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SPIRULINA PLATENSIS AT TENUATES STRESS-INDUCED PHYSICAL AND BIOCHEMICAL CHANGES IN RATS

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ABSTRACT: The aim of present study on *Spirulina platensis* (Spirulina) was to evaluate the anti-stress activity. The anti-stress activity was assessed by tail suspension method andcold immobilization induced change of biochemical parameters. Acute toxicity (LD_{50}) of *Spirulina platensis* values was also studied. Duration of immobilization time and cold immobilization induced alteration of biochemical parameters (liver functioning profiles like- AST, ALT, ALP and liver tissue anti-oxidant profiles like- MDA, SOD, GST) were used as a stress indicator. *Spirulina platensis* administered orally at two different doses of 200 and 400 mg/kg respectively. Diazepam, a well-known anti- stress drug (2 mg/kg i.p.) was taken as standard drug. The dose of 200 and 400 mg/kg of *Spirulina Platensis* significantly decreased the duration of immobilization time and also provide protection against stress induced alteration of biochemical parameters. It is concluded that the 200 and 400 mg/kg dose of *Spirulina platensis* have anti-stress activity.

INTRODUCTION: Stress may be defined as nonspecific response of the body which directly affects the normal physiological balance resulting in threatened homeostasis [1]. Industrialization, civilization and modern lifestyle are the main roots of stress which have been detected worldwide. It is reported that Stress involved in the patho-physiologically of a various range of illness, ranging from neurodegenerative disorders such as parkinsonism, depression, alzheimer and anxiety and they cause other type of disorders such as Rheumatoid arththritis, Male sexual dysfunction, Myocardial infarction, Peptic ulcer, diabetes mellitus.

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The stress indicates in the prominent metabolic rate which could increase synthesis of reactive oxygen species like- hydrogen peroxide (H_2O_2), hydroxyl radicals ($HO^{\cdot}$) and superoxide anion radicals (O_2^{-}). Reactive oxygen species also contributes in damaging biomembranes, due to increased lipid peroxidation, thereby negotiating cell integrity and function. During stress, the ability of the body defense system to combat the oxidative stress gets diminished which may be observed by body anti-oxidant enzyme markers like lipid peroxidation (LPO), super-oxide dismutase (SOD), reduced glutathione (GSH). [2 - 3].

There are many hypotheses which state that the stress induced changing level of anti- oxidant enzyme has been investigated in different tissues (liver) of animal [4].

Stress is the non-specific response of the body which is observed by altering of immune defence system. It has been observed that many physical and chemical stressors such as trauma, milk,

radiation etc. produce the stressful condition in body [5]. *Spirulina* is microscopic and filamentous blue green alga which has been used up by man since ancient times.

There has been a long history of Spirulina as a food supplement that takes up major proportion of 50-70% protein. It also contains high source of Vitamin B complex, Vitamin E, chlorophyll, gamma-linolenic acid, carotenoids and some minerals like - copper, magnesium, zinc, potassium and iron [9]. Spirulina is being used worldwide as a nutraceutical worldwide as well as potential source of pharmaceutical targets [10].

Spirulina has chromophoric group composed of phycobilisomes as light-collecting protein–pigment complexes. Phycobiliproteins are main part of phycobilisomes which present in the form of phycocyanin and allophycocyanin and are responsible for anticarcinogenic and antioxidant effects [11]. It was hypothesized that *Spirulina* also possess potent anti-viral activity [12 - 13], hypo-cholesterolemic and hypolipidemic [14], anti-diabetic activity [15].

Finally, the goal of the research was to expand the usefulness of *Spirulina* as an anti-stress and adaptogenic agent.

MATERIALS AND METHODS:

Animals: All conditions were kept according to CPCSEA norms. The animals of either sex were chosen randomly from animal house having uniform weight 160±20 gm. The room temperature was maintained at 22±2°C with food (Lipton India Ltd. pellets) and water *ad libitum*. The animals were transferred to the laboratory at least 1h before beginning of the experiment. The experiments were performed during day (08:00-16:00 h).

The institutional animal ethical committee has approved to the study protocol (Approve Ref No. PBRI/IAEC/2010/PN67, CPCSEA Reg No. 1283/c/ 09/CPCSEA). The experiment was conducted as per the permission of Institutional animal ethical committee.

Drugs and Chemicals: Sodium carboxy methyl cellulose (Loba Chemie, Mumbai, India), diazepam (Calmpose, Ranbaxy Laboratories, India), Spirulina spray dried powder(M/S Parry Neutraceuticals, Chennai, India).

Administration of the Extracts: Sodium carboxy methyl cellulose (0.3% w/v) as a suspending agent is used for the preparation of oral suspension of Spirulina in distilled water. The oral suspension of Spirulina was administered in a dose of 200 and 400 mg/kg p.o. respectively, 60 min before starting of the experimental protocol. Vehicle (0.3% w/v Na CMC solution) was administered to the control groups in volume equivalent to that of the oral suspension and drugs.

Acute Toxicity Test: The LD_{50} and signs of toxicity of the Spirulina was estimated by oral feeding dose 1. The doses (100 to 5000 mg/kg) of the Spirulina were administered in five different groups of mice (six mice per group) maintained under the same condition.

One group was given only saline (10mg/kg), while other group (2-5) received different dose of the drug.

Death within 24h was recorded. The LD_{50} was calculated from the graph of percent mortality against log-dose drug.

Pharmacological Evaluation:

Preliminary Neuropsycho - Pharmacological Screening: The spirulina was administered to group of mice and grossly monitored behaviour effects were noted and quantified as described by Irwin [15]. After the treatment with drugs, mice were observed at 1/2, 1, 1½, 3, 6 and 24 h for studying behavioral changes.

Before the treatment with Spirulina, we conducted the test with some standard reference drugs that have known effects on CNS.

Tail Suspension Test: Swiss albino rat weighing 160– 180 gm were used preferentially. They were housed in plastic cages for at least 10 days prior to testing in a 12 h light cycle with food and water freely available. Animals were transported from the housing room to the testing area in their own cages and allowed to adapt to the new environment for 1 h before testing. Groups of 10 animals were treated with the test compounds or the vehicle by intra-peritoneal injection 30 min prior to testing. For the test, the mice were suspended on the edge of a shelf 58 cm above a table top by adhesive tape placed approximately 1 cm from the tip of the tail.

The duration of immobility was recorded for periods of 5 min. Mice were considered immobile when they hung passively and completely motionless for at least 1 min [16].

Cold Immobilization: Here, cold immobilization model was used to induce stress. Priorly, the rats were separated into five groups of six animals each of either sex. By placing the rats in plastic restraint boxes which were further placed in a refrigerator at a temperature of 4 - 7 °C for 2 hours. They were immobilized. After 2 hrs they were returned to their home cages and the procedure was repeated for 10 days. Normal control was administered at 0.3% Na CMC in saline and stress was not exposed to them, stress control animals were administered 0.3% Na CMC in saline and were exposed to stress, Spirulina dose 1 animals and Spirulina dose 2 animals were administered Spirulina 200 mg/kg p.o. and 400 mg/kg p.o. respectively and drug (diazepam 1 mg/kg) was administered to standard drug group animals. For 10 days the rats were treated and subjected to stress, and the animals were overnight fasted on the 10th day.

The blood was obtained from the retro orbital sinus and rats were scarified by cervical dislocation on the 11th day [17]. Liver function marker (AST, ALT and ALP) was estimated in collected blood.[18 - 19]. In the ice cold normal saline, the liver tissue was separated and anti-oxidants profile was estimated using liver tissues [20 - 21].

Statistical Analysis: The results are representing as a mean ± S.E.M. The statistical significance was analyzed one-way analysis of variance (ANOVA) followed by Bonferroni's test. Significant of P value was to be considered when difference of P value was less than 0.05.

RESULTS:

Acute Toxicity Studies: Tests showed that the LD_{50} of the Spirulina by i.p. route in mice was 5000 mg/kg orally.

Preliminary Neuropsycho - Pharmacological Screening: Spirulina showed decrease depressant activity as well as an increase in spontaneous motor activity, muscle tone, palpebral ptosis and ipsilateral flexor reflex.

Effect of Spirulina and Standard Drugon Tail Suspension Test: Resultant data showed that 200 and 400 mg/kg dose of *Spirulina* could significantly reduce immobilization time as compared to standard drug - Diazepam (1 mg/kg).

TABLE 1: EFFECT OF *SPIRULINA* AND STANDARD DRUG ON THE DURATION OF IMMOBILIZATION TIME IN ALBINO RATS

S. no.	Treatments (Dose : mg/kg)	Duration of immobilizationtime (s)
1	Vehicle (0.3% Na CMC in normal saline)	132.2±9.266
2	Spirulina (200 mg/kg)	99.2±3.42*
3	Spirulina (400 mg/kg)	73±6.98**
4	Diazepam (1mg/kg)	58.6±3.8**

one way ANOVA followed by Bonferroni's multiple comparison tests
*Significant variation as compared to vehicle control treated group (P<0.5)
**Significant variation as compared to standard drug treated group (P<0.05)

TABLE 2: EFFECT OF *SPIRULINA* AND DIAZEPAM ON COLD IMMOBILIZATION STRESS INDUCED SERUM LIVER ENZYME IN RATS

S.no.	Groups	Treatment (dose: mg/kg)	AST (IU/L) (Mean ± SEM)	ALT (IU/L) (Mean ± SEM)	ALP (IU/L) (Mean ± SEM)
1	Normal Control	0.3 % Na CMC in normal saline	98.72±9.14	122.8±3.74	11.78±0.48
2	Stress Control	0.3 % Na CMC in normal saline	178.2±12.3	261.6±10.57	24.99±1.64
3	Drug treated	Spirulina (200 mg/kg)	150.1±8.8 *	224.1±6.13*	21.06±0.91*
4	Drug treated	Spirulina (400 mg/kg)	128.1±8.1 **	189.6±4.02 **	19.68±1.2 **
5	Standard treated	Diazepam (1 mg/kg)	107.8±6.5 **	159±4.1 **	14.49±1.1 **

one way ANOVA followed by Bonferroni's multiple comparison tests
*Significant variation as compared to vehicle control treated group (P<0.5)
**Significant variation as compared to standard drug treated group (P<0.05)
AST = Aspartate Aminotransferase, ALT = Alanine aminotransferase, ALP = Alkaline Phosphate,

TABLE 3: EFFECT OF *SPIRULINA*AND DIAZEPAM ON COLD IMMOBILIZATION STRESS INDUCED LIVER ANTIOXIDANT ENZYME IN RATS

S. no.	Groups	Treatment (dose: mg/kg)	MDA (nmol/mg) (Mean ± SEM)	GSH (mg/g protein) (Mean ± SEM)	SOD (units/mg protein) (Mean ± SEM)
1	Normal Control	0.3 % Na CMC in normal saline	1.65±0.07	6.84±0.07	125.68±6.22
2	Stress Control	0.3 % Na CMC in normal saline	4.89±0.18[a]	2.39±0.086 [a]	42.77±3.56 [a]
3	Drug treated	Spirulina (200 mg/kg)	3.13±0.06 [*]	4.89±0.088 [*]	80.59±5.91[*]
4	Drug treated	Spirulina (400 mg/kg)	2.35±0.1 [**]	5.13±0.102 [**]	96.16±3.43 [**]
5	Standard	Diazepam (1 mg/kg)	2.07±0.13 [**]	5.64±0.096 [**]	106.81±4.131 [**]

MDA= Malondialdehyde, GSH = Glutathione, SOD = Superoxide Dismutase
one way ANOVA followed by Bonferroni's multiple comparison tests
*Significant variation as compared to vehicle control treated group (P<0.5)
**Significant variation as compared to standard drug treated group (P<0.05)

DISCUSSION AND CONCLUSION: Stress alters all endocrinal and physiological balance and affects homeostasis mechanism in body. On long term condition of stress, depression and anxiety condition are beginning [22].

Generally, tail suspension model are used to evaluate behaviour changes as well as immobilization time. Normally depletion of neurotransmitters like serotonin, nor-adrenaline to leads to depression in body [23]. Depression has been linked to perturbations in the neurotransmission involving brain 5-HT, nor-epinephrine (NE) and dopamine activity [27 - 28] (Maes and Meltzer, 1995; Posener *et al.*, 1994). Clinical studies show that combined 5-HT and NE reuptake inhibitor is more effective than used alone (Anderson, 1998; Nelson *et al*., 2004). Based on these observations, we evaluated the role of NE in the antidepressant effect of Spirulina [29 - 30].

Tail suspension test results point out clearly that the Spirulina (200 mg/kg) reduced immobilization time as well as enhanced the overall performance in rats. However, the Spirulina reduced immobilization time in rat as compared to the vehicle treated animal and possess a mood stabilizing activity due to attribution of carotenoids and amino acid because *Spirulina platensis* have rich source of amino acid like - Tryptophan, Phenylalanine, Tyrosine. It is precursor of Serotonin, Nor-adrenaline, and Dopamine in the body [31 - 32].

The cold stress exposed to rat lead to elevated levels of ALT, AST and ALP due to secretion of corticosterone from cortex, adrenaline from medulla and nor-adrenaline from sympathetic nerve terminals which supply substrate for energy metabolism and the confirm of availability of ATP demand in the muscles, CNS, and organ of demand. Epinephrine and corticosterone shows hyperglycemic effect by this mechanism because increased level of glucose occurs by gluconeogenesis process. In stress condition, secondary substrate as a fat used for glucose formation and gluconeogenesis proceed in response to cortico-sterone. The transfer of γ-amino groups of alanine and aspartate are being catalyzed by ALT and AST enzymes respectively, to γ- keto group of keto-gluterate, resulting to the formation of oxalo acetic acid and pyruvic acid.

In contrast to ALT, that is present in liver and AST found in some of the tissue. Amino acid can enter the citric acid cycle and can perform the intermediary metabolism of carbohydrate and lipids due transamination [24].

The data results show that spirulina dose (200 mg/kg) showed a protective action and Spirulina dose (400 mg/kg) and diazepam (1mg/kg) showed revert action on the animals against the alterations of biochemical changes due to cold stress such as changes in the serum levels (liver functional enzyme markers) by blocking to attenuating the activation of HPA axis.

MDA, GSH and SOD are oxidative stress marker in stress rats. The *spirulina platensis* are having better capability to detoxify lipid peroxidation products which are cytotoxic and reactive aldehyde metabolites such as MDA. It is accumulated during cold immobilization stress. Cellular functions (nucleotide and protein synthesis) are impaired due to their cytotoxic products. In response to cold stress, the SOD level was decreased because superoxide radical participates which known to generate highly noxious hydroxyl

radical through its reaction with H_2O_2 (Haber Weiss reaction). It is hypotheses that in turn decrease the SOD through an alteration in histidine residue located in the active site of the enzyme. Additionally GSH was decreased during cold immobilization stress. It could be described by the consumption of GSH due to scavenging of the rapidly generated hydrogen peroxide and lipid peroxides [25].

Thus Spirulina dose (200 and 400 mg/kg) were protective effect on cold immobilization stress rat. It was significantly reduced MDA level and also improved levels of SOD and GSH. *Spirulina platensis* contains phycobiliproteins (phycocyanin and allophycocyanin) and β- carotene that are responsible for its antioxidant activity which is scavenging all reactive free radicals [26].

Thus, it can be concluded that *Spirulina platensis* is a capable anti-stress and adaptogenic agent.

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