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*RESEARCH ARTICLE*

# Adaptogens as Anti-stress Agents in Reducing increased Plasma Cortisol Level during Stress.

**Anju*, and Ashis Kumar Ghosh.**

Department of Biochemistry, Patna University, Patna – 800 005, Bihar, India.

**ABSTRACT**

The stress response involves the activation of both the sympathetic-adrenal response and the hypothalamic-pituitary-adrenal axis. During times of increased stress, the adrenal gland is stimulated to produce increased levels of hormones. Cortisol, the main hormone involved in the stress response, is secreted in increased amounts within minutes of a perceived stressor. Cortisol secretion can increase as much as 20-fold and has several important physiological effects. Short-term surges in cortisol levels can suppress inflammation and at the same time suppress immune function. Though inflammation control is important, surges of sustained levels of cortisol are not healthy and ultimately lead to premature aging, degenerative disease, and increased susceptibility to cancer. Studies show that psycho-social stress activates the hypothalamus-pituitary-adrenal axis causing an increase in morning cortisol levels, which correlated to the subjects reports of increased fatigue and anxiety. Although this stress response is important for survival during an acute stressor, prolonged activation of the stress response may lead to adrenal exhaustion in which cortisol levels drop to insufficient levels resulting in fatigue or illness. Many herbs have been shown to impact adrenal function. Adaptogens are plants that produce a non-specific response improving the physiological resistance to stressors. These herbs are often used in the context of adrenal support formulas to balance adrenal hormone levels. It is believed that adaptogenic herbs can increase low levels of adrenal hormone or decrease levels that are elevated. Additionally, these herbs provide balancing activity on many body systems that are impacted by stress, such as the immune response and blood sugar control. In the present study, we have evaluated the efficacy of ethanolic extract of *Ocimum sanctum* 47mg/kg p.o, *Withania somnifera* 23 mg/kg p.o and *Bacopa monnieri* 23 mg/kg p.o on plasma cortisol level in mice subjected to swim endurance test and cold restraint stress. The standard group was administered water soluble root powder of *Panax ginseng* 100 mg/kg p.o and the stress control group was administered distilled water orally for 7 days. It was found that mice pretreated with ethanolic extracts of *Ocimum sanctum*, *Withania somnifera* and *Bacopa monnieri* showed a fall in the plasma cortisol level. The standard group also showed a significant decrease in the plasma cortisol level compared to the stress and normal control groups.

**Keywords:** Adaptogens, *Bacopa monnieri*, Cortisol, Hypothalamic-pituitary-adrenal axis, *Ocimum sanctum*, *Panax ginseng, Withania somnifera*

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***Corresponding author***

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## INTRODUCTION

Adaptogens are defined as herbs or roots that possibly act like a 'stress vaccine' in the human body. Ultimately, they are proposed to reduce chronic stress and fatigue through the adaptation of stress. Adaptogens have been around in Chinese functional medicine and Indian Ayurveda medicine since ancient times [16]. They became more popular during world war II when Russian scientists were looking to improve soldiers stamina through herbal medicine [1-11].

Adaptogens are similar to catecholamines, neurotransmitters involved in stress situations. Common catecholamines are adrenaline, epinephrine, dopamine and norepinephrine. Since, adaptogens are mild stressors the theory is that they build the body's immunity up to be able to adapt and control future severe and moderate stress situations. These stress-protective effects mainly help the Hypothalamus-Pituitary-Adrenal Axis and SAS system. They are responsible for controlling the body's stress responses during times of stress – mental health disorders, traumatic injury, exercise, eating disorders, surgery, malnutrition and low blood sugar amongst other conditions [44]. The adaptogens have been shown to influence the hypothalamus-pituitary-adrenal axis (HPA axis) to confer immunostimulatory actions, and to activate cognitive functions [22]. A number of herbs can be described as adaptogens based upon both clinical and in vitro research.

The mechanism of adaptogens appears to involve the hypothalamic-pituitary-adrenal axis with resultant decrease or normalizing of nitric oxide or cortisol, which are increased during times of stress [45].

Holy basil (*Ocimum sanctum*) is a herb that has been extensively studied. Besides the normal adaptogen properties holy basil can protect against the damaging effects of ionizing radiation. It reduces cortisol levels when elevated by stress and lowers blood sugar in type – 2 diabetes [14]. With its neuroprotective properties it enhances circulation to the brain helping with memory and foggy thinking. Combined with ginkgo holy basil is indicated for mental cloudiness and poor memory during the menopausal transition [12-15].

Ashwagandha (*Withania somnifera*) an Ancient Ayurvedic healing herb has been used for centuries to heal a wide variety of conditions. This powerful herb is best known for its restorative benefits. Ashwagandha's ability to lower cortisol levels has a powerful effect when it comes to have a successful experience with weight loss attempts [30]. That is because of cortisol, a hormone that is secreted by the adrenal glands as a response to stress can occasionally become severly elevated or elevated for a prolonged period of time. This happens when the body is under a significant amount of stress. When cortisol levels are healthy and average our bodies emanate that through are balanced well-being. However, when they are elevated particularly for a prolonged period of time the body starts to experience some of the most commonly recognized symptoms of stress: headache, insomnia, stress and more. If heightened cortisol levels are severe in an individual, they are at increased risk for a variety of health problems including things such as stroke, heart disease, metabolic syndrome, diabetes and obesity [17-19].

*Bacopa monnieri* has been used by Ayurvedic medical practioners for centuries for a variety of purposes, including improving memory, reducing anxiety and treating epilepsy. *Bacopa monnieri* is considered an adaptogenic herb, meaning that it increases the body's resistance to stress [42]. Research suggests that *Bacopa monnieri* helps reduce stress and anxiety by elevating the mood and reducing levels of cortisl, a hormone that is closely linked to stress levels [32-36]. In fact, research shows that it may boost brain function and alleviate anxiety and stress among other benefits [37-40].

## MATERIALS AND METHODS

Plant material leaves of *Ocimum sanctum, Withania somnifera* and roots 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 Rotavapour 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 ± 5 g. They were acclimatized to laboratory condition 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°C ± 2°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+*O.sanctum*), (Stress+*W.somnifera*), (Stress+*B.monnieri*) treatment group which were fed with ethanolic extract of *Ocimum sanctum*, *Withania somnifera, Bacopa monnieri* (p.o) for 7 days.

## STRESS PROCEDURE

**Swim Endurance Test:** The mice in group IV were given ethanolic extract of *Ocimum sanctum* 47 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 8$^{th}$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 *Ocimum sanctum* 47 mg/kg, *Withania somnifera* 23 mg/kg, *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 for 7 days, orally.

On the 8$^{th}$ 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 plasma cortisol was determined by Enzyme Linked Immunosorbent Assay (ELISA) [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.

## RESULT

Acute toxicity studies with extract revealed that $LD_{50}$ *Ocimum sanctum* is 4.5g /kg, $LD_{50}$ *Withania somnifera* is 1750 mg/kg, $LD_{50}$ *Bacopa monnieri* is 17g/kg body weight (p.o). As shown in figure 1, the extract of *Ocimum sanctum* improves swim duration in mice. Mice pretreated with ethanolic extract of *Ocimum sanctum* 47 mg/kg *Withania somnifera*23 mg/kg *Bacopa monnieri*27 mg/kg 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+*O.sanctum*, $P<0.01$; SC vs S+*P.ginseng*, $P<0.01$; One way ANOVA, $P<0.01$, F = 41.336; Fig. 1). (n = 6 in all groups, SC vs S+*W.somnifera*, $P<0.01$; SC

vs S+*P.ginseng*, *P*<0.01; One way ANOVA, *P*<0.01, F = 41.336; Fig. 1). (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).

The induction of cold restraint stress led to a rise in plasma cortisol level. All the four treatments produced a significant reduction in plasma cortisol level. The plasma cortisol level which was found to be elevated in the animals subjected to cold restraint stress was significantly reduced by all the four treatments (*P*<0.01), compared to controls. (n = 6 in all groups, NC vs SC, *P*<0.01; SC vs S+*O.sanctum*, *P*<0.01; SC vs S+*P.ginseng*, *P*<0.01; One way ANOVA, *P*<0.01, F = 92.616; Fig. 4).(n = 6 in all groups, NC vs SC, *P*<0.01; SC vs S+*W.somnifera*, *P*<0.01; SC vs S+*P.ginseng*, *P*<0.01; One way ANOVA, *P*<0.01, F = 92.616; Fig. 4). (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).

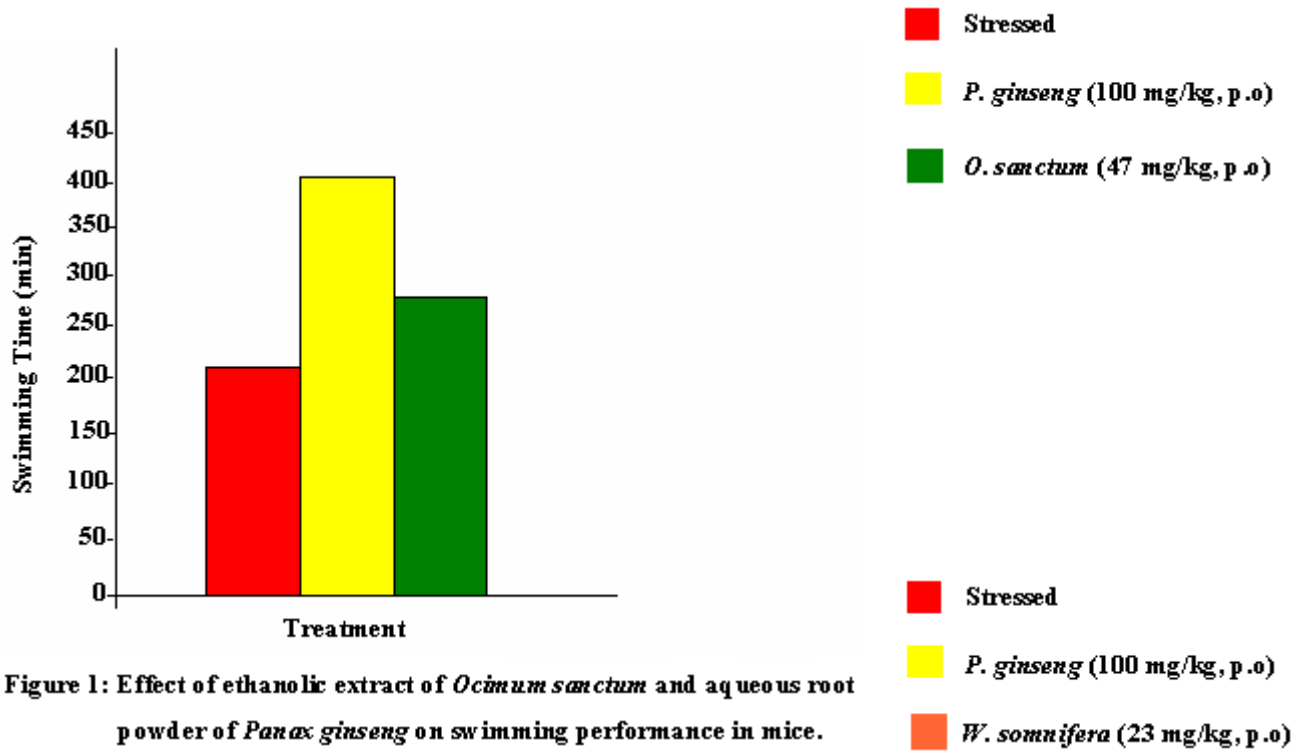


**Figure 1: Effect of ethanolic extract of *Ocimum sanctum* and aqueous root powder of *Panax ginseng* on swimming performance in mice.**

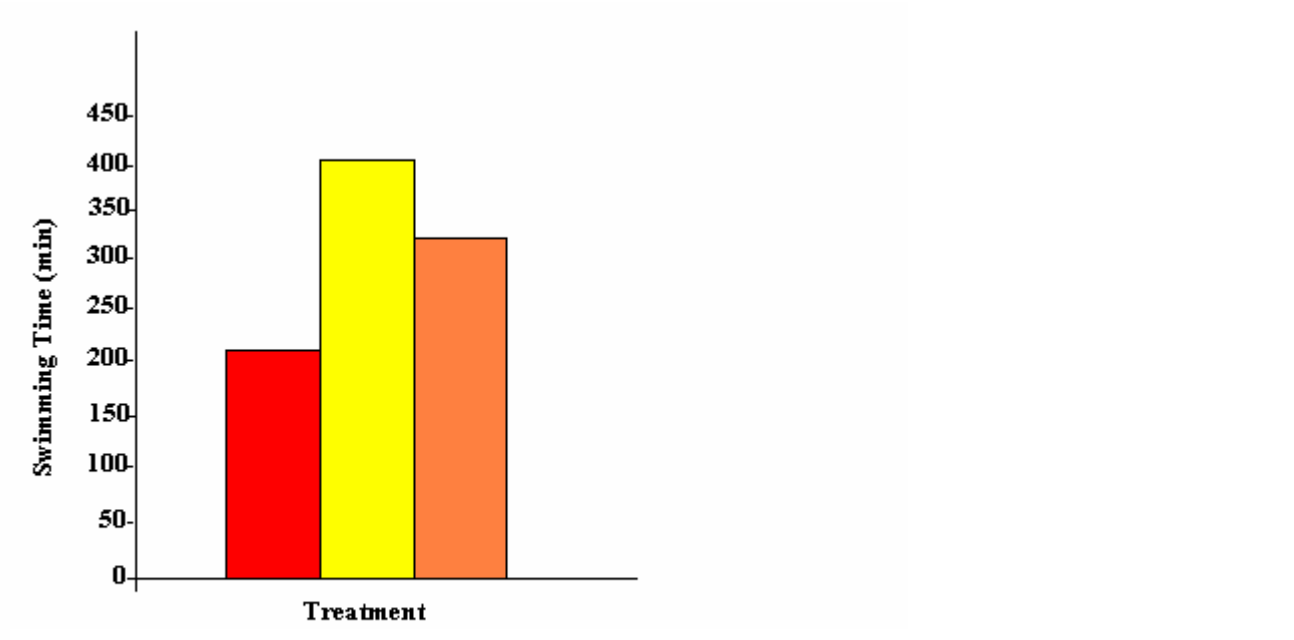


**Figure 1: Effect of ethanolic extract of *Withania somnifera* and aqueous root powder of *Panax ginseng* on swimming performance in mice.**

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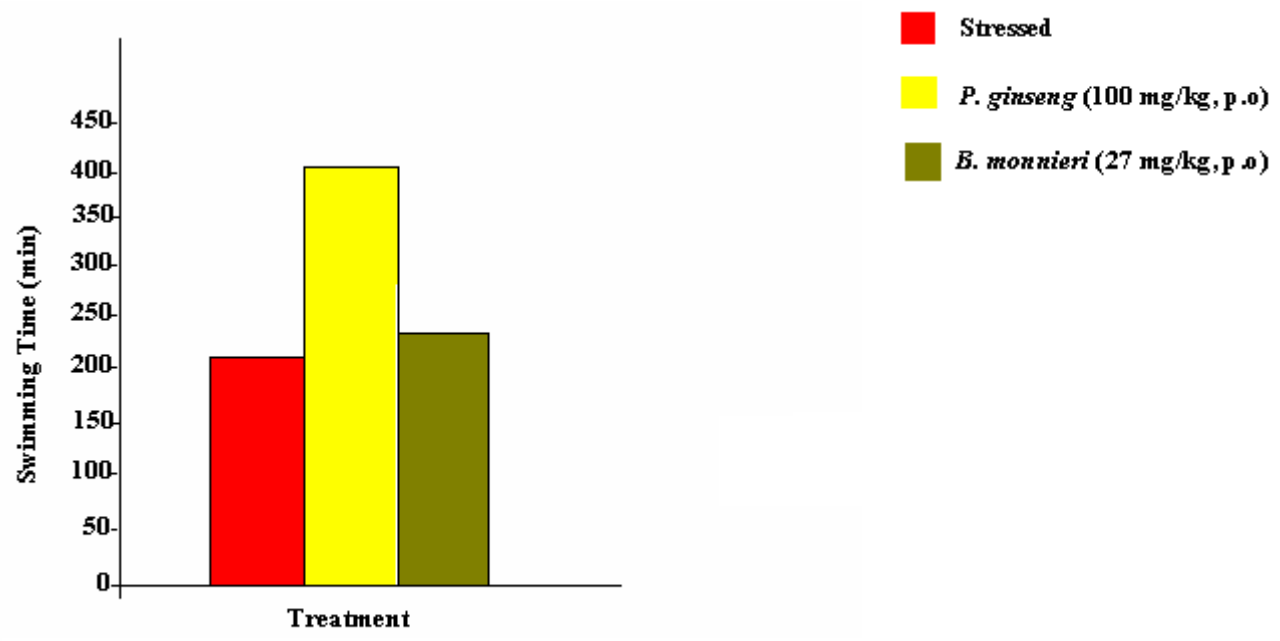


**Figure 1: Effect of ethanolic extract of *Bacopa monnieri* and aqueous root powder of *Panax ginseng* on swimming performance in mice.**

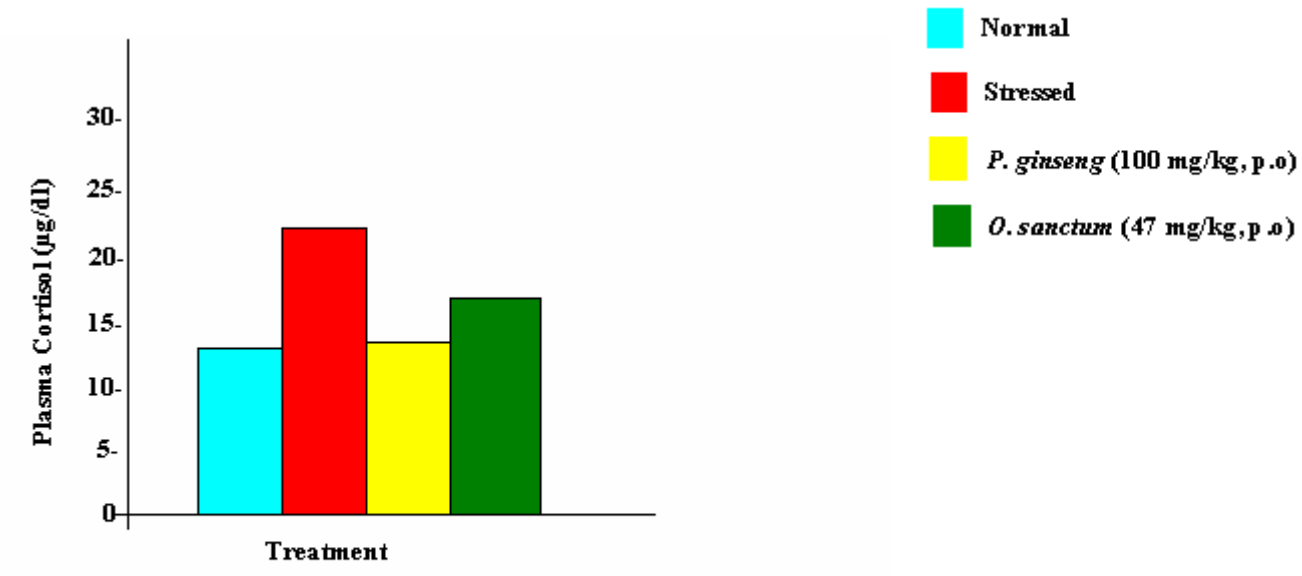


**Figure 4: Effect of ethanolic extract of *Ocimum sanctum* and aqueous root powder of *Panax ginseng* on cold restraint stress induced changes in plasma cortisol level in mice.**

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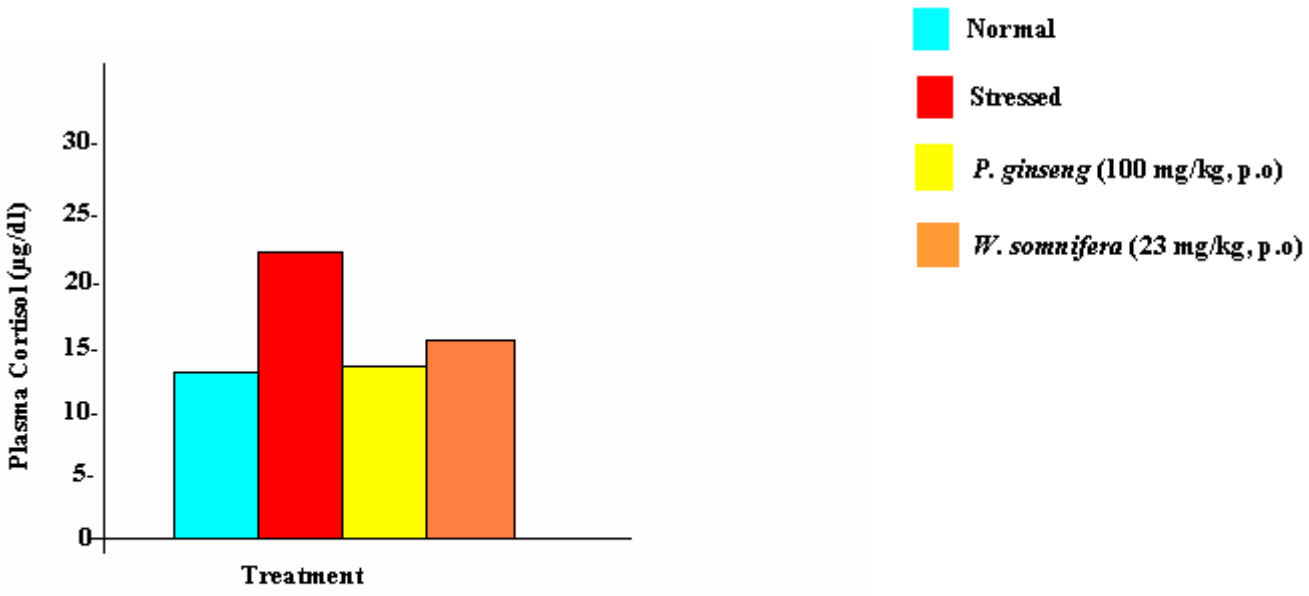


**Figure 4: Effect of ethanolic extract of *Withania somnifera* and aqueous root powder of *Panax ginseng* on cold restraint stress induced changes in plasma cortisol level in mice.**

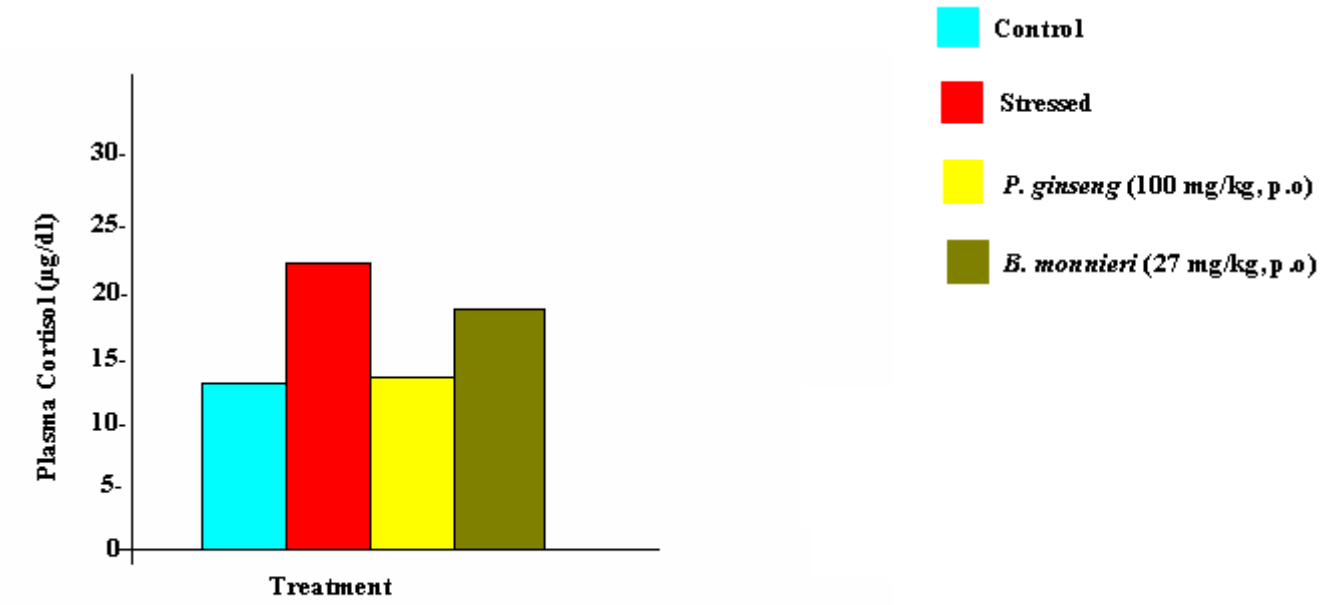


**Figure 4: Effect of ethanolic extract of *Bacopa monnieri* and aqueous root powder of *Panax ginseng* on cold restraint stress induced changes in plasma cortisol level in mice.**

## DISCUSSION

Stress causes an increase in the corticosterone level in the blood. Treatment of stressed animals with ethanolic extract of *Ocimum sanctum* 47 mg/kg (p.o), has been shown to prevent the changes in the plasma level of cortisol induced by exposure to acute stress, indicating the anti-stressor property of the plant against swim endurance test and cold restraint stress [12-15]. Pretreatment with the ethanolic extract of the roots of *Withania somnifera* 23 mg/kg (p.o), increased swimming endurance in mice and

significantly reduced the cold restraint stress induced changes in mice [17-20]]. 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 [32-36].

Cold restraint stress induced elevations of plasma cortisol. *Ocimum sanctum* was found to lower plasma cortisol level. Stress induced increase in plasma cortisol and blood glucose level were blocked by the administration of *Withania somnifera* to mice, while swimming time was increased [30], [34-46]. 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], [28-29]. The increased cortisol level, increased blood glucose and triglyceride levels are reversed by anti-stress agents (Sen et al., 1992). 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 [38-40].

## CONCLUSION

These herbs are representative of the more commonly used adaptogenic herbs. In response to perceived stress, adaptogens balance the HPA axis as well as influence other body systems including the immune, neurologic and reproductive systems [45]. Research shows adaptogens also have antioxidant activity, protecting the body from oxidative stress and free radical formation [13]. These herbs aid in normalizing many physiologic mechanisms, such as regulating blood pressure, blood sugar, cellular energy production and immune alterations in the day-to-day management of stress. Augmenting the body's own physiologic processes, adaptogens function to maintain homeostasis [25-26].

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