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# Adaptogenic and anti-stress activity of *Ocimum sanctum* in mice

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## ABSTRACT

Forced swimming, a good physical exercise model, is considerd as a physical stressor also. During physical exercise, oxygen utilization increases 10 -15 folds and it is well established that reactive oxygen species (ROS) generation is a direct function of the rate of oxygen utilization. Oxygen reperfusion is another process of ROS imposition due to physical exercise like swimming though there is also a controversy about oxidative stress development due to exercise that focuses on the elevation in antioxidant defense system due to regular exercise. The present study has been designed to find out the effects of forced swimming and cold restraint stress-induced physical stress imposition in male Swiss albino mice. Ethanolic extract of the leaves of *Ocimum sanctum* was tested for anti-stress, adaptogenic activity in mice using swim endurance test and cold restraint stress. The extract exhibited significant anti-stress, adaptogenic activity by improving the swim duration and restoring the cold restraint stress altered biochemical changes. The aim of the work is to find out the adaptogenic effect of ethanolic extract of the leaves of *Ocimum sanctum* in enhancement of performance during stress and also to search out the potentiality of the above mentioned plant products.

**Keywords:** *Ocimum sanctum*, Anti-stress, Adaptogenic, Swim endurance test, Cold restraint stress.

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## INTRODUCTION

Adaptogens are herbs that are non-toxic, produce a non-specific defensive response to stress, and have a normalizing influence on the body. Adaptogens help the body adapt to stress, support its normal functions, and restore balance. They increase the body's resistance to physical, biological emotional and environmental stressors [1]. They are unique from other substances in their ability to balance endocrine hormones and the immune system. All adaptogens display effects that help to regulate the neuroendocrine and the immune systems, provide a defense against stress, and increase the ability of a person to maintain optimal homeostasis [2]. A number of plants possess adaptogenic activity due to diverse classes of chemical compounds [3-11]. *Ocimum sanctum* is a plant that is known in India as Tulsi, and "Holy Basil". It has gained a solid place as a tonic in traditional Indian medicine. *Ocimum sanctum* leaves contain an essential oil of varying compositions. The chief components are eugenol, methylchavicol, alpha and beta bisabolen. Additional constituents are the flavonaglyca luteolin, epigenin and their 7-O-glucuronides as well as the C-glycosides orientin and molludistin and the triterpene acid ursolic acid [12-15]. The physical endurance of mice, after i.p application of the extract, was strengthened, without any increase in the weight of the adrenal gland, and without a lowering of the ascorbic acid – content of the adrenal glands [23], [24], [26].

*Ocimum sanctum* is an adaptogenic herb, one of the primary botanicals used in ayurvedic medicine to modulate a physiological stress response and to increase adaptive energy [17]. Traditional ayurvedic use for anti-stress activity has been verified by numerous animal clinical studies [22]. A variety of studies have also shown sacred basil to support the health of the cardiovascular, immune and digestive systems, acting as an effective remedy to help ease stress-related conditions, including hypertension, mood disorders and peptic ulcers [25], [34-35]. The present study has been undertaken to evaluate the anti-stress and adaptogenic activity of the ethanolic extract of the leaves of *Ocimum sanctum* in mice [18], [19].

## MATERIALS AND METHODS

Plant material leaves of *Ocimum sanctum* 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 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).

**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*), treatment group which were fed with ethanolic extract of *Ocimum sanctum* (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 (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$^{o}$C for 2 hours. The blood was collected by orbital sinus veinpuncture method in a heparinised tube and the following investigations were carried out.

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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) [33], serum triglyceride was determined by GPO-POD method [31], [32], total cholesterol was determined by CHOD-POD method and HDL cholesterol was determined by CHOD-PAP 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.

**RESULT**

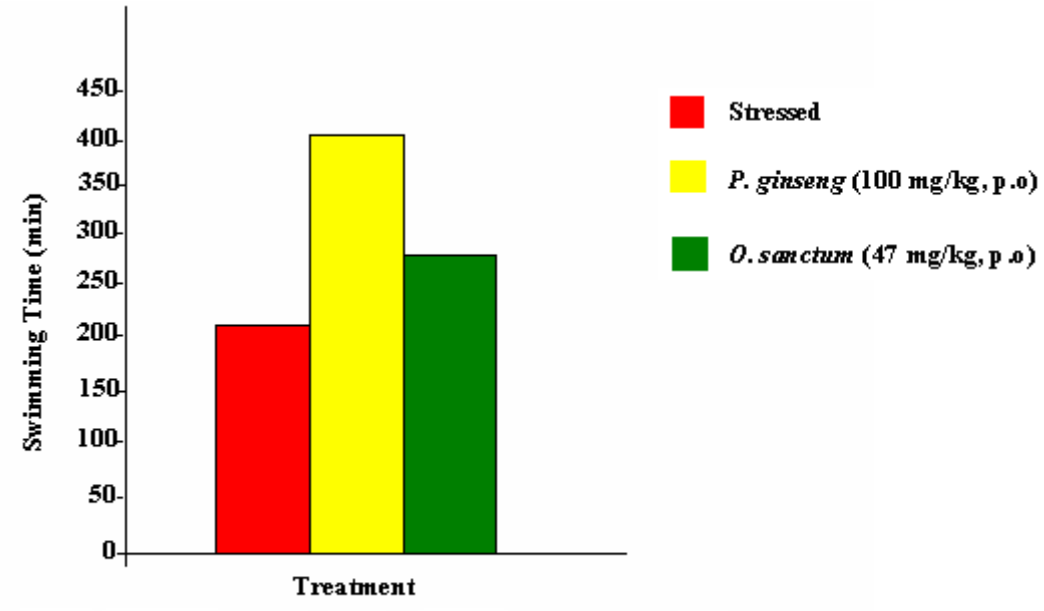


**Figure 1: Effect of ethanolic extract of *Ocimum sanctum* and aqueous root powder of *Panax ginseng* on swimming performance in mice.**

Acute toxicity studies with extract revealed that $LD_{50}$ is 4.5g /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 (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+*O.sanctum*, *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 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+*O.sanctum*, $P$<0.01; SC vs S+*P.ginseng*, $P$<0.01; One way ANOVA, $P$<0.01, F = 6.006; Fig. 2).

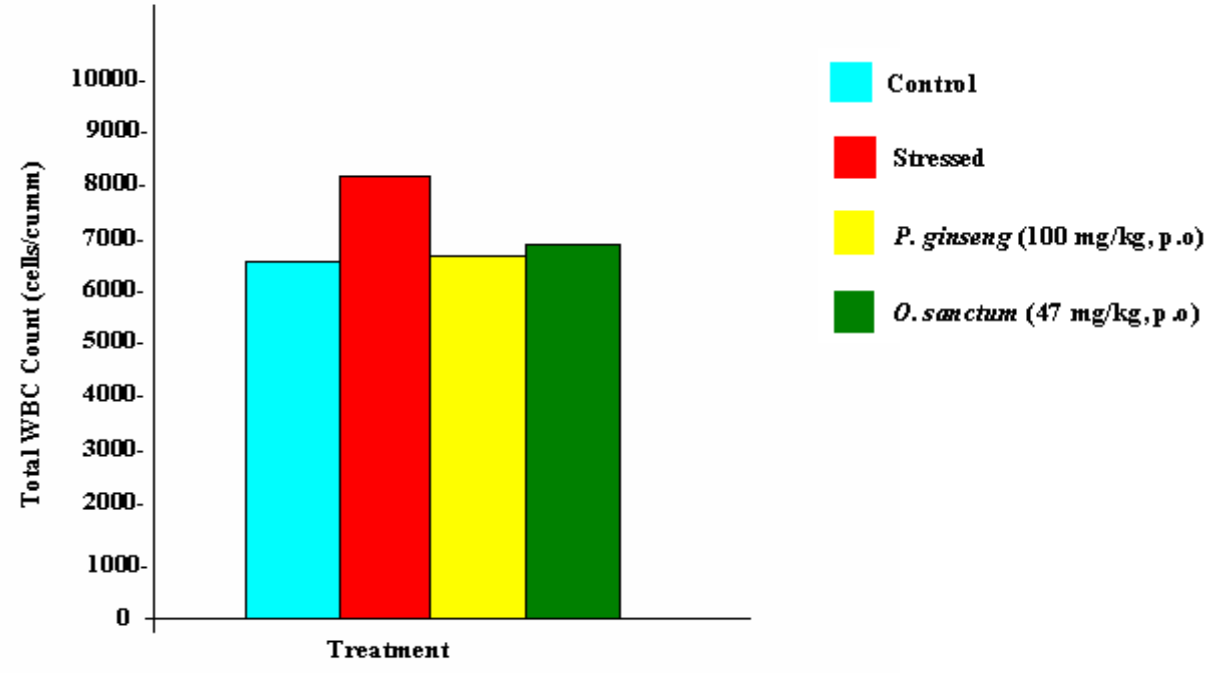


**Figure 2: Effect of ethanolic extract of *Ocimum sanctum* and aqueous root powder of *Panax ginseng* on cold restraint stress induced changes in total WBC count in mice.**

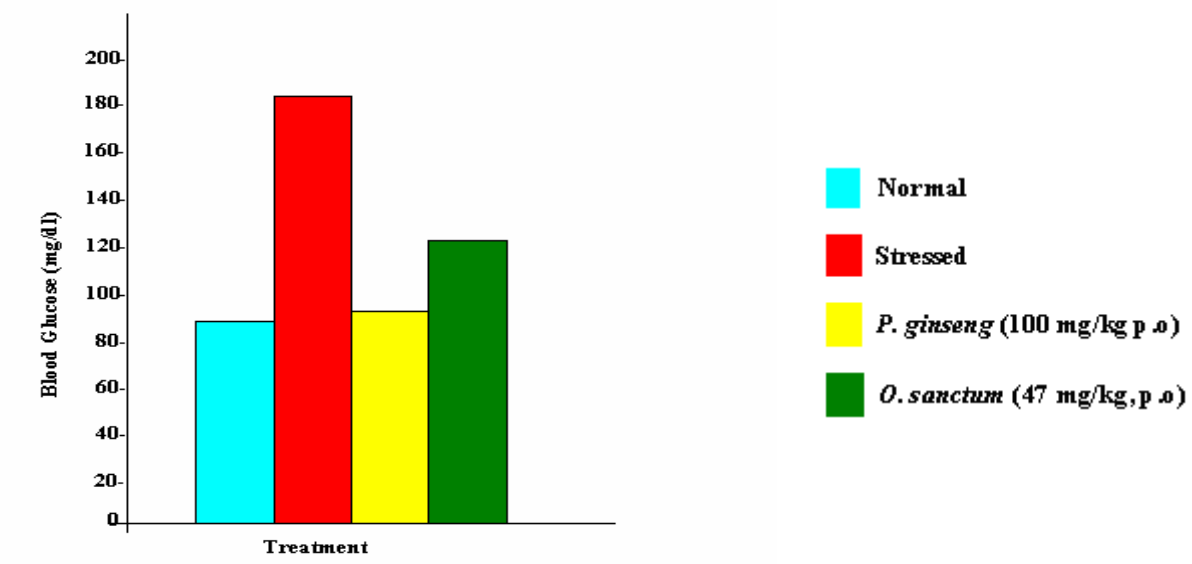


**Figure 3: Effect of ethanolic extract of *Ocimum sanctum* and aqueous root powder of *Panax ginseng* on cold restraint stress induced changes in blood glucose level in mice.**

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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 (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).

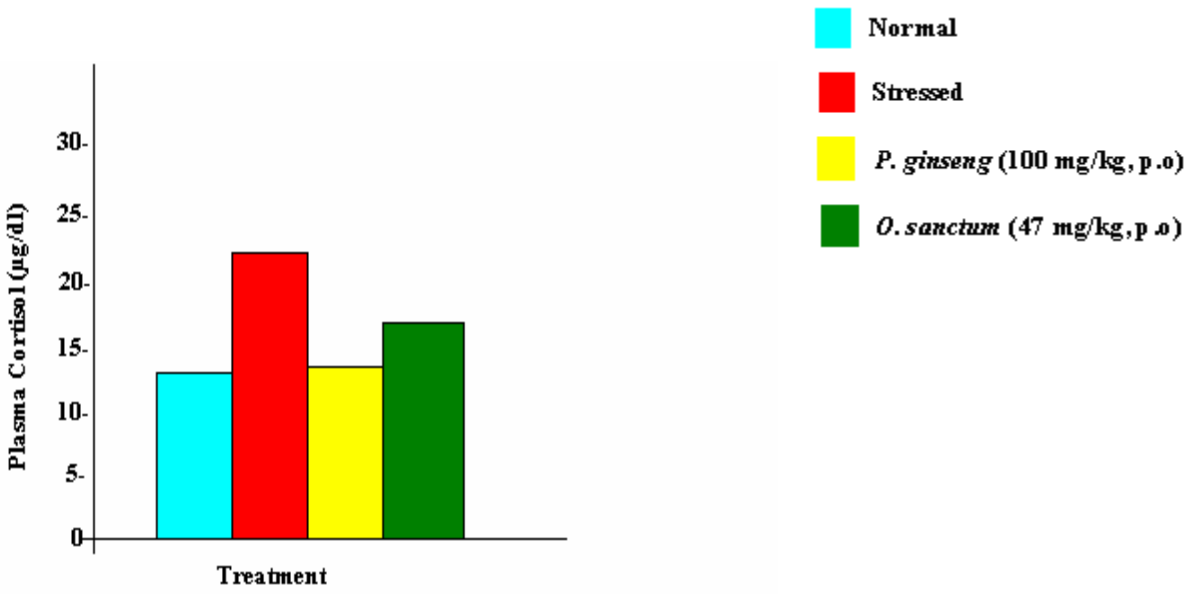


**Figure 4: Effect of ethanolic extract of *Ocimum sanctum* and aqueous root powder of *Panax ginseng* on cold restraint stress induced changes in plasma cortisol level in mice.**

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).

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 *Ocimum sanctum* 47 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+*O.sanctum*, *P*<0.01; SC vs S+*P.ginseng*, *P*<0.01; One way ANOVA, *P*<0.01, F = 98.553; Fig. 5).

Therefore, on the basis of the above findings it is concluded that the extract of *Ocimum sanctum* 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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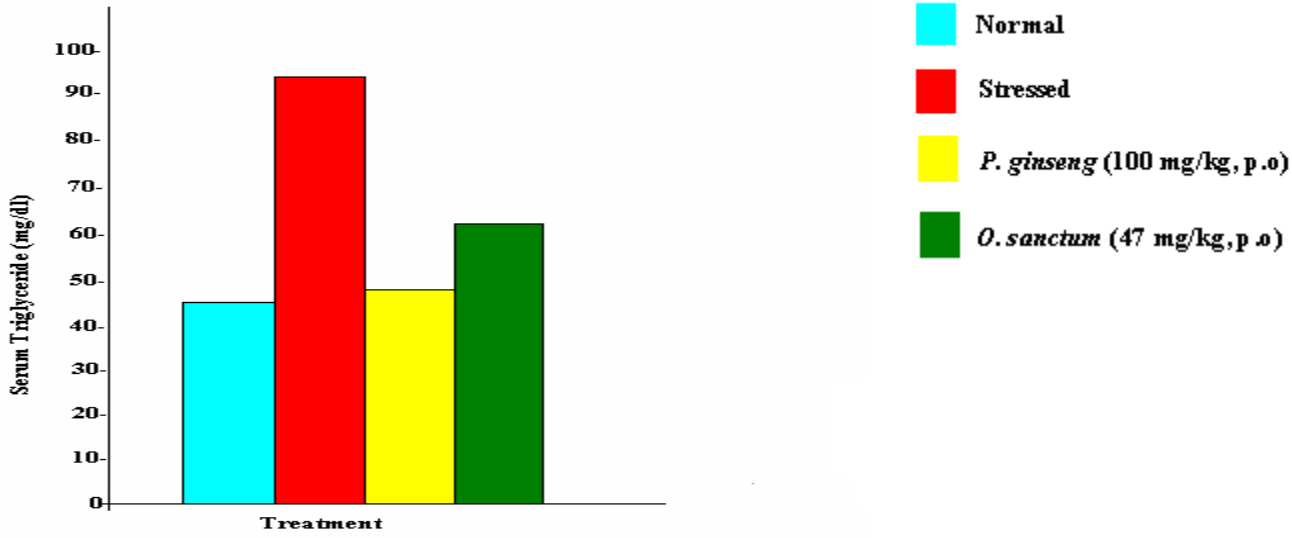


**Figure 5: Effect of ethanolic extract of *Ocimum sanctum* and aqueous root powder of *Panax ginseng* on cold restraint stress induced changes in serum triglyceride level in mice.**

## 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 [21], [40-42]. Humoral immunologic response was increased by the ethanolic extract of *Ocimum sanctum* leaves in mice indicating its immunostimulatory capability. Studies with ursolic acid, a major constituent in *Ocimum sanctum*, has shown that it protects the hippocampal neurons from kianic acid induced injury [34].

Cold restraint stress induced elevations of plasma cortisol, blood glucose and triglyceride levels, increase in total leucocyte count, eiosnophils and basophils [16], [20], [36], [37]. No significant increase was observed in the serum cholesterol level. *Ocimum sanctum* was found to lower plasma cortisol, blood glucose and triglyceride levels and also decreased the stress-induced increase in total WBC count, without affecting other biochemical parameters in mice [29], [30]. A 95% ethanolic extract of *Ocimum sanctum*, 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 [27], [28], [38-42].

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