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ASSESSMENT OF ANXIOLYTIC POTENTIAL OF *SPIRULINA PLATENSIS*

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ABSTRACT: *Spirulina platensis*, planktonic blue-green algae, has been used as a source of protein (60-70%) and vitamin supplement for more than 15 years without any unknown adverse events. Traditionally, *Spirulina platensis* has been used as an antioxidant agent. However, no pharmacological work has been done to evaluate the anti-anxiety activity. The present study was premeditated for the evaluation of the anti-anxiety activity of *Spirulina platensis.* Elevated plus maze (EPM) and light-dark models were used to evaluate the anti-anxiety potential of *Spirulina platensis* in Swiss albino mice. Albino mice were treated orally with diverse doses of the *Spirulina platensis* (*i.e.*, 300 and 400 mg/kg). Diazepam (2 mg/kg, IP) was used as a standard drug. In conclusion, 400 mg/kg dose of *Spirulina platensis* showed significant anti-anxiety activity ($P<0.05$) while the control group was near to standard group. It concludes that *Spirulina platensis* could be useful as an anti-anxiety agent for prospects.

INTRODUCTION: Anxiety is an exaggerated feeling of apprehension, uncertainty, and fear. It is an unpleasant state of tension with anticipation of imminent danger [1]. Anxiety affects most of the population nearly one-eighth of the total population worldwide. Benzodiazepines are being a major class of compounds used for the treatment of anxiety. However, the clinical uses of benzodiazepines are limited by their side effects such as psychomotor impairment, potentiating of other central depressant drugs, and dependence liability.

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It has prompted researchers to investigate alternative medicine having less undesirable effects [2]. Spirulina also called arthospira, which is a microscopic and filamentous cyanobacterium (blue-green algae) that has a long history of use as food. *Spirulina* contains 50-70% protein by weight and is a rich source of vitamins especially vitamin B_{12} (β- carotene provitamin A), vitamin E. It also contains carbohydrates like- rhamnose, fructose, ribose, mannose and some minerals like copper, magnesium, zinc, potassium, and iron [3].

Several studies have shown the ability of *Spirulina* or its extracts as a potent anti-viral, [4-6] anti-cancer, [7, 8] hypocholesterolemic, hypolipidemic, [9] anti-diabetic [10], and health improvement agent. *Spirulina platensis* is gaining attention as a nutraceutical and a source of potential pharmaceutical [11]. *Spirulina platensis* also contains phycobilisomes as light-harvesting protein-pigment complexes.

Phycobilisomes are mainly (80–85%) composed of brilliantly colored polypeptides named phycobiliproteins. The more important biliproteins in these microalgae are phycocyanin, allophycocyanin and phycoerythrin having the same chromophoric group.[12] Therefore, the present study was designed to evaluate the anti anxiety activity of *Spirulina Platensis* using the EPM and dark and light model, and exteroceptive behavior on animal model.

MATERIALS AND METHODS:

Animals: Swiss albino mice of both sexes weighing 20–30 gm were used for the study. The animals were housed in groups of six under standard laboratory conditions of temperature (25 ± 2 ºC), lighting (0800–2000 h), and relative humidity (50 ± 5%), with food and water freely available. All experiments were carried out during the light period (0800–1600 h). The institutional animal ethical committee approved the protocol of the study.

All the procedures were performed by the institutional ethical committee constituted as per the directions of the committee for control and supervision of experiments on animals (CPCSEA No. 1283/c/09) under the ministry of animal welfare division, the government of India, New Delhi.

Drugs and Chemicals: Spirulina in the form of spray dried powder (M/S Parry Neutraceuticals, Chennai, India), Diazepam (Calmpose, Ranbaxy Laboratories, India), Sodium carboxymethyl cellulose (Loba Chemie, Mumbai, India).

Administration of the Extracts: Oral suspensions of the *Spirulina platensis* were prepared in distilled water using 0.3% w/v sodium carboxymethyl cellulose (sodium CMC) as a suspending agent. In brief, the weighed the amount of sodium CMC was solubilized distilled water with trituration. Subsequently, *Spirulina platensis* powder was added into an above slurry with mixing to get a uniform suspension. The oral suspension was administered in a dose of 300 and 400 mg/kg to mice by oral route, 60 min before the test procedures. Control groups were given only the vehicle (0.3% w/v Na CMC vehicle suspension) in volume equivalent to that of the oral suspension and drugs.

Assessment of Anti-Anxiety Activity:

Elevated Plus Maze Test: The elevated plus-maze consists of two open (16×5 cm) and two closed arms (16×5×12 cm), an open roof arranged in such a manner that the two open arms were opposite to each other. The maze is elevated to a height of 25 CM mice were divided into four groups of six each.

Group 1 animals received vehicle (0.3% Na CMC in saline),

Group 2 and **Group 3** animals received Spirulina dose (300 mg/kg P.O. and 400 mg/kg P.O.), and Group 4 animals received standard diazepam (2mg/kg I.P.). The test drugs were administered 60 minutes before the experiment by oral route, and the standard drug was administered 30 minutes before the experiment by I.P. route. The mice were then placed in the center of the maze facing one of the open arms.

During the next 5 minutes, the number of entries in the open arm and the time spent in the open arm were recorded. The experiment was conducted in a sound-proof room[13].

Light-Dark Model: The apparatus consisted of a rectangular wood box (46×27×30 cm), divided into one small (18×27cm) and one large (27×27cm) areas, with a door-like opening (7.5×7.5) in the center of separation. The small compartment was painted in black and light-free, whereas the large one was white.

Each animal was individually placed in the center of the bright compartment (facing away from the door), and the following parameters were noted for 5 min: time spent in the light compartment and the number of crossings between the light and dark compartments.

The test was performed in a quiet and darkened room (red bulb), and mice were kept in this room for at least 1 h before the session.[14]

Statistical Analysis: The results are given as mean ± S.E.M. The data obtained were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni's test. Differences were considered significant at the 5% level.

RESULTS

Elevated Plus Maze (EPM) Model: The two doses of *Spirulina platensis* (300 mg/kg & 400mg/kg) were administered by oral route 60 min before placing in the apparatus. The mice treated with dose (400 mg/kg) of *Spirulina Platensis*, were spent 144.35 ± 2.62 seconds (s) in the open arm **Fig. 1a** and **Table 1** and a number of the entry in the open arm was found 7.5± 0.6 **Fig. 1b** and **Table 1**. The 400 mg/kg doses of *Spirulina Platensis* having a significant effect (P<0.05) in term of time spent in open chamber and number of entries in the open chamber when compared of the vehicle control group.

TABLE 1: ANXIOLYTIC ACTIVITY OF *SPIRULINA PLATENSIS* USING ELEVATED PLUS MAZE MODEL IN MICE

S. no.	Treatment	No. of entries in open arm (Mean ± SEM)	Time spent in open arm (sec.) (Mean ±SEM)
1	Vehicle (0.3% Na CMC in saline)	4.28 ± 0.45	90.34 ± 4.73
2	Spirulina 300 mg/kg P.O.	6.16 ± 0.47*	120.68 ± 4.18*
3	Spirulina 400 mg/kg P.O.	7.5 ± 0.66**	144.35 ± 2.62**
4	Diazepam 2 mg/kg I.P.	11.16 ± 0.94*	189.3 ± 4.14*

one way ANOVA followed by Bonferroni's multiple comparison tests; * Significant variation as compared to vehicle control treated group (P<0.05); ** Significant variation as compared to standard drug-treated group (P<0.05)

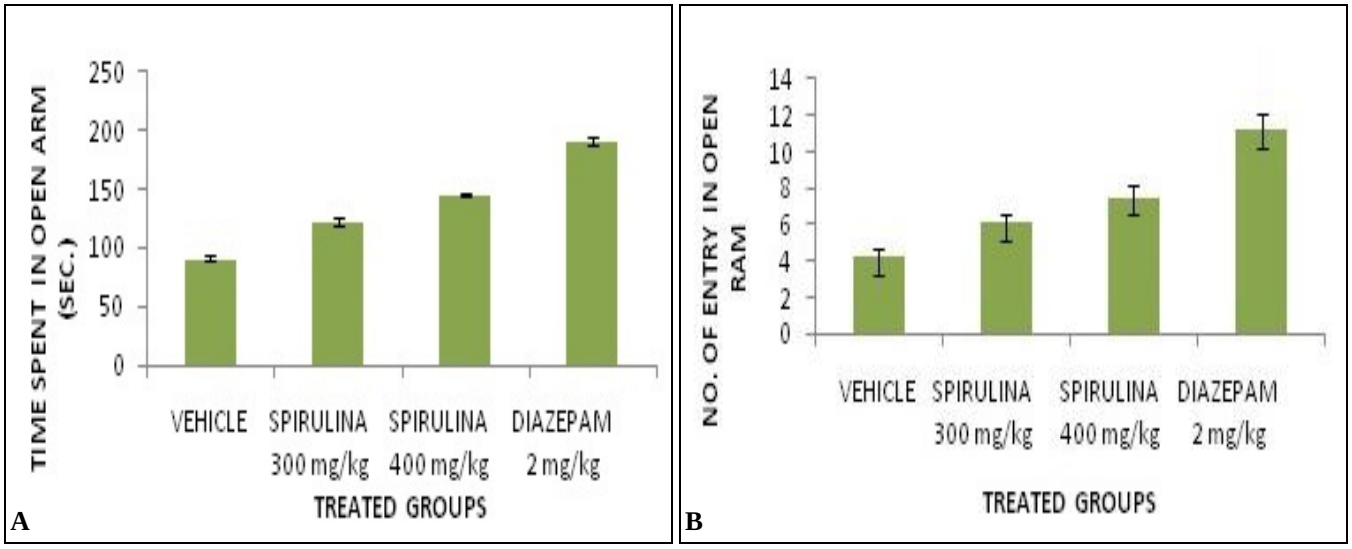

FIG. 1: (A) EFFECT OF SPIRULINA PLATENSIS ON TIME SPENT IN OPEN ARM (B) EFFECT OF *SPIRULINA PLATENSIS* ON NUMBER OF ENTRY IN OPEN ARM

One way ANOVA followed by Bonferroni's multiple comparison tests
Significant variation as compared to vehicle control treated group (P<0.05)
Significant variation as compared to standard drug-treated group (P<0.05)

Light and Dark Model: The two doses of *Spirulina platensis* (300 mg/kg & 400 mg/kg) were administered by oral route 60 min before placing in the apparatus. Mice treated with 400 mg/kg dose of *Spirulina Platensis* were spent 59.9 ± 1.11 s in open chamber **Fig. 2b** and **Table 2**. Moreover, 5.16 ± 0.41 number of entries were counted in the open chamber **Fig. 2b** and **Table 2**. The 400 mg/kg doses of *Spirulina platensis* having a significant effect (P<0.05) in term of time spent in open chamber and number of entries in the open chamber when compared of the vehicle control group.

TABLE 2: ANXIOLYTIC ACTIVITY OF *SPIRULINA PLATENSIS* USING LIGHT AND DARK MODEL IN MICE

S. no.	Treatment	No. of entries in the light chamber (Mean ± SEM)	Time spent in the light chamber (sec.) (Mean ± SEM)
1	Vehicle (0.3% Na CMC in Saline)	2.75 ± 0.47	28.09 ± 1.67
2	Spirulina 300 mg/kg P.O.	3.76 ± 0.42*	40.14 ± 2.53*
3	Spirulina 400 mg/kg P.O.	3.83 ± 0.53**	47.91 ± 1.50**
4	Diazepam 2 mg/kg I.P.	5.16 ± 0.47*	59.9 ± 1.11*

One way ANOVA followed by Bonferroni's multiple comparison tests
* Significant variation as compared to vehicle control treated group (P<0.05)
**Significant variation as compared to standard drug-treated group (P<0.05)

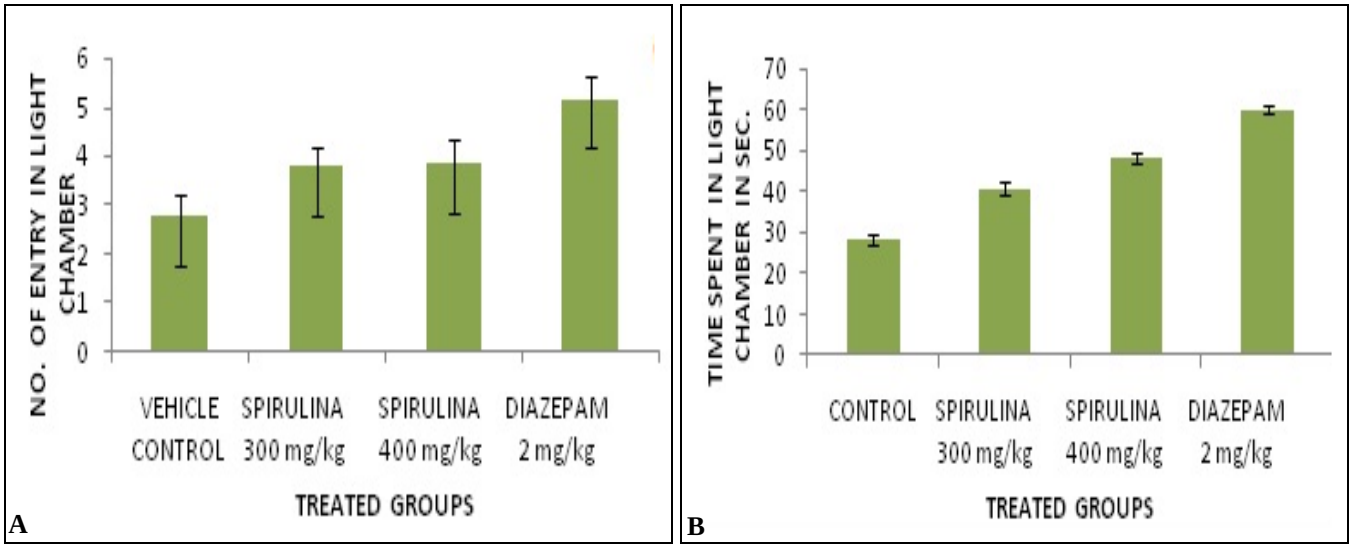

FIG. 2: (A) EFFECT OF SPIRULINA PLATENSIS ON NUMBER OF ENTRY IN LIGHT CHAMBER (B) EFFECT OF *SPIRULINA PLATENSIS* ON TIME SPENT IN LIGHT CHAMBER

One way ANOVA followed by Bonferroni's multiple comparison tests
Significant variation as compared to vehicle control treated group (P<0.05)
Significant variation as compared to standard drug-treated group (P<0.05)

DISCUSSION: The EPM test is based on a premise where the exposure to an EPM evoked an approach-avoidance conflict that was considerably stronger than that evoked by the exposure to an enclosed arm. The decrease in aversion to the open arm is the result of an anxiolytic effect, expressed by the increased time spent and entries in the open arm, [15] while the fear due to height induces anxiety in the animals when placed on the EPM.

The ultimate manifestation of anxiety and fear in the animals is exhibited by a decrease in the motor activity and preference to remain at safer places. Anxiolytic agents are expected to increase the motor activity, which is measured by the time spent by the animal in the open arms [16].

The dose of *Spirulina Platensis* (400mg/kg) significantly increased (P<0.05) the percentage of average time spent by the animals in the open arms. Anxiolytic-like- activity was also observed in a light/dark box. It is an ethological based approach-avoidance conflict test. The number of transition between the light and dark compartment as well as the time spent in the light side is recognized as anxiety indices. It despite the transition parameter being highly dependent on locomotors activity [17].

The dose of *Spirulina Platensis* (400mg/kg) significantly increased (P<0.05) the percentage of average time spent by the animals in the open chamber. The earlier reports on the chemical composition of *Spirulina Platensis* suggested that *Spirulina Platensis* containing important biliproteins (phycocyanin, allophycocyanin, and Phycoerythrin). It acted as a potent anti oxidant agent. To explore the mechanism mediating the anxiolytic properties of *Spirulina Platensis*, phycocyanin is interaction with the $GABA_A$ receptor. $GABA_A$ receptor may modulate by phycocyanin.

However, the mechanism of interaction of phycocyanin with $GABA_A$ receptor remains unknown. Conclusively, phycocyanin acts on the anxiety by its antioxidant potential. The results obtained in this study suggest that *Spirulina Platensis* possesses anxiolytic properties.

CONCLUSION: From the experimental study, *Spirulina Platensis* was exhibited significant anti-anxiety activity as evidenced by EPM and Light and Dark model. In the present study, the dose of *spirulina platensis* (400 mg/kg) showed significant anti-anxiety activity with the comparison of the standard drug (2mg/kg). Finally, it is concluded that *Spirulina Platensis* showed significant anti-anxiety activity. Thus, *Spirulina Platensis* has potential clinical application in the management of anxiety disorder.

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CONFLICT OF INTEREST: Nil

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