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Original Article

# Efficacy and safety of a herbal drug of *Coccinia grandis* (Linn.) Voigt in patients with type 2 diabetes mellitus: A double blind randomized placebo controlled clinical trial

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ABSTRACT

*Background:* Several lines of preclinical studies have shown promising antidiabetic effects of the aqueous leaves extract of *Coccinia grandis* (Linn.) Voigt (Cucurbitaceae) *in vivo* and *in vitro*.
*Purpose:* : The present study was conducted to evaluate the efficacy and safety of a newly developed herbal formulation of *C. grandis* in newly diagnosed patients with type 2 diabetes mellitus (T2DM).
*Study design:* : A three months long, randomized, double blind, placebo controlled clinical trial in patients with newly diagnosed T2DM.
*Method:* : Based on fasting plasma glucose (FPG) concentration, a total number of 158 newly diagnosed patients with T2DM (45 ± 15 years age) were recruited for the present trial from the University Medical Clinic, Teaching Hospital, Karapitiya, Galle, Sri Lanka. They were randomly assigned to the test or placebo group to receive 500 mg of herbal drug (n = 79) or placebo drug (n = 79) once daily for three months. Patients and investigators were blinded for the treatment. Percentage of glycated hemoglobin ($HbA_{1C}$ %), insulin and lipid profile parameters were estimated at the base line and at the end of the intervention. Serum concentration of fructosamine was assessed at every other visit of the trial. The homeostatic model assessment for insulin resistance (HOMA-IR), atherogenic index (AI), cardio-protective index (CPI) and coronary risk index (CRI) were calculated. Furthermore, fasting plasma glucose concentration, renal and liver toxicity parameters, hematological parameters, blood pressure (BP) were assessed throughout the study in two weekly intervals till the end of three months.
*Results:* : Out of 158, a total number of 145 patients completed the entire clinical trial period successfully. Mean (SD) changes of variables from the baseline to the end of the intervention in test and placebo groups were 0.65 (0.54) and 0.08 (0.66) for $HbA_{1C}$ % ($p < 0.001$), 1.91 (3.07) and -1.28 (9.77) for insulin ($p < 0.001$), 0.02 (0.03) and -0.01 (0.04) for frucosamine ($p < 0.001$), 1.51 (0.49) and 0.05 (0.50) for FPG ($p < 0.001$), 1.73 (1.36) and -0.37 (3.38) for HOMA-IR ($p < 0.001$), 0.16 (0.18) and -0.04 (0.42) for TG ($p < 0.001$), 0.07 (0.08) and -0.02 (0.19) for VLDL-C ($p < 0.001$), respectively. However, the herbal drug of *C. grandis* was unable to change other outcome variables significantly when compared to the placebo ($p > 0.05$). All the renal, liver and toxicity parameters, hematological parameters and BP were within the normal physiological reference ranges at each visit.

*Abbreviations:* γ-GT, gamma glutamyl transferase; AI, atherogenic index; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; *C. grandis*, *Coccinia grandis*; CPI, cardio-protective index; CRI, coronary risk index; DBP, diastolic blood pressure; FPG, Fasting plasma glucose concentration; $HbA_{1C}$, glycated hemoglobin; HDL-C, high density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment for insulin resistance; LDL-C, low density lipoprotein cholesterol; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; OGTT, oral glucose tolerance test; PCV, packed cell volume; RBC, red blood cells; SBP, systolic blood pressure; T2DM, Type 2 diabetes mellitus; TC, total cholesterol; TG, triglycerides; VLDL-C, very low density lipoprotein cholesterol; WBC, white blood cells; WC, waist circumference.

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*Conclusion:* : Treatment with herbal drug of *C. grandis* (500 mg per day) for three months for patients with newly diagnosed T2DM significantly improved their glycemic and selected lipid profile parameters with well tolerated safety.

## Introduction

Type 2 diabetes mellitus (T2DM), the most prevalent type of diabetes, is a metabolic disorder resulting from insulin resistance and/or relative deficiency of insulin (IDF, 2019). The emerging prevalence of T2DM and its associated complications pose a major global health burden. According to the estimations of the International Diabetes Federation (IDF) in 2019, 463 million adults aged 20-79 years are living with diabetes globally and the estimation is projected to reach 700 million by 2045 (IDF, 2019). The rising prevalence of diabetes is driven by several factors including aging of population, sedentary lifestyle, greater consumption of unhealthy food, rapid urbanization etc. (Zheng et al., 2018). Despite the availability of several hypoglycemic drugs such as biguanides, sulfonylureas, meglitinides, thiazolidinediones, α-glucosidase inhibitors, dipeptidyl peptidase-IV inhibitors etc. for the management of hyperglycemia in patients with T2DM, growing impact of diabetes and its associated complications lead to one death per every eight second (IDF, 2019; ADA, 2020). Although available hypoglycemic agents control blood glucose levels, these have failed to reduce the mortality rate in patients with T2DM. Further, many currently used hypoglycemic drugs are associated with several adverse effects such as hypoglycemia, weight gain, gastrointestinal distress such as diarrhea, nausea, vomiting, etc. (Luna and Feinglos, 2001; Shrestha et al., 2017). As, diabetes is a major health care burden, there is a need for novel antidiabetic drugs with improved efficacy and less side/adverse effects.

Phytomedicines have evolved from a plethora of medicinal plants with therapeutic potentials. Medicinal plants as key elements in phytomedicines have been recognized as rich sources of drug leads for the management of diabetes acting through diverse mechanisms. These are good resources of phamacophores which could be successfully blended with scientific scrutinization for the development of novel hypoglycemic agents. Several phytomedicines have targeted in depth pathophysiology of diabetes through multiple mechanisms with an aim of developing safe, effective and novel antidiabetic agents (Choudhury et al., 2018; Salehi et al., 2019).

*Coccinia grandis* (Linn.) Voigt, which belongs to the Cucurbitaceae family, is an edible perennial climber. From time immemorial, it has been widely used as an ingredient in Sri Lankan traditional herbal medicine for the treatment of several diseases including diabetes mellitus, skin diseases, urinary tract infections, bronchitis, ulcers etc. (Ediriweera and Ratnasooriya, 2009). The plant is named as Scarlet gourd in English and Kem wel or Kowakka locally. A cross sectional survey conducted among adults with T2DM using an interviewer based questionnaire has reported that the leaves of *C. grandis* were used as a complementary medicine for the management of diabetes in Sri Lanka (Medagama et al., 2014). The antidiabetic potential of leaves of *C. grandis* has been investigated in streptozotocin induced diabetic rats and via α-amylase and α-glucosidase inhibition assays (Attanayake et al., 2015a; Mohammed et al., 2016; Sutradhar et al., 2011; Pulbutr et al., 2017). Its safety assessment conducted according to the Organization for Economic Co-operation and Development (OECD) guidelines has been established *in vivo* (Attanayake et al., 2013). A reduction in postprandial blood glucose concentration measured by oral glucose tolerance test (OGTT) in healthy subjects upon the treatment of raw leaves of *C. grandis* has also been observed (Munasinghe et al., 2011). Antioxidant, anti-inflammatory and antihyperlipidemic properties of *C. grandis* were also scientifically proven in animal models. (Attanayake et al., 2015b; Deshpande et al., 2011; Mohammed et al., 2016). A study conducted on leaves of *C. grandis* of Sri Lankan origin has revealed that one of the mechanisms of antihyperglycemic activity of leaves of *C. grandis* as presumably by restoration of function of pancreatic β-cells (Attanayake et al., 2019). Further, the *in vitro* glycation induced protein cross-linking inhibitory effect of the leaf extract of *C. grandis* has been proven (Perera and Handuwalage, 2015). Several other possible mechanisms such as reduction in the absorption of glucose in small intestine, increment of glucose uptake by peripheral tissues and etc., might cause antihyperglycemic activity of *C. grandis* (Attanayake et al., 2016b). The presence of secondary metabolites as polyphenols, alkaloids flavonoids, saponins, etc. of *C. grandis* may contribute to its antidiabetic activity as suggested by preclinical data (Al-Madhagy et al., 2019; Attanayake et al., 2016a). Cucurbitacins B and D, Cephalandrol, Cephalandrin A and B and related analogs have been identified as bioactive compounds responsible for the glucose lowering effect in the standardized extract of *C. grandis* (Subbiah, 2008). In addition, taxaterone, tinsoporin compound A and B, ferulic acid, methyl caffeate, ligstroside, trans-p- coumaric acid, kaempferol-3-O-β- D-glucoside have been identified (Bindurani and Singh, 2019; Al-Madhagy et al., 2019). However, the exact antidiabetic principle of the aforementioned bioactive compounds has not been reported and further research is warranted. Based on the promising results in preclinical studies, Attanayake and co-workers (2016a) have recommended the use of aqueous leaf extract of *C. grandis* for the development of commercially viable antidiabetic herbal drug.

Despite the presence of preclinical evidences on *in vitro* and *in vivo* antidiabetic activity of *C. grandis* leaves extract, efficacy of the leaves of *C. grandis* in a form of hypoglycemic drug, the clinical utility and the safety assessment of the herbal drug of *C. grandis* in patients with diabetes remains untapped to date. We herein, report the antidiabetic efficacy and safety of the herbal drug of *C. grandis* through a randomized double blind, placebo controlled clinical trial in newly diagnosed T2DM patients with an aim of developing a commercially viable antidiabetic phytomedicine targeting the effective management of T2DM in clinical practice.

## Materials and method

### *Ethics*

Ethical clearance for the study was granted by Faculty of Medicine, University of Ruhuna, Sri Lanka (14.06.2017:3.9). The clinical trial protocol was registered in the clinical trial registry, Sri Lanka (SLCTR/2018/012). Written informed consent was obtained from all study subjects after explaining the study protocol and informing of that the output of the intervention would be published in scientific journals without their identities.

### *Plant material*

The leaves of *C. grandis* were collected from the Southern region of Sri Lanka. The plant was authenticated at the National Herbarium comparing authentic samples at the Herbarium, Royal Botanical Gardens, Peradeniya, Sri Lanka. A voucher specimen was preserved at the Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Sri Lanka (B/2017/Wasana 01).

### *Preparation of herbal drug of C. grandis and placebo*

Previously standardized aqueous refluxed (4 h) plant extract of *C. grandis* (Attanayake et al., 2016) was concentrated to obtain a powder (yield of 30% w/w) using a freeze dryer (Model: Telstor LyoBeta 4 ps). The standard freeze-drying conditions were optimized by freezing the

hot water extract at – 40 °C for 3 h, followed by vacuum chamber preparation for one hour (60 °C) and condenser preparation for 1 h (pressure set up to 56 μbar). The drying was carried out continuously for 14 h at -40 °C. Final sample was kept for defrosting for two hours before taking from the freeze dryer.

Corn starch (500 mg/capsule) and 100% genuine freeze-dried powder of *C. grandis* (500 mg/capsule) aqueous leaves extract were filled into opaque gelatin capsules using an automated capsule filling machine to prepare placebo and herbal capsules respectively. We independently confirmed herbal drug content using high performance liquid chromatography (HPLC). The HPLC fingerprint profile of the herbal drug of *C. grandis* is shown in Supplementary file, Fig 1. Prior to perform HPLC analysis, the content of the herbal drug of *C. grandis* was dissolved in water:acetonitrile (5:95) mixture, sonicated for 30 min and filtered through 0.45 μm syringe cartridge filter. The filtrate was introduced for the HPLC analysis.

### *Study design*

This phase II clinical trial was a single center with balanced randomization (1:1), double blind, placebo controlled, parallel group study with seven visits. All visits of the study were scheduled within 14 days of each other. The study was clearly explained to study subjects by the researchers and an individual questionnaire including socioeconomic and demographic data was completed for each patient through comprehensive interviews before the commencement of the trial. All study subjects were given a subject diary to record adverse events experienced during the study period and to note down the daily use of study medication. At visit 1, recruited patients were randomly assigned following a simple randomization procedure using random number table into two groups in 1:1 ratio either to receive the herbal capsule of *C. grandis* or the placebo capsule for two weeks by an independent Medical Officer. The herbal and placebo drugs were prepacked in bottles and consecutively numbered for each patient according to the randomization schedule. Each patient was assigned an order number and received the capsules in the corresponding prepacked bottle. During the trial, the researchers and patients were blinded to the treatment assignments due to the identical shape, color, weight and size of the herbal and placebo capsules and their containers. Subjects were advised to take one capsule per day after lunch. Accordingly, patients were given herbal or placebo capsules at their second, third, fourth, fifth and sixth visits for two weeks ahead. The records of the diary and the number of remaining capsules in the bottle at the end of every two weeks throughout the trial period were used as a measure of direct and indirect compliance respectively. The study subjects who consumed more than 90% of capsules and with the due recordings in their diaries, had good compliance and they were included for statistical analysis.

### *Patients*

Patients with newly diagnosed T2DM, who presented and referred to the University Medical Clinic, Teaching Hospital, Karapitiya, Galle, Sri Lanka, were screened by the repeat assessment of FPG concentration. Galle is a major commercial city in Sri Lanka, which is a developing

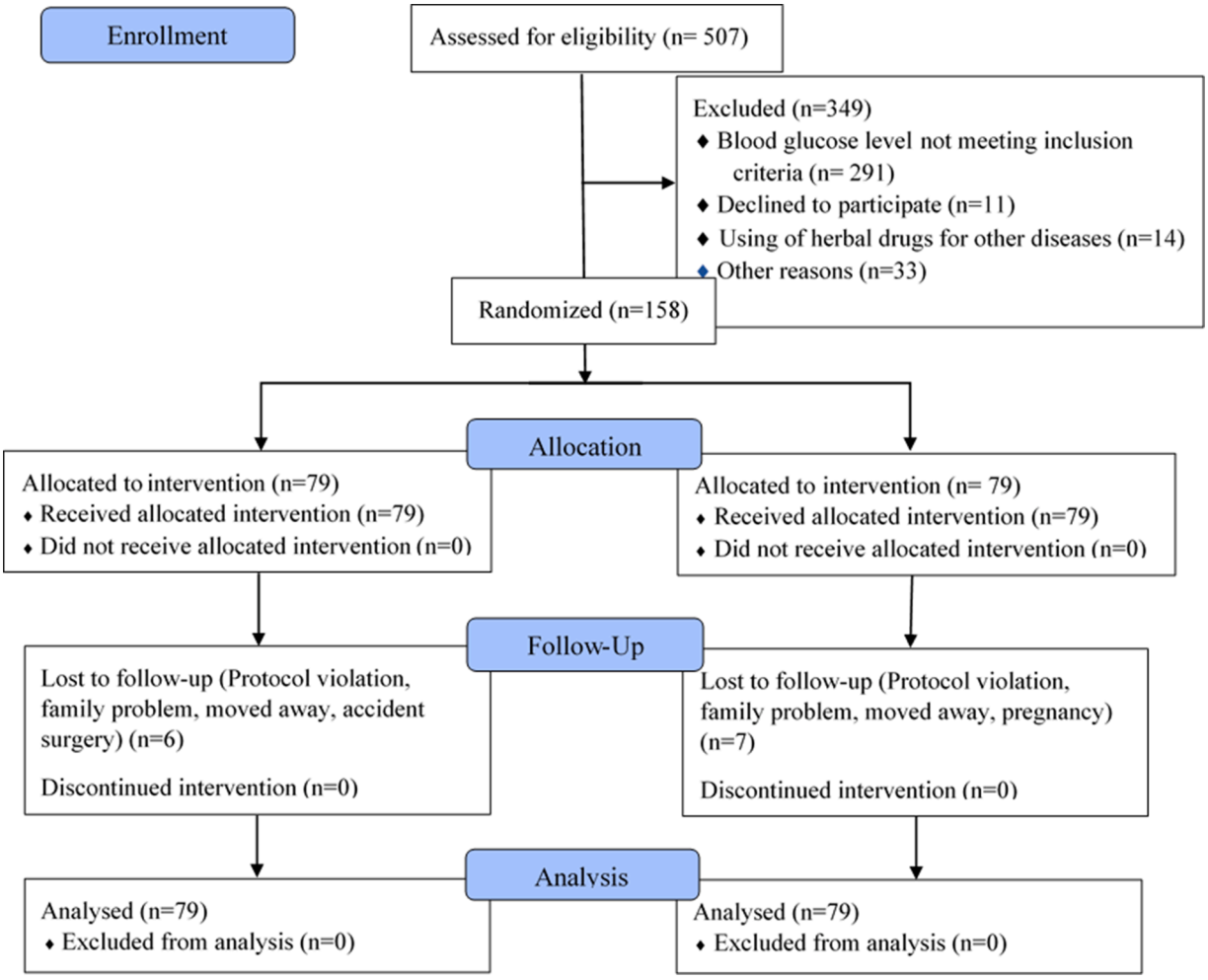


**Fig. 1.** CONSORT follow chart of the clinical trial.

country in South East Asian region with 8.6% prevalence of diabetes. Among the screened subjects, the patients with newly diagnosed T2DM (detected to have diabetes for the first time), who are having FPG concentration between 6.99-8.88 mmol/L were invited to attend the Clinical Investigation Unit, Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka on the second day of their first screening. On that day, after repeating FPG test, the patients with FPG concentration; 6.99-8.88 mmol/L and/or glycated hemoglobin ($HbA_{1C}$) percentage; 6.3 – 7.5% were enrolled for the study under the supervision of consultant physician as one of the investigators in the present trial. Other inclusion criteria for the study participants included being able to communicate effectively with the study personnel and to understand the nature of the study and willing to give written informed consent at the enrollment. None of the enrolled patients received any other anti-diabetic pharmacotherapies during the clinical trial period. They only received general instructions on physical activities recomended by the ADA and they were advocated by a single qualified dietician at every other visit during the clinical trial period. The exclusion criteria are summarized in Supplementary file, Table 1. By considering 5% reduction in the mean of $HbA_{1C}$ % in herbal drug of *C. grandis* treated group, confidence interval of 95%, $\alpha = 0.05$ and power of 80%, 72 patients with newly diagnosed T2DM were proposed per group. Considering the possible dropouts of 10% of the subjects during the study period, 79 patients were proposed to each group.

*Outcomes*

According to the objectives of the study, primary outcome was the determination of antidiabetic efficacy of the herbal drug of *C. grandis* by estimation of percenatge of $HbA_{1C}$, FPG concentration and fasting serum concentration of fructosamine, insulin and calculation of insulin resistance in newly diagnosed patients with T2DM. The secondary outcome was the determination of antihyperlipidemic efficacy of the herbal drug in terms of serum levels of total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) and calculation of the atherogenic, cardio-protective and coronary risk indices in newly diagnosed patients with T2DM. The tertiary outcome was the determination of renal, hepatic and hematological safety of the herbal drug of *C. grandis* in the study subjects. Renal toxicity was evaluated based on serum concentration of creatinine. Liver toxicity was assessed based on the serum concentration of gamma glutamyl transferase (γ-GT, EC 2.3.2.2), alanine aminotransferase (ALT, EC 2.6.1.2), aspartate aminotransferase (AST, EC 2.6.1.1) and alkaline phosphatase (ALP, EC 3.1.3.1). The hematological parameters such as white blood cells (WBC), red blood cells (RBC), hemoglobin, platelet count, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were assessed for the herbal drug of *C. grandis* in recruited patients.

*Blood sampling and biochemical measurements*

Overnight fasting (8-10 h) venous blood samples (8-10 ml) were collected into plasma tubes and gel tubes from each participant before 9:00 am at each visit by a trained phlebotomist. Serum and plasma were separated by centrifugation at 2,500 rpm for 10 min and immediately stored at -80 °C. The biochemical assessments were performed on collected serum or plasma samples.

*Glycemic parameters*

$HbA_{1C}$ percentage in blood was determined by resin exchange method using Stanbio glycohemoglobin test kit (USA) at the baseline and at the end of the intervention. FPG concentration was assessed at each visit by glucose oxidase method (Biorex, UK). Serum concentration of fructosamine was quantified at every other visit by enzymatic method (MaxDiscovery™, USA). Serum concentration of insulin was measured by ELISA method (DRG, USA) and insulin resistance was calculated using homeostasis model assessment (HOMA-IR) (Matthews et al., 1985) at the baseline and at the end of the intervention.

*Lipid profile parameters*

Serum concentration of TC, TG and HDL-C were estimated using enzymatic methods (Stanbio, USA) and serum concentration of LDL-C

**Table 1**
Baseline characteristics of the study subjects who were randomized.

| Baseline characteristics | Test group (n=79) | Placebo group (n=79) |
|---|---|---|
| Socio-demographic data | | |
| Age (years) | 48.41 (6.97) | 48.87 (7.20) |
| Sex (male) | 32 (40.5) | 30 (38.0) |
| Anthropometric data | | |
| Body mass (kg) | 62.10 (11.46) | 61.23 (11.62) |
| Height (cm) | 152.28 (34.28) | 156.66 (10.26) |
| BMI ($kg/m^2$) | 25.55 (3.91) | 24.95 (3.93) |
| WC (cm) | 89.66 (8.72) | 88.49 (8.89) |
| Blood pressure (mm Hg) | | |
| Systolic | 122.30 (15.55) | 126.13 (18.08) |
| Diastolic | 79.08 (8.46) | 78.77 (9.45) |
| Clinical data | | |
| $HbA_{1C}$ (%) | 6.32 (0.58) | 6.49 (0.64) |
| Insulin (mIU/l) | 18.92 (10.23) | 16.99 (10.37) |
| Fructosamine (mmol/l) | 0.34 (0.11) | 0.35 (0.12) |
| FPG (mmol/l) | 7.51 (0.79) | 7.33 (0.57) |
| HOMA-IR | 6.43 (3.61) | 5.57 (3.48) |
| TC (mmol/l) | 4.74 (0.80) | 4.73 (0.82) |
| HDL-C (mmol/l) | 1.21 (0.41) | 1.18 (0.29) |
| TG (mmol/l) | 1.44 (0.41) | 1.49 (0.45) |
| LDL-C (mmol/l) | 2.86 (0.87) | 2.84 (0.87) |
| VLDL-C (mmol/l) | 0.66 (0.19) | 0.67 (0.22) |
| AI | 3.31 (1.50) | 3.27 (1.28) |
| CPI | 0.52 (0.43) | 0.48 (0.29) |
| CRI | 4.31 (1.50) | 4.27 (1.28) |
| Creatinine (μmol/l) | 84.83 (12.36) | 80.68 (13.26) |
| ALT (U/l) | 22.68 (8.96) | 20.28 (9.10) |
| AST (U/l) | 19.53 (10.70) | 18.26 (7.27) |
| ALP (U/l) | 55.81 (16.42) | 58.57 (18.00) |
| γ-GT (U/l) | 23.67 (11.08) | 22.03 (10.26) |
| WBC (Cumm) | 7195.70 (1746.39) | 6832.28 (1700.92) |
| RBC (Million/μl) | 4.56 (0.45) | 4.55 (0.38) |
| Hemoglobin (g/dl) | 13.36 (1.69) | 13.40 (1.22) |
| Platelet count/$10^4$ (Cumm) | 28.03 (7.00) | 27.89 (6.12) |
| PCV (%) | 39.79 (4.26) | 38.80 (3.87) |
| MCV (FL) | 85.76 (4.81) | 86.27 (4.11) |
| MCH (pg) | 29.10 (2.35) | 29.47 (1.69) |
| MCHC (g/dl) | 33.85 (1.42) | 34.14 (1.09) |
| Marital status | | |
| Single | 5 (6.3) | 11 (13.9) |
| Married | 71 (89.9) | 66 (83.5) |
| Divorced | 1 (1.3) | 1 (1.3) |
| Widowed | 2 (2.5) | 1 (1.3) |
| Education | | |
| Not schooled | - | 1 (1.4) |
| Primary education | 6 (7.6) | 8 (10.1) |
| Secondary education | 60 (75.9) | 59 (74.7) |
| Certificate/Diploma | 10 (12.7) | 8 (10.1) |
| Graduate | 2 (2.5) | - |
| Any other | 1 (1.4) | 3 (3.8) |
| Occupation | | |
| Professional | 4 (4.1) | 5 (6.3) |
| Skilled | 18 (19.2) | 21 (26.6) |
| Unemployed | 19 (26.0) | 21 (26.6) |
| Any other | 38 (50.7) | 32 (40.5) |
| Monthly income (Sri Lankan Rupees) | | |
| < 5 000 | - | 6 (7.6) |
| 5 000-15 000 | 8 (10.1) | 11 (13.9) |
| 15 000-30 000 | 36 (45.6) | 38 (48.1) |
| > 30 000 | 35 (44.3) | 24 (30.4) |

* Data are mean (SD) or number (%).

and VLDL-C were calculated using the Friedewald equation (Friedewald et al., 1972) at the baseline and at the end of the intervention.

Atherogenic, cardio-protective and coronary risk indices were calculated using serum lipid profile parameters (Azmi and Qureshi, 2012; Parsaeyan, 2012).

*Toxicity parameters*

Serum concentration of creatinine, γ-GT, ALT, AST, and ALP were estimated by enzymatic reactions (Stanbio, USA). Hematological parameters were assessed using a hematological analyzer (Mindray BC5150, China). All the toxicity parameters except γ-GT were estimated at each visit and the assessment of γ-GT was carried out at the baseline, visit 4 and at the end of the intervention.

All laboratory tests were quality controlled and biochemical estimations were performed in duplicates using the same kits.

FPG concentration, percentage of $HbA_{1C,}$ serum concentration of TC, TG, HDL-C, ALT, AST, ALP, γ-GT and creatinine were performed on UV visible spectrophotometer (Spectra max, USA).

*Blood pressure measurement*

Blood pressure was monitored at each visit of the intervention; both systolic and diastolic blood pressure (SBP and DBP) were measured three times. The average of three measurements was used for the analysis. Monitoring of blood pressure was performed on the same arm throughout the trial period. Hypertension was defined as a SBP $\geq$ 140 mm Hg and a DBP $\geq$ 90 mm Hg on three repeated measurements.

*Anthropometric assessments*

Anthropometric parameters such as height, mass and waist circumference (WC) of the study subjects were measured at the base line and end of the intervention. Body mass was measured in the fasting state using a portable scale with light clothes and without shoes to the nearest 0.1 kg accuracy. Height was measured in standing position without shoes and while keeping the shoulder in erect position near to wall, using a stadiometer to the nearest 0.1 cm accuracy. Body mass index (BMI) was calculated as mass in kilogram divided by the height in meter squared ($kg/m^2$). WC was measured after locating and palpating the bony landmarks of the lowest rib and the iliac crest in a horizontal plane around the abdomen by using a non-stretching, flexible tape meter nearest 0.1 cm accuracy. Same equipment was used for the measurements throughout the trial.

*Statistical analysis*

All the data were analyzed using SPSS software version 25.0. Continuous data were expressed as mean (SD) while the categorical data were expressed as frequencies and percentages. Normality of the data sets was assessed using D'Agostino-Pearson omnibus $K^2$ test. The comparison of the differences in the mean (SD) changes from baseline to end of the intervention between the treatment and the placebo group was performed for the primary, secondary and tertiary endpoints. This was supplemented by a repeated measures analysis. The between group changes were assessed using unpaired sample *t*-test and Mann–Whitney *U* test for normally and non-normally distributed data, respectively. The within group changes were compared using paired sample *t*-test or Wilcoxon signed-rank test for normally and non-normally distributed data, respectively. Intention-to-treat analysis was performed for the primary, secondary and tertiary endpoints. $p \leq 0.05$ was considered as the statistically significant at each of the case.

## Results

A total number of 507 subjects (30-60 years in both gender) with newly detected T2DM were screened and among them, 158 patients were enrolled for the study during February 2018 to September 2019. Baseline characteristics of the enrolled patients from the time when diagnosis of 2 ± 1 days for test group and placebo group are shown in Table 1.

A total number of 73 patients in test group and 72 patients in placebo group completed the three months of study period. Figure 1 represents the CONSORT follow chart of the present trial.

No side effects were observed in any of the patient and they had good compliance for the intervention.

*Effect of herbal capsule on glycemic parameters*

The mean changes of glycemic parameters from baseline to end of the intervention of both test and placebo group are shown in Table 2. At the base line, there were no statistically significant differences in selected glycemic parameters as $HbA_{1C}$, insulin, fructosamine, FPG and in HOMA–IR between test group and placebo group ($p > 0.05$). At the end of the intervention, there were significant differences in $HbA_{1C}$ ($p < 0.001$), fructosamine ($p = 0.005$) and FPG ($p < 0.001$) between test group and placebo group. Indeed, there were significant differences ($p < 0.05$) in FPG between test group and placebo group from the visit 4 onward to the end of the intervention. The change of FPG and serum concentration of fructosamine in test group and placebo group during the intervention period are shown in Figure 2 and Figure 3, respectively. There were significant mean (SD) changes ($p < 0.001$) of all the glycemic parameters from baseline to end of the intervention (visit 7) between the treatment and the placebo group. In addition, there were significant mean (SD) changes of FPG concentration from baseline to visit 2 ($p = 0.002$), visit 3 ($p < 0.001$), visit 4 ($p < 0.001$), visit 5 ($p < 0.001$) and visit 6 ($p < 0.001$) between the treatment and placebo group while with the significant mean (SD) changes of serum concentration of fructosamine from baseline to visit 5 ($p < 0.001$).

*Effect of herbal capsule on serum lipid profile, atherogenic, cardio-protective and coronary risk indices*

Changes in serum lipid profile, AI, CPI and CRI in patients during the study period are shown in Table 3. No significant changes in lipid profile parameters and any of the selected indices were observed between test group and placebo group at the base line ($p > 0.05$). However, there were significant changes in TG ($p < 0.001$) and VLDL-C ($p < 0.001$)

**Table 2**
Glycemic parameters in study subjects baseline to three months.

| Variables | Group | Baseline mean (SD) | After intervention mean (SD) | Mean (SD) changes | *p* value |
|---|---|---|---|---|---|
| $HbA_{1C}$ (%) | Test | 6.32 (0.58) | 5.66 (0.67) | 0.66 (0.52) | <0.001 |
| | Placebo | 6.49 (0.64) | 6.43 (0.74) | 0.06 (0.64) | |
| Insulin (mIU/l) | Test | 18.92 (10.23) | 17.02 (8.64) | 1.91 (2.95) | <0.001 |
| | Placebo | 16.99 (10.37) | 18.27 (12.39) | -1.28 (9.32) | |
| Fructosamine (mmol/l) | Test | 0.34 (0.11) | 0.32 (0.11) | 0.02 (0.03) | <0.001 |
| | Placebo | 0.35 (0.12) | 0.36 (0.12) | -0.01 (0.03) | |
| FPG (mmol/l) | Test | 7.51 (0.79) | 6.08 (0.66) | 1.43 (0.55) | <0.001 |
| | Placebo | 7.33 (0.57) | 7.30 (0.61) | 0.04 (0.48) | |
| HOMA-IR | Test | 6.43 (3.61) | 4.70 (2.59) | 1.73 (1.31) | <0.001 |
| | Placebo | 5.57 (3.48) | 5.94 (4.18) | -0.37 (3.22) | |

* Data are mean (SD).

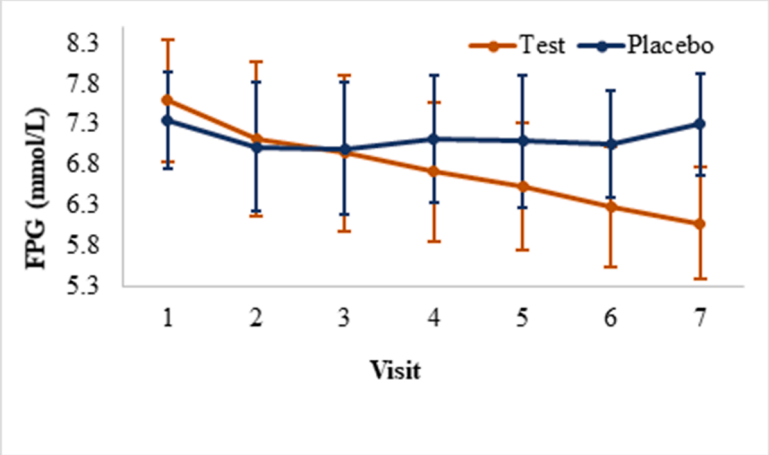


**Fig. 2.** Changes in mean values of FPG in test group and placebo group. Each data point represents mean (SD).

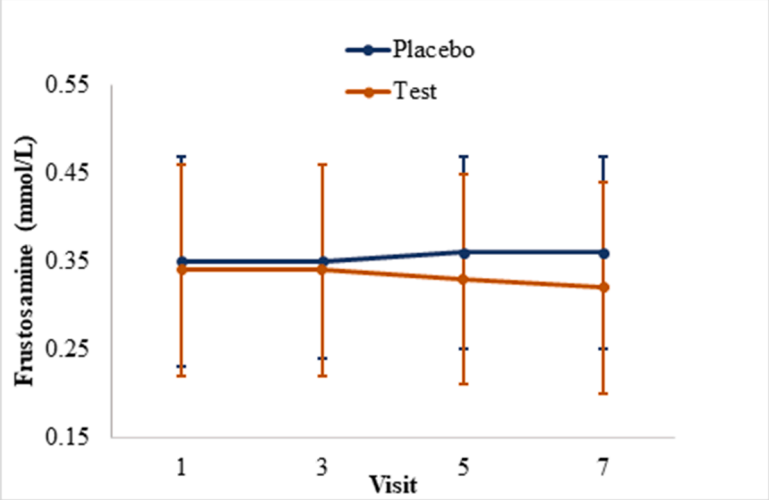


**Fig. 3.** Changes in mean values of fructosamine in test group and placebo group. Each data point represents mean (SD).

between test group and placebo group at the end of the intervention. Further, there were significant mean (SD) changes ($p < 0.001$) of TG and VLDL-C from the baseline to end of the intervention between the treatment and the placebo group.

*Effect of herbal capsule on safety profile*

The comprehensive laboratory assessments including renal, hepatological and hematological toxicity parameters at visit 1 and visit 7 are summarized in Table 4. All the tested parameters were within the reference range at the base line, through-out the intervention and even at the end of the intervention in both groups.

*Effect of herbal capsule on blood pressure measurements and anthropometric assessments*

The changes of blood pressure measurements and anthropometric parameters of BMI and WC in study subjects at the visit 1 and visit 7 are shown in Table 5. There were no significant changes observed upon the treatment of herbal drug of *C. grandis*.

## Discussion

One of the most challenging conundrums in drug discovery is the establishment of a well-balanced efficacy-safety profile of commercially viable new drugs. The present study aimed to determine the clinical utility of a newly developed herbal drug of *C. grandis* by means of efficacy and safety in newly diagnosed patients with T2DM, through a randomized, double blind, placebo controlled clinical trial. Randomized, double blind, placebo controlled clinical trials are considered as the gold standard of epidemiologic studies that may assess efficacy of a treatment in a group of patients by comparing the outcomes of patients receiving a placebo (Misra, 2012). The dose of the herbal drug (500 mg per day) was determined based on the recommendations of Ayurvedic physicians, outputs of the pre-clinical studies and percentage yield of the refluxed hot water extract of *C. grandis* leaves.

The rising burden of microvascular complications in diabetes is associated with poor glycemic control as measured by $HbA_{1C}$ as the gold standard parameter in monitoring the glycemic control (Sabanayagam et al., 2009). Hence, the assessment of $HbA_{1C}$ percentage is recommended as a standard of care for testing and monitoring of T2DM and its associated microvascular complications (WHO, 2011). The short-term glycemic control of the herbal drug of *C. grandis* in newly diagnosed patients with T2DM was assessed in the present study mainly in terms of

**Table 3**
Changes of lipid profile parameters, atherogenic, cardio-protective, and coronary risk indices in study subjects baseline to three months.

| Variables | Group | Baseline mean (SD) | After intervention mean (SD) | Mean (SD) changes | *p* value |
|---|---|---|---|---|---|
| TC (mmol/l) | Test | 4.74 (0.80) | 4.65 (0.79) | 0.09 (0.69) | 0.055 |
| | Placebo | 4.73 (0.82) | 4.81 (0.73) | -0.08 (0.81) | |
| HDL-C (mmol/l) | Test | 1.21 (0.41) | 1.29 (0.39) | -0.08 (0.30) | 0.646 |
| | Placebo | 1.18 (0.29) | 1.30 (0.44) | -0.12 (0.39) | |
| TG (mmol/l) | Test | 1.44 (0.41) | 1.28 (0.38) | 0.16 (0.17) | <0.001 |
| | Placebo | 1.49 (0.45) | 1.55 (0.51) | -0.06 (0.41) | |
| LDL-C (mmol/l) | Test | 2.86 (0.87) | 2.77 (0.90) | 0.09 (0.78) | 0.650 |
| | Placebo | 2.84 (0.87) | 2.81 (0.83) | 0.03 (0.87) | |
| VLDL-C (mmol/l) | Test | 0.66 (0.19) | 0.59 (0.18) | 0.07 (0.08) | <0.001 |
| | Placebo | 0.67 (0.22) | 0.71 (0.23) | -0.04 (0.20) | |
| AI | Test | 3.31 (1.50) | 2.98 (1.43) | 0.33 (1.11) | 0.213 |
| | Placebo | 3.27 (1.28) | 3.24 (1.79) | 0.03 (1.37) | |
| CPI | Test | 0.52 (0.43) | 0.58 (0.46) | -0.07 (0.44) | 0.342 |
| | Placebo | 0.48 (0.29) | 0.58 (0.46) | -0.09 (0.40) | |
| CRI | Test | 4.31 (1.50) | 3.98 (1.43) | 0.33 (1.11) | 0.213 |
| | Placebo | 4.27 (1.28) | 4.24 (1.79) | 0.03 (1.37) | |

* Data are mean (SD).

reduction in $HbA_{1C}$ percentage and serum fructosamine concentration. The reduction in mean $HbA_{1C}$ percentage and fructosamine level implicate a proper glycemic control during the period of intervention in patients with T2DM. The determination of the herbal drug of *C. grandis* on glycemic parameters in patients with diabetes was evaluated at each visit for the entire study period using a commonly available test of FPG which measures plasma glucose concentration at a given point of time. With the treatment of herbal drug of *C. grandis*, mean FPG was significantly decreased ($p < 0.05$) from the visit 4 onward to the end acquiring a satisfactory glycemic control in recently diagnosed diabetic patients with signifying the antihyperglycemic properties of the herbal drug. The results of the study revealed that the treatment with herbal drug of *C. grandis* for three months was able to produce a difference in the mean change of $HbA_{1C}$ as 0.60% (Test; 0.66% - placebo; 0.06%) and of FPG concentration as 1.39 mmol/l (Test; 1.43 mmol/l - placebo; 0.04 mmol/l). Importantly, the reduction in FPG and $HbA_{1C}$ are comparable with previously reported studies on the improvement of glycemic indices by oral hypoglycemic agents. Indeed, a multicenter, double blind, dose response study of metformin treatment caused a mean changes of FPG at 7 week to 14 week with a placebo by 19 mg/dl (for 500 mg) to 84 mg/dl (2000 mg) in patents with T2DM. Further differences in $HbA_{1C}$ % ranged from 0.6% to 2.0% in the metformin at a dose of 500 mg to 2000 mg daily, in the above study group, respectively (Garber et al., 1997). In a randomized, parallel group, placebo controlled trial conducted for 12 weeks revealed that administration of dapagliflozin at a dose of 5 to 50 mg reduced FPG from -0.89 to – 1.72 mmol/l when compared to placebo, respectively (List et al., 2009). Accordingly, the results of the present study are in line with the previously reported studies on improvement of glycemic indices by available anti-diabetic pharmacotherapies. Even though, there were no significant change of fasting insulin concentration observed in test group when compared to placebo group at the end of the intervention, within the test group analysis revealed that herbal drug of *C. grandis* was able to reduce fasting insulin concentration significantly ($p < 0.001$) and thereby increased the insulin sensitivity in diabetic patients. Further, a significant reduction of HOMA-IR index in test group when compared to its base line value also indicated the probable insulin sensitizer effect of the herbal drug of *C. grandis* in patients with T2DM.

**Table 4**
Changes of safety parameters in study subjects baseline to three months.

| Variables | Group | Baseline mean (SD) | After intervention mean (SD) | Mean (SD) changes | *p* value |
|---|---|---|---|---|---|
| Creatinine (μmol/l) | Test | 84.83 (12.36) | 83.09 (12.48) | 1.74 (11.42) | 0.867 |
| | Placebo | 80.68 (13.26) | 79.98 (13.46) | 0.70 (9.48) | |
| ALT (U/l) | Test | 22.68 (8.96) | 21.29 (8.19) | 1.39 (5.43) | 0.479 |
| | Placebo | 20.28 (9.10) | 19.38 (9.09) | 0.90 (6.98) | |
| AST (U/l) | Test | 19.53 (10.70) | 18.97 (9.64) | 0.56 (5.35) | 0.312 |
| | Placebo | 18.26 (7.27) | 16.38 (5.10) | 1.88 (7.10) | |
| ALP (U/l) | Test | 55.81 (16.42) | 53.96 (16.66) | 1.86 (9.57) | 0.134 |
| | Placebo | 58.57 (18.00) | 58.98 (17.71) | -0.41 (9.37) | |
| γ-GT (U/l) | Test | 23.67 (11.08) | 20.92 (10.14) | 2.76 (4.25) | 0.386 |
| | Placebo | 22.03 (10.26) | 18.26 (8.05) | 3.77 (5.75) | |
| WBC (Cumm) | Test | 7195.70 (1746.39) | 6909.29 (1463.85) | 286.41 (1360.20) | 0.331 |
| | Placebo | 6832.28 (1700.92) | 6762.33 (1585.47) | 69.95 (1426.99) | |
| RBC (Million/μl) | Test | 4.56 (0.45) | 4.57 (0.42) | -0.01 (0.33) | 0.804 |
| | Placebo | 4.55 (0.38) | 4.53 (0.33) | 0.02 (0.22) | |
| Hemoglobin (g/dl) | Test | 13.36 (1.69) | 13.37 (1.62) | -0.01 (0.77) | 0.640 |
| | Placebo | 13.40 (1.22) | 13.45 (1.28) | -0.05 (0.68) | |
| Platelet count/$10^4$ (Cumm) | Test | 28.03 (7.00) | 27.13 (5.66) | 0.90 (4.98) | 0.551 |
| | Placebo | 27.89 (6.12) | 27.27 (6.34) | 0.62 (3.00) | |
| PCV (%) | Test | 39.79 (4.26) | 39.73 (4.60) | 0.06 (2.36) | 0.628 |
| | Placebo | 38.80 (3.87) | 39.34 (3.38) | -0.54 (3.09) | |
| MCV (FL) | Test | 85.76 (4.81) | 85.59 (5.24) | 0.17 (2.11) | 0.649 |
| | Placebo | 86.27 (4.11) | 86.50 (3.56) | -0.23 (2.04) | |
| MCH (pg) | Test | 29.10 (2.35) | 29.16 (2.39) | -0.06 (0.88) | 0.765 |
| | Placebo | 29.47 (1.69) | 29.42 (2.06) | 0.06 (1.36) | |
| MCHC (g/dl) | Test | 33.85 (1.42) | 34.07 (1.48) | -0.22 (1.25) | 0.731 |
| | Placebo | 34.14 (1.09) | 34.28 (1.35) | -0.14 (1.46) | |

* Data are mean (SD).

Dyslipidemia in patients with T2DM is a major risk factor for cardiovascular diseases. Results of our study showed a significant reduction in serum concentration of TG and VLDL-C in herbal drug treated patients compared to placebo treated patients at the end of the intervention. Also it was observed as significant increment in HDL-C compared to its' base line value in the herbal drug of *C. grandis* treated group. Indeed, the results of the present study revealed that there is a difference in the mean change for TG as 0.22 mmol/l (test; 0.16 mmol/l– placebo; -0.06

**Table 5**
Changes in blood pressure and anthropometric parameters in study subjects baseline to three months.

| Variables | Group | Baseline mean (SD) | After intervention mean (SD) | Mean (SD) changes | *p* value |
|---|---|---|---|---|---|
| Blood pressure (mm Hg) | Test | SBP; 122.30 (15.55) | SBP; 122.28 (9.54) | SBP; 0.03 (12.69) | 0.250 |
| | | DBP; 79.08 (8.46) | DBP; 80.15 (6.45) | DBP; -1.08 (8.72) | 0.371 |
| | Placebo | SBP; 126.13 (18.08) | SBP; 123.29 (17.56) | SBP; 2.84 (24.35) | |
| | | DBP; 78.77 (9.45) | DBP; 78.28 (9.83) | DBP; 0.49 (12.89) | |
| BMI (kg/m$^2$) | Test | 25.55 (3.91) | 25.20 (3.74) | 0.35 (1.34) | 0.684 |
| | Placebo | 24.95 (3.93) | 24.60 (3.74) | 0.35 (1.15) | |
| WC (cm) | Test | 89.66 (8.72) | 88.76 (7.88) | 0.90 (4.73) | 0.928 |
| | Placebo | 88.49 (8.89) | 88.03 (8.65) | 0.47 (3.45) | |

* Data are mean (SD).

mmol/l) and VLDL-C as 0.11 mmol/l (test; 0.07 mmol/l - placebo;-0.04 mmol/l) upon the three months of treatment of the herbal drug of *C. grandis* in patients with T2DM. Interestingly, the herbal drug of *C. grandis* was able to control elevations of lipid profile parameters along with control of glycemic status in patients with T2DM. It is known that decrement of VLDL-C lowers the risk of developing plaque deposits on arterial walls by allowing a non-restricted blood flow. Further a low level of TG is important in the management of cardiovascular diseases. The preclinical study reported by Singh et al. (2007) mentioned that the polyprenol, an isolated compound from the leaves of *C. grandis*, exerted a significant potential to lower serum concentration of TG and TC followed by a beneficial effect on HDL-C in dyslipidemic hamster model. The indices AI, CPI and CRI which are calculated from lipid profile parameters are strong markers for predicting the risk of atherosclerosis and coronary heart diseases. There were no significant differences observed in the mean changes of these indices from baseline to the end of the intervention between test and placebo group. However, within the group analysis results showed that a significant reduction of AI and CRI ($p = 0.014$) and a significant increment of CPI ($p = 0.008$) in the test group when compared to the base line values. This dual therapy of action by means of antihyperglycemic and an improvement in lipid profile parameters of the herbal drug of *C. grandis*, is important for the development of antidiabetic drugs targeting the management of diabetes mellitus and its' one important complication as dyslipidemia.

The per protocol analysis for the primary outcome measures of the present study also revealed that there were significant mean (SD) changes ($p < 0.001$) of all glycemic parameters and indices from the baseline to the end of the intervention between the treatment and the placebo group (Supplementary file, Table 2). Thus, the clinical effectiveness of the herbal drug of *C. grandis* against mild hyperglycemia is further supported with the efficacy in newly diagnosed patients with T2DM. Furthermore, results of the per protocol analysis performed for the secondary outcome measures revealed that there were significant mean (SD) changes ($p < 0.001$) of TG and VLDL-C from the baseline to end of the intervention between the treatment and the placebo group like in the case of intention-to-treat analysis (Supplementary file, Table 3). Even though results of the intention-to-treat analysis revealed that there was a borderline significant mean change ($p = 0.055$) in TC concentration from the baseline to end of the intervention between the treatment and the placebo group, however, per protocol analysis revealed that there was no significant mean change ($p = 0.170$) of serum TC concentration (Supplementary file, Table 3). All the above findings implicate the clinical utility and efficacy of the herbal drug of *C. grandis* in the management of hyperglycemia and serum lipid profile changes in newly diagnosed patients with T2DM.

Toxicity assessment of the present study showed that the daily use of 500 mg of herbal drug of *C. grandis* for three months by newly diagnosed T2DM patients was safe. There were no clinically meaningful differences in the overall incidence of the adverse experiences and major or minor episodes of hypoglycemia which lead to discontinuation of the trial in the test group compared with the placebo group. Only the patients who violated the protocol and faced accidental situations such as surgery, family problems and moving away from the study area were the causes for dropouts in the trial.

The strengths of the present clinical trial include the conductance of a double blind, placebo controlled, randomized protocol which is a strong advantage on controlling research bias and the protocol is rarely achievable during clinical trials of newly developed drugs. The trial preserved a high rate of adherence to the herbal drug of *C. grandis*, suggesting a high translational potential for the use by newly diagnosed diabetic patients. The enrollment of newly diagnosed patients with T2DM for the clinical trial delivers a fine advantage as their glycemic and lipid profile parameters have not been affected by lifestyle modifications or pharmacological interventions. Collectively all these strengths led to demonstrate real causality of the clinical trial. The limitations of the trial include not performing OGTT in the assessment of glycemic parameters, the short term nature of the trial with providing three months of utilization of the herbal drug of *C. grandis*. In the study we didn't assess the comparative efficacy of newly developed herbal drug of *C. grandis* with an active agent or their adorn action. Therefore, it is good to assess comparative efficacy or adorn action of *C. grandis* with an active agent in future.

## Conclusion

The results of the three months long, randomized, double blind, placebo controlled clinical trial revealed that the administration of newly developed herbal capsule of *C. grandis* (500 mg per day) improved glycemic indices and lipid profile parameters in newly diagnosed patients with T2DM. The herbal drug would be a therapeutic promise against development and progression of cardiovascular diseases and the drug may exert a potential on weight neutral or no significant weight gain in newly diagnosed patients with T2DM. This dual therapy of action by means of antihyperglycemic activity and improvement of lipid profile together with safety and tolerability of the herbal drug of *C. grandis* could be beneficial as a new therapeutic approach for the early management of patients with T2DM.

## Author contributions

Keddagoda Gamage Piyumi Wasana: Methodology, Validation, Formal analysis, Investigation, Data Curation and Writing - Original draft.

Anoja Priyadarshani Attanayake: Conceptualization, Methodology, Validation, Writing - Review and Editing, Supervision and Project administration.

Thilak Priyantha Weerarathna: Conceptualization, Methodology, Validation Writing - Review and Editing and Supervision

Kamani Ayoma Perera Wijewardana Jayatilaka: Review and Editing of the paper and Supervision.

All data were generated in-house, and no paper mill was used. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy.

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## Declaration of interest

None.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phymed.2020.153431.

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