The effect of a biscuit containing the extract of *Salacia reticulata* on the glycaemia in patients with Type 2 Diabetes Mellitus

A single center randomized triple blind placebo controlled two period two-sequence crossover clinical trial

Study title for general public

The effect of a biscuit containing *Kothala Himbutu* on blood glucose in patients with diabetes; A randomized control study

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Project proposal

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1. INTRODUCTION:

Herbal medicines have been in use for millennia. In recent years, there has been increasing interest in herbal medicines as they have increased in popularity in the developed world. The use of self-prescribed herbal medicines in USA increased from 2.5 to 12.1% between 1990 and 1997 and frequency of consultations with practitioners of herbal medicine rose from 10.2 to 15.1% (1). A similar increase has been reported in Europe (2). The justification for using these remedies appears to be based on traditional use rather than evidence from clinical trials.

Many herbal preparations are used in the treatment of diabetes mellitus in Sri Lanka. As in Europe, many patients use them concurrently with prescribed medication but do not tell their doctor about it (3). There is anecdotal evidence of such patients showing improved control and even an increased incidence of hypoglycemia in such patients. There is some evidence from animal studies and non-randomized uncontrolled studies in humans for the efficacy of some of these remedies (4).

The alpha glucosidase inhibitor acarbose is used in clinical practice (5). An alpha glucosidase inhibitor kotalanol has been identified in one commonly used herb, *Salacia reticulata* called Kothala Himbutu in Sinhalese by traditional physicians (6). Data on the efficacy of the herbal preparation from randomized controlled clinical trials is not available. Traditionally this preparation is ingested as an herbal tea (Kothala Himbutu tea), where the herbal mixture is ingested in a cup of boiled water and in the form of a tea bag prepared using a formula passed on in an oral tradition to members of the family of the Ayurvedic physician. Efficacy of tea bags containing *Salacia reticulata* in lowering blood glucose in people with type 2 diabetes and its safety in a double blind randomized cross over trial was demonstrated in 2006 (7). Recently biscuit containing extract of *Salacia reticulata* (Kothala Himbutu biscuit) was tested and found to have low glycaemic index compared to a biscuit not containing *Salacia reticulata* (8). This biscuit is available in the market in Sri Lanka and consumes by customers.

2. OBJECTIVE:

We intend to conduct a triple blind randomized clinical trial to investigate the effect of a biscuit containing the extract of *Salacia reticulata* on the fasting blood glucose levels and

HBA_{1C} in patients with Type 2 diabetes mellitus and also to find out whether there are any side effects on other vital organs such as kidneys and liver.

3. PATIENTS AND METHODS:

Patients with diabetes mellitus will be selected for screening from those seeking treatment at university departmentsof Medicine in University Rajarata. One hundred and sixty patients (112) with diabetes mellitus Type 2 will be included in a triple blind randomized placebo controlled two-period two-sequence cross over study (AB/BA).

3a. Selection criteria:

Male and female patients who have been diagnosed as having Type 2 diabetes mellitus for a minimum period of 6 months, who are interested in seeking Ayurvedic treatment as sole treatment or as complimentary treatment to western medical treatment and meet the following criteria will be selected to participate in this study.

1. Onset of diabetes mellitus for six months.
2. Aged between 30 and 65 years
3. Stable glycaemic control over the preceding 2-3 months. They will be initially selected from a range of HBA_{1C} varying from 7 – 14 and the individual subject should not have a variation of >20% between the previous HBA_{1C} and the HBA_{1C} done at the commencement of the study

3b. Exclusion Criteria:

The following patients will be excluded from the study.

Exclusion Criteria:

1. Chronic kidney disease with estimated Glomerular filtration rate less than 30ml/hour
2. Severe valvular heart disease or heart failure.
3. Those on Insulin therapy
4. Severe liver disease (AST > 10 X times the upper limit)

4. RECRUITMENT

Patients who seek treatment for diabetes mellitus will be screened for participation in the study. The subjects will be provided with a detailed patient information sheet supplemented by a verbal explanation and reviewed at a later date to obtain written consent in order to give them an opportunity to discuss their participation with family and other relevant persons whose advice is deemed of value by the subject.

Type I Diabetes will be excluded by clinically. Participant will have two HBA_{1C} within the two months period to ensure stability of glycemic level. This is done to exclude recruiting patients who have rapidly changing glycaemic values. As this trial is a crossover trial stability of the glycemic control over time needs to be assured to prevent excessive variation of HBA_{1C} interfering with study outcome. A medical officer will perform a complete history and clinical examination with blood pressure. Blood and urine samples will be tested for baseline investigations such as FBC, UFR, LFT, CRP, renal & liver profiles.

4a Primary outcome variable

I. Glycaemic control evaluated with HBA_{1C} carried out centrally with ion exchange HPLC and the units will be NGSP standard.
II. Serious liver and kidney adverse events when taking the biscuits containing the extract of *Salacia reticulata*.

4b Secondary outcome variable

Fasting blood glucose measured by glucose oxidase method.

5. THE STUDY PRODUCT

A biscuit containing the extract of *Salacia reticulata* is the active treatment. The study product is available in the market. Both study biscuit containing *Salacia reticulata* and the placebo will be wrapped in identical covers for concealment.

1. To ensure the uniformity of the product between successive batches, Ceylon Biscuits Ltd (CBL) adheres to strict quality control measures during the production process and prior to release of the product to the market.
2. Formulation of the biscuit (study product): By CBL.
    a. Placebo biscuit & formulation: - A biscuit identical to the study product, without the extract of *Salacia reticulata*. The fiber content of the biscuit containing Kothala himbutu extract and the placebo will be identical.
    b. Contents of the study biscuit

Name of ingredient	Quantity (As %)
Wheat flour	73.51
Yeast	1.06
Vegetable fat	12.82
Wheat bran	8.12
Leavening agents	0.82
Salt	1.48

Soya lecithin 0.15

Water extract of Kothala himbutu

6. SAMPLE SIZE & RANDOMIZATION

1. Consecutive patients who seek treatment for diabetes mellitus will be screened for eligibility.
2. Those who are eligible will be invited for the study.
3. Sample size calculation:

Sample size was initially calculated by using a formula specific for crossover studies (9). The standard deviation (0.9) to calculate the standard error was taken from the baseline data (7). Clinically significant improvement of HbA1c in the intervention group over the placebo group was estimated as 0.5 units of HbA1c (difference in means). As this gave very low sample size (13 in each arm) we calculated the sample size for parallel studies according to Pocock (10). Significance level (alpha) 0.05 and power (1-beta) = 0.8 giving rise total of 51 patients were in each arm. With anticipated dropout rate of 10%, 112 patients were estimated sample size for a single center study

6b Randomization

SBA will generate randomization code (A or B) using a computer and prepared sealed, serially numbered, opaque envelopes. All eligible participants will be identified and given a serial number on the day of randomization (after run-in period). A study assistant will open the envelope and assign the patient to either biscuit A (placebo) or B (KH) and deliver the biscuit boxes to the patient. The manufacturers (Ceylon Biscuits Ltd) held randomization code.

6C. Allocation concealment

Investigators, patients and those assessing the analysis will be masked to the group outcome. Both Kothala Himbutu (KH) and placebo biscuits are identical in size, wrapped in same cover, and packed in identical boxes.

Patients participating in the trial will be asked about prior consumption of KH biscuits bought from the market (to exclude contamination). At the end of each period patients were asked whether they could assume the type of biscuit (KH biscuit or placebo) given to them and their preference for the type.

7. STATISTICAL ANALYSIS (10)

1. Being a cross over trial, the analysis will be done as a paired analysis.
2. Intention to treat analysis.

3. Formal tests for period effect, treatment effect, carryover effect and interaction between treatment and period will be carried out.

8. PROCESS OF THE TRIAL

After running period where stability of HBA_{1C} is assured patients will be randomized to receive either a standard preparation of Kothala himbutu biscuits or a placebo, a similar biscuit without the extract of *Salacia reticulata*. The study product and the placebo will be given to the participants and the investigators and the participants will be blinded to the treatment.

Four biscuits will be taken as a mid morning and afternoon snack, at 10am and 4 pm respectively. Oral hypoglycemic treatment for diabetes taken at the time of recruitment will be continued but subject to change if the condition of the patient changes, as determined by treating clinician.

Patients will continue treatment for a period of seven months separated by a washout period of one month at cross over. On completion of the first three months on either treatment or placebo patients will not receive either placebo or biscuit with the active ingredient during a wash out period of one month. The wash out period is determined as one month to minimize the carry over bias. In the event that significant difference exists between the two groups at the end of three months, the study will be terminated at that point.

During this time the subjects will have a minimum of eight to ten visits inclusive of the booking visit and will meet with a medical officer at each visit for review of glycaemic control and other possible complications. A six-point blood glucose profile (pre and post breakfast, pre and post lunch, pre and post dinner samples) will be performed on one day during the week prior to each visit.

HBA_{1C} will be performed at recruitment, at three months and on completion of the study at seven months. Existing oral medication and doses for diabetes mellitus will be adjusted as requested during the study.

In the event that the clinical investigators feel that significant deterioration of glycaemic control has occurred or that the patient would require a step up to insulin, patients will be withdrawn from the study.

Specific written instructions will be provided for the timing of snacks in the standard dosage. Counting the unused biscuits will assess compliance. All adverse events will be recorded. Patient preference for the biscuits will also be assessed.

8 a. Visit Schedule

V1 - Selecting patients and Recruitment

Check for inclusion and exclusion criteria

Informed written consent form signed

Obtain demographic data

History and examination

Dietary advice

Screening investigations

FBC, liver profile, renal profile, FBS, HBA_{1c}, ECG, Urine for microalbumin

V2 - 8 weeks after V1

Review investigation reports of V1 in relevance with exclusion criteria

2^{nd} HbA_{1c}, Lipid profile, UFR

V3 Trace laboratory reports

Provision of a glucometer

Glucometer Training and self-monitoring of blood glucose

V4 Randomization

Checking for glucometer capability

Review the blood sugar series

History and examination

Providing biscuit

V5 - May be over the phone (1 week after providing biscuit)

Evaluate the compliance for the biscuit

V6 - 1month after providing biscuit

Evaluate the blood sugar series

History and examination

Review dosage of other drugs

Provide stock of biscuit for 1 month

SGPT, SGOT, Serum creatinine

V7 - 2months after providing biscuit

Evaluate the blood sugar series

Trace laboratory reports

History and examination

Review dosage of other drugs

Provide stock of biscuit for 1 month

SGPT, SGOT, Serum creatinine

V8 - 3 months after providing biscuit

Stop biscuits

History and examination

Trace the laboratory reports

Review dosage of other drugs

SGPT, SGOT, Serum creatinine, HbA_{1c}, FBS, Urine for microalbumin

1 month washout period

V9 - 1 month after stopping biscuits

Evaluate the blood sugar series

History and examination

Trace the laboratory reports

Review dosage of other drugs

Change to other group of biscuit (provide biscuit for 1 month)

SGPT, SGOT, Serum creatinine

V10 1 month after changing biscuits

Evaluate the blood sugar series

History and examination

Trace laboratory reports

Review the dosage of other drugs

SGPT, SGOT, Serum creatinine

Provide stock of biscuit for 1 month

V11 2 month after changing biscuits

Evaluate the blood sugar series

History and examination

Trace laboratory reports

Review dosage of other drugs

SGPT, SGOT, Serum creatinine

Provide stock of biscuit for 1 month

V12 3 month after changing biscuits

History and examination

Trace laboratory reports

Review dosage of other drugs

SGPT, SGOT, Serum creatinine, HbA_{1c}, FBS, Urine for microalbumin

V13 9 months after changing biscuits

HbA_{1c}

8a. Ethics and informed consent

Written informed consent will be obtained from the patients and ethical clearance obtained from the Ethics Review Committees of Peradeniya Medical Faculty and Rajarata Medical Faculty.

The trial will be registered with the International Standard Randomized Controlled Trial Number Register.

8. TRIAL MONITORING

8a. Data monitoring & Safety Board

Independent data and safety monitoring board will regularly review interim data from the trial. The composition of this body is as follows.

1. Dr NJ Dahanayake MBBS MD, Senior Lecturer & head Department of Medicine FMAS, RUSL.
2. Prof Samath Dharmarathne MBBS MD MSc Associate Professor in Community Medicine Peradeniya Medical Faculty.

8b. Trial Steering Committee

1. Prof. Sisira Siribaddana – MBBS MD FRCPE FCCP
2. Dr. Arjuna Medagama - MBBS MD MRCP
3. Dr Suneth Agampodi MBBS, MSc, MPH, MD
4. Prof. Devaka Fernando. MBBS, MD, FRCP, FRCPE

8c Conduct of trials:

The trial will be conducted at the medical clinic of the Department of Medicine, Faculty of Medicine & Allied Sciences, and Rajarata University of Sri Lanka at Teaching Hospital Anuradhapura under the direct supervision of Prof. Sisira Siribaddana.

9. DATA AND SAMPLE COLLECTION

The data will be entered into a structured questionnaire and will contain demographic data as well as a detailed history regarding the course, treatment and control of diabetes mellitus in individual patients. All blood samples will be immediately transported to a pre-planned, selected laboratory for analysis. Each sequential data item will be added to the patient records maintained in written (structured questionnaire) and computer data base forms (Microsoft Access) either on line or uploaded to the central database within 5 working days of receipt.

10. DATA ANALYSIS AND PRESENTATION:

There will be interim analysis of data after the end of first treatment period. to observe the progress of each patient to ensure good glycaemic control. Standard statistical packages will be used in data analysis. Patient flow will be presented in a CONSORT diagram.

11. FUNDING:

The cost of the study will be met by an unreserved unconditional research grant from Ceylon Biscuits Ltd. The investigators as a group have the right to publish their findings in peer reviewed journals. Individual centers will not present or publish findings independently but will do so as part of a two center collaborative research group.

References & Notes:

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