



Research Article

EVALUATION OF A HIGHLY STANDARDIZED *WITHANIA SOMNIFERA* EXTRACT ON ENDOTHELIAL DYSFUNCTION AND BIOMARKERS OF OXIDATIVE STRESS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS: A RANDOMIZED, DOUBLE BLIND, PLACEBO CONTROLLED STUDY

Pingali Usharani^{1*}, Nishat Fatima², Chiranjeevi Uday Kumar³, P.V. Kishan³

¹Additional Professor, Department of Clinical Pharmacology and Therapeutics, Nizam's Institute of Medical Sciences, Hyderabad, Telangana, India.

²Research Scholar, Department of Clinical Pharmacology and Therapeutics, Nizam's Institute of Medical Sciences, Hyderabad, Telangana, India.

³Senior Resident, Department of Clinical Pharmacology and Therapeutics, Nizam's Institute of Medical Sciences, Hyderabad, Telangana, India.

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ABSTRACT

Background: Type 2 Diabetes mellitus is a multisystem disorder with oxidative stress and endothelial dysfunction. *Withania somnifera* Dunal (*Ashwagandha*) is shown to have potent antioxidant, hypoglycemic and hypolipidemic effects in several studies. The present study was planned to compare the effect of *Withania somnifera* on endothelial dysfunction and biomarkers in patients with diabetes mellitus. **Materials and Methods:** After taking IEC approval and written informed consent, 66 eligible patients, who are on metformin therapy, were randomized to receive either one capsule of highly standardized aqueous extract of *Withania somnifera* 250mg twice daily, one capsule of *Withania somnifera* 500mg twice daily or Placebo for a duration of 12 weeks. Primary efficacy parameter was a change in endothelial function (measured as change in reflection index of more than 6 %) performed by salbutamol challenge test at baseline and after 12 weeks of treatment. Secondary end points were change in biomarkers of oxidative stress (malondialdehyde, nitric oxide and glutathione), high sensitivity C-reactive protein and change in lipid profile. Safety lab parameters were measured, at baseline and after 12 weeks of treatment. **Results:** A total of 60 patients completed the study. Twelve weeks of treatment with *Withania somnifera* 250mg and 500mg produced significant reduction in reflection index (-2.52±1.32% to -7.49±3.49%) and (-2.24±1.00% to -9.03± 2.42%) respectively, suggesting improvement in endothelial function versus placebo (-2.11±1.62% to -0.81± 2.86%). Similarly a significant improvement in biomarkers of oxidative stress, systemic inflammation, lipid parameters and HbA1c levels, compared to baseline and placebo, was observed with *Withania somnifera*. All treatments are well tolerated. **Conclusion:** *Withania somnifera* showed significant improvement in endothelial function, reduction in biomarkers of oxidative stress and systemic inflammation and can be used as a therapeutic adjunctive in patients with type 2 Diabetes mellitus.

KEYWORDS: *Withania somnifera*, *Ashwagandha*, Oxidative stress, Endothelial dysfunction.

INTRODUCTION

Cardiovascular diseases (CVD), comprising coronary heart disease (CHD) and cerebro-vascular diseases, are the leading cause of death globally, accounting for 21.9 per cent of total deaths and are projected to increase to 26.3 per cent by 2030^[1]. For India this increase is

estimated to be 58%, from 51 million people in 2010 to 87 million in 2030^[2]. Among the risk factors, diabetes and its predominant form, type 2 diabetes mellitus (T2DM), has a distinctive association with CHD. Atherosclerosis accounts for approximately 80% of all diabetic mortality

and about 75% of this is a consequence of coronary artery disease, the remaining 25% results from accelerated cerebrovascular and peripheral vascular disease^[3]. Endothelial dysfunction is believed to be important in the pathogenesis of microvascular and macrovascular disease especially leading to a marked increase in atherosclerotic vascular disease^[4]. Endothelial dysfunction results from reduced bioavailability of the vasodilator nitric oxide (NO) mainly due to accelerated NO degradation by reactive oxygen species^[5]. Oxidative stress, through a single unifying mechanism of superoxide production, is the common pathogenic factor leading to insulin resistance, β -cell dysfunction, impaired glucose tolerance and ultimately to type 2 DM and has been implicated as the underlying cause of both the macrovascular and microvascular complications associated with it. It follows that therapies aimed at reducing oxidative stress would benefit patients with type 2 DM and those at risk for developing diabetes^[6,7]. The spectrum of dyslipidemia in diabetes mellitus can include all the various types of dyslipidemia identified in the general population^[8]. Drugs like statins, ACE-Inhibitors etc have been effectively tried for endothelial dysfunction, but are associated with side effects. Some herbal formulations are reported to possess potent antioxidant, anti-inflammatory and cardio-protective properties and are used by patients with increased risk of cardiovascular morbidity and mortality. *Withania somnifera*, also known as Ashwagandha is an important herb in the Ayurvedic and indigenous medical system. The active principles of *Withania somnifera* sitoindosides VII-X and Withaferin A (glycowithanolides) have shown to possess antioxidant effects. Several studies indicated that *Withania somnifera* has antitumor, antistress, anti-inflammatory and rejuvenating properties^[9].

The present study was thus undertaken to evaluate the effect of highly standardized aqueous extracts of *Withania somnifera* 250mg, *Withania somnifera* 500mg and Placebo on endothelial function in patients with type 2 diabetes mellitus and further study its probable mechanism of action.

MATERIALS AND METHODS

The present study was a randomized, double blind, placebo controlled, parallel design conducted in the Department of Clinical Pharmacology and Therapeutics, Nizam's Institute of Medical Sciences, Hyderabad, India. Sixty six patients were enrolled in the study which was approved by the Institutional Ethics Committee. All subjects gave written Informed consent prior to participation in the study. Patients of either gender, aged 18-65 years, fasting plasma glucose between 110 -126 mg/dL, a glycosylated hemoglobin (HbA1c) between 6.5 % and 8% and taking stable dose of anti-diabetic treatment (Metformin 1500-2500 mg/day^[10]) for the past 8 weeks prior to the screening visit; and having endothelial dysfunction defined as \leq 6% change in reflection index (RI) on post salbutamol challenge test were included in the study. Patients with severe uncontrolled hyperglycemia, uncontrolled hypertension, cardiac arrhythmia, impaired hepatic or renal function, history of malignancy or stroke, smoking, chronic alcoholism, any other serious disease requiring active treatment and treatment with any other herbal supplements, were excluded from the study.

Study Medication

In the present study we used SENSORIL® an aqueous extract of the roots of *Withania somnifera* (Ashwagandha) and is highly standardized by HPLC as shown in Fig. 1, containing not less than 10% withanolide glycosides, not more than 0.5% of Withaferin-A and not less than 32% of oligosaccharides in the dose of 250mg twice daily and 500mg twice daily. Identical matching placebo capsules were used.

Estimation of Withanolide glycosides, aglycones and Oligosaccharides in *Withania somnifera* extracts (Sensoril®)

Withania somnifera extract (roots), used in this study, was standardized to contain withanolide glycosides (not less than 10% w/w), Oligosaccharides (not less than 32% w/w) and aglycones (Withaferin-A) (not more than 0.5% w/w) by HPLC using external standards (isolated from *Withania somnifera* root extract by column chromatography). Briefly, *Withania somnifera* extract (200 mg) was dissolved in distilled water (20 ml), sonicated for 10 min and

heated on steam bath for 10 min followed by centrifugation at 8500 rpm for 12 min. The water soluble portion (supernatant) was diluted with millipore water to get 2.5 mg/ml for Withanolide glycosides and aglycones quantification, whereas 10mg/ml was used for oligosaccharide quantification. This solution was filtered through 0.2 µm syringe filter and 20µl was used for HPLC analysis using the following conditions.

- (i). Withanolide glycoside and aglycones estimation: Waters HPLC system (equipped with 515 model pump, Waters TM 2996 photodiode array detector and empower software); the column used was Lichrocart RP C18 250X4 mm 5µm (Merck; LO 10138433) using Acetonitrile:Water (1:1) as mobile phase at a flow rate of 0.6 ml/min and detection wavelength 225 nm. The percentage content of Withanolide glycosides and aglycones were calculated using area of the respective peaks and the linear regression equation ($y = 3000006x + 45860$) of the external standard.
- (ii). Oligosaccharide estimation: Waters HPLC system (equipped with 515 model pump, Waters TM 2414 Refractive Index detector and empower software); the column used was Carbohydrate analysis 300X3.9 mm (Waters: WAT 084038) column using Acetonitrile:Water (80:20) as mobile phase at a flow rate of 2 ml/min. The percentage content of Oligosaccharides were calculated using area of the oligosaccharide peaks and the linear regression equation ($y = 10142x+51551$) of the external standard.

Methodology

After screening, all the eligible subjects were randomized to receive either one of the three treatments, one capsule of *W.somnifera* 250mg twice daily or *W.somnifera* 500mg twice daily or Placebo twice daily for 12 weeks. Subjects were reviewed for follow up at 4, 8 and 12 weeks of therapy. At each visit they were evaluated for efficacy and safety. Pharmacodynamic evaluation for endothelial function was conducted before and at the end of the treatment. Blood samples were collected for evaluation of biomarkers at baseline and end of treatment. Similarly safety lab investigations for hematological, hepatic and renal biochemical parameters were conducted before and at the end of the study and also as and when required in case of any adverse drug reaction (ADR).

Subjects were enquired for the presence of ADR and the same was recorded in the case report form. Compliance to therapy was assessed by pill count method.

Primary and Secondary Efficacy Parameters

The primary efficacy measure was a change in endothelial dysfunction as assessed by more than 6% change in reflection index at 12 weeks in all the treatment groups. Secondary efficacy parameters include change in oxidative stress markers, Serum levels of nitric oxide, hsCRP (high sensitivity C-reactive protein) and lipid profile at 12 weeks in all the treatment groups. Additionally, safety and tolerability assessment of the test medications were also conducted.

Assessment of Endothelial Function

A salbutamol challenge test employing digital volume plethysmography was used to assess endothelial function as reported by Chowieńczyk et al^[11] and Naidu et al^[12]. Patients were examined in supine position after 5 minutes of rest. A digital volume pulse (DVP) was obtained using photo plethysmograph (Pulse Trace PCA2, PT200, Micro Medical, Kent, UK) transmitting infrared light at 940 nm, placed on the index finger of right hand. The signal from the plethysmograph was digitized using a 12 bit analogue to digital converter with a sampling frequency of 100 Hz. DVP waveforms were recorded over 20 second period and the height of the late systolic / early diastolic portion of the DVP was expressed as a percentage of the amplitude of the DVP to yield the reflection index (RI), as per the procedure described in detail by Millaesseau et al^[13]. After DVP recordings had been taken, three measurements of reflection index (RI) were calculated and the mean value was determined. Patients were then administered 400µg of salbutamol by inhalation. After 15 minutes three measurements of RI were obtained again and the difference in mean RI before and after administration of salbutamol was used for assessing endothelial function. A change of ≤6% in RI post salbutamol was considered as endothelial dysfunction.

Evaluation of Biomarkers and Safety Parameters

The levels of Nitric oxide^[14], MDA^[15], Glutathione^[16] were estimated spectrophotometrically and hsCRP (high sensitivity C-reactive protein) by ELISA method. Samples were collected after an overnight fast

for determination of hemoglobin, blood urea and serum creatinine, liver function test, lipid profile [Total cholesterol, High density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein (VLDL-C) and Triglycerides] using appropriate standard techniques.

Data Analysis

The data are expressed as mean \pm standard deviation. ANOVA and Paired t- test were used for analysis. A p-value < 0.05 was considered to be statistically significant. All statistical analyses were performed using the

Graph pad prism version 4 (Graph pad Software, La Jolla, CA, USA).

RESULTS

A total of 66 subjects were screened out of which 4 patients were excluded because of abnormal lab investigation, 2 patients relocated, hence unable to continue the study and 60 subjects completed the study. Twenty subjects each in *W.somnifera* 250mg, *W.somnifera* 500mg and Placebo group completed the study. There was no significant difference between treatment groups in baseline demographic characteristics and the sample was homogenous as shown in Table 1.

Table1: Demographic characteristics of all study groups (All Values expressed as Mean \pm SD)

Parameter	<i>W.somnifera</i> 250mg	<i>W.somnifera</i> 500mg	Placebo
Total No	20	20	20
Age in Yrs	55.40 \pm 8.07	57.30 \pm 9.40	57.45 \pm 8.85
Gender (M/F)	14/6	13/7	12/8
Bodyweight (Kg)	68.07 \pm 6.51	67.30 \pm 6.16	66.09 \pm 5.56
BMI (Kg/m ²)	24.89 \pm 2.03	25.01 \pm 2.92	24.82 \pm 1.86

As shown in Table 2 and Fig. 2, at baseline, there was no significant difference of mean absolute change in RI between all the three treatments. Treatment with *W.somnifera* 250 mg and *W.somnifera* 500mg produced significant change in RI compared to baseline and placebo ($p<0.001$). Though there was an apparent better response with 500mg compared to 250mg, the difference was however not statistically significant.

Table 2: Effect of *W.somnifera* 250, *W.somnifera* 500mg and Placebo on RI after 12 weeks of treatment (All Values expressed as Mean \pm SD)

Parameter	<i>W.somnifera</i> 250mg (n=20)		<i>W.somnifera</i> 500mg (n=20)		Placebo (n=20)	
	Pre Treatment (A)	Post Treatment (B)	Pre Treatment (C)	Post Treatment (D)	Pre Treatment (E)	Post Treatment (F)
Mean	-2.52	-7.49 *	-2.24	-9.03*	-2.11	-0.81
SD	\pm 1.32	\pm 3.49	\pm 1.00	\pm 2.42	\pm 1.62	\pm 2.86

Notes: * $p<0.001$ compared to baseline & placebo (F), NS -D and B, F and E

Nitric oxide, Malondialdehyde, and Glutathione levels were used to assess oxidative stress. As shown in Table 3, Treatment with *W.somnifera* 250 mg and 500 mg significantly reduced malondialdehyde levels and increased nitric oxide and glutathione levels, suggesting improvement in antioxidant status and significant reduction in the levels of systemic inflammatory biomarker, highly sensitivity C-reactive protein, after 12 weeks of treatment when compared to baseline and placebo. *W.somnifera* 250mg and 500mg showed

significant increase in NO ($p<0.05$, $p<0.001$), GSH ($p<0.05$, $p<0.001$) and a significant decrease in MDA ($p<0.05$, $p<0.001$) respectively compared to baseline. *W.somnifera* 500 mg showed a significant decrease in MDA ($p<0.05$) and a significant increase in NO ($P<0.05$), GSH ($p<0.01$) in comparison to *W.somnifera* 250 mg. There was a significant decrease in hsCRP for both active treatment groups ($p<0.001$) as compared to baseline and placebo and between *W.somnifera* 250 mg, 500 mg ($p<0.05$).

Table 3: Effect of *W. Somnifera* 250 mg, *W. Somnifera* 500 mg and Placebo on Biomarkers of oxidative stress after 12 weeks of treatment (All Values expressed as Mean \pm SD)

Parameter	<i>W.somnifera</i> 250 mg (n=20)		<i>W.somnifera</i> 500mg (n=20)		Placebo (n=20)	
	Pre treatment (A)	Post treatment (B)	Pre treatment (C)	Post treatment (D)	Pre treatment (E)	Post treatment (F)
NO (μ M/L)	32.23 \pm 20.37	34.60 \pm 18.07	29.57 \pm 5.94	39.21 \pm 10.07	32.62 \pm 12.75	31.17 \pm 12.62
MDA (nM/ml)	3.60 \pm 1.00	3.26 \pm 0.73	3.47 \pm 0.99	2.64 \pm 0.85	3.58 \pm 0.57	3.62 \pm 0.51
GSH (μ M/L)	423.85 \pm 221.72	474 \pm 226.24	404.00 \pm 67.37	527.58 \pm 103.84	420.31 \pm 138.97	419.47 \pm 134.93
hsCRP (mg/L)	2.31 \pm 0.76	1.38 \pm 0.75	2.82 \pm 1.27	0.97 \pm 0.25	2.27 \pm 0.99	2.25 \pm 1.04

Notes:

NO, $p < 0.05$ for B versus A and D versus B, $p < 0.001$ for D versus C and NS for F versus E

MDA, $p < 0.05$ for B versus A and D versus B, $p < 0.001$ for D versus C and NS for F versus E

GSH, $p < 0.05$ for B versus A and $p < 0.01$ for D versus B, $p < 0.001$ for D versus C and NS for F versus E

hsCRP, $p < 0.001$ for B versus A and D versus C, $p < 0.05$ for D versus B, NS for F versus E

The mean percentage increase in nitric oxide for *W.somnifera* 250 mg and 500mg was 15.29 % and 33.75% respectively when compared to baseline. Both treatments were found to be significant when compared to placebo as shown in Figure 3. The mean percentage decrease in malondialdehyde for *W.somnifera* 250 mg and 500mg was 6.36 %, 21.39 % respectively when compared to baseline. There was a significant decrease for *W.somnifera* 500 mg when compared to placebo as shown in Fig. 3.

The mean percentage increase in glutathione for *W.somnifera* 250 mg and 500mg

was 14.72%, 31.48 % respectively when compared with baseline. Both treatments were found to be significant when compared to placebo as shown in Figure 4. *W.somnifera* 250 mg and 500 mg produced a mean percentage decrease of 41.22 % and 57.71 % respectively, for high sensitivity C-reactive protein compared with baseline. Both treatments were found to be significant when compared to placebo as shown in Fig. 4

In the present study, *W.somnifera* demonstrated lipid lowering effect in patients with type 2 diabetes. The results of lipid profile are shown in Table 4.

Table 4: Effect of *W.somnifera* 250 mg, *W.somnifera* 500 mg and Placebo on lipid profile after 12 weeks of treatment (All values expressed as Mean \pm SD)

Parameter	<i>W.somnifera</i> 250mg (n=20)		<i>W.somnifera</i> 500mg (n=20)		Placebo (n=20)	
	Pre treatment	Post treatment	Pre treatment	Post treatment	Pre treatment	Post treatment
Total Cholesterol TC (mg/dl)	174.9 \pm 32.97	160.3 \pm 33.32 \$	186.8 \pm 19.31	158.0 \pm 15.91 \$*	177.2 \pm 22.73	190.3 \pm 22.47
HDL-C(mg/dl)	40.1 \pm 5.08	40.5 \pm 4.45 NS	40.20 \pm 7.46	45.3 \pm 5.44 \$@	37.9 \pm 5.44	37.4 \pm 5.90
LDL-C(mg/dl)	126.75 \pm 40.81	109.7 \pm 28.84 #	118.9 \pm 16.50	96.25 \pm 21.13 \$	125.3 \pm 38.79	136.05 \pm 44.16
Triglycerides (mg/dl)	170.2 \pm 42.28	147.4 \pm 38.59 #	171.9 \pm 60.12	131.8 \pm 40.24 \$	166.5 \pm 16.62	170.3 \pm 17.09
VLDL-C(mg/dl)	32.45 \pm 7.27	31.20 \pm 7.62 NS	31.55 \pm 11.79	28.5 \pm 8.68 NS	29.25 \pm 6.70	29.0 \pm 7.19

Notes:

$p < 0.01$ compared to baseline, placebo, \$ $p < 0.001$ compared to baseline, placebo,

* $p < 0.05$ compared to *W.somnifera* 500mg vs. 250mg, @ $p < 0.01$ compared to *W.somnifera* 500mg vs. 250mg, NS- compared to baseline, placebo. In placebo group NS for all parameters compared to baseline.

Treatment significantly reduced the levels of total cholesterol, low density lipoprotein cholesterol and Triglycerides as compared to the baseline for *W.somnifera* 250 mg ($p<0.01$) and *W.somnifera* 500 mg ($p<0.001$). However no significant change was found in very low density lipoprotein cholesterol levels compared to baseline in both active treatment groups. Treatment with *W.somnifera* 500mg showed significant increase in high density lipoprotein cholesterol levels compared to baseline and though there was an increase in *W.somnifera* 250 mg, it was not statistically significant. There was a significant change between the two active treatment groups for Total cholesterol ($p<0.05$) and HDL-C ($p<0.01$), further the reduction in LDL-C, Triglycerides and VLDL-C levels between *W.somnifera* 250mg and 500mg was not statistically significant. The mean percent reduction in total cholesterol for *W.somnifera* 250 mg and 500mg were 8.48 % and 14.8 % respectively compared to baseline. The mean percent reduction in LDL-C for *W.somnifera* 250 mg and 500 mg were 10.74 % and 18.14 % respectively compared to baseline. The mean percentage reduction in triglycerides for *W.somnifera* 250 mg and 500mg were 11.8 % and 20.9% respectively compared to baseline.

The mean percent reductions in total cholesterol, LDL-C and triglycerides for *W.somnifera* 250 mg and 500mg were found to be significant compared to placebo. The mean percent reduction in VLDL-C for *W.somnifera* 250 mg and 500mg were 3.05 % and 7.16 % respectively compared to baseline, but were not significant when compared to placebo. The mean percentage increase in HDL-C for *W.somnifera* 250 mg and 500 mg were 1.46 % and 15.00 % respectively compared to baseline. A significant increase in HDL-C was found only with *W.somnifera* 500 mg in comparison to placebo. However when compared between *W.somnifera* 250 mg and 500mg, statistical significance was achieved for increase in HDL-C and decrease in total cholesterol.

DISCUSSION

In the present study, we evaluated the effect of *W.somnifera* 250 mg, *W.somnifera* 500 mg and placebo on endothelial function in patients with diabetes. Both the active treatments have shown a beneficial effect on

endothelial function, along with a significant improvement in biomarkers of oxidative stress (Nitric oxide, Glutathione, Malondialdehyde) and systemic inflammation (High sensitivity C Reactive Protein levels).

Diabetes is associated with accelerated atherosclerosis and microvascular complications, which are the major causes of morbidity and mortality. Endothelial dysfunction is one of the early prognostic markers of atherosclerosis which eventually results in cardiovascular disease^[17] and it has been reported that endothelial dysfunction occurs in patients with diabetes much earlier than the clinical manifestations of vascular complications of the disease^[18]. In our earlier study, we reported the presence of endothelial dysfunction in diabetic patients assessed by salbutamol challenge test, indicating a decrease of 6% in RI, which is a marker of endothelial-dependent vasodilatation^[19]. In another study by us in diabetic patients, on treatment with polyherbal formulation, containing *W.somnifera* 100mg as one of the constituent, there was a significant decrease in RI indicating improvement in endothelial function^[20]. Similarly, in this study, treatment with *W.somnifera* 250mg and 500mg showed significant decrease in RI in type 2 diabetes mellitus compared to baseline and placebo.

Endothelial dysfunction associated with diabetes has been attributed to a lack of bio available nitric oxide due to reduced ability to synthesize nitric oxide from L-arginine ^[21] Previous studies show that increased oxidative stress has the potential to impair NO bioavailability in several ways^[22] in which superoxide anion may react with NO to form peroxynitrite and eliminate the biological activity of NO. Peroxynitrite is a highly reactive oxidant that may alter the catalytic activity of eNOS in endothelial cells and guanylyl cyclase in vascular smooth muscle cells. As a result, peroxynitrite reduces both the production of NO and the responsiveness of target tissues to NO^[23]. In our study we observed a significant increase in NO levels in both active treatment groups suggesting a possible role in decreasing the endothelial dysfunction compared to baseline and placebo. Oxidative stress in diabetes mellitus owing to an increase in the production of oxygen free radicals, such as super oxide ($O_2\bullet^-$), hydrogen peroxide (H_2O_2)

and hydroxide (OH \bullet -) radicals and deficiency in antioxidant defense mechanisms. Increased non-enzymatic and auto oxidative glycosylation is one of the possible mechanisms that contribute to the formation of free radicals and free radical-induced lipid peroxidation in diabetes mellitus^[24] Cellular enzymatic (superoxide dismutase) and non enzymatic (glutathione) antioxidants act as the primary line of defense to counteract the deleterious effects of these free radical species. Nakhjavani M et al. demonstrated a significant rise in serum MDA, oxidative stress marker in diabetics as compared to healthy controls^[25]. In the present study we observed high MDA levels at baseline, which was significantly decreased on treatment with *W.somnifera*. A significant increase in glutathione levels with both active treatments was also recorded compared to baseline and placebo indicating improvement in antioxidant status. In another study by us with a polyherbal formulation (Cardipro[®]) with *W.somnifera* as one of the constituent, significant increase in GSH and a decrease in MDA levels were observed in patients with diabetes mellitus^[19]. Previous research suggests a positive relationship between components of the metabolic syndrome and markers of inflammation, such as C-reactive protein in the Atherosclerosis Risk in Communities study, in which there was a positive link found between systemic inflammation and the development of type 2 diabetes mellitus and its cardiovascular complications^[26]. In a study, Tajiri et al. reported that increase in high-sensitivity C-reactive protein a marker of systemic inflammation could be an important pathophysiological link between the metabolic syndrome and coronary artery disease^[27]. In another study, FJ del Canizo Gomez et al. showed that increased serum hsCRP was one of the independent risk factors for the development of retinopathy in patients with type 2 diabetes mellitus^[28]. In our study, treatment *W.somnifera*, showed a significant decrease in hsCRP compared to baseline and placebo.

Diabetes being a metabolic disorder, lipid metabolism is also deranged leading to hyperlipidemias. Hypercholesterolemia along with hyperglycemia is a major risk factor for the development of atherosclerosis and is associated with coronary and peripheral vascular disease^[29] Management of dyslipidemia, a well-recognized and modifiable

risk factor, is a key element in the multifactorial approach to prevent CVD in individuals with type 2 diabetes. Andallu B et al. observed significant decrease in serum cholesterol, triglycerides, LDL (low density lipoproteins) and VLDL (very low density lipoproteins) cholesterol in six mild NIDDM subjects and six mild hypercholesterolemic subjects, treated with the powder of roots of *W. somnifera* for 30 days indicating its potential as a natural source with hypolipidemic effects^[30]. In another study by Ashwini kumar et al on 18 healthy volunteers for safety, tolerability and activity of *W.somnifera*, they observed a significant decrease in total cholesterol and decreasing trend was observed in triglycerides. However, no significant change in serum HDL, LDL, and VLDL cholesterol was seen.^[31] In our study *W.somnifera* 500mg significantly decreased the total cholesterol, LDL Cholesterol, and triglyceride levels which are more likely to be raised in diabetic patients.

CONCLUSION

In the present study, treatment with highly standardized aqueous extract of *W.somnifera* 250mg and *W.somnifera* 500mg for 12 weeks produced significant improvement in endothelial function in diabetic patients. A significant reduction in levels of biomarker for oxidative stress and systemic inflammation were observed suggesting an improvement in endothelial function. *W.somnifera* 500mg showed a significant reduction in lipid profile. All the treatments were well tolerated. It is suggested that *W.somnifera* could be further evaluated for its therapeutic role as an adjunctive in the management of diabetes mellitus associated with endothelial dysfunction.

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***Address for correspondence**

Dr. Pingali Usharani

Additional Professor

Department of Clinical Pharmacology and Therapeutics

Nizam's Institute of Medical Sciences

Punjagutta, Hyderabad- 500082

Telangana, India.

Phone: +919849574143

Email: ushapingali@yahoo.com

PHOTOGRAPHS

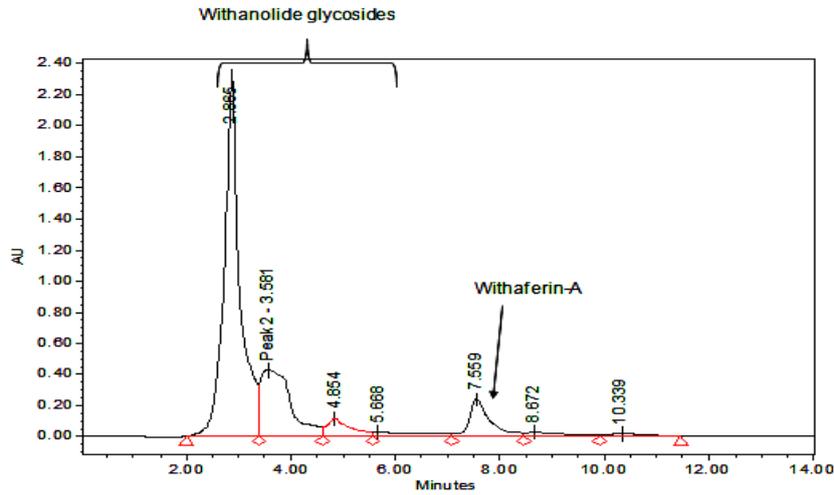


Figure 1: HPLC Chromatogram of SENSORIL® (W.somnifera) with peaks for its active constituents, Withanolide glycosides and Withaferin-A.

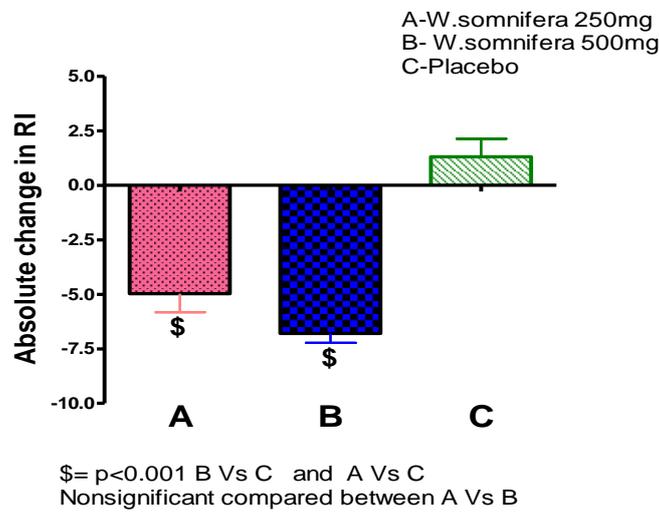


Figure 2: Absolute Change in RI after 12 weeks of Treatment

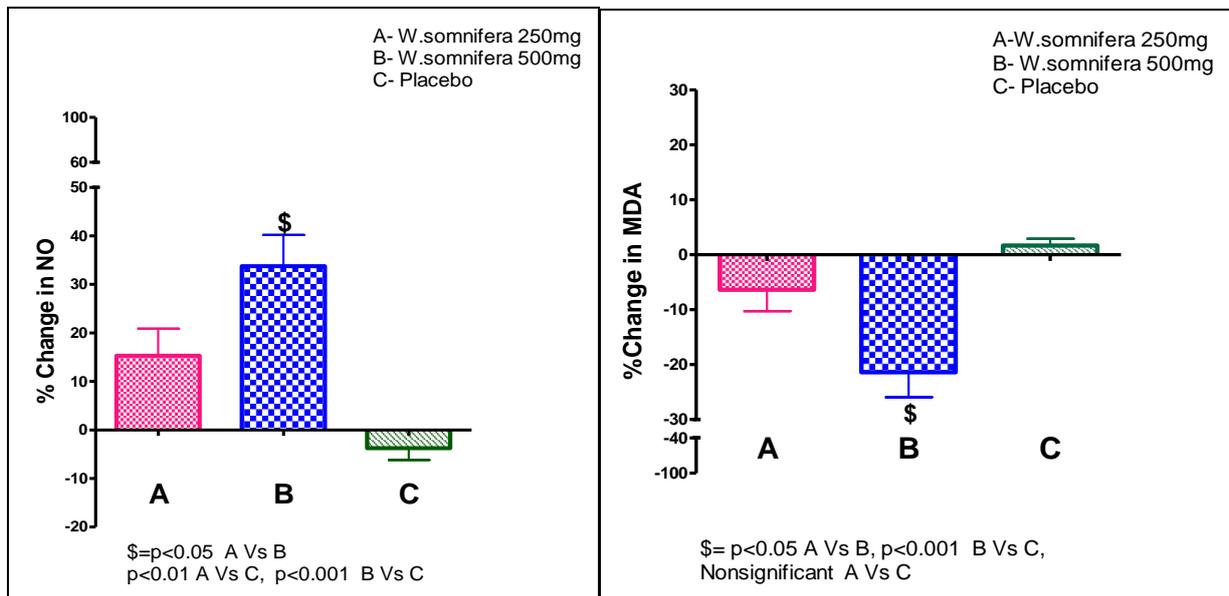


Figure 3: Mean Percent Change in NO, MDA Levels after 12 weeks of Treatment

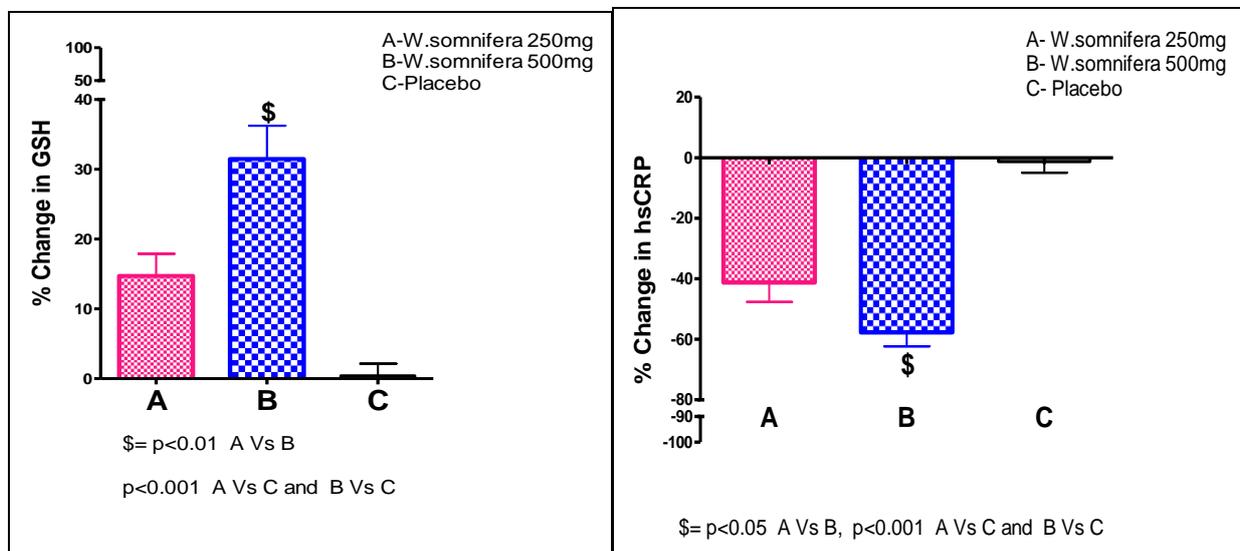


Figure 4: Mean Percent Change in GSH, hsCRP Levels after 12 weeks of Treatment