Prevention of Cold Stress Induced Adrenal Hypertrophy by *Spirulina platensis*

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**Abstract**
*Spirulina platensis* (Spirulina) was investigated on an acute stress (4h cold stress, 4±1°C) induced biochemical alteration in albino rats. Gastric ulcerations, adrenal gland weight, adrenal gland ascorbic acid content, and histopathology were also used as stress indices. *Panax ginseng* (ginseng) was used as standard adaptogenic agent for comparison. Acute cold stress resulted in elevation of blood glucose levels, increase in adrenal gland weight, and reduction in adrenal gland ascorbic acid content and histological changes. Spirulina (100 mg/kg, 200 mg/kg and 500 mg/kg p.o.) and ginseng (100 mg/kg p.o.) attenuated all these cold stress induced perturbations. Results demonstrated the anti-stress activity of Spirulina, qualitatively comparable to ginseng, against a variety of biochemical and histological perturbations induced by acute cold stress.

**INTRODUCTION**
Stress is known to alter the physiological homeostasis of the organism and complex mechanisms contributing to the breakdown in adaptational processes (George et al., 1992). It has been postulated that stress is involved in etiopathogenesis of a variety of diseases like depression and anxiety, immunosuppression, endocrine disorders, male potency and cognitive dysfunctions to the diseases like peptic ulcers, hypertension and ulcerative colitis (Goel, 1991). Using agents, which could include a state of nonspecific increase to resistance to affect the internal homeostasis, can provide the answer to this problem. Spirulina is a rich blend of proteins, lipids and carbohydrates (Sheshadri and Umesh, 1992; Johnson and Shubert, 1986), minerals (Zinc, Magnesium, Manganese, Selenium) vitamins (β-carotene, riboflavin, cyanocobalamine, α-tocopherol) and α-linoleic acids (Denise, 1993; Takeuchi, 1978). Spirulina is known to support the human immune system and promote cellular health that is primarily affected in stress (Fukino et al., 1990). The objective of the study was to explore the usefulness of Spirulina as an anti-stress and adaptogenic agent.

**MATERIALS AND METHODS**

**Animals**
Male Swiss albino rats (200-250 g) were obtained from registered breeders (Haffkine Institute, Parel, Mumbai, India).

**Drugs, Reagents, Solutions and Biochemical Kits**
*Spirulina* spray dried powder (M/S Parry Neutraceuticals, Chennai, India), ginseng (Glenmark Ltd., Mumbai, India), sodium carboxy methyl cellulose (Loba Chemie, Mumbai, India), glacial acetic acid, formalin, trichloroacetic acid, hydrochloric acid, sulfuric acid and 2,4-dinitrophenyl hydrazine (S.D. Fine chemicals, Mumbai, India.), heparin, thio urea (Biological E. Ltd., Mumbai, India), ascorbic acid (Glaxo Ltd. Mumbai, India) glucose diagnostic kit (E. Merck India Ltd).

**Stress Induction**
The rats were divided into 6 groups, each group containing six rats. All animals
were pre-treated for 15 d with the respective test drugs, after which the rats were exposed
to cold stress at 4±1°C for 4h.

**Group I**
Unstressed rats received 1 mL/kg p.o. of 0.3% Na. CMC in saline; served as normal group.

**Group II**
Stressed rats received 1 mL/kg p.o. of 0.3% Na. CMC in saline; served as negative control.

**Group III**
Stressed rats were treated with standard oral drug (ginseng); served as positive control.

**Group IV-VI**
Stressed rats in groups IV, V, and VI received Spirulina suspended in 0.3% Na. CMC solution. The doses of Spirulina used for pre-treatment were 100, 250 and 500 mg/kg p.o. respectively.

Animals were sacrificed following stress induction, under the influence of ether anesthesia. 5 mL of blood was collected in heparinised tubes from each animal and plasma obtained by centrifugation (Superspin R-V/FM, Plastocraft, India) for estimation of glucose and ascorbic acid levels. Adrenal glands from each rat were decapsulated for determination of adrenal gland weight, adrenal gland ascorbic acid content and histopathological studies. Anti-ulcerogenic effect of Spirulina was also evaluated by isolating the stomach from the rats. The isolated stomachs were cut open along the greater curvature, washed with cold water and examined microscopically (X 10). The mean percent ulcer and the severity of gastric mucosal lesions (cumulative length in mm) were determined (Sairam et al., 2001).

**Determination of Plasma Glucose Levels**
The plasma glucose was estimated as described earlier (Philip, 1994). 10 µL of plasma was mixed with 1 mL of Glucose oxidase-Peroxidase (GOD-POD) reagent. The resulting mixture was incubated at 37°C for 15 min. Absorbance of test and standard was measured at 540 nm (Merck biochemical analyzer, Microlab 200, Vital Scientific, Netherlands).

**Determination of Plasma Ascorbic Acid Levels**
In a centrifuge tube containing 6 mL of 6% trichloroaceticacid, 2 mL of plasma was added drop by drop to form fine suspension. It was allowed to stand for 5 min and centrifuged (2500 rpm, 15 min). To the supernatant fluid, 0.5 g of acid washed norit (activated charcoal) was added, stirred vigorously and then filtered through a filter paper. To each 1 mL of norit filtrate one drop of 10% thiourea and 0.25 mL of 2,4-dinitrophenylhydrazine reagent were added. The mixture was incubated for 3 h at 37°C and placed in a beaker containing ice. To each of these tubes, while in ice bath, 1 mL of 85% H₂SO₄ was added drop by drop. Tubes were shaken thoroughly to ensure complete mixing. After 30 min, tubes were wiped and read in UV-Visible Spectrophotometer (Jasco V530, Japan) at 540 nm. Appropriate standards were run along with the unknown tubes. Standard solutions of ascorbic acid varying from 1-25 µg/mL in 4% trichloroacetic acid were prepared for standard curve of ascorbic acid. The ascorbic acid content per 100 g of adrenal gland for each group was then calculated (Roe and Kuether, 1949).

**Adrenal Gland Histopathology**
The separated adrenal glands were preserved in saline formalin. Microscopic sections of these adrenal glands were prepared and sent to Bombay Veterinary College, Parel, Mumbai, India for histopathological studies.

**Data Analysis**
The data were analyzed using two-tailed student’s t-test.
RESULTS

Effect of Spirulina on Plasma Glucose Levels in Cold Stressed Rats
Cold stress caused elevation in the plasma glucose levels by 33% as compared to normal animals. Pretreatment of rats with Spirulina (100, 200, 500 mg/kg, p.o.) inhibited hyperglycemic response rise as compared to stress group (Table 1).

Effect of Spirulina on Plasma Ascorbic Acid Levels in Cold Stressed Rats
The cold stress (4°C, 4 h) increased the plasma ascorbic acid levels by 20% as compared to normal. Animals receiving ginseng showed lower ascorbic acid levels in plasma as compared to negative control. Pre-treatment of animals with Spirulina prevented the rise in plasma ascorbic acid levels resulting due to stress (Table 1).

Effect of Spirulina on Adrenal Gland Weight in Cold Stressed Rats
Cold stress increased the adrenal gland weight in negative control animals as compared to the normal (non-stressed) animals. Ginseng administration negated the stress effects by bringing back the adrenal gland weight to normal (Table 1). Pre-treatment with Spirulina reverted the increase in adrenal gland weight obtained in stressful conditions.

Effect of Spirulina on Adrenal Gland Ascorbic Acid Content in Cold Stressed Rats
The cold stress reduced the adrenal gland ascorbic acid content. The effect was however reversed by the administration of both Spirulina and ginseng (Table 1).

Effect of Spirulina on Histological Changes in Adrenal Gland Due to Cold Stress
Distortion of the cords, loss of architecture and swelling in cortical region was observed in this group as a result of stress (Fig. 4) compared to normal (Fig. 1). Zona-fasciculata had large to small vacuolations with coalesced areas. Zona-reticularis also revealed vacuolations. The initial layer of zona-glomerulosa was discontinuous. Lipid-rich cells seen in zona-fasciculata layer of normal group were converted to eosinophilic cytoplasm relatively devoid of lipid. Zona-reticularis was widened into region of zona-glomerulosa. Medulla revealed the zones of coalesced empty foci (Fig. 4). Treatment with Spirulina caused increase in depleted lipid content. Distortion of the cords, loss of architecture and swelling in cortical region was reduced due to the pre-treatment with Spirulina (Fig. 3). Large vacuolations in Zona-fasciculata and Zona-reticularis, which were observed in stressed rats, were found to reduce (Fig. 3). With ginseng pre-treatment Zona-fasciculata and Zona-reticularis revealed small vacuoles. Zona-glomerulosa appeared with thin layer of cells and had normal appearance. Medullary vacuolations were mostly small to medium. Architecture appeared to be non-distorted (Fig. 2).

Gastric Ulceration
Cold stress markedly increased the incidences, number and severity of gastric ulcers. Pre-treatment with Spirulina and ginseng reduced these stress-induced indices (Table 1). Animals treated with Spirulina showed dose dependent reduction in ulcer formation.

DISCUSSION
The brain and pituitary gland respond to stress by releasing adrenocorticotropic hormone (ACTH) (Torellas et al., 1980). This stimulates adrenals to increase production of the hormones adrenaline, noradrenaline, and corticosteroids (Rang and Dale, 1996). Adrenaline raises blood sugar, corticosteroid stimulates glycogenolysis and gluconeogenesis to produce and release more glucose and cholesterol into the blood (Sharma and Godhawani, 1970). Spirulina significantly prevented the rise in plasma glucose levels caused by cold stress (Table 1). This effect may be due to its protective effect on adrenal gland resulting in lesser release in catecholamines and corticosteroids. Interestingly, in line with the findings by (Bhattacharya et al., 2000), Spirulina
pretreatment countered the depletion of adrenal gland ascorbic acid content during stress (Table 1). Apparently, corticosteroid secretion results in release of ascorbic acid in plasma from its deposit sites such as adrenal gland and liver to neutralize the free radicals produced during stress. Therefore ascorbic acid levels in plasma serves as the indication of amount of stress experienced by the individual (Bhattacharya et al., 2000). Spirulina treated animals showed significant reduction in the rise of plasma ascorbic acid (Table 1). This indicated that the animals treated with Spirulina could combat stress effectively.

During stress, pituitary gland secretes greater amount of ACTH, this stimulates adrenal gland to synthesize cortical hormones (Ortiz et al., 1974). The reasons for an increased requirement of adrenal cortical hormones in response to stress are not well understood, but adrenal corticosteroids do somehow reduce the mortality of animals (Alan and Clemston, 1989). The individual or combined weights of adrenal glands may increase under the influence of ACTH with near doubling in weights (Wortsman et al., 1984; Natelson et al., 1981). The increased requirement of adrenal cortical hormones during stress may be one of the reasons of increased adrenal weights. Pre-treatment with Spirulina results in prevention of increase in adrenal gland weight (Table 1) caused due to stress, thus inhibiting the basic signs of stress response.

The morphological changes associated with stress occur mainly in the cortical region. Essentially there was cortical cell lipid depletion as a result of stress (Fig. 4). This does not reflect adrenal exhaustion, but rather conversion of relatively inactive vacuolated cells to the synthesis of steroids. Pre-treatment with Spirulina reduced the distortion of cords and prevented the swelling of cells in cortical region. Increase in depleted lipid content was observed (Fig. 3). All these signs indicate recovery from stress on treatment with Spirulina.

Due to cold stress, gastric ulceration was formed in the glandular part of the stomach in rats. The lesions varied from epithelial shedding to elongated areas of erosion. These manifestations were prevented by pretreatment with Spirulina and standard adaptogen ginseng (Table 1). Spirulina showed dose dependent inhibition of ulcer formation and had better effect than ginseng at 500 mg/kg dose.

The present investigation indicates that Spirulina has significant adaptogenic activity as shown by its mitigating effects on several cold stress induced biochemical and histological perturbations, comparable to that of ginseng, the popular adaptogenic agent.

**Literature Cited**


Roe, J.H. and Kuether, A. 1949. The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid. J. Biol. Chem. 147:399-407.

Tables

Table 1. Effect of Spirulina on plasma glucose, plasma ascorbic acid levels, adrenal gland weight, adrenal ascorbic acid content and ulcer index in cold stressed rats. Values are mean ± SD of 6 animals per group.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plasma glucose levels (mg/dl)</th>
<th>Plasma ascorbic acid levels (µg/mL)</th>
<th>Adrenal gland weight in mg/100 g of body weight</th>
<th>Adrenal gland ascorbic acid content in mg/100 g of gland weight</th>
<th>Ulcer Index</th>
<th>Percent Reduction in Ulcer Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Cold Stress</td>
<td>100.72 (± 8.38)</td>
<td>85.57 (± 5.29)</td>
<td>13.05 (± 3.01)</td>
<td>283.1 (± 21.22)</td>
<td>2.0</td>
<td>100%</td>
</tr>
<tr>
<td>Cold Stress 4°C for 4 hrs (C.S.)</td>
<td>133.5 (± 5.08)</td>
<td>102.68 (± 6.17)</td>
<td>24.53 (± 2.82)</td>
<td>154.8 (± 12.51)</td>
<td>14.83</td>
<td>---</td>
</tr>
<tr>
<td>SP 100 mg/kg + C.S.</td>
<td>129.58 (± 5.23)*</td>
<td>97.5 (± 5.12)*</td>
<td>16.42 (± 1.72)**</td>
<td>203.67 (± 9.87)**</td>
<td>10.33</td>
<td>35 %</td>
</tr>
<tr>
<td>SP 200 mg/kg + C.S.</td>
<td>113.89 (± 5.88)**</td>
<td>92.25 (± 3.31)**</td>
<td>13.96 (± 0.81)**</td>
<td>227.8 (± 10.20)**</td>
<td>7.83</td>
<td>54 %</td>
</tr>
<tr>
<td>SP 500 mg/kg + C.S.</td>
<td>120.74 (± 3.86)**</td>
<td>90.05 (± 1.09)**</td>
<td>15.20 (± 0.81)**</td>
<td>234.8 (± 9.80)**</td>
<td>5.33</td>
<td>74 %</td>
</tr>
<tr>
<td>PG 100 mg/kg + C.S.</td>
<td>109.38 (± 4.36)**</td>
<td>87.91 (± 1.27)**</td>
<td>13.06 (± 1.27)**</td>
<td>257.5 (± 12.44)**</td>
<td>5.58</td>
<td>72 %</td>
</tr>
</tbody>
</table>

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

Fig. 1. Microscopic section of adrenal gland of normal rat.

Fig. 2. Microscopic section of adrenal gland of group treated with ginseng and subjected to cold stress.
Fig. 3. Microscopic section of adrenal gland of group treated with Spirulina and subjected to cold stress.

Fig. 4. Microscopic section of adrenal gland subjected to cold stress.