TASMANIAN SCIENTIFIC RESEARCH ADVISORY COMMITTEE

STUDY PROTOCOL PRO FORMA

TITLE

The effect of nutraceutical supplement (Thompson's Super Bioflavonoid Complex®) on blood glucose levels in prediabetes and type 2 diabetes – A Pilot Study.

AIMS

Aim 1-: To determine the effect of nutraceutical supplement (Thompson's Super Bioflavonoid Complex®) as natural DPP-4 inhibitor on glycaemic control in prediabetes and type 2 diabetes mellitus (T2DM).

HYPOTHESES

Hypothesis 1-: Thompson's Super Bioflavonoid Complex® *(two capsules per day for 12 weeks*) will significantly reduce DPP-4 activity in prediabetes and type 2 diabetes , increasing incretin hormone levels, and improving the blood glucose response to a glucose challenge and reduce the degree of oxidative stress.

This study will investigate the potential of commercially available nutraceutical supplements (Thompson's Super Bioflavonoid Complex®, 500 mg (Citrus Bioflavonoid capsules, two capsules/day) to reduce blood glucose levels in patients with prediabetes and T2DM. The action of these supplements is thought to be similar to the dipeptidyl peptidase-4 (DPP-4) inhibitor group, also known as gliptins, which are second line anti-diabetic drugs after metformin and sulfonylureas. The gliptins block the action of DPP-4, an enzyme which inhibits a group of gastrointestinal hormones called incretins [1]. Incretins help stimulate the production of insulin when it is needed (e.g. post meal) and reduce the production of glucagon by the liver when it is not needed (e.g. during digestion). By protecting incretins, DPP-4 inhibitors help regulate blood glucose levels. Studies have shown that gliptins possess side effects which include (but not limited to) flu-like illness, joints pain, and pancreatitis [1]. Naturally derived DPP-4 inhibitors may offer an alternative to prescription based gliptins, or as "add on" therapy to first line products and decrease the risk of developing diabetic complications. This will also reduce the financial burden (total annual cost of the antidiabetic medicine is around AU$193.8 million) [2].

BACKGROUND

Since T2DM is a progressive condition, most patients will eventually require further treatment with more than one anti-diabetic drug to maintain their glycaemic goals [3]. Clinical evidence from the A Diabetes Outcome Progression Trial (ADOPT) and the United Kingdom Prospective Diabetes Study (UKPDS) shows that many T2DM patients on monotherapy will fail to maintain glycaemic control after just a few years, or even sooner, depending on disease duration prior to diagnosis and treatment adherence [3]. Given the failure of current treatments to provide long-term glycaemic control in a substantial proportion of patients, there is strong evidence for the need for new, better optimized treatment options [4], including evidenced based nutraceuticals. It should be noted that drugs of natural origin have a good track record in T2DM drug development; a recent review of all drugs approved worldwide since 1981 found 15 of 19 antidiabetic drugs (excluding biologicals such as insulin) have been either natural products or derived from natural product pharmacophores [5]. The chemically known anti-diabetics "dipeptidyl peptidase-IV (DPP-4) Inhibitors", also known as gliptins, control blood glucose levels via inhibiting the DPP-4 enzyme action on incretin hormones, and hence improve insulin secretion [6]. Recent studies have explored

the naturally related active compounds which possess DPP-4 Inhibitor activity. These can be generally divided into two main types: phytosterols and bioflavonoids [7].

Bioflavonoids are classified as organic polyphenolic compounds, which are found in various plant-based foods in different concentrations, like berries and citrus fruits. Citrus bioflavonoids can be specified as plant pigments available in various sources such as oranges, lemons and grapefruits. They have the capability of enhancing the absorption levels as well as the utilisation of vitamin C [8]. Thompson's Super Bioflavonoid Complex® contains citrus bioflavonoid extract-500 mg, ascorbic acid-390 mg, rutin-100 mg, and sodium ascorbate-125.3 mg (equiv 110 mg Ascorbic Acid). Lemon bioflavonoid is the source of eriocitrin [9], which possesses a lipid lowering activity as seen in animal models [9]. Orange bioflavonoid comprises hesperetin, hesperidin and naringenin [10]. These possess potent antioxidant activity as seen in previous studies [11]. Other *in-vivo* studies signified the impact of naringenin and hesperidin in suppressing macrophage infiltration [11], and reducing blood glucose levels [12]. The grapefruit bioflavonoid contains mainly naringenin, which is beneficial in reducing arterial stiffness in post-menopausal women [13], and helps improving hyperglycaemia [14]. A more recent study investigated the effects of diabetinol (nobiletin-49%, tangeretin-13%, including limonoids, tocotrienols and vitamin E) on glycaemic control in patients with impaired fasting glucose [15].

METHODS

Design

Participants: Fifty participants recently diagnosed with prediabetes and/or T2DM living in Hobart/Launceston regions will be recruited for this study. The study will be conducted in two clinical areas: 1- Medical Science Precinct, University of Tasmania (UTAS), Hobart. 2- School of Health Sciences, Newnham campus, Launceston. Potential participants will be identified by local media, flyers, local General Practices, as well as by the Diabetes clinic at the Royal Hobart Hospital and Launceston General Hospital. Participants will undergo the following:

1. Medical & lifestyle questionnaires (general health, diet & physical activity questionnaires).
2. Anthropometrics (body weight, height, waist & hip circumference).
3. Blood tests to measure blood glucose, HbA1c, serum lipids, insulin, & oxidative markers (8-hydroxy deoxy Guanosine (8-OHdG), and resistin).
4. DPP-4 activity in the blood before and after 12 weeks.
5. Glucose metabolism (2hr oral glucose tolerance test).
6. Haemodynamic measures (blood pressure, heart rate & ECG).
7. Urinary electrolytes & kidney function.

Research design: This is a randomized double-blinded placebo-controlled study. The total duration of the recruitment process is 12 weeks.

Participants will be randomly allocated into two study groups (25 participants in each study group): Study group (Group-1) include participants who use the nutraceutical supplement (Thompson's Super Bioflavonoid Complex®) for 12 weeks and continue on their current anti-diabetic treatment with no interruption. Control group (Group-2) include participants who use the placebo capsules for 12 weeks and continue on their current anti-diabetic treatment with no interruption.

The placebo capsules would contain the same excipients as the active capsules, but the active herbal extract would be replaced by excipients.
The excipients in the Thompson's Super Bioflavonoid Complex are microcrystalline cellulose (BP), calcium hydrogen phosphate dehydrate (USP), silica – colloidal anhydrous (BP), crospovidone (BP) and magnesium stearate (BP).

As per the instruction provided, the supplements will need to be stored below 30 ◦C in a dry place.

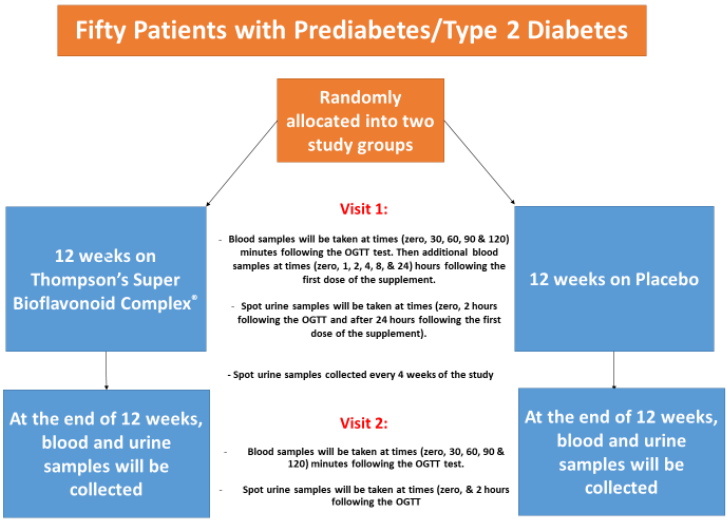


Figure 1: Overview of the study.

Setting

The study will be conducted in clinical areas at: 1- Medical Science Precinct, University of Tasmania (UTAS), Hobart, and 2- The Clinical rooms at the School of Health Sciences, Newnham campus, Launceston.

Participants

Inclusion Criteria-:
For all participants

1. Have been clinically diagnosed with prediabetes and/or early stage type 2 diabetes (on diet and lifestyle intervention or metformin only treatment).
2. Aged between 25 – 75 years.
3. Lean, overweight or obese (BMI 19-35 kg/m^2).
4. Have normal or controlled blood pressure (Seated brachial blood pressure <140/90 mmHg).
5. Have given signed informed consent to participate in the study.
6. Willing to take nutraceutical supplement (Thompson's Super Bioflavonoid Complex®), for 12 weeks.

Exclusion Criteria-:
For all participants

1. Age <25 yrs or >75 yrs
2. Morbidly obese with a BMI ≥35 kg/m2
3. Not on diet and lifestyle or metformin only treatment for their diabetes (e.g. insulin injections, sulphonylureas, GLP-1 receptor agonists).
4. History of myocardial infarction or stroke
5. History of malignancy within past 5 years (except for non-melanoma skin cancers)
6. Identification of any medical condition requiring immediate therapeutic intervention
7. Uncontrolled hypertension (resting brachial blood pressure ≥160/100 mmHg)
8. History of severe liver disease
9. History of drug or alcohol abuse
10. Elective major surgery during the course of the study
11. Pregnancy/lactation
12. Currently consuming nutraceutical supplements (especially vitamin C and D)
13. Participation or intention to participate in another clinical research study during the study period.
14. Not willing to take nutraceutical supplements (Thompson's Super Bioflavonoid Complex®), for 12 weeks.
15. Participants with impaired renal function.

Participants will be invited from Hobart/Launceston regions who are able to come to the clinical areas at Medical Science Precinct/School of Health Sciences, UTAS. Venous blood samples & urine samples will be collected at the start of the study. Thompson's Super Bioflavonoid Complex® supplement will be provided to the participants for 12 weeks, and samples will be collected at the end of the study to compare the results of the selected markers aiming to identify the metabolic response to these nutraceutical supplements.

Biomarkers of metabolic response included in this study are 8-hydroxy deoxyguanosine (8-OHdG) (oxidative DNA damage), total lipid peroxidation (lipid peroxidation), interleukin-6 (Inflammatory response) and resistin (adipocytokine response).

Sample size Justification
Our previous data shows that we had statistical significant changes in the levels of 8-OHdG and antioxidants using 40 T2DM patients [16]. Based on these data and assuming 20-25% withdrawal rate from the study, for a two sample test (t-test) with a Type I error of 5% ($\alpha = 0.05$) and 80% power, we will require 50 participants in each group.

Recruitment
Potential participants will be identified by radio, flyers, local General Practices, and by the Diabetes clinic at the Royal Hobart Hospital/Launceston General Hospital. Potential participants may be contacted using the National Diabetes Services Scheme (NDSS) through Diabetes Tasmania. Participants from previous studies may be contacted if they have given consent to be contacted for future studies.

Participants who meet the inclusion/exclusion criteria will receive the participant information sheet/consent form inviting them to take part. Contact details of the study coordinator will be given, so that participants can raise any queries or concerns that they have and inform of their interest in participating in the study.

Participants from previous studies will be contacted via telephone, email or mail regarding the study and will be given the opportunity to contact the study coordinator if they are interested. Participants responding to online, print media and radio advertisements will be invited to contact the study coordinator by telephone or email. Initial screening will be based on a health history questionnaire and questions prior to attendance to rule out exclusion criteria.

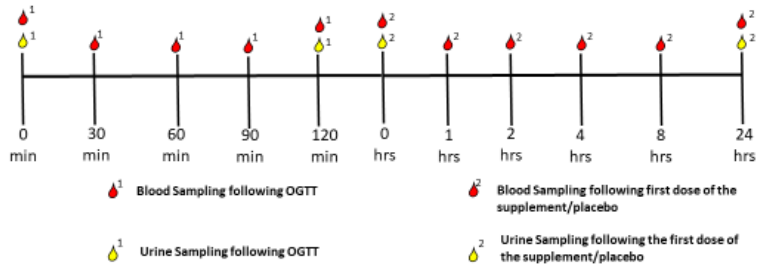
Measures

The interview questionnaire is attached.

Data Collection

Timeline of Testing: (see diagrams below).

1- Vistit 1:



2- Visit 2:

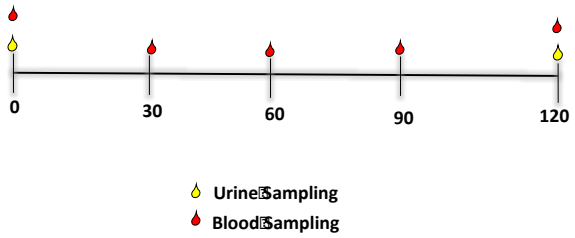


Figure 2: Timeline for blood and urine sample collection.

Eligibility and consent visit

Participant will only be given appointment after they have been comprehensively assessed for this trial, and will be required to come to Medical Science Precinct/ School of Health Sciences, UTAS. Signed consent will be obtained prior to performing any study related procedures. Participants will undergo the following procedures:

i) Anthropometrics: We will measure body weight, height, waist and hip circumference.
ii) Lifestyle: Potential participants will complete a general health questionnaire (attached) which includes questions regarding family history of T2DM and a physical activity questionnaire (attached). Participants will be provided with a 3-day food record (attached) to complete at home and brought to the next clinic visit (if deemed eligible to continue with the study).
iii) Blood pressure: Brachial blood pressure will be recorded with a validated Omron HEM 907 device. Measures will be taken with a correct sized cuff, feet flat on floor, back supported and no talking (as per recommendations). After five minutes rest, measures will be taken in duplicate (one minute apart). A second set of duplicate measures (one minute apart) will be taken after 10 minutes rest, and the average of these last two values will be used for analysis. We have recently found that this is the optimal, and most clinically relevant, time to acquire brachial and central BP.

VISIT 1:

Finger Prick Method

A finger prick will be performed on the participants to test their blood glucose level using glucometer

Participants will undergo a variety of metabolic and blood pressure measurements. Participants will fast (≥12 hours) and refrain from caffeine and alcohol (≥12 hours) and refrain from exercise for 48hrs prior to attending the clinic at 8:00-10:00am. T2D participants on metformin will be instructed to discontinue their medication for treating hyperglycaemia on the morning of the study. Testing will be conducted on participants in a quiet, temperate room and in the supine position. A polyethylene catheter will be placed in an antecubital vein for blood sampling. Menstruating females will only be tested in the early follicular phase.

Clinical Biochemistries

A blood sample (0.33M EDTA tubes) will be taken to measure clinical chemistries (glucose, insulin, lipids and HbA1c). A second blood sample will be taken, centrifuged at 4ºC for 10 minutes, and plasma/serum samples stored at –80ºC for analysis of other metabolite, inflammatory markers

Urine sample

We will also measure urinary electrolytes (e.g. sodium, potassium, creatinine, albumin), markers of oxidative stress (e.g. 8-Hydroxy-2'-deoxyguanosine) and kidney function. A spot urine sample will be taken at rest and 2hrs following the OGTT.

In addition, spot urine samples will be taken every 4 weeks during the study.

Oral glucose tolerance test (OGTT)

A 2-hour OGTT will be performed to assess glucose tolerance and insulin sensitivity and is a routine clinical test for determining insulin resistance and diabetes. A fasting blood sample (time: −10 minutes) will be taken prior to ingestion of a drink containing 25g of glucose (time: 0 minutes). Blood samples will also be taken at specific time points post-glucose ingestion (0, 30, 60, 90 and 120 minutes), thereby enabling measurement of 2-hour area under the curve (AUC) for glucose and insulin and insulin sensitivity indices.

Whole-body insulin sensitivity will be calculated according to the Matsuda index (17):

Insulin sensitivity = 10,000 / √ [(FPG × FPI) × (mean OGTT glucose × mean OGTT insulin)]

Here, FPG represents fasting plasma glucose, FPI- fasting plasma insulin, and mean values the average of 0, 30, 60, 90and 120-minute plasma concentrations.

Insulin sensitivity of muscle and hepatic tissues will be calculated according to equations validated by Abdul-Ghani et al. (18):

Muscle insulin sensitivity index = (dG/dt) divided by (mean plasma insulin).

Hepatic insulin sensitivity index = glucose $AUC_{0\text{-}60}$ × insulin $AUC_{0\text{-}60.}$

Here, dG/dt refers to the rate of decrease in plasma glucose from peak to nadir (slope of least square fit; excluding any rebound increase in glucose) and $AUC_{0\text{-}60}$ refers to area under the curve during the first 60-minutes of the OGTT.

In addition to these estimates based on dynamic testing, insulin sensitivity derived from fasting steady-state measurements will be calculated by the homeostasis model assessment index (HOMA-IR); that is, fasting plasma insulin × fasting plasma glucose / 22.5

During the OGTT, blood samples will be collected and transferred to ice-cold tubes containing 10µl of 0.33M EDTA, immediately centrifuged at 4ºC for 10 minutes and plasma samples stored at –80ºC for further analysis.

Thompson's Super Bioflavonoid Complex® supplementation/ Placebo:
Participants will be randomly divided into the two study groups. Study group participants (Group-1) will be asked to take 2 capsules of Thompson's Super Bioflavonoid Complex® daily before the meal for 12 weeks (total of 168 capsules per participants). Control group participants (Group-2) will be asked to take placebo capsules for the whole 12 weeks. The Thompson's Super Bioflavonoid Complex®/Placebo capsules will be provided free of charge, for the entire length of the study. However, if participants skip 42 capsules (equivalent to 3 weeks) over the course of the intervention, they will be excluded from the study.

ARTG Product Link-:http://search.tga.gov.au/s/search.html?collection=tga-artg&profile=record&meta_i=251014

Australian Register of Therapeutic Goods (ARTG) Entry- 251014	
Supplement Facts- Serving Size- 1 Tablet	***Amount Per Serving***
Thompson's Super Bioflavonoid Complex®	1100 mg
Citrus Bioflavonoid Extract	500 mg

Ascorbic Acid (Vitamin C) & Sodium ascorbate (125.3mg) equiv. Ascorbic Acid (Vitamin C)110mg)	390 mg + 110= 500mg
Rutin	100 mg

Participants will be asked to complete a logbook to record the day and time they take the capsules. Participants will be asked not to change any other lifestyle factors (e.g. diet or level of physical activity). During the last week of the Thompson's Super Bioflavonoid Complex® supplementation/Placebo study, they will be asked to repeat the food questionnaire and the physical activity questionnaire. This will be filled out at home and brought to the clinic on visit 2.

Pharmacokinetics/pharmacodynamic study:
During visit 1, and after the first dose of Thompson's Super Bioflavonoid Complex® supplementation/ Placebo, additional blood and urine samples will be collected following the first dose of the supplement to measure the pharmacokinetics/pharmacodynamics characteristics of a single dose administration of the citrus bioflavonoid in human body. A series of blood and urine samples will be collected at times (0, 1, 2, 4, 8 and 24) hours. Participants will be able to move around and stay within the clinical area for the first 4 hours, and they can leave and come back for the 8 hrs and 24 hrs time of collection.

Commented [HA1]: All, please amend this as you think more appropriate.

VISIT 2 - WEEK 12 TESTING:
Following the 12 weeks of Thompson's Super Bioflavonoid Complex® supplementation/Placebo, participants will be asked to fast overnight (i.e. not eat food and only drink water on the morning of the study) and stop taking metformin and then at Medical Science Precinct/School of Health Sciences, UTAS. They will undergo an oral glucose tolerance test to measure your glucose tolerance and insulin sensitivity. This will require the participants to drink 25g of glucose (sugar drink) and then the sample of blood will be taken from their vein before and at 0, 30, 60, 90 and 120 min after drinking the glucose solution. Blood pressure and vascular health will also be measured using a blood pressure cuff placed on your upper arm of the participants as well as urine sample will be collected to measure kidney function, electrolytes and oxidative stress. The body composition measurement (height, weight, waist and hip circumference) will also take place.

Quality control and feasibility

It is worth to mention that this study was reviewed and updated several times during the successive meetings held between the PhD candidate and his supervisor. Besides, it has been discussed during the regular diabetes lab meeting and amended based on the feedback received from the researchers.

Mr. Gupta undertook a phlebotomy course to upgrade his knowledge about the procedure and learned more about the risk management in this study. In addition, there will be experienced staff available in the clinical areas who will be willing to help and support in any form necessary for the study.

Data analysis

Data analysis will be done using SPSS program (IBM SPSS Statistics version 22), and Microsoft Excel 2010. All values will be expressed as mean ± SEM. Statistical analyses will be performed using a one-way ANOVA followed by unpaired Student's t test and paired students t-test (individual results before and after the course of supplements)-LSD- (Least significant difference) (non-parametric, with two-tailed) will be used for non-parametric results (to compare patients versus control results). Results will be considered significant at P equal or < than 0.05.

REFERENCES

REFERENCES

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