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## Antistress, Adoptogenic and Immunopotentiating Activity Roots of *Boerhaavia diffusa* in Mice

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**Abstract:** Ethanolic extract of roots of *Boerhaavia diffusa* was evaluated for antistress, adoptogenic activity in albino mice, by swim endurance test and cold restrain stress. The extract improved the stress tolerance by significantly increasing the swim duration and reducing the elevated WBC, blood glucose and plasma cortisol. Immunomodulatory activity was evaluated by carbon clearance assay and delayed hypersensitivity test. The extract significantly increased carbon clearance, indicating the stimulation of reticuloendothelial system. The extract produced an increase in DTH response to SRBC in mice, which was comparable with that of Levamisol, indicating stimulatory effects on lymphocytes and accessory cell types required for the expression of reaction.

**Key words:** *Boerhaavia diffusa*, swim endurance test, cold restrain stress, cortisol, delayed hypersensitivity, carbon clearance assay

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### 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. (Seyle, 1973). 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 immunopotentiality-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 (Raison and Miller, 2001). Correlations between

depression and elevated susceptibility for infections or mortality rates have been observed and are associated with immune suppression (Irwin, 2002). 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 (Sapolsky *et al.*, 2000). Stressful stimulation influences antigen-specific as well as nonspecific reactions (Ader and Cohen, 1993).

Anti stress activity has been studied in a number of plants. *Boerhaavia diffusa* (common name: Punarnava) family Nyctaginaceae is a important indigenous medicine for the treatment of dyspepsia, jaundice, enlargement of spleen, abdominal pain, arthritis and asthma (Kirtikar and Basu, 1956). The roots of *Boerhaavia diffusa* are reported to be laxative, expectorant and useful in oedema, haemorrhage, anaemia, scanty urine, leprosy and skin diseases, insomnia, rheumatism, ocular disorders and in snake poisoning and rat-bite infection ([www.agri-history.org](http://www.agri-history.org)). The alcoholic extract of *Boerhaavia diffusa* was found to be devoid of any teratogenic effect (Singh *et al.*, 1991). The plant possesses diuretic and cardiogenic activity (Devi *et al.*, 1986), hepatoprotective (Rawat *et al.*, 1997), hypotensive (Hansen *et al.*, 1995), immunomodulating (Pandey *et al.*, 2005), antioxidant (Satheesh and Pari, 2004), anti-hemorrhaging (Barthwal and Srivastava, 1991), antispasmodic (Borrelli *et al.*, 2006), antimicrobial (Hilou *et al.*, 2006), pain-relieving (Hiruma-Lima *et al.*, 2000), cytotoxic (Leyon *et al.*, 2005)

and anticancerous activity (Bharali *et al.*, 2003). The present study is taken up to investigate the antistress, adaptogenic and immunomodulatory activity of roots of *Boerhaavia diffusa*.

## MATERIALS AND METHODS

**Animals:** Eight-week old Swiss albino mice of both sexes, weighing 20-25 g, maintained on natural light/dark cycle, at a temperature of  $25\pm 2^\circ\text{C}$ , commercial pellet diet (Lipton India, Bangalore) and water *ad libitum* were used in study. IAEC's permission was obtained before starting the experiments.

**Plant material and drugs:** Roots of *Boerhaavia diffusa* were collected from Hillgreen Pvt Ltd, Bangalore, in December 2003, dried in shade, coarsely powdered and subjected to Soxhlet extraction, using 40% ethanol, at a temperature below  $60^\circ\text{C}$  for 24 h. The extract was concentrated by distilling the solvent and air-dried. (12.5% w/w, reddish brown, solid). The extract was subjected to qualitative phytochemical analysis for presence of various constituents like alkaloids, sterols, sugars, glycosides, phenols and tannins, fixed oils and fats, flavonoids, saponins and gums and mucilages. A solution of the extract ( $10\text{ mg mL}^{-1}$ ) was prepared by dissolving it in distilled water for oral administration to animals. A water-soluble powder of Ashwagandha (Natural remedies, Bangalore) in a dose of  $100\text{ mg kg}^{-1}$  P.O. and Levamisole (Khandelwal Labs) in a dose of  $25\text{ mg kg}^{-1}$ , P.O. was used as reference standard anti-stress drugs (Dhuley, 2000) for comparison in this study.

**Acute toxicity:** The nature and extent of the untoward reactions following the administration of extract in graded doses up to  $3\text{ g kg}^{-1}$  body weight orally, was studied in albino mice. (OECD guidelines 423).

### Experimental

**Swim endurance test:** Group I mice ( $n = 6$ ) were administered extract  $100\text{ mg kg}^{-1}$  orally, using oral gavage, for 7 days. Group II mice were administered water-soluble powder of Ashwagandha  $100\text{ mg kg}^{-1}$  orally. On the 8th day, the animals were allowed to swim till exhausted in a propylene tank of dimension  $37\times 37\times 30\text{ cm}$ , filled with water to a height of 25 cm. The end point was taken when the animals drowned and swimming time for each animal was noted.

**Cold restraint stress:** Group I mice were administered extract  $100\text{ mg kg}^{-1}$  orally, using oral gavage, for 7 days. Group II mice were administered water-soluble powder of Ashwagandha (Natural remedies, Bangalore)  $100\text{ mg kg}^{-1}$

orally. On the 8th day, animals were individually placed in plastic containers of capacity 350 mL. They were immobilized in their normal position, using adhesive tape. The containers were placed in a fridge for 2 h. The blood was collected by heart-puncture method, in a heparinised tube and WBC count was done, blood glucose was determined and plasma cortisol level was determined (Varley *et al.*, 1984).

**Immunomodulatory activity:** Carbon clearance assay- Group I (Control) mice received 2% acacia gum, orally, Group II animals received Levamisole (Khandelwal Labs) in a dose of  $25\text{ mg kg}^{-1}$ , orally and Group III animals received the extract ( $100\text{ mg kg}^{-1}$ ), for 10 days prior to the injection of 0.1 mL of carbon suspension intravenously through tail vein. Blood samples were collected from retro-orbital plexuses just before and at 5, 10, 15 and 30 min, after injection of carbon suspension. An aliquot of 25  $\mu\text{L}$  of blood sample was lysed with 3 mL of distilled water and absorbance was measured spectrophotometrically at 650 nm using pre-injection blood sample as blank. The graph of absorbance against time was plotted for each animal in respective group. The rate of carbon clearance, termed as Phagocytic index is the slope of time concentration curve. The mean Phagocytic index was then calculated for each group (Pallabi *et al.*, 1998).

**Delayed type hypersensitivity-**Animals of group I, II and III were immunized on day 0 by an intraperitoneal administration of 200  $\mu\text{L}$  of  $5\times 10^9$  SRBC  $\text{mL}^{-1}$  and challenged by a subcutaneous administration of 25  $\mu\text{L}$  of  $5\times 10^8$  SRBC  $\text{mL}^{-1}$  into the right hind footpad on day +7. Group I (Control) mice were administered orally 2% acacia gum, Group II animals were administered Levamisole (Khandelwal Labs) in a dose of  $25\text{ mg kg}^{-1}$ , orally and Group III animals were administered orally the extract ( $100\text{ mg kg}^{-1}$ ), from day -14 until day +6. Delayed type hypersensitivity response was determined at 24 h after SRBC Challenge on day +7.

**Statistical analysis:** All the values are expressed as mean  $\pm$ SEM and data was analyzed by one-way ANOVA, using Graphpad INSTAT. The post-hoc analysis was carried out by Dunnet's multiple comparison test to estimate the significance of difference between individual groups.

## RESULTS

Qualitative phytochemical analysis of ethanolic extract of roots of *Boerhaavia diffusa* revealed that it contains alkaloids, carbohydrates, glycosides, triterpenoids, steroids and phenols and tannins. Acute

Table 1: Effect of *Boerhaavia diffusa* roots on stress (values are Mean±SEM)

Treatments	Swimming time (min)	Total WBC count (cells cumm <sup>-1</sup> )	Blood glucose (mg dL <sup>-1</sup> )	Plasma cortisol (µg 100 mL <sup>-1</sup> )
Control	-	5620.00±80.63	86.24±3.86	13.04±0.24
Stress control	419.81±10.55	7126.50±35.73*	137.50±4.43*	21.41±0.56*
<i>Boerhaavia diffusa</i> (100 mg kg <sup>-1</sup> ) (Group I)	523.07±16.05	5640.20±34.08**	87.55±2.83**	14.82±0.14**
Ashwagandha (100 mg kg <sup>-1</sup> ) (Group II)	716.17±27.28	5679.20±62.98**	85.85±3.52**	13.97±0.38**
F	45.011	241.48	333.33	44.798

n = 6, \*: p<0.001v/s control, \*\*: p<0.001v/s stress control

Table 2: Macrophage phagocytic activity: Mean absorbance-time data

Treatments	Time (min)				Mean
	5	10	15	30	
Control	0.786±0.060	0.542±0.010	0.383±0.050	0.278±0.001	0.032±0.0065
Levamisole (25 mg kg <sup>-1</sup> p.o)	0.691±0.070	0.387±0.025	0.218±0.018	0.101±0.051	0.0504±0.0031
<i>Boerhaavia diffusa</i> (100 mg kg <sup>-1</sup> p.o)	0.679±0.012	0.407±0.076	0.286±0.014	0.114±0.030	0.046±0.0025

toxicity studies with extract revealed that LD<sub>50</sub> is more than a dose of 3 g kg<sup>-1</sup> body weight. As shown in Table 1, the extract improves significantly (p<0.001) the swim duration and reduces the elevated WBC, blood glucose and plasma cortisol levels.

Table 2 shows that in control, Levamisole treated and *Boerhaavia diffusa* treated group, mean absorbance at 650 nm was found to reach its peak value at 5 minutes after injection of carbon suspension which progressively decrease up to 30 min. To assess the functional changes in macrophage, in response to treatment, their phagocytic ability was determined. The phagocytic index of Levamisole and *Boerhaavia diffusa* is found to be 1.57 and 1.44, respectively, which indicate that Levamisole and *Boerhaavia diffusa* caused stimulation of reticuloendothelial activity markedly.

## DISCUSSION

Rodents, when forced to swim in a restricted space become immobile after an initial period of vigorous activity. This immobility signifies behavioral despair, resembling a state of mental depression (Thiebot *et al.*, 1992). The increase in total swimming time of *Boerhaavia diffusa* treated mice indicates better stress tolerance (Archana and Namasivaya, 1999). Cold stress typically increases the total leukocytes count, eosinophils and basophils (Sundaresan *et al.*, 1990). Plant adaptogens are smooth prostressors which reduce the reactivity of host defense system and decrease the damaging effects of various stressors due to increased basal levels of mediators involved in the stress response (Panossian *et al.*, 1999). Since the stress induced increased total WBC count is decreased by the extract of *Boerhaavia diffusa*, it indicates antistress, adaptogenic activity. Increased swim duration and decrease in total leucocyte in mice pretreated with *Boerhaavia diffusa* are similar to the changes produced by reference drug Ashwagandha.

During stress, blood glucose level increases (Dominiczak, 1999), which is found to be significantly reduced in *Boerhaavia diffusa* treated mice. Lowering of stress induced hyperglycemia is an indication of antistress, adaptogenic activity of plant (Sen *et al.*, 1992). In response to stress, ACTH is released, which acts on the adrenal cortex to stimulate the synthesis and release of cortisol (Sadock and Sadock, 2003). Increased plasma cortisol influences the mobilisation of stored fat and carbohydrate reserves (Tache and Selye, 1976), which in turn increases blood glucose level. The increased cortisol levels are reversed by anti-stress agents (Sen *et al.*, 1992). *Boerhaavia diffusa* significantly decreased stress induced elevated levels of cortisol. The reference drug in this study, Ashwagandha, also produced similar results.

Immune system plays a pivotal role in stress (Anisman *et al.*, 2002). The Reticuloendothelial system (RES) is a multiorgan system whose primary function is phagocytosis. The rate of removal of colloidal carbon by sessile intravascular phagocytes in the Liver and Spleen is a measure of Reticuloendothelial phagocytic activity. A significant increase of Carbon Clearance by RES was seen after administration of extract and Levamisole in the present study, indicating the stimulation of RES phagocytic activity. This phagocytic activity, measured as phagocytic index is actually a measure of the phagocytic activity of the macrophages of the RES. Activated macrophages secrete a number of cytokines such as GM-CSF and IL-1 which in turn stimulate other immunocytes like neutrophils (Drews *et al.*, 1982). Delayed Type Hypersensitivity (DTH) is a cell mediated response (mediated by sensitized lymphocytes), which manifest after 2-3 days of exposure to the sensitizing antigen. It induces a local inflammatory response, due to the influx of macrophages and neutrophils. These cells are recruited under the influence of cytokines, which collectively induce extravasation and chemotaxis of circulating monocytes and neutrophils, myelopoiesis and activate macrophages (Cotran *et al.*, 1994).

Administration of Levamisole, *Boerhaavia diffusa* produced an increase in DTH response to SRBC in mice which, corresponds to cell mediated immunity and indicates that the extract of *Boerhaavia diffusa* has stimulatory effects on lymphocytes and accessory cell types required for the expression of reaction. Our results agree with the previous reports (Godhwani *et al.*, 1988; Subramoniam *et al.*, 1996) of the immunopotentiating effects of *W. somnifera*, *O. sanctum* and *E. officinalis*.

### CONCLUSION

Present study indicates that roots of *Boerhaavia diffusa* posses antistress, adoptogenic and immunopotentiating activity.

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