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METHODOLOGY

The methodology pertaining to the present research entitled "**Hypoglycemic effect of bitter gourd (*Momordica charantia L.*) on prediabetics and Type II diabetics**" is presented under the following headings:

PHASE I - Baseline survey on consumption pattern of bitter gourd

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B. Selection of Type II diabetics
C. Formulation of tool and collection of baseline data

PHASE II - Development and standardization of bitter gourd recipes

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C. Assessment of nutritional status of the selected prediabetics
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PHASE I - Baseline survey on consumption pattern of bitter gourd

A. Selection of locale

The present study was conducted in the diabetic clinics in and around Coimbatore city, due to easy accessibility and availability of sufficient diabetics visiting the hospital periodically and also the willingness to co-operate for the study. The Doctors and dietitians were interested in the research work related to dietary management of diabetes. The diabetologists were much interested in research work related to food-based approach for the management of diabetes. Also these hospitals had well equipped clinical laboratory of their own for blood analysis needed for the study.

B. Selection of Type II diabetics

Purposive sampling, one of the most common sampling strategies which group, participants according to preselected criteria relevant to a particular research question (Santha *et al.*, 2015). In this sampling method, subjects were selected carefully based on the study purpose with the expectation that each participant will provide exclusive and value rich information required for the study. As a result, members of the accessible population who were not interchangeable were selected and sample size is determined by data saturation (Suen *et al.*, 2014). Based on their willingness to participate in the study, about 332 Type II diabetic subjects of both male and female belonging to the age group of 30 - 65 years were selected by purposive sampling method.

C. Formulation of tool and collection of baseline data

A well structured interview schedule (Appendix I) was framed and pre tested to elicit information about age, sex, composition of the family, educational status, occupation, dietary pattern, exercise pattern and life style habits. Awareness of the subjects about the control and management of diabetes, knowledge and use of bitter gourd was also obtained through the interview schedule from all the selected 332 diabetics.

Anthropometric measurements namely height and weight were recorded and Body Mass Index (BMI), Waist Circumference (WC), Waist to Height Ratio (WHtR)

and Conicity Index(CI) were calculated for all the surveyed Type II diabetics. Fasting, post prandial blood glucose levels, glycosylated haemoglobin levels and lipid profile values were also noted from the medical reports at the time of personal interview.

PHASE II - Development and standardization of bitter gourd recipes

Momordica charantia, is extensively cultivated in Asia, Africa and South America and widely used in folk remedy for diabetes, specifically in India, China and Central America (Grover *et al.,* 2002). The active components present in bitter gourd includes vicine, charatin and polypeptide P thought to be structurally similar to human insulin. Although bitter gourd has many culinary uses, especially in South East and East Asia, it is used extensively in folk medicine (Upadhyay, 2015). Bitter gourds are cooked with other vegetables, stuffed, stir fried or added in smaller quantities to beans and soups to provide a slightly bitter flavor. However, for most recipe preparations, bitter gourd are blanched, parboiled or soaked in salt water before cooking to reduce the bitterness (Behera *et al*., 2010).

In general, vegetables are prepared at home based on the convenience and taste preference rather than retention of nutrients and health-promoting compounds (Masrizal *et al.,* 1997). The cooking method, duration of cooking and temperature induce significant changes in the composition, affecting the bioavailability and the content of chemo preventive compounds in vegetables. Hence, the common domestic cooking methods like boiling, pan frying or stir frying and braising for 5 to 15 minutes, compared with raw bitter gourd were used to evaluate the effect on antioxidant content using ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) method.

A. Training at AVRDC-The World Vegetable Center, Taiwan

This clinical trial is part of an International project entitled “A better bitter gourd: Exploiting bitter gourd (*Momordica charantia L*.) to increase incomes, manage Type II diabetes and promote health in developing countries” funded by the Federal Ministry of Economic Cooperation and Development (Germany) and monitored by AVRDC-The World Vegetable Center, Taiwan. The investigator was appointed as

Research Assistant in the project and due approval was obtained from the project manager and other funding authorities for using the project data for Ph.D research work.

During August to October 2012, the investigator underwent training at Nutrition laboratory in AVRDC-The World Vegetable Center, Shanhua, Taiwan. Investigator developed and standardized several bitter gourd recipes to popularize the same among the diabetics and other population. In addition, the investigator worked on the antioxidant properties of bitter gourd samples prepared using different methods of cooking common in India.

a. Bitter gourd sample preparation and extraction protocol

. The following schematic diagram represents the bitter gourd sample preparation protocol (Figure 1).

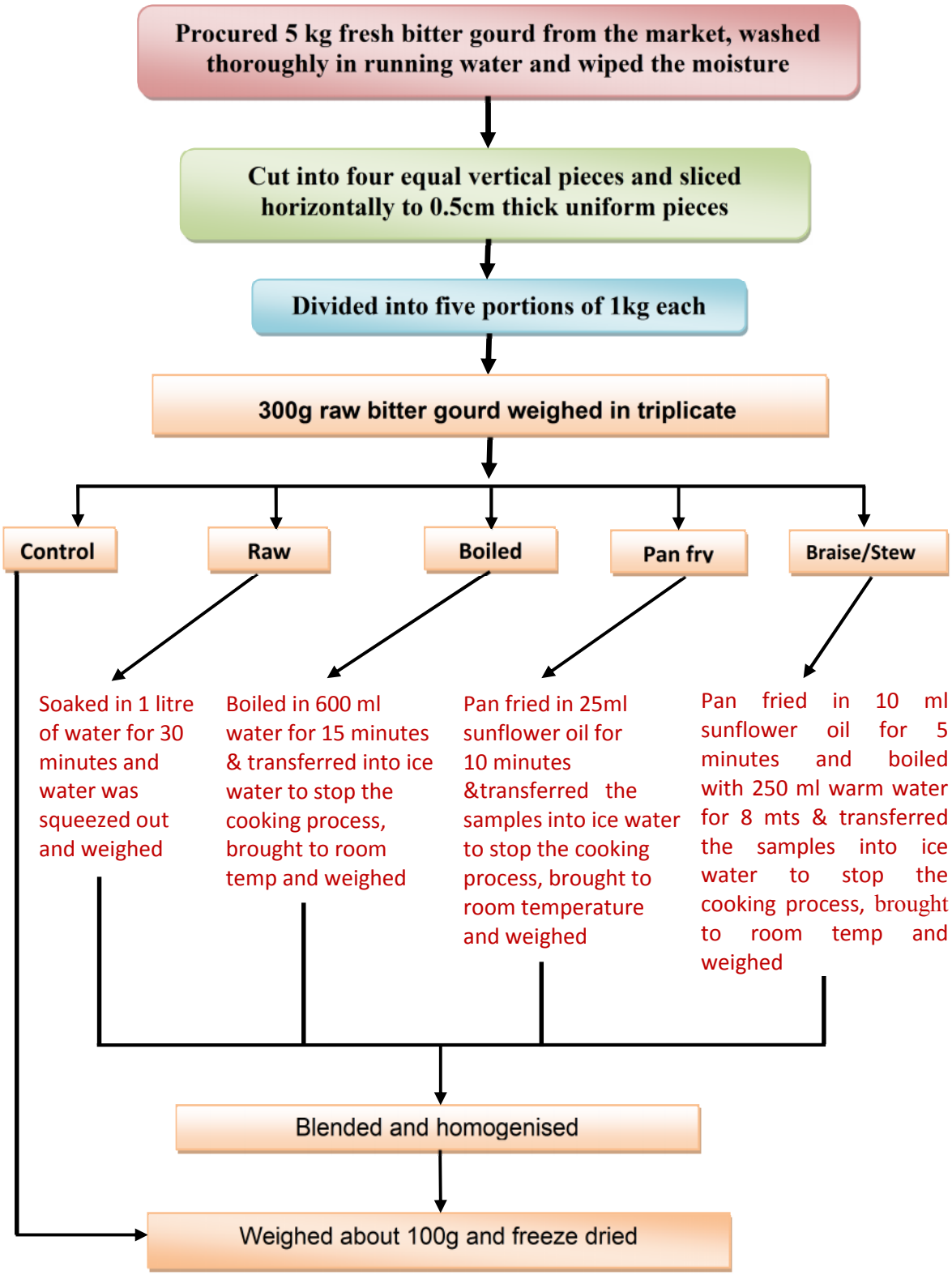


FIGURE 1. BITTER GOURD SAMPLE PREPARATION WITH DIFFERENT COOKING METHODS

b. Extraction protocol

0.2 g of freeze dried bitter gourd powder was mixed with 19.8 ml methanol (1:99). The solution was shaken in the dark for two hours at 10000 rpm at 25°C using a Wisdom® Orbital Shaker Incubator 501R for 2 hrs and then centrifuged at 10000 rpm for 10 minutes and the supernatant was collected and stored at - 20º C for antioxidant analysis.

c. Analysis of total antioxidant activity

Bitter gourd extracts possess potent antioxidant and free radical scavenging activities which could be derived from compounds such as flavonoids and phenols. These antioxidant activities could have contributed to the therapeutic benefits of certain traditional claims of wild bitter gourd (Wu and Ng, 2010) and may be directly or indirectly responsible for its hypoglycemic property. ABTS method, a frequently used assay to measure the antioxidant capacities of foods (Arnao *et al*., 2001) was used with slight modifications (Yang *et al*., 2006). Bitter gourd extract 20 µL with 1mM ABTS, 0.03 per cent H_2O_2, 0.25 µM horseradish peroxidase was taken in 50 mm sodium phosphate buffer at pH 7.5 and made up to 2 ml in the assay temperature of 25ºC.

The reaction was monitored until stable in 730 mm and then aqueous phase added. The decrease in absorbance, which is proportional to the ABTS radical cation quenched, was determined after 5 minutes. The TEAC (Trolex Equivalent Antioxidant Capacity) value is expressed as µmol TE g−1. The test was done with triplicates and the values were recorded for the bitter gourd samples.

B. Development and organoleptic evaluation of bitter gourd recipes

Around 15 different bitter gourd recipes were prepared using the most common Indian cooking methods namely raw, boiling, pan fry, stir fry, braising and microwave cooking (Table II) and standardized.

Sensory evaluation provides an index of overall acceptability of foodstuffs which depends on its appearance, flavor, taste, texture, aftertaste and overall acceptability. To ensure the acceptability of the modified recipes, they were subjected to evaluation of composite scoring for their sensory qualities. All recipes were prepared fresh in the food laboratory in the Department of Food Science and Nutrition, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore and presented to a panel of 10 each of non diabetic and Type II diabetic adult taste panel members. Three trials of organoleptic evaluation was conducted for all the standardized 15 recipes using nine point hedonic rating scale.

TABLE II

BITTER GOURD RECIPES PREPARED USING DIFFERENT COOKING METHODS

Cooking methods	Recipes
Raw	Amla bitter gourd juice Bitter gourd juice
Boiling	Bitter gourd soup Bitter gourd lentil soup
Pan frying	Stuffed karela Karela roll Bitter gourd finger chips
Stir frying	Bitter gourd fried rice Bitter gourd noodles Bitter gourd pachadi
Braising	Karela tomato curry Bitter gourd mixed vegetable curry Bitter gourd paruppusili
Micro wave	Crispy karela Bitter gourd dhal powder

C. Crafting the bitter gourd recipe book

To promote the better and frequent usage of bitter gourd in Indian cuisine a bitter gourd recipe book was developed. As lifestyle and eating habits in India have been changing in the recent decades, cases of Type II diabetes have been increasing at a fast pace, While dietary management is an essential component of managing diabetes, the project focused on developing healthy and nutritious bitter gourd recipes for Indian populations. The recipes were designed to suit local food preparation methods, ingredients, materials and taste while modified for reduced sugar, salt and oil contents and cooking time. Each dish has been carefully planned and taste tested to ensure delicious and easy to prepare dishes that can delight the palate of everyone in the family. From soups to main dishes, appetizers to snacks, there were recipes suitable for everyday meal.

This recipe book was developed to promote the usage of bitter gourd in Indian cuisine. The recipes follow healthy dietary guidelines and incorporate bitter gourd in everyday dishes. The recipe book was published in English as well as one of the Indian local language Tamil (Annexure 2) by the investigator. The training ceretificate of the investigator from AVRDC, The World Vegetable Center is given Annexure 3

PHASE III - Screening of prediabetics and data collection

A. Selection of area for screening the prediabetics

Before developing Type II diabetes, majority have "prediabetes" stage in which the blood sugar levels are higher than normal but not yet high enough to be diagnosed as diabetes, with no clear symptoms of prediabetes. Hence healthy volunteers (office administrators, employees, trainees and authorities) between the age of 25-60 years were selected from Avinashilingam Jan Shikshan Sansthan, a Central Government funded center situated half a km from Avinashilingam University for Women and from the following five private industries for screening the prediabetic subjects:

- Sharp Electrodes and Trendys, Kalappati
- Sharp Tools , Kalappati

- Best Engineering Pvt Ltd, Kovilmedu,
- Enbest Pumps India Pvt Ltd, Somayapalayam and
- Gowri Metals, TVS Nagar

The authorities in these organizations were health conscious, aware of physical fitness, provided nutritious working lunch for their employees and hence allotted time in working hours for conduct of the study. In addition, the administrators rendered excellent cooperation for screening all their employees by permitting the subjects for clinical trial, distributing the supplements weekly and also sparing time to collect blood samples every week, which facilitated the smooth conduct of the clinical trial.

B. Screening using Indian Diabetic Risk Score (IDRS)

The study design, duration of the study and expected outcome of the study was explained and oriented to all the 948 participants by a formal meeting in their work premises itself. This was arranged with the support of the authorities of concerned industries and informed written consent was obtained from all the participants before the start of the study. Informed consent is one of the most important tools for ensuring respect for persons during research and understand what it means to participate in a particular research study so participants can decide in conscious, deliberate way whether he/she want to participate (ICMR, 2006).

The IDRS has a sensitivity of 72.5 per cent and specificity of 60.1 per cent and is derived based on the largest population based study on diabetes in India CURES (Joshi, 2005). IDRS is a simple, very low-cost screening tool developed by Mohan *et al.* (2005) having multiple potential applications in clinical and epidemiological settings in India and was used to identify the prediabetics in the study. The screening of prediabetics was done using the Indian Diabetic Risk Score (IDRS-Table III).

TABLE III

INDIAN DIABETES RISK SCORE*

Criteria Assessed	
Age (Yrs)	Std. Points
<35	0
35-49	20
≥50	30
Waist circumference (cm)	
Waist <80(female),<90 (male)	0
Waist 80-89 (female), 90 -99 (male)	10
Waist ≥90(female),≥100 (male)	20
Physical activity	
Vigorous regular exercise or manual work at home/work	0
Moderate regular exercise or physical activity at home/work	10
Mild regular exercise or physical activity at home/work	20
No exercise and sedentary activities at home/work	30
Family history of diabetes	
No diabetes in parents	0
One parent diabetic	10
Both parents diabetic	20

* Mohan *et al.,* (2005)

Subjects with an IDRS risk points <30 were categorized as low risk, between 30 and 50 points as medium risk and those with ≥60 points are high risk for developing diabetes. Details of the screened participants (N-948) for the clinical trial are presented in Table IV.

TABLE IV

CLASSIFICATION OF SCREENED SUBJECTS AS PER IDRS

Details	Low risk vulnerabilities		Medium risk vulnerabilities		High risk vulnerabilities		Total	
	No	%	No	%	No	%	No	%
Male	14	2.6	337	62.4	189	35	540	57
Female	4	1	256	62.7	148	36.3	408	43
Total	18	1.9	593	62.6	337	35.5	948	100

Among a total of 948 subjects screened, 540 (57 per cent) were males and 408 (43 per cent) were females. About 35 percent male and 36.3 percent female subject, were having an IDRS risk score of ≥ 60 and were categorized as subjects with high risk vulnerabilities. Higher percentages (62.6 %) of the subjects were in the medium risk and only 1.9 per cent of subjects were in low risk catogery.

All the subjects with high risk vulnerabilities were further tested for their Fasting Blood Glucose (FBG) level to confirm prediabetic status. The fasting blood glucose was analysed from capillary blood after 12 hours fasting with Accucheck active glucometer (Roche Diagnostics, Germany) and the blood glucose values were recorded. Breakfast was provided for all the participants involved in FBG screening. Table V reveals the distribution of screened subjects as per blood glucose level.

TABLE V

DISTRIBUTION OF SCREENED SUBJECTS AS PER BLOOD GLUCOSE LEVEL

Sex	Normal subjects		Prediabetics		Diabetics		Total
	No	%	No	%	No	%	No
Male	113	59.7	64	33.8	12	6.34	189
Female	92	62.2	56	37.8	0	0	148
Total	205	60.8	120	35.6	12	3.6	337

From the high risk vulnerabilities group, around 60.8 per cent of the subjects were having desirable blood glucose level of <100 mg/dl. About 120 (35.6 %) subjects(64 male and 56 female) had fasting blood glucose level ranging between 100-125 mg/dl and identified as prediabetics as per American Diabetes Association (2013). Around 12 subjects who were not diagnosed before as diabetics were also identified during the screening and adviced to monitor their blood glucose level further with a diabetologist and confirm their status.

C. Socio-economic profile of the prediabetics

Among the 120 prediabetics identified, based on the willingness and ready to give written consent, 90 prediabetics (male 53 and female 37) were selected from various venue to participate in the clinical trial. Same pre tested interview schedule with slight modification was administrated to collect the demographic profile namely age, sex, education, occupation, family income, area of residence and behavioral measures such as dietary habits, meal pattern, use of tobacco, consumption of oil, physical activity and family history of diabetes of the 90 prediabetics.

PHASE IV - Conduct of clinical trial on prediabetics

The study design was registered in the Clinical Trial Registry of India (CTRI), Indian Council of Medical Research (ICMR). CTRI registration number is CTRI/2013/06/003759. To conduct the clinical trial, the methodology of the present study was presented before the Institutional Human Ethical Committee (IHEC) of Avinashilingam University and the approval number AUW/IHEC-2013 AP-01 was obtained.

A. Preparation of the supplement for clinical trial

Within the same International project, an animal study conducted with forty mice revealed that bitter gourd treated mice groups had significantly lower mean fasting blood glucose and HbA1c levels than the placebo treated mice.

The daily dosage of 2.5 g freeze dried bitter gourd powder (50 g fresh equivalent) was calculated according to Human Equivalent Dosage (HED) by the Center for Drug Evaluation and Research (CDER 2005). According to this, a dosage

of 2.5 g in human is equivalent to 461.61 mg/kg of body weight in mice, assuming a body weight of 70 kg for humans and 30 g for mice:

HED = animal dose in mg/kg x (animal weight in kg / human weight in kg) x 0.33

In an animal trial, a dosage of 500 mg bitter gourd powder per kg body weight was tested which improved insulin sensitivity in high fat diet fed mice and is equivalent to 2.5 g per day in humans. A daily dosage of up to 4.8 g per day has been proven to be safe for humans (Tsai *et al.,* 2012).

On the basis of the animal trial conducted, the supplement was prepared for human feeding trial also. The bitter gourd variety NS1020 was grown and harvested at AVRDC – The World Vegetable Centre in Taiwan. Fresh bitter gourd were washed with clean water and then dipped into water containing 1-2% hydrogen peroxide. After washing the whole fruits, they were chopped, freeze dried and ground to fine powder (80mesh). To mask the bitter taste of the bitter gourd, alpha-cyclodextrin and 0.75 g cucumber powder was added to 2.5 g bitter gourd powder in the bitter gourd sachets named as Mary. Both bitter gourd and placebo sachets contained 1.5 g alpha-cyclodextrin with strawberry flavor, equivalent to 1% (w/v) of the final drink preparation, which is approved by the Food and Drug Administration (FDA) as a stabilizer for flavors in food. The placebo sachets named John consisted of 3.25 g of raw cucumber powder. Processing of the fruits was done by “Challenge Bioproducts Co., LTD, Taiwan” and packing the powders in sachets were done by Tai Won Food Industrial Co., Ltd (Taiwan). Both were ISO 22000 and HACCP certified companies. The same procedure was adopted for the cucumber and used was as placebo.

Freeze dried bitter gourd and cucumber powders 2.5 g was packed in aluminum sachets (6 x 10 cm) sealed and tested for food grade quality. Required quantity of bitter gourd and placebo sachets were shipped from Taiwan to India (study area) to conduct the clinical trial and there it was stored at -20°C in a deep freezer.

B. Overview of the study design and conduct of clinical trial

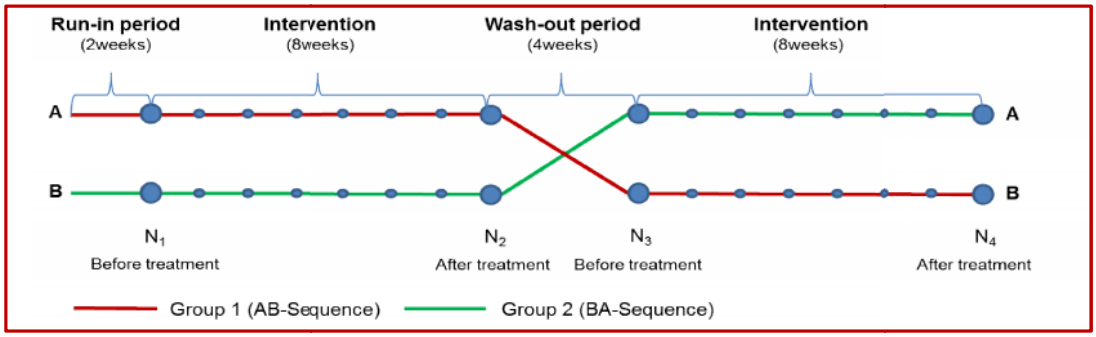
A single blinded, placebo-controlled, randomized, crossover designed intervention study was conducted with freeze dried bitter gourd powder to find out its

hypoglycemic effect. Crossover trial involves two treatments which are consecutively administered in each patient enlisted in the study. The main intention served by the design is to provide a basis for separating treatment effects from period effects (Wellek, 2012). For the clinical trial, around 53 male and 37 female subjects were selected using the inclusion and exclusion criteria given below:

Inclusion criteria: Prediabetics aged >25 and having FBG between 100 – 125 mg/dl on two separate days or a HbA1c >5.7-6.4% and those willing to participate in the study with written consent were included in the study.

Exclusion criteria: Those having a diagnosed disease, taking any sort of medication, high blood pressure (>160/>100), low blood pressure (<90/<60), being mentally ill, pregnant or planned for pregnancy and breast feeding were excluded from the study.

Clinical trial started after run in period of two weeks. Run-in period is a period before a clinical trial is commenced when no treatment is given.In this period the preface data was collected from the clinical trial participants. From the 3rd week onwards the first phase of intervention was conducted for eight weeks. Second phase of intervention was started after four weeks of wash out period to complete the total intervention. Wash out period is a period of time without active treatment, usually scheduled prior to initiation of placebo and/or active treatment arms. This can refer to a protocol required period of withdrawal from current treatment before active treatment starts. Figure 2 illustrates the overview of the intervention study.



Venous blood samples for FPG, HbA1c, insulin, lipid profile & anthropometrics, blood pressure and body fat

Capillary blood samples for FPG and anthropometrics

FIGURE 2. OVERVIEW OF THE INTERVENTION STUDY

Clinical trial was carried out as AB-BA sequence. In the first phase Group 1 (AB) consisting of 18 male and 27 female started the intervention with bitter gourd juice (A) followed by placebo (B), while the Group 2 (BA) with 35 male and 10 female pre diabetics started with the supplementation John(B) followed by Mary(A). The intervention continued for a period of eight weeks. Mary or John sachets were mixed freshly with 150 ml water and consumed during their lunch time throughout the eight week period of respective intervention phase. Participants were provided with sachets on a weekly basis to ensure the intake. In order to measure 150 ml of water, measuring cups were given to the participants.

Between the two arms, 4 weeks were left as wash-out period. This is to minimize the carry-over effect of one phase to the other. Cross over was done after this washout period.

C. Assessment of nutritional status of the selected prediabetics

Nutrition assessment is the most crucial step in diabetes management. The assessment forms the basis for developing the intervention plan and identifying potential changes to the lifestyle and health habits that will improve health. The main purpose of an assessment is to gather information needed to assist in the development of individual nutrition goals and subsequently establish an appropriate nutrition intervention.

1. Anthropometric measurements

Anthropometry is the hallmark technique and it is important in health assessments. Anthropometric measurements are associated with the presence of chronic conditions such as diabetes and hypertension or dyslipidaemia in different populations.

a. Measurement of height

Height was measured to 0.1 nearest millimeter using anthropometric rod. The subjects were asked to stand erect on a flat surface with heels together and upper limbs hanging closely to the sides of the body (ICMR, 2011). For the prediabetics in the intervention the height was measured initially (N1), after eight weeks (N2), after wash out period (N3) and finally (N4).

b. Measurement of weight

Body weight is a more sensitive measure of nutritional adequacy than that of height and it reflects recent nutritional status. Weight management is one of the most vital therapeutic tasks for Type II diabetic individuals (Anderson *et al*., 2003). To record the actual body weight, the subjects were made to stand on the platform of the digital SALTER electronic balance without footwear with minimal clothing (ICMR, 2010). For the selected subjects (N=948) the weight was recorded to nearest 100 g every week using the above mentioned procedure.

c. Body Mass Index (BMI)

Measuring BMI is important to identify individuals who may have undiagnosed diabetes or may be at increased future risk of diabetes (Hsu *et al.*, 2015). In addition, measuring BMI also is important for managing diabetes for the purpose of weight control. Body Mass Index was computed as the weight (kg) divided by height in m^2. Measuring both BMI and waist circumference is a better way to predict weight-related risk.

d. Waist Circumference (WC)

The prognostic importance of high waist circumference has been recognized within the diagnostic criteria to identify individuals with features of the metabolic syndrome (Despres, 2006). In the recent past, particularly with increasing incidence of obesity, considering the significance of abdominal adiposity in diet related chronic diseases, waist circumferences were used to evaluate the abdominal adiposity in subjects.

The subjects were made to stand erect with weight evenly balanced on both feet, which are placed about 25 to 30 cm apart. Using a non stretchable fiber glass tape waist circumference was measured around the waist horizontally midway between the lowest rib margin and iliac crest up to the nearest 0.5 mm and measured at the initial period, after eight weeks, after wash out period and at the final stage of intervention.

e. Waist to Height Ratio (WHtR)

Waist-to-Height Ratio (WHtR) have been evaluated for predictive value and has demonstrated a positive correlation for diabetes and metabolic risk in Asian populations (Xin *et al.,* 2012). A WHtR under 50 per cent is generally considered healthy and was calculated using the recorded values for height and waist circumference.

f. Conicity Index (CI)

Another index for abdominal adiposity is the Conicity Index (CI). This has a theoretical range, includes a built-in adjustment of waist circumferences for height and weight and does not require the hip circumference to assess fat distribution.

$$C\ I = \text{Waist Circumference (m)} / [0.109 \times \sqrt{\{\text{Bodyweight (kg)} / \text{Height (m)}\}}]$$

where 0.109 is a constant which results from the conversion of units of volume and mass into units of length (Valdez *et al*., 1993). Cutoff values for male is 1.25 and for females is 1.18 were used to classify conicity index into normal and high categories and this was calculated at the initial period, after eight weeks, after a washout period and at the final stage.

All the anthropometric measurements were done to the selected 948 healthy volunteers and also for 332 diabetic subjects.

2. Dietary Survey

The value of nutritional assessment is greatly enhanced when it is supplemented with food consumption pattern. Diet history plays a vital role to determine the nutritional status. Therefore in order to assess the food consumption of the selected prediabetic subjects, 24 hours recall method was used. By using the ICMR (2010) food consumption table, the mean food and nutrient intake of the

slected male and female prediabetcs were calculated and compared with the ICMR recommended dietary allowances to find out the adequacy of food consumption.

3. Blood pressure and body fat percentage

Blood pressures of the participants were measured in the seated position for 5 minutes, in the right arm using the digital Omron BP monitor (Omron HEM 7117, Hoofddorp, Netherlands). Blood pressure was measured at the initial period (N1), after eight weeks (N2), after wash out period (N3) and at the final (N4).

Visceral fat is directly linked with higher total cholesterol and LDL (bad) cholesterol, lower HDL (good) cholesterol and insulin resistance. Body fat was measured using body fat analyzer (Omron HBF-306C). The subjects were made to stand with both feet slightly apart. Instructed to hold the grip electrodes and wrap their middle finger around the groove of the handle, place the palm between the top and bottom of the electrodes and put their thumbs up, resting on top of the unit. The arms were straightened out, at a 90 degree angle to their body and asked the subjects not to move during the measurement and were measured at the initial period, after eight weeks, after wash out period and at the final.

4. Biochemical estimation

Hyperglycemia is the biochemical characteristic of diabetes. Fasting plasma glucose as the recommended method for diagnosing asymptomatic diabetes. As HbA1C is used more commonly to diagnose diabetes in individuals with risk factors, it will also identify those at higher risk for developing diabetes in the future. International Expert Committee of ADA in 2009 stressed that those with HbA1C levels above the laboratory “normal” range but below the diagnostic cut point for diabetes (6.0 to <6.5%) are at very high risk of developing diabetes (ADA, 2013). The HbA1C has several advantages like greater pre analytical stability and less day-to-day perturbations during stress and illness.

Glycemic status of the subjects was monitored through blood sugar levels by measuring the capillary blood every week after 12 hours fasting with Accucheck active glucometer manufactured by Roche Diagnostics, Germany.

Venous blood samples were analyzed for all the selected 90 prediabetic subjects in a NABL accredited laboratory. A trained technician from the laboratory collected the venous blood after 12 hours of fasting to analyze the bio chemical parameters. All the below mentioned biochemical parameters were measured at the initial period (N1), after eight weeks (N2), after wash out period (N3) and at the final (N4): were estimated by their respective methods.

- Fasting blood glucose (Spectrophotometry)
- HbA1c (HPLC method)
- Serum insulin (Carbonyl metallo immuno assay)
- Fructosamine (Spectrophotometry) and
- Lipid profile (Spectrophotometry)

D. Evaluation of the hypoglycemic effect of bitter gourd among prediabetics

The hypoglycemic effect of bitter gourd was evaluated and compared with the anthropometric changes including body weight, waist circumference, WHtR, CI, blood pressure, body fat percentage and biochemical estimations during bitter gourd and placebo intervention of the selected prediabetic subjects.

PHASE V - Conduct of dietary intervention on Type II diabetics

A. Formulation of supplement with bitter gourd

Freeze dried foods are quite expensive due to the specialized procedure and equipments used. Freeze dried foods also take up almost as much space as fresh foods, while dehydrated foods take up less space. In India, unfortunately, many people do not know how to use freeze dried foods to prepare meals at home. A food based approach was planned for diabetic subjects which should be effortlessly consumed and simple process to follow throughout their routine day to day activity. Several bitter gourd recipes were standardized and organoleptically evaluated and from that bitter gourd dhal powder was selected because of its high acceptability. These bitter gourd dhal powder is ready to eat convent food without further preparation. It can be prepared easily, portable and distributed to the diabetics. The keeping quality is also good compared to the other recipes.

For the preparation of bitter gourd dhal powder, firm, green colour bitter gourd without any insect infestation was procured from local supermarket, washed thoroughly in running water drained and cut in to circles. Subsequently the fruit pulp were sun dried till it became crispy and brittle, ground and made into fine powder. One kg of fresh bitter gourd on an average yield 60-75 g dried bitter gourd powder.

Four variations of bitter gourd dhal powder was prepared using weighed quantities of bengal gram dhal, red gram dhal, roasted bengal gram dhal , asafotida, red chillies in. All these ingredients were roasted in low flame for few minutes till it gets a good aroma and uniform heat and was homogenized to a fine powder. Bitter gourd powder with different variations was added to the dhal powder as given in Table VI.

TABLE VI

COMPOSITION OF THE BITTER GOURD DHAL POWDER (15g)

Variation	Ingredients (g)					
	Dried Bitter gourd powder	Bengal gram powder	Roasted bengal gram powder	Red gram powder	Red Chilli	Salt
I	2	6	3	3	0.5	0.5
II	3	5	3	3	0.5	0.5
III	4	4	3	3	0.5	0.5
IV	5	3	3	3	0.5	0.5

Four different variations of bitter gourd dhal powder were developed and acceptability trial was carried out with 10 semi trained taste panel members. The mean acceptability scores revealed that the acceptability, taste, texture, flavor and overall acceptability was higher for variation II when compared to all other variations. Hence, variation II was selected for intervention study with diabetics with raw equivalent 50 g of bitter gourd. According to Tsai *et al.,*(2012) 4.8 g per day bitter gourd powder can be used without any adverse effects..

For the selected bitter gourd dhal powder (variation II) various nutrients were estimated and the values are recorded as moisture (7.5 per cent), total ash (5.6 per cent), acid insoluble ash (0.17 per cent), fat (6.6 per cent), crude fiber (1.3 per cent) protein (20.7 per cent) and carbohydrate (59.6 per cent) was analysed for selected bitter gourd dhal powder (Variation II) selected for dietary intervention.

B. Dietary intervention to Type II diabetics

Among the 332 Type II diabetic subjects, 40 Type II diabetic subjects were selected for dietary intervention using the following inclusion criteria:

- Fasting blood glucose level within the range of 140-200 mg/dl,
- Post prandial blood glucose level within the range of 160-260 mg/dl
- HbA1c level 7 - 8.5%
- Willing to give written consent and participate in the dietary intervention

The selected forty Type II diabetic subjects were randomly divided into two groups namely an intervention group (N=20) and a nutrition education group (N=20). For the intervention group bitter gourd dhal powder was supplemented for a period of eight weeks along with nutrition education. The selected bitter gourd dhal powder was prepared and packed in zip lock covers containing 15g each and the subjects were advised to eat along with their breakfast or lunch. This packet was distributed at seven days interval to ensure their intake thus monitoring the subjects. For the nutrition education group nutrition and health education on dietary management of diabetes, healthy eating, and consequence of life style modification and importance of dietary fiber was explained and educated once in 15 days.

C. Evaluation of the hypoglycemic effect of bitter gourd among Type II diabetics

Impact of intervention is evaluated through anthrpometric measurements and biochemical parameters were estimated before and after the 60 days of intervention as per the standard procedures mentioned earlier.

The mean food and nutrient intake of male and female diabetics in both experimental and control group also calculated from the the 24 hr recall method and compared with the RDA.

D. Analysis of data and interpretation of the results

Data collected were classified, tabulated and analyzed using Statistical Package for the Social Sciences, version 20 (SPSS Inc., Chicago, IL, USA). All data were expressed as mean±SD. Student's *t*-test was performed for each experimental group. Data were also compared by Analysis of Variance (ANOVA) and only values with $P<0.05$ were considered as significant. Correlation analysis was done for the socioeconomic status and biochemical parameters of the study population.