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RESEARCH ARTICLE

Effect of Adaptogens on Glycemic Index in Stress- induced Mice

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ABSTRACT

Diabetes mellitus is a common name for a group of chronic diseases whose common symptoms is the inability to regulate blood sugar levels. It occurs with an uncontrolled increase in post - meal glucose (hyperglycemia) and a possible decrease to unconsciousness (hypoglycemia) when injected with insulin. There are two basic types of diabetes: Type 1 diabetes and Type 2 diabetes. Type 1 diabetes is the autoimmune – white blood cell of the patient destroying Langerhan's Pancreatic β – cells which are responsible for insulin production. Type 2 diabetes is caused by the pancreas producing insulin, but the tissue does not respond to it, they react poorly. When stressed, the body prepares itself by ensuring that enough sugar or energy is readily available. Insulin levels fall, glucagon and epinephrine (adrenaline) levels rise and more glucose is released from liver. At the same time, growth hormones and cortisol levels rise, which causes body tissues (muscle and fat) to be less sensitive to insulin. As a result, more glucose is available in the bloodstream. In type 2 diabetes low blood sugars from too much medication or insulin are a common cause of stress. The hormonal response to a low blood sugar includes a rapid release of epinephrine and glucagon, followed by a slower release of cortisol and growth hormone. These hormonal responses to the low blood sugar may last for 6-8 hours – during that time the blood sugar may be difficult to control. The phenomena of a low blood sugar followed by a high blood sugar is called a "rebound' or 'somogyi' reaction.In the present study, we have evaluated the efficacy of ethanolic extract of *Ocimum sanctum* 47 mg/kg p.o, *Withania somnifera* 23 mg/kg p.o and *Bacopa monnieri* 23 mg/kg p.o on blood glucose 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 extracs of *Ocimum sanctum*, *Withania somnifera* and *Bacopa monnieri* showed reduced blood glucose level. The standard group also showed significant decrease in blood glucose level when subjected to stress.

Keywords: Diabetes mellitus, β-cells, Cortisol, Epinephrine, Glucagon, Hyperglycemia, Hypoglycemia.

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INTRODUCTION

Adaptogens have been defined as natural bioregulators that increase the ability to adapt to environmental factors and avoid the damage caused by those factors. More specifically, adaptogens are non-toxic herbs, roots and fungi (eg. mushrooms) that help the body resist or adapt to damaging stressors and promote or restore normal physiological functioning [7]. They help us to better handle mental or physical stress, improve immunity, ward off depression and anxiety and basically feel better overall [1-11].

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 level 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*) is one of the most popular adaptogenic herbs. Although, it has an energizing effect, it possesses a more calming action than other adaptogens. This makes it useful for anxiety, stress induced insomnia and nervous exhaustion. As an anti-proliferative herb it has been used in the treatment of cancer. Ashwagandha contains a significant amount of iron supporting its benefit in treating iron deficient anemia [30]. 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 [17-19].

Bacopa monnieri also called as brahmi has been used by ayurvedic medical practioners for centuries for a variety of purposes, including improving memory, reducing anxiety and treating epilepsy. *Bacopa monnieri* help to prevent anxiety and stress. It is considerd an adaptogenic herb, meaning that it increases the body's resistance to stress [42]. Research suggests that *Bacopa monnieri* help reduce stress and anxiety by elevating the mood and reducing levels of cortisol, a hormone that is closely linked to stress levels [32-36].

Bacopa monnieri contains powerful compounds that may have antioxidant effects. For example, bacosides the main active compound in *Bacopa monnieri*, have been shown to neutralize free radicals and prevent fat molecules from reacting with free radicals. When fat molecules react with free radicals, they undergo a process called lipid peroxidation. Lipid peroxidation is linked to several conditions, such as Alzheimer's, Parkinson's and other neurodegenerative disorders [28]. *Bacopa monnieri* may help prevent damage caused by this process [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 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 bodyweight of 35 $\pm$ 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^{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).

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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 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 *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 8th day, the animals were individually placed in plastic container 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 blood glucose level was determined by GOD/POD method.

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} *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/kgand 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 blood glucose level. All the four treatments produced a significant reduction in the blood glucose level. 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 *Ocimum sanctum* 47 mg/kg, *Withania somnifera* 23 mg/kg, *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+*O.sanctum*, $P<0.01$; SC vs S+*P.ginseng*, $P<0.01$; One way ANOVA, $P<0.01$, F = 60.373; Fig. 3).(n = 6 in all groups, NC vs SC, $P<0.01$; SC vs S+*W.somnifera*, $P<0.01$; SC vs

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S+*P.ginseng*, *P*<0.01; One way ANOVA, *P*<0.01, F = 60.373; Fig. 3). (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).

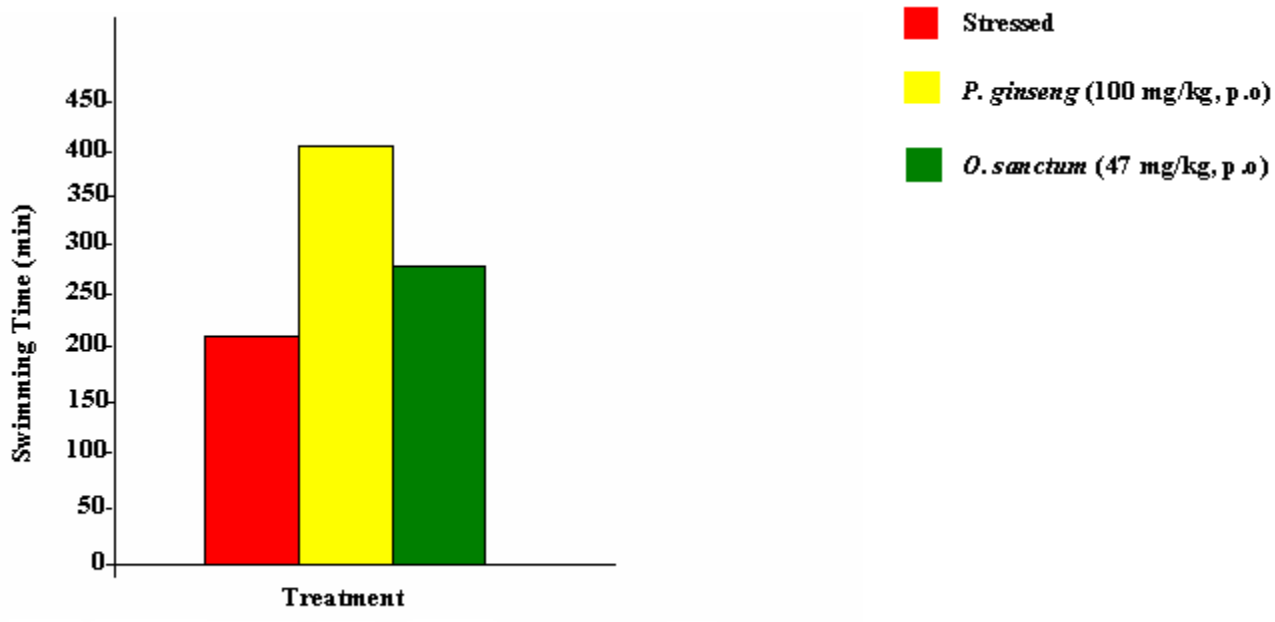

Figure 1: Effect of ethanolic extract of *Ocimum sanctum* and aqueous root powder of *Panax ginseng* on swimming performance in mice.

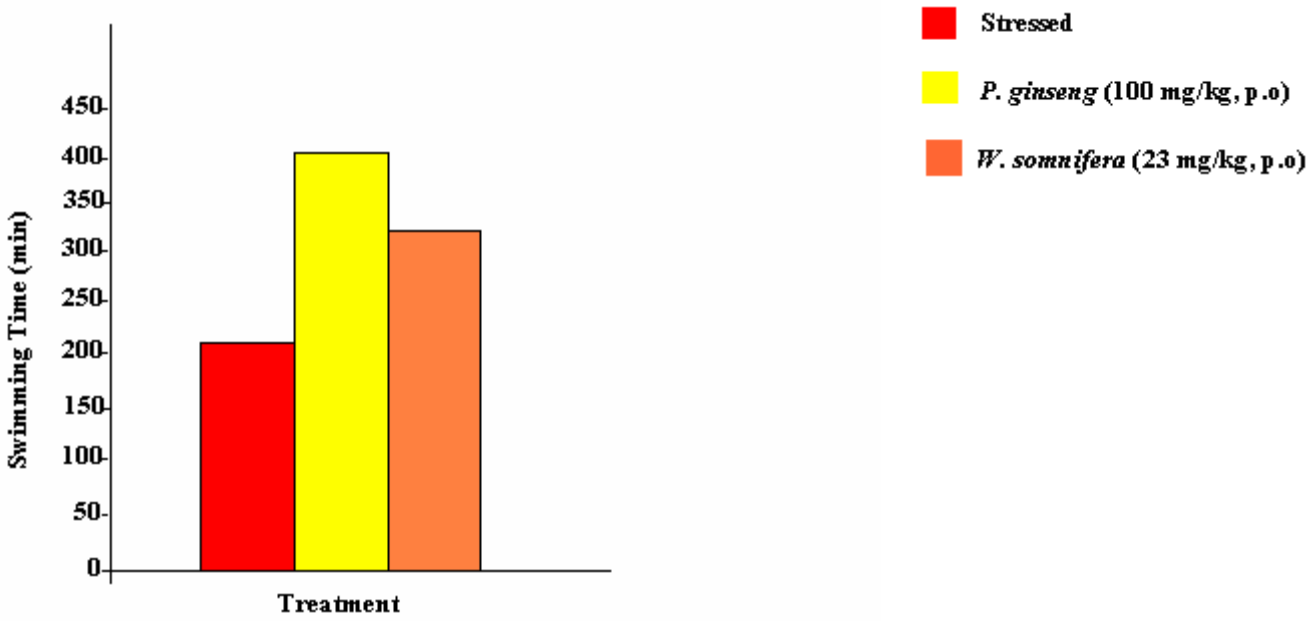

Figure 1: Effect of ethanolic extract of *Withania somnifera* and aqueous root powder of *Panax ginseng* on swimming performance in mice.

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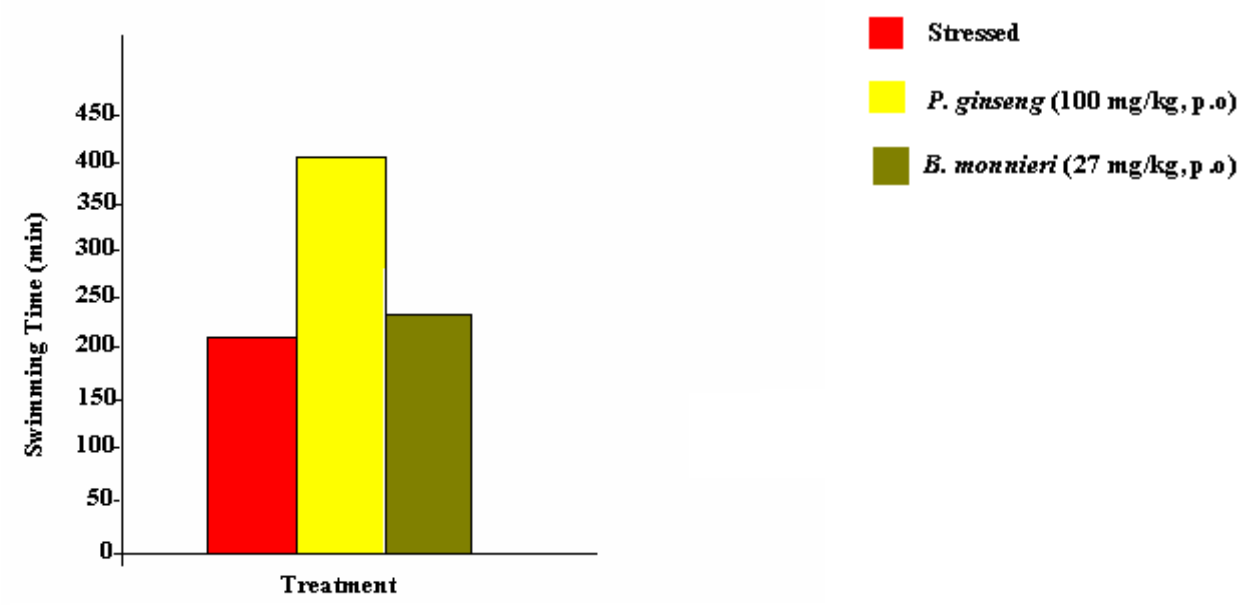

Figure 1: Effect of ethanolic extract of *Bacopa monnieri* and aqueous root powder of *Panax ginseng* on swimming performance in mice.

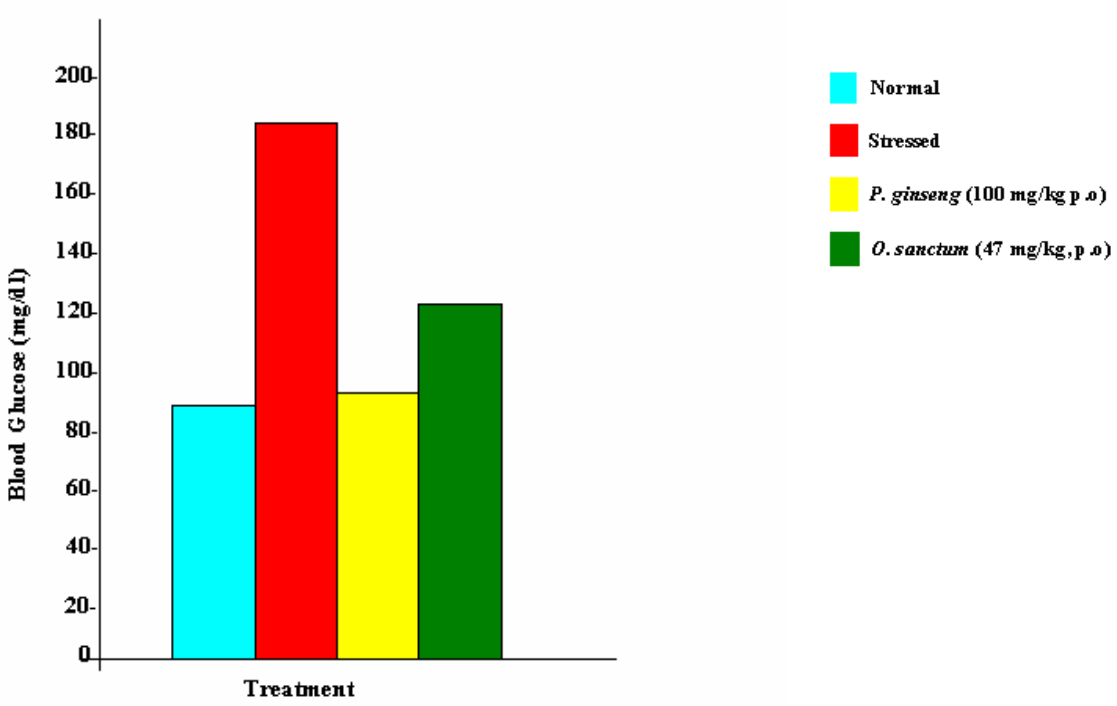

Figure 3: Effect of ethanolic extract of *Ocimum sanctum* and aqueous root powder of *Panax ginseng* on cold restraint stress induced changes in blood glucose level in mice.

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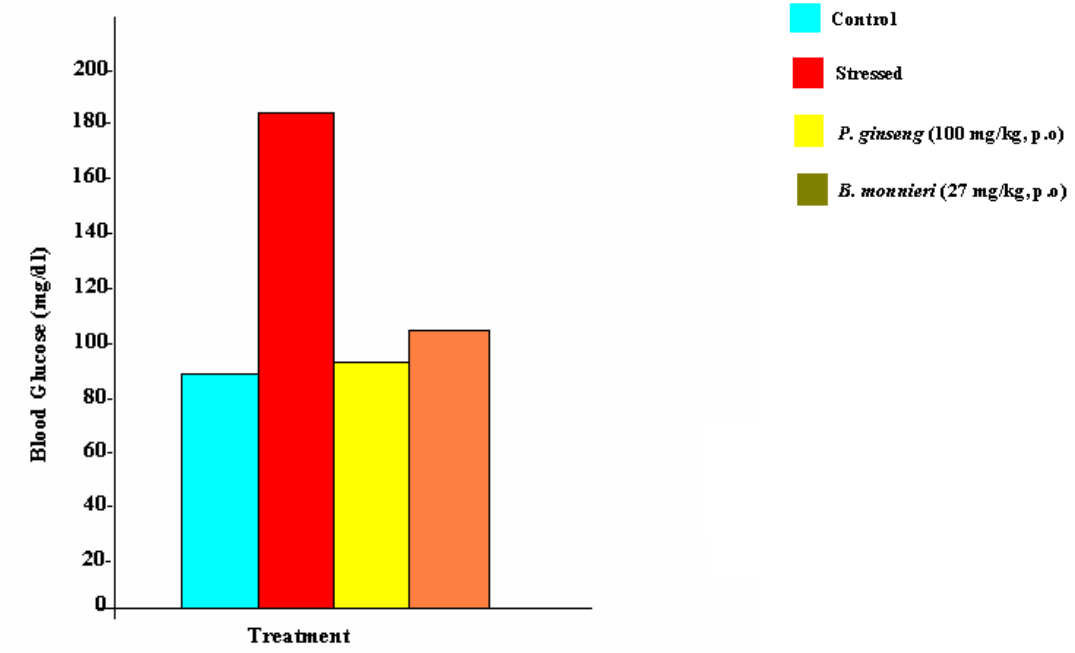

Figure 3: Effect of ethanolic extract of *Withania somnifera* and aqueous root powder of *Panax ginseng* on cold restraint stress induced changes in blood glucose level in mice.

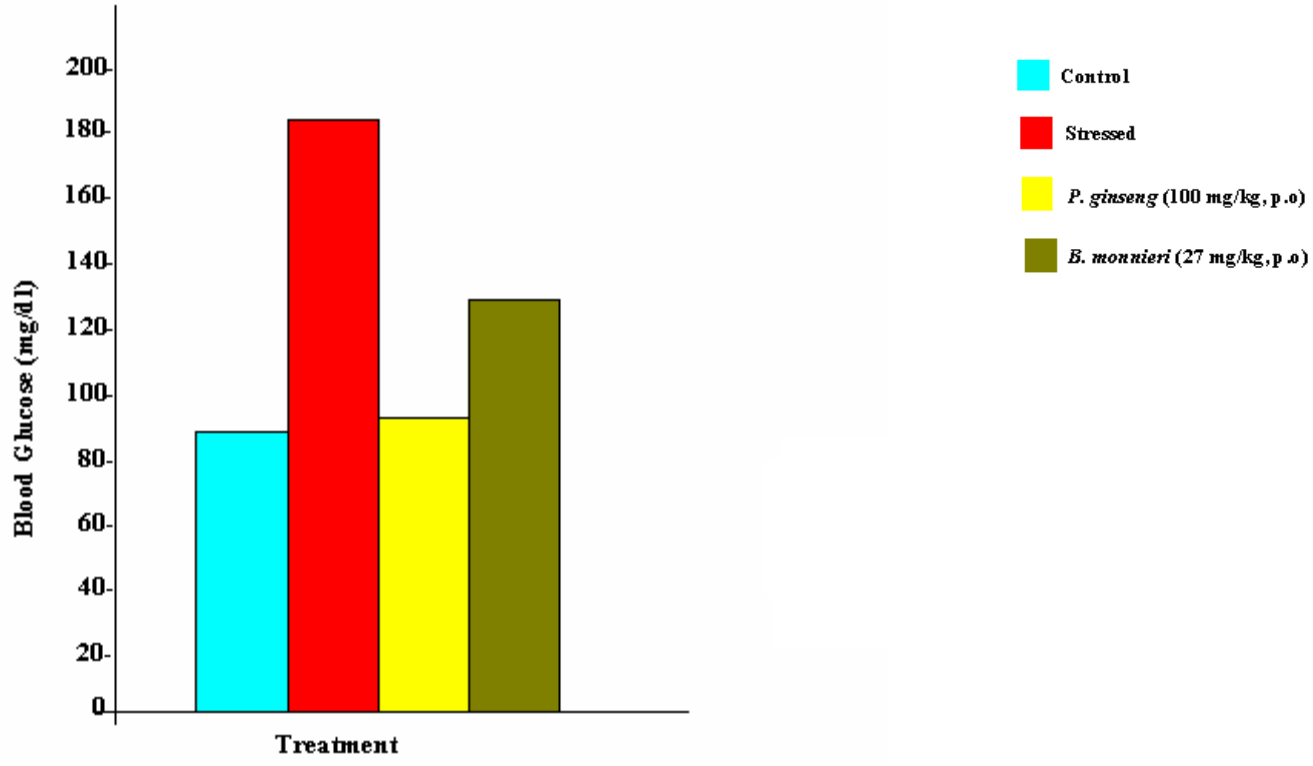

Figure 3: Effect of ethanolic extract of *Bacopa monnieri* and aqueous root powder of *Panax ginseng* on cold restraint stress induced changes in blood glucose level in mice.

Stress causes an increase in the blood glucose level in the blood. Treatment of stressed animals with ethanolic extract of *Ocimum sanctum* 47 mg/kg, *Withania somnifera* 23 mg/kg, *Bacopa monnieri* 27 mg/kg (p.o), has been shown to prevent the changes in the blood glucose level induced by exposure to acute stress, indicating the anti-stressor property of the plant against swim endurance test and cold restraint stress [12-15]. *Ocimum sanctum* 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]. In addition to reducing potentially dangerous cortisol levels, *Withania somnifera* aid in the stabilization of blood sugar levels [17-20]. When cortisol levels are higher they trigger the release of insulin in the body. The anti-diabetic effects of *Bacopa monnieri* is clearly evident from the results of the present study, moreover the efficacy of *Bacopa monnieri* in maintaining normal levels of glycosylated hemoglobin is indicative of good control over blood glucose levels as this particular parameter reflects diabetes management and prognosis [33-36].

Cold restraint stress induced elevations of blood glucose levels [19]. *Ocimum sanctum, Withania somnifera, Bacopa monieri* was found to lower blood glucose levels and also decreased the stress-induced increase in other biochemical parameters in mice [13-14], [17-19] [33-36]. A 95% ethanolic extract of *Ocimum sanctum*, *Withania somnifera, Bacopa monnieri* administered to Swiss albino mice had a normalizing action on discrete regions of the brain and controlled the alteration in neurotransmitter levels due to stress, emphasizing the anti-stressor potential of this herb [13], [19], [39-40].

CONCLUSION

Adaptogens are used to minimize stress reactions and provide protection against the long-term effects of stress. These effects may be related to adaptogens effects on glucose metabolism [16]:

1. Increase blood glucose level by stimulating the liver to convert glycogen to glucose.
2. Enhance the entry of glucose into cells.
3. Enhance the utilization of glucose within cells.

Pretreatment with adaptogens appears to alter endocrine functions of the pituitary adrenal gland axis [44]. Regular pretreatment with adaptogens causes a normalization of stress hormone levels and a generally decreased predisposition to stress. Other body systems also respond to the direct or indirect regulatory influences of adaptogens [26]. For example, adaptogens have been shown to influence the hypothalamic-pituitary-adrenal axis (HPA axis), to confer immunostimulatory actions, and to activate cognitive functions [41].

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