SUMMARY AND CONCLUSIONS

Diabetes poses great challenge to the world's health care system. The global prevalence of diabetes was 387 million in 2015. This number is projected to increase to 552 million by 2030, or 9.9% of adults (IDF Diabetes Atlas, 2015). In India this figure will rise from 61.3 to 101.2 million by 2030 (IDF Diabetes Atlas, 2015).

The present study focused on the anti-diabetic properties of the leaves of *Aegle Marmelos (L.) Correa* (Bael leaves). Several animal studies have focussed on the hypoglycaemic effect of *Aegle marmelos (L.) Correa* (Alam et al, 1990; Prakash, 1992; Rao, 1992; Ponnachan, Paulose and Panikar, 1993; Das et al., 1996; Sharma et al., 1996, Sachdeva et al., 2001; Upadhya et al., 2004). *Aegle marmelos* is also reported to have a high margin of drug safety and no short term toxicity (Miyazaki, 2007). Though the review is replete with extensive animal model studies and use of bael in ancient traditional Indian system of medicine, limited data is available on documented human clinical trial evidence to support these claims and to fill the gap in the existing knowledge, the present study was designed with broad and specific objectives.

Broad objective

To study the impact of *Aegle marmelos (L.) Correa* leaf juice supplementation in Type 2 Diabetes Mellitus subjects

Specific objectives

i. To study in-depth antioxidant profile, proximate composition, trace elements and heavy metals in the cultivated variety ('*Gomayasi*') and wild variety of *Aegle marmelos (L.) Correa* leaves

ii. To conduct knowledge, practice and use (KPU) of *Aegle Marmelos (L.) Correa* leaves with practitioners of Ayurveda and Naturopathy.

iii. To assess the impact of *Aegle Marmelos (L.) Correa* (Bael) leaf juice supplementation on blood sugar levels, lipid profile, liver and kidney functions of type II Diabetes subjects

This study was carried out in 3 phases

Ethical Clearance

The study was approved by ethical committee of Department of Foods and Nutrition, The Maharaja Sayajirao University of Baroda. The Ethical Clearance No. is IECHR/2013/20.

The details of methods and results of the 3 phases based on the objectives are mentioned below

Phase 1 In-Depth Antioxidant Profile Of Aegle Marmelos (L.) Correa Leaves

Methods

Samples for *'Gomayasi'* leaves of *Aegle Marmelos (L.) Correa* **(**a newly developed spineless variety of bael fruit), was procured from Central Horticultural Experiment Station (CIAH), Vejalpur (Godhra), Panchmahal, Gujarat, India. Sample for the wild variety was collected from the identified zone (21004'49.7"N 70035'10.6"E) of Gir forest, Talala, Gir Somnath, Gujarat, India. Standard methods were used to assess the proximate principles, mineral and heavy metal content, antioxidant activity, polyphenol content and individual phenol content.

Observations

a) Proximate Composition of *Aegle Marmelos (L.) Correa* Leaf

Total ash, moisture, protein, carbohydrate, fat and crude fiber content of wild variety of Gir and cultivated variety called *'Gomayashi*' was 6.5% and 6%; 53.9% and 52.1%; 7.6% and 2.2% ; 10.3% and 5.4%; 8.1% and 12.7% and 30.1% and 25.1% respectively. The nutritive value of wild variety was found to be better than cultivated variety *'Gomayasi'* particularly in proteins, carbohydrates and crude fibre.

b) Mineral Content in *Aegle Marmelos (L.) Correa* Leaf

Total iron (181; 165 ppm), manganese (62; 56 ppm), zinc (49; 38 ppm) and copper (12; 11 ppm) for wild and cultivated variety and many trace elements were detected in both the varieties when subjected to X-ray Flourescence instrument for mineral analysis.

Heavy Metal Analysis of *Aegle Marmelos (L.) Correa* Leaf

Heavy metals were analyzed through Atomic Absorption Spectrophotometer (AAS) and none of the metals like Cadmium, Arsenic, Lead and Mercury were detected in our study for both the varieties.

c) Antioxidant Capacity of *Aegle Marmelos (L.) Correa* Leaf

Free radical reducing power as assessed by FRAP was 14.65 µmol/l and 11.80µmol/l and IC50 value (DPPH) for wild and cultivated variety was 437 µg/ml and 620µg/ml respectively. Wild variety showed slightly higher reducing power (FRAP) and free radical inhibiting power (µg/ml DPPH) as compared to cultivated variety. The wild variety is having more antioxidant capacity than the cultivated one (437 µg/ml<620µg/ml).

d) Total and Individual Phenol Content of *Aegle Marmelos (L.) Correa* Leaf

Results for TPC of wild and cultivated *Aegle marmelos* leaf samples were 76 mg GAE/g and 65 mg GAE/g of dry extract which came to be 7.6% and 6.56% respectively. TPC of wild variety was more than cultivated variety.

HPLC analysis for quantification of individual polyphenol revealed the presence of Gallic acid, chlorogenic acid and Ferullic acid in wild variety whereas Gallic acid, Ferullic acid and pyrocatechol was found in cultivated variety.

Conclusions

Presence of important nutrients, trace elements, no heavy metals and rich source of antioxidant properties, total polyphenols, and individual phenols in both the varieties of *Aegle Marmelos (L.) Correa* leaf may be of value in managing diabetes and its associated conditions but wild variety being superior to cultivated variety was selected for supplementation in clinical trial.

Phase 2 To study the knowledge, practice and use of Aegle Marmelos (L.) Correa leaves with the practitioners of Ayurveda and Naturopathy in Gujarat

Methods

Key informant interviews were conducted with the help of semi-structured open-ended questionnaire in Gujarat for gathering data on knowledge, practice and use of *Aegle marmelos (L.) Correa* in various diseases and diabetes in particular from 10 practitioners each from Ayurveda and Naturopathy using snow ball sampling method based on their consent to be part of this study. Desk review on the same was done through

a) personal visits to Gujarat Agricultural Universities namely: 1) Junagadh Agricultural University (JAU), 2) Sardar Patel Agricultural University , 3) Anand Aromatic Plant Research Centre and 4) Jamnagar Ayurveda College Jamnagar 5) Bael Vejalpur Research Station CSIR Panchmahal Godhra; b.) Pub Med and other web sources

Observations

The Ayurveda practitioners used *Aegle marmelos (L.) Correa* leaves for the treatment of diabetes and the dosage for the treatment ranged from 5-10g/day in various forms such as powder, decoction and dry extract in the form of capsules (1 capsule contains 0.5mg of extract which is equivalent to 5mg of leaf powder). The Naturopathic practitioners used the *Aegle marmelos (L.) Correa* leaves for the treatment of diabetes and the dosage ranged from 20-40 fresh leaves in the form of juice mixed with water to make a volume of around 100ml.

Several Ayurvedic formulations were available commercially in the market of various reputed brands such as Dabur, Himalaya, Oshidhi, Patanjali etc. selling *Aegle Marmelos (L.) Correa* leaves in the form of various powder, tablets, capsules and these formulations were selling the products and drugs in poly herbal formulations (PHF) rather than single drug.

Conclusions

Both Ayurveda and naturaopathy practitioners used the AM leaves for treatment of diabetic subjects. However, since availability of fresh AM leaves was not a problem, based on the Naturopathic practitioners, 10-30 g of fresh *Aegle marmelos* leaves were selected for the standardization of various doses of *Aegle marmelos* juice prepared using 10g, 20g and 30g leaves for making 100ml juice which were subjected to sensory evaluation to type II diabetic subjects, to be used for the clinical human trials in the next phase.

Phase 3 To assess the impact of Aegle Marmelos (L.) Correa leaf juice supplementation on blood sugar levels, lipid profile, liver and kidney functions of type II diabetes subjects.

Methods

Selection of the dosage of supplementation of *Aegle marmelos (L.) Correa* fresh leaf juice for the clinical trial

Aegle Marmelos (L.) Correa fresh leaf juice (bael) (100ml) was standardized using varying amounts of leaves (10, 20, 30g) and was subjected to sensory evaluation (9-point hedonic scale) to a panel of semi trained 30 T2DM subjects (42-69 y; F-11:M - 19). The mean scores obtained for the coded Sample A, Sample B and Sample C were 6.8±1.25, 7.28±1.51 and 6.68±2.13 respectively. Thus, *Aegle Marmelos (L.) Correa* leaf juice made from 20 g leaves in 100ml water, was selected for the supplementation in clinical trial.

Methods For Supplementation of *Aegle marmelos (L.) Correa* fresh leaf juice for the clinical trial

Sixty confirmed T2DM subjects from a private hospital in Veraval city, dist Gir Somnath, Gujarat, India who gave their written consent for the study were selected. Subjects were enrolled having FBS> 125mg/dl and HbA1c > 7% on oral hypoglycaemic drugs with regular follow-up in the hospital and were randomly divided into two groups, Experimental group (n=30) and Control group (n=30).

Baseline Information were collected from both the groups regarding general information, medical history using pre-tested structured questionnaire, their health related quality of life (using QOL questionnaire-MDRTC), anthropometry (Weight, BMI), dietary intake (24 Hr dietary Recall Method and Food Frequency Questionnaire). Their biochemical parameters (Glycemic and lipid profile, serum creatinine, total proteins, HbA1c, Hs-CRP, SGOT, SGPT and serum FRAP) were estimated. Biochemical analysis (Pre and Post analysis) was performed by private local laboratory in Veraval city.

For the experimental group (n=30), 3000ml (100ml/subject) fresh juice was prepared daily using 600g bael leaves (20g/subject) using a kitchen mixer grinder and served daily between 7:45 am to 8:15 am in a spiritual center at Veraval city for a period of 60 days. During study period none of the subjects took other complementary or alternative medicine.

Impact Analysis (Post Data) Post data were collected after 60 days with respect to same parameters as pre data.

Observations

Baseline information of the subjects (SES, anthropometric, bio-physical and bio-chemical parameters)

The mean age of the subjects was 51 years (M-50.80±9.13y; F-51.74±7.52y). Ninety four percent were Hindus. Thirty percent of the total subjects were illiterate and average family size was 5.6 members. Average monthly family income was between Rs 3000-25000. Twenty five percent of the total subjects were obese BMI≥30). About 31 % (F 18 %; M 13 %) were hypertensive with 40% of diabetic subjects having strong family history of diabetes mellitus. Twenty four hour dietary recall revealed that there was no significant difference between the mean nutrient intake between ($p>0.05$) males and females. The calories coming from carbohydrates, protein and fat was found to be 54%, 12% and 34% respectively. Food frequency questionnaire revealed that the mean intake of the fruits was once in a week (45%) which reflected poor intake of protective foods. However, there was no significant difference among males and females ($p>0.05$) in the baseline mean±SD values for all the biochemical parameters of the screened type II diabetes mellitus subjects.

Impact Of *Aegle Marmelos (L.) Correa* leaf juice supplementation on Experimental group

Impact on diet, bio-physical and anthropometric profile

There was no significant statistical difference in the mean macronutrients like energy, fat, carbohydrate, proteins and micronutrients like minerals and vitamins intake of the male and female subjects of Experimental group but in Control group there was significant statistical difference ($p < 0.05$) in the mean pre and post macronutrients intake like energy, fat, carbohydrate and proteins intake of the male and female subjects. There was significant decrease in weight ($p<0.05$) and BMI ($p<0.05$) and body fat ($p<0.001$) in the subjects of Experimental group. Whereas no such trend was recorded in Control group. There was significant decrease in systolic blood pressure ($p<0.001$) (5-6 %) and DBP (5.3%) (Pre-post Expt./Control Group SBP values: 138.8±19.1mmHg- 129.8±19.0mmHg/134.3±20.2-127.1±18.0) and (Pre-post DBP mean±SD values: 86.6±10.0mmHg/82.0±11.6 mmHg).

Impact of *Aegle Marmelos (L.) Correa* leaf juice supplementation on the biochemical parameters of the Experimental group

***Aegle Marmelos (L.) Correa* leaf juice supplementation resulted in remarkable reduction in the following biochemical parameters:-**

FBS 20 % (Expt. group: Mean pre/post FBS values- [174.7±41/140.1±46.3; Cont. group (15% increase): 168.3±37.3/193.7±41.5], HbA1c 20% [Expt. group: Mean pre/post HbA1c values- 9.8±1.2/7.9±1.1; Cont. group (7% increase): 8.5±1.1/9.1±1.1], LDL 15% [Expt. group: Mean pre/post LDL values- 140.7±41.9/119.7±34.7; Cont. group (7.5% increase): 120.6±32.3/129.7±28.3], TG 10.9% [Expt. group: Mean pre/post TG values- 152.8±44.5/137.4±42.2; Cont. group (9.6% increase): 144.9±4.8/158.9±41.1], VLDL 12.6% [Expt. group: Mean pre/post VLDL values- 31.0±10.0/27.2±8.3; Cont. group (11.2% increase): 28.4±8.1/31.6±8.6], Serum FRAP 17.7% rise [Expt. group: Mean pre/post serum FRAP values- 1.6±0.3/1.9±0.3; Cont. group (15% drop): 168.3±37.3/193.7±41.5], SGPT 13% [Expt. group: Mean pre/post SGPT values- 25.3±7.8/21.7±5.7; Cont. group (15% increase): 25.9±8.9/29.8±8.5], SGOT 19% Expt. group: Mean pre/post SGOT values- 29.0±9.9/23.3±8.6; Cont. group (14.4% increase): 26.3±7.9/30.1±9.8].
Maximum difference was noted in Glycemic profile i.e. FBS, HbA1c, PPBS and serum antioxidant (Serum FRAP) values, lipid profile like TG, LDL and VLDL values and liver enzymes (SGPT and SGOT) while minimum difference was recorded in serum creatinine and HsCRP values. There was no difference in total proteins values post supplementation.

Conclusions

The findings of the present study indicated that supplementation of 20 g *Aegle Marmelos (L.) Correa* (bael) leaf juice supplementation had beneficial impact on blood sugar values and lipid profile as well as liver functions and it can be supplemented along with oral hypoglycaemic drugs to keep the above parameters in control.