Restraint Stress Induced Changes and Their Modification by *Spirulina platensis* in Albino Rats: an Experimental Study

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**Abstract**

*Spirulina platensis* is a blue green algae containing vital nutrients (proteins, lipids and carbohydrates), minerals (zinc, magnesium, manganese, selenium), vitamins (β–carotene, riboflavin, cyanocobalamine, α-tocopherol), and α–linoleic acid, reported to promote physical health, improve defense mechanisms of the human body and enhance longevity of life. These attributes are similar to the modern concept of adaptogenic agents, which are known to afford protection of the human physiological system against diverse stressors. Present study investigated the adaptogenic activity of *S. platensis* against chronic restraint stress induced perturbations in glucose metabolism and immunosupression. Adrenal gland weight, corticosterone content of adrenal gland, plasma corticos terone levels, and histopathological studies of adrenal gland were used as stress indices. Ginseng (*Panax ginseng*) was used as the standard adaptogenic agent for comparison.

Chronic restraint stress induced marked increase in plasma glucose levels, significant increase in adrenal gland weight and plasma corticosterone levels with concomitant decrease in adrenal gland corticosterone content. Restraint stress resulted in distortion of cords, loss of architecture and formation of lesions in the cortex of adrenal gland. These effects were attenuated by *S. platensis* (100, 200 and 500 mg/kg, p.o.) and ginseng (100 mg/kg, p.o.) administered once daily over a period of 14 d (prior stress period) and continued for next 7 d (during the period of stress induction). Results indicate that *S. platensis* has adaptogenic activity, qualitatively comparable to ginseng, against a variety of biochemical, physiological and histological perturbations induced by restraint stress.

**INTRODUCTION**

Blue green algae (*Spirulina platensis*) is rich in proteins, lipids and carbohydrates, minerals (zinc, magnesium, manganese, selenium), vitamins (β–carotene, riboflavin, cyanocobalamine, α-tocopherol), and α–linoleic acid (Sheshadri and Umesh, 1992), reputed to promote physical health and improve resistance of the body and other external factors tending to perturb the homeostasis of humans and promote revival of physiological functions after debilitating diseases (Brehkman and Dardymov, 1969).

There is comprehensive experimental and clinical evidence that stress alters the physiological homeostasis of an organism. Complex mechanisms contribute to the breakdown in adaptational processes resulting in various visceral, behavioral and endocrinological changes. Hypothalamic-pituitary-adrenal (HPA) axis and adrenal glands are particularly crucial for the regulation of stress physiology (Munck et al., 1995).

Restraint stress (RS) is widely used to study the stress-induced changes in experimental animals. Preliminary studies have shown that chronic RS significantly increased the weight of adrenal glands (Bhattacharya et al., 2000). Therefore the effect of *S. platensis* pretreatment on chronic RS induced changes in adrenal gland was studied extensively. The parameters such as plasma glucose, cholesterol and corticosterone levels, adrenal gland corticosterone content and histological changes in adrenal gland cellular structure were used to determine the stress indices in this study.
MATERIALS AND METHODS

Animals
Male Swiss albino rats (200-250 g) were obtained from registered breeder (Haffkine Institute, Parel, Mumbai, India). Three animals were housed per cage under standard conditions (23 ± 1.0°C; relative humidity 55 ± 10%; 12h/12h light/dark cycle), fed standard pellet diet (M/S Amrut Ind. Ltd. Pune, India) and purified water, ad libitum.

Drug Treatment
Spray-dried blue green alga (S. platensis) (M/S Parry Neutraceuticals, Chennai, India) was orally administered as 0.3% carboxy methylcellulose (CMC) suspension, in the doses of 100, 200, 500 mg/kg/d (p.o.) for 14 consecutive days. Stress induction was initiated on 15th d and continued for next 7 days. Treatment was continued during the stress period. Ginseng was used as standard adaptogenic agent for comparison. Control animals were treated with vehicle (0.3% CMC suspension).

Induction of Stress
The rats were randomly assigned to the unstressed control, stress and drug treated stress groups. Those assigned to vehicle or drug treated groups were subjected to immobilization, done by stretching and firmly tying the limbs to iron rod with the help of thread and keeping the animals in inverted position for half an hour. Animals were sacrificed on day 7, immediately on completion of stress procedure.

Assessment of Stress Intensity
1. Estimation of Blood Glucose Levels (Philip, 1994). The plasma was analyzed for estimation of glucose; in brief 10 µL of plasma was mixed with 1 mL Glucose Oxidase-Peroxidase (GOD-POD) reagent. The resulting mixture was incubated at 37°C for 15 min. Absorbances of test and standard were measured at 540 nm (Merck biochemical analyzer, microlab 200, Vital Scientific, Netherlands).

\[
\text{Glucose} + \text{O}_2 + \text{H}_2\text{O} \xrightarrow{\text{GOD}} \text{Gluconic acid} + \text{H}_2\text{O}_2
\]

\[
2 \text{H}_2\text{O}_2 + 4\text{-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4 \text{H}_2\text{O}
\]

2. Estimation of Plasma Cholesterol. The cholesterol levels on first day and on seventh day, after completion of stress period, were determined by using CHOD-PAP method (Richmond, 1974); 10 µL plasma was mixed with 1 mL reagent containing peroxidase, cholesterol oxidase, cholesterol esterase and 4-aminoantipyrine. Cholesterol and its esters are released from lipoproteins by detergents. Cholesterol esterase hydrolyzes the esters. In the subsequent enzymatic oxidation by cholesterol oxidase, H$_2$O$_2$ is formed. This is converted into colored quinonimine, which was read at 546 nm (Merck biochemical analyzer, microlab 200, Vital Scientific, Netherlands).

3. Estimation of Plasma Corticosterone Levels. On seventh day after completion of stress period the rats were anesthetized and sacrificed. Blood was collected in tubes containing EDTA-Na$_2$, centrifuged and the separated plasma stored at -20°C. The concentrations of corticosterone in plasma were estimated by RIA method (Yalow and Berson, 1971).

4. Adrenal Gland Function Test. On seventh day both the adrenals of each rat were isolated and weighed. Mean weight of adrenals per 100 g of body weight for each group was calculated. One of the two adrenals was preserved for histopathological studies in formalin, while the other was used for estimation of corticosterone content. Corticosterone was estimated by using RIA method (Yalow and Berson, 1971). One adrenal gland, of each rat, preserved in formalin was sent to micron laboratory for histopathological studies.
5. Total Leukocyte Count. Blood collected on first and seventh day was diluted with white blood cell (WBC) diluting fluid and counting done using a Neubauer’s chamber (Feinoptik, Blankenberg, Germany).

RESULTS

Plasma Glucose Levels
Chronic stress markedly increased the plasma glucose levels. Pre-treatment with ginseng and S. platensis inhibited rise in plasma glucose levels (Table 1).

Plasma Cholesterol Levels
Seven days of stress increased the cholesterol levels. Prior treatment with ginseng and S. platensis lowered plasma cholesterol levels due to chronic stress. S. platensis at 200 mg/kg and 500 mg/kg (p.o.) showed better activity compared to ginseng (Table 1).

Plasma Corticosterone Levels
Chronic RS caused increase in plasma corticosterone levels. Both ginseng and S. platensis reduced the elevation in plasma corticosterone levels, compared to negative control (Table 2).

Adrenal Gland Weight and Corticosterone Content of Adrenal Glands
Seven days of RS increased the adrenal gland weight and reduced corticosterone levels of stressed animals compared to the unstressed. Pre-treatment with ginseng and S. platensis inhibited stress induced alterations in adrenal gland weight and adrenal corticosterone content (Table 2).

Histopathological Studies
Restraint stress resulted in distortion of cords, loss of architecture and formation of lesions in the cortex of adrenal gland. S. platensis and ginseng treatment was found to attenuate this stress induced alterations in adrenal gland histology (Fig. 1-4).

Immunological Study
Total leukocyte count of stressed animals was reduced compared to the unstressed. Ginseng, a known immunostimulant, caused an increase in total leukocyte count. S. platensis reverted the reduction in total leukocyte count (Table 3).

DISCUSSION
The two main systems involved in response to stress are the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (Best and Taylor, 1991). Triggered primarily by an area in the brain stem (lowest part of brain) called the locus coeruleus, the sympathetic nervous system secretes catecholamines. The hypothalamus is a major integrating center for receiving messages from different centers and converting them to hormonal signals, via the control of the pituitary gland and by neural pathways. The main CNS nucleus involved in the regulation of the pituitary-adrenal axis is the para-ventricular nucleus (PVN) of the hypothalamus. The PVN is the principal CNS source of corticotropin-releasing factor (CRF), which is the major physiological regulator of pituitary adrenocorticotropic hormone (ACTH) secretion, and of vasopressin (VP), which interacts with CRF to promote ACTH release. Secretion of ACTH occurs via posterior pituitary, which is also responsible for controlling the secretion of various peptide hormones including vasopressin, oxytocin and corticotropin releasing factor (CRF). This system is now known as the hypothalamic-pituitary-adrenal (HPA) axis. Thus, activation of this HPA system results in secretion of CRF, ACTH, β-endorphin and glucocorticoids into the circulation (Saito et al., 1984).

Release of adrenocorticotropic hormone (ACTH) in stress stimulates adrenals to increase production of hormones epinephrine, norepinephrine and corticosteroids (Biswa
et al., 2001). These hormones have profound effect on metabolic functions. Catecholamines (epinephrine and nor-epinephrine), stimulates the heart, constrict certain blood vessels to increase blood flow to muscles and brain and to decrease it to the digestive tract and internal organs. Catecholamines also raise blood sugar by releasing glucose into the blood, so cells will have the energy to ward off the stressful stimuli. The glucocorticoids have an effect on glucose, protein and fat metabolism (Hoffman, 1996). Corticosteroids stimulate gluconeogenesis, decrease glucose utilization by the cells, elevate blood glucose concentration and give rise to the condition called adrenal diabetes (Natelson et al., 1981). S. platensis and ginseng were found to inhibit stress induced rise in blood glucose levels. The effect observed may be attributed to their protective effect on adrenal glands as shown by the histopathological studies i.e. reduction in the distortion of cords, prevention of swelling of cells in cortical region and increase in depleted lipid content of adrenal cortex.

The CNS has enormous regulatory power over the immune system. The acute stress stimuli may be beneficial since it elevates the immune system nonspecifically to fight against it, but the delicate balance between CNS and immune system can be thrown off by chronic stress. The corticosteroids secretion, during chronic stress, decreases the number of eosinophils and lymphocytes in blood (Stein et al., 1985). Likewise, chronic stress causes significant atrophy of all lymphoid tissue throughout the body, which in turn decreases the output of both T-cells and antibodies from lymphoid tissue. As a result the level of immunity for almost all foreign invaders of the body is decreased (Nagaraj and Jaganathan, 1999). S. platensis and ginseng reverted this immunosupressing effect caused due to stress indicated by significant increase in WBC count.

Both S. platensis and ginseng prevented chronic stress-induced immunosuppression. Increase in plasma corticosterone, concomitant fall in adrenal gland corticosterone content, increase in adrenal gland weight and change in adrenal gland histology are consequences of chronic stress, which were reversed by S. platensis and P. ginseng. Rise in plasma glucose and cholesterol levels were also inhibited by S. platensis and P. ginseng. The findings indicate that, like standard adaptogen P. ginseng, S. platensis can attenuate chronic stress induced biochemical, hormonal and histological changes in rats. S. platensis possibly exhibits its adaptogenic (anti-stress) effect by preventing the hyperactivity of adrenal cortex.

**Literature Cited**


Chemistry in Diagnosis and Treatment, Oxford press, England.

Tables

Table 1. Effect of pre-treatment with ginseng (*Panax ginseng*) and blue green algae (*Spirulina platensis*) (SP) on plasma glucose and cholesterol levels in restraint stressed (RS) rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Glucose Levels (mg/dL)</th>
<th>Cholesterol Levels (mg %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (No RS)</td>
<td>106.43 (± 9.56)</td>
<td>97.56 (± 18.5)</td>
</tr>
<tr>
<td>Restraint stress (RS)</td>
<td>188.33 (± 15.78)</td>
<td>152.97 (± 17.21)</td>
</tr>
<tr>
<td>SP 100 mg/kg + RS</td>
<td>167.54 (± 11.49)**</td>
<td>143.4 (± 15.32)*</td>
</tr>
<tr>
<td>SP 200 mg/kg + RS</td>
<td>144.4 (± 10.39)**</td>
<td>128.4 (± 14.75)**</td>
</tr>
<tr>
<td>SP 500 mg/kg + RS</td>
<td>140.82 (± 16.2)**</td>
<td>127.2 (± 13.64)**</td>
</tr>
<tr>
<td>PG 100 mg/kg + RS</td>
<td>138.1 (± 13.82)**</td>
<td>139.2 (± 9.05)**</td>
</tr>
</tbody>
</table>

* p<0.5, ** p<0.05 (compared to respective control)

Table 2. Effect of pre-treatment with ginseng (*Panax ginseng*) and blue green algae (*Spirulina platensis*) (SP) on plasma and adrenal corticosterone levels in restraint stressed (RS) rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plasma corticosterone (µg/dL)</th>
<th>Adrenal gland weight (mg/100 g body wt)</th>
<th>Adrenal corticosterone (µg/100 g gland weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (No RS)</td>
<td>21.94 (±2.62)</td>
<td>13.05 (± 3.01)</td>
<td>4.34 (±1.50)</td>
</tr>
<tr>
<td>Restraint stress (RS)</td>
<td>49.47 (±3.13)</td>
<td>29.53 (± 6.82)</td>
<td>1.23 (±0.3)</td>
</tr>
<tr>
<td>SP 100 mg/kg + RS</td>
<td>36.59 (±1.91) **</td>
<td>19.52 (±5.32) **</td>
<td>4.17 (±2.1) **</td>
</tr>
<tr>
<td>SP 200 mg/kg + RS</td>
<td>37.12 (±1.7) **</td>
<td>22.11 (±8.02) **</td>
<td>2.89 (±0.4) **</td>
</tr>
<tr>
<td>SP 500 mg/kg + RS</td>
<td>30.12 (±2.15) **</td>
<td>18.24 (±5.61) **</td>
<td>3.29 (±0.9) **</td>
</tr>
<tr>
<td>PG 100 mg/kg + RS</td>
<td>24.75 (±3.12) **</td>
<td>19.34 (± 6.29) **</td>
<td>3.45 (±1.13) **</td>
</tr>
</tbody>
</table>

* p<0.5, ** p<0.05 (compared to respective control)
Table 3. Effect of pre-treatment with ginseng (*Panax ginseng*) and blue green algae (*Spirulina platensis*) (SP) on white blood cell (WBC) count in restraint stressed (RS) rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>WBC count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (No RS)</td>
<td>10575 (± 685)</td>
</tr>
<tr>
<td>Restraint stress (RS)</td>
<td>9687 (± 730)</td>
</tr>
<tr>
<td>SP 100 mg/kg + RS</td>
<td>10483 (± 386)**</td>
</tr>
<tr>
<td>SP 200 mg/kg + RS</td>
<td>9940 (± 377)*</td>
</tr>
<tr>
<td>SP 500 mg/kg + RS</td>
<td>10214 (± 753)**</td>
</tr>
<tr>
<td>PG 100 mg/kg + RS</td>
<td>10305 (± 578)**</td>
</tr>
</tbody>
</table>

* p<0.5, ** p<0.05 (compared to respective control)

Figures

Fig. 1. Microscopic section of normal adrenal gland.

Fig. 2. Microscopic section of adrenal gland of rat subjected to restraint stress.
Fig. 3. Microscopic section of adrenal gland of rat pretreated with Spirulina and subjected to restraint stress.

Fig. 4. Microscopic section of adrenal gland of rat pretreated with Ginseng and subjected to restraint stress.