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Adaptogenic and Anti-stress Activity of *Withania somnifera* in Stress Induced Mice

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ABSTRACT

The aim of the study was to evaluate the effect of ethanolic extract of roots of *Withania somnifera* 23 mg/kg (p.o), on acute stress induced biochemical and immunological perturbations in mice. The standard group was administered water soluble root powder of *Panax ginseng* 100 mg/kg (p.o), while the stress control group was administered distilled water orally. After 7 days of pretreatment with the extract, the animals were concomitantly exposed to swim endurance test and cold restraint stress (4^{o}C for 2 hours). Cold restraint stress resulted in significant increase in adrenal gland weight with concomitant decrease in spleen weight in stress control group which was significantly reverted by pretreatment with the extract of *Withania somnifera*. The activation of HPA system results in secretion of corticotropin-releasing hormone, adrenocorticotropic hormone (ACTH), β-endorphin and glucocorticoids into the circulation. Pretreatment of animals with *Withania somnifera* extract 23 mg/kg (p.o), improved the swim duration in mice and significantly restored back the stress induced alterations in plasma cortisol, blood glucose and triglyceride levels.

Keywords: HPA system, *Withania somnifera* , Adrenocorticotropic hormone, Cortisol, Swim endurance test, Cold restraint stress.

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INTRODUCTION

Adaptogens are naturally occurring substances found in rare plants and herbs. It is absolutely safe and non-toxic to the human body and increases the body's non-specific resistance to internal and external stimuli and brings the dysfunctioning body's system back into balance. Adaptogens can successfully combat the negative effects of stress, improve health and well-being, and enhance body's performance[1-10, 13].

Withania somnifera (Ashwagandha) has been used for thousands of years as a popular remedy for many conditions. Perhaps its main use, as described in Ayurvedic literature, is as a "rasayana" or rejuvenating drug. The word Ashwagandha indicates the equine (of horses) odour of the plant. Another name Avarada suggests the application of this plant for enhancing longevity. The root drug is considered a tonic and roborant. The root of *Withania somnifera* is used to make the Ayurvedic sedative and diuretic "Ashwagandha", which is also considered an adaptogen. It is said to "protect the organism from illness", through maintaining the healthy balance of the physical energies [16, 31]. The root contains the steroid lactone withaferin A and related withanolides, besides various alkaloids [38, 39]. The sitoindosides IX and X isolated by Ghosal et al., represent C-27-glycowithanolides, the sitoindosides VII and VIII, acyl-esteryl glucosides. The sitoindosides VII, VIII, IX and X represent the adaptogenic active substances of *Withania somnifera*, in spite of diverse steroidal structures [11, 15].

Ashwagandha is one of the main herbs for promoting ojas and rejuvenating the body. It is well-known semen promoter and it treats impotency and infertility. It increases physical endurance and improves sexual function [17, 19-26] . It is a rejuvenative general tonic, which stimulates immune system. Ashwagandha has adaptogenic, immunomodulatory and anti-inflammatory effects [11, 12, 28]. It regenerates hormonal system and has anti-stress properties. It is used in many general tonics and preparations, such as chayavana prash [14], [15]. The present study has been undertaken to find out the mechanism of anti-stress activity of *Withania somnifera* in stress induced mice.

MATERIALS AND METHODS

Plant material roots of *Withania somnifera* 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+*W.somnifera*), treatment group which were fed with ethanolic extract of *Withania somnifera* (p.o), for 7 days.

Stress Procedure

Swim Endurance Test

The mice in group IV were given ethanolic extract of *Withania somnifera*, 23 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 *Withania somnifera* 23 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. 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) [32], serum triglyceride was determined by GPO-POD method [27], total cholesterol was determined by CHOD-POD method and HDL cholesterol was determined by CHOD-PAP method.

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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 1750 mg/kg body weight, (p.o). As shown in figure 1, the extract of *Withania somnifera* improves swim duration in mice. Mice pretreated with ethanolic extract of *Withania somnifera* 23 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+*W.somnifera*, *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 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+*W.somnifera*, *P*<0.01; SC vs S+*P.ginseng*, *P*<0.01; One way ANOVA, *P*<0.01, F = 6.006; Fig. 2).

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 *Withania somnifera* 23 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+*W.somnifera*, *P*<0.01; SC vs S+*P.ginseng*, *P*<0.01; One way ANOVA, *P*<0.01, F = 60.373; Fig. 3).

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+*W.somnifera*, *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 *Withania somnifera* 23 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+*W.somnifera*, *P*<0.01; SC vs S+*P.ginseng*, *P*<0.01; One way ANOVA, *P*<0.01, F = 98.553; Fig. 5).

Thus, on the basis of the above findings it is concluded that the extract of *Withania somnifera* 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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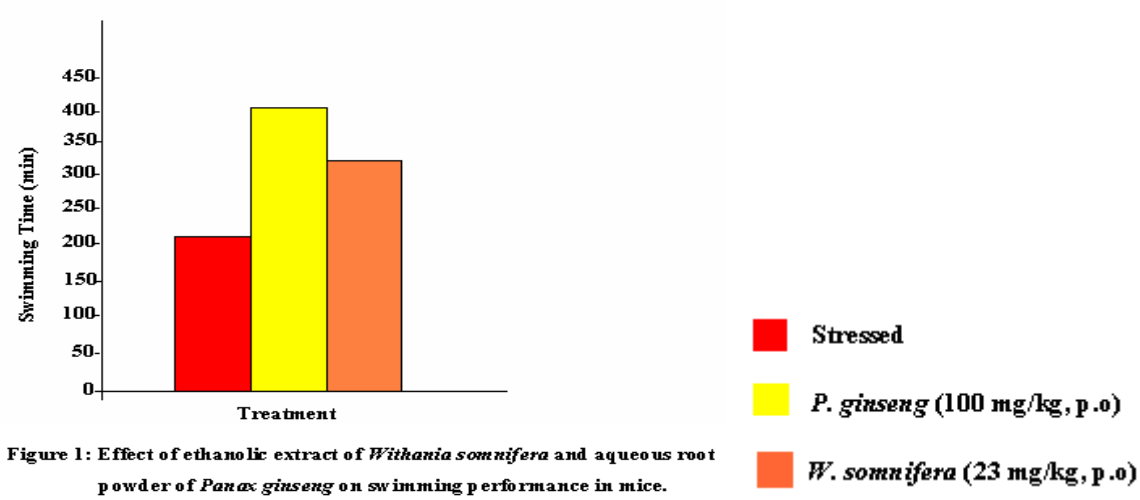

Figure 1: Effect of ethanolic extract of *Withania somnifera* and aqueous root powder of *Panax ginseng* on swimming performance in mice.

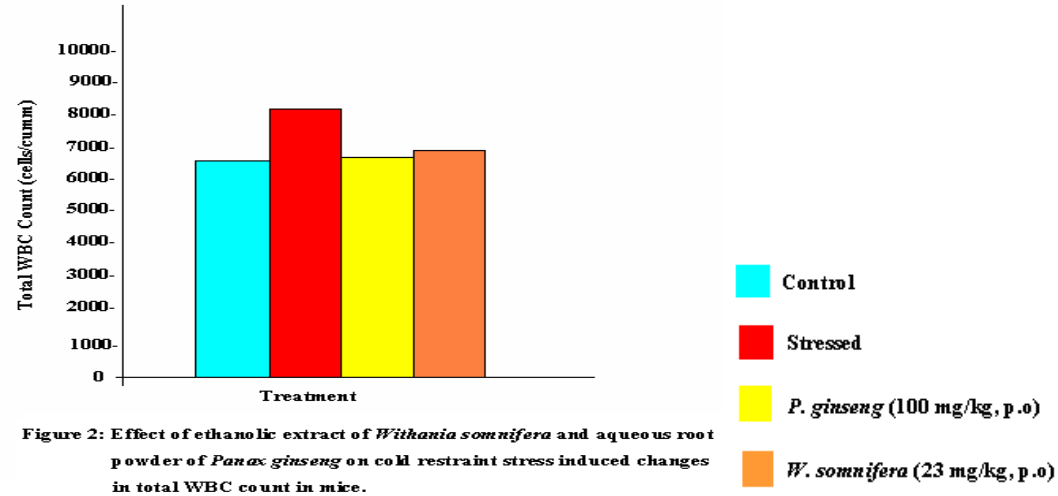

Figure 2: Effect of ethanolic extract of *Withania somnifera* and aqueous root powder of *Panax ginseng* on cold restraint stress induced changes in total WBC count in mice.

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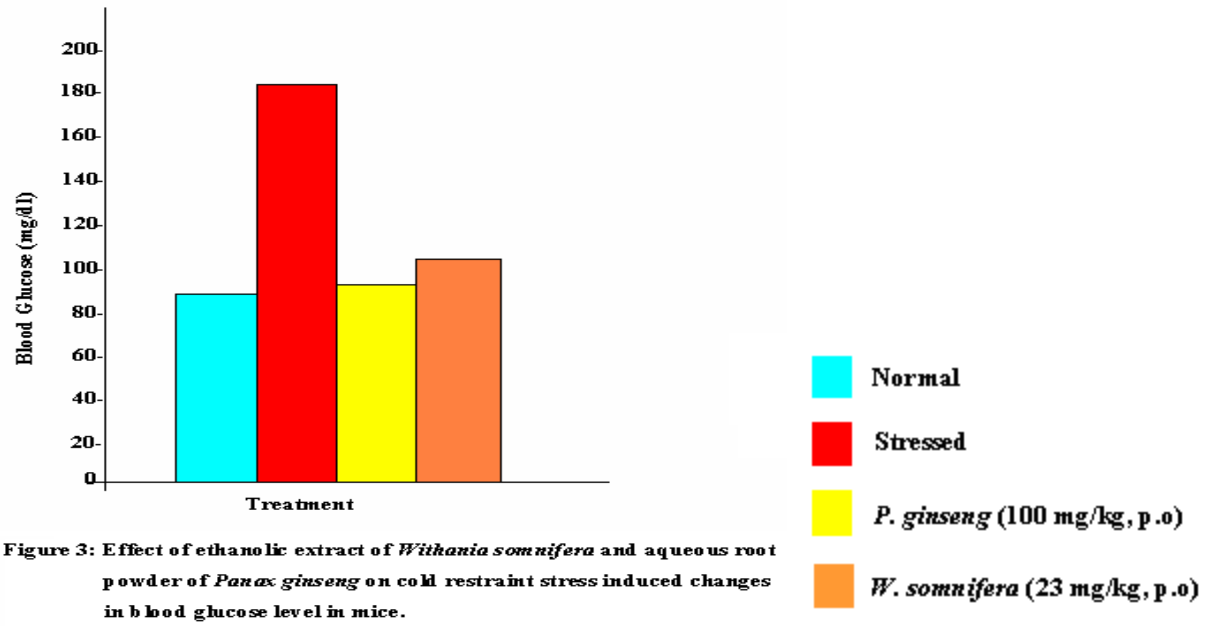

Figure 3: Effect of ethanolic extract of *Withania somnifera* and aqueous root powder of *Panax ginseng* on cold restraint stress induced changes in blood glucose level in mice.

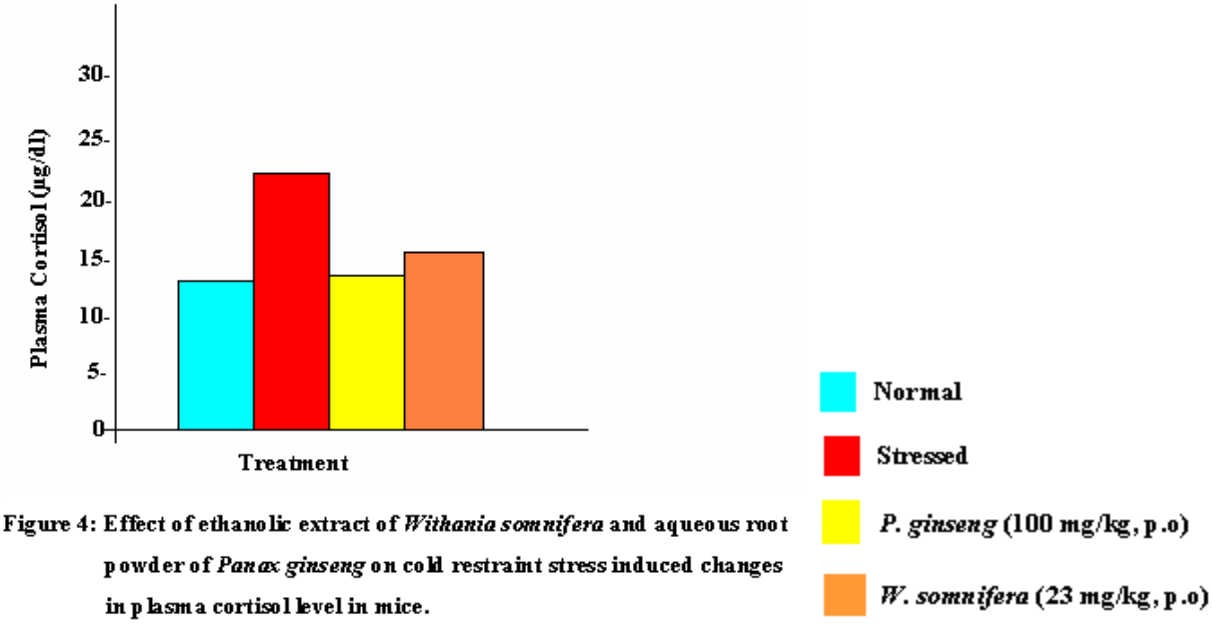

Figure 4: Effect of ethanolic extract of *Withania somnifera* and aqueous root powder of *Panax ginseng* on cold restraint stress induced changes in plasma cortisol level in mice.

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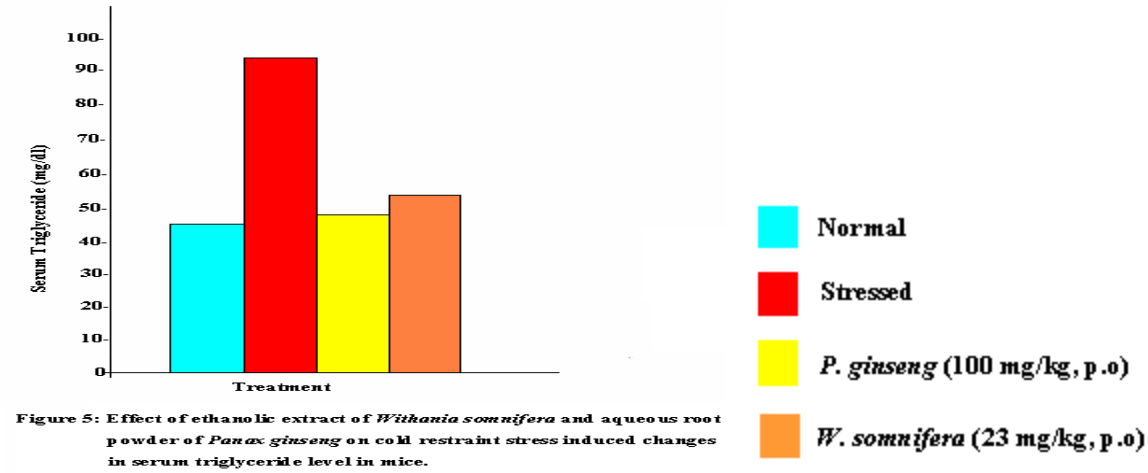

Figure 5: Effect of ethanolic extract of *Withania somnifera* and aqueous root powder of *Panax ginseng* on cold restraint stress induced changes in serum triglyceride level in mice.

DISCUSSION

The testing of the physical endurance of mice, after pre-treatment with *Withania* extract showed a near doubling of the length of perseverance in the swimming test. 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 [18, 33]. No significant change in serum cholesterol level was observed. Stress induced increase in plasma cortisol, total WBC count, blood glucose and serum triglyceride level were blocked by the administration of *Withania somnifera* to mice, while swimming time was increased [29, 30, 34-46].

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