S.E.M. The findings were statistically evaluated by one-way ANOVA, finding $P < 0.05$ to be significant.

3. Results and Discussion

3.1. Forced Swim Test (FST)

This confirmatory test predicts the status of depression with the time of immobility. In the current study, 50% hydroethanolic extract of *S. bispinosa* produced significant antidepressant effect in albino rats in forced swim tests and its efficacy was found to be similar to standard drug imipramine. In forced swim test, albino rats are forced to swim in restricted space from which they cannot escape. This induces a condition of behavioural dejection in animals, which is claimed to replicate a condition similar to human depression. The results show that *S. bispinosa* extracts can decrease immobility time in forced swim test. After 30 days of treatment, it is found that the *S. bispinosa* produce a higher antidepressant activity of plant extract (Fig. 1). It has been previously suggested by Jawaid*et al.* [14] that an increase in both swimming and climbing behaviors in the FST.

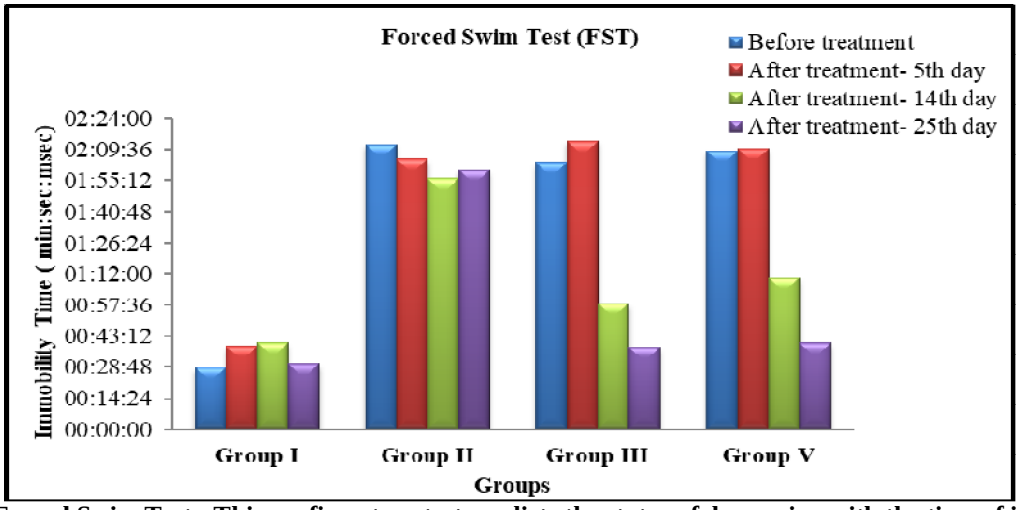

Figure 1: Forced Swim Test - This confirmatory test predicts the status of depression with the time of immobility

3.2. Biochemical analysis

3.2.1. Determination of superoxide dismutase (SOD)

In the present study, there was a significant decrease in SOD activity of depressed rats (group II) when compared to the normal rats (group I). During treatment, the significant values were found to be reversed. Group IV were treated with plant extract of *S. bispinosa* where it showed a significant increase in the levels of SOD in brain of rats when compared to that of depressed (group II) rats. In rats treated with standard drug (group III) imipramine showed increased activity of SOD in the brain which is close to the normal control (group I) are shown in Table 2.

Table 2: Effect of *Sesbania bispinosa*on SOD levels in the brain tissue

Groups	Condition	Concentration of SOD (Mg/Dl)
Group I	Normal Control	56.33±2.34
Group II	Depression induced	14±1.79 ^{a*}
Group III	Drug treated-Imipramine	55.67±1.51 ^{b*}
Group IV	Plant treated-High dose	55.17±1.72 ^{c*}

Values are expressed by mean±SD of six samples * Significant at P< 0.05

An earlier study also reported the similar results: Anxiolytic and antidepressant profile of the methanolic extract of *Piper nigrum*fruits. Treatment with this plant significantly increases the SOD levels [15]. In depressed groups, the decreased SOD activity

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may be due to the progress of highly reactive oxygen metabolites (ROMs). The decrease in Erythrocyte SOD levels was suggested to be a hallmark for depression [16].

3.2.2.Determination of catalase

The catalase activity was decreased in group II (depressed albino rats) when compared to the group I (normal albino rats). After treatment, activities were found to be reverted. plant extract of *S. bispinosa* treated group showed significant increase in the levels of catalase in a brain of rats when compared to that of group II. In rats treated with imipramine, the activity of catalase in the brain was increased which is close to the group I are shown in Table 3.

Table 3: Effect of *Sesbania bispinosa*on catalase levels in the brain tissue

Groups	Condition	Conc. Of Catalase (amount of hydrogen peroxide consumed/min/mg protein)
Group I	Normal Control	87.94±0.99
Group II	Depression induced	43.16±2.93 ^{a*}
Group III	Drug treated-Imipramine	85.53±1.45 ^{b*}
Group IV	Plant treated-High dose	80.21±0.98^{c*}

Values are expressed by mean±SD of six samples. * Significant at P< 0.05

A previous study also reported similar results. Treatment with *Citrus macroptera*significantly increased the catalase activity in rat brain tissue [17]. Antioxidant study in brain tissue of depressed rats revealed a decrease in catalase levels denoting that the effect of different stressor triggered free radical generation [18].

3.2.3.Determination of glutathione peroxidase (GPx)

The activity of GPx decreased in depression induced rats (group II) as compared to the normal control (group I). Significant increase in the activity of GPxwas noticed in experimental groups such as group III, group IV. Thus, the present study showed statistical significance as indicated in Table 4.

Table 4: Effect of *Sesbania bispinosa*on GPx levels in thebrain tissue

Groups	Condition	Concentration of GPx (µg of glutathione reduced/min/mg protein)
Group I	Normal Control	24.48±2.65
Group II	Depression induced	7.69±1.15 ^{a*}
Group III	Drug treated-Imipramine	21.67±0.87 ^{b*}
Group V	Plant treated-High dose	20.70±1.79 ^{c*}

Values are expressed by mean±SD of six samples. * Significant at P< 0.05

The similar study was accounted for by Leelavathiand Doss [18] using *Meliaazedarach*on depression induced rat brain tissue. Studies show that the activity of glutathione peroxidase (GPx) is reduced which could be due to its exhausted adoptive response to counter the effect of increased oxidative stress [19].

3.2.4.Determination of Ascorbic acid

Table 5 indicated that Ascorbic acid is significantly decreased in group II when compared to that of group I (normal control). In the plant treated group IV, the Ascorbic acid activity was significantly increased than the group II rats. In the group III (standard drug treated) rats the activity of Ascorbic in the brain was increased more than the normal rats.

Table 5: Effect of *Sesbania bispinosa*on Ascorbic acid levels in thebrain tissue

Groups	Condition	Concentration of Ascorbic acid (µg)
Group I	Normal Control	5.34±0.49

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Group II	Depression induced	1.78±0.37 [a*]
Group III	Drug treated-Imipramine	5.3±0.27 [b*]
Group IV	Plant extract treated	5±0.04 [c*]

Values are expressed by mean±SD of six samples. * Significant at P< 0.05

The Vitamin C is an anti-stress vitamin and may counter too much adrenaline. Vitamin C blocks the behavioural response to dopamine and enhances the effect of neuroleptic drugs. States of depression and anxiety are associated with psychiatric disorders which are attenuated by adequate intake of Vitamin C [20].

4.Conclusions

In this current study, depression was induced to an albino rats by CUMS protocol. The status of depression was assessed by the forced swim test which was a confirmatory test of depression. In Group II, the level of all antioxidants such as SOD, Catalase, GPx and Ascorbic acid shows a low enzymic and nonenzymatic antioxidant activity when compared to normal rats. These levels were significantly increased when treated with 50% hydroethanolic leaf extract of *S. bispinosa*and antidepressant drug Imipramine. Thus, it can be concluded that the plant *Sesbania bispinosa*has potent antidepressant activity.

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