

Evaluation of effect of highly standardized aqueous extract of roots and leaves of *Withania somnifera* on cold pressor test induced cardiovascular changes in healthy human subjects

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ABSTRACT

Background: Stress is an important cardiovascular risk factor. Cold pressor test (CPT) is a simple, validated, non-invasive test used to measure stress induced changes in cardiovascular parameters. The objective of this study was to evaluate effect of *Withania somnifera* extract on cold pressor stress test induced changes on cardiovascular parameters and aortic wave reflections in healthy human subjects.

Methods: This was a double-blind, placebo controlled, crossover study. Participants were randomized to receive either two capsules of *Withania somnifera* extract 250 mg or two capsules of placebo twice daily for 14 days. Pharmacodynamic parameters heart rate, aortic pressure, augmentation index (AIx), subendocardial viability ratio (SEVR), radial and aortic blood pressure (BP) were recorded before and after CPT at baseline and at end of treatment. After washout period of 10 days, subjects crossed over to other treatment group and same procedure was repeated. Safety assessments were done at baseline and at end of treatment.

Results: A total of 20 volunteers completed the study. Compared with baseline and placebo, *Withania somnifera* extract produced a significant decrease in mean percent change of arterial stiffness indices (AIx, radial and aortic BP). SEVR with CPT increased, however it was non-significant compared to baseline and placebo. Both treatments were well-tolerated and no serious adverse events were reported.

Conclusions: *Withania somnifera* extract showed a significant decrease in cold pressor stress test induced changes on aortic wave reflections, suggesting its beneficial effects in reducing stress induced cardiovascular changes. However, further clinical studies are warranted to evaluate these effects in patients with cardiovascular and other associated diseases.

Keywords: Augmentation index, Cardiovascular disease, Cold pressor test, *Withania somnifera*

INTRODUCTION

The maintenance of life is critically dependent on keeping the internal milieu constant in the face of a changing environment.¹ This is known as "homeostasis."² According to Selye, "stress" represents the effects of anything that seriously threatens homeostasis. Severe, prolonged stress responses might lead to tissue damage and disease.³ Allostasis refers to the maintenance of homeostasis through the constant adjustment and

balancing of hypothalamic pituitary adrenal (HPA) axis and sympathoadrenal medullary (SAM) pathways in the process of adapting to challenge.⁴⁻⁶

The relationship between stress and cardiovascular disease is complex. Type A pattern individuals, who are generally stressed out, tend to be associated with a greater cardiovascular risk due to increased sympathetic activity and enhanced catecholamine responses to stress.⁷ Free radicals generated by stress cause oxidative stress

damage to cardiovascular tissues leading to imbalance between oxidant/antioxidant systems.⁸ The existing evidence support the view that oxidative stress may play a crucial role in different types of cardiovascular diseases and that the antioxidant therapy may prove beneficial in combating these problems.⁹

Antioxidants play an important protective role against reactive oxygen species. *Withania somnifera* (family Solanaceae), also called as “Ashwagandha”, is used widely in traditional medicine for its anti-inflammatory, anti-tumour, anti-stress, anti-oxidant, immunomodulatory, haemopoetic and rejuvenating properties.¹⁰ The anti-oxidative property of WS mainly by increasing the free radical scavenging enzymes like superoxide dismutase, catalase and glutathione peroxidase levels.¹¹ Augmentation of endogenous antioxidants, maintenance of the myocardial antioxidant status and significant restoration of most of the altered haemodynamic parameters may contribute to its cardio protective effect.¹²

Acute cold stress contributes to elevations in blood pressure and heart rate. Studies report that a sudden exposure to the cold can increase the blood pressure of humans by 20 mmHg, especially when there exists cold induced pain at the same time.^{13,14} The effects of cold induced stress on cardiovascular changes in normal healthy subjects can be evaluated by cold pressor test, which is a simple, non-invasive and validated test for sympathetic activation.¹⁵

The present study was hence undertaken to evaluate the effect of *Withania somnifera* on cold pressor stress test induced changes on cardiovascular parameters and aortic wave reflections in healthy human subjects.

The objective of this study was to evaluate the effect of *Withania somnifera* extract on cold pressor stress test induced changes on cardiovascular parameters and aortic wave reflections in healthy human subjects.

METHODS

This was a prospective, randomized, double blind multi-dose, cross-over, placebo controlled study conducted after approval of the Institutional Ethics Committee of Nizam's Institute of Medical Sciences (NIMS), Hyderabad, India. All subjects gave written informed consent prior to participation in the study. The cold pressor stress test model equipment used in the present study was designed and validated by the department of Clinical Pharmacology and Therapeutics, NIMS, Hyderabad.¹⁶

Twenty six healthy male subjects, aged 20-35 years were screened for their eligibility to participate in the study with complete medical history, physical examination, hematological and biochemical screening, electrocardiogram and chest X-ray. History of smoking

and drug abuse was excluded. The subjects were excluded from study if there was any evidence of physical illness, drug abuse or abnormal laboratory parameters. Eligible subjects were trained on the test procedure on two prior occasions to make them familiar with the testing device and test procedure. All the recordings were carried out in the morning between 7.30 am and 10:00 am after a light breakfast.

The study medications included capsules of Sensoril® (250 mg strength) and placebo. Each Sensoril® 250 mg capsule consisted of highly standardized aqueous extract of roots and leaves of *Withania somnifera* (Sensoril®) containing not less than 10% withanolide glycosides, not less than 32% oligosaccharides and not more than 0.5% of Withaferin-A. Each placebo capsule contained microcrystalline cellulose (49.7% w/w), lactose (49.5%) and magnesium stearate (0.69% w/w). Both study medications were supplied by Natreon, Inc. USA.

Study procedure

The study used a double blind placebo controlled crossover design, with participants randomized to receive either two capsules of *W.somnifera* 250 mg twice a day or two capsules of placebo twice a day for 14 days in Run I. Subjects arrived at the study site following an overnight fast and abstinence of caffeine containing beverages and alcohol for 12 hours. Before initiating the test procedure, the subjects were rested in a supine position for 20 minutes in a quiet, temperature-controlled ($26^{\circ}\pm 1^{\circ}\text{C}$) room. Subjects were asked to breathe normally and to remain still during cardiovascular measurements. They were permitted to listen to music and to read, except during the periods of cardiovascular measurements. The test measurements were performed at baseline and at the end of 14 days. All the measurements were recorded 3 hours post drug administration. The baseline arterial stiffness was recorded with Sphygmocor, after which cold pressor test (CPT) was performed. The arterial stiffness was again recorded within 2 minutes of performing CPT.

Recording of vital parameters

Brachial BP and heart rate were measured with an automated digital BP monitor (OMRON, SEM-1) and a mean of three readings was taken. All readings were taken with cuff placed on the subject's non-dominant arm positioned at heart level with the forearm resting on a table.

Cold pressor test (CPT)

Cold pressor test is a potent stimulus for eliciting large elevations in blood pressure. The recording was performed in a temperature controlled room at 24°C . After 10 minutes of rest, subject's BP was recorded. Then the subject's non-dominant hand was immersed till the wrist in the water bath maintained at 35°C for 2 minutes.

15 seconds before the end of this 2 minute period, a pressure of 20 mm less than the normal diastolic pressure of that subject was applied using BP cuff on the lower part of non-dominant hand. Immediately after applying the pressure the subject was asked to immerse his non-dominant hand till the wrist in to the cold water bath maintained at 4-5°C, till he was able to sense unbearable pain. The subject was then asked immerse his hand again in water bath maintained at 35°C for 1 minute for normalization of temperature.¹⁶

Measurement of wave reflection indices

Arterial stiffness 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 non-invasive recording and analysis of the arterial pulse.¹⁷⁻¹⁹ Augmentation index (AIx) and augmented pressure of the central (aortic) pressure waveforms were measured as indices of wave reflections. The AIx (defined as augmented pressure divided by pulse pressure and expressed as a percentage) is a composite measure of the magnitude of wave reflections and arterial stiffness, which affects timing of wave reflections. Because the augmentation index is influenced by changes in heart rate (HR), it was also accordingly corrected. The central (aortic) arterial pressure was derived from radial artery recordings, 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. Wave forms of radial pressure were calibrated according to sphygmomanometric systolic and diastolic pressure measured in 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. The subendocardial viability index, an indicator of myocardial workload and perfusion (O₂ supply vs. demand) was calculated as the ratio of the integral of diastolic pressure and time to the integral of systolic pressure and time.²⁰

After the baseline test measurements, the subjects were discharged with study medications for 14 days and were instructed to take 2 capsules twice daily with 240 ml of water. The same test procedure was again repeated after 14 days of treatment to obtain post treatment values. A washout period of 10 days was given between the treatments. Subjects were then crossed over to receive the second formulation of 2 capsules twice daily for 14 days for run II. All the test procedures described for run I were repeated pre and post treatment.

Statistical analysis

The study has been carried out in 26 healthy volunteers as a pilot study. Two-way ANOVA and paired t-test were used for the analysis of study parameters. A $p < 0.05$ was

considered to be statistically significant. All statistical analyses were performed using Graphpad Prism 5, Sandiego, CA, USA.

RESULTS

A total of twenty six male subjects were screened, out of whom twenty eligible male subjects were randomized into the study to receive the study medications in a crossover design. All the randomized subjects completed the study and were evaluated for cardiovascular measurements. Mean age, height and weight of the randomized subjects were 24.89±4.90 years, 170±4.14 cm, 64.63±5.29 kg respectively.

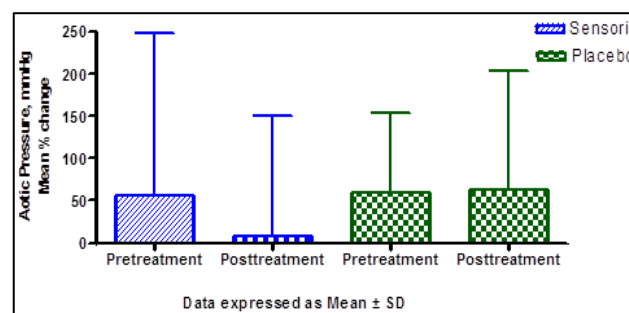


Figure 1: Mean % change in aortic pressure induced by cold pressor test Withania somnifera and placebo (n=20).

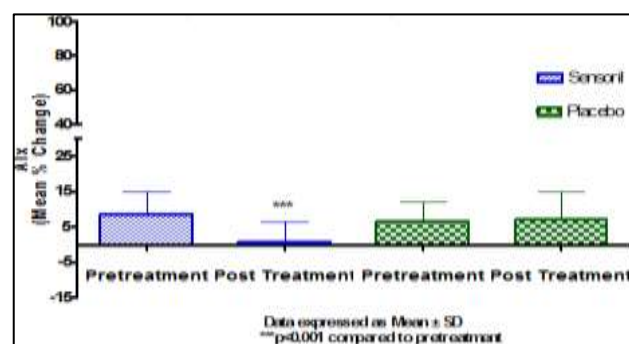


Figure 2: Mean % change in augmentation index induced by cold pressor test Withania somnifera and placebo (n=20).

Effect of cold pressor test on wave reflections

The results of effect of cold pressor test on wave reflections are shown in (Table 1). *W. somnifera* produced a statistically significant decrease in aortic pressure ($p < 0.05$) (Figure 1), augmentation index ($p < 0.001$) (Figure 2), radial systolic blood pressure ($p < 0.05$), aortic systolic blood pressure ($p < 0.01$), radial and aortic mean blood pressures ($p < 0.05$) and radial pulse pressure ($p < 0.05$), compared to baseline. An increase in heart rate and SEVR were observed, which were not statistically significant ($p > 0.05$). Also, a non-significant decrease in radial and aortic diastolic blood pressures and aortic pulse

pressure were observed ($p>0.05$). When compared with placebo, statistically significant differences ($p<0.05$) were observed with *W. somnifera* in aortic pressure, augmentation index, radial and aortic systolic blood pressures, radial and aortic diastolic blood pressures, radial and aortic mean blood pressures and radial pulse

pressure. No significant differences were observed in heart rate, SEVR and aortic pulse pressure ($p>0.05$). All subjects tolerated both treatments well and none of the subjects discontinued the study due to adverse events. Safety laboratory parameters were within normal values.

Table 1: Effects of *Withania somnifera* and placebo on cold pressor induced changes in waveform reflections, (Data are expressed as the mean \pm SD).

	<i>Withania somnifera</i>				Placebo			
	Pre treatment		Post treatment		Pre treatment		Post treatment	
	Baseline (Before CPT)	Within 2 minutes of hand immersion	Baseline (Before CPT)	Within 2 minutes of hand immersion	Baseline (Before CPT)	Within 2 minutes of hand immersion	Baseline (Before CPT)	Within 2 minutes of hand immersion
HR (bpm)	71 \pm 7.15	68 \pm 6.79	71 \pm 6.28	70 \pm 6.96 ^{NS}	70 \pm 4.94	69 \pm 3.52	70 \pm 4.08	69 \pm 4.60
AP (mmHg)	2.9 \pm 3.72	6.35 \pm 3.99	2.27 \pm 3.20	3.21 \pm 3.01 ^{*#}	3.80 \pm 3.90	5.95 \pm 3.72	4.25 \pm 4.16	7.45 \pm 4.41
AIx (%)	114.11 \pm 18.02	124 \pm 21.86	109.86 \pm 14.34	110.54 \pm 14.26 ^{***#}	116 \pm 19.75	123 \pm 19.26	117.25 \pm 19.84	125 \pm 19.14
SEVR (%)	147.5 \pm 19.22	149.37 \pm 16.21	144 \pm 19.89	151 \pm 18.66 ^{NS}	144 \pm 16.68	148 \pm 13.55	143 \pm 14.27	147 \pm 15.3
SBP	113 \pm 4.41	122 \pm 7.11	112 \pm 4.15	114 \pm 4.70 ^{*#}	112.9 \pm 4.02	120 \pm 5.33	114 \pm 2.97	122 \pm 4.78
DBP	69 \pm 5.06	72 \pm 8.16	70 \pm 3.66	71 \pm 4.81 ^{NS#}	71.2 \pm 4.42	74 \pm 4.08	71 \pm 3.64	73.9 \pm 3.27
MBP	85 \pm 4.66	89 \pm 7.92	83 \pm 3.22	85 \pm 4.62 ^{*#}	85 \pm 3.74	90 \pm 4.00	85 \pm 2.96	90 \pm 3.13
PP	44 \pm 5.20	50 \pm 8.18	42 \pm 5.24	43 \pm 4.55 ^{*#}	42 \pm 4.46	46 \pm 4.55	43 \pm 3.69	48 \pm 4.72
SBP	98 \pm 4.76	106 \pm 8.46	98 \pm 3.77	99 \pm 5.05 ^{*#}	98 \pm 4.63	104 \pm 5.25	99 \pm 4.87	106 \pm 5.35
DBP	69.41 \pm 5.52	72.95 \pm 8.0	69 \pm 4.27	70 \pm 5.16 ^{NS#}	70 \pm 4.75	73 \pm 5.55	71 \pm 3.58	74 \pm 3.55
MBP	81 \pm 5.98	87 \pm 8.87	81 \pm 5.07	81 \pm 4.91 ^{*#}	79 \pm 3.65	84 \pm 4.44	80 \pm 2.99	85 \pm 2.93
PP	28 \pm 4.67	32 \pm 6.11	29 \pm 3.71	30 \pm 4.13 ^{NS}	28 \pm 6.29	31 \pm 6.72	28 \pm 5.81	32 \pm 6.49

HR: Heart rate; AP: Aortic Pressure; AIx: Augmentation index; SEVR: Subendocardial viability ratio; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; MBP: Mean blood pressure; PP: Pulse pressure; * $p<0.05$, compared to baseline; ** $p<0.01$, compared to baseline; *** $p<0.001$, compared to baseline; NS-not significant, compared to baseline; # $p<0.05$, compared to placebo.

DISCUSSION

In this study, 20 healthy male subjects were evaluated for the effect of treatment with *Withania somnifera* and placebo on stress induced cardiovascular changes. Physiological parameters have been used as risk factors to predict cardiovascular events.²¹ Accordingly, increased aortic stiffness, enhanced wave reflection, increased systolic pressure and increased central pulse pressure have been identified as independent components of cardiovascular risk.²² In this study, cold pressor test (CPT) was used as a model to produce stress induced cardiovascular changes in healthy subjects.

The cold pressor test which is considered to be a sympatho excitatory maneuver is a simple, non-invasive and validated test of sympathetic activation.²³ It induces a vascular sympathetic activation and a subsequent increase in blood pressure in healthy subjects.²⁴ The principle behind CPT is that sudden and increasingly painful cold stress causes massive discharge of the sympathetic nervous system and release of norepinephrine. This sympathetic discharge triggers responses in the cardiovascular system that includes arteriolar constriction, increased heart rate, and increased cardiac contractility. These responses combine to increase the blood pressure. This is known as the pressor response.²⁵ Also, CPT has

been shown to cause mild to moderate activation of the HPA axis.²⁶ The cold exposure also increases aortic augmentation index (AIx), a measure of wave reflection, leading to augmented central systolic pressure.²⁷

Cold pressor test conducted in healthy individuals demonstrated that stress exerted through the cold pressor for a minute stimulated the sympathetic nervous system resulting in accelerated heart rate and elevated blood pressure, both systolic and diastolic, in comparison to those recorded before the foregoing test in all the normotensive volunteers.²⁸ In our study, similar observations have been recorded. In our study, there was an increase in aortic pressure, augmentation index, radial and aortic systolic and diastolic BP after CPT. These stress induced changes on cardiovascular parameters were attenuated on treatment with *W.somnifera* extract. However, treatment with placebo did not show any significant change in response to CPT induced stress.

Augmentation index (AI) determined from either a directly measured or a derived central arterial pressure waveform has been proposed as a measure of aortic stiffness and wave reflection.²⁹ Because of the dependence of timing of wave reflection on pulse wave velocity (PWV), reductions in AI after an intervention have been interpreted as evidence of comparable

reductions in PWV and aortic stiffness.²⁹ In our study, *W.somnifera* significantly attenuated the rise in augmentation index induced by CPT, thus suggesting its cardio protective effect.

Withania somnifera has been shown to possess cardio protective properties. The pharmacological effects of the roots and leaves of *W. somnifera* are attributed to the presence of Withanolides, a group of steroidal lactones and alkaloids.^{30,31} Experimental studies have demonstrated the hypotensive and bradycardiac effects of *Withania* alkaloids. Further it was found that the hypotensive effect was due to autonomic ganglion blocking action and depressant action on the higher cerebral centers. The alkaloids produced immediate but short lived cardio-depressant effects and a weak but prolonged cardiostimulant effects.³²

Oxidative stress has been shown to play an important role in the causation of cardiovascular diseases and evidence shows that antioxidant therapy may prove beneficial in combating these problems.³³ Pre-clinical studies have demonstrated that the cardio protective activity of *Withania somnifera* is mainly due to its antioxidant and anti-apoptotic activity, both of which constitute the anti-stress properties.³⁴ It increases the level of antioxidant enzymes such as glutathione peroxidase, superoxide dismutase, catalase, creatinine phosphokinase and lactate dehydrogenase. It also inhibits lipid-peroxidation, comparable to vitamin E, a known cardio protective antioxidant.^{35,36} In a study by Thirunavukkarasu et al, *W.somnifera* was found to possess energy boosting properties in ischaemia-reperfusion compromised heart and it was further recommended to be used as a dietary supplement for cardio protection.³⁷ In another study, a polyherbal formulation containing *W. somnifera* has been found cardio protective and antioxidant in isoproterenol-induced ischemic rats.³⁸ Apart from the antioxidant effects, *W.somnifera* has also demonstrated its effect as an adaptogen that works on a nonspecific basis to normalize physiological function, working on the HPA axis and the neuro-endocrine system and thus reducing the stress related changes.³⁹ These well-established antioxidant and cardio protective actions of *Withania somnifera* explain the possible mechanism of *W. somnifera* extract in reducing cardiovascular changes produced by CPT.

CONCLUSION

The *Withania somnifera* extract used in the present study showed significant decrease in cold pressor stress test induced changes on aortic wave reflections, suggesting the beneficial effects of this formulation in reducing stress induced cardiovascular changes. However, further clinical studies are warranted to evaluate the beneficial effects of *Withania somnifera* in patients with cardiovascular and other associated diseases.

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