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EFFICACY OF *SHARBAT AFTIMOON* (POLYHERBAL FORMULATION) IN ARTERIAL STIFFNESS: A RANDOMIZED CONTROLLED TRIAL

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***ABSTRACT*:**
In recent years, many studies have emphasized the role of arterial stiffness in the development of hypertension, and have made clear that arterial stiffness especially aortic stiffness may antedate to the development of hypertension, which is a leading cause of potentially preventable disability and premature death. **Aims:** The main aim of this study is to evaluate the efficacy of *sharbat aftimoon* in decreasing arterial stiffness. **Methodology:** Eighty patients of primary hypertension of both gender with pulse wave velocity >12m/s, between the age group of 30 to 65 years were selected and randomly assigned to control and test group; comprising 40 patients in each group. Test group was treated with *Sharbat aftimoon* 25 ml orally twice a day before food, whereas control group was given losartan, 1 tablet each 50 mg once a day for 60 days. **Results:** After 60 days of treatment period, Carotid –femoral PWV, CPP, and Aix decreased significantly compared with baseline, however significant difference was found between the groups with more reduction in control group. **Conclusions:** This study reveals that the test formulation has good response in decreasing arterial stiffness. However further research is needed to confirm these results by adopting robust study designs.
***Index terms*- arterial stiffness, sharbat aftimoon, polyherbal formulation, Unani medicine**

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INTRODUCTION:

Hypertension is a progressive cardiovascular syndrome characterized by the presence of BP elevation to a level that places patients at increased risk for target organ damage in multiple vascular beds.[1] It is a complex, multifactorial disease and accounts for about 90-95% of all cases of hypertension.[2] It tends to be familial and is likely to be the consequence of an interaction between environmental and genetic factors.[1-2] It is becoming an important public health problem in the world and its prevalence is rapidly increasing in developing countries and considered one of the leading causes of death and disability among the elderly.[3] According to WHO, hypertension is one of the most important causes of premature deaths worldwide, and was estimated that it causes 7.5 million deaths worldwide, about 12.8% of the total of all annual deaths, which is responsible for 3.7% of global Disability Adjusted Life Years (DALY) loss.[4-5] Although, we have achieved a milestone in managing hypertension by finding effective antihypertensive drugs; but still are short of truly overcoming this disease.[6] Moreover, over the past 50 years, antihypertensive therapies were designed to alter the peripheral

vascular resistance and cardiac output, without taking into account the fact that arterial stiffness plays a vital role in the development of hypertension.[7] Many studies have made clear that arterial stiffness especially aortic stiffness may antedate and may contribute to the development of hypertension, which is a leading cause of potentially preventable disability and premature death.[8-9] In order to achieve true control over this common human malady, novel antihypertensive therapies is needed to be designed and evaluated by considering the pathomechanism of arterial stiffness associated with increased blood pressure. Unani scholars have attributed black bile to the genesis of arterial stiffness and have been using sharbat aftimoon for several therapeutic effects in medical conditions like palpitations, dizziness, and hard swellings and are getting favorable results.[10-15] Despite having such favorable effects, the de-obstruent, antihypertensive and anti-inflammatory efficacy of different ingredients of this formulation have been established in various models either in vitro or in vivo or in clinical studies.[16-18]

MATERIALS AND METHODS

Study design

This prospective open-label randomized controlled trial was conducted at department of Moalajat, National Institute of Unani Medicine, Bengaluru, Karnataka from June 2016 to November 2017. Study was approved by Institutional Ethical Committee under IEC No: NIUM/IEC/2014-15/031/PhD/Moal/02 and was conducted as per the principles of the Declaration of Helsinki. The trial was registered in the clinical trial registry of India under no. CTRI/2017/11/010656.

Sample Size Estimation

The study was powered to demonstrate differences in primary outcome between individual study subjects. The Sample size was calculated by using previous literature and through survey experts with effect size of 10 mmHg from mean systolic B.P, alpha error of 0.05 and allowable power of 0.8. Considering a dropout rate of 10%, the sample size required was exactly 80 (40 per group).

Participants

Patients of both gender, between the age group of 30 to 65 years, with stage- 1 primary hypertension ranging systolic pressure 140 to 159 mmHg and diastolic pressure 90 to 99 mmHg and pulse wave velocity > 12m/s were recruited from OPD/IPD and evaluated for the consideration as a research subjects. The diagnosis was confirmed by repeated blood pressure measurements, Medical history, Physical examination, Laboratory and instrumental investigations. Every subject was completely informed of the experimental procedures and had signed an informed consent statement before joining in the trial. Certain investigations were carried out with an aim to exclude the patients with pathological conditions mentioned under exclusion criteria like: Patients with cardiac arrhythmias (chronic atrial fibrillation, frequent premature ventricular and atrial contractions, ventricular or supraventricular tachycardia, tachycardia [heart rate > 100 beats per minute]), atrioventricular block, malignant hypertension, diabetes mellitus, Patients with the episodes of marked arterial hypotension (systolic BP < 90 mmHg), myocardial infarction or acute cerebrovascular events, stable angina or vasospastic angina, valvular diseases, chronic liver disease, acute or chronic renal failure, Bronchial asthma and Diabetes mellitus, pregnant and lactating mothers.

Intervention

The intervention was a Unani pharmacopeial (*Qarabadine Nuskha*) polyherbal formulation with potential ingredients like Cuscuta reflexa, Mellisa officinalis, Carthamus tinctorius, Narcissus jonquil, Polipodium vulgare, Borago officinalis, Doronicum hookeri, and Ocimum basilicum. The ingredients were procured from the market of Bangalore except Narcissus jonquil and Mellisa officinalis which were procured from Kashmir. All the ingredients were identified and authenticated from Foundation of Revitalisation of Local Health Traditions and were submitted to the Ilmul Advia Museum vide voucher number 72/M/Res/2019, before the formulation was prepared in the Pharmacy of National Institute of Unani Medicine Bangalore. The formulation which is in the form of syrup was prepared as per the guidelines mentioned in National formulary of Unani medicine. The syrup was packed in 350 ml bottles. One bottle was given to each patient for 1 week. The standard control was losartan with a trade name of losakind, manufactured by Mankind Pharmaceuticals Ltd.

Study procedure

Known cases of hypertensive patients, fulfilling the inclusion criteria, were selected from the OPD/IPD and randomly allocated into two Groups viz. Group –A (test group) and Group -B (control group) comprising 40 patients in each group, after obtaining a written voluntary consent. Patients who were randomly assigned to the Test group were instructed to take *Sharbat Aftimoon* 25 ml twice daily, morning and evening before food with a

cup of lukewarm water. The Control group patients were instructed to take Losartan 50 mg once daily. Treatment period in both the groups was fixed as 60 days. The efficacy of the test and control group was assessed based on objective parameters. Objective parameter includes arterial stiffness, which is evaluated through Pulse wave velocity (PWV), Central pulse pressure (CPP) and Augmentation index (Aix) was assessed at an interval of 15 days (4 visits of follow up) using periscope (Genesis medical system Pvt. Ltd, Hyderabad). The safety of the treatment was assessed by clinical examination performed at every visit and by biochemical investigations like: SGOT, SGPT, blood urea, serum creatinine and Lipid profile carried out before and after the treatment. Efficacy analysis was performed on an intension to treat (ITT) basis by last observation carried forward (LOCF) method on participants that received at least one dose of *Sharbat Aftimoon* or Losartan 50 mg and that underwent at least one assessment post baseline.

Statistical analysis:

Both descriptive and inferential statistical analyses were carried out in the present study. Results on continuous measurements were presented on Mean ± SD (Min-Max) and results on categorical measurements were presented in Number (%). Significance was assessed at 5 % level of significance. Student t test (two tailed, independent) was used to find the significance of study parameters on continuous scale between two groups (Inter group analysis) at each visit on metric parameters. Leven's test for homogeneity of variance was performed to assess the homogeneity of variance. Student t test (two tailed, dependent) was used to find the significance of study parameters on continuous scale within each group between 0 and 8 weeks. Chi-square/ Fisher Exact test was used to find the significance of study parameters on categorical scale between two or more groups, Non-parametric setting for Qualitative data.

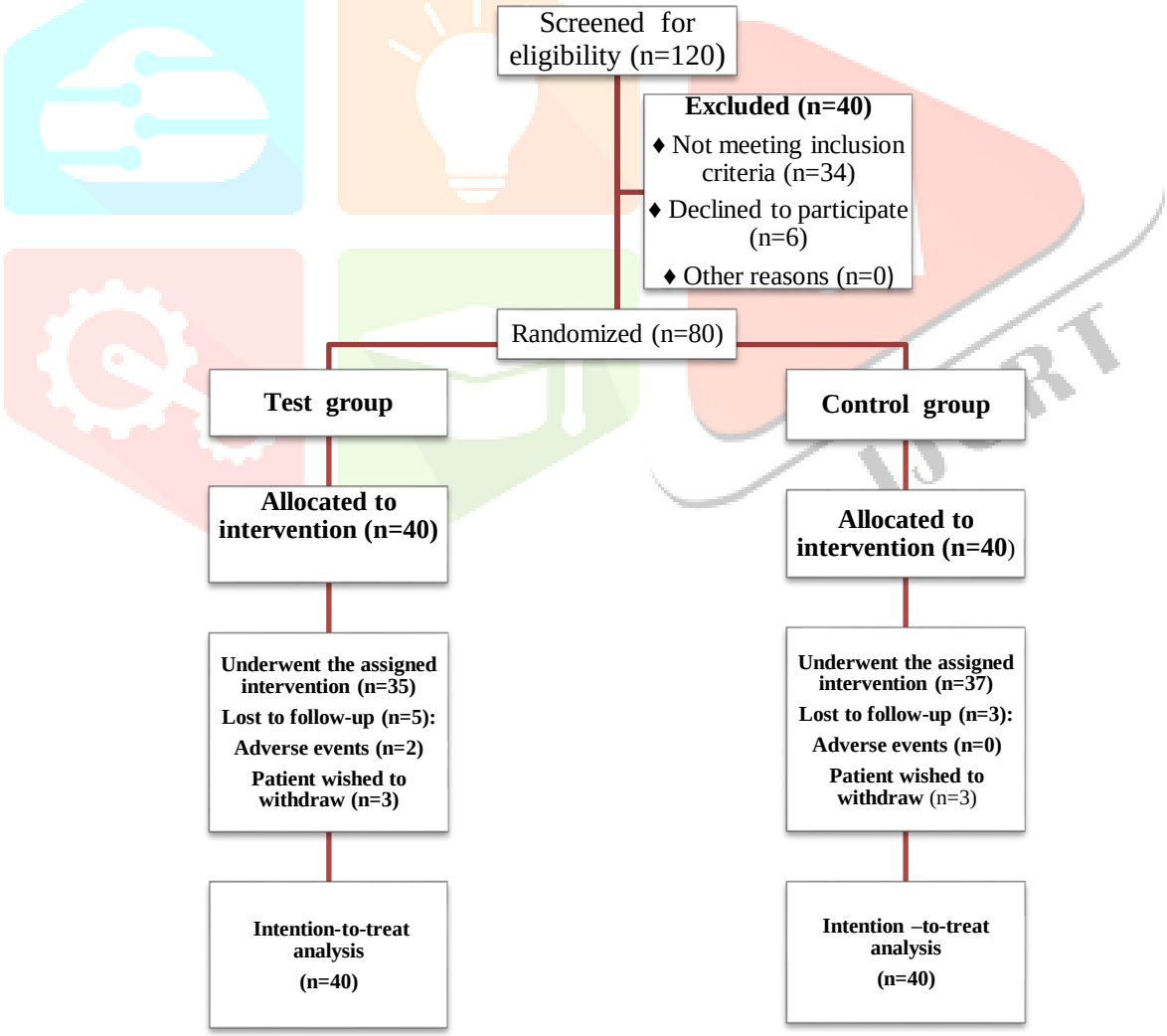

Fig. 1: Consort diagram of the study.

RESULTS

Baseline characteristics

Patient dispositions and Baseline characteristics

Total 120 patients were screened for inclusion in the study, of whom 80 patients met the eligibility criteria and were enrolled in the study. They were randomly allocated into test (Group A) and control (Group B) groups respectively. Most of the patients (n=72; 90%) completed the study and few patients had dropped out of the study (n=8; 10%). Two patient in the test group left the trial because of adverse events and one had a bike accident, and another two left because of traveling. In the control group, one patient traveled abroad and two patients moved back to their native villages during the treatment period.

The descriptive statistics comparing demographic variables and baseline outcome measures are provided in table 1. The demographic variables and baseline outcome parameters of two groups, one receiving sharbat aftimoon 25 ml and other receiving losartan 50 mg did not differ significantly at baseline.

Clinical efficacy

Arterial stiffness, which is considered as useful measure for the prediction of cardiovascular risk especially in hypertensive patients, can be measured noninvasively at low cost with: Pulse wave velocity (PWV), Augmentation index (Aix), and Central pulse pressure (CPP).

Effect on pulse wave velocity

After 60^{th} day of treatment, A substantial decrease in PWV with a mean difference of 4.148 m/s with respect to baseline was found (19.68±8.80 vs. 15.54±7.36; p<0.001). In control group, the mean differences in the pulse wave velocity at each visit were found significant, with a post treatment mean difference of 4.48 m/s with respect to baseline (18.72±9.77 vs. 14.23±8.66; p<0.001). However, no significant difference was found between test group and control group.

Effect on Central pulse pressure

In test group, we observed a significant reduction in CPP at 45^{th} day from baseline (61.95±6.29 vs. 59.98±5.90; p=0.005). Compared with baseline values, CPP shows a significant decrease with a mean difference of 2.525 mmHg after 60^{th} day of treatment (59.43±6.61 vs. 61.95±6.29; p= 0.008). In control group, a significant reduction in CPP was observed at 15^{th} day from baseline (60.55±4.64 vs. 58.83±4.57; p= 0.009). Compared with baseline values, CPP shows consistent and significant decrease after 30^{th}, 45^{th}, and 60^{th} day of treatment (56.60±6.37, 53.13±6.98, and 50.00±8.11 vs. 60.55±4.64; p<0.001 for all).When test group was compared with control group, a moderate significant difference was observed at 15^{th} day (61.18±5.78 vs. 58.83±4.57; p=0.047). Which became strongly significant at 30^{th} day (61.30±5.45 vs. 56.60±6.37; p=0.001). Thereafter, the difference increases consistently and significantly at 45^{th} and 60^{th} of treatment (59.98±5.90 vs. 53.13±6.98, and 59.43±6.61 vs. 50.00±8.11; p=0.001 for both).

Effect on Augmentation index

In test group, a significant reduction in augmentation index was observed at 15^{th} day from baseline (23.51±4.11 vs. 24.04±4.09; p=0.004). After 30^{th}, 45^{th} and 60^{th} day of treatment a consistent and significant decrease was observed in augmentation index when compared with baseline (23.32±4.21, 23.08±4.54, and 22.21±4.64 vs. 24.04±4.09; p=0.003, 0.001, and <0.001 respectively). In control group, losartan treatment demonstrated a significant decrease in augmentation index at 15^{th} day when compared with baseline (21.71±4.77 vs. 23.98±3.33; p=0.006). When compared with baseline, augmentation index showed a consistent and significant decrease after 30^{th}, 40^{th}, and 60^{th} day of treatment (20.78±3.82, 19.25±3.33, and 18.26±3.89 vs. 23.98±3.33; p<0.001 for all).

Biochemical evaluations

As a part of the safety evaluation, laboratory tests were performed for assessment of different biochemical and hematological parameters. Statistical analyses of these parameters did not indicate any significant changes in the test group when compared to the control group. However, surprisingly significant changes in some of the safety parameters were observed in the test group when analyzed over the 60 days period with those of baseline. In test group, after 60 days of treatment, SGOT, SGPT, serum cholesterol, and triglycerides have significantly moved towards the lower limit of interval with respect to baseline (20.95±6.00 vs. 25.50±10.06; 24.00±9.07 vs. 28.20±14.17; 189.83±30.54 vs. 204.70±35.94; 159.80±56.61 vs. 186.70±62.28; (p= 0.011,0.025,0.012,0.007, respectively). (data not shown).

Table 1: Baseline characteristics of the study groups.

	Group A	Group B	p
Gender (male/female)	20/20	23/17	0.501
Age (year)	49.03±9.13	48.75±8.88	0.892
BMI (kg/m)	28.58±5.51	27.65±4.38	0.406
Smoke (%)	30%	32.5%	0.809
Alcohol (%)	12.5%	17.5%	0.531
Vegetarian diet	12.5%	27.5%	0.094
Mixed diet	87.5%	72.5%	0.094
Family history of HTN	42.5%	50%	0.501
Serum Cholesterol (mg/dl)	204.70±35.94	191.45±32.80	0.0890
TGL (mg/dl)	186.70±62.28	164.88±47.5	0.082
HDL (mg/dl)	40.35±6.73	39.80±7.35	0.728

The values are presented as means ± SD or %. BMI, body mass index; TGL, triglyceride; HDL, high-density lipoprotein.

Table 2: Pulse wave velocity in two groups of patients studied

Pulse wave velocity	Group A	Group B	Total	P value
Results				
• **Baseline**	19.68±8.80	18.72±9.77	19.20±9.25	0.644
• **15th day**	19.32±8.69	16.93±8.96	18.13±8.85	0.229
• **30th day**	18.16±7.94	15.56±8.83	16.86±8.44	0.169
• **45th day**	16.70±7.62	14.71±8.63	15.71±8.15	0.277
• **60th day**	15.54±7.36	14.23±8.66	14.88±8.01	0.470
Difference from Baseline				
• **15th day**	0.360	1.78	1.074	-
• **30th day**	1.520	3.16	2.340	-
• **45th day**	2.980	4.00	3.494	-
• **60th day**	4.148	4.48	4.316	-
P value from baseline				
• **15th day**	<0.001**	<0.001**	<0.001**	-
• **30th day**	<0.001**	<0.001**	<0.001**	-
• **45th day**	<0.001**	<0.001**	<0.001**	-
• **60th day**	<0.001**	<0.001**	<0.001**	-

The values are presented as means ± SD.

Table 3: Central pulse pressure in two groups of patients studied

Central pulse pressure	Group A	Group B	Total	P value
Results				
• **Baseline**	61.95±6.29	60.55±4.64	61.25±5.53	0.260
• **15th day**	61.18±5.78	58.83±4.57	60.00±5.31	0.047*
• **30th day**	61.30±5.45	56.60±6.37	58.95±6.35	0.001**
• **45th day**	59.98±5.90	53.13±6.98	56.55±7.29	<0.001**
• **60th day**	59.43±6.61	50.00±8.11	54.71±8.75	<0.001**
Difference from Baseline				
• **15th day**	0.775	1.725	1.250	-
• **30th day**	0.650	3.950	2.300	-
• **45th day**	1.975	7.425	4.700	-
• **60th day**	2.525	10.550	6.538	-
P value from baseline				
• **15th day**	0.077+	0.009**	0.001**	-
• **30th day**	0.262	<0.001**	<0.001**	-
• **45th day**	0.005**	<0.001**	<0.001**	-
• **60th day**	0.008**	<0.001**	<0.001**	-

The values are presented as means ± SD.

Table 4: Augmentation index in two groups of patients studied

Augmentation index	Group A	Group B	Total	P value
Baseline	24.04±4.09	23.98±3.33	24.01±3.71	0.945
• **15th day**	23.51±4.11	21.71±4.77	22.61±4.51	0.075+
• **30th day**	23.32±4.21	20.78±3.82	22.05±4.19	0.006**
• **45th day**	23.08±4.54	19.25±3.33	21.16±4.40	<0.001**
• **60th day**	22.21±4.64	18.26±3.89	20.23±4.69	<0.001**
Difference from Baseline				
• **15th day**	0.535	2.275	1.405	
• **30th day**	0.720	3.205	1.963	
• **45th day**	0.965	4.730	2.848	
• **60th day**	1.830	5.728	3.779	
P value from baseline				
• **15th day**	0.004**	0.006**	<0.001**	
• **30th day**	0.003**	<0.001**	<0.001**	
• **45th day**	0.001**	<0.001**	<0.001**	
• **60th day**	<0.001**	<0.001**	<0.001**	

The values are presented as means ± SD.

DISCUSSION:

In the present study, the administration of Sharbat aftimoon for 60 days offers significant reduction in arterial stiffness in hypertensive patients. The effect of test formulation on arterial stiffness (*slabat sharaeen*) is due to its ingredients like Aftimoon (*Cuscuta reflexa*), Badranjboya (*Mellisa officinalis*), Tukhme Qurtum (*Carthamus tinctorius*), Barge Gaouzuban (*Borago officinalis*, Gule Nasreen (*Narcissus jonquil*) , having the property to expel the abnormal *balgham* (phlegm) and *sauda* (black bile) from the body, responsible for the stiffness of vessels.[10,12] This observation is also supported by various studies, which provide evidence that these ingredients have the potential to decrease stiffness of blood vessels. Katsuya S et al. in a double blind, placebo-controlled study have found significant beneficial results of long-term supplementation with Safflower seed extract on arterial stiffness in human subjects.[19] Yui S. et al in a comparative clinical trial have found significant reduction in pulse wave velocity (brachial- ankle), on giving lemon balam tea compared to barley tea for 6- weeks. This effect is believed to be due to the active components present in lemon balam extract particularly rosmaric acid, which potently inhibits activity against the formation of pentosidine- which is directly related with the degree of arterial stiffness. The degree of arterial stiffness is known to be associated with various markers of inflammation like monocyte chemoattractant protein-1, TNF-α, interleukin-1, interleukin-17 and interleukin-6.[20] Udavant PB et al. have evaluated the antiiflammatory and cytotoxic activities of cuscuta reflexa, and found that methonolic extract of cuscuta reflexa and its ethyl acetate soluble fraction showed significant anti-inflammatory and cytotoxic activities, by using human red blood stabilizing activity.[21] Borage officinalis contains approx 20-25% γ-linolic acid (GLA), which is believed to promote the synthesis of anti-inflammatory metabolites.[22] Guivernau M et al, have found that Platelet aggregation induced by low concentrations of adenosine diphosphate (ADP) and epinephrine, and serum thromboxane B2 was decreased by 45% in both humans and animals after GLA supplementation, and cocluded that these effects may contribute to the prevention of atherosclerotic diseases and cardiovascular protection.[23] Abdel-Halim et al found that Lycorine (one of the alkaloid of narcasis) inhibits murine macrophage production of tumor necrosis factor alpha (TNF-a), and shows inhibitory effects on nitric oxide production and induction of inducible nitric oxide synthase (NOS) in lipopolysaccharide-activated macrophages.[24] In test group, the incidental findings in the safety parameters were anticipated, because most of the ingredients of test formulation has long been used in Unani medicine to treat hepatic disorders and several studies have been conducted to prove their hepato-protective and hypolipidemic properties. Bokent et al, observed a significant reduction in total cholesterol, total lipid and SGOT, SGPT levels in serum and LPO levels in liver tissue in hyperlipidemic rats by administration of an extract of mellisa officinalis.[25] Hamed et al; have proved that ethanolic extract of *Borago officinalis* and *Ocimum basilicum* has potential hepatoprotective effects on CCl4-induced hepatic injury in rats.[26] In another animal study,El-Gengaihi et al proved that borage officinalis oil induced marked decrease in the values of the different lipid parameters.[27] Rahimi et al; observed a significant decrease in total total cholesterol, triglycerides, SGOT, SGPT and ALP in alloxan induced diabetic rats after treatment with 200 mg/kg safflower seed oil for 28 days.[28]

CONCLUSION:

The present study shows that *sharbat aftimoon* is an effective and safe drug in the treatment of arterial stiffness in primary hypertension patients with no clinically or statistically significant side effects or toxicity reported during or after the trial. Thus, it may be considered as safe alternative treatment in arterial stiffness. However considering a double blind design and more than two months duration for future studies may be a reasonable approach to prove its even more authenticity.

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CONFLICT Of INTEREST: **None declared**

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