International Journal of Herbal Medicine
Available online at www.florajournal.com

E-ISSN: 2321-2187
P-ISSN: 2394-0514
www.florajournal.com
IJHM 2021; 9(4): 119-128
Received: 19-05-2021
Accepted: 21-06-2021

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Effect of multi-component water-based herbal nutritional supplement in hypertension management

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Abstract
Hypertension (HTN) prevalence in India now estimated at 29.8%. HTN accounts for 57% of all stroke deaths and 24% of all coronary heart disease (CHD) deaths in India. The present study aims to determine the effect of a multi-component water extract on systolic and diastolic blood pressure levels in hypertensive subjects and corresponding lowering of cardio vascular risk factors. The components of the herbal water extract were Black Cumin, Cinnamon, Turmeric, Ginger, Cardamom, Nutmeg, Clove, Guava Leaves, Flax Seeds, Cumin, Fenugreek, Fennel Seeds, and Licorice. A single-center, prospective, open-labeled, non-interventional, observational study was performed with 50 hypertensive subjects for 180 days. Five visits with intermediary evaluations on the 30th and 90th day were carried out. The systolic and diastolic blood pressure, C-reactive protein (CRP), HbA1c were monitored. Significant and gradual decrease (average of 48 mm Hg) in systolic blood pressure (SBP), a decrease (average of 34 mm Hg) in diastolic blood pressure (DBP), and a reduction (average of 9.1%) in C-reactive protein (CRP) were observed after 180 days. All 50 subjects in the study have shown reduction in SBP and DBP with minimum reduction of 29 mm Hg and 17 mm Hg by day 180 versus baseline values respectively. The findings are independent of their medication/s profile with anti-hypertensive drugs. The herbal extract is effective in reducing the SBP and DBP of all the subjects when administrated as an adjuvant therapy.

Keywords: hypertension, systolic BP, diastolic BP, C-reactive protein, herbal extract, adjuvant therapy

1. Introduction
Hypertension (HTN) a major risk factor for development of stroke, heart disease, heart failure, and renal disease in India and throughout the world. According to a 2020 ecological study among 182 countries, the prevalence of HTN ranged from 13% to 41% [1]. The fourth national family health survey evaluated hypertension in a large population-based sample (n=799,228) in India and reported HTN in 13.8% men vs. 8.8% women (overall 11.3%) aged 15–49 and 15–54 respectively [2].

The European guidelines [3] retain the previous definition of hypertension (i.e., BP >140/90 mm Hg) whereas the American guidelines [4] lowered the threshold. But overall, the guidelines define hypertension when systolic blood pressure (SBP) is ≥140 mm Hg and/or their diastolic blood pressure (DBP) is ≥90 mm Hg after repeated examination. Many factors such as genetics, obesity, life style patterns and diet are considered as risk factors for development of HTN. The treatment protocols are dependent on the degree of hypertension and presence of comorbidities such as Diabetes mellitus, stroke, Chronic kidney disorder and coronary artery disease. The treatment also needs stress alleviation, dietary and lifestyle changes to get more effective control of the disease.

Various multidirectional approach and multiple drug combinations includes angiotensin receptor blockers (ARB); calcium channel blockers (CCB); beta-blockers (BB); and diuretics (DU) [5] which are routinely administered for treatment of HTN as the control of HTN is difficult and often requires trials of various combination and doses of drugs to suit an individual requirement. About 70% of hypertensive subjects require the combination of at least two antihypertensive agents to achieve recommended goals. The use of combinations of drugs allows for action on several different mechanisms to control the HTN. In a meta-analysis of 42 clinical trials, it was found that by combining two drugs with different mechanisms of action, an antihypertensive effect of approximately two to five times greater than that obtained by monotherapy is possible [6, 7]. Increasing the dose of monotherapy reduces coronary events by 29% and cerebrovascular events by 40%, while combining two antihypertensive agents with a different mechanism of action reduces coronary events by 40% and cerebrovascular events by 54% [8].

Medicinal plant products may play a major role in the prevention and treatment of HTN and act as simple adjuvants to HTN therapy thereby reducing dosage and adverse effects of hypertension apart from playing a role in cutting the costs of therapy [9].

In view of this, we evaluated an antihypertensive nutritional supplement (HNS) for managing HTN. The supplement is in an aqueous format and is composed of spices and herbs used in day-to-day preparation of Indian Asian culinary preparations. We conducted a study to evaluate the efficacy and safety of this antihypertensive nutritional supplement in hypertensive subjects.

2. Material and Methods

2.1 Study design

The current study was a single-center, prospective, open-labeled, non-interventional, observational study conducted for 6 months (i.e., 180 days). Ethics committee approval was obtained from the Vijaya Hospital, SPSR Nellore District, Andhra Pradesh, India before the initiation of the clinical trial. The study was performed following the principles of "The Declaration of Helsinki" (World Medical Association) and Good Clinical Practice (GCP) Guidelines issued by the Indian Council of Medical Research (ICMR) &Drugs Controller General of India (DCGI).The nature and proposal of the study design (aims, methodology, potential risks, and anticipated benefits)and participation in the study were fully explained to each subject and written consent was obtained prior to entering the study. The trial was registered on the clinical trial registry of India (CTRI) website at http://ctri.nic.in/ (CTRI number: CTRI/2020/04/024646). The total 5 phases of clinical trials are Phase 0- Screening Visit 0 (day 0); Phase 1- Enrolment Visit I (day 2); Phase 2- Initial follow-up Visit I (day 0 to day 30); Phase 3- Mid follow-up Visit III (day 0 to day 90); and Phase 4- End of Study Visit IV (on day 180).

2.2 Herbal nutritional supplement for hypertension (HNS)

A water based herbal nutrition supplement was provided by M/s Diabliss Consumer Products Pvt Ltd., Chennai, India

2.2.1 Ingredients of HNS: Ingredients of the HNS from Diabliss are: *Nigella sativa* (Black Cumin)[10,11], *Cinnamomum verum* (Cinnamon) [12], *Curcuma longa* (Turmeric) [13], *Zingiber officinale* (Dry Ginger) [12, 14], *Elettaria cardamomum* (Cardamom) [12, 15], *Myristica fragrans* (Nutmeg) [16], *Syzygium aromaticum* (Clove) [17], *Psidium guajava* (Guava Leaves) [18], *Linum usitatissimum* (Flax Seeds) [19, 20], *Cuminum cyminum* (Cumin) [21], *Trigonella foenum-graecum* (Fenugreek) [22], *Foeniculum vulgare* (Fennel Seeds) [23], and *Glycyrrhiza glabra* (Licorice) [24, 25, 26] are the ingredients of HNS.

In our HNS preparation, licorice was one of the ingredients, and 0.08 g/day was used and at this low intake level it is considered safe without requiring notification of side effects [25, 26]. All these ingredients are known to have anti-inflammatory, anti-diabetic, anti-hypertensive, and antioxidant properties and useful in reducing abnormal blood pressure or HTN, regulating cholesterol levels, and reduces the risk of developing cardiovascular diseases.

2.2.2 Preparation and intake of herbal solution

A volume of 15 ml of HNS was mixed with 985ml of water and subjects consumed all 1000 ml solution in a day. Every patient consumed equal proportions of the water mixed with HNS at breakfast, lunch and dinner during the 180-day period. All subjects were regularly monitored by the physician during the study period. The body weight, body mass index, and blood pressure of subjects were recorded.

2.3 Subjects

The current study included fifty subjects (27 male and 23 female subjects), age >45 years, with the diagnosis of HTN and diastolic blood pressure (DBP) of >110 mmHg and systolic blood pressure (SBP) of >150 mm Hg. Among the 50 subjects, 25 subjects were long-term hypertensives.>2 years with HTN, and 25 subjects with new hypertensives, <1 Year with HTN. All the Subjects were also diabetic and followed their current HTN, diabetes medications and initiated supplemental intake of HNS. Inclusion and exclusion criteria are listed in table 1. HTN medication profile of subjects included ARB (1 out of 50), CCB (8/50), ARB with CCB (3/50), BB (6/50), CCB with BB (4/50) and DU with ARB (28/50). Diabetes medication profile included Biguanides or Sulfonylureas with Biguanides.

Table 1: Inclusion and Exclusion criteria for a clinical trial with HNS.

Inclusion criteria	Exclusion criteria
▪ Male/Females subjects between >45years of age ▪ Subjects are able to communicate effectively ▪ Subjects are willing to provide written informed consent ▪ In the judgment of the Principal Investigator, subjects are able to comply with protocol requirements ▪ Expressed ability and willingness to schedule and attend monthly visits for the duration of the study ▪ Subjects with a diagnosis of hypertension (DBP 110 mm Hg and SBP>150mm Hg) ▪ Subjects who are hypertensive for <1 Year and for>1 year with standard HTN medication	▪ Patients are not ready to refrain from taking treatment with other drugs that could interfere with the evaluation of efficacy and tolerability. ▪ Patients with secondary HTN (eg. due to renal artery stenosis), acute or severe bronchial asthma, renal or hepatic disease or serum electrolyte abnormalities, uncontrolled diabetes mellitus, significant cardiovascular or cerebrovascular disease or cardiac surgery, or percutaneous trans luminal coronary angiography in the previous ≥ 3 months ▪ Pregnant or breast-feeding women, and women of childbearing age not using an adequate method of contraception ▪ History of: alcohol consumption or drug abuse, or HIV infection or epilepsy or active hepatitis B or C within the past 3 months ▪ Participation in another trial concurrently or within 4 weeks prior to the Screening Visit. ▪ Contra-indications or hypersensitivity to study product, History of medical condition/disease according to the discretion of the Investigator ▪ Any other condition that, in the opinion of the investigator, would adversely affect the subject's ability to complete the study or its measures

2.4 Screening Tests

The following tests were performed for all the subjects on very first day before treatment, designated as day 0, day 30, day 90, and day 180- the last day of the study. Further, daily blood pressure levels, pulse rate as measured by a standardized sphygmomanometer (Omron BP device calibrated with inbuilt storage). Readings were taken at 8 am and 6pm for all subjects. Three readings separated by one minute were taken in the morning and evening. The average of morning and evening readings was calculated.

HbA1c was separated and estimated by the high-performance liquid chromatography method [27].

C - reactive protein (CRP) was estimated by latex-enhanced nephelometry [28].

Liver function tests: Total bilirubin was estimated by the vanadate oxidation method [29]. Alkaline phosphate was assayed as per the modified method [30]. Aspartate [31] and alanine [32] transaminase activities were also assayed as per the methods referred.

Renal function tests: Creatinine amidohydrolase was used to measure serum creatinine [33]. Uric acid was assayed using the uricase-peroxidase method [34].

Lipid profile: Total cholesterol was assayed using cholesterol oxidase and peroxidase Trinder CHOD/POD End Point method. Triglycerides assay was performed by anenzymatic colorimetric method; HDL and LDL were assayed as per the methods referred [35]. The complete blood count was estimated by the spectrophotometric method in an automated analyzer [36].

The scheduled activity that was followed or all the 50 participants is listed in table 2.

Table 2: The scheduled activity that was followed for all the participants of a clinical trial with HNS.

Scheduled activity	Screening Visit 0 (Day 0)	Enrolment Visit I (Day 2)	Follow-up Visit II (Day 0 to 30)	Follow-up Visit III (Day 0 to 90)	End of Study Visit IV (Day 180)
Patient information &Written Informed Consent	X	-	-	-	-
Demography and Medical History	X	-	-	-	-
Vital Parameters and Clinical Examination	X	X	X	X	X
Screening and Provisional Eligibility (Inclusion/Exclusion)	X	-	-	-	-
Enrolment and Final Eligibility (Inclusion/Exclusion)	-	X	-	-	-
Study Assessment, Concomitant Illness, Concomitant medication and Adverse Event record	X	X	X	X	X
Blood Pressure (DBP and SBP)	X	X	X	X	X
Complete blood profile, Lipid profile, Liver and kidney function tests (Serum AST, ALT, ALP, uric acid, urea, and creatinine)	X	-	-	-	X
C-Reactive Protein	X	-	X	X	X
Glycated hemoglobin - HbA1c	X	-	-	X	X
Compliance	-	X	X	X	X
Study Completion/Termination	-	X	X	X	X

3. Results and Discussion

3.1 Impact on Systolic BP (SBP), Diastolic BP (DBP) and C - reactive protein (CRP)

All participants completed the study, and in terms of compliance, all participants consumed 100% of the prescribed HNS. No side effects were reported during the study.

The data of HTN parameters of 50 subjects are presented in figure 1. With regards to mean SBP (figure1a), there was a gradual decrease following the consumption of HNS. Mean SBP showed a decrease of 22 mm Hg on day 30, 34 mm Hg decrease on day 90, 48 mm Hg decrease at the final visit on day 180. A similar trend was found with regards to mean DBP (figure 1b). Mean DBP decreased by 20 mm Hg on day 30, 24 mm Hg decrease on day 90 and 34 mm Hg decrease on day 180. Mean CRP (figure 1c) also showed continuous reduction: 2.8% reduction on day 30, 6.3%reduction on day 90 and9.1% decrease on day 180. Figures 2 & 3plots individual SBP and DBP values and shows every individual showed lower SBP and DBP when we compare day 0 and day 180 readings. Figure 4 is CRP values of subjects on day 0 and day 180. 48 of the 50 subjects showed lower CRP values whereas 2 of the subjects showed no change in CRP values. Further, the two subjects whose CRP did not change during the course of the clinical trial showed lowering of SBP and DBP.

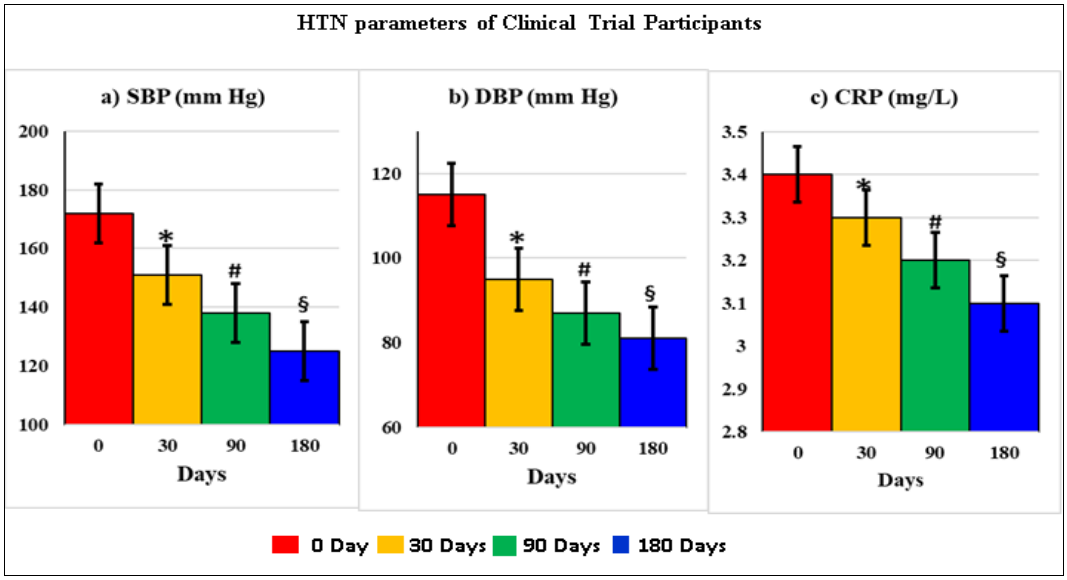

Fig 1: HTN parameters of clinical trial participants. Values were expressed as Mean ± S.D. of 50 participants. (a) SBP (b) DBP and (c) CRP.The values that were highlighted with special symbols were statistically significant in comparison with the respective day 0 (visit I) values at least by * ($p< 10^{-2}$), # ($p< 10^{-8}$), and § ($p<10^{-15}$).

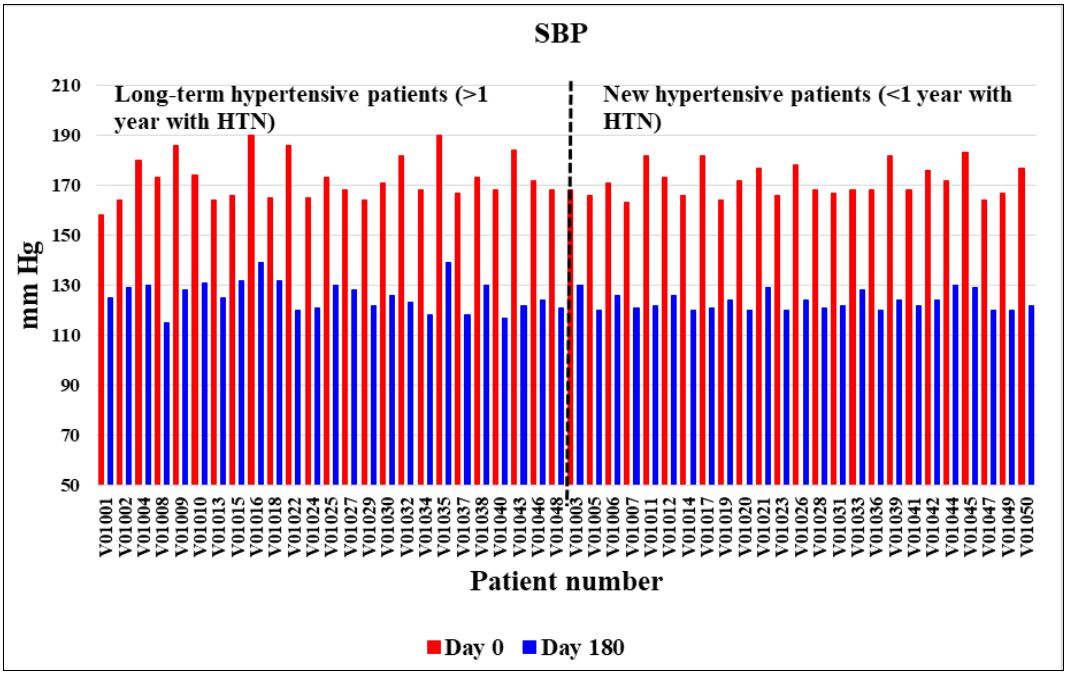

Fig 2: Individual Systolicblood pressure (SBP-mm Hg) levels of 0 day Vs 180 day

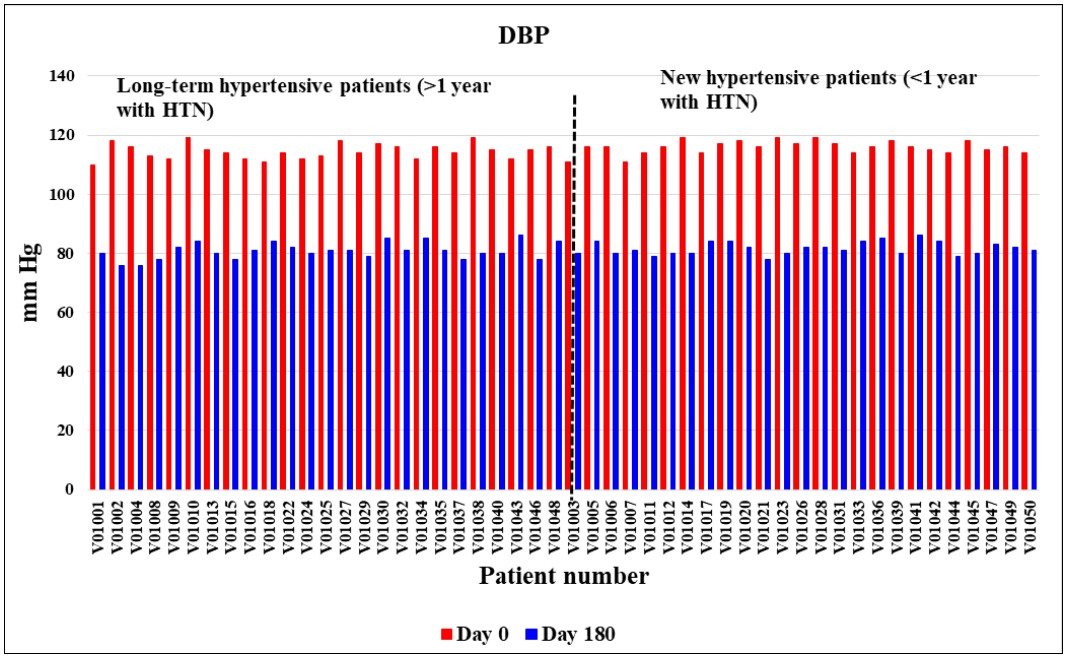

Fig 3: Individual Diastolicblood pressure (DBP-mm Hg) levels of 0-day Vs 180 days

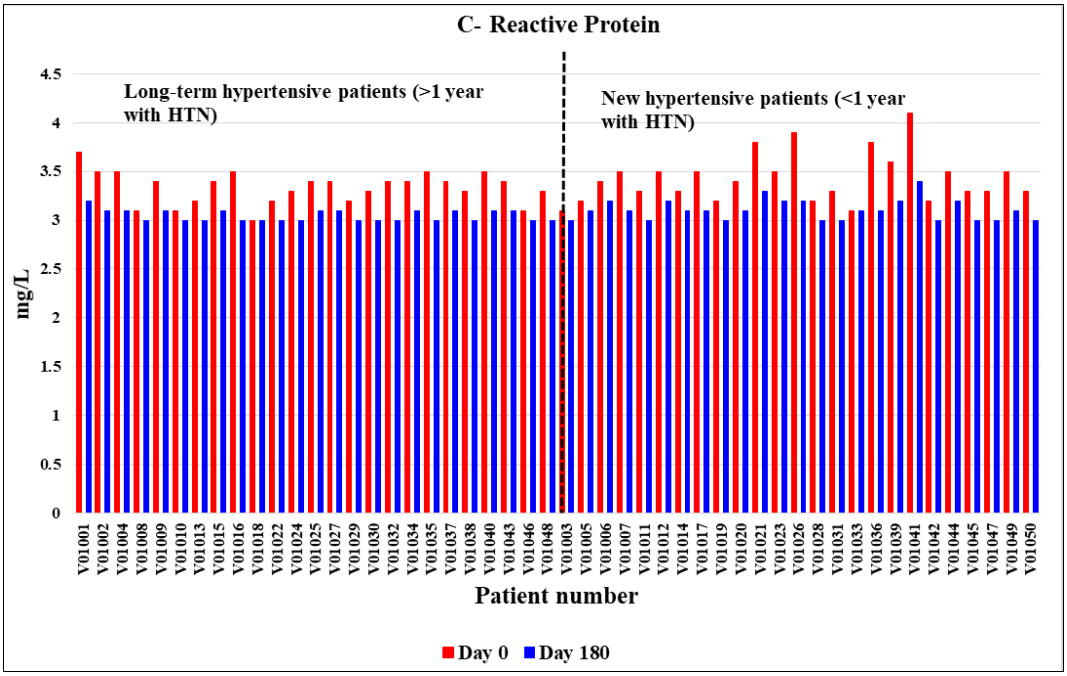

Fig 4: Individual C-reactive protein (CRP-mg/L) levels of 0-day Vs 180 days.

3.2 Impact of HTN Medications on SBP and DBP

The three HTN parameters were tabulated as a function of the medication/s profile of the subjects in table 3. The absolute values and percentage decrease of all the 3 parameters studied compared to the corresponding day 0. They were found to be remarkably persistent, especially at days 90 and 180, and independent of which combination of the medication was being used.

With regards to the 3 HTN parameters, the minimal statistical significance at day 30 for SBP was in the order of 10^{-20}, for DBP was in the order of 10^{-37}, and for CRP was in the order of 10^{-2}. The statistical significance for SBP, DBR and CRP was 10^{-58}, 10^{-85}, and 10^{-15} respectively on day 180.

Table 3: Summary of the HTN parameters (Means of SBP, DBP, and CRP) of the 50 clinical trial participants based on the anti-hypertensive medications used

Medication used with Supplement	No. of Subjects	Day 0	Day 30	Day 90	Day 180	Mean (mm Hg) Difference Vs 0 Day		
		Mean SBP (mm Hg)				Day 30	Day 90	Day 180
ARB	1	166	152	140	132	14	26	34
CCB	8	171	149	136	124	22	35	47
ARB + CCB	3	174	148	134	126	26	40	48
BB	6	172	155	139	124	17	33	48
CCB + BB	4	172	152	137	124	20	35	48
DU + ARB	28	173	150	139	125	23	34	48
		Mean DBP (mm Hg)				Mean (mm Hg) Difference Vs 0 Day		
ARB	1	114	92	86	78	22	28	36
CCB	8	115	98	88	81	17	27	34
ARB + CCB	3	117	95	90	84	22	27	33
BB	6	116	94	88	81	22	28	35
CCB + BB	4	114	95	89	84	19	25	30
DU + ARB	28	115	94	86	81	21	29	34
		Mean CRP (mg/L)				Mean % Difference Vs 0 Day		
ARB	1	3.4	3.3	3.1	3.1	-3%	-9%	-9%
CCB	8	3.5	3.4	3.2	3.1	-3%	-9%	-11%
ARB + CCB	3	3.6	3.4	3.3	3.1	-6%	-8%	-14%
BB	6	3.4	3.3	3.1	3.1	-3%	-9%	-9%
CCB + BB	4	3.3	3.1	3.2	3.1	-6%	-3%	-6%
DU + ARB	28	3.4	3.3	3.2	3.1	-3%	-6%	-9%

3.3 HbA1c Trends

Mean Glycated hemoglobin, HbA1c showed 0.2%% reduction on day 90 ended and 0.4% reduction on day 180. The individual HbA1c data of the final visit at 180 days in figure 5. The statistical significance for HbA1c was found to be 10^{-6} on day 90 and 10^{-19}on day 180.

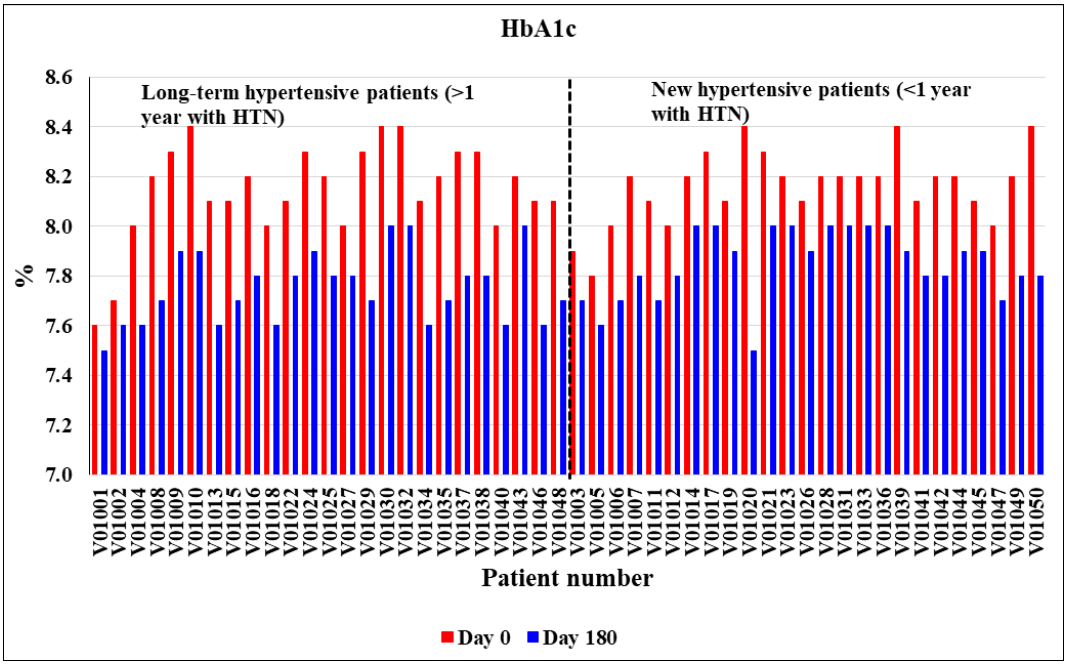

Fig 5: Individual HbA1c (%) levels of visit I (0-day) and final visit (180 days)

3.4 Daily SBP and DBP Profiles

The daily average SBP and DBP blood pressure data of all subjects are shown in Figure 6. A review of individual daily BP curves showed a range of BP profiles. Figures 7 and 8 show some of the varied BP reduction profiles. In figure 7 we see a more or less continuous reduction in SBP and a stepped reduction in DBP. In figure 8 more distinct stepped reductions in SBP and DBP followed by flat regions can be seen. These could be an indication of different BP-lowering mechanisms at play. However, the literature on such BP lowering regimes was not found. This is an area for further investigation to understand the mechanisms at play.

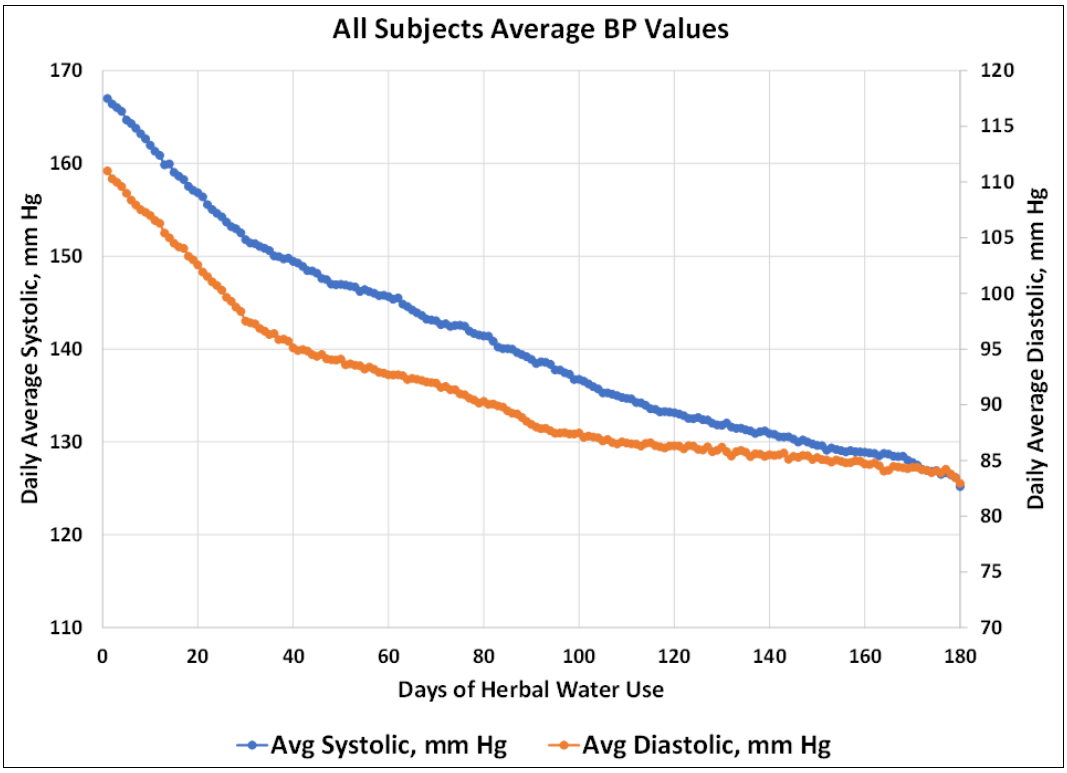

Fig 6: Average Systolic and Diastolic BP values of all subjects from individual daily average readings of morning and evening BP measurements using a standardized sphygmomanometer (Omron BP device calibrated with inbuilt storage)

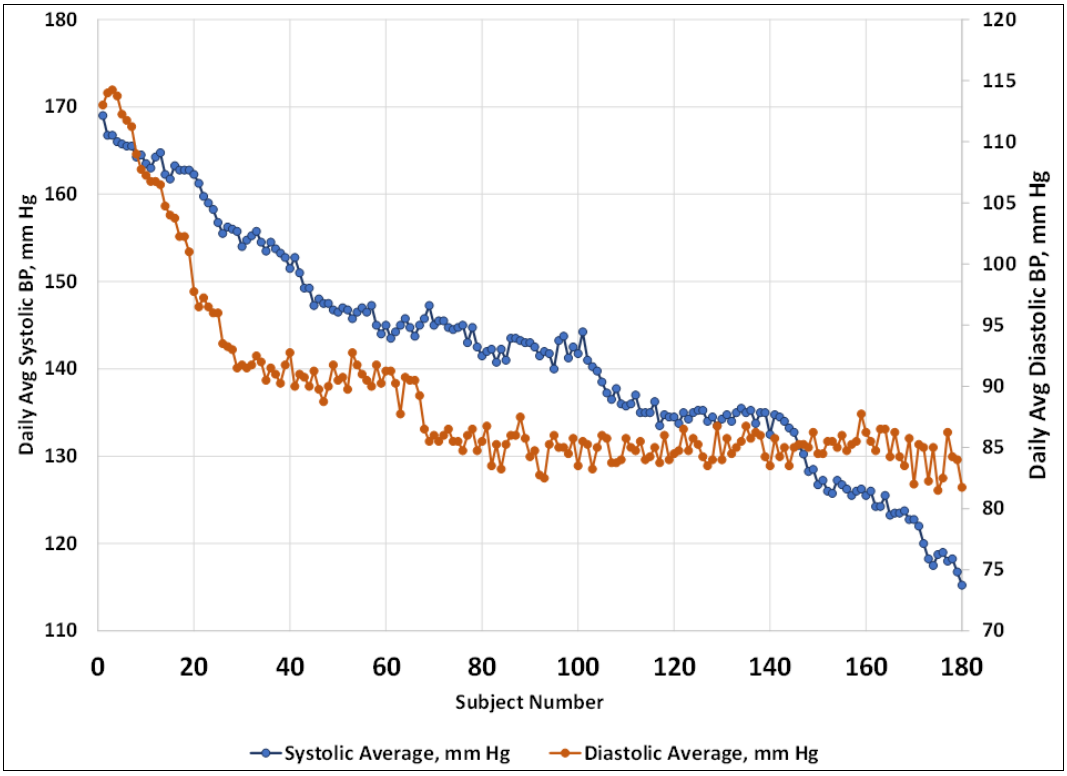

Fig 7: Subject V01008 Daily average BP values shows a more or less continuous SBP reduction and 2 or 3 regimes of DBP reduction.

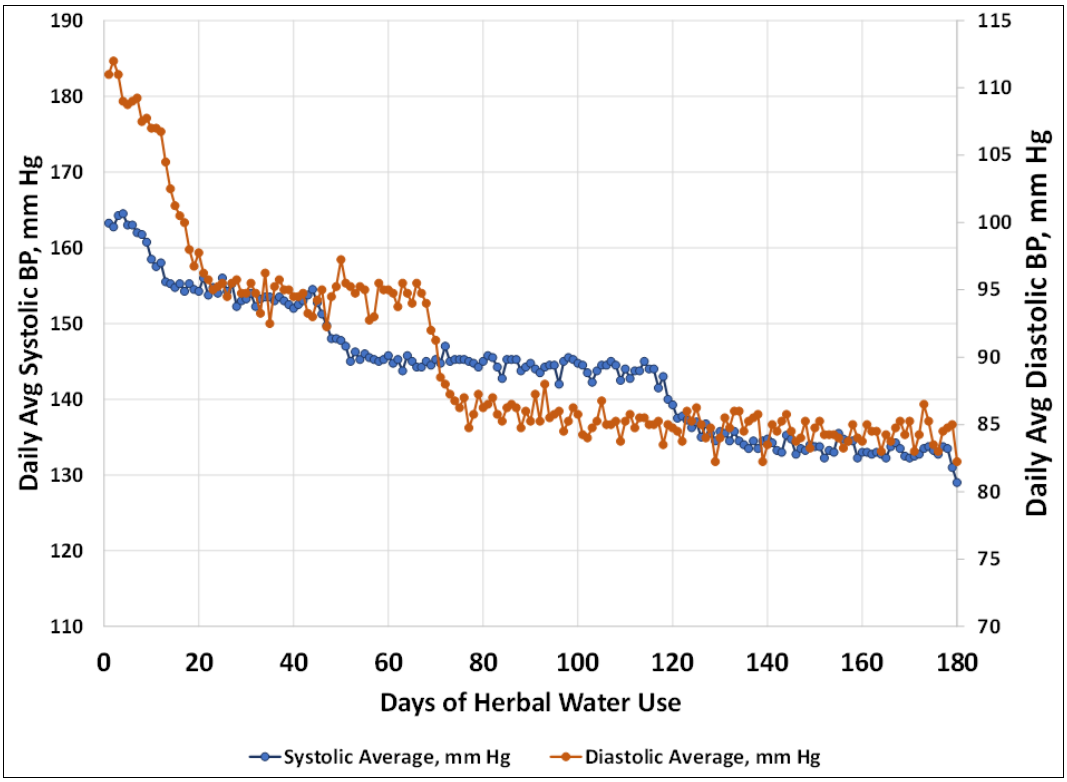

Fig 8: Subject V01027 shows multiples regimes of SBP and DBP reduction

3.5 Statistical Analysis

Statistical summary of all the 3 HTN parameters studied for all the 50 subjects for all the visits are presented in table 4. They include maximum, minimum, range, mean, standard deviation, median and mode along with the assessments of data distribution in terms of skewness, kurtosis and outlier determination.

SBP range (the difference between the maximum and minimum) of 32 at Day 0, increased to 40 and 48 at Day 30 and Day 90 respectively but came down to 24 by the final visit on day 180, a good indication for the effect of the supplement in the long term. Similarly, for DBP, the range of 9 at Day 0, indeed, increased to 27and 18 at Day 30 and Day 90 respectively and decreased to 10on Day 180. With regards to CRP, the range value was 1.1 on Day 0, and consistently decreased to 0.8, 0.5, and 0.4 on Day 30, Day 90 and Day 180 respectively. These trends are an indicator of the long-term impact of supplementation of HNS.

Skewness is a measure of the asymmetry of the distribution of the population of a random variable about its mean, while the

positive sign indicates the right shift to the mean, the negative sign is for the left shift. In the current data, the distribution is mostly symmetric.

Kurtosis is a statistical measure used to describe the degree to which variables cluster either in the tails or in the peak of a distribution. In the current data, very few kurtosis values, especially, those >1, indicating moderate tails hinting at outliers. Outliers are determined by Tukey's [39] method using the Interquartile range (IQR) and the lack of or very few outliers found are consistent with the data of kurtosis.

Overall, very few aberrations were noticed in the statistical analysis, indicating the positive benefits of HNS on the 3 HTN parameters studied in the long term.

Table 4: Statistical summary of the 3 HTN individual parameters (SBP, DBP, and CRP) of the clinical trial participants of all the visits.

Statistical parameter	SBP (mm Hg)				DBP (mm Hg)				CRP (mg/L)			
	Day 0	Day 30	Day 90	Day 180	Day 0	Day 30	Day 90	Day 180	0Day	Day 30	Day 90	Day 180
Maximum	190	168	168	139	119	108	98	86	4.1	3.8	3.5	3.4
Minimum	158	128	120	115	110	81	80	76	3	3	3	3
Range	32	40	48	24	9	27	18	10	1.1	0.8	0.5	0.4
Mean	172	150	138	125	115	95	87	81	3.4	3.3	3.2	3.1
Std Dev	8	10.5	10.6	5.2	2.41	6.52	4	2.5	0.22	0.02	0.1	0.1
Median	170	152	139	124	116	94	86	81	3.4	3.3	3.2	3.1
Mode	168	152	140	120	116	90	86	80	3.5	3.2	3.1	3
Coefficient of variation	4.5	6.9	7.6	4.1	2.1	6.8	5.1	3	6.3	4.8	3.6	2.9
% Decrease compared to 0 Day	0	12.5	19.9	27.6	0	17.7	24.3	29.5	0	2.8	3.6	3
Skewness	0.71*	-0.24	0.39	0.78*	-0.20	-0.002	0.52*	0.08	1*	1*	0.8	1.3*
Kurtosis	-0.37	-0.71	0.1	0.58	-0.72	-0.64	-0.25	-0.51	1.8	1.5	0.4	2.05
Q1 (25 Percentile)	166.3	143	130.5	121	114	90	84	80	3.3	3.2	3.1	3
Q3 (75 Percentile)	177	158	143	128	117	100.5	91	83.8	3.5	3.4	3.2	3.1
Q3-Q1	10.8	15	12.5	7.8	3	10.5	7	3.8	0.3	0.2	0.1	0.1
1.5*IQR	16	22.5	18.8	11.6	4.5	15.8	10.5	5.6	0.5	0.3	0.2	0.2
Lower outlier fence	150.1	120.5	111.8	109.4	109.5	74.3	73.5	74.4	2.8	2.9	3	2.9
Upper outlier fence	193.1	180.5	161.8	140.4	121.5	116.3	101.5	89.4	3.9	3.7	3.4	3.3
Number of Outliers	0	0	1	0	0	0	0	0	1	1	3	2

3.6 Subject Progression with respect to AHA Hypertension Categorization

Table 5 is American Heart Association (AHA) hypertension categorization along with clinical trial participant distribution during the course of the trial. Based on the classification, we can see at day 0, 0% of the subjects were in Normal, Elevated and High BP-HTN Stage 1 categories, 80% of the subjects were in High BP-HTN Stage 2 and 20% of the subjects were in HTN Crisis category. At the end of the clinical trial on day 180, 4% of the subjects were in Normal BP category, 60% were in Elevated category, and 36% High BP-HTN Stage 1 category, 0% of the subjects were in High BP-HTN Stage 2 and 0% of the subjects were in HTN Crisis category.

Other parameters such as complete blood picture (hemoglobin, total and differential leukocyte counts, platelet count, erythrocyte sedimentation rate), blood pressure, liver function tests (bilirubin, alkaline phosphatase, aspartate, and alanine aminotransferases) renal functional tests (creatinine, uric acid), lipid profile (total-, high density-, low density- and very low density- lipoprotein cholesterols, triglycerides) electrocardiograms, were performed for all the 50 subjects as shown in table 2 have not shown any particular variations during the course of the clinical trial.

Table 5: American Heart Association (AHA) Hypertension Categorization & Clinical Trial Participant Distribution.

Blood Pressure Category			No. of subjects (% of subjects) in each HTN category				
Blood Pressure level	Systolic(mm Hg)	Diastolic(mm Hg)	Day 0	Day 2	Day 30	Day 90	Day 180
Normal	< 120	< 80	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (4%)
Elevated	120-129	< 80	0 (0%)	0 (0%)	0 (0%)	0 (0%)	30 (60%)
High BP-HTN Stage 1	130-139	80-89	0 (0%)	0 (0%)	1 (2%)	24 (48%)	18 (36%)
High BP-HTN Stage 2	≥ 140	≥ 90	40 (80%)	47 (94%)	49 (98%)	26 (52%)	0 (0%)
HTN Crisis	> 180	> 120	10 (20%)	3 (6%)	0 (0%)	0 (0%)	0 (0%)
Total			50 (100%)				

4. Conclusion

The continuous and significant reduction of SBP and DBP among all subjects and consistent reduction irrespective of various medications/combination medications used makes HNS a very useful supplement for hypertension management. The entire study population saw reduction in SBP and DBP. The study demonstrated HNS based nutraceuticals may be considered as an effective adjunctive therapy for HTN.

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