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A COMPARATIVE STUDY TO EVALUATE THE EFFECT OF HIGHLY STANDARDISED AQUEOUS EXTRACTS OF *PHYLLANTHUS EMBLICA, WITHANIA SOMNIFERA* AND THEIR COMBINATION ON ENDOTHELIAL DYSFUNCTION AND BIOMARKERS IN PATIENTS WITH TYPE II DIABETES MELLITUS

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Keywords:

Endothelial dysfunction, Biomarkers, Oxidative stress, *Phyllanthus emblica, Withania somnifera*, hs-CRP levels

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ABSTRACT:

Introduction: Biomarkers of oxidative stress and endothelial dysfunction play an important role in the pathogenesis of type II Diabetes Mellitus (DM). The present study was planned to compare the effect of highly standardised aqueous extract of *Phyllanthus emblica*, *Withania somnifera* and their combination on endothelial dysfunction and biomarkers of oxidative stress in patients with type II DM.

Methods: After taking IEC approval and written informed consent, eligible patients were randomized to receive either one of the three treatments, one capsule of *Phyllanthus emblica* 500 mg twice daily, one capsule of *Withania somnifera* 500 mg twice daily or one capsule of combination of *Phyllanthus emblica* 250mg+*Withania somnifera* 250mg twice daily for a duration of 12 weeks. Efficacy end points were change in endothelial function, change in biomarkers of oxidative stress and systemic inflammation measured at baseline and after 12 weeks of treatment.

Results: Thirty patients completed the study. Twelve weeks treatment with *Phyllanthus emblica, Withania somnifera* and their combination produced significant reduction in Reflection index (RI) compared to baseline, (-2.17±0.72% Vs -10.09±0.86), (-2.29±0.91% Vs -9.4±1.80%) and (-2.18±1.01% Vs -9.21±1.22%) respectively, suggesting improvement in endothelial function. There was also significant reduction in the biomarkers of oxidative stress and systemic inflammation. All three treatments were well tolerated.

Conclusion: *Phyllanthus emblica* and *Withania somnifera* and their combination have shown significant improvement in endothelial function, reduction in biomarkers of oxidative stress and systemic inflammation in patients with type II DM. On further analysis, *Phyllanthus emblica* has shown a better response compared to other treatment groups.

INTRODUCTION: Diabetes has emerged as a major healthcare problem in India. According to Diabetes Atlas published by the International Diabetes Federation (IDF) [1], there were an estimated 40 million persons with diabetes in India in 2007 and this number is predicted to rise to almost 70 million people by 2025.

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The real burden of the diabetes is however due to its associated complications which lead to increased morbidity and mortality among which cardiovascular disease is the major cause [2].

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 [3]. Endothelial dysfunction, present at disease onset, may be the cause of atherogenesis that is present throughout the course of diabetes mellitus (DM) and associated with late-stage adverse outcomes [4].

This endothelial dysfunction results from reduced bioavailability of the vasodilator nitric oxide (NO) mainly due to accelerated NO degradation by reactive oxygen species [5]. A currently favored hypothesis is that oxidative stress, through a single unifying mechanism of superoxide production, is the most common pathogenic factor leading to insulin resistance, β-cell dysfunction, impaired glucose tolerance (IGT) and ultimately to type II DM.

Furthermore, this mechanism has been implicated as the underlying cause of both the macrovascular and microvascular complications associated with type II DM. It follows that therapies aimed at reducing oxidative stress would benefit patients with type II DM and those at risk for developing diabetes [6, 7]. High-sensitivity C-reactive protein (hs-CRP) is a predictor of early atherosclerotic damage and is related to increased adiposity (mainly abdominal), insulin resistance, and dyslipidemia [8]. hs-CRP also directly impairs production of nitric oxide, resulting in endothelial dysfunction [9].

Many herbs possess potent antioxidant, anti-inflammatory and cardio-protective properties and are used by patients with increased risk of cardiovascular morbidity and mortality. Amla (*Phyllanthus emblica*) is widely used in Indian medicine for the treatment of various diseases. There is some published evidence that *Phyllanthus emblica* has significant hypoglycemic and lipid lowering affects in diabetic patients [10, 11, 12].

In vitro and animal studies have indicated that *Phyllanthus emblica* has potent antioxidant effects on superoxide and hydroxyl radicals, scavenging activity, and an ability to augment antioxidant enzymes [10, 13]. *Withania somnifera* (Ashwagandha) is widely used in Ayurvedic medicine for its cardio protective potential due to its therapeutic potential as antioxidant, hypoglycemic and hypolipidemic agent [14, 15].

The present study was thus undertaken to evaluate and compare the effect of highly standardised aqueous extracts of *Phyllanthus emblica*, *Withania somnifera* and their combination on endothelial function and biomarkers in patients with type II diabetes mellitus and further study its probable mechanism of action.

MATERIALS AND METHODS: The present study was a prospective, randomized, double blinded and parallel group study conducted in the Department of Clinical Pharmacology and Therapeutics, Nizam's Institute of Medical Sciences, Hyderabad. Forty two 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 sex, aged 18-65 years, fasting blood glucose of ≥110 mg/dL, a glycosylated haemoglobin (HbA1c) between 6.5 to 8.0 % and taking stable dose of anti-diabetic treatment (Metformin 1500-2500 mg/day) for the past 8 weeks prior to the screening visit; and having endothelial dysfunction defined as ≤ 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.

After screening, all the eligible subjects were randomized to receive either one of the three treatments as per prior randomization schedule for a total duration of 12 weeks i.e., one capsule of *Phyllanthus emblica* (CAPROS®) 500 mg twice daily; one capsule of *Withania somnifera* (SENSORIL®) 500 mg twice daily; or one capsule of a combination of *Phyllanthus emblica* and *Withania somnifera* (CAPSORIL® 500 mg - CAPROS® 250 mg +SENSORIL® 250 mg) twice daily. CAPROS® is an aqueous extract of the edible fruits of *Phyllanthus emblica* (Amla), containing not less than 60% of low molecular weight hydrolysable tannins (LMwHT) comprising Emblicanin-A, Emblicanin-B, Punigluconin and Pedunculagin as the bioactives and is highly standardized by high-performance liquid chromatography (HPLC).

SENSORIL® is an aqueous extract of the roots plus leaves of *Withania somnifera* (Ashwagandha), containing not less than 10% withanolide glycosides, not more than 0.5% of Withaferin-A

and not less than 32% of oligosaccharides and is highly standardized by HPLC. The HPLC Chromatograms for both *Phyllanthus emblica* and *Withania somnifera* are shown in **Figure 1a and Figure 1b** respectively.

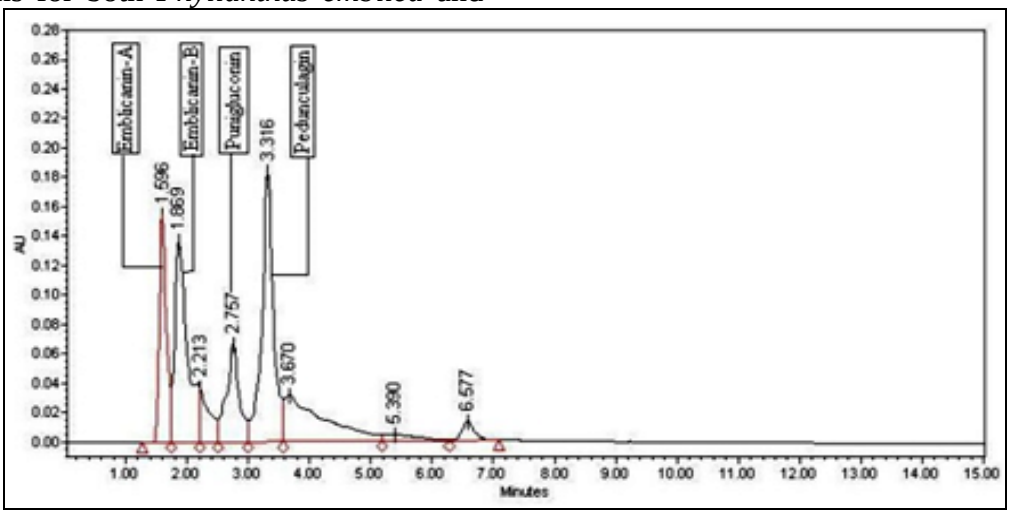

FIGURE 1A: HPLC CHROMATOGRAM OF *PHYLLANTHUS EMBLICA* SHOWING PEAKS FOR EMBLICANIN-A, EMBLICANIN-B, PUNIGLUCONIN AND PEDUNCULAGIN

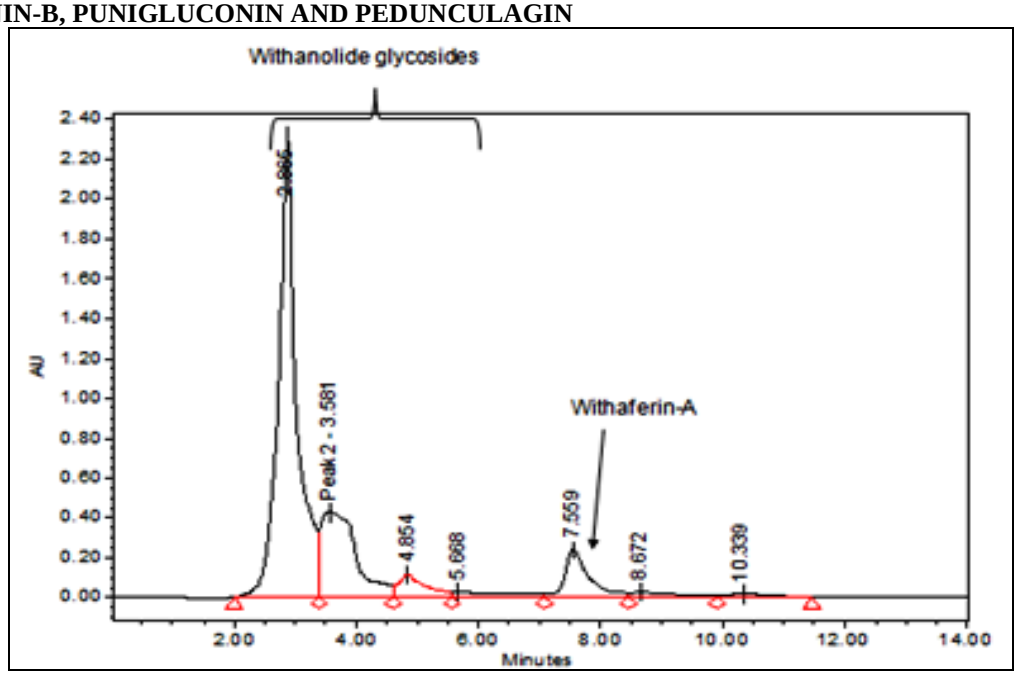

FIGURE 1B: HPLC CHROMATOGRAM OF *WITHANIA SOMNIFERA* SHOWING PEAKS FOR WITHANOLIDE GLYCOSIDES AND WITHAFERIN-A

Subjects were reviewed at 4 weeks, 8 and 12 weeks of therapy. Pharmacodynamic evaluation for endothelial function was conducted at baseline and after 12 weeks of therapy. Blood samples were collected for evaluation of biomarkers before and at the end of treatment. Safety lab investigations for haematological, 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 (RI) at 12 weeks in all the treatment groups. Secondary efficacy parameters include change in biomarkers of oxidative stress, systemic inflammation, changes in lipid profile and HbA1c levels in all the treatment groups.

Additionally, safety and tolerability assessment of the test medications were also done.

Assessment of Endothelial Function: New non-invasive methods can detect endothelial function by indirect measure such as Reflection Index (a Pharmacodynamic biomarker of vascular dysfunction) to enable the detection, prevention, and treatment of diabetes and its complications. A salbutamol challenge test employing digital volume plethysmography was used to assess endothelial function as reported by Chowienczyk *et al* [16] and Naidu *et al* [17].

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 infra-red light at 940 nm, placed on the index finger of right hand (**fig. 2**).

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 seconds and the height of the late systolic / early diastolic portion of the DVP was expressed as a percentage of the amplitude of DVP to yield the reflection index (RI) as shown in Figure 2, as per the procedure described in detail by Millasseau *et al* [18] After the DVP recordings were 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.

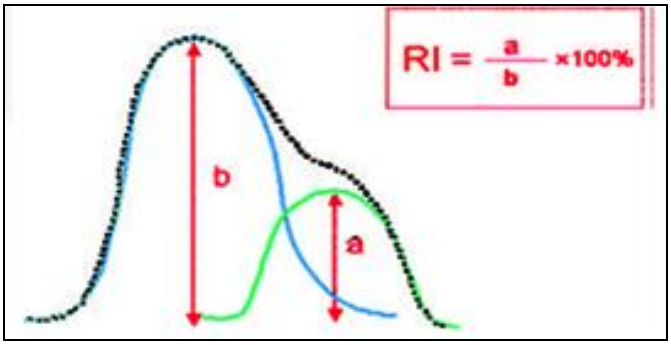

FIGURE 2: PULSE ANALYZER (MICRO MEDICAL) WITH PHOTO PLETHYSMOGRAPH (PPG) TRANSDUCER AND A DIGITAL VOLUME PULSE (DVP) WAVEFORM

Evaluation of Biomarkers and Safety Parameters: Nitric oxide [19], MDA [20] and Glutathione (GSH) levels [21] were estimated using UV spectrophotometer and hs-CRP (high sensitivity C-reactive protein) by enzyme-linked immunosorbent assay (ELISA) method. Lipid profile is a biomarker for hyperlipidemia induced cardiovascular morbidity and mortality. All the subjects underwent complete physical examination, safety lab evaluations at baseline and at the end of treatment. Samples were collected after an overnight fast of 12 hours after the last dose of medication for determination of haemoglobin, blood urea and serum creatinine, liver function tests, lipid profile [Total cholesterol, High density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C)] using appropriate standard techniques.

Data Analysis: Data is expressed as Mean ±SD. ANOVA and paired and unpaired t- test were performed for within group and between groups analysis respectively. A *p*-value < 0.05 was considered to be statistically significant. All statistical analysis was performed using the Graph pad Prism, Version 4, USA.

RESULTS: A total of 36 subjects were screened and 30 eligible subjects have completed the study. Ten subjects each in *Phyllanthus emblica* 500 mg, *Withania somnifera* 500 mg and combination of *P.emblica* and *W.somnifera* 250+250 mg groups have completed the study. Detailed demographic characteristics of the three study groups are shown in **Table 1**. There was no significant difference between treatment groups with respect to demographic variables.

TABLE 1: DEMOGRAPHIC CHARACTERISTICS OF ALL STUDY GROUPS

Parameter	*Phyllanthus emblica*	*Withania somnifera*	Combination of ***P.emblica*** and ***W.somnifera***
Total No.	10	10	10
Age	58.60±10.54	60.10±6.471	57.30±9.23
Sex (M/F)	9/1	8/2	9/1
Weight	67.93±6.35	70.10±5.64	68.95±6.625
BMI (kg/m^2)	24.83±2.20	25.65±3.517	24.97±2.65

RI was used to assess endothelial function. As seen from **Figure 3A**, compared to the pre-treatment value, a significant improvement in RI was observed in all the three treatment groups after 12 weeks (p<0.001). Although the absolute change in RI was apparently high with *Phyllanthus emblica*, there was no significant difference between the three treatment groups. The comparison of absolute change in RI with all the three treatments is shown in **Figure 3B**.

	Pre treatment	Post treatment	Absolute Change	*p* Value compared to baseline
Phyllanthus emblica	**-2.17±0.72**	**-10.09±0.86**	**-7.92±1.24**	***p*<0.001**
Withania somnifera	**-2.29±0.91**	**-9.40±1.80**	**-7.11±1.49**	***p*<0.001**
Combination of *P.emblica* and *W.somnifera*	**-2.18±1.01**	**-9.21±1.22**	**-7.03±1.45**	***p*<0.001**

FIGURE 3A: EFFECT OF *PHYLLANTHUS EMBLICA* 500mg, *WITHANIA SOMNIFERA* 500mg AND COMBINATION OF *P. EMBLICA* AND *W. SOMNIFERA* ON RI AFTER 12 WEEKS OF TREATMENT (All Values expressed as Mean ± SD)

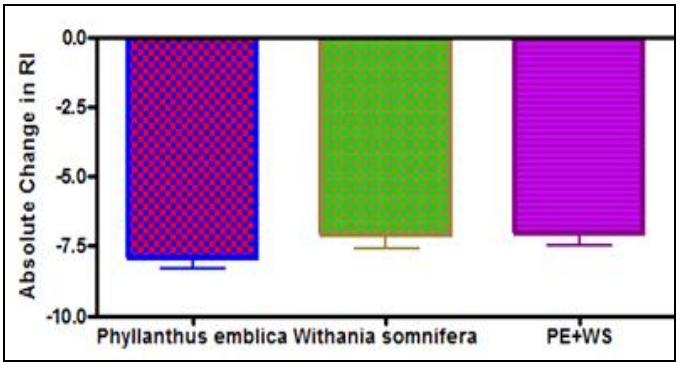

FIGURE 3B: COMPARISON OF ABSOLUTE CHANGE IN RI WITH ALL THE THREE TREATMENTS

Nitric oxide, malondialdehyde and glutathione levels were used to assess oxidative stress. As seen from Table 2, treatment with *Phyllanthus emblica* 500 mg, *Withania somnifera* 500 mg and combination of *P. emblica* and *W. somnifera* 250+250 mg has shown statistically significant improvement in the levels of oxidative stress biomarkers such as nitric oxide (NO) & glutathione (GSH) and significant reduction in the levels of MDA after 12 weeks of treatment when compared to pre-treatment levels.

The *p* value compared to baseline with *Phyllanthus emblica* 500 mg, *Withania somnifera* 500 mg and combination of *P. emblica* and *W. somnifera* 250+250 mg for NO were <0.001, <0.05 and <0.001 respectively, and the same for MDA and GSH levels in all the three treatment groups was <0.01.

The mean percentage change for biomarkers of oxidative stress was shown in Figure 4. The mean percent improvement was seen for NO and GSH levels whereas mean percent reduction was observed for MDA. On comparison of absolute change in biomarkers of oxidative stress between the three treatment groups, statistical significance was achieved only for NO with *Phyllanthus emblica* 500 mg when compared to *Withania somnifera* 500 mg and combination of *P. emblica* and *W. somnifera* 250+250 mg (p<0.01) as shown in Table 2. However, statistical significance was not achieved for MDA and GSH when compared between the three treatment groups (p=NS).

TABLE 2: EFFECT OF *PHYLLANTHUS EMBLICA* 500mg, *WITHANIA SOMNIFERA* 500 MG AND COMBINATION OF *P. EMBLICA* AND *W. SOMNIFERA* ON BIOMARKERS OF OXIDATIVE STRESS AFTER 12 WEEKS OF TREATMENT

Biomarkers of Oxidative Stress in different groups	Pre treatment	Post treatment	Absolute Change	Mean % Change	*p* Value compared to baseline	*p* Value for absolute change compared between the groups
Phyllanthus emblica group **NO (µM/L)**	24.68±2.21	44.71±4.56	20.03±4.34	82± 20.48	$p<0.001$	$p<0.01$ compared to *W somnifera* & combination
Phyllanthus emblica group **MDA (nM/ml)**	3.27±0.91	2.18±0.92	-1.08±0.72	-32.67±19.58	$p<0.01$	NS
Phyllanthus emblica group **GSH (µM/L)**	363.7±130.3	541.6±154.4	178.0±137.8	62.70±61.32	$p<0.01$	NS
Withania somnifera group **NO (µM/L)**	27.85±4.68	37.13± 10.63	9.27±10.37	35.32± 40.02	$p<0.05$	NS
Withania somnifera group **MDA (nM/ml)**	3.32± 1.31	2.13± 0.94	-1.18±1.10	-32.47±25.45	$p<0.01$	NS
Withania somnifera group **GSH (µM/L)**	352.8± 91.6	503.61±113.60	150.79±127.59	61.08± 90.05	$p<0.01$	NS
Combination of *P. emblica* and *W. somnifera* group **NO (µM/L)**	28.45± 5.11	39.26± 4.44	10.81±6.73	41.35±26.80	$p<0.001$	NS
Combination of *P. emblica* and *W.somnifera* group **MDA (nM/ml)**	3.02± 1.04	2.25± 0.91	-0.77±0.64	-24.63±17.80	$p<0.01$	NS
Combination of *P. emblica* and *W.somnifera* group **GSH (µM/L)**	365.6±186.5	534.9±160.8	169.27±155.29	62.32±60.69	$p<0.01$	NS

(All Values expressed as Mean ± SD). **NS –** Non Significant.

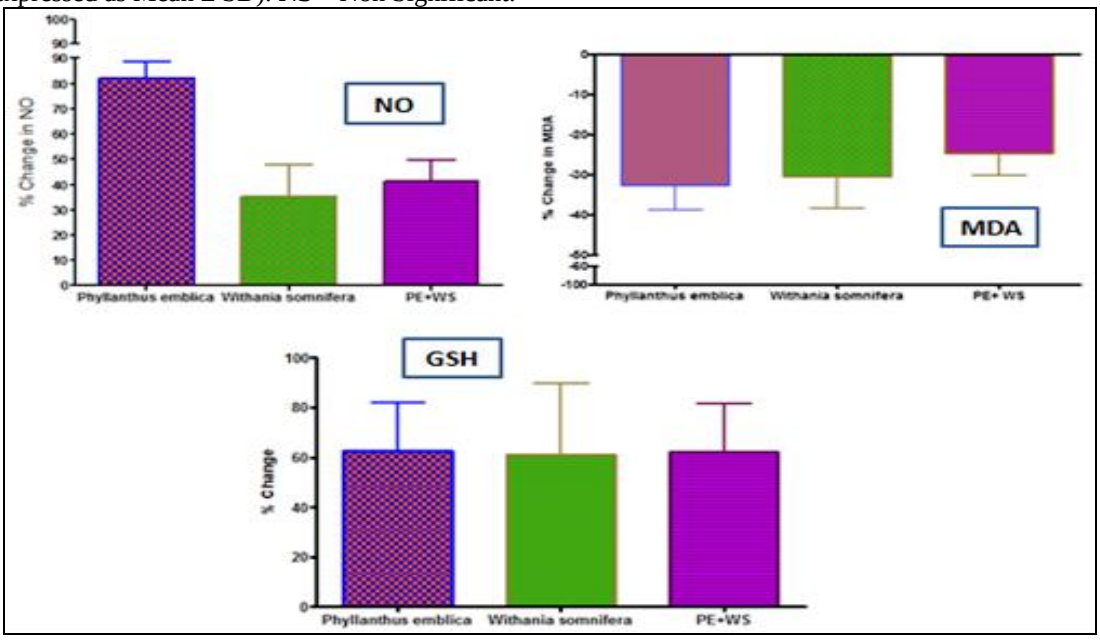

FIGURE 4: Mean percentage change in nitric oxide, MDA, and glutathione levels with *phyllanthus emblica* 500 mg, *withania somnifera* 500 mg and combination of *p. emblica* and *W. somnifera* at the end of 12 weeks treatment

hs-CRP was used as an inflammatory biomarker. As seen from **Figure 5A**, treatment with *Phyllanthus emblica* 500 mg, *Withania somnifera* 500 mg and combination of *P. emblica* and *W. somnifera* 250+250 mg has shown significant reduction in hs-CRP levels after 12 weeks of treatment when compared to pre-treatment levels (p<0.001). The mean percentage reduction in hs-CRP levels for all the three treatment groups was shown in **Figure 5B**. Comparison of absolute change in hs-CRP levels was found to be non-significant between the three treatment groups (p=NS).

	Pre Treatment	Post Treatment	Absolute Change	Mean %Change	*p* Value
Phyllanthus emblica	3.68±1.03	1.19±0.38	-2.49±0.82	-66.9 ±7.17	p<0.001
Withania somnifera	3.77±1.05	0.99±0.35	-2.78±1.02	-71.44 ±12.65	p<0.001
Combination of *P.emblica* and *W.somnifera*	3.62±1.18	1.09±0.24	-2.53±1.14	-66.06 ±14.43	p<0.001

FIGURE 5A: EFFECT OF *PHYLLANTHUS EMBLICA* 500 mg, *WITHANIA SOMNIFERA* 500 MG AND COMBINATION OF *P. EMBLICA* AND *W.SOMNIFERA* ON INFLAMMATORY BIOMARKER hs-CRP (mg/l) AFTER 12 WEEKS OF TREATMENT. (All Values expressed as Mean ± SD)

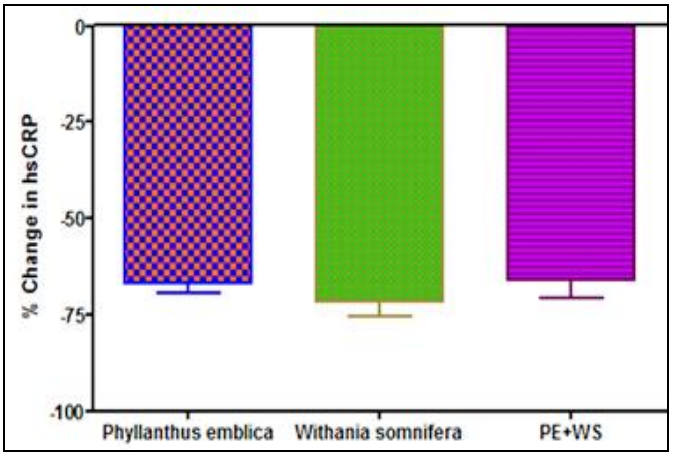

FIGURE 5B: MEAN PERCENTAGE CHANGE IN hs-CRP LEVELS FOR ALL THE THREE TREATMENT GROUPS

Both *Phyllanthus emblica* and *Withania somnifera* are reported to have potent hypolipidemic activity. In the present study, we demonstrated their lipid-lowering effect individually and in combination in type II diabetics. The results on the lipid profile are shown in **Table 3**. Treatment with *Phyllanthus emblica* 500 mg, *Withania somnifera* 500 mg and combination of *P. emblica* and *W. somnifera* 250+250 mg significantly reduced the levels of total cholesterol, low-density lipoprotein (LDL) cholesterol and triglycerides and increased high-density lipoprotein (HDL) cholesterol levels compared with baseline at the end of 12 weeks.

Though there was an apparent reduction in mean percentage change of total cholesterol and triglycerides in all the three treatment groups, the difference among the groups was however not statistically significant.

The mean percentage increase in HDL cholesterol was found to be significant for *Phyllanthus emblica* 500 mg when compared to *Withania somnifera* 500 mg and combination of *P. emblica* and *W. somnifera* 250+250 mg (p<0.05).

The mean percentage reduction in LDL cholesterol was found to be significant for *Phyllanthus emblica* 500 mg when compared to *Withania somnifera* 500 mg and combination of *P. emblica* and *W. somnifera* 250+250 mg (p<0.001 between *Phyllanthus emblica* and combination of *P. emblica* and *W. somnifera*; p<0.05 between *Phyllanthus emblica* and *Withania somnifera*).

TABLE 3: EFFECT OF *PHYLLANTHUS EMBLICA* 500 MG, *WITHANIA SOMNIFERA* 500 mg AND COMBINATION OF *P.EMBLICA* AND *W.SOMNIFERA* 250+250 mg ON LIPID PROFILE

Parameter	*P. emblica* 500 mg n=10		*W. somnifera* 500 mg n=10		Combination of *P emblica* and *W somnifera* (250+250) n=10	
	Pre treatment	Post treatment	Pre treatment	Post treatment	Pre treatment	Post treatment
Total Cholesterol	195.8±29.4	158.2±23.7 #	194.1±21.1	163.8±13.4 $	188.9±17.5	160.3±17.9 $
HDL-C (mg/dl)	40.70±7.04	50.80±5.9 #	41.50±7.5	45.30±6.7 *	39.00±7.9	42.10±5.8 *
LDL-C (mg/dl)	127.3±17.3	72.60±6.2 #	123.7±17.6	90.90±21.04 #	118.8±20.9	96.40±16.3 $
Triglycerides (mg/dl)	163.6±78.7	117.6±43.6 $	166.6±57.4	122.3±29.2$	164.5±44.4	125.4±35.9 #

*- p<0.05 compared to baseline. $-p<0.01 compared to baseline. #-p<0.001 compared to baseline

There were no significant changes in laboratory safety parameters (haematological, renal and hepatic parameters) in all the three treatment groups. All the three study medications were well tolerated by the subjects. There were no serious adverse events recorded in the study except for dyspepsia reported by three patients in the *Phyllanthus emblica* 500 mg group. None of the subjects discontinued the study prematurely because of these adverse events.

DISCUSSION: In the present study, we evaluated the effects of highly standardised aqueous extracts of *Phyllanthus emblica* 500 mg, *Withania somnifera* 500 mg and combination of *P.emblica* and *W.somnifera* 250+250 mg on endothelial function and biomarkers in diabetic patients. All the three treatments have shown a beneficial effect on endothelial function, along with a significant improvement in biomarkers of oxidative stress and inflammation, including nitric oxide, glutathione, malondialdehyde and hs-CRP levels. Further, the three treatments significantly decreased total cholesterol, triglycerides, and low-density lipoprotein cholesterol, and increased high-density lipoprotein cholesterol. However, the combination does not seem to have any synergistic effect.

Endothelial dysfunction is one of the early prognostic markers of atherosclerosis, and may eventually result in cardiovascular disease. Risk factors such as dyslipidemia, hypertension, smoking, and type II diabetes are associated with impaired endothelial function. Diabetes is associated with accelerated atherosclerosis and microvascular complications, which are the major causes of morbidity and mortality. It has been reported that endothelial dysfunction occurs in patients with diabetes much earlier than the clinical manifestations of vascular complications of the disease [22]. In an earlier study, we reported the presence of endothelial dysfunction assessed by salbutamol challenge indicating a decrease of 6% in RI, which is a marker of endothelial-dependent vasodilatation in diabetic patients [17, 23].

Endothelial cell dysfunction is emerging as a key component in the pathophysiology of the cardio-vascular abnormalities associated with diabetes mellitus. The intact endothelium promotes vasodilatation principally via the release of nitric oxide, originally known as endothelium-derived relaxing factor. Nitric oxide has been recognized as a key determinant of vascular homeostasis, regulating several physiologic properties, including vascular permeability, and has antithrombotic properties.

Decreased production or increased metabolism of nitric oxide may lead to inadequate amounts of nitric oxide within the vasculature and its patho biologic consequences leading to atherosclerosis.[24] The 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.[5]

Vascular injury in diabetes as a result of hyperglycemia has been associated with oxidative stress. Oxidative stress also plays an important role in the etiology of atherosclerosis and coronary heart disease, and is one of the main mechanisms

involved in endothelial dysfunction [7]. Cellular enzymatic (superoxide dismutase) and nonenzymatic (glutathione) antioxidants act as the primary line of defence to counteract the deleterious effects of these free radical species.[13] Reduced GSH levels, a measure of anti-oxidant capacity and MDA levels, a measure of oxidative lipid damage are the main biomarkers for oxidative stress. C-reactive protein is a sensitive marker for systemic inflammation. A relationship between inflammation and development of atherosclerotic disease, in particular coronary heart disease, has recently been demonstrated in epidemiologic studies. Previous research suggests a positive relationship between diabetes and markers of inflammation, such as CRP in the Atherosclerosis Risk in Communities study, [25] in which there was a positive link found between systemic inflammation and the development of type 2 diabetes mellitus and its cardiovascular complications.

Hypercholesterolemia along with hyperglycemia is a major risk factor for the development of atherosclerosis and is associated with coronary and peripheral vascular disease [26]. Reduction of hyper-cholesterolemia has been associated with improvement of coronary artery disease, and intensive interventions, including diet, exercise, and use of hypolipidemic and anti-inflammatory drugs, are recommended. However, some patients cannot tolerate the adverse effects of these drugs, necessitating the use of safer therapeutic alternatives [27].

The use of herbal products by practitioners of Ayurvedic medicine to manage the cardiovascular complications in diabetic patients has given a new lead to the use of natural products like *Phyllanthus emblica*, *Withania somnifera* and their combination to identify less expensive and safer strategies for managing cardiovascular disease in diabetics. The tannoid principles of P. emblica have been reported to have antioxidant activity in vitro and in vivo. In a study conducted in rats, it was seen that fresh P. emblica juice enriched with emblicanin A and emblicanin B showed antioxidant activity in an ischemia-reperfusion model of oxidative stress in the rat heart [28]. *Phyllanthus emblica* extract has also been reported to have cytoprotective effects on account of their antioxidant effects on lipid peroxidation [29].

An earlier study by Antony *et al* [13] also demonstrated the beneficial antioxidant activity of amla fruit on atherosclerosis and dyslipidemia. In another study by Anila *et al* [30], the flavonoid content of amla was analyzed for its biological activity and found to have a potent hypolipidemic effect.

Akhtar et al [12] evaluated the antihyperglycemic and lipid-lowering properties of amla in healthy volunteers and diabetic patients. Significant decreases in total cholesterol and triglycerides and improvement in high-density lipoprotein cholesterol were observed in both normal volunteers and diabetic subjects receiving 2–3 g of *P. emblica* powder per day. In their study of *P. emblica,* Antony *et al* [13] also reported a significant reduction in total cholesterol, low-density lipoprotein cholesterol, and triglycerides, as well as a significant elevation of high-density lipoprotein cholesterol.

Although the exact mechanism by which amla exerts this beneficial effect is presently not clear, it seems likely that it brings about favorable changes in the lipid profile via several mechanisms, including interference with cholesterol absorption [29], inhibition of HMG-CoA reductase activity, and an increase in lecithin cholesterol acyl transferase activity [28]. In an earlier study done by us, we have concluded that *P. emblica* significantly improved endothelial function and reduced biomarkers of oxidative stress and systemic inflammation in patients with type 2 diabetes mellitus [11]. Further it was concluded that *P. emblica* extract may be a good therapeutic alternative to statins in diabetic patients with endothelial dysfunction because it has the beneficial effects of the statins but without the well-known adverse effects of these agents, including myopathy and hepatic dysfunction [11].

A study by Shahid *et al* [31], found that fasting blood sugar and HbA1c levels were significantly elevated whereas serum nitric oxide levels were significantly depressed in normotensive diabetics and hypertensive patients compared with controls. In a recent study by Udaya Kumar R *et al* [32], they have evaluated the hypoglycaemic and hypolipidemic effects of extracts of *Withania somnifera* root and leaves in alloxan induced diabetic rats and found them to be effective.

In a study with *W. somnifera* by Anwer *et al* [33], animal models have shown significant reductions in blood glucose and HbA1c levels and Insulin resistance. In another study with a polyherbal formulation (Cardipro) in which *Withania somnifera* is an active constituent done by us, we concluded that there was a significant increase in the level of NO and decrease in RI thereby reflecting an improvement of endothelial function in patients with type II diabetes mellitus [34].

CONCLUSION: In the present study, treatment with highly standardised aqueous extracts of *Phyllanthus emblica* 500 mg, *Withania somnifera* 500 mg and their combination (250+250 mg) for 12 weeks produced significant improvement in endothelial function in diabetic patients as measured by RI compared to baseline. Reduction in the levels of biomarkers of oxidative stress and inflammation were observed suggesting improvement in endothelial function in diabetic patients. All the three treatment groups also showed significant improvement in the lipid parameters.

All the three treatments were well tolerated. On further analysis, *Phyllanthus emblica* 500 mg has shown a better response in terms of improvement in endothelial function, reduction in the levels of biomarkers of oxidative stress and improvement in lipid profile compared to *Withania somnifera* 500 mg and their combination. It is suggested that further studies are needed to be undertaken for exploring the beneficial effects of these compounds in diabetic patients with endothelial dysfunction.

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References:

1. International Diabetes Federation. Diabetes Atlas. 4th edn. Brussels: International Diabetes Federation; 2007. Online version of Diabetes Atlas available at: http:// www.eatlas.idf.org.
2. Parving H-H, Mauer M, Ritz E: Diabetic Nephropathy, chapter 36, The Kidney- vol II, 8th ed., edited by Brenner BM, Philadelphia, Saunders, 2008; pp 1265-1298.
3. Stehouwer CD, Lambert J, Donker AJ, Hinsbergh VW. Endothelial dysfunction and pathogenesis of diabetic angiopathy. *Cardiovasc Res*. 1997; 34:55–68.
4. Maria AP, Sara G, Carmela N, Carratu MR, Monica M. Endothelial dysfunction in diabetes: From mechanism to therapeutic targets. Current Medicinal Chemistry. 2009; 16 (1): 94-112.
5. Assunta P, Elena A. Chronic hyperglycemia and nitric oxide bioavailability play a pivotal role in pro-atherogenic vascular modifications. Genes Nutrition. 2007; 17 (2): 195-208.
6. Silvio E, Inzucchi MD. Oral antihyperglycemic therapy for type 2 diabetes. Scientific review and clinical applications. J of American Medical Association. 2002; 287 (3):360-372.
7. Wright E, JL Scism-Bacon JL, Glass LC. Oxidative stress in type 2 diabetes: The role of fasting and postprandial glycemia. Int J of Clinical Practice. 2006; 60 (3): 308-314.
8. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest*. 2003; 112:1796–1808.
9. Rahat K, Chatwal S, Arora S, et al. Antihyperglycemic, antihyperlipidemic, anti-inflammatory and adenosine deaminase-lowering effects of garlic in patients with type 2 diabetes mellitus with obesity. *Diabetes Metab Syndr Obes*. 2013; 6:50–56.
10. Muhammad SA, Ayesha R, Amanat A, Ahmad M. Effect of amla fruit *(Emblica officinalis Gaertn.)* on blood glucose and lipid profile of normal subjects and type 2 diabetic patients. Int J of Food Sciences and Nutrition. 2011; 62(6): 606-616.
11. Usharani et al. Effects of *Phyllanthus emblica* extract on endothelial dysfunction and biomarkers of oxidative stress in patients with type 2 diabetes mellitus: a randomized, double-blind, controlled study. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy (Dovepress) 2013:6 275–284.
12. Akhtar MS, Ramzan A, Ali A, Ahmad M. Effect of amla fruit (Emblica officinalis Gaertn.) on blood glucose and lipid profile of normal subjects and type 2 diabetic patients. *Int J Food Sci Nutr*. 2011; 62:606–616.
13. Antony B, Benny M, Kaimal TN. A pilot clinical study to evaluate the effect of Emblica officinalis extract (AmlamaxTM) on markers of systemic inflammation and dyslipidemia. *Indian J Clin Biochem*. 2008; 23: 378–381.
14. Sharma V, Sharma S, Pracheta, Paliwal R. *Withania somnifera* a rejuvenating ayurvedic medicinal herb for the treatment of various human ailments. Int J of Pharm Tech Research. 2011; 3(1): 187-192.
15. Lakshmi CM, Betsy BS, Simon D. Scientific basis for the therapeutic use Withania somnifera (Ashwagandha): Alternative Medicine Review. 2000; 5(4): 334-346.
16. Chowienezyk PJ, Kelly RP, and MacCallum H, et al. Photoplethysmographic assessment of pulse wave reflection: blunted response to endothelium dependent beta2-adrenergic vasodilation in type II diabetes mellitus. J Am Coll Cardiol. 1999; 34:2007–2014.
17. Naidu MUR, Sridhar Y, Usharani P, Mateen AA. Comparison of two β2 adrenoceptor agonists by different routes of administration to assess human endothelial function. Indian J Pharmacol. 2007; 39 (3):168-169.
18. Millaesseau SC, Kelly RP, Ritter JM, Chowienczyk PJ. Determination of age related increases in large artery

stiffness by digital pulse contour analysis. Clinical Science. 2002; 103: 371-377.
19. Vidyasagar J, Karunaka N, Reddy MS, Rajnarayan K, Surender T, Krishna DR. Oxidative stress and antioxidant status in acute organophosphorous insecticide poisoning. Indian J of Pharmacol. 2004; 36(2): 76-79.
20. Katrina MM, Michael GE, David AW. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Biology and Chemistry. 2001; 5(1): 62–71.
21. Elman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959; 82:70-77.
22. Schalkwijk CG, Stehouwer CDA. Vascular complications in diabetes mellitus: the role of endothelial dysfunction. Clin Sci. 2005; 109: 143–159.
23. Usharani P, Mateen AA, Naidu MU, Raju YS. Effect of NCB-02, atorvastatin and placebo on endothelial function, oxidative stress and inflammatory markers in patients with type 2 diabetes mellitus: a randomized, parallel-group, placebo-controlled, 8-week study. Drugs R D. 2008; 9:243–50.
24. Duncan B, Meeking D, Kenneth S, Cummings M. Endothelial dysfunction and pre-symptomatic atherosclerosis in type 1 diabetes – pathogenesis and identification. Br J Diabetes Vasc Dis. 2003; 3:27–34.
25. Koenig W, Sund M, Frohlich M, *et al*. C-reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men. Circulation. 1999; 99:237–242.
26. Raja Babu Panwar, Rajeev Gupta, Bal Kishan Gupta, Sadiq Raja, Jaishree Vaishnav, Meenakshi Khatri & Aachu Agrawal. Atherothrombotic risk factors & premature coronary heart disease in India: A case-control study. Indian J Med Res July 2011, 134, pp 26-32.
27. Suanarunsawat T, Devakul Na Ayutthaya W, Songsak T, Thirawarapan S, Poungshompoo S. Antioxidant activity and lipid-lowering effect of essential oils extracted from Ocimum sanctum leaves in rats fed with a high cholesterol diet. J Clin Biochem Nutr. 2010; 1:52–59.
28. Bhattacharya SK, Bhattacharya A, Sairam K, Ghosal S. Effect of bioactive tannoid principles of Emblica officinalis on ischemia-reperfusion-induced oxidative stress in rat heart. Phytomedicine. 2002; 9: 171–174.
29. Mathur R, Sharma A, Dixit VP, Varma M. Hypolipidemic effect of fruit juice of Emblica officinalis in cholesterol-fed rabbits. J Ethnopharmacol. 1996; 50:61–68.
30. Anila L, Vijayalaxmi NR. Flavanoids from Emblica officinalis and Magnifera indica. Effectiveness for dyslipidemia. J Ethnopharmacol. 2002; 79:81–87.
31. Shahid SM, Mahboob T. Diabetes and hypertension: correlation between glycosylated hemoglobin (HbA1c) and serum nitric oxide (NO). Aust J Basic Appl Sci. 2009; 3:1323–1327.
32. Udaykumar R, Sampath K, Thankaraj SM, Rajesh M, Anbazhagan VR, Kim SC et al. Hypoglycaemic and hypolipidemic effects of Withania somnifera root and leaf extracts on alloxan –induced diabetic rats. Int J Mol Sci. 2009; 10: 2367-2382.
33. Anwer T et al. Effect of Withania somnifera on insulin sensitivity in non-insulin dependent diabetes mellitus rats. Basic Clin Pharmacol Toxicol 2008. Jun; 102(6):498-503.
34. Nishat Fatima, Pingali Usharani, N.Muralidhar. Randomised double blind placebo controlled study to evaluate the effect of cardipro on endothelial dysfunction and biomarkers in patients with type 2 diabetes mellitus. Innovative Journal of Medical and Health Science 2: 6 Nov – Dec (2012) 122 – 128.

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