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DETERMINATION OF THE EFFECT OF FUNCTIONAL HERBAL WATER BLEND IN TYPE-II DIABETES MELLITUS MANAGEMENT

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ABSTRACT

Diabliss functional beverages are provided in a water-soluble format to deliver the required efficacy for general health benefits and wellness. Management of type 2 diabetes and controlling high blood sugar levels and their complications is one such health benefits, which is the focus of the current study. Preclinical sub-acute toxicity studies were performed with functional herbal water blend (FHWB) in Wistar rats based on which the human equivalent safe dose was found to be 3.88 g/day. To evaluate the efficacy of FHWB in patients of type 2 diabetes mellitus, single centric, open-label study with 45 subjects for 180 days in five visits was performed with intermediary evaluations at 30 and 90 days as well. Significant and gradual mean reductions with a maximum of 48% in fasting blood sugars (FBS), 44% in postprandial blood sugars and 20.2% in HbA1c in the present study after 180 days indicate the potent efficacy of herbal solution supplementation in the management of type 2 diabetes mellitus among the patients participated in the clinical trial independent of their medication/s profile with anti-diabetic drugs. All other blood, or liver or renal functional and/or lipid profile parameters did not exhibit any significant difference from the corresponding initial visit (0 day) values. FHWB is quantitatively effective and has no side effects and it is easy to store and administer.

KEYWORDS: Diabetes mellitus, functional food and beverage, HbA1c, fasting blood sugar, postprandial blood sugar, sub-acute toxicity study.

INTRODUCTION

Diabetes mellitus (DM) is an endocrine disorder, which is growing at a frightening rate both in developing and developed countries. The major types of diabetes mellitus are type 1, type 2, gestational, and maturity-onset diabetes mellitus.[1]

Autoimmune diabetes mellitus or type 1 DM (T1 DM) is an autoimmune disease with the destruction of insulin-producing pancreatic β-cells, after an inflammatory process leads to a chronic deficiency of insulin in genetically susceptible individuals. In 1997, the Committee of Experts of the American Diabetes Association (ADA) recommended dividing T1DM into type 1A diabetes (i.e., autoimmune-mediated) and type 1B (i.e., other forms of diabetes with severe insulin deficiency but without the proof of autoimmune etiology hence, also known as idiopathic).[2] Type 2 is the most common type of DM, which usually occurs in adults and accounts for about 90% of the diabetic population.[3,4] When insulin receptors start failing to respond to insulin, termed as insulin resistance, owing to the modifications of insulin receptors, lead to increases in blood glucose level.[5] If not intervened, either by lifestyle changes in diet along with exercise or pharmacologically at this stage, the situation leads to more serious consequences. Responding to the continuously increasing levels of blood glucose, pancreatic β-cells for some more time continue to overproduce insulin, leading to hyper-insulinemia,[6] which also fails to clear hyperglycemia due to continued, unattended insulin resistance. Ultimately, it leads to a situation of pancreas getting exhausted, ending with 'no insulin' production, which is clinically termed as type 2 DM, necessitating insulin injections. Gestational diabetes mellitus, is a condition in which hormones secreted by the placenta prevent some of the mothers' bodies from using insulin effectively, a condition referred to as insulin resistance. Even though this type of diabetes typically disappears following delivery, 40% of the women with gestational diabetes have more chances to develop type 2 DM later in life.[7]

The most common long-term diabetes-related health problems are damage to the large blood vessels of the heart, brain, legs (macrovascular complications), damage to the small blood vessels, causing problems in the eyes, kidneys, feet, and nerves (microvascular complications). Old people with diabetes are at high risk of cognitive decline and institutionalization.[8]

The ADA released new research estimating the total costs of diagnosed diabetes in the USA rose to $327 billion in 2017 from $245 billion in 2012, representing a 26% increase over a

five-year period.[9] The economic and social impact of diabetes in India had an annual estimated cost of 10 to 12,000 crores of rupees (1.33 to 1.6 billon USD) in 2003, which is likely to witness a scaling up to 126,000 crores of rupees (16.8 billion USD) by 2025.[10]

The global diabetes prevalence in 2019, estimated at 9.3% of population (463 million people) is projected to go up to 10.2% (578 million) by 2030 and to 10.9% (700 million) by 2045.[11] Among the US population overall, crude estimates for 2018 were: 34.2 million people of all ages or 10.5% of the US population had diabetes. Approximately, 88 million American adults, i.e., more than 1 in 3 have prediabetes.[12] In India, the potential epidemic status of diabetes seems to be quite high. According to the World Health Organization (WHO) reports, there were 32 million people affected by diabetes in the year 2000, 50.8 million in 2010. It increased to 77 million in 2018 in India and this number is predicted to rise to 134 million by 2045[13] as per the International Diabetes Federation (IDF).

Prediabetes-Importance on Blood Glucose Control

Prediabetes is an asymptomatic condition with glycemic parameters above the normal levels but below the diabetes threshold. Insulin resistance, impaired incretin action, and insulin hypersecretion are central to the pathophysiology of prediabetes.[14] Impaired glucose tolerance is defined as blood glucose levels of 140 to 199 mg/dl during a 75 g for oral glucose tolerance test (normal <140 mg/dl), and impaired fasting glucose is defined as blood glucose levels of 100 to 125 mg/dl, although the WHO has a narrower threshold of between 110 and 125 mg/dl.[15] Impaired fasting glucose also may be diagnosed with impaired glucose tolerance, but many have normal responses to a glucose tolerance test. Fasting glucose helps in identifying prediabetes.[16]

Management of Diabetes through Pharmacological agents

The most important goal in the management of type 2 diabetes is to control high blood sugar levels and their complications. Type 2 DM is typically controlled with precise medical therapy and a stepwise approach, which includes initial lifestyle modifications with regards to exercise and diet.

Oral antidiabetic drugs are usually introduced when lifestyle modifications fail to satisfactorily control hyperglycemia.[17] Oral antidiabetic drugs act on the organs such as the pancreas, liver, and skeletal muscle are very useful for managing high blood glucose especially, in the early stages of the disease, achieving typical HbA1c reductions of 0.5% to

2%. The commonly used drugs are Sulphonyl ureas (Glibenclamide), Biguanides (Metformin), α-glucosidase inhibitors (Acarbose), and Thiazolidinediones (Pioglitazone, Rosiglitazone). Human insulin (injected) has a slower onset of action and a prolonged effect compared to endogenous insulin when it is injected subcutaneously at mealtime.[18]

Management through Medicinal plants

Several medicinal plants have been identified to possess antidiabetic potential. Most of the herbal preparations are reported to have minimal or no side effects. However, some of these herbal moiety drugs raise bioavailability issues.[19] The current study is with one of such functional herbal water blend unique by Diabliss for managing blood sugar delivered in an aqueous format to minimize bioavailability issues.[20]

MATERIAL AND METHODS

Functional Herbal Water Blend (FHWB)

Functional beverages are provided in a water-soluble format to deliver the required efficacy for general health benefits and wellness. When FHWB was specifically designed to manage blood sugar, and the required active principle is delivered in a consumable format of approximately, 15 ml water, instead of 2.5 g/day (Calculation on page 12) of powders which may require 7 tablets when consumed in a 500 mg tablet dosage form. Further, as active principle is delivered in an aqueous format, it is generally expected to enhance bioavailability,[21] and the clinical data support this contention.

Ingredients of FHWB

Phyllanthus emblica (Amla),[22,23] *Syzygium cumini* (Black Jamun),[24,25] *Trigonella foenum-graecum* (fenugreek),[26] *Cinnamomum cassia* (Cinnamon),[27] *Curcuma longa* (turmeric),[28] *Syzygium aromaticum* (Clove),[29] *Piper nigrum* (Black pepper),[30] *Zingiber officinale* (Ginger),[31] *Coriandrum sativum L.* (Coriander Seeds), [32, 33]*Illicium verum* (Staranise),[34,35], *Cuminum cyminum* (Cumin),[36] Nigella sativa (Black Cumin),[37] *Senna alexandrina* (Indian Senna),[38] *Psidium guajava* (Guava Leaves),[39,40] *Piper Nigrum* (White Pepper),[41] *Trachyspermum ammi* (Ajwain),[42] *Foeniculum vulgare* (Fennel Seeds),[43,44] are the ingredients of FHWB. Based on the literature, all these ingredients are used in either delaying glucose absorption, or enhance insulin activity and sensitivity. They are expected to increase glycogen storage in the liver and thereby reduces blood glucose and lower HbA1c levels.

Directions for use: Make up 15 ml of FHWB to 500 ml with water. Consume throughout the day, preferably at breakfast, lunch, and dinner in equal proportions.

METHODS

Preclinical studies

Acute and sub-acute toxicity studies were conducted as per the guidelines of OECD Nos.423 and 407 respectively at Sugen Life Sciences Pvt. Ltd., Andhra Pradesh, India.

The required quantity of FHWB was weighed and mixed with sterile distilled water before dosing (oral, single dose). Three female Wistar rats per step were treated with the test item in steps 1 and 2 at the dose level of 300, 2000 mg/kg body weight (bw) respectively. The animals of steps 1 and 2 were observed for clinical signs, morbidity and mortality for 14 days, if any. Post-dosing parameters evaluated included body weight (days 7 and 14) and gross pathological examination. Three animals of step 3, similar to step 2 were treated with 2000 mg/kg bw were kept for confirmation of the results of the highest dose. Observation of clinical signs and pathological examination were performed as before for 14 days.

In sub-acute 28-day repeated dose toxicity study, six groups of Wistar rats (5 males and 5 females/each group) were administered with FHWB at the doses of 0, 100, 200, 400 mg/kg bw; as vehicle control, low dose, mid-dose and high dose respectively. Another 0 and 400 mg/kg bw as vehicle control and 'high dose recovery' groups respectively were maintained for 28 days. Vehicle control recovery group and high dose recovery group of animals after the initial 14 days of administration with FHWB, were prolonged for another 14 days (without treatment) to observe any delayed clinical signs, morbidity and/or mortality.

Clinical Trial Study details

Ethical approval was obtained from the Ethics Committee of Vijaya Hospital, SPSR Nellore District, Andhra Pradesh, India. The study was performed following the current version of the declaration of Helsinki (52nd WMA General Assembly, Edinburgh, Scotland, October 2000). The trial was conducted in agreement with the International Conference on Harmonization (ICH) guidelines on Good Clinical Practice (GCP).

Study Design: A single centric, open-label study to evaluate the efficacy of FHWB in Type 2 Diabetes mellitus patients.

All patients provided written informed consent to participate in the study before being screened. The patient information sheet detailed the procedures involved in the study (aims, methodology, potential risks, anticipated benefits) and the investigator explained these to each patient. The patient signed the consent form to indicate that the information had been explained and understood. The patient signed and dated the informed consent form to indicate that they fully understood the information, and volunteered to participate in the study.

Preparation and intake of Herbal Solution

The ingredients of FHWB were used in proportionate ratio and converted into a final consumable form by adding distilled water. A volume of 15 ml of FHWB was dissolved to a final volume of 500 ml with water and patients should take all 500 ml solution in a day. Every patient should take 75 ml out of 500 ml final FHWB just before breakfast and another 75 ml just after breakfast. Similarly, 75 ml each just before and after lunch and another 75 ml each just before and after dinner and finally, 50 ml at bedtime every day for 180 days. All the patients were regularly monitored by the physician during the study period of 180 days. The body weight, body mass index and blood pressure of patients were noted and recorded.

Experimental procedures

A total of 45 Type 2 Diabetes Mellitus patients (21 males and 24 females) were selected with an age group ranged from 35-55 years with fasting blood glucose levels between 150-250 mg/dl besides surviving with standard medication/s during clinical trial study for 6 months (180 days). Inclusion and exclusion criteria were listed in table 1. As per the medication/s profile: they have been taking either Metformin (11 out of 45) or Metformin with Gliclazide (17/45) or with Glimepiride (15/45) or with Vildagliptin (2/45). The total 5 phases of clinical trials are Phase 0- Screening Visit 0 (day -3 to day 0); Phase 1- Baseline Visit I (day 0); Phase 2- Initial follow-up Visit II (day 1 to day 30); Phase 3- Mid follow-up Visit III (day 1 to day 90); and Phase 4- End of Study Visit IV (on day 180).

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

Inclusion criteria	Exclusion criteria
• Male/Females subjects between 35-55 years 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 fasting blood glucose levels between 150-250 mg/dl • Subjects who are diabetic for one year with standard Diabetic medication • Subjects with HbA1c Levels of 8%-10%	• Contra-indications or hypersensitivity to study product, History of medical condition/disease according to the discretion of the Investigator • No contra-indications to exercise as outlined by the American Endocrine Society, pregnant women and patients with Chronic Cardiovascular diseases. • Patients with Fasting glucose levels more than 250 mg/dl, diabetes-associated retinopathy, nephropathy and neuropathy. • Subjects having a history of high alcohol intake (>2 standard drinks per day), abnormal findings on complete blood count, and HIV positive • Subjects having a history of psychiatric disorder that may impair the ability of subjects to provide written informed consent, Subjects participated in any clinical study within thirty (30) days before screening • Any other condition that, in the opinion of the investigator, would adversely affect the subject's ability to complete the study or its measures

Screening Tests

The following tests were performed for all the subjects on, very first day before treatment and hence, designated as 0 day, and on day 30, day 90, and on 180th – the last day of the study, and reports were recorded for: blood sugar levels: both fasting (FBS) and postprandial blood sugar (PPBS) levels were estimated by glucose oxidase and peroxidase method.[45] HbA1c was separated and estimated by high-performance liquid chromatography method[46] using a Bio-Rad variant-II turbo instrument.

Liver function tests: Total bilirubin was estimated by vanadate oxidation[47] method. Alkaline phosphate was assayed as per modified International Federation of Clinical Chemists (IFCC) method.[48] Aspartate[49] and alanine[50] transaminase activities were also assayed as per the methods referred.

Renal function tests: Creatinine amidohydrolase was used to measure serum creatinine.[51] Uric acid was assayed using uricase-peroxidase method.[52]

Lipid profile: Total cholesterol was assayed using cholesterol oxidase and peroxidase Trinder CHOD/POD End Point method.[53] Triglycerides assay was performed by an enzymatic colorimetric method; HDL and LDL were assayed as per the methods referred.[53]

The complete blood count was estimated by spectrophotometric method in an automated analyzer. Urine pregnancy rapid test device is used for the detection of human chorionic gonadotropin (hCG) in urine specimens for women.[54]

Scheduled activity that was followed for all the 45 participants of clinical trial with FHWB was listed in table 2.

Table 2: Scheduled activity that was followed for all the participants of clinical trial with FHWB. Except for fasting, postprandial blood sugars and HbA1c, other parameters studied didn't show any significant difference from the corresponding values of 0 day (visit I).

Scheduled Activity	Screening Visit 0 (Day -3 to-1)	Visit I (Day 0)	Visit II (Day 30±3)	Visit III (Day 90±3)	End of Study Visit IV (Day 180±3)
Written Informed Consent	X	-	-	-	-
Demography and Medical History	X				
Vital Signs and Clinical Examination	X	X	X	X	X
Blood tests for Hb, TLC, DLCs, PLC, ESR	X	-	-	-	X
Liver and kidney function tests (Serum AST, ALT, and creatinine)	X	-	-	-	X
Glycated hemoglobin - HbA1c	X	-		X	X
Fasting and postprandial blood sugars	X	-	X	X	X
Lipid profile	X	-			X
Routine Urine Examination including Urine pregnancy tests for female and ECG at screening	X	-	-	-	X
Eligibility criteria assessments	X	-	-	-	-
Questionnaires	-	X	X	X	X
Concomitant medication record	-	X	X	X	X
Dispense Study Medication	-	X	X	X	
Adverse Event record	-	X	X	X	X

RESULTS

Preclinical studies

Results of oral acute toxicity study (for 14 days) showed that functional herbal water blend (FHWB) did not produce any mortality, behavioral changes and no signs of toxicity were observed up to the dose of 2000 mg/kg in rats including the confirmatory repeat with high dose. Hence, under the classifications of GHS, it is labeled as category 5 which may have

LD_{50} in the range of 2000- 5000 mg/kg bw and is safe for human consumption and is classified as generally regarded as safe (GRAS).

In repeated dose (sub-acute) 28-day oral toxicity study, FHWB did not produce any signs of toxicity or any changes in behavioral parameters as shown in table 3.

Table 3: Morbidity and mortality summary of animals following sub-acute toxicity study. No morbidity or mortality was observed in either sex, at any of the doses administered for 28 days (groups 1-4) including the recovery group of animals (groups 5 & 6) up to 42 days.

Group (5 animals/group)	**Day of Observation**				
	1	**8**	**15**	**22**	**28**
Male					
G1- Vehicle control	1	1	1	1	1
G2-Low-dose	1	1	1	1	1
G3-Mid-dose	1	1	1	1	1
G4-High-dose	1	1	1	1	1
Female					
G1- Vehicle control	1	1	1	1	1
G2-Low-dose	1	1	1	1	1
G3-Mid-dose	1	1	1	1	1
G4-High-dose	1	1	1	1	1

Note: 1- Normal

Group (5 animals/group)	**Day of Observation**						
	1	**8**	**15**	**22**	**29**	**36**	**42**
Male							
G5-Vehicle control recovery	1	1	1	1	1	1	1
G6-High-dose recovery	1	1	1	1	1	1	1
Female							
G5-Vehicle control recovery	1	1	1	1	1	1	1
G6-High-dose recovery	1	1	1	1	1	1	1

Note: 1- Normal

It did not affect cellular as well as non-cellular aspects of the blood studied. Serum biochemical profile and histological study clearly indicated that the test formulation is not likely to produce any serious changes at the highest dose studied (400 mg/kg bw) including the recovery study group after discontinuation of the FHWB. Hence, 400 mg/kg bw can be considered as NOAEL (No-observed adverse effect level) in rats.

Interspecies allometric scaling for dose conversion from animal to human studies is one of the most critical areas in clinical pharmacology. Allometric approach considers the differences in body surface area, which is associated with animal weight while extrapolating the doses of therapeutic agents among the species. This review provides information about translation of doses between species and estimation of starting dose for clinical trials using allometric scaling.[55]

Hence, based on the current results, the human equivalency of dose from rats sub-acute toxicity study was calculated to be 3.88 g/day for an average human of 60 kg bw (400 x 0.162 x 60 mg/day/adult).[56] This laid the basis for human clinical trial with FHWB for managing blood sugar which was considered safe for human consumption, especially in the safer range at 2.5 g/day in 15 ml of FHWB for managing blood sugar that was used in the clinical trials with 45 human subjects.

The data of diabetic parameters of 45 subjects (both males and females together) were presented in figure 1. With regards to mean FBS (figure1a), there was a gradual decrease following the consumption of FHWB. After 30 days, FBS showed a decrease of 12.6%, which was 20.6% by day 90, with 48% decrease at the final visit or 180 days. A similar trend was found with regards to PPBS (figure 1b) also. At day 30, mean PPBS was decreased by 11.4%, with 22.8% decline at day 90 and finally with a decrease of 44.3% at 180 days. Pertaining to HbA1c (figure 1c) as well, a 16.9% reduction (mean) at day 90 ended finally with 20.2% decrease at 180 days.

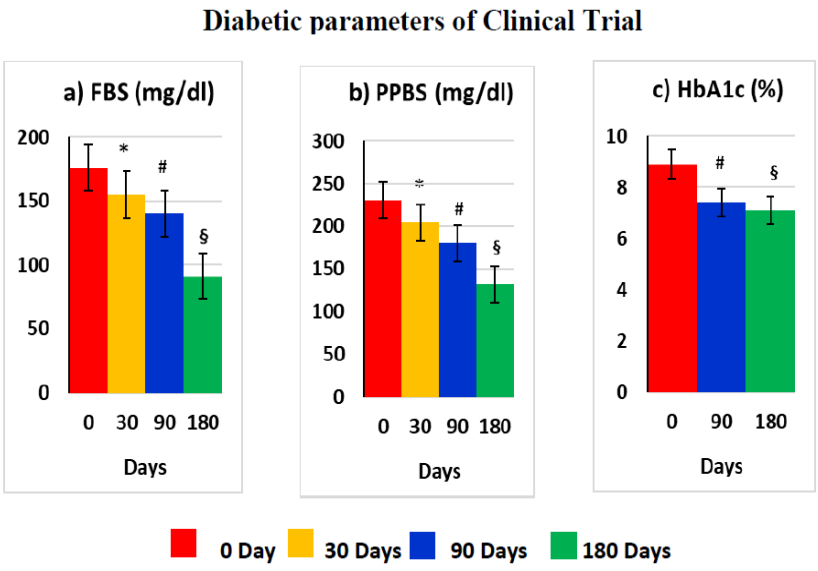

Figure 1: Diabetic parameters of clinical trial participants.

Values were expressed as Mean ± S.D. of 45 participants. (a) FBS, (b) PPBS and (c) HbA1c. The values that were highlighted with special symbols were statistically significant in comparison with the respective day 0 (visit I) values at least by * ($p< 10^{-5}$), # ($p< 10^{-17}$) and § ($p<10^{-31}$).

While the participants continued with their anti-diabetic medication/s during the trial period of 180 days as they have been doing before the trial, one might speculate whether there was any overlapping effect of medication/s with FHWB supplement. Hence, all the three diabetic parameters were tabulated as per each of the medication/s profile of the patients/participants in table 4.

Table 4: Summary of the Diabetic parameters (Means of FBS, PPBS and % HbA1c) of the 45 clinical trial participants based on the anti-diabetic medication/s profile. NT- Not tested. The effect of FHWB was found to be independent of the medication/s profile on diabetic parameters.

Medication used with Supplement	No. of Subjects	Day 0	Day 30	Day 90	Day 180	Mean % Difference Vs 0 Day		
						Day 30	Day 90	Day 180
		Mean FBS (mg/dl)						
Metformin	11	173	147	132	92	-15%	-24%	-47%
Metformin + Gliclazide	17	178	153	139	93	-14%	-22%	-48%
Metformin + Glimepiride	15	174	154	139	88	-11%	-20%	-49%
Metformin + Vildagliptin	2	174	176	168	92	1%	-3%	-47%
		Mean PPBS (mg/dl)						
Metformin	11	231	192	171	133	-17%	-26%	-42%
Metformin + Gliclazide	17	242	216	189	131	-11%	-22%	-46%
Metformin + Glimepiride	15	236	214	183	134	-9%	-22%	-43%
Metformin + Vildagliptin	2	224	215.5	201	132	-4%	-10%	-41%
		Mean % HbA1c						
Metformin	11	8.6	NT	7.0	6.8	NT	-18%	-21%
Metformin + Gliclazide	17	9.1	NT	7.5	7.3	NT	-17%	-20%
Metformin + Glimepiride	15	8.9	NT	7.3	7.0	NT	-19%	-21%
Metformin + Vildagliptin	2	8.9	NT	8.4	8.2	NT	-6%	-8%

It is evident that the percentages decrease of all the 3 parameters studied compared to the corresponding 0 day, were found remarkably persistent, especially, more at days 90 and 180, irrespective of which combination of the medication was being used. However, the combination of Metformin + Vildagliptin, did not match the percentages of the other 3 medication profiles, in spite of showing significant decrease at 180 days compared to 90 days, which might be mostly due to the low sample number of 2. Hence, it can certainly be

claimed that the effect of FHWB that was observed during the 180 day period is independent of the medication/s profile of the participants, who have been using them for years before.

With regards to the 3 diabetic parameters, the minimal statistical significance found for PPBS was in the order of 10^{-6} (at 30 days) and for FBS, 10^{-12} (at 30 days) and for HbA1c, 10^{-15} (at 90 days). One may argue that very high number of subjects [58] might have contributed to such a very high level of statistical significance. However, as it could be tedious to present the individual data points at every visit of all the three parameters, we presented the individual data of 3 parameters of the final visit at 180 days for FBS, PPBS and HbA1c in figures- 2, 3 and 4 respectively, where quite high levels of statistical significance was observed in the orders of 10^{-60}, 10^{-40}, 10^{-31} respectively.

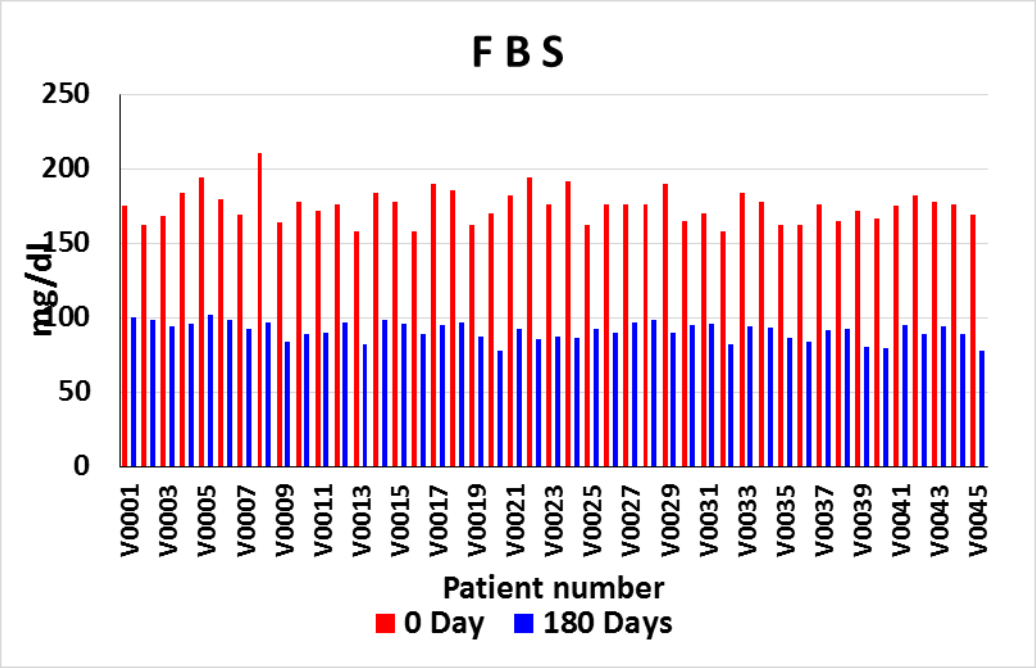

Figure 2: Individual fasting blood sugar (FBS-mg/dl) levels of 0 day Vs 180 days.

Fasting blood sugar (FBS- mg/dl) levels were presented in an individually comparative manner of 0 day (visit I) vs 180 days (final visit) for all the 45 subjects participated in current clinical trial with FHWB. Every individual showed significantly reduced FBS at 180 days compared to that of 0 day and no aberrations were noticed.

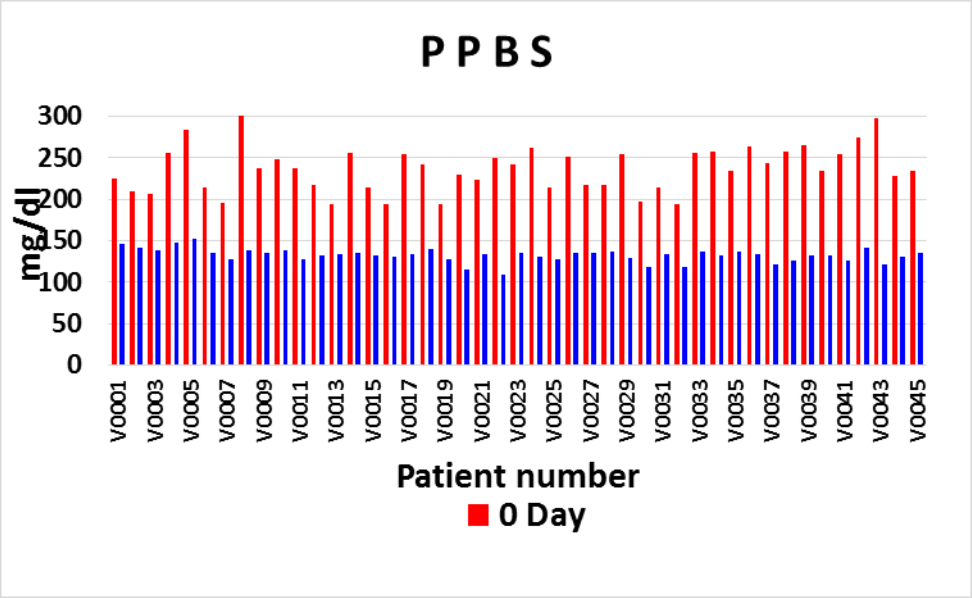

Figure 3: Individual postprandial blood sugar (PPBS-mg/dl) levels of 0 day Vs 180 days. Individual postprandial blood sugar (PPBS mg/dl) levels were presented in an individually comparative manner of visit I (0 day) vs final visit (180 days) for all the 45 subjects participated in the current clinical trial with FHWB. Every individual showed significantly reduced PPBS at 180 days compared to that of 0 day and no aberrations were noticed.

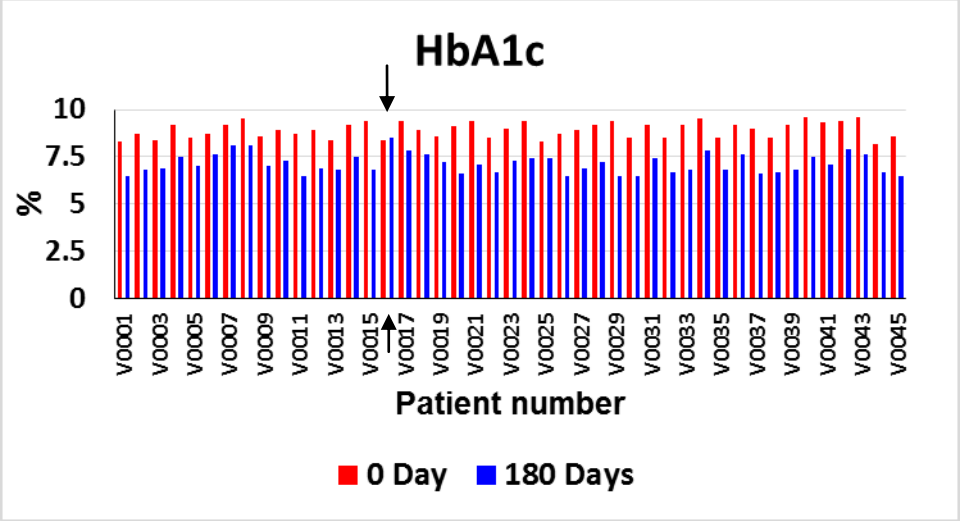

Figure 4: Individual HbA1c (%) levels of visit I (0 day) and final visit (180 days): Individual comparison of HbA1c (%) levels between I (0 day) and final visit (180 days) of all 45 subjects in the above figure shows that % HbA1c is appreciably reduced by the final visit when compared with that of visit 1, except for the number 16th individual indicated with arrows.

In spite of a very few individual aberrations existed, overall, majority of the subjects showed the improved effect of treatment and stayed close to the trends of FBS, PPBS and HbA1c presented in figure 1. To substantiate the same, the statistical summary of all the 3 diabetic parameters studied for all the 45 subjects participated in clinical trial for all the visits from I to IV were presented in table 5, such as: the maximum, the minimum, the range, the mean, standard deviation, median and mode along with the assessments of data distribution in terms of skewness, kurtosis and outlier determination. Ideally, for all of them, the mean and median values are almost similar and mode values were not far from them.

Table 5: Statistical summary of the 3 diabetic individual parameters (FBS, PPBS and HbA1c) of the clinical trial participants of all the visits.

Statistical Parameter	F B S (mg/dl)				P P B S (dl)				HbA1c (%)		
	0 Day	30 Day	90 Day	180 Day	0 Day	30 Day	90 Day	180 Day	0 Day	90 Day	180 Day
Maximum	210	192	179	102	302	298	242	152	9.6	8.7	8.5
Minimum	158	134	106	78	194	158	136	109	8.0	6.0	7.0
Range	52	58	73	24	108	140	106	43	1.6	2.7	1.5
Mean	175	153	139	91	237	210	183	132	8.9	7.4	7.1
Std Dev	11	14	14	6	27	27	19	8	0.4	0.6	0.5
Median	176	152	137	92	238	210	184	134	8.9	7.3	7.0
Mode	176	146	133	98	194	196	192	134	9.2	7.0	6.5
Coefficient of Variation	6.3	9.2	10.1	6.6	11.4	12.9	10.4	6.1	4.5	8.1	7.0
% Decraese compared to mean of 0 Day	0.0	12.6	20.6	48.0	0.0	11.4	22.8	44.3	0.0	16.9	20.2
Skewness	0.69*	0.78*	0.34	-0.40	0.26	0.69*	0.27	-0.45	-0.05	0.27	0.65*
Kurtosis	0.85	0.31	0.77	-0.57	-0.31	1.63	1.07	1.18	-1.35	-0.57	-0.25
Q1 (25 percentile)	165.5	146	130	86	215	192	168	128	8.5	6.9	6.7
Q3 (75 percentile)	184	163	146.5	96.5	256	225.5	195.5	137	9.3	7.8	7.5
Q3-Q1	18.5	41	17	33.5	16.5	27.5	10.5	9	0.8	0.9	0.8
1.5*IQR	27.75	61.5	25.5	50.25	24.75	41.25	15.75	13.5	1.1	1.4	1.2
Lower Outlier Fence	137.75	120.5	105.25	70.25	153.5	141.75	126.75	114.5	7.4	5.6	5.5
Upper Outlier Fence	211.75	188.5	171.25	112.25	317.5	275.75	236.75	150.5	10.4	9.2	8.7
Number of Outliers	0	1	1	0	0	1	1	2	0	0	0

Basically, Coefficient of Variation (CV) <10 is very good, and 10-20 is good, 20-30 is acceptable. The current data, with 4.5-12.9 range of CV, can be termed good to very good with regards to CV.

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 and if it is < -1 or $> +1$, the distribution is highly skewed and if it is between -1 and -0.5 or between $+0.5$ and $+1$, the distribution is moderately skewed.

However, if skewness is between −0.5 and +0.5, the distribution is approximately symmetric indicating even distribution on either side of the mean. In the current data, highlighted with * are moderately symmetric and the rest are all symmetric. (General acceptable range of skewness is -3 to +3).

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. That is, data sets with high kurtosis tend to have heavy tails, or outliers. Data sets with low kurtosis tend to have light tails, very few or lack of outliers. Appropriate Kurtosis range is -10 to +10).

- A **positive value** indicates heavy-tails (i.e., a lot of data in the tails).
- A **negative value hints** light-tails (i.e., little data in the tails).

So, while HbA1c at the end of day 180 was not different from starting values, it is possible this subject 16 may require a longer time frame to see lowering of HbA1c as the subjects' FBS and PPBS started showing significant lowering by day 180.

Indeed, the range of 52 (difference between the maximum and minimum) for FBS at visit I, increased to 58 and 73 at visits II and III respectively, but came down to 24 by final visit/180 days, a good indication for the effect of the supplement in the long term. Similarly, for PPBS, the 0 day/visit I range of 108, indeed, increased to 140 by visit II at 30 days, but gradually decreased to 106 and 43 for visits III/90 days and IV/ 180 days, respectively showing how profoundly effective was the supplementation of FHWB. With regards to HbA1c as well, the range value enhanced from a visit I/0 day value of 1.6 to 2.7 by visit III at 90 days, but came down to 1.5 by the final visit at 180 days. Hence, whatever the ranges of maximum and minimum were to start with at 0 day/visit I, all the 3 parameters mostly either decreased or stayed as such by the final visit/180 days, however, with a gradual but a sustained decrease in the mean values of all the 3 diabetic parameters, owing to the obvious efficacy of FHWB for managing blood sugar. These trends can be considered as other indicators that in the long term, the supplementation of FHWB can be much more effective, irrespective of the initial variations the patients may have.

In the current data, the distribution is mostly symmetric and only occasionally, it was moderately symmetric (table 5), which was evident by kurtosis as well. In the current data (table 5), very few kurtosis values, especially, those >1, indicating moderate tails hinting

outliers. Outliers are determined by Tukey [57] method using Interquartile range (IQR) and the lack of or very few outliers found are consistent with the data of kurtosis.

Very few aberrations that were noticed might be due to some other conditions, habits such as lack of exercise and diet control and/or the genetic makeup of those individuals concerned. Hence, it may be claimed that the profound effect of FHWB was evident among most of the individuals for all the 3 diabetic parameters studied especially, in the long term.

DISCUSSION

The hyperglycemic parameters that lead to as well to determine diabetes have been fasting (FBS) and postprandial blood sugar (PPBS) which remain high compared to a normal or a desired level. However, there has been a nagging issue about the reliability of these parameters based on the blood test results for quite some time. For example, a variety of factors, such as a short burst of physical activity just before a blood test for sugar, which can escalate blood sugar abruptly but temporarily, can raise a question about their dependence and hence, the reliability for decision making. In spite of this issue, these two parameters have been for a long time remained as the basic benchmarks to assess the hyperglycemic situation of an individual in the clinical set up. At the same time, there has been an ongoing search for another reliable and less varying parameter within a day such as FBS or PPBS and relatively more stable marker. Such a factor emerged out to be the glycated hemoglobin or HbA1c, which stays stable and reliable for at least a period of 3-4 months unlike FBS or PPBS that can vary within a day, and can be assessed drawing blood at any time of the day irrespective of fasting or not. The hyperglycemic condition leads to a situation where critical proteins of physiological importance such as hemoglobin are subjected to hyper or abnormal level of glycation following unattented/uninterfered hyperglycemia, and so, leads to lowered functional efficiency of those proteins. Glycated HbA1c has been considered more reliable and emerged out as a parameter to assess the hyperglycemic conditions in addition to FBS and PPBS. A measure of HbA1c at 6.5% is recommended as the cut off point for diagnosing diabetes. A value less than 6.5% does not exclude diabetes diagnosed using glucose tests and stringent quality measures have to be employed in estimating HbA1C.[58] In general, HbA1c and its corresponding blood sugar levels are considered to represent average blood sugar levels in contrast to FBS, and will be less than PPBS, which rises to the maximum possible after a meal (in a normal person) and 6.5% HbA1c corresponds to an average blood sugar level of 140 mg/dl.[59]

Indeed, more than the durability advantage to assess the hyperglycemic situation, HbA1c- the glycated hemoglobin has a very critical physiological role. In normal, healthy persons HbA1c ranging from 4 - 5.9%, facilitates normal delivery of oxygen to the variety of the tissues in the human body. However, during hyperglycemic/diabetic conditions, non-enzymatic glycation increases hemoglobin-oxygen affinity and reduces oxygen delivery to tissues by altering the structure and function of hemoglobin.[60] Any value of HbA1c >7, gradually increases affinity of hemoglobin with oxygen and reduces its ability to deliver it to the tissues and will reflect upon the health of the effected patient/person with diabetes in a wide variety of ways systemically. Hence, any drug/supplement that reduces HbA1c significantly, such as FHWB in the current report should be considered as a very useful one for improving the overall normal function of the tissues, especially in the diabetic patients, as it improves the oxygen delivering ability of hemoglobin reducing HbA1c levels, which is the major consequential effect which should not be overlooked and/or underestimated.

Other parameters

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 45 subjects - see table 2. For all these parameters, mostly, no difference was observed when compared to the corresponding visit I/0 day values at any visit until the final. If any, a moderate reduction in each of the lipid profile parameters was observed, however, they were found to be statistically not significant (data not shown).

Hence, the current regimen of 15 ml of FHWB (equivalent to 2.5 g/day) for managing blood sugar has been found to successfully lower the three critical diabetic parameters, such as FBS, PPBS and HbA1c in the human limited clinical trial with 45 subjects as per the criteria described above, independent of the medication/s profile that patients have been continuing to take during the trial period. Also, as per the acute and sub-acute toxicity studies with rats, it emerged out to be a safe one for human consumption as all other liver, renal functional tests and other blood parameters studied not showing any variations compared to the baseline. Therefore, 15 ml of FHWB could be considered as an effective adjuvant therapy in pre-diabetic and diabetic patients.

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