EFFECT OF *Withania somnifera* EXTRACT ON MENTAL STRESS INDUCED CHANGES IN HEMODYNAMIC PROPERTIES AND ARTERIAL WAVE REFLECTIONS IN HEALTHY SUBJECTS

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**ABSTRACT:** Mental stress is known to contribute to the risk for hypertension and coronary atherosclerosis. *Withania somnifera* is well known for its anti-stress and antioxidant activity. The present study was done to assess the effect of *Withania somnifera* extract on acute mental stress induced changes in hemodynamics and arterial wave reflection properties in human participants. In this double-blind, placebo-controlled, randomized, crossover study, 20 healthy participants received 500 mg twice daily of an encapsulated dried aqueous extract of roots and leaves of *Withania somnifera* or matching placebo for 14 days with a wash out period of 14 days. Blood pressure and central arterial wave reflections were measured noninvasively using Sphygmocor before and after a standardized mental stress test. The results demonstrated an acute effect of mental stress on blood pressure and arterial wave reflections. *Withania somnifera* extract produced a statistically significant decrease in aortic pressure, augmentation index, radial and aortic SBP, radial and aortic DBP and significant increase in the subendocardial viability ratio (SEVR) compared to baseline and placebo. A significant decrease in hs-CRP, MDA, serum cortisol levels is seen with *Withania somnifera* extract treatment compared to baseline and placebo. These results suggest that beneficial properties of *Withania somnifera* extract can mitigate the effects of stress and deserves further investigation in patients with associated diseases.

**KEY WORDS:** Arterial properties, Augmentation index, Mental stress, *Withania somnifera* extract

**INTRODUCTION**

Stress is defined as the state in which individuals are faced with the need to make difficult or undesirable changes in order to adapt to events and situations in their lives. The body reacts to these changes with physical, mental, and emotional response. Psychological stress has been linked with a broad array of adverse health outcomes. Studies have demonstrated that stress heightens risk for upper respiratory infection, accelerates the progression of coronary artery disease, and exacerbates the course of autoimmune disorders (Miller et al. 2002).

Although many different types of potential stressors exist, mental and psychosocial stressors are apparently powerful and exert profound effects on the circulatory system. The connection between mental stress and coronary heart disease (CHD) was first investigated by Friedman and Rosenberg in 1959, who described that stress-prone individuals (said to have a “Type A Behavioral Pattern”) more often than others suffer from CHD (Friedman and Rosenman, 1959). Mental stress has been shown to impact cardiovascular health and related morbidity and mortality (Krantz and Manuck, 1984). Conditions such as atherosclerosis, hypertension, stroke and hyperlipidemia have been linked to chronic and repetitive acute stress (Carroll et al.1998; Rozanski et al. 1999; Strike and Steptoe, 2003; Rozanski et al. 1988). Mental stress can be induced by several different methods like mental arithmetic, numbers reading backward, computer quiz, speech stress (public speaking) and reaction time stress tests (stroop’s color word conflict test) in the laboratory (Aubert et al. 2010; Carter, 2008). These tests are known to induce circulatory reactions resembling that evoked by the classical “defense reaction”. This hemodynamic pattern includes increase in blood pressure, heart rate and cardiac output, vasoconstriction in the splanchnic and kidney regions and an increased blood...
flow in skeletal muscle (Freysschuss et al. 1988; Lindvall et al. 1991). This is probably due to vasoconstriction, triggered by enhanced sympathetic nervous system activity. Stress has been shown to increase inflammation primarily through the cytokine interleukin-6 and C-reactive protein (CRP) (Papanicolaou et al. 1988; Yudkin et al. 2000). The secretion of cortisol after stress has been shown to be a reliable marker of psychological stress. Exposure to stress situations has been proposed to impair antioxidant defense system, leading to oxidative damage by changing the balance between oxidant and antioxidant factors, which have been implicated in many human diseases. The preventive role of antioxidants was reported in modifying the major disease conditions related to stress. *Withania somnifera* (Ashwagandha) also known as winter cherry is one of the important ayurvedic rejuvenative botanicals, that improves the body's ability to maintain physical effort and helps the body adapt to various types of stress. It is especially beneficial in stress related disorders such as hypertension, diabetes, arthritis etc (Singh et al. 2010). The roots of *Withania somnifera* are used to promote physical and mental health and used to stabilize mood in patients with behavioral disturbances (Bhattacharya et al. 2000). Research using animal models indicates that supplementation with ashwagandha moderates the stress response when exposed to chronic environmental stressors, including attenuation of symptoms such as depression, increased blood sugar, glucose intolerance, increased cortisol, cognitive deficits and stomach ulcers. The aim of the present study was to investigate the effect of *Withania somnifera* extract on the cardiovascular and haemodynamic response to mental stress in healthy human subjects.

**MATERIALS AND METHODS**

**Study Medication**

Each capsule contains either an inert placebo or 250 mg of highly standardized aqueous extract of the roots and leaves of *Withania somnifera* (SENSORIL® Natreen Inc, USA). The *Withania somnifera* extract was derived from a withaferin A and corresponding withanolide glycoside-predominant, genetically uniform chemotype, which was cultivated in the central and northern provinces of India. Each capsule of *Withania somnifera* used in the present study contains aqueous extract of the roots plus leaves of *Withania somnifera* (Ashwagandha) highly standardised by HPLC containing not less than 10% withanolide glycosides, not more than 0.5% of Withaniferin-A and not less than 32% of oligosaccharides. The placebo capsules were identical in appearance and contain 49.7% (w/w) of microcrystalline cellulose, 49.5% (w/w) of lactose and 0.69% (w/w) of magnesium stearate.

**Study Population**

The study population consisted of twenty-four healthy male individuals.

All subjects were nonsmokers, nonobese (body mass index <23 kg/m²) and they did not have hypertension, diabetes, or hyperlipidemia. They were clinically well and not taking antioxidant vitamin supplementation over past three months. All the subjects abstained from nicotine, caffeine and alcohol intake for at least 24 hrs, prior to and during the test day. Ethical committee of Nizam’s Institute of Medical Sciences, Hyderabad, approved the study protocol and all participants gave written informed consent before study related procedure. All the participants were trained on study procedures on at least two occasions prior to the study to introduce them to the test procedure and to make them familiar with the testing device.

**Study Methodology**

In this double blind placebo controlled crossover study, participants were randomized to receive either *Withania somnifera* extract or placebo capsules in Run I. After an overnight fast, participants were asked to relax in supine position for 20 minutes before initiation of test procedures in the clinical research unit. All the recordings were obtained in an adequately lit, quiet experimental room with an ambient temperature of 22°C ± 2°C to avoid distraction and increase the comfort level of the subject. Brachial blood pressure (BP) and heart rate (HR) were measured with an automated digital BP monitor (OMRON, SEM-1), and the mean of three readings was calculated. Then arterial stiffness was recorded with sphygmocor. The mental stress test was performed as described below. Both blood pressure and arterial stiffness are recorded again within 2 minutes of performing stress test. Subjects then received the study medication and were asked to take 2 capsules twice a day of the study medication allocated to them as per prior randomization schedule with 240 ml of water daily for 14 days. All the measurements were recorded 3 hrs post drug administration on Day-15. A washout period of 14 days was given between the treatments. Subjects then crossed over to receive the second treatment 2 capsules twice daily for 14 days in Run II. All the test procedures described for Run I was repeated pre and post treatment. Participants were asked to report any side effects with either treatment. About 15 ml venous blood samples were collected in heparinized tubes before dosing at Day-1 (baseline) and on Day-15 (End of treatment) for safety and biomarker assessments. If there was any adverse event, it was noted down in the case record form. The drug assignments for each subject were prepared by a staff member otherwise not involved in the present study using a randomization program (Statistica: Stat Soft, Inc, USA). After the study, the drug assignments were revealed for statistical analysis.

**Mental Stress Test**

The mental arithmetic test is a validated and widely accepted test that can induce a considerable degree of perceived stress (Vlachopoulos et al. 2006). In the present study mental stress was induced by asking the participants to perform computerized psychometric tests like choice discrimination test, digit symbol substitution test, digit vigilance test quickly
and as accurately as possible (Raveendranadh et al. 2013). Each test was done thrice and the total duration of the stress test was 10 min. During the test, a metronome was played loudly with headphones as a distracter. This also acted as a source of stress.

Measurement of Wave Reflection Indices

Augmentation index (AIX) and augmented pressure of the central (aortic) pressure waveform was measured by using a validated, commercially available system (SphygmoCor; AtCor Medical, Australia) that employs the principle of applanation tonometry and appropriate acquisition and analysis software for noninvasive recording and analysis of the arterial pulse. The technique has been described in detail previously (Vlachopoulos and O’ourke, 2000). In brief, from radial artery recordings, the central (aortic) arterial pressure was derived with the use of a generalized transfer function that has been shown to give an accurate estimate of the central arterial pressure waveform and its characteristics. Waveforms of radial pressure were calibrated according to sphygmomanometric systolic and diastolic pressure measured at the brachial artery because there is practically negligible pressure pulse amplification between the brachial and the radial artery. The subendocardial viability index was calculated as the ratio of the integral of diastolic pressure and time to the integral of systolic pressure and time.

Measurement of hs-CRP, MDA and Cortisol

The samples were centrifuged after collection at 3500 rpm, 4°C for 10 min. Immediately after centrifugation, the supernatant was stored at – 80°C. Serum levels of hs-CRP and cortisol were measured by ELISA method (Diagnostic Biochem, Canada) and MDA levels were estimated spectrophotometrically using standard methods.

Statistical analysis

All the data were presented as Mean ± SD. All variables were tested for homogeneity of variance and normal distribution, before any statistical analysis was applied. Within subject and pair-wise comparisons between the two treatments (Withania somnifera extract vs. Placebo) were tested for statistical significance using ANOVA and paired t-test. Statistical significance was at p<0.05. Data analysis was performed using Graphpad prism software, Version 4 (Graphpad software Inc. Sandiego, California, USA).

RESULTS

Total twenty-four subjects were screened, out of which four subjects were excluded because of abnormal laboratory investigations. The mean age, height and weight of twenty four subjects were 25.10 ± 2.29 yrs, 169 ± 5.04 cm, and 65.51 ±3.22 kg respectively. All subjects completed both treatments and no participant discontinued the study.

In healthy human subjects, mental stress is known to increase blood pressure. In the present study, mental stress induced a

<table>
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<th>TABLE 1. Effects of placebo and Withania somnifera extract on mental stress induced changes in waveform reflections. The data are presented as Mean ± SD; n=20; *p&lt;0.05, †p&lt;0.01, ‡p&lt;0.001; Post Stress – Pretreatment (Placebo) group vs. Post Stress – Post Treatment (W. somnifera treated group); ¶p&lt;0.05, §p&lt;0.01, ‡p&lt;0.001; Post Stress – Post Treatment (Placebo) group vs. Post Stress – Post Treatment (W. somnifera treated group)</th>
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Withania somnifera and cardiovascular effects

A sustained increase in radial and aortic systolic and diastolic pressure, when compared to baseline in both the groups. However the heart rate remained unchanged. Augmentation index and aortic pressure showed a sustained increase with mental stress denoting an increase in wave reflections. Subendocardial viability ratio is an indicator of myocardial perfusion is reduced by mental stress as compared to baseline.

Compared to baseline and placebo treatment, Withania somnifera extract produced a statistically significant decrease in aortic pressure (from 5.0±2.57 to 3.0±2.7) and augmentation index (from 118±14.71 to 113±12.67) (Table 1). There was a significant increase in the subendocardial viability ratio (SEVR) from 143±18.61 to 158±17.34 on treatment with Withania somnifera extract compared to baseline and placebo. Both radial and aortic SBP as well as radial DBP were significantly decreased with Withania somnifera extract compared to baseline and placebo, however the decrease in aortic DBP is found to be nonsignificant (Table 1).

Compared to placebo, there was a 4% decrease in augmentation index (Figure 1) and significant increase in SEVR. Radial SBP, aortic SBP and DBP also decreased significantly (Figure 2,3). It is well established that in stressful conditions, inflammatory process is accompanied by elevated levels of hs-CRP. Treatment with Withania somnifera extract significantly decreased the elevated levels of hs-CRP induced by mental stress, compared to baseline and placebo (Table 2).

Cortisol hormone released during stress accelerates the generation of free radicals and suppresses the immune function. In the present study serum cortisol and MDA levels, decreased significantly with Withania somnifera extract treatment compared to baseline and placebo. (Table 2).

Compliance to study medication was good and all the subjects in the Withania somnifera extract group and in the placebo group took more than 90% of the study drugs. Safety laboratory parameters were within normal values and none of the treatments affected the safety lab parameters.

### TABLE 2. Effect of placebo and Withania somnifera extract on stress induced changes in Biomarkers measured at Day 1 (Basal) and Day 15 (Post Treatment). The data are presented as Mean ± SD; n=20; *p<0.05, **p<0.01; Post Stress – Pretreatment (Placebo) group vs. Post Stress – Post Treatment (W. somnifera treated group); *p<0.05, **p<0.01; Post Stress – Post Treatment group vs. Post Stress – Post Treatment (W. somnifera treated group)

<table>
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<tr>
<th>Biomarkers</th>
<th>Placebo Pretreatment</th>
<th>Placebo Post stress</th>
<th>Withania somnifera extract Pretreatment</th>
<th>Withania somnifera extract Post stress</th>
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</thead>
<tbody>
<tr>
<td>Hs-CRP (mg/l)</td>
<td>1.05 ± 0.99</td>
<td>1.06 ± 0.93</td>
<td>1.02 ± 0.96</td>
<td>0.63± 0.56**</td>
</tr>
<tr>
<td>MDA (nmol/ml)</td>
<td>5.05±0.56</td>
<td>5.09±0.46</td>
<td>5.07±0.62</td>
<td>4.45±0.55k,d</td>
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<tr>
<td>Serum Cortisol (µg/dl)</td>
<td>30.22±1.13</td>
<td>29.93±1.72</td>
<td>29.55±1.33</td>
<td>26.79±1.88 h,e</td>
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**FIGURE 1. Mean % change in augmentation index induced by mental stress before and after intake of Placebo and Withania somnifera extract. **p<0.01 compared to baseline, ***p<0.01 compared to placebo**

**FIGURE 2. Mean % change in radial BP induced by mental stress before and after intake of Placebo and Withania somnifera extract. ***p<0.001 compared to baseline, **p<0.01 compared to placebo**

**FIGURE 3. Mean % change in aortic BP induced by mental stress before and after intake of Placebo and Withania somnifera extract. *p<0.05, **p<0.01 compared to baseline; *p<0.05 compared to placebo**
DISCUSSION

Studies have demonstrated that psychological stress accelerates the progression of coronary artery disease and exacerbates the course of autoimmune disorders. This is the first study, to the best of our knowledge, to show that administration of *Withania somnifera* extract can ameliorate the negative change in cardiovascular parameters associated with mental stress. The increase in blood pressure and wave reflections with mental stress in the present study were in accordance with previous studies. Mental stress is known to result in rapid changes in systemic hemodynamics (Steptoe et al. 1996) mediated by sympathetic activation (Noll et al. 1996). In humans, arterial wall distensibility can be reduced by an increased sympathetic drive, as shown by the effects on the radial and carotid arteries during cold pressor and mental arithmetic tests (Boutouyrie et al. 1994) and smoking (Failla et al. 1997) i.e. manoeuvres that cause sympathetic activation. A few recent studies have provided further valuable insight into the cardiovascular and haemodynamic responses to sympathetic activation by examining the effects of environmental stress on the magnitude and timing of aortic wave reflection and central pressure. For example Edwards et al. (2006), have demonstrated that cold exposure and the resulting peripheral vasoconstriction increase wave reflection and central SBP. Vlachopoulos et al. (2006), have also shown increased aortic stiffness with premature wave reflection during acute mental stress. A possible underlying mechanism for the unfavorable effect of mental stress on arterial stiffness could be catecholamine release (Strike and Steptoe, 2003; Saitoh et al. 1995; Reims et al. 2004). It has been consistently shown that acute mental stress results in a substantial increase in the circulating levels of catecholamines, i.e., epinephrine, norepinephrine, and dopamine.

The roots and leaves of *Withania somnifera* has been reported to have adaptogenic, immunomodulatory, antioxidative and neurological effects (Sharma et al. 2011). *Withania somnifera* possesses a potent anti-stress properties and is reported to alleviate stress induced changes and provides cardio-protection in ischemic rats similar to the properties ascribed to adaptogens like panax ginseng by increasing the swimming time during a swimming endurance test. A significant increase in relative heart weight and glycogen content in the myocardium and liver was also observed in the withania group (Dhuley, 2000a; 1998b). The active principles of ashwagandha, for instance the sitoindosides VII–X and Withaferin-A, have been shown to have significant anti-stress activity against acute models of experimental stress.

In the present study, *Withania somnifera* extract produced a beneficial effect on arterial function by reducing aortic stiffness, wave reflections and aortic pulse pressure. Indeed, increased aortic stiffness, enhanced wave reflection, increased systolic pressure and pulse pressure (and especially central pulse pressure) has been identified as independent components of cardiovascular risk (Weber et al. 2004; Vlachopoulos et al. 2000; Blacher, 1999). *Withania somnifera* is reported to induce a significant decrease in the diastolic blood pressure in “normotensive” pentobarbitral anaesthetized dogs (Ahumada et al. 1991). In our study, treatment with *Withania somnifera* extract produced a significant decrease in radial and aortic SBP and DBP. A significant decrease in systolic and diastolic BP and heart rate is reported in chronically stressed individuals treated with *Withania somnifera* as shown by Biswajit et al. (2008). In contrast Sandhu et al. reported that *Withania somnifera* didn't show any effect on resting systolic and diastolic BP in healthy subjects treated with 500 mg for 8 weeks (Sandhu et al. 2010).

Subendocardial viability ratio (SEVR) is an index of myocardial oxygen supply and demand. Low SEVR has been shown to be associated with coronary artery disease, decreased coronary flow reserve in patients with healthy coronary arteries, severity of type I and type II diabetes, decreased renal function, and a history of smoking (Doonan et al. 2011). The decrease in SEVR following exposure to stress in the present study, may be probably due to vasoconstriction and decreased myocardial perfusion. In the present study, *Withania somnifera* extract produced a significant increase in SEVR indicating improvement in myocardial perfusion.

An increase in the inflammatory marker C-reactive protein (CRP) has been associated with a higher risk of incident coronary heart disease (CHD). In the present study, *Withania somnifera* extract decreased hs-CRP indicating a decline in systemic inflammation. Cortisol has an important effect on the glucose, protein and fat metabolism and cardiovascular reactivity (Venkataraman et al. 2007; Widmaier et al. 2003). Chronic rise of cortisol level due to hypothalamic-pituitary-adrenal axis stress response has been associated with the pathogenesis of several disorders such as immunosuppression, obesity, cardiovascular disease, DM, stroke and osteoporosis (McEwen et al. 1997; Raff et al. 1999). High levels of serum cortisol were reported to increase blood pressure in healthy adults (Ward et al. 2003) and in healthy older men (Phillips, 1998). The present study showed a significant decrease in cortisol level on treatment with *Withania somnifera* extract. Our study results are in accordance with previous studies. Biswajit et al. (2008) reported significant decrease in hs-CRP, and cortisol levels in chronically stressed individuals treated with *Withania somnifera*. The therapeutic activity of *Withania somnifera* may be attributed to its hypothalamic pituitary adrenal axis, which regulates the serum cortisol concentration.

The measurement of MDA is widely used as an indicator of lipid peroxidation and increased levels of the peroxidation products have been associated with a variety of acute, chronic pathophysiological processes in both humans and animal models (Draper et al. 1990; Romero et al. 1998). High MDA level is indicative of higher lipid peroxidation as observed in coronary heart disease patients. The decrease in MDA level after treatment with *Withania somnifera* extract in the present study is clearly indicative of antioxidant activities of the drug. Bhattacharya SK, et al. (1997), reported a dose-related increase in superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) levels in the rat brain frontal.
cortex and striatum following administration of *Withania somnifera* (10 or 20 mg/kg intraperitoneally were given once daily for 21 days). These results indicate that *Withania somnifera* perhaps has an antioxidant effect in the brain which may be responsible for its diverse pharmacological properties. In another study (Dhuley, 1998), an aqueous suspension of *Withania somnifera* root extract prevented an increase in stress-induced lipid peroxidation (LPO) in mice and rabbits.

**CONCLUSION**

Mental stress for a brief period results in aortic stiffness and alterations in wave reflections. In the present study *Withania somnifera* extract decreased the mental stress induced changes on aortic wave reflections measured by sphygmocor in normal healthy subjects, suggesting the beneficial effects of this formulation in reducing the cardiovascular pharmacodynamic effects of mental stress and hence in decreasing the cardiovascular morbidity. Further studies are required to assess whether favorable effects of *Withania somnifera* extract decrease mental stress induced changes in patients with associated diseases.

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