Antistress activity of ethanolic extract of *Asparagus racemosus* Willd roots in mice

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Ethanolic extract of the roots of *A. racemosus* improved the stress tolerance in chemical writhing test and swimming endurance test at all the doses as compared to stress control group. Restraint stress induced elevation of blood glucose, triglyceride and cholesterol levels were significantly lowered by pretreatment with extract. Moreover, stress induced variations in levels of lipid peroxidation, nitric oxide, protein and glutathione content in mouse brain were significantly ameliorated by pretreatment with extract. The extract attenuated the elevated weight of adrenal glands and increased the reduced weight of the spleen during stress. In conclusion, the results suggest antistress property of *Asparagus racemosus* in different model of stress.

**Keywords:** Antistress, *Asparagus racemosus*, Restraint stress, Root extract, Saponins

Stress, a pattern of metabolic and behavioral reactions occurs in response to physical, chemical, biological and emotional changes and helps in strengthening the organism. If the stress is extreme, the homeostatic mechanisms of the organism become deficit and the survival of the organism is threatened. Stress triggers a wide range of the body changes called General Adaptation Syndrome (GAS) and induce a marked rise in the brain levels of biogenic amines such as adrenaline and nor-adrenaline which assist the organisms to cope with stress. Endocrine response to stress is mediated by secretion of glucocorticoids. However, increased and prolonged severe stress is responsible for fatigue, reduced stamina, lowered mood and in the etiopathogenesis of variety of diseases like depression, anxiety, immunosuppression, endocrine disorders, male impotency, cognitive dysfunction, peptic ulcer, hypertension and ulcerative colitis.

Moreover, there are reports suggesting the contribution of stress in oxidant production in brain. Restraint stress in animal results in oxidative stress i.e. increased production of reactive oxygen species which can cause tissue damage by reacting with lipids, DNA and proteins. In today’s world, oxidative stress has been implicated in over hundreds of disease states which range from arthritis and connective tissue disorder to carcinogenesis, aging, physical injury, infection and acquired immunodeficiency syndrome.

*Asparagus racemosus* Willd. (Liliaceae), commonly known as *Satavari* or *Satawar* is used by the Indian traditional system of medicine (*Ayurveda* and *Yunani*) for the treatment of various ailments. The roots are used in dysentery, tumors, inflammation, neuropathy, bronchitis, hyperacidity, conjunctivitis, spasm, chronic fever, rheumatism, tuberculosis, epilepsy, leprosy and skin diseases. The major active constituents of *A. racemosus* roots are steroidal saponins (Shatavarin I-IV). Quantitative analysis of *A. racemosus* root extract revealed the presence of flavonoids (36.7±3.9 mg/100 mL), polyphenols (88.2±9.3 mg/100 mL) and vitamin C (42.4±5.1 mg/100 mL). In *Ayurveda*, *Asparagus racemosus* is described as a ‘rasayana’ herb. ‘Rasayana’ is a group of plant drugs known to promote physical and mental health, improve defence mechanisms of the body and enhance longevity.

Chronic stress leads to many degenerative diseases, as well as premature aging, hence there is a great need for safe and effective prevention strategies to combat the ravages of stress on the nervous system. In an attempt to identify herbal drug with potent antistress activity, the present study has been undertaken to

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report antistress property of ethanolic extract of *Asparagus racemosus* using different models.

**Materials and Methods**

**Chemicals**—Sodium chloride (0.9%), disodium hydrogen phosphate, sodium dihydrogen phosphate, Tris-HCl Buffer, trichloroacetic acid (TCA), thiobarbituric acid (TBA), NaOH, sodium potassium tartrate, KI, sulphosalicylic acid (4%), HPO₃, sulphanilamide and naphthyl ethylene diamine dihydrochloride were purchased from Loba Chemie Pvt. Ltd, (Mumbai, India) and 5-5'-dithio-bis-2-nitrobenzoic acid (DTNB) from HiMedia Laboratories Pvt. Limited, (Mumbai, India). All other chemicals and reagents used were of analytical grade. The extract of *Withania somnifera* (Ashwagandha) was generously gifted by Dr. Hemant Kumar Pandey (Defence Research Development Organization, Pithoragarh, 262501, India). Different doses of *A. racemosus* extract used in the study were prepared in distilled water.

**Plant material and extraction**—*A. racemosus* roots were obtained from the herbal garden of the Defence Research Development Organization, Pithoragarh, 262501, India. The plant sample was further verified from Botanical Survey of India, Dehradun (Accession No. BSI-93450). Dried and powdered roots of *A. racemosus* (200 g) were successively extracted thrice with 800 mL of 70% ethanol. The filtrate obtained from all the three extractions were pooled and concentrated using Hedolph Rotatory Vacuum Film Evaporator to yield a semisolid residue. The yield of the extract (EEAR) was 12.9% (w/w).

**Animals**—Albino laca mice of either sex (20 ± 5 g) were obtained from the Animal House of the Department. They were kept in clean cages placed in well ventilated housed condition with food and water *ad libitum* and maintained on a natural 12:12 h L:D cycle. The procedures used in the study were formed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Ethics Committee of the institute (6/2010).

**Antistress activity**

**Chemical induced stress in mice**—Mice were randomly divided into 5 groups of 6 animals each. Group I received distilled water (0.1 mL/100 g). Animals in Groups II, III and IV were treated with different doses (100, 200, 400 mg/kg, po) of EEAR. Animals in Group V received diazepam (2 mg/kg, ip) and served as positive control. All the treatments were given continuously for 15 days. On day 15, one hour after the drug treatment all the animals received 0.1 mL of 6% (v/v) glacial acetic acid ip and number of writhes was observed in all the groups for 20 min.

**Swimming endurance test in mice**—Mice were randomly divided into 5 groups of 6 animals each, as discussed above. All the treatments were given chronically for 10 days. On day 10, one hour after the drug treatment all the animals were allowed to swim individually in a glass tank filled with water. The immobility time of each mouse was recorded for 30 min.

**Restraint stress**—Mice were randomly divided into 6 groups of 6 animals each. Groups I and II received distilled water (1mL/100 g). Group III animals received *Withania somnifera* (Ashwagandha) root extract (100 mg/kg, po; positive control). Animals in Groups IV, V and VI received 100, 200 and 400 mg/kg, po doses of EEAR. All the treatments were given continuously for 12 days. On day 12, one hour after the last treatment, the forelimbs and hind limbs of the mice in Groups II, III, IV, V and VI were tied with adhesive tape thereby immobilizing them for 2 h. After the induction of stress for 2 h, the adhesive tapes were removed and blood was collected from retroorbital plexus of the stressed and nonstressed mice. The mice were then sacrificed and their brain, adrenal glands and spleen were removed. The adrenal glands and spleen were weighed. The blood obtained from retroorbital plexus was centrifuged and the serum obtained was used for the estimation of blood glucose, triglyceride and cholesterol levels using commercial kits.

**Assessment of oxidative stress**—Mouse brain was rinsed with 0.9% ice-cold normal saline and processed to get 10% homogenate in cold buffer using glass Teflon homogenizer. The homogenates were used to estimate lipid peroxidation, reduced glutathione (GSH), protein and nitrite levels.

**Statistical analysis**—Results were expressed mean±SE. The intergroup variation was measured by One Way Analysis of Variance (ANOVA) followed by Tukey’s test. Statistical significant difference was considered at *P*<0.05. The statistical analysis was done using Jandal sigma stat statistical software version 2.0.

**Results**

**Effect of EEAR against chemical induced stress**—*A. racemosus* root extract significantly decreased the
number of writhes in mice compared to the vehicle control group and a dose dependent effect was observed (Table 1). The percentage inhibition in number of writhes was found to be 58.0, 74.7 and 80.9% with 100, 200 and 400 mg/kg doses of A. racemosus respectively. Diazepam produced 87% inhibition in number of writhes.

Effect of EEAR in swimming endurance test—In swimming endurance test, all the three doses of EEAR significantly and dose dependently decreased the immobility time as compared to the vehicle control group. Diazepam also significantly reduced the immobility time compared to the vehicle control (Table 1).

Effect of EEAR on different biochemical parameters in restraint stress model—Restraint stress significantly elevated the level of glucose, triglycerides and cholesterol in mice as compared to vehicle control (non stressed). Mice treated with EEAR (100 mg/kg) did not significantly reduced the blood glucose level elevated by stress, however 200 mg/kg and 400 mg/kg doses significantly reduced the blood glucose level as compared to stress control (Table 2). Cholesterol and triglyceride levels (Table 2) were significantly lowered by all the three doses of EEAR as compared to stress control. Reduction of increased cholesterol level following stress by EEAR (400 mg/kg) was comparable to vehicle control.

Effect of EEAR on different oxidative stress parameters in brain—Stress increased the level of lipid peroxidation and nitric oxide in mouse brain and decreased the level of protein and glutathione (Table 3). EEAR at doses 400 mg/kg significantly reduced the elevated levels of lipid peroxidation in the mice brain but insignificant effect was observed at 100 mg/kg and 200 mg/kg doses. Elevated nitric oxide levels following stress was significantly reduced by all the three doses of EEAR. Ashwagandha (positive control) significantly reduced both lipid peroxidation and nitric oxide levels in mouse brain (Table 3). Reduced protein levels observed following stress was significantly increased

Table 1—Effect of Asparagus racemosus on chemical induced stress and swimming endurance test
[Values are mean±SE from 6 animals in each group. Figures in parentheses represents % inhibition]

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of writhes</th>
<th>Immobility time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle Control</td>
<td>42±6.696</td>
<td>27.02±0.81</td>
</tr>
<tr>
<td>EEAR, 100 mg/kg</td>
<td>17.6±6.592*</td>
<td>23.49±1.03* (13.09)</td>
</tr>
<tr>
<td>200 mg/kg</td>
<td>10.6±3.501*</td>
<td>22.32±0.656* (17.38)</td>
</tr>
<tr>
<td>400 mg/kg</td>
<td>8±3.764*</td>
<td>21.37±0.388* (20.93)</td>
</tr>
<tr>
<td>Diazepam (2 mg/kg)</td>
<td>5.4±0.2±1.159*</td>
<td>17.4±0.568* (35.6)</td>
</tr>
</tbody>
</table>

P values:<0.05* significantly different from vehicle control

Table 2—Effect of Asparagus racemosus on different biochemical parameters in blood after restraint stress
[Values are mean±SE from 6 animals in each group]

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood glucose (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle Control</td>
<td>75.34±6.13</td>
<td>60.46±1.04</td>
<td>36.38±2.49</td>
</tr>
<tr>
<td>Stress control</td>
<td>159.29±7.83**</td>
<td>134.66±1.94**</td>
<td>98.46±2.26**</td>
</tr>
<tr>
<td>EEAR, 100 mg/kg</td>
<td>137.36±4.78</td>
<td>116.85±2.58*</td>
<td>81.16±1.32*</td>
</tr>
<tr>
<td>200 mg/kg</td>
<td>99.21±8.79*</td>
<td>102.90±2.90</td>
<td>76.90±1.40*</td>
</tr>
<tr>
<td>400 mg/kg</td>
<td>86.43±15.02*</td>
<td>70.44±5.64*</td>
<td>51.54±0.99*</td>
</tr>
<tr>
<td>Ashwagandha (100 mg/kg)</td>
<td>108.93±13.61*</td>
<td>93.02±2.51*</td>
<td>68.32±1.90*</td>
</tr>
</tbody>
</table>

P values:<0.05; significantly different from * stress control; ** vehicle control

Table 3—Effect of Asparagus racemosus on different biochemical parameters in brain after restraint stress.
[Values are mean±SE from 6 animals in each group]

<table>
<thead>
<tr>
<th>Group</th>
<th>Lipid peroxidation (n moles of MDA/mgpr)</th>
<th>Glutathione (n moles of GSH/mgpr)</th>
<th>Protein (µg/dl)</th>
<th>Nitric oxide (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle Control</td>
<td>1.28±0.05</td>
<td>30.4±0.92</td>
<td>77±1.84</td>
<td>81±8.42</td>
</tr>
<tr>
<td>Stress control</td>
<td>12.71±1.12 **</td>
<td>4.75±0.48 **</td>
<td>7.75±0.47 **</td>
<td>180±0*</td>
</tr>
<tr>
<td>EEAR, 100 mg/kg</td>
<td>10.10±1.04</td>
<td>7.75±0.63*</td>
<td>8.25±0.47</td>
<td>147.50±4.78*</td>
</tr>
<tr>
<td>200 mg/kg</td>
<td>9.45±0.134</td>
<td>9.25±1.31†</td>
<td>9.0±0.40</td>
<td>135.00±5.00*</td>
</tr>
<tr>
<td>400 mg/kg</td>
<td>5.69±1.33†</td>
<td>19.0±2.74*</td>
<td>11.8±1.11*</td>
<td>100.00±8.16*</td>
</tr>
<tr>
<td>Ashwagandha (100 mg/kg)</td>
<td>7.197±0.64*</td>
<td>18.2±3.79*</td>
<td>12.5±1.44*</td>
<td>127.50±7.50*</td>
</tr>
</tbody>
</table>

P values:<0.05; significantly different from * stress control; ** vehicle control
by 400 mg/kg dose of EEAR. However glutathione levels were increased significantly by all the three doses of EEAR. *Ashwagandha* was effective in increasing both the protein levels and glutathione levels in brain of stressed animals (Table 3).

**Effect of EEAR on weight of adrenal glands and spleen**—Restraint stress caused a significant increase in the weight of adrenal glands and decreased the weight of spleen. All the three doses of EEAR significantly reduced the weight of adrenal glands and increased the weight of spleen in a dose dependent manner (Table 4).

**Discussion**

Central neurotransmitters are functionally involved in the regulation of stress responses and are released in response to stress to strengthen the organisms by providing resistance against the stressful events, a process known as adaptation. Prolonged severe stress creates ineffective adaptation, which results in reduced stamina and mood. Studies have shown reduced brain levels of adrenaline and noradrenaline in animals exposed to stress such as the swimming test and immobilization stress. Under stressful conditions, utilization and synthesis of these amines are increased in various regions of the brain. However, if the stress persists and becomes uncontrollable, the utilization of the amines exceeds synthesis thereby resulting in their depletion.

The present study demonstrated that ethanolic extract of roots of *A. racemosus* reduced the number of writhes significantly as compared to vehicle control, thus demonstrating antistress activity. Increased swimming endurance in mice, pretreated with adaptogens has been reported and this model is used to evaluate agents with adaptogenic properties. In swimming endurance paradigm, animals forced to swim in water eventually assume a characteristic immobile posture which reflects a state of tiredness, fatigue, reduced stamina or depressed mood. These signs represent the core symptoms observed in depressed patients and in individual under intense stress. Drugs with anti-stress property reduce the duration of immobility in animals. In the present study EEAR also reduced the immobility time in swimming endurance test and thus further supports its antistress potential. Diazepam used as a positive control significantly reduced the chemical writhing and immobility time in both chemical induced stress and swimming endurance test. As discussed earlier the chemical constitution of *Asparagus racemosus* consists of steroidal saponins, flavonoids and polyphenols. Steroidal saponins have glucocorticoid like effects and thus they are responsible for reducing the stress by fighting fatigue and adapting the body to cope with the stress. Activation of the hypothalamic-pituitary-adrenal axis results in secretion of corticotrophin hormone, adrenocorticotropic hormone, and glucocorticoids into the circulation. Release of adrenocorticotropic hormone in stress stimulates adrenals to increase production of epinephrine and norepinephrine and these hormones have profound effect on metabolic functions. Increased plasma cortisol influences the mobilization of stored fat and carbohydrate reserves which in turn increase blood glucose, cholesterol and triglyceride levels. When mice were subjected to restraint stress in the present study, their blood glucose, cholesterol and triglyceride levels increased. *A. racemosus* root extract significantly reduced the rise in blood glucose. Stress induced rise in cholesterol and triglyceride levels were also reduced by all the three doses of *A. racemosus*. A number of studies have shown that saponins from different sources lower serum cholesterol levels in a variety of animals including human subjects. The nervous system is extremely sensitive to peroxidative damage following restraint stress as it has high oxygen tension and is rich in oxidizable substrates and low in antioxidant capacity. Lipid peroxidation enhances depletion of intracellular

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight of adrenal glands (mg)</th>
<th>Weight of spleen (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle Control</td>
<td>4.680±0.46</td>
<td>213.400±5.92</td>
</tr>
<tr>
<td>Stress control</td>
<td>14.575±1.51**</td>
<td>121.225±8.82**</td>
</tr>
<tr>
<td>EEAR, 100 mg/kg</td>
<td>10.175±0.80*</td>
<td>171.950±14.09*</td>
</tr>
<tr>
<td>200 mg/kg</td>
<td>6.900±0.50†</td>
<td>202.000±9.32†</td>
</tr>
<tr>
<td>400 mg/kg</td>
<td>5.725±0.18†</td>
<td>209.775±4.44†</td>
</tr>
<tr>
<td>Ashwagandha (100 mg/kg)</td>
<td>7.050±0.40*</td>
<td>187.275±2.28*</td>
</tr>
</tbody>
</table>

*p* values: <0.05; significantly different from * stress control; **vehicle control*
glutathione content and can be related to oxidative damage as glutathione serves as a first line of defense as an endogenous non-enzymatic antioxidant. It was found that lipid peroxidation and nitric oxide levels were highly increased in the stress control group as a result of oxidative damage. EEAR at 400 mg/kg dose reduced the lipid peroxidation but no significant effect was observed at doses 100 and 200 mg/kg. *A. racemosus* significantly lowered the nitric oxide level at all three doses. Restraint stress caused depletion in the glutathione and protein content of the brain in mice. The reduced glutathione level was significantly increased by all three doses of EEAR but protein level was increased only by 400 mg/kg dose.

Adrenal glands contain relatively large amounts of ascorbic acid and cortisol, which are markedly decreased and cause hypertrophy of adrenal glands when they are stimulated by stress or injection of adrenocorticotropic hormone. The weights of adrenal glands were thus increased following restraint stress. Pretreatment with *A. racemosus* extract might have prevented the depletion of ascorbic acid and cortisol and eventually decreased hypertrophy of adrenal glands. It was also effective in decreasing the atrophy of spleen. From the result of the present study it is concluded that *Asparagus racemosus* has potential protective effect against different paradigms of stress and the antistress property of *Asparagus racemosus* could be attributed to the presence of constituents like polyphenols, saponins and flavonoids.

**Acknowledgement**

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**References**


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