



ANTISTRESS ACTIVITY OF *BOERHAAVIA DIFFUSA* ROOT EXTRACT AND A POLYHERBAL FORMULATION CONTAINING *BOERHAAVIA DIFFUSA* USING COLD RESTRAINT STRESS MODEL

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ABSTRACT

Hydroethanolic extract (80%) of *Boerhaavia diffusa* (HEBD) and a polyherbal formulation (Punarnava mandur) PHF-09 containing *Boerhaavia diffusa* were compared for their antistress activity using Cold restraint stress model. Punarnava mandur contains *Boerhaavia diffusa*, mandur bhasm, varun, bharangi. Stress was induced by subjecting animals to Cold restraint. Due to Cold restraint stress there was an imbalance in the levels of biochemical parameters like glucose, triglycerides, cholesterol, SGOT, SGPT which were near normalized following the administration of HEBD and PHF-09. HEBD and PHF-09 were found to have comparable antistress activity.

Keywords: HEBD, Punarnava mandur, Cold restraint stress

INTRODUCTION

In this modern era, stress has become an integral part of human life.¹ It is vital that stress is kept under control and normal functioning is not hampered due to excessive stress.² Stress is considered to be any condition which results in perturbation of the body's homeostasis.³ If the level of stress is extreme, the homeostatic mechanisms of the organism become deficit and the survival of the organism is threatened.⁴ Stress has been postulated to be involved in the etiopathogenesis of a variety of disease states, viz; hypertension, peptic ulcer, diabetes, immunosuppression, reproductive dysfunctions and behavioural disorders like anxiety due to involvement of the central nervous system (CNS), endocrine system, and metabolic system.⁵

Drugs having antistress properties induce a state of non-specific resistance against stressful conditions. Drugs like benzodiazepines, certain CNS stimulants such as amphetamines and caffeine as well as some anabolic steroids are routinely used by people to combat stress. The incidence of toxicity and dependence has limited the therapeutic usefulness of these drugs.⁶

Herbal formulations have been in use for many years not only in Asian countries but also globally for human well-being. The herbal formulations claimed to enhance physical endurance; mental functions and non-specific resistance of the body have been termed as adaptogens.⁷ The potential utility of safer and cheaper herbal medicines as antistress agents have been reported as they can withstand stress without altering the physiological functions of the body. Various herbs like *Withania somnifera*, *Emblica officinalis*, *Asparagus racemosus*, *Ocimum sanctum*, *Tribulus terrestris* and *Piper longum* are claimed to have immunomodulatory, adaptogenic, anabolic effects and the ability to improve vital energy.⁸

Herbal medicines are known to act synergistically in combination. Polyherbal formulation (Punarnava mandur) PHF-09 is composed of punarnava, mandura bhasm, varun and bharangi.

Boerhaavia diffusa (Family: *Nyctaginaceae*) is commonly known as "Punarnava" in the Indian system of medicine. The roots of *Boerhaavia diffusa* have been found to have anti-inflammatory, fibrinolytic and anti-convulsant activities.⁹ The plant is also reported to have adaptogenic and antistress activity.¹⁰

OBJECTIVE

The main objective of present study is to establish the antistress activity of hydroethanolic extract of *Boerhaavia diffusa* (HEBD) and to compare its antistress potential to that of a polyherbal formulation (PHF-09).

MATERIALS AND METHODS

Dried root powder of *Boerhaavia diffusa* was obtained from Yucca enterprises, Dadar, Mumbai. Extraction of finely powdered roots of *Boerhaavia diffusa* was carried out by using 80% ethanol as a solvent in a Soxhlet apparatus at 70°C. The extract was then dried on the water bath at 70°C and stored in air tight container at 4°C. This HEBD was compared with PHF-09 for its anti-stress activity.

Biological study

Albino rats of wistar strain (weighing 150-200 g) of either sex, were obtained from registered breeder Haffkine Institute for training, research and testing, Parel, India. They were housed under standard conditions of temperature (24±1°C), relative humidity (65±10 %), 10-h light and 14-h dark cycle and fed with standard pellet diet with water *ad libitum*. All experimental protocols involving animal studies were approved after careful scrutiny by the Institutional Animal Ethics Committee (IAEC).

Drugs

Hydroethanolic extract of *Boerhaavia diffusa* was administered at a single dose level and PHF-09 was administered at two dose levels. Normal saline water was used as control. Diazepam was used as standard.

Experimental method

Cold restraint stress model was used for evaluating antistress activity. Animals were divided into six groups of six animals each. Group 1 negative control (Normal) group did not receive any stress or treatment, Group 2 positive control (stress) group did not receive any treatment, Group 3 received standard (Diazepam 2 mg/kg p.o.), Group 4 received punarnava extract (300 mg/kg p.o.), Groups 5 and 6 received 300 mg/kg p.o. & 600 mg/kg p.o. of PHF-09 respectively.

Stress was induced to all groups except normal control group by subjecting animals to Cold restraint stress by immobilizing them at 4°C for 4 hours daily in cylindrical cage over a period of 7 days. Standard and test drugs were administered to the respective groups for all 7 days.¹¹ Animals were humanely sacrificed on the 7th day using ether. Blood (2-4ml) was withdrawn by cardiac puncture and serum was separated to study various biochemical parameters like Glucose (GLU), Triglycerides (TG), Cholesterol (CHOL), SGOT and SGPT. Adrenal glands were removed aseptically and relative adrenal gland weight was measured:

Relative adrenal gland weight= Adrenal gland weight / body weight.

Statistical analysis

All the values were expressed as mean \pm S.E.M (n=6). Statistical evaluation of the results was carried out using Analysis of Variance (ANOVA) followed by Dunnett's test. P values <0.05 were considered significant.

RESULTS AND DISCUSSION

The HEBD and PHF-09 showed antistress activity in dose dependent manner. Cold restraint stress resulted in significant decrease in glucose & triglyceride levels. Triglyceride level was near normalized following the administration of HEBD and PHF-09, the effect being more profound following treatment with PHF-09 at 600 mg/kg p.o. as compared to HEBD treatment. A significant increase was observed in the levels of cholesterol, SGOT, SGPT in stress control group as compared to normal control group as shown in Figures 1 and 2.

Decrease in blood glucose level was probably due to depletion of energy stores due to repeated stress. There is a close relationship between catecholamines and triglycerides. Therefore, it could be suggested that the decrease in the level of serum triglyceride is mediated via adrenal medullary secretions and through activation of sympathetic nervous system. Both catecholamines and corticosteroids have a lipolytic effect on the adipose tissue, stimulating fat mobilization and increasing circulating free fatty acids¹² which probably results in an increase in plasma cholesterol concentration after Cold restraint stress.

Cold restraint stress resulted in significant increase in enzyme levels namely SGOT and SGPT and this pattern was reversed by HEBD and PHF-09 treatment. SGOT and SGPT are markers of hepatic functional status as they indicate parenchymal liver damage. SGOT and SGPT levels were increased in stress group possibly because of the alterations in the membrane permeability which might occur in the cells during stress. These changes also represent a functional alteration in the cell membrane due to steroidal storms, which occur during stress.¹³

Relative adrenal gland weight was increased due to Cold restraint stress which was near normalized following the administration of HEBD and PHF-09. Exposure to Cold restraint stress resulted in adrenal hypertrophy, indicating the active involvement of the hypothalamic-pituitary-adrenal (HPA) axis, which is highly responsive to stress. Adrenal hypertrophy takes place in response to the secretion of adrenocorticotrophic hormone (ACTH) from the pituitary due to increased corticosterone levels from cortical cells to combat stress¹⁴ as shown in Table 1. Both HEBD and PHF-09 exhibited comparable antistress activity.

Phytochemical constituents like flavonoids, alkaloids, glycosides and sterols have been reported to be present in the alcoholic root extracts of *Boerhaavia diffusa*. The antistress activity of *Boerhaavia diffusa* is mainly attributed to these constituents with established antioxidant activity.¹⁰

Table 1: Antistress activity of hydroethanolic extract of *Boerhaavia diffusa* and polyherbal formulation (PHF-09) on relative adrenal gland weights in Cold restraint stress model

Groups/Parameter	Normal group	Stress group	Standard	Punarnava extract	Polyherbal formulation	
			(Diazepam 2mg/kg)	300mg/kg	300mg/kg	600mg/kg
Relative Adrenal gland weight=	0.0001011 \pm 0.002216	0.0001987 \pm 0.000006994 ^a	0.0001385 \pm 0.00001721 ^{**}	0.0001116 \pm 0.000007215 [*]	0.000115 \pm 0.00001485 [*]	0.001295 \pm 0.00001689 [*]

All values expressed as Mean \pm SEM, n = 5;

One-way ANOVA followed by Dunnett's test is applied for statistical analysis, All treated groups were compared with stress group.

* Significant at p < 0.01, ** Significant at p < 0.05

Stress control group was compared with normal control group at ^ap<0.01.

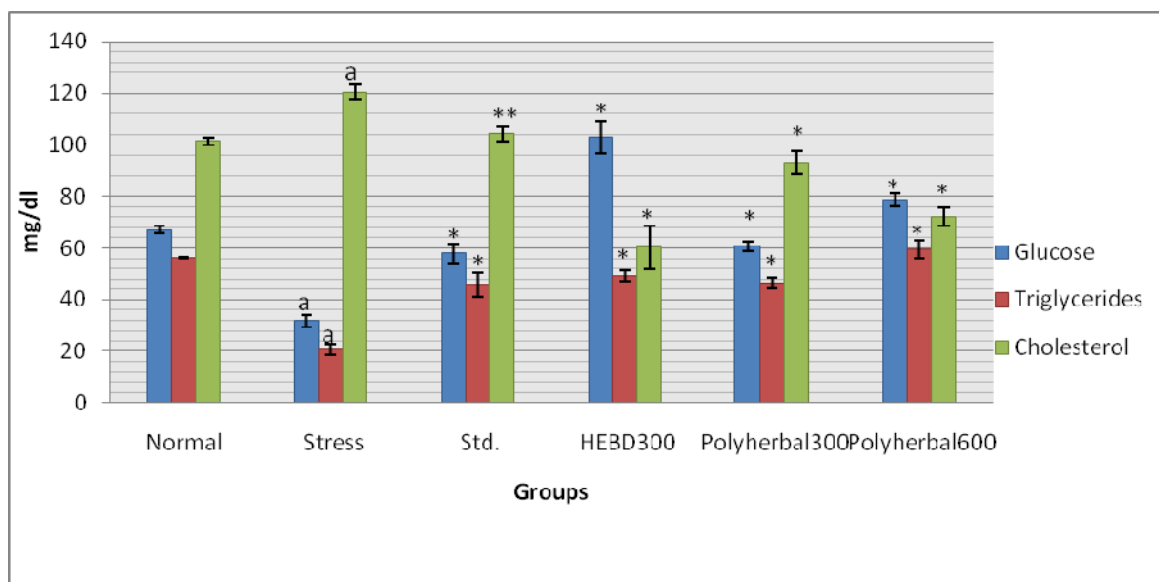


Fig. 1: Antistress activity of hydroethanolic extract of *Boerhaavia diffusa* and polyherbal formulation (PHF-09) in Cold restraint stress model

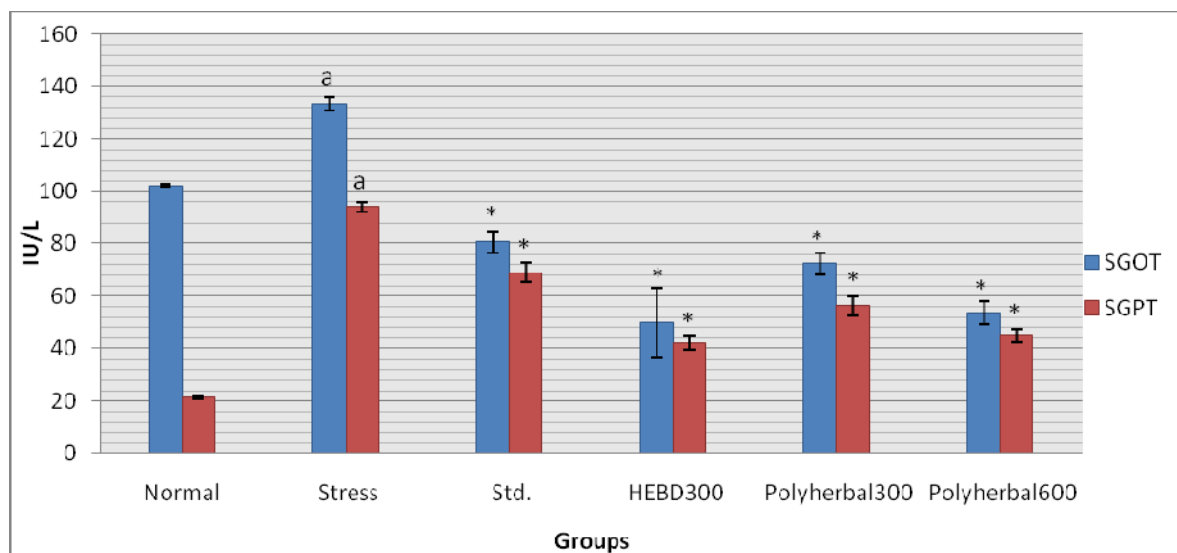


Fig. 2: Antistress activity of hydroethanolic extract of *Boerhaavia diffusa* and polyherbal formulation (PHF-09) in Cold restraint stress model

All values expressed as Mean \pm SEM, n = 6;

One-way ANOVA followed by Dunnett's test is applied for statistical analysis,

All treated groups were compared with stress group.

* Significant at $p < 0.01$, ** Significant at $p < 0.05$

Stress control group was compared with normal control group at ^a $p < 0.01$.

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