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A STUDY OF *TERMINALIA CHEBULA* EXTRACT ON ENDOTHELIAL DYSFUNCTION AND BIOMARKERS OF OXIDATIVE STRESS IN PATIENTS WITH METABOLIC SYNDROME.

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ABSTRACT

Background: Metabolic syndrome subjects have endothelial dysfunction via increased oxidative stress increasing the risk of atherosclerosis and coronary heart disease. *Terminalia chebula* (*T.chebula*) is known for its antioxidant and antihyperlipidemic activity. The present study compared the effects of an aqueous extract of *T.chebula* 250 and 500mg versus placebo on endothelial dysfunction and biomarkers of oxidative stress in patients with metabolic syndrome. **Methods:** Eligible patients were randomized to receive either *T.chebula* 250 mg, *T.chebula* 500 mg or placebo twice daily for 12 weeks. The primary efficacy parameter was the change in endothelial function at baseline and after 12 weeks of treatment. Secondary efficacy parameters were changes in biomarkers of oxidative stress (malondialdehyde, nitric oxide, and glutathione), high sensitivity C-reactive protein levels and lipid profile. Laboratory safety parameters were measured at baseline and after 12 weeks of treatment. **Results:** Fifty six patients completed the study. Treatment with *T.chebula* 250 mg and 500 mg for 12 weeks produced significant reductions in the reflection index (−2.25%±0.70% to −3.72%±1.35% versus −2.35%±0.85% to −6.14%±1.01% respectively), suggesting improvement in endothelial function compared with baseline. There was a significant improvement in biomarkers of oxidative stress and systemic inflammation compared with baseline and placebo. Further, the treatments significantly improved the lipid profile compared with baseline and placebo. All treatments were well tolerated. **Conclusion:** Both *T.chebula* 250 and 500mg significantly improved endothelial function and reduced biomarkers of oxidative stress and systemic inflammation in patients with metabolic syndrome, without any significant changes in laboratory safety parameters. However, *T.chebula 500mg* produced better improvement in endothelial function than *T.chebula 250mg.*

KEYWORDS: *Terminalia chebula*, endothelial dysfunction, oxidative stress, metabolic syndrome.

INTRODUCTION

Metabolic syndrome (MetS) is a cluster of various interrelated cardio metabolic risk factors. MetS confers a two to fourfold increased risk for cardiovascular disease (CVD) and fivefold increased risk of diabetes.[1] The prevalence of metabolic syndrome is rapidly increasing in India and other South Asian countries, leading to increased mortality and morbidity due to CVD and type 2 diabetes.[2,3] Endothelial dysfunction has been associated with the presence of the metabolic syndrome in adults.[4] Endothelial dysfunction is frequently defined as reduction of the bioavailability of vasodilator biomarkers such as nitric oxide along with the increase of the mediators of vasoconstriction.[5] This adds to the burden of the genetic predisposition to cardiovascular disease. Hence, any impairment in vascular endothelial cells results in arterial stiffness and remodeling leading to development of cardiovascular disorders.[6] The factors contributing to endothelial dysfunction are hyperinsulinemia, hyperglycemia, increase in fatty acid levels, hypertriglyceridemia, decrease in HDL-cholesterol, and increase in small dense LDL-cholesterol.[7,8]

Traditional medicine has been described in literature as having therapeutic potential in many diseases. *Terminalia chebula* (*T.chebula*) belonging to the family Combretacea, is a popular traditional medicine used extensively in the preparation of many Ayurvedic formulations in India, South-East Asia, China and Taiwan.[9] It is commonly known as black Myroblans in English and Harad in Hindi. It is used in traditional

medicine due to the wide spectrum of pharmacological activities associated with the biologically active chemicals present in this plant. It is used for the treatment of number of diseases like cancer, cardio vascular diseases, ulcers, leprosy, arthritis, gout, epilepsy etc. It has been reported as antioxidant[10] and antidiabetic[11] activity.

MATERIALS AND METHODS

Materials

The test product used in the present study comprised a highly standardized aqueous extract of *T.chebula* (Natreon Inc, New Brunswick, NJ, USA), containing chebulagic acid, chebulinic acid and other low molecular weight hydrolysable tannins. The matching placebo capsules contained microcrystalline cellulose (49.7% w/w), lactose (49.5% w/w), and magnesium stearate (0.69% w/w) as excipients, and were also supplied by Natreon Inc.

Methods

The present study was a prospective, randomized, double blind trial conducted in the Department of Clinical Pharmacology and Therapeutics, Nizam's Institute of Medical Sciences, Hyderabad, India. Fifty 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 included in the study, were of either gender, aged 30-68 years, having endothelial dysfunction defined as ≤ 6% change in reflection index (RI) on post salbutamol challenge test and having metabolic syndrome according to "The International Diabetes Federation" guidelines 2006.[12] Patients with severe uncontrolled hyperglycemia, uncontrolled hypertension, cardiac arrhythmia, impaired hepatic or renal function, history of malignancy or stroke, chronic alcoholism or any other serious disease requiring active treatment, concomitant medication known to alter endothelial function, and treatment with any other herbal supplements were excluded from the study. Pregnant and lactating women were also excluded.

All the eligible subjects were randomized to receive either one capsule of *T.chebula* 250mg twice daily or one capsule of *T.chebula* 500mg twice daily or one capsule of matching placebo twice daily. Subjects were reviewed after 4, 8, and 12 weeks of therapy. At each visit, they were evaluated for efficacy and safety. Pharmacodynamic evaluation of endothelial function was done at baseline and at the end of 12 weeks of treatment. Blood samples were collected for evaluation of biomarkers at baseline and at the end of treatment. A laboratory safety evaluation, including hematology and hepatic and renal biochemistry, was performed before and at the end of the study, and as needed in the event of an adverse drug reaction. Subjects were asked about the occurrence of adverse events, which were recorded on the case report form. Compliance was assessed by tablet count.

The primary efficacy measure was a change in endothelial dysfunction as reflected by a change in RI compared with baseline at 12 weeks in all the treatment groups. Secondary efficacy parameters were changes in biomarkers of oxidative stress (malondialdehyde, nitric oxide, and glutathione), high sensitivity C-reactive protein levels and changes in lipid profile after 12 weeks in all the treatment groups.

Assessment of endothelial function

A salbutamol challenge test involving digital volume plethysmography was used to assess endothelial function as per the procedure described by Chowienczyk et al[13] and Naidu et al[14]. Patients were examined in the supine position after 10 minutes of rest. A digital volume pulse was obtained using a photoplethysmographic pulse tracer (Pulse Trace PCA2, PT200, Micro Medical, Gillingham, Kent, UK) transmitting infrared light at 940 nm, which was placed on the index finger of the right hand. Signals from the plethysmograph were digitized using a 12-bit analog to digital converter at a sampling frequency of 100 Hz. Digital volume pulse waveforms were recorded over a 20-second period, and the height of the late systolic/early diastolic portion of the digital volume pulse was expressed as a percentage of the amplitude of the digital volume pulse to yield the RI, as per the procedure described in detail by Millasseau et al.[15] The RI was obtained from the digital volume pulse recording. The mean of three such recordings was considered as the representative value. Patients were then administered 400 µg of salbutamol by inhalation. After 15 minutes, three measurements of RI were obtained and the difference in mean RI before and after administration of salbutamol was used as a measure of endothelial function. A change in RI of ≤6% post salbutamol was considered to indicate endothelial dysfunction.

Evaluation of biomarkers

Serum malondialdehyde,[16] nitric oxide,[17] and glutathione[18] levels were estimated spectrophotometrically and high sensitivity C-reactive protein by enzyme-linked immunosorbent assay.

Assessment of pharmacodynamics and safety

A complete physical examination was performed at every visit. Vital parameters, including blood pressure and heart rate, were recorded using a multiparameter Galaxy L&T monitor (Larsen and Toubro Ltd, Mumbai, India) at baseline and at the end of treatment. Blood samples were collected after an overnight fast of 12 hours for determination of hemoglobin, complete blood profile, blood urea, creatinine, liver function and lipid profile, all of which were measured using standard techniques. Any change in laboratory safety parameters was investigated and any adverse drug reactions were also recorded.

Statistical analysis

The data are expressed as the mean ± standard deviation. The paired *t*-test was performed within the groups and the unpaired *t*-test was performed between the groups. A p value of less than 0.05 was considered to be statistically significant. All statistical analyses were performed using Graph Pad Prism version 6 (Graph Pad Software, La Jolla, CA, USA).

RESULTS

Total of 65 subjects were screened and 56 eligible subjects completed the study. Eighteen subjects in *T.chebula* 250mg, 20 in *T.chebula* 500mg and 18 in placebo group completed the study.

Detailed demographic characteristics of the three study groups are shown in Table No.1**.** There was no significant difference between treatment groups in baseline characteristics including age, weight and body mass index indicating that the study population was homogenous, with no statistically significant differences between the treatment groups with respect to demographic variables.

Table No.1 Demographic characteristics of all study groups

Parameter	***T.chebula* 250mg**	***T.chebula* 500mg**	**Placebo**
Total No	18	20	18
Age in Yrs	58.44±5.29	56.72±6.21	56.89±7.39
Gender (M/F)	12/6	13/7	14/4
Bodyweight (Kg)	78.94±6.72	77.3±8.15	78.17±5.94
Height (cm)	162.28±3.06	161.67±3.53	161.17±2.77
BMI (Kg/m^2)	30.00±2.83	29.59±3.02	30.11±1.72

Nine patients in *T.chebula* 250mg, nine patients in *T.chebula* 500mg and 13 in placebo group had body mass index (BMI) > $30kg/m^2$. Lipid abnormalities were present in all patients; eleven patients in *T.chebula* 250mg, ten in *T.chebula* 500mg and five in placebo group had raised blood pressure at baseline. Eight patients in *T.chebula* 250mg, eight in *T.chebula* 500mg and five in placebo group had fasting plasma glucose >100mg/dL at baseline.

The RI was used to assess endothelial function. Daily treatment with *T.chebula* 250 mg and 500 mg significantly reduced the RI compared with baseline and placebo (Table 2). Further, *T.chebula* 500 mg achieved a highly significant improvement in endothelial function compared with *T.chebula* 250 mg. The mean absolute change in RI was significant for the active treatments compared with placebo (Figure 1).

Table No.2- Effect of *T.chebula* 250mg, *T.chebula* 500mg and Placebo on marker of endothelial function (RI %)

Parameter	***T. chebula 250mg* (n=18)**		***T. chebula* 500mg (n=20)**		**Placebo (n=18)**	
ΔRI (%)	PreTT	Post TT	PreTT	Post TT	PreTT	Post TT
Mean	-2.25	-3.72 **$**	-2.35	-6.14 **$**	-2.27	-0.97
SD	0.70	1.35	0.85	1.01	1.22	2.45

$-p<0.001 compared to baseline

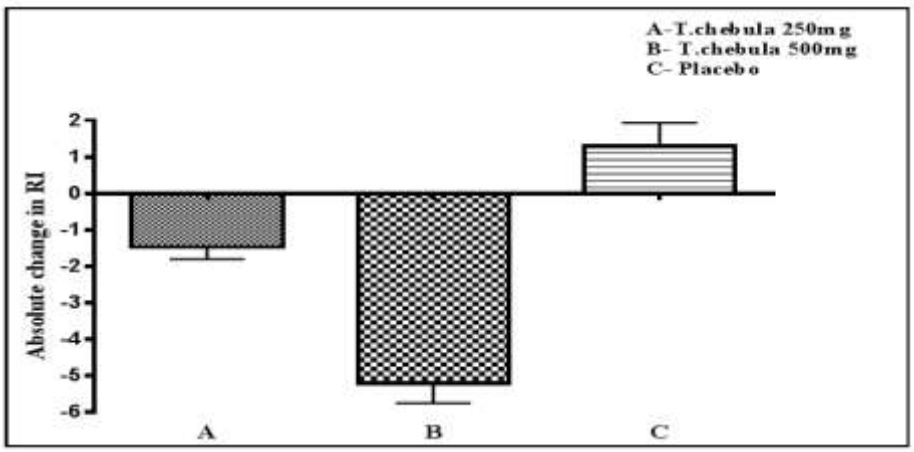

Figure 1: Absolute change in RI after 12 weeks of treatment

Notes: *P* < 0.001 when compared between A and B, B and C, p <0.01 when compared between A and C
Abbreviations: RI, reflection index; *T.chebula, Terminalia chebula.*

Malondialdehyde (MDA), Nitric oxide (NO), and Glutathione (GSH) levels were used to assess oxidative stress. Daily treatment with *T.chebula* 250 mg and 500 mg significantly reduced malondialdehyde levels and increased glutathione levels, suggesting improvement in antioxidant status. Earlier studies have demonstrated that endothelial dysfunction is associated with reduced bioavailability of nitric oxide. Daily treatment with *T.chebula* 250 mg and 500 mg significantly increased nitric oxide levels compared with baseline and placebo. The effect of the trial medications on biomarkers is shown in Table 3, indicating that *T.chebula* 250 mg and 500 mg significantly decreased high sensitivity C-reactive protein (hsCRP) levels compared with baseline and placebo. Further analysis showed that *T.chebula* 500 mg had a better effect than *T.chebula* 250 mg on all the biomarkers evaluated. Similar changes were also noted with the other biomarkers.

Table No 3: Effect of *T.chebula* 250mg, *T.chebula* 500mg and Placebo on Biomarkers of Oxidative Stress and Inflammation

Parameter	***T.chebula* 250mg (n=18)**		***T.chebula* 500mg (n=20)**		**Placebo(n=18)**	
	Pre TT	Post TT	Pre TT	Post TT	Pre TT	Post TT
NO(µM/L)	30.29±2.74	35.57±2.42 #	31.11±2.23	38.81±3.17 #	32.97±4.02	32.58±3.88
GSH(µM/L)	383.37±64.1	422.05±69.92#	390.70±56.41	452.56±67.0 #	421.24±61.1	429.3±62.4
MDA(nmol/ml)	3.40±0.36	3.21±0.38 #	3.37±0.67	2.92±0.68 #	3.79±0.67	3.90±0.60
hsCRP(mg/L)	3.45±0.36	3.26±0.42 @	3.48±0.48	2.75±0.73 #	3.61±0.74	3.66±0.61

#-p<0.001 compared to baseline, @-p<0.05 compared to baseline

The mean percent change in biomarker values was analyzed for each of the study treatments. As seen in Figure 2, the mean increase in nitric oxide was 18.1% for *T.chebula* 250 mg and 25.25% for *T.chebula* 500 mg compared to baseline. The mean increase in glutathione was 10.51% and 16.36%, respectively for *T.chebula* 250 mg, *T.chebula* 500 mg compared to baseline (Figure 3).The mean reduction in malondialdehyde was 5.77% in the *T.chebula* 250 mg group and 13.35% in the *T.chebula* 500 mg group compared to baseline (Figure 4). Similarly, *T.chebula* 250 mg and *T.chebula* 500 mg produced a mean decrease in high sensitivity C-reactive protein of 5.5% and 18.85% respectively, compared to baseline (Figure 5).

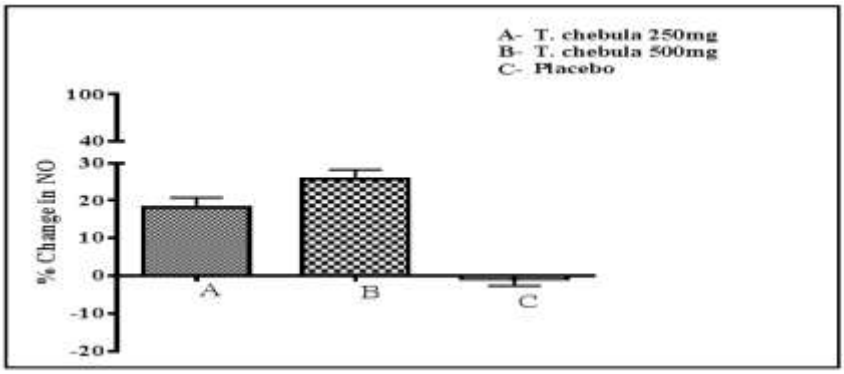

Figure 2: Mean Percent change in NO after 12 weeks of treatment

Notes: p < 0.001 compared between all three groups. NO; Nitric oxide

Abbreviations: NO, Nitric oxide; *T.chebula*, *Terminalia chebula*

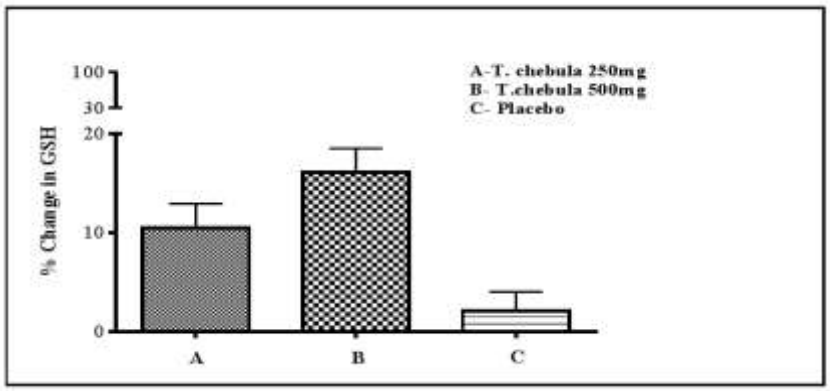

Figure 3: Mean Percent change in GSH after 12 weeks of treatment

Notes: p < 0.001 compared between all three groups.

Abbreviations: GSH; Glutathione; *T.chebula*, *Terminalia chebula*

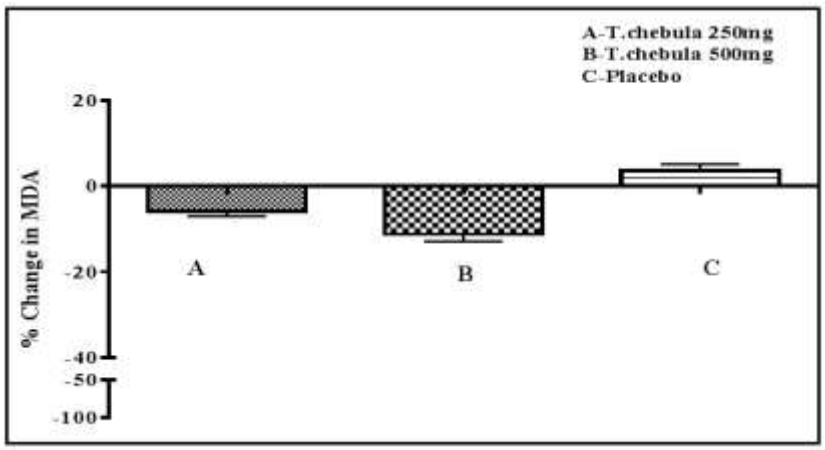

Figure 4: Mean Percent change in MDA after 12 weeks of treatment

Notes: p < 0.001 between A and C, B and C. non-significant between A and B
Abbreviations: MDA: Malondialdehyde *T.chebula, Terminalia chebula*

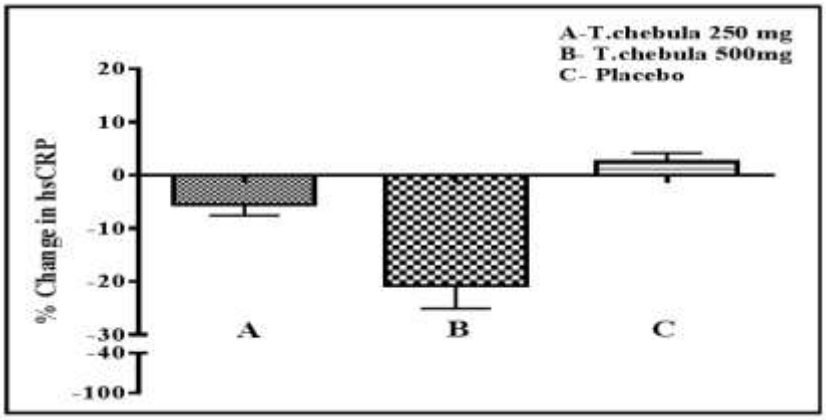

Figure 5: Mean Percent change in hsCRP after 12 weeks of treatment

Notes: p < 0.001 between A and C, B and C, p < 0.05 between A and B
Abbreviations: hsCRP: high sensitivity C-reactive protein T.chebula, *Terminalia chebula*

In the present study, we demonstrated its lipid-lowering effect in patients with metabolic syndrome. The results on the lipid profile are shown in Table 4. Treatment with *T.chebula* 250 mg and 500 mg significantly reduced the levels of total cholesterol, low-density lipoprotein (LDL-C) cholesterol, and triglycerides, and increased high-density lipoprotein cholesterol (HDL-C) levels compared with baseline and placebo. The mean reduction in total cholesterol was 7.21% and 15.49% with *T.chebula* 250 mg and 500 mg respectively, and low-density lipoprotein cholesterol decreased by 8.94% and 14.05% respectively. There was a similar mean decrease in triglyceride and very low-density lipoprotein cholesterol levels and a mean increase in high-density lipoprotein cholesterol levels by 11.9% and 15.8% with *T.chebula* 250 mg and 500 mg respectively.

Table No.4: Effect of *T.chebula* 250mg, *T.chebula* 500mg and Placebo on Lipid profile

Parameter	*T.chebula* 250mg (n=18)		*T.chebula* 500mg (n=20)		Placebo (n=18)	
	Pre TT	Post TT	Pre TT	Post TT	Pre TT	Post TT
Total Cholesterol (mg/dl)	178.50±34.7	165.8±34.76**$**	172.39±27.62	138.17±19.20 **$**	179.16±18.2	184.16±20.8
HDL-C(mg/dl)	38.33±5.19	40.16±5.11 **@**	35.0±3.22	41.3±3.63 **$**	33.28±2.74	33.29±2.79
LDL-C(mg/dl)	129.22±18.5	116.44±13.16 **#**	134.83±22.07	115.67±18.82 **$**	136.7±16.02	141.6±14.79
Triglycerides (mg/dl)	172.5±29.07	153.0±32.00**$**	172.9±36.91	141.0±35.17 **$**	184.3±19.82	184.7±21.62

$ -p<0.001 compared to baseline, #-p<0.01 compared to baseline,
@-p<0.05 compared to baseline

There was no significant change in laboratory safety parameters for any of the study treatments compared with baseline. All the medications were well tolerated and no subject discontinued the study prematurely because of adverse drug reactions.

DISCUSSION

In the present study, we evaluated the effect of *T.chebula* 250 mg, *T.chebula* 500 mg and placebo on endothelial function in patients with MetS. The two active treatments showed a beneficial effect on endothelial function, along with a significant improvement in biomarkers of oxidative stress, including nitric oxide, glutathione, and malondialdehyde. Further, the two active treatments significantly decreased total cholesterol, triglycerides, and low-density lipoprotein cholesterol, and increased high-density lipoprotein cholesterol, whereas placebo did not have any significant effect on endothelial function or any of the other study parameters. Laboratory safety parameters remained within the normal ranges in all the treatment groups, and no subject discontinued the study due to adverse drug reactions.

It is well-established that the vascular endothelium exerts various anti-atherogenic properties, mainly via NO bioavailability. More specifically, it plays critical role in the inhibition of platelet aggregation, inflammation, thrombosis, leukocyte adhesion, as well as smooth muscle contraction. In turn, impaired endothelial function, affecting NO bioavailability, significantly contributes to the initiation and progress of atherosclerosis through several functions of endothelial cells.[19] However, both traditional and novel cardiovascular risk factors including smoking, aging, hypercholesterolemia, hypertension, hyperglycemia, and a family history of premature atherosclerotic disease are all associated with alteration in endothelial function. Other mechanisms responsible for the development of endothelial dysfunction include reduced generation of endothelium derived hyperpolarizing factor[20] and increased production of endothelium-dependent constricting factors such as endothelin-1.[21,22]

Insulin resistance and MetS may be linked to endothelial dysfunction by several mechanisms. Insulin resistance is associated with endothelial dysfunction in that hyperinsulinemia causes the release of the potent vasoconstrictor endothelin. Also, the increased production of cytokines, low-grade inflammation (as reflected by elevated plasma levels of CRP), defects in insulin signaling pathways, activation of the renin-angiotensin system (RAS), and increased oxidative stress are associated with insulin resistance and could contribute to endothelial dysfunction.[23] In a study by Halcox et al, of the individual components of MetS, the presence of obesity was a consistent determinant of vascular endothelial dysfunction in the coronary microcirculation.[24] Several other investigators have also shown both in adults and children that obesity is independently associated with impaired peripheral endothelial function.[25,26,27] In the present study, 12 weeks of treatment with *T.chebula* 250 mg and *T.chebula* 500 mg significantly increased nitric oxide levels in patients with MetS compared with placebo.

In recent years many Ayurvedic and Traditional Chinese Medicinal drugs are being investigated for their possible therapeutic roles in various diseases. For cardiovascular diseases, herbal treatments have been used in patients with congestive heart failure, systolic hypertension, angina pectoris, atherosclerosis, cerebral insufficiency, venous insufficiency, and arrhythmia.[28]

Increase in oxidative and inflammatory stress play a vital role in the initiation and progression of atherosclerotic vascular disease. MetS, which often accompanies obesity, has also been independently linked with increased oxidative stress and inflammatory burden.[29,30] A potential mechanism underlying the increased cardiovascular risk in obese adults with MetS may be augmented oxidative stress and inflammatory burden. The tannoid principles of *T. chebula* have been reported to have antioxidant activity. A study by Lee et al demonstrated the beneficial antioxidant activity of *T. chebula* in vivo and in vitro.[31] Cytotoxic effects of methanolic extract of *T. chebula* have also been demonstrated on malignant cell lines.[32]

Dyslipidemia is widely established as an independent risk factor for cardiovascular disease.[33] Low HDL cholesterol and hypertriglyceridemia have been found to be independently and significantly related to myocardial infarction/stroke in patients with MetS.[34] Reduction of hypercholesterolemia has been associated with improvement of coronary artery disease, and intensive interventions, including diet, exercise, and use of antihypercholesterolemic and anti-inflammatory drugs, are recommended. However, some patients cannot tolerate the adverse effects of these drugs, necessitating the use of safer therapeutic alternatives.[35] In the present study, we found that 12 weeks of treatment with *T.chebula* 250 mg and *T.chebula* 500 mg achieved a significant improvement in the lipid profile. Although the exact mechanism by which *T.chebula* exerts this beneficial effect is presently not clear, it seems likely that it brings about favorable changes due to the high amount of saponins, phytosterols, chebulinic acid and corilagin.[36]

C-reactive protein is a sensitive marker for systemic inflammation. Epidemiological studies have shown an association between moderately elevated CRP levels in coronary heart disease.[37] Patients with metabolic syndrome are at increased risk of coronary artery disease. In our study, 12 weeks of treatment with *T.chebula* 250 mg and *T.chebula* 500 mg significantly reduced high sensitivity C-reactive protein levels, suggesting that both medications probably exert their beneficial effects via reducing systemic inflammation and acting as oxygen

free radical scavengers, thereby improving endothelial function.

CONCLUSION

Impaired endothelial function metabolic syndrome may be due to reduced bioavailability of nitric oxide and increased oxidative stress. In the present study, proprietary *T.chebula* extract containing chebulagic acid and chebulinic acid as bioactives achieved significant improvement in endothelial function and a reduction in biomarkers of oxidative stress and systemic inflammation. However, extensive clinical studies are required in larger numbers of patients to establish the efficacy and safety of *T.chebula* in the management of endothelial dysfunction.

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DECLARATIONS

Conflict of interest: None declared.

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