Antistress activity of ethanolic extract of *Emblica officinalis* fruits in stress induced biochemical and physiological perturbations

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ABSTRACT

*Emblica officinalis* is a plant with diverse ethnical medicinal uses. The plant has been explored for diverse pharmacological actions; here it is planned to screen fruit extract for Adaptogenic activity. Ethanolic extract of fruits of *Emblica officinalis* was investigated on acute anoxia stress tolerance test in Swiss mice. Further wistar rats were subjected to chronic cold restraint stress to gauge the antistress potential of the extract. Stimulation of hypothalamus pituitary adrenal axis in stressful condition alters plasma glucose, triglyceride, cholesterol and BUN. There is also alteration in blood cell counts. Pretreatment with extract significantly ameliorated the stress-induced variations in these biochemical levels and blood cell counts in both acute and chronic stress models. The extract treated animals showed increase in anoxia tolerance time in anoxia stress model. Treatment groups also reverted back increase in liver, adrenal gland weights and atrophy of spleen caused by cold chronic stress stress model. The results indicate that ethanolic extract of *Emblica officinalis* has significant Adaptogenic activity against variety of biochemical and physiological perturbations.

Key Words: Adaptogenic activity, Ethanolic extract, *Emblica officinalis*, anoxia tolerance, Cold restraint stress.

INTRODUCTION

Stress basically is a reaction of mind and body against change in the homeostasis. The productive stress is called Eustress while harmful stress is called Distress. If the stress is extreme, the homeostatic mechanisms of the organism become deficit and the survival of the organism is threatened. Under these conditions, stress triggers a wide range of body changes called General Adaptation Syndrome (GAS). The stimuli, which produce GAS, are called the Stressors and range from physical to psychological factors including cold, heat, infection, toxins, major personal disappointment etc. In the stress-filled environment we live in, successful adaptation to stress is a prerequisite for survival. In the indigenous system of medicine, there are many herbal drugs and formulations recommended to enable one to withstand stress without altering the physiological functions of the body. This, drug induced state of
resistance against aversive stimuli is termed as adaptogenic activity and the drugs, named adaptogens. Stress alters the equilibrium of various hormones which have a significant impact on the immune response in general. The status of immune system-immunosuppression versus immunopotentiation-will depend upon the net effect of these changes. Stress and depression have been shown to affect immune system functioning, with both immunosuppression and immune activation. [2] Correlations between depression and elevated susceptibility for infections or mortality rates have been observed and are associated with immune suppression.[3] The physiological reaction to stress involves alteration in the autonomic nervous system, the endocrine system and the immune system. The secretion of Glucocorticoids is a classic endocrine response to stress. Stressful stimulation influences antigen-specific as well as nonspecific reactions. [4] Many herbs reported in ancient literature have potent antistress activity and their utilities in current scenario need to be unveiled. Emblica oficinalis Gaertn., commonly known as amla (Hindi) and gooseberry (English), has been used in the traditional system of medicine to reduce fever, alleviate asthma, treat constipation and enhance digestion, strengthen the heart, benefit the eyes, enhance intellect and as a health tonic. [5] The fruits of Emblica oficinalis have potent antioxidant activity due to the presence of tannoids, tannins, vitamin C and flavonoids. [6] The pharmacological studies on Emblica officinalis fruit have revealed that it has good antioxidant [7], cytoprotective and immunomodulatory [8], antidiabetic [9], hypolipidemic [10], antitusssive [11], anticancer [12], cardioprotective [13], antulcerogenic [14], antiepileptic and hepatoprotective activity [15]. The root contains ellagic acid and lupeol and bark contains leucodelphinidin. The seeds yield a fixed oil (16%) which is brownish-yellow in color. It has the following fatty acids: linolenic (8.8%), linoleic (44.0%), oleic (28.4%), stearic (2.15%), palmitic (3.0%) and myristic (1.0%). Since Emblica officinalis has a number of medicinal properties and is a potent anti-oxidant, the present study was undertaken to evaluate the potential usefulness of fresh fruits of Emblica officinalis for antistress and adaptogenic activity in experimental animals. Withania somnifera, an established ayurvedic herb used as an adaptogen is used as reference standard [16].

MATERIALS AND METHODS

Plant material and extraction

Fresh fruits of Amla were collected from the local market of Rangareddy District, Hyderabad and the botanical authentication was done by Dr.Ram Chandra Reddy, Head, Department of Botany, Osmania University and Hyderabad and voucher specimen no. MRCP/07 is lodged in our research laboratory for further reference. The fresh fruits were sliced using a home slicer and the slices obtained were shade-dried, pulverized and passed through a 20-mesh sieve. The dried, coarsely powdered plant material was extracted with 99% ethanol using Soxhlet apparatus at a temperature below 60°C for 24 hours. The solvent was evaporated under vacuum, which gave semisolid mass (yield: 26% w/w) with respect to the dried powder. Oral suspensions containing 100mg/ml, 200mg/ml and 400mg/ml of the ethanol extract of Emblica officinalis were prepared in 1% w/v gum acacia and were used for the evaluation of Adaptogenic activity.

Animals

Swiss albino mice weighing 20-25 g and Albino Wistar rats weighing 150-250 g of either sex, 4 months of age were used for this study. The experimental animals were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at 25±3°C and 35-60% humidity). Standard pelletized feed and tap water were provided ad libitum. The Institutional Animal Ethical Committee (IAEC) of Malla Reddy College of Pharmacy, Hyderabad, approved the study.

Anoxia stress tolerance test in mice

Swiss mice of either sex were divided randomly into 5 groups, each group containing 6 mice. Group I mice received 0.1% gum acacia in saline; (vehicle control). Group II, III and IV mice were treated with ethanolic extract at doses of 100, 200 and 400 mg/kg, p.o. and stress. Group V mice were treated with Withania somnifera (100 mg/kg, p.o.) and stress. The drug treatment was carried out daily for a period of 21 days. At the end of each week i.e. 1st, 2nd and 3rd
weeks of drug treatment, the animals were exposed to the anoxia stress and anoxia tolerance time was noted. Hermetic vessel of one litre air capacity was used to induce anoxia stress \[17\]. Each animal was kept in the hermetic vessel and the time to show the first sign of convulsion was noted, and were immediately removed from the vessel and resuscitated if needed.

**Chronic cold restraint stress**

Rats of either sex (200-250g) were used for chronic cold restraint stress. Group I rats received 0.1% gum acacia in saline; (vehicle control). Group II rats were treated with 0.1% gum acacia in saline and stress; (negative control). Group III, IV and V rats were treated with ethanolic extract at 100, 200 and 400 mg/kg, p.o. and stress. Group VI rats were treated with *Withania somnifera* (100 mg/kg, p.o.) and stress (positive control). Rats were subjected to cold stress by exposing them to 4 ± 1°C, daily for 2hrs for a period of 10 days. \[17\]. Animals were sacrificed at the end of the study period and blood was collected for estimation of various biochemical parameters such as serum glucose levels, RBC count, total leukocyte count as well as lipid profile. Similarly the weights of organs i.e. liver, spleen and adrenal glands were also recorded.

**Statistical analysis**

All the values are expressed as mean ±SEM and data was analyzed by one-way ANOVA, using Graph pad INSTAT. The post-hoc analysis was carried out by Dunnett’s multiple comparison test to estimate the significance of difference between individual groups.

**RESULTS**

**Effect of ethanol extract in anoxia stress tolerance test:**

In the anoxia tolerance test (Fig.1) the extract at 100, 200 and 400 mg kg\(^{-1}\) doses statistically produced a dose dependent significant (P<0.05) increase in mean time to convulsion in mice subjected to anoxia stress.

**Effect of ethanol extract in cold restraint stress:**

In cold restraint stress, ethanol extract at 100, 200 and 400mg/kg offered significant (P<0.05) protection against the change in the weights of liver, spleen and adrenal gland (Fig 2, 3, 4) when compared to stress control. The extract dose dependently reduced the elevated levels of biochemical parameters (P<0.05) (Table 1).

**DISCUSSION**

Adaptogens are the substances meant to put the organisms into a state of non-specific heightened resistance in order to better resist stressor and adapt to extraordinary challengers. They normalize body functions, strengthen systems and functions that are compromised by stress and have a protective effect against a wide variety of environmental and emotional stress.

In present study, in acute and chronic stress models, the significant increase in blood glucose level was observed because; under stressful conditions adrenal cortex secretes cortisol in man and corticosterone in rats. Hyper secretion of cortisol helps in maintenance of internal homeostasis through the process of gluconeogenesis and lipogenesis \[18\]. Pretreatment with the *Emblica officinalis* as well as reference standard drug *Withania somnifera* significantly (P<0.05) reduced the elevated glucose levels indicating their suppressant effect on hyper activity of adrenal cortex and maintained the homeostatic mechanism.

The marked increase in serum cholesterol, triglycerides and BUN levels in stress induced animals is due to stimulation of hypothalamo-pituitary axis (HPA) and sympathetic system, resulting in, liberation of catecholamines and glucocorticosteroids, which inhibits the immune system at multiple sites like liver, kidney \[19\]. *Emblica officinalis* as well as reference standard drug *Withania somnifera* significantly (P<0.05) reduced the elevated serum cholesterol, triglycerides and BUN levels, which may be due to inhibition of stimulation of sympathetic nervous system.

The increase in weight of adrenals in stressed animals is due to the stress induced adrenomedullary response leading to increased production of corticotropic hormone that leads to increase in weight of adrenals \[18\]. *Emblica officinalis* and *Withania somnifera* has significantly (P<0.01) reduced the liver and adrenal gland weight, this may be due to the reversal of the stress induced adrenomedullary response and hence
decreased production of corticotrophic hormone. The decrease in weight of spleen may be due to
recruitment of lymphocytes to blood from spleen which results in squeezing of the spleen. The pretreatment with the Emblica officinalis and reference standard Withania somnifera significantly (P<0.01) increased the spleen weight. This may be
due to inhibition of recruitment of lymphocytes to blood from spleen.
During stress, heart rate, blood pressure and blood flow rate increases. To meet these extra demands RBC and WBC counts will be increased. In the present study the extract has decreased the elevated levels of RBC and WBC in both swimming endurance and cold restraint stress models. This study has also shown that the extract prolonged mean time to convulsion, which therefore demonstrate antistress property. The prolongation of

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glucose (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>BUN (mg/dl)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>79.03±0.01</td>
<td>88.8±3.63</td>
<td>72.9±1.85</td>
<td>25.03±1.69</td>
</tr>
<tr>
<td>Cold stress</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>122.5±0.04</td>
<td>157.03±2.14</td>
<td>105.4±2.65*</td>
<td>70.15±0.83</td>
</tr>
<tr>
<td>EO extract 100 mg/kg p.o.</td>
<td>112.35±0.7*</td>
<td>100±2.32*</td>
<td>91.4±2.58*</td>
<td>61.7±1.04*</td>
</tr>
<tr>
<td>EO extract 200 mg/kg p.o.</td>
<td>108.06±0.02*</td>
<td>88.14±4.52*</td>
<td>86.4±3.05*</td>
<td>56.17±1.25*</td>
</tr>
<tr>
<td>EO extract 400 mg/kg p.o.</td>
<td>104.8±0.08**</td>
<td>6.6±1.89**</td>
<td>81.08±1.25**</td>
<td>42.25±2.01**</td>
</tr>
<tr>
<td>W. Somnifera 100 mg/kg p.o.</td>
<td>103.2±0.02**</td>
<td>62.9±4.02**</td>
<td>91.89±1.32**</td>
<td>37.45±1.54**</td>
</tr>
</tbody>
</table>

The rats were treated with Withania somnifera (100 mg/kg), EO (100, 200, 400 mg/kg, p.o.) once daily for 10 days. Control rats were given gum acacia. Cold restraint stress was induced by exposing them to 4 ± 1°C, daily for 2 hrs for a period of 10 days. The animals were sacrificed; the blood collected and the biochemical parameters were estimated. The values are expressed as mean±SEM, n=6 in each group. "P<0.05 significant as compared to control, ""P<0.05, significant as compared to stress control, statistical test employed is ANOVA followed by dunnett’s t test.
Fig. 1. The mice were pretreated with *Withania somnifera* (100 mg/kg), EO (100, 200, 400 mg/kg, p.o.) once daily for 21 days. Control mice were given saline. Anoxia was induced at the end of 1st, 2nd and 3rd weeks and the anoxia stress tolerance time was noted. The values are expressed as mean±SEM, n=6. Significance at *P<0.05* when compared to control, **P<0.05** when compared to control as determined by ANOVA followed by Dunnett’s t test.

![Mean duration of tolerance time](image)

Fig 2. Effect of ethanolic extract of *Emblica officinalis* on Organ weights (Liver) in cold restraint stress in rats. The rats were pretreated with *Withania somnifera* (100 mg/kg), EO (100, 200, 400 mg/kg, p.o.) once daily for 10 days. Control rats were given gum acacia. Cold restraint stress was induced by exposing them to 4 ± 1°C, daily for 2hrs for a period of 10 days. The animals were sacrificed, the organs were isolated from the animals and the weights were noted. The values are expressed as mean±SEM, n=6 in each group. *P<0.05* significant as compared to control, **P<0.05**, significant as compared to stress control, statistical test employed is ANOVA followed by dunnet’s t test.

![Weight of liver](image)
Fig 3. Effect of ethanolic extract of *Emblica officinalis* on Organ weights (Spleen) in cold restraint stress in rats. The rats were pretreated with *Withania somnifera* (100 mg/kg), EO (100, 200, 400 mg/kg, p.o.) once daily for 10 days. Control rats were given gum acacia. Cold restraint stress was induced by exposing them to 4 ± 1°C, daily for 2hrs for a period of 10 days. The animals were sacrificed, the organs were isolated from the animals and the weights were noted. The values are expressed as mean±SEM, n=6 in each group. *P<0.05 significant as compared to control, **P<0.05, significant as compared to stress control, statistical test employed is ANOVA followed by dunnet’s t test.

![Weight of Spleen](image)

Fig 4. Effect of ethanolic extract of *Emblica officinalis* on Organ weights (Adrenal gland) in cold restraint stress in rats. The rats were pretreated with *Withania somnifera* (100 mg/kg), EO (100, 200, 400 mg/kg, p.o.) once daily for 10 days. Control rats were given gum acacia. Cold restraint stress was induced by exposing them to 4 ± 1°C, daily for 2hrs for a period of 10 days. The animals were sacrificed, the organs were isolated from the animals and the weights were noted. The values are expressed as mean±SEM, n=6 in each group. *P<0.05 significant as compared to control, **P<0.05, significant as compared to stress control, statistical test employed is ANOVA followed by dunnet’s t test.

![Weight of Adrenal gland](image)
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REFERENCES