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Bacopa monnieri – a Preliminary Study Evaluating Its Anti-Stress Activity in Swiss Albino Mice

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ABSTRACT

Investigations were carried out on the adaptogenic property of an ethanolic extract of *Bacopa monnieri* against acute stress model in mice. Male mice were exposed to swim endurance test and cold restraint stress (4^0C for 2 hours) after 7 days of pretreatment with the extract. Panax root powder (*Panax ginseng* 100 mg/kg, p.o) was taken as a standard and the control group was administered distilled water. Mice were sacrificed immediately after stress, the blood was collected, plasma and serum were separated out for biochemical estimation. Adrenals, spleen and thymus were dissected for organ weight. Acute exposure significantly increased the adrenal gland weight, plasma cortisol, blood glucose, triglycerides, total WBC count, eiosinophils and basophils but significantly decreased the spleen weight. However no significant change was observed in the serum cholesterol level. Pretreatment with *Bacopa monnieri* 27 mg/kg (p.o), significantly reduced the acute state increase in the adrenal gland weight, plasma cortisol, blood glucose, triglycerides and total WBC count. Panax root powder significantly reversed stress-induced increase in adrenal gland weight. On the basis of our result, it is concluded that the ethanolic extract of *Bacopa monnieri* possesses a potent adaptogenic activity.

Key words: Adaptogenic, *Bacopa monnieri*, *Panax ginseng*, Adrenal gland, Cortisol.

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INTRODUCTION

Adaptogenic herbs are used to strengthen the body's immune response and increase an individual's ability to cope up with physical and mental stress. They are also used to increase overall vitality. The use of adaptogens eliminates or significantly decreases the classical signs of the prolonged stress reaction. Adaptogens help to maintain homeostasis in the face of stress by regulating the body's adaptive reactions. Adaptogens help the body utilize fuel more efficiently, with fewer toxic or waste byproducts (like lactic acid), which can contribute to fatigue and reduced function. A number of plants possess adaptogenic activity due to diverse class of chemical compounds [1], [7].

Bacopa monnieri, also known as Water Hyssop (Brahmi), is prominently used in Ayurveda, a holistic system of medicine originating from India [32]. Historically, the use of *Bacopa monnieri* dates back to approximately 6th century AD. Today practitioners of Ayurveda recognize *Bacopa monnieri* as an adaptogen, a physiological agent that naturally increases the body's resistance to physical and emotional stress [2-4], [17].

The brahmi herb contains saponins as bacoside A and B; sapogenins-bacogenins A1-A4, betulic acid and alkaloids as principal constituents. The alkaloids are brahmine, herpestine, hydrocotyline, dihydrocotyline and centoloides along with 3 bases like B-1-oxalate, B-2-acetate and B-3-chloroplantinate. The saponins are - bacoside-A, bacoside-B, brahmoside, brahminoside, asiaticoside, monnierin, hersaponin and madecacoside. Besides these α-amirin, three sugars, two glucose, rhymnose, arabinose, arabinosal, bacogenins, δ-mannitol, β-sitosterol, stigma sterol, tannins along with acids as brahmic acid, isobrahmic acid, betulic acid, centoic acid and centolic acids. Two common flavonoids, luteolin and apigenin, are present in *Bacopa monnieri*. The presence of a-alanine, aspartic acid, glutamic acid and serine is also reported [6].

Brahmi is the main revitalizing herb for the nerves and the brain cells. The herb is known for its ability to build and improve mental performance. Improves both short-term and long-term memory. Strengthens adrenal processes that facilitate carbohydrate metabolism [5], [6]. It increases intelligence, longevity, circulation in the brain; it decreases senility and ageing. It fortifies the immune system, both cleansing and feeding it. At the same time, it is a powerful blood purifier and is specific for chronic skin diseases, including leprosy and syphilis, as well as eczema and psoriasis. It is valuable in intermittent or periodic fevers, like malaria. It is a relaxant to the whole nervous system and increases sex drive; it is cerebrovascular stimulating, a circulatory stimulant, and it accelerates wound healing [8-15]. The present study has been undertaken to evaluate the anti-stress activity of *Bacopa monnieri* in Swiss albino mice.

MATERIALS AND METHODS

Plant material leaves of *Bacopa monnieri* were collected, dried in shade, and finely powdered. The powder was soaked in absolute ethanol (95%) and left for 48 hours. The

supernatant was collected and the residue was further soaked in absolute ethanol (95%) for 24 hours. The supernatant was collected and filtered. The filtrate was subjected to Rota vapour extraction at a temperature below 60^{o}C for 24 hours. The concentrated form of the extract was obtained and freeze-dried.

The study was conducted on healthy, adult, male albino mice having a body weight of 35 $\pm$ 5 g. They were acclimatized to laboratory conditions for
2 weeks prior to experimentation. Animals were housed in propylene cages (6 mice/cage) in a mice experimentation laboratory at a temperature of 25^{o}C $\pm$ 2^{o}C with 12 – 12 h dark - light cycle. They were provided with standard food and water ad libitum. Institutional animal ethical committee (I.A.E.C) approval was obtained before the experiment and care was taken to handle the mice in humane manner. All the chemicals used in the present study were obtained from Euro Diagnostics (Mumbai, India), India Scientific Company (Patna, Bihar) and Bihar Scientific Corporation (Patna, Bihar).

Experimental

The adult animals (8 weeks old) were divided into 4 groups (n = 6 in each group) as follows: Group I consisted of Normal control (NC), these mice remained undisturbed in the home cage throughout the experimental period. Group II consisted of Stress control (SC), which were fed with equivolume of distilled water orally for 7 days. Group III (Stress+*P.ginseng)* consisted the standard group, these mice were fed with aqueous root powder of *Panax ginseng* (p.o), for 7 days. Group IV consisted of (Stress+*B.monnieri*), treatment group which were fed with ethanolic extract of *Bacopa monnieri* (p.o), for 7 days.

Stress Procedure

Swim Endurance Test: The mice in group IV were given ethanolic extract of *Bacopa monnieri* 27 mg/kg (p.o), for 7 days. The standard group (III) was administered water soluble root powder of *Panax ginseng* 100 mg/kg (p.o), while the stress control group (II) was administered distilled water orally, for 7 days.

On the 8th day, the animals were allowed to swim till exhausted in a propylene tank of dimension 24 cm* 17 cm* 14 cm, filled with water to a height of 10 cm. The end point was taken when the animals drowned and 'swimming time'
for each animal was noted. The mean swimming time for each group was calculated and the data was statistically analyzed (Kumar et al., 1999).

Cold Restraint Stress: The mice in group IV were given ethanolic extract of *Bacopa monnieri* 27 mg/kg orally (p.o), for 7 days. The standard group (III) was administered water soluble root powder of *Panax ginseng* 100 mg/kg (p.o), while the stress control group (II) was administered distilled water for 7 days, orally.

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On the 8th day, the 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 cold chamber maintained at 4°C for 2 hours. The blood was collected by orbital sinus veinpuncture method in a heparinised tube and the following investigations were carried out. Total WBC count was done using Neubauer's chamber, blood glucose was determined by GOD/POD method, plasma cortisol was determined by Enzyme Linked Immunosorbent Assay (ELISA) [20], serum triglyceride was determined by GPO-POD method, total cholesterol was determined by CHOD-POD method and HDL cholesterol was determined by CHOD-PAP method [33].

Statistical Analysis

Data was analyzed by the application of one way analysis of variance (ANOVA) using Graph pad in stat software. *P*<0.01 was considered to be significant.

RESULTS

Acute toxicity studies with extract revealed that LD_{50} is 17g/kg body weight, (p.o). As shown in figure 1, the extract of *Bacopa monnieri* improves swim duration in mice. Mice pretreated with ethanolic extract of *Bacopa monnieri* 27 mg/kg (p.o), and water soluble root powder of *Panax ginseng* 100mg/kg (p.o), show significant improvement in the swimming time (*P*<0.01), as compared to control. (n = 6 in all groups, SC vs S+*B.monnieri*, *P*<0.01; SC vs S+*P.ginseng*, *P*<0.01; One way ANOVA, *P*<0.01, F = 41.336; Fig. 1).

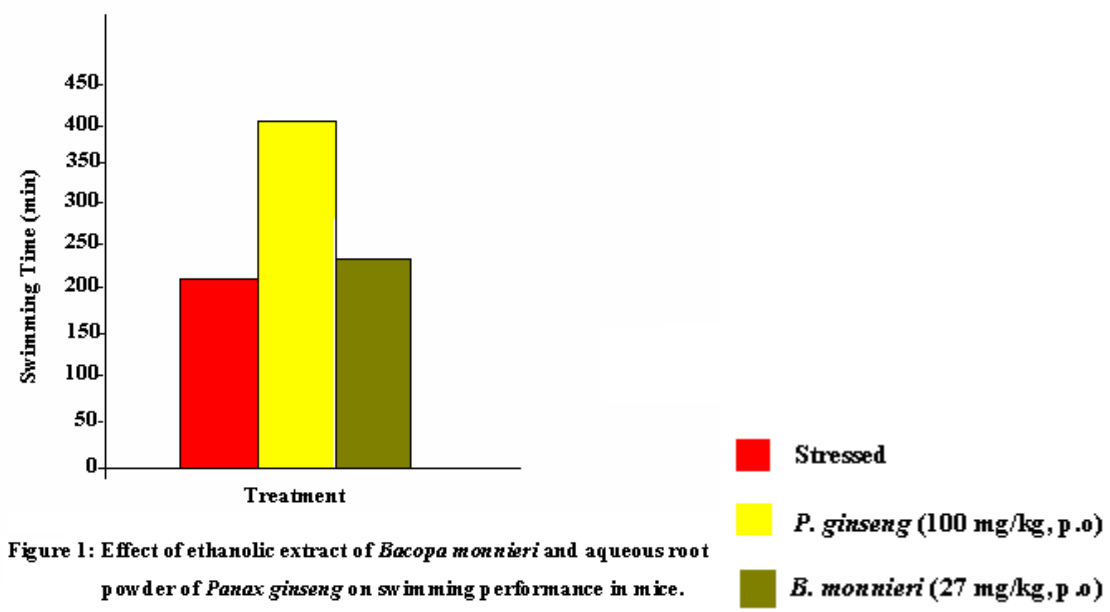

Figure 1: Effect of ethanolic extract of *Bacopa monnieri* and aqueous root powder of *Panax ginseng* on swimming performance in mice.

The induction of cold restraint stress led to a rise in total WBC count, blood glucose, plasma cortisol and serum triglyceride levels. All the two treatments produced a significant

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reduction in total WBC count (P<0.01), as compared to controls. (n = 6 in all groups, NC vs SC, P<0.01; SC vs S+*B.monnieri*, P<0.01; SC vs S+*P.ginseng*, P<0.01; One way ANOVA, P<0.01, F = 6.006; Fig. 2).

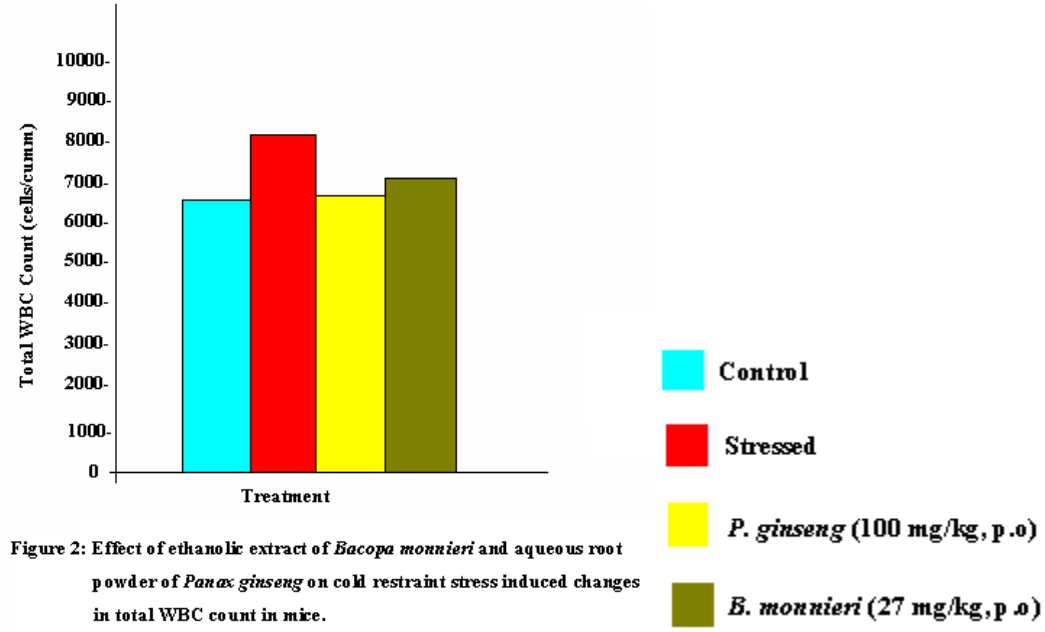

Figure 2: Effect of ethanolic extract of *Bacopa monnieri* and aqueous root powder of *Panax ginseng* on cold restraint stress induced changes in total WBC count in mice.

The blood glucose was significantly increased, when the animals were subjected to cold restraint stress compared to control (P<0.01). Pretreatment of animals with the extract of *Bacopa monnieri* 27 mg/kg (p.o), or water soluble root powder of *Panax ginseng* 100 mg/kg (p.o), prevented this (P<0.01). (n = 6 in all groups, NC vs SC, P<0.01; SC vs S+*B.monnieri*, P<0.01; SC vs S+*P.ginseng*, P<0.01; One way ANOVA, P<0.01, F = 60.373; Fig. 3).

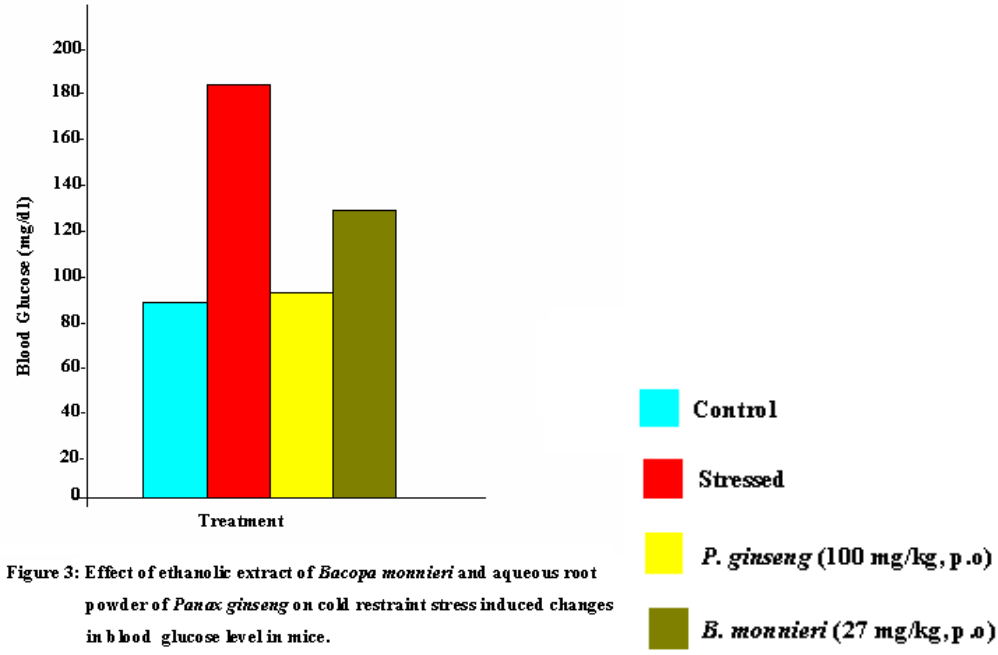

Figure 3: Effect of ethanolic extract of *Bacopa monnieri* and aqueous root powder of *Panax ginseng* on cold restraint stress induced changes in blood glucose level in mice.

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The plasma cortisol level which was found to be elevated in the animals subjected to cold restraint stress was significantly reduced by all the two treatments (P<0.01), compared to controls. (n = 6 in all groups, NC vs SC, P<0.01; SC vs S+*B.monnieri*, P<0.01; SC vs S+*P.ginseng*, P<0.01; One way ANOVA, P<0.01, F = 92.616; Fig. 4).

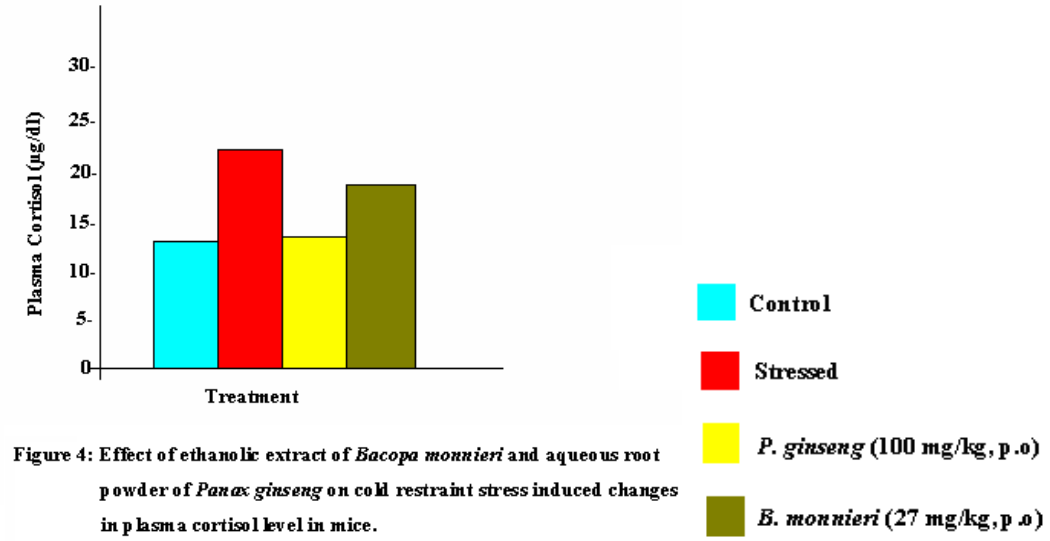

Figure 4: Effect of ethanolic extract of *Bacopa monnieri* and aqueous root powder of *Panax ginseng* on cold restraint stress induced changes in plasma cortisol level in mice.

The triglyceride level was increased in the animals subjected to cold restraint stress compared to control (P<0.01). However, no significant change in the serum cholesterol level was observed. Treatment of animals with the extract of *Bacopa monnieri* 27 mg/kg (p.o), or water soluble root powder of *Panax ginseng* 100 mg/kg (p.o), before subjecting them to cold restraint stress, prevented the increase in serum triglyceride levels (P<0.01). (n = 6 in all groups, NC vs SC, P<0.01; SC vs S+*B.monnieri*, P<0.01; SC vs S+*P.ginseng*, P<0.01; One way ANOVA, P<0.01, F = 98.553; Fig. 5).

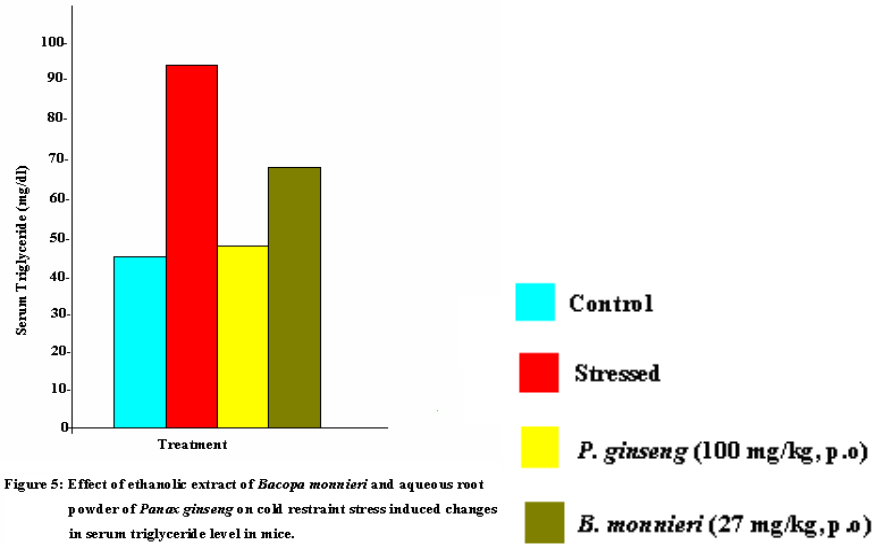

Figure 5: Effect of ethanolic extract of *Bacopa monnieri* and aqueous root powder of *Panax ginseng* on cold restraint stress induced changes in serum triglyceride level in mice.

Therefore, on the basis of the above findings it is concluded that the extract of *Bacopa monnieri* improves the swim duration in mice and prevented the increase in total WBC count, blood glucose, plasma cortisol, and serum triglyceride levels.

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DISCUSSION

Increased swimming endurance in mice which is pretreated with adaptogens has been reported and the test system is used to evaluate agents with adaptogenic properties. In conclusion, mice treated with ethanolic extract of *Bacopa monnieri* show significant improvement in the swimming time, suggesting a central nervous system stimulant and/or anti-stress activity [16], [31].

Cold stress typically increases total leucocyte count, eiosinophils and basophils (Sundaresan et al., 1990) [21]. Adaptogens work at the cellular level to help the body cope up with stress-related situations. When the body is placed under stress, molecules known as beta-lipoproteins increase in number and block the key enzyme hexokinase, which is responsible for transforming glucose to be used by the cells for energy. When cells do not receive an adequate supply of energy, they are unable to function properly [19]. Adaptogens are able to thwart this effect by preventing the formation and accumulation of beta-lipoproteins and allowing the hexokinase enzyme to freely convert glucose into energy for the cells (Wahlstrom, 1987). Since the stress induced total WBC count is decreased by the extract of *Bacopa monnieri* 27 mg/kg (p.o), the plant possess anti-stress, adaptogenic activity [22-30], [40], [41].

Cortisol is released in response to neural stimuli, caused by chronic stress (Simmons, 1998). Increased plasma cortisol influences the mobilization of stored fat and carbohydrate reserves (Tache and Selye, 1976), which in turn increases blood glucose level and triglycerides [18], [28]. The increased cortisol level, increased blood glucose and triglyceride levels are reversed by anti-stress agents (Sen et al., 1992), while no significant change was observed in the serum cholesterol level. The extract of *Bacopa monnieri* 27 mg/kg (p.o), significantly reduced the acute state increase in the adrenal gland weight, plasma cortisol, blood glucose and triglyceride levels, exhibiting anti-stress activity [34-41].

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