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EVALUATION OF ANTIDEPRESSANT ACTIVITY OF SPIRULINA BY USING EXPERIMENTALLY INDUCED DEPRESSED ANIMALS

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ABSTRACT

*To investigate the antidepressant activity**of** the spray dried powder of Spirulinaplantensis which was used for the treatment of depression in different doses and Imipramine is used as standard drug in experimental animals. The evaluation is done by conductingthe followingtests like5-HT induced head twitches, forced swim test, Tail suspension test, Clonidine induced aggression behaviour, L-dopa induced hyper activity and aggressive behavior. The animal models used for conducting the tests are albino mice (20-30 g) of either sex. From the results of all the experimental models it was observed that Spirulina is showing the antidepressant activity which was dose dependent.*

Key words: *Spirulina, Depression, Imipramine, 5-HT, Clonidine, L-dopa.*

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INTRODUCTION

The monoamine biochemical theory of depression is the monoamine hypothesis, which states that depression caused by a functional deficit of monoamine transmitters at certain sites in the brain, while mania results from functional excess.[1-2] Mood disorders are among the most prevalent forms of mental illness. Severe forms of depression affect 2%–5% of the U.S. population, and up to 20% of the populations suffer from milder forms of the illness. Depression is almost twice as common in females as males. Another roughly 1%–2% are afflicted by bipolar disorder (also known as manic-depressive illness), which affects females and males equally. Mood disorders are recurrent, life threatening (due to the risk for suicide), and a major cause of morbidity worldwide [3].

Human studiesas well as animal studies have suggested that major depression produced by CMS is associated with elevated lipid peroxidation levels [4]. On the other hand, it has been reported that increased ROS production may cause the destruction of phospholipids and altered viscosity of neuron membranes, and consequently the changes in membrane viscosity may affect serotonergic and catecholaminergic receptor functions. In addition, MDA directly exerts inhibitory effect on serotonin binding areas on the receptor. An intricate relationship exists between serotonin metabolism and oxidative stress.

The effect of increasing monoamine levels (dopamine, 5-HT and NE) on BDNF and growth factors may be one mechanism that produces the antidepressant response. The antidepressant effects such as the naphthodianthrone hypericin, the phloroglucinol derivative hyperforin [5], and the flavonoids hyperoside, miquelianin, and isoquercitrin. Furthermore,flavonoids as well as hypericin had an effect on HPA axis function and related gene expression in both stressed and unstressed animals after repeated administration.

The barks of Magnolia *officinalis* have been used in traditional Chinese medicine to treat a variety of mental disorders including depression [6]. These results suggested that the mixture of honokiol and magnolol possessed potent antidepressant-like properties in behaviors involving the normalization of biochemical abnormalities in the serotonergic system in rats[7]. In animal models of depression rely on one of two principles: actions of known antidepressants or responses to stress [8, 9]. these behavioral tests have not normally been utilized in depression research and may offer new insights into the neurobiological mechanisms involved [10].

Consequently, CRH hyper function, as well as monoamine hypo function, may be associated with depression [11].

MATERIALS AND METHODS

Plant materials

The spray dried powder of *Spirulina* whichis used in this experiment was obtained from Nihal traders, Hyderabad.

Experimental animals

Wistar strain albino rats (150-200 g) and albino mice (20-30 g) of either sex were obtained from Albino labs,Hyderabad with**CPCSEA/IAEC/EXP/25/50/2014/EXP-2014**. The animals were housed in polypropylene cages at an ambient temperature of 25 °C± 1°C and 45–55% RH, with a 12:12 h light/dark cycle. The animals had free access to commercial food pellets and water *ad libitum* unless stated otherwise. Animals were acclimatized for at least one week before using them for experiments and exposed only once to every experiment. The care and maintenance of the animals were carried out as per the approved guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), the research protocols were approved by the Institutional Animal Ethical Committee (IAEC).

ACUTE TOXICITY STUDY

Acute toxicity study – up and down procedure – was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD). If animal dies at particular dose, lower dose was given to next animal and if animal survives at a particular dose next higher dose was given for remaining animals. The maximum upper limit dose 2000 mg/kg of *SP* was administered orally to mice. Animals were observed individually after dosing. Observation included mortality and clinical signs, such as changes in skin fur, eyes and mucous membranes.

GROSS BEHAVIOUR IN RATS

The gross behaviors like body position, locomotion, rearing, tremors and gait was observed. The effect of *Spirulina*on passivity, grip strength, pain response, stereotypy, vocalization, righting reflex, and body weight and water intake was assessed [12]. Pilot study was carried out with various doses (50, 100, 200 and 400 mg/kg, per oral route to rats) of *SP*. At doses of 100, 200 and 400 mg/kg, it was active and at 50 mg/kg it was inactive. Based on this observations three different doses (100, 200 and 400 mg/kg) of *SP* were selected in the present studies.

TREATMENT SCHEDULE

Animals were divided into five groups containing six animals each except in antioxidant studies, where six groups of animals (n= 6) were taken. The animals were pretreated orally with 0.3% Carboxymethylcellulose suspension of *SP* for 7 days daily at the doses of 100, 200 or 400 mg/kg/day. All the experimental procedures were started on day 7, 1 h after the drug administration. In case of FST (8 days) the drug administration was continued till the end of the experimental schedule. Control (I group) rats received the vehicle (0.3% CMC suspension), Group II, III and IV rats received the *SP* at the doses of 100, 200 and 400 mg/kg/day, p.o. respectively and group V rats received either IMP (15mg/kg, i.p.) or Lorazepam (2.5 mg/kg, i.p.).

***IN VIVO* MODELS EMPLOYED IN THE STUDY**

Forced swimming test (FST)

The procedure [13] swimming sessions were conducted by placing rats in individual glass cylinders (45 cm high×20 cm in diameter) containing (25±2 °C) water 38 cm deep, so rats could not support themselves by touching the bottom with their feet. Two swimming sessions were performed between 12:00 h and 19:00 h, an initial 15 min pretest followed 24 h later by a 6 min test. Rats were divided into 5 groups (n=6)

Group-1 : vehicle (0.3% CMC)
Group-2 : Spirulina (100 mg/kg, p.o.)
Group-3 : Spirulina (200 mg/kg, p.o.)
Group-4 : Spirulina (400 mg/kg, p.o.)
Group-5 : Imipramine (15 mg/kg, i.p.)

Doses were given once daily for 7 days. On the 7th day rats were subjected to 15 min. After 15 min, in the water the rats were removed and allowed to dry in a heated enclosure (32 °C) before being returned to their home cages. They were again placed in the cylinder 24 h later and the total duration of immobility was measured during a 6 min test. Floating behavior during this 6 min period had been found to be reproducible in different groups of rats. The animal was judged to be immobile whenever it remains floating in water in a slightly hunched but upright position, its nose just above the surface. The total immobility time for the period of 6 min was recorded with the help of stopwatch.

5-HTP induced head twitches in mice

Procedure: Mice divided into 5 groups (n=6)

Group-1 :vehicle (0.3% CMC)
Group-2 :Spirulina (100 mg/kg, p.o.)
Group-3 : Spirulina (200 mg/kg, p.o.)
Group-4 : Spirulina (400 mg/kg, p.o.)
Group-5 : Imipramine (15 mg/kg, i.p.)

Doses were given once daily for 7 days. On the 7th day, 1hr after the administration of the test and standard drugs, mice were treated with 5-HTP (100 mg/kg i.p.) and the numbers of head twitches performed by each mice was counted by staggering method using three 2 min periods (19–21 min), (23–25 min), (27– 29 min) after 5-HTP administration and number of head twitches were scored live by a blind observer.

Clonidine-induced aggression in mice

Mice were divided into 5 groups of 8 each (n=8), each group contain 4 pairs of mice, two pairs from each sex (each pair contained same sex of mice).

Group-1 : vehicle (0.3% CMC)
Group-2 : Spirulina (100 mg/kg, p.o.)
Group-3 : Spirulina (200 mg/kg, p.o.)
Group-4 : Spirulina (400 mg/kg, p.o.)
Group-5 : Lorazepam (2 mg/kg, i.p.)

Doses were given once daily for 7 days. On the 7th day, Clonidine was given 1 h after the administration of the test and standard drugs. The animals were then caged in bell shaped glass jar with a floor area of approximate 16 cm^2. The biting/fighting episodes were recorded live by a blind observer over a period of 30 min, in each pair.

Tail suspension test (TST)

Mice were divided into 5 groups (n=6)

Group-1 : vehicle (0.3% CMC)
Group-2 : Spirulina (100 mg/kg, p.o.)
Group-3 : Spirulina (200 mg/kg, p.o.)
Group-4 : Spirulina (400 mg/kg, p.o.)
Group-5 : Imipramine (15 mg/kg, i.p.)

Doses are given once daily for 7 days. On the 7th day, 1hr after the administration of the test and standard drugs, mice were suspended on the edge of a table 50 cm above the floor by the adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6 min period .Animal was considered to be immobile when it did not show any movement of body and hanged passively.

L-DOPA induced hyper activity and aggressive behavior in mice (LHA)

Mice were treated with L-DOPA (100 mg/kg i.p.) and were divided into 5 groups of 8 each (n=8), each group contain 4 pairs of mice, two pairs from each sex (each pair contained same sex of mice).

Group-1 : vehicle (0.3% CMC)
Group-2 : Spirulina (100 mg/kg, p.o.)
Group-3 : Spirulina (200 mg/kg, p.o.)
Group-4 : Spirulina (400 mg/kg, p.o.)
Group-5 : Lorazepam (2 mg/kg, i.p.)

Doses were given once daily for 7 days. On the 7th day, L-DOPA was given 1 h after the administration of the test and standard drugs, Stages of activity and aggressive behavior were recorded live every 10 min for 30 min after L-DOPA administration by the blind observer. The different parameters of observation were piloerection, salivation, increase in motor activity, irritability, reactivity, jumping squeaking, and aggressive fighting.

The scores were graded in the following manner:
0-No effect;
1-Piloerection, slight salivation, slight increase in motor activity;
2-Piloerection, salivation, marked increase in motor activity and irritability;
3-Piloerection, profuse salivation, marked increase in motor activity, reactivity, jumping, squeaking and aggressive fighting.

STATISTICAL ANALYSIS

Results were expressed as mean ± S.E.M. Statistical analysis was performed using one-way analysis of variance (ANOVA). If the overall *P*-value was found statistically significant ($P < 0.05$), further comparisons among groups were made according to Newman Keuls test.

RESULTS AND DISCUSSION

Forced Swim Test (FST) results showed that both *SP* (100, 200 and 400 mg/kg, p.o.) and imipramine (15 mg/kg, i.p.) significantly decreased the duration of immobility time in a dose dependent manner in FST model. Post-hoc analysis showed that the *SP* (100, 200 and 400 mg/kg) and Imipramine (IMP) treated groups were significantly different (p<0.001) from the vehicle treated group.

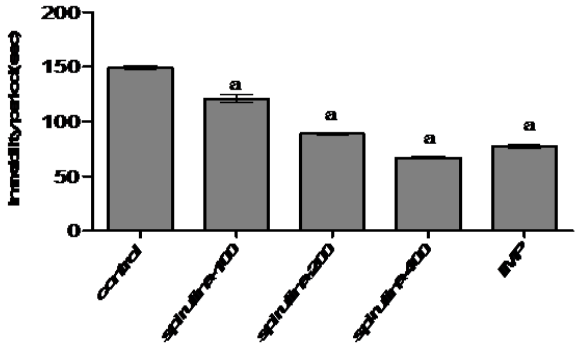

Figure 1. Effect of *SP* on forced swim test (FST), the values were Mean ± S.E.M. (n = 6). a = p<0.001 compared to control

Tail Suspension Test (TST) results showed that both *SP* (100,200,400 mg/kg, p.o.) and imipramine (15 mg/kg, i.p.) significantly decreased the duration of immobility time in a dose dependent manner in TST model. Post-hoc analysis showed that the *SP* (100, 200 and 400 mg/kg) and IMP treated groups were significantly different (p<0.001) from the vehicle treated group.

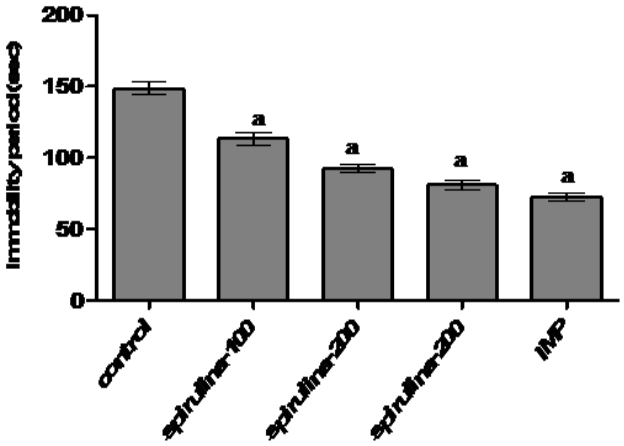

Figure. 2. Effect of *SP* on tail suspension test (TST) in mice, the values were Mean ± S.E.M. (n = 6). a = p<0.001 compared to control.

5-HTP induced head twitches in mice were represented the effect of *SP* and IMP on 5-HTP-induced head twitches in mice. Post-hoc analysis revealed that three doses of *SP* (100, 200 and 400 mg/kg, p<0.01, p<0.001) significantly increased the 5-HTP-induced head twitches in comparison to control group. Further, the dose of 400 mg/kg was more effective than 100, 200 mg/kg. Similarly, IMP treated group showed significant increase (p<0.001) in the 5-HTP-induced head twitches compared to control. However, the effect of 400 mg/kg of *SP* was significantly higher than IMP (p<0.001).

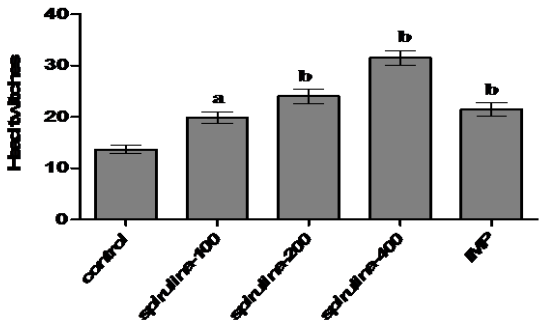

Figure. 3. Effect of *SP* on 5-HTP-induced head twitches in mice, the values were Mean ± S.E.M. (n = 6). a = p<0.001 compared to control.

L-DOPA induced hyperactivity and aggressive behavior in mice results were reveals the effect of *SP* and lorazepam on L-DOPA-induced hyperactivity and aggressive behavior is shown in Table 1.

Table–1: Effect of *SP* and lorazepam on L-DOPA-induced hyperactivity and aggressive behavior in mice.

Group no.	Treatment (dose in mg/kg)	Behavioral score(MEAN ± SEM)
I	Control (0.3% CMC)	1
II	Spirulina (100 mg/kg, p.o.)	2.29 ± 0.152[a]
II	Spirulina (200 mg/kg, p.o.)	2.38 ± 0.129[a]
IV	Spirulina (400 mg/kg, p.o.)	2.77 ± 0.152[a]
V	Lorazepam (2.5 mg/kg, i.p.)	2.28 ± .148[a]

The values were expressed Mean ± S.E.M. (n = 6). a = p < 0.001 compared to induced group.

Post-hoc analysis revealed that three doses of *SP* (100,200 and 400 mg/kg, p<0.001) significantly increased the L-DOPA-induced hyperactivity and aggressive behavior (LHA) in comparison to control group.

Table – 2: Effect of *SP* and lorazepam on clonidine induced aggression in mice.

Group no.	Treatment (dose in mg/kg)	MEAN ± SEM	
		Latency to 1st attack	No. of bouts
I	Control (0.3% CMC)	340.97 ± 11.4	22.05 ± 1.27
II	Spirulina (100 mg/kg, p.o.)	373.49 ± 4.502 a	18.07 ± 0.654 a
III	Spirulina (200 mg/kg, p.o.)	395.37 ± 1.109 b	16.53 ± 0.654 b
IV	Spirulina (400 mg/kg, p.o.)	476.32 ± 8.04 b	11.95 ± 1.109 b
V	Lorazepam (2.5 mg/kg, i.p.)	499.13 ± 4.126 b	12.06 ± .653 b

The values were expressed Mean ± S.E.M. (n = 6). a = p < 0.001 compared to induced group.

Results were indicates the effect of *SP* (100, 200 and 400 mg/kg, p.o.) and lorazepam (LA; 2.5 mg/kg) on the latency to first attack and the number of bouts in the clonidine induced aggressive behavior in mice. Post-hoc analysis showed that *SP* (p<0.001) significantly increased the latency to first attack and decrease the no. of bouts compared to control.

Indicates the effect of *SP* (100, 200 and 400 mg/kg, p.o.) and lorazepam (LA; 2.5 mg/kg) on the latency to first attack and the number of bouts in the clonidine induced fighting behavior in mice. Post-hoc analysis showed that *SP* (p<0.001) significantly increased the latency to first attack and decrease the fighting responses compared to control.

Table – 3: Effect of *SP* and lorazepam on clonidine induced fighting response in mice.

Group no.	Treatment (dose in mg/kg)	MEAN ± SEM	
		Latency to 1st attack	No. of bouts
I	Control (0.3% CMC)	99 ± 12.2	99 ± 1.24
II	Spirulina (100 mg/kg, p.o.)	107.8 ± 4.527 [a]	80.672 ±0.721 [a]
III	Spirulina (200 mg/kg, p.o.)	113.61 ± 1.149 b	71.17± 0.652 b
IV	Spirulina (400 mg/kg, p.o.)	140.16 ± 7.29 b	57.976 ± 1.114 b
V	Lorazepam (2.5 mg/kg, i.p.)	146.97 ± 4.217 b	54.57± 0.653 b

The values were expressed Mean ± S.E.M. (n = 6). a = p<0.001 compared to induced group

CONCLSION

The results from the present study shows the antidepressant activity of *Spirulina,*since it reduces the immobility in FST and TST. In the study, *Spirulina* increased the frequency of head twitches and Clonidine induced aggression and alsohyperactivity which is induced by L-DOPA. The aggressive behavior indicates that it enhanced activity of serotonergic, noradrenergic and dopaminergic pathways respectively. The results also confirmed that the serotonergic, noradrenergic and dopaminergic pathways are involved in depression. Pretreatment of the animal with *Spirulina* increased the levels of SOD and Catalase with simultaneous decrease in LPO levels in the rat brain, which shows its strong antioxidant activity. Since oxidative stress is reported to play a major role in depression, the antioxidant activity

of *Spirulina*might be a part of the mechanism for its antidepressant activity.

Results from the experiment indicate that the antidepressant activity of *Spirulina platensis* may be due to the facilitatory effect on serotonergic, noradrenergic and dopaminergic systems apart from the antioxidant activity.

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