Bitter gourd reduces elevated fasting plasma glucose levels in an intervention study among prediabetics in Tanzania

Michael B. Krawinkel[a],[*],[1], Christine Ludwig[a],[1],[2], Mark E. Swai[b], Ray-yu Yang[c], Kwok Pan Chun[d], Sandra D. Habicht[a],[c]

[a] Justus Liebig University Giessen, Institute of Nutritional Sciences, Wilhelmstrasse 20, D-35392 Giessen, Germany
[b] Kilimanjaro Christian Medical Centre, Moshi, United Republic of Tanzania (MES)
[c] The World Vegetable Center (WorldVeg), P.O. Box 42, Shanhua, 74151 Tainan, Taiwan (ROC)
[d] Hong Kong Baptist University, Faculty of Social Science, Department of Geography, Hong Kong, China

ARTICLE INFO

ABSTRACT

Ethnopharmacological relevance: Impaired glucose tolerance and diabetes mellitus have become major health issues even in non-industrialized countries. As access to clinical management is often poor, dietary interventions and alternative medicines are required. For bitter gourd, Momordica charantia L., antidiabetic properties have been claimed.

Aim of the study: The main objective of the intervention study was to assess antidiabetic effects of daily bitter gourd consumption of 2.5 g powder over the course of eight weeks among prediabetic individuals.

Materials and methods: In a randomized placebo-controlled single blinded clinical trial, 52 individuals with prediabetes were studied after consuming a bitter gourd or a cucumber juice. For reducing the impact of between subject differences in the study population, a crossover design was chosen with eight weeks for each study period and four weeks washout in between. Fasting plasma glucose was chosen as the primary outcome variable.

Results: Comparing the different exposures, the CROS analysis (t = -2.23, p = 0.031, r = 0.326) revealed a significant difference in the change of FPG of 0.31 mmol/L (5.6 mg/dL) with a trend ($R^2 = 0,42387$). The number of 44 finally complete data sets achieved a power of 0.82, with a medium-to-large effect size (Cohen's d 0.62). The effect was also proven by a general linear mixed model (estimate 0.31; SE: 0.12; p: 0.01; 95%CI: 0.08; 0.54). Not all participants responded, but the higher the initial blood glucose levels were, the more pronounced the effect was. No serious adverse effects were observed.

Conclusions: Bitter gourd supplementation appeared to have benefits in lowering elevated fasting plasma glucose in prediabetes. The findings should be replicated in other intervention studies to further investigate glucose lowering effects and the opportunity to use bitter gourd for dietary self-management, especially in places where access to professional medical care is not easily assured.

1. Introduction

Incidence rates of prediabetes and type 2 diabetes mellitus have been increasing worldwide in recent decades and 80% of diabetes patients occur in middle-income countries (IDF, 2013). For Sub-Saharan Africa, the current estimated prevalence of diabetes mellitus is 3.2%, with an expected increase to 4.2% by 2040. Many new diabetes patients (67%) go undiagnosed (IDF, 2015). Although prediabetes does not always progress to type 2 diabetes mellitus, up to 25% of cases do within three years after the diagnosis of impaired glucose tolerance (IGT) (Ministry of Health and Social Welfare, Tanzania, 2013). In the United Republic of Tanzania, recent rates of diabetes mellitus and prediabetes, the latter defined as IGT were 3.5% (IDF, 2015) and 9.1% (IDF, 2013).

In Tanzania, management and treatment facilities for diabetes are

insufficient (Ministry of Health and Social Welfare, Tanzania, 2013; Peck et al., 2014; Robertson et al., 2015). Thus, prevention of diabetes is critical to reduce the burden that arises from increased prevalence and uncontrolled diabetes with all its complications (Wyne, 2008). In addition to lifestyle interventions, which have been shown to positively influence glucose metabolism (Jenkins and Hagberg, 2011; Samjoo et al., 2013), medicinal plants with antidiabetic properties (Khan et al., 2012; Parikh et al., 2014; Tag et al., 2012; Yakubu et al., 2015) might be used to lower elevated blood glucose levels and, thus, to prevent or delay the onset of diabetes. *Momordica charantia,* also known as bitter gourd or bitter melon, is often consumed and also used in traditional Asian medicine to manage diabetes mellitus. The unripe fruit gets harvested in many Asian countries, but also in some African, South American, and Caribbean countries (Basch et al., 2003; Krawinkel and Keding, 2006). Although bitter gourd has been shown to have anti-diabetic effects in many in-vitro and in-vivo studies, results from clinical studies of diabetes patients are inconsistent. Effects among people with prediabetes have been rarely studied (Habicht et al., 2014; Efird et al., 2014).

The aim of the current study was therefore to assess antidiabetic effects of a dietary supplement of 2.5 g dried bitter gourd powder per day over an 8-weeks period among prediabetic participants in Moshi, Tanzania. Prediabetes was chosen for ethical reasons because frank diabetes mellitus deserves immediate effective treatment whilst in prediabetes, lifestyle changes are a solid management option.

2. Materials and methods

2.1. Subjects and study location

The study was conducted at the Kilimanjaro Clinical Research Institute (KCRI) located in Moshi Municipality, Kilimanjaro Region in northern Tanzania, with a regional population of 1640,087 in 2012 (National Bureau of Statistics, 2013).

Screening for prediabetes was performed using the following inclusion criteria: Fasting plasma glucose (FPG) values between 5.6 and 6.9 mmol/L (100–125 mg/dL) on two days or on one day in addition to HbA_{1c}-values between 5.7% and 7.5% (39–58 mmol/mol), BMI of 27–35 kg/m^2, blood pressure (BP) between 90/60 and 160/110 mmHg, age between 30 and 65 years, and a waist circumference > 80 cm for women and > 90 cm for men. Exclusion criteria were any clinically diagnosed chronic disease, taking medication regularly, having an identified glucose-6-phosphatase-dehydrogenase (G6PD) deficiency, heavy alcohol consumption, pregnancy, and lactation.

The sample size was calculated using G*Power 3.1 (Faul et al., 2007). The calculation was based on an expected difference of 0.56 mmol/l (10 mg/dl) in mean FPG between groups (two sample *t*-test), a standard deviation of 0.56 mmol/l (10 mg/dl) in each group, an alpha level of 0.05 and a power of 0.95. The calculated sample size was 54 participants. After screening, sixty-one eligible participants were included into the randomization process and placed into sequence groups. Randomization was performed using the Mersenne Twister random number generator (Matsumoto and Nishimura, 1998). To ensure equal distributions of female and male participants, both sexes were separately randomized to each group. All participants were instructed not to change their eating habits or physical activities during the intervention study. For details see CONSORT-diagram in Fig. 1 (Matsumoto and Nishimura, 1998).

2.2. Study design

The study was a randomized, single blinded, placebo-controlled, cross-over intervention study with the total study population divided into an AB-BA sequence. As this was the first study to examine anti-diabetic effects of bitter gourd among prediabetics, no double blinding was chosen to adequately intervene in cases of hypoglycemia due to the

supplement. "Group 1" (AB) started with bitter gourd supplementation, followed by placebo after a washout period of four weeks. "Group 2" (BA) started with placebo followed by bitter gourd supplementation with the same washout period in between. A four-week washout period has previously been shown to prevent carry-over effects (Tsai et al., 2012). Assessments were done before and after each supplementation phase and during weekly checkup visits of participants. The study flow is illustrated in Fig. 2.

2.3. Plant material

Plant material used was bitter gourd as treatment and cucumber as placebo. The exact botanical name of bitter gourd is *Momordica charantia L.*, which can be found under http://www.theplantlist.org as accepted name, it belongs to the *Cucurbitaceae* family.

The bitter gourd variety used in the study was NS1020 from Namdhari Seeds Pvt. Ltd® (India) and was grown and harvested at the AVRDC premises in Tainan, Taiwan, between July and September 2012. The cucumber variety used as placebo and as an additive to bitter gourd sachets was MALANI™ from Seminis® (India), also grown at AVRDC fields. The procedure to produce supplement and placebo sachets were standardized. Bitter gourd and cucumber fruits were washed with clean water and dipped into water containing 1–2% hydrogen peroxide. Fruits, including seeds and skin, were chopped, freeze-dried, and ground into powder (80 mesh). Processing was performed by Challenge Bioproducts Co., Ltd. (Taiwan), an ISO 22000 and HACCP certified company.

2.4. Treatment

The daily dosage of bitter gourd powder was 2.5 g, based on results of a previous animal trial in which the equivalent dosage of 500 mg/kg body weight improved insulin sensitivity in mice exposed to a high-fat-diet. In the same trial, raw bitter gourd was more effective than cooked bitter gourd (unpublished data). A daily dosage of up to 4.8 g has been found to be safe for humans (Tsai et al., 2012). To mask the bitter taste, 0.75 g alpha-cyclodextrin mixed with 2% lemon peel oil (CAS Number 8008-56-8), 75 mg beta-cyclodextrin, 15 mg steviol glyscoside, and 0.75 g cucumber powder were added to the bitter gourd powder. For the placebo, 3.25 g cucumber powder were mixed with the same amounts of the aforementioned ingredients. Alpha-cyclodextrin was purchased from SEI CHENG CHEMICAL CO., LTD. Company (Taiwan), beta-cyclodextrin was purchased from Baolingbao Biology Co., Ltd (China), and steviol glycoside was purchased from YIH YUAN FOOD ADDITIVES & CHEMICAL INDUSTRIAL CO., LTD. (Taiwan). All concentrations were in accordance with regulations of the US-Food and Drug Administration (FDA, 2004, 2001). Mixing of powders with additives and packing was performed by TAI WON FOOD INDUSTRIAL CO., LTD (Taiwan), certified according to ISO 22000 and HACCP.

Sachets were shipped to Tanzania after obtaining permission from Tanzanian Food and Drug Authority (TFDA) and stored in a cold room until the time of distribution. Participants received sachets and bottled drinking water on a weekly basis during their regular checkup appointments. A closable cup, with a marking at 150 mL, was provided to mix the powder and water. Participants were advised to mix powder and water freshly and consume the drink after their main meal.

2.5. Data assessment and outcome measures

Before and after each intervention period, a 5 mL fasting venous blood sample was drawn from participants by a certified nurse. Fasting plasma glucose, high density cholesterol (HDL), triglycerides (TG), cholesterol (Chol), glutamate pyruvate transaminase (GPT), and creatinine were analyzed using ReflotronPlus®, Roche Germany. Reagents were purchased from Roche Germany. Insulin was analyzed using a BioTek® Human Insulin (INS) ELISA Kit manufactured by Bioassay

Fig. 1. CONSORT study flow diagram.
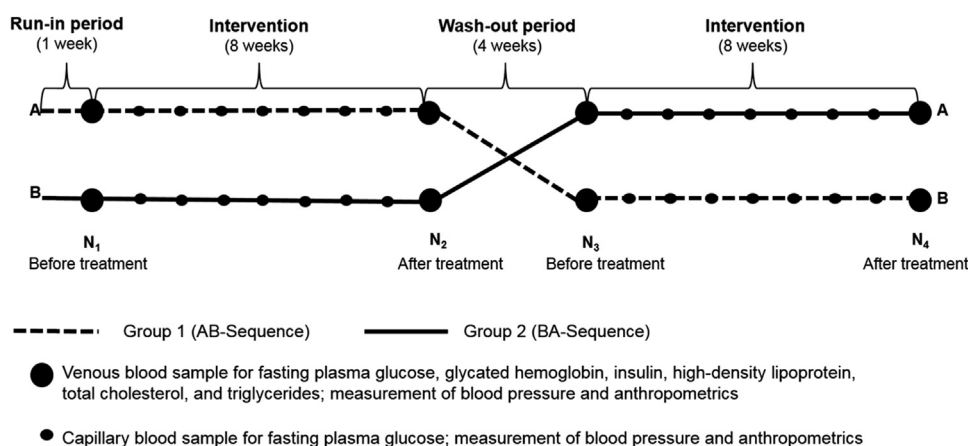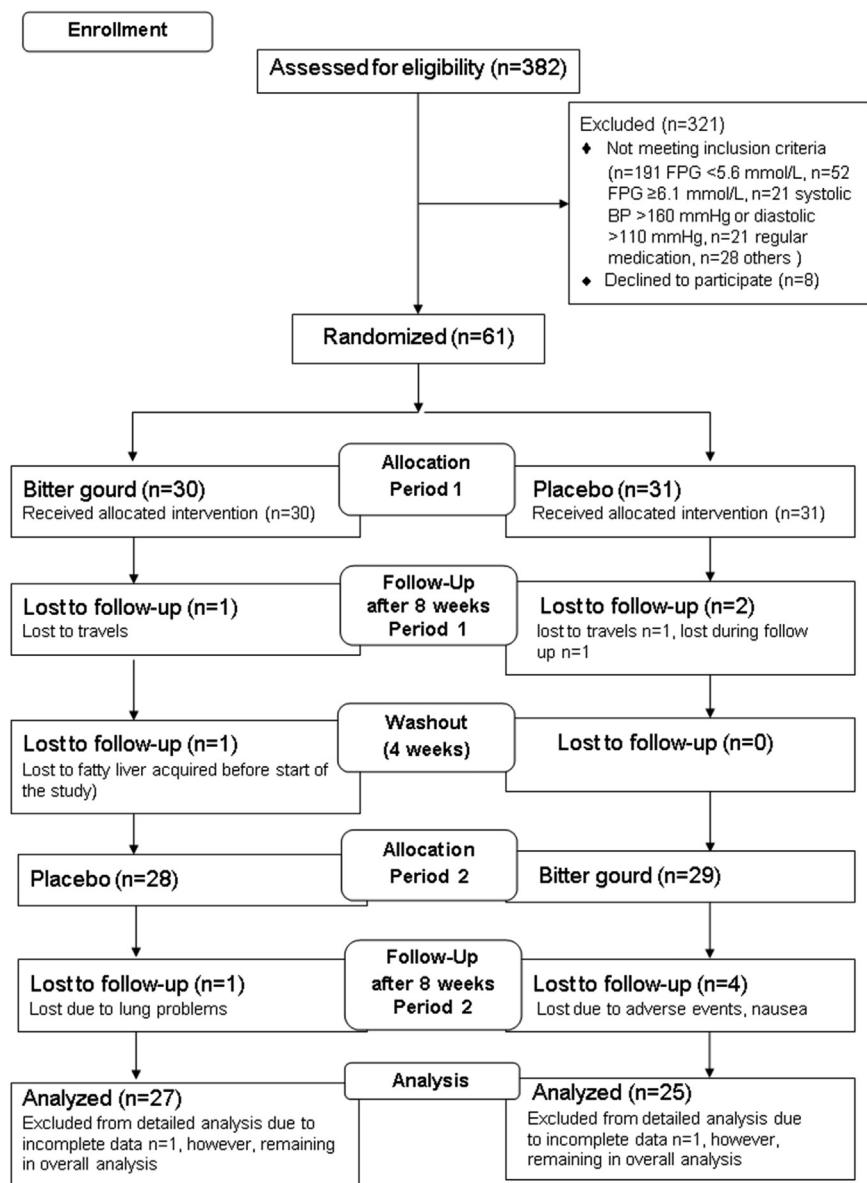
Fig. 2. Study design and study flow with measurements.

Technology Laboratory, Shanghai, China. A Homeostasis Model Assessment (HOMA) index was calculated (21). In addition, the second blood drop from the capillary sample was used to measure HbA_{1c} with HemoCue® 501 analyzer (HemoCue® GmbH, Germany). Results were available after five minutes.

At the baseline assessment, further socioeconomic, demographic, and relevant medical data were obtained using a standardized questionnaire. All questionnaires were compiled in English and then

forward and backward translated to check for inconsistencies. Interviews were conducted in Swahili, the national language of Tanzania.

On all examination days, i.e. baseline, endline and weekly assessments, capillary blood samples were obtained from a dry fingertip after local disinfection to assess FPG with Accu-Chek Aviva® (Roche Diagnostics, Switzerland) (data not shown). Results were available after a few seconds. Body weight and BP were assessed before and after each supplementation period and at every weekly visit of the participants. After five minutes rest in a seated position, each participant's BP was measured using a digital blood pressure monitor on the left arm (Visomat Double Comfort, Uebe Medical GmbH, Germany). During the five minutes rest, participants were initially asked about their age, current medical status, alcohol consumption as well as pregnancy and lactation among women. During the ongoing study, the five minutes rest was used to apply a regular check-up questionnaire concerning adverse events and usage of any medication. Body weight was measured to the nearest 0.1 kg using a digital scale (Seca 877, Germany). Body height was measured (only at baseline) to the nearest 0.5 cm using a portable stadiometer (Seca 217, Germany). Body mass index was calculated as body weight in kg divided by body height in m^2. Waist circumference and hip circumference (data not shown) were measured twice using a retractable measurement band. If the difference between the two measurements was greater than 1 cm, a third measurement was performed. The mean of the measurements was then recorded. During the run-in period one week before the start of the intervention, G6PD deficiency was assessed using CareStart™ G6PD (Access Bio, USA), a rapid test kit for capillary blood samples.

After each assessment, participants were provided with a small snack and reimbursement for their travelling expenses.

To assess changes in food intake and physical activity, a food frequency questionnaire (FFQ) and physical activity questionnaire (PAQ) were conducted. Participants were interviewed during the run-in period and at the end of the second intervention period. The FFQ addressed the food intake of the previous month. To compare food intake data, a monthly food variety score was calculated. Regarding changes in physical activity, only the sports section of the PAQ was compared. Both questionnaires were pre-tested in a study in 2012 in the same study area and adjusted for locally available and consumed foods. Additionally, the weekly regular checkup questionnaire included questions on whether participants changed their food intake or physical activity during the previous week and, if yes, what kinds of changes were made.

2.6. Ethics

The study protocol was approved by the Medical Research Coordinating Committee of the National Institute of Medical Research, Tanzania, TFDA, and the institutional review boards of the Kilimanjaro Christian Medical College, the Regional Medical Office in Moshi, and the Faculty of Medicine at Justus-Liebig-University, Giessen, Germany. All participants enrolled for the study gave their written informed consent. In case of the unlikely event of an adverse reaction needing compensation, the participants were ensured under the National Insurance Cooperation (NIC).

A data transfer agreement was obtained to allow the data to be analyzed outside of Tanzania. The study was registered under the number DRKS00005131 in the German Clinical Trial Registry (https://drks-neu.uniklinik-freiburg.de/drks_web/).

2.7. Statistics

Double data entry and checking were performed using SPSS version 21 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.). Data were analyzed using SPSS version 22 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Descriptive data are reported as mean

and SD. Per protocol analysis was performed for data from venous blood samples, anthropometrics, HbA_{1c}, and BP. Socioeconomic data and capillary FPG values were analyzed for all 52 participants who completed the study in March 2014, though some blood sample data were missing. As no capillary FPG levels were found hypoglycemic and no participant showed signs of hypoglycemia, venous FPG values < 3.3 mmol/L were considered as measurement errors and, thus, excluded. For other variables, one extreme outlier was excluded from the analysis of TG values. Exclusion of this outlier did not influence the statistical significance of the findings.

The difference in the primary outcome (FPG) between bitter gourd and placebo groups was analyzed with a CROS analysis and general linear mixed model. The CROS analysis (Freeman, 1989; Senn, 2002) is based on the Hills-Armitage approach (Hills and Armitage, 1979), which compares calculated mean period and cross-over differences and can detect a treatment effect despite the existence of a period effect.

Second, a mixed model for fixed effects (Type III) was applied to examine treatment, period, and carry-over effects (Allison, 2009). The fixed effects in this model were treatment (bitter gourd or placebo), period (period one or period two), and carry-over effects. The general linear model detected a period effect and a significant influence of the baseline FPG on the endline FPG. Thus, for differences between bitter gourd and placebo, the change of FPG from baseline to endline was analyzed. However, differences between baseline_1 and baseline_2 levels between Group 1 and Group 2 were checked with a two-sample t-test or Mann-Whitney test.

3. Results

3.1. Characteristics of the study population

The study started with sixty-one participants in October 2013 and ended with 52 participants in March 2014 (dropout rate 15%). Among the participants who finished the study, not all had complete data for the endline assessment. Reasons for dropouts are stated in Fig. 1. socioeconomic, demographic characteristics, and family-related data of the overall study population are presented in Table 1.

Overall mean age was 47.5 ± 8.7 years. The majority was married and had either finished primary education or gone to college; most were working in the business sector or in offices. Only 4% stated smoking or having smoked, and about 50% said that they consume alcoholic beverages. Fifty percent had a family member with high blood pressure and 15% reported having a family history of diabetes mellitus. There were no significant differences between Group 1 and Group 2 at the start of the first intervention period regarding BMI, fasting plasma glucose (FPG), HbA_{1c}, insulin and HOMA for insulin resistance (Table 2). Based on a cut-off HOMA-score of ≥ 2.5 (21), around 40% of participants were classified as insulin resistant or severely insulin resistant, around two thirds were classified as having normal insulin levels.

Before the start of the intervention study, namely between screening and baseline assessments, mean capillary FPG significantly decreased by 0.3 mmol/L from 5.97 to 5.67 mmol/L (Wilcoxon Signed Rank Test, z = − 3.276, p = 0.001), and systolic BP by 6 mmHg from 128 to 122 mmHg (paired t-test, t(51) = 3.845, p = 0.001). After the washout period, Group 1 started with a significantly lower FPG level compared to Group 2. All other variables did not differ. Comparing the treatment groups, i.e. bitter gourd vs cucumber-based placebo, the CROS analysis (t = − 2.23, p = 0.031, r = 0.326) revealed a significant difference in the change of FPG: there was a decrease in FPG in the bitter gourd group and an increase in the placebo group. Overall, the treatment difference was 0.31 mmol/L (5.6 mg/dL).

Although the calculated sample size of 54 was not achieved, the number of 44 complete data sets achieved a power of 0.82, with a medium-to-large effect size Cohen's d of 0.62. The significant treatment effect was also proven by results of the general linear mixed model (estimate 0.31; SE: 0.12; p: 0.01; 95%CI: 0.08;0.54). All other outcomes

Table 1
Selected characteristics of study population.

Variable	Group		
	Overall (n = 52)	Group 1 (AB) (n = 28)	Group 2 (BA) (n = 24)
Age [years]	47.5 ± 8.7	48.2 ± 8.4	46.6 ± 9.1
Mean household size	4.4 ± 2.7	4.5 ± 3.1	4.1 ± 2.1
Female/Male-ratio	27:23	27:23	27:23
Fam. history Dm [%]	15	18	12
Fam. history high BP [%]	50	46	54
Education [%]			
No formal education	2	4	0
Finished primary	38	43	33
Finished secondary	13	14	13
College	35	25	46
Other	12	7	4
Occupation [%]			
Farmer	6	11	0
Business sector	27	30	25
Student	6	7	4
Teacher	10	7	13
Nurse	6	11	0
Police officer	8	4	13
Office workers or other	37	30	45
Others			
Motorized work access [%]	56	52	61
Smoking [%]	4	4	4
Drinking alcohol [%]	52	39	67

did not differ between treatments as shown in Table 3.

3.2. Study outcomes

The change in FPG differed between subjects as not all had a decrease in FPG during bitter gourd supplementation. In Group 1, FPG of 15 participants responded and in Group 2, FPG of eleven participants responded. Overall, the change in FPG had a range from −1.75 to 0.92 mmol/L. The decrease in FPG between baseline and endline was found to be greater with a higher baseline FPG level (Fig. 3).

This observation was confirmed by the general linear mixed model: baseline FPG level had a significant effect on the outcome of endline FPG level. To control for changes in food intake during the study period a FFQ was assessed during a run-in period and at endline_2. Data of the two FFQs and derived food variety scores were available for 48 participants. Food variety scores did not differ between run-in and endline

assessment (51.8 ± 11.3 vs. 50.7 ± 11.4). The physical activity questionnaire was used to check whether participants changed their sports activities during the intervention study. At run-in, 31% said they engaged in sports whilst at endline this number slightly decreased to 27%.

Prior to the study period, nine participants reported adverse events such as headache, stomach pain, nausea, and flatulence. No serious health events eventually related to the intervention were observed. Some participants reported loose stools, diarrhea, flatulence, stomach rumbling, nausea or vomiting. These symptoms were reported by the bitter gourd group more often than the placebo group (mean numbers per symptom were n = 9 vs n = 5). However, it was difficult to establish a clear causal relationship between the intake of bitter gourd and the occurrence of the symptoms.

Due to the cold season 21 participants (39%) reported an intake of oral analgetics for headache or flu symptoms during the week before the venous blood sampling at the beginning of period 1 and 9 (17%) at the end of period 1.

4. Discussion

In this study, bitter gourd supplementation showed potential to lower elevated FPG compared to placebo. Although participants were screened for prediabetes, some had normal FPG levels at baseline. This may have affected the outcome of the study and reduced the mean glucose-lowering effect. The observed effect was associated with the baseline FPG level. Participants with higher FPG levels at baseline showed a greater change in FPG than those with lower FPG levels. Therefore, it is expected that bitter gourd is even more effective in lowering glucose levels when participants start at high value, i.e. when diabetes mellitus is manifest already. In the current study, no effect on insulin or HbA_{1c} was found. Further research is needed to explore bitter gourd's potential to lower glucose in prediabetes and its mechanisms.

The current study used whole fruit powder mixed with water as a supplement drink. Whole bitter gourd fruit was used in other studies, either as pill (John et al., 2003) or capsule (Tsai et al., 2012; Srivastava et al., 1993). Others used clear juice from the flesh (Fuangchan et al., 2011) or aqueous extract (Srivastava et al., 1993). In the study by John and colleagues, supplementation of 6 g dried powder per day for four weeks as an adjunct therapy for 26 diabetic patients had no significant effect on outcome parameters despite having higher mean baseline FPG levels of 8.33 ± 1.49 mmol/L. The authors assumed that patients might have maintained their normal dietary regimens or intake of oral antidiabetic agents (John et al., 2003). A supplementation of 100 mL aqueous bitter gourd extract per day, for seven weeks among patients with mild to severe diabetes significantly decreased postprandial glucose levels by 54%, and lowered HbA_{1c} levels from 8.37 ± 0.39% to

Table 2
Data of the treatment-sequence groups at baseline for each period.

Variable	Period 1			Period 2		
	Group 1 (AB)	Group 2 (BA)	p	Group 1 (AB)	Group 2 (BA)	p
BMI [kg/m²]	29.1 ± 2.0	30.2 ± 2.5	n.s.	29.5 ± 2.1	30.2 ± 2.8	n.s.
FPG [mmol/L]	5.27 ± 0.44	5.40 ± 0.53	n.s.	4.98 ± 0.49	5.39 ± 0.61	0.039[a]
HbA_{1c} [%]	5.85 ± 0.43	5.85 ± 0.43	n.s.	5.86 ± 0.39	5.89 ± 0.43	n.s.
Insulin [μU/mL]	23.9 ± 16.2	25.0 ± 17.1	n.s.	27.5 ± 16.1	26.6 ± 16.1	n.s.
HOMA-Index	6.07 ± 4.51	6.60 ± 4.41	n.s.	6.03 ± 3.38	7.50 ± 4.14	n.s.
Chol [mmol/L]	4.26 ± 0.91	4.49 ± 0.98	n.s.	4.10 ± 1.22	4.42 ± 0.77	n.s.
HDL [mmol/L]	0.95 ± 0.35	0.91 ± 0.24	n.s.	0.91 ± 0.31	0.85 ± 0.25	n.s.
TG [mmol/L]	1.70 ± 0.94	1.32 ± 0.55	n.s.	1.76 ± 0.71	1.54 ± 0.40	n.s.
Systolic BP [mmHg]	120.7 ± 15.4	122.5 ± 16.2	n.s.	115.5 ± 16.3	116.4 ± 13.1	n.s.
Diastolic BP [mmHg]	82.5 ± 12.4	83.9 ± 9.0	n.s.	79.8 ± 10.9	80.9 ± 8.3	n.s.

FPG: fasting plasma glucose, HOMA: Homeostasis Model Assessment, Chol: cholesterol, HDL: high density lipoprotein, TG: triglycerides, BP: blood pressure, n.s. non-significant.
Number of available data varied between parameters in Group 1: BMI (n = 26), FPG (n = 24), HbA_{1c} (n = 26), insulin and HOMA-Index (n = 25), Chol, HDL, and TG (n = 26), systolic and diastolic BP (n = 25), in Group 2: BMI (n = 24), FPG (n = 20), HbA_{1c} (n = 24), insulin and HOMA-Index (n = 21), Chol and HDL (n = 24), TG (n = 23), systolic and diastolic BP (n = 25).
[a] Two-Samples T-Test.

Table 3
Study variables of the treatment groups at baseline (T_1) and endline (T_2).

Variable	Bitter gourd			Placebo			Diff	n
	T_1	T_2	Change	T_1	T_2	Change		
FPG [mmol/L]	5.3 ± 0.52	5.1 ± 0.5	−0.2 ± 0.6	5.2 ± 0.5	5.3 ± 0.6	0.10 ± 0.5	0.31*	88
HbA$_{1c}$ [%]	5.9 ± 0.43	5.8 ± 0.3	−0.1 ± 0.3	5.9 ± 0.4	5.9 ± 0.4	0.00 ± 0.3	0.05	100
Insulin [μU/mL]	25.1 ± 15.94	26.0 ± 14.8	0.9 ± 6.2	26.4 ± 16.5	26.3 ± 14.5	−0.13 ± 6.5	1.04	92
Chol [mmol/L]	4.3 ± 0.85	4.3 ± 0.8	−0.1 ± 0.7	4.3 ± 1.12	4.4 ± 1.1	0.09 ± 0.8	0.16	100
HDL [mmol/L]	0.9 ± 0.3	0.9 ± 0.4	−0.0 ± 0.2	0.9 ± 0.3	0.9 ± 0.3	0.02 ± 0.2	0.05	100
TG [mmol/L]	1.6 ± 0.7	1.57 ± 0.53	−0.1 ± 0.7	1.6 ± 0.7	1.7 ± 0.6	0.14 ± 0.6	0.19	98
BMI [kg/m^2]	29.6 ± 2.5	29.6 ± 2.4	0.0 ± 0.6	29.8 ± 2.3	29.7 ± 2.4	−0.1 ± 0.5	0.12	102
Syst. BP [mmHg]	119 ± 14	117 ± 14	−2 ± 11	119 ± 17	116 ± 13	−2 ± 11	0.1	100
Diast. BP [mmHg]	82 ± 11	80 ± 9	−2 ± 8	82 ± 10	80 ± 9	−2 ± 6	0.1	100

Diff: Difference between Treatments, FPG: fasting plasma glucose, Chol: cholesterol, HDL: high density lipoprotein, TG: triglycerides, BP: blood pressure, Syst: Systolic, Diast: Diastolic, n.s. non-significant, * $p \leq 0.01$ (general linear mixed model).
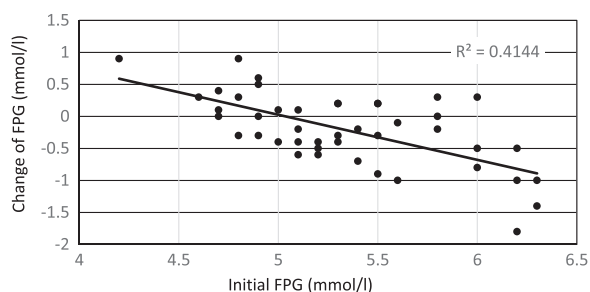

Fig. 3. Association between initial fasting plasma glucose (FPG) and change under bitter gourd supplementation.

6.95 ± 0.46%. In the same setting, five diabetes patients were supplemented with 5 g fruit powder per day in tablet form for three weeks with no antidiabetic effect being observed (Srivastava et al., 1993). Tsai *et al.* reported an improvement in log HOMA, Quicki, and McAuley values – although statistically not significant - after supplementation of 4.8 g of fruit powder capsules per day for three months among participants with at least three symptoms of the metabolic syndrome. Incidence rates of metabolic syndrome and higher waist circumference significantly decreased after three months by 19% and 2.50 ± 0.86 cm respectively. Follow up visits showed sustained results until one month after the end of the study (Tsai et al., 2012). Other studies have used different kinds of bitter gourd fruit parts and also varied in terms of dosage and study duration. Some studies reported significant antidiabetic effects after bitter gourd supplementation with improvements in glucose tolerance (Welihinda et al., 1986) and reductions in fructosamine levels (Fuangchan et al., 2011), fasting and postprandial glucose levels (Tongia et al., 2004; Kochhar and Nagi, 2011), HbA$_{1c}$ levels (Zänker et al., 2012), and usage of oral antidiabetic drugs (Kochhar and Nagi, 2011). However, other researchers did not find significant effects of bitter gourd supplementation among diabetic study populations (Dans et al., 2007).

In the current study, around 60% of the participants responded to the bitter gourd supplementation. This rate was lower compared to another study with 73% (n = 13) of diabetic participants responding to bitter gourd supplementation (Welihinda et al., 1986). Non-respondence may be due to normal FPG levels or genetic factors.

Many different compounds in bitter gourd have been investigated in cell and animal studies. Some of the isolated compounds are p-insulin (polypeptide-p), momordicosides, oleanic acid, trehalose, and momordin (Habicht et al., 2014; Joseph and Jini, 2013; Singh et al., 2011). Three major pathways are responsible for the glucose-lowering effect of bitter gourd, i.e. decreasing intestinal glucose absorption, increasing insulin secretion, and increasing glucose uptake in peripheral tissues (Habicht et al., 2014). Other pathways include an inhibition of adipocyte differentiation (Nerurkar et al., 2010) and a suppression of key

gluconeogenic enzymes (Singh et al., 2011; Shibib et al., 1993).

No significant differences were observed in this trial regarding BMI, blood lipids, and blood pressure between bitter gourd and placebo groups. This was similar to the study by Dans et al. (2007) as well as Tsai et al. (2012) who found no effect on total cholesterol and body weight, and blood pressure. In an animal study, bitter gourd treatment showed a significant reduction in body weight gain after bitter gourd treatment compared to control (Chan et al., 2005; Huang et al., 2008; Klomann et al., 2010). The absence of an effect of bitter gourd on plasma cholesterol, HDL, and triglycerides in this study might be due to the fact that blood lipids of most participants were within normal range anyway.

Some mild adverse effects after bitter gourd consumption were reported, e.g. flatulence, loose stools, mild diarrhea, headache, nausea, and vomiting, but no hypoglycemia. Four female participants dropped out due to nausea and vomiting after consumption of bitter gourd. Similar effects were also reported in other studies (Tsai et al., 2012; Fuangchan et al., 2011; Dans et al., 2007).

The low dropout rate of 15% during the six months study period demonstrates participants' interest in their health and well-being, as well as a tolerance for the study product. In the current setting, there was no significant influence of bitter gourd supplementation on renal and liver function. Other studies already reported no alteration of aspartate aminotransferase or alanine aminotransferase by a bitter gourd treatment (Tsai et al., 2012; Fuangchan et al., 2011; Zänker et al., 2012; Dans et al., 2007). However, and for reasons not shown here, bitter gourd consumption is not recommended for pregnant and lactating women as well as people with G6PD-deficiency.

5. Limitations

Screening for eligible study participants was performed once only and limited to FPG and HbA1$_c$. However, under the restricted conditions in the current setting, measurement of FPG could be performed easily and is recognized as a valid method of assessing glycemic status. The point-of-care device used did not require permanent power supply or extensive training of health staff. HbA$_{1c}$ values carry limited information due to methodological problems (different charges) and intervention periods of less than 3 months.

The study was a cross-over designed trial to limit between-subject variability, but within-subject variance could not be excluded and eventually had an influence on the study outcomes. Pre-study lifestyle changes between screening and baseline assessment resulted in a FPG-lowering effect.

Although only 52 participants finished the study and only 44 venous blood samples were analyzed for the primary outcome only, the final sample size achieved a power of 0.82 (two-sample *t*-test) and further statistical tests revealed a treatment difference despite the presence of a period effect.

6. Conclusions

The consumption of 2.5 g dry bitter gourd (equivalent to 50 g fresh bitter gourd) may depict an effective approach to lower elevated blood glucose levels in individuals with prediabetes. The glucose lowering effect of bitter gourd was found higher among participants who started with higher baseline FPG levels. As the current study included pre-diabetic participants only, the glucose-lowering effect of bitter gourd is expected to be even more pronounced among patients with higher glucose levels. However, more studies are needed to support these findings.

Acknowledgements

Funding

Conflict of interest (COI) statement

None of the authors declares a conflict of interest.

Authors' contributions

Author contributions: MBK, RYY and SDH designed the research and managed the project; RYY was responsible for quality of bitter gourd samples for the supplementation; CL and MES conducted the research; KPC and CL analyzed data; CL wrote the paper; MBK had primary responsibility for final content, all authors contributed to finalizing the manuscript.

References

Allison, P.D., 2009. Fixed Effects Regression Models. Quantitative Applications in the Social Sciences. SAGE, Los Angelos, USA, pp. 123.

Basch, E., Gabardi, S., Ulbricht, C., 2003. Bitter melon (*Momordica charantia*): a review of efficacy and safety. Am. J. Health Syst. Pharm. 60, 356–359.

Chan, L.L.Y., Chen, Q., Go, A.G.G., Lam, E.K.Y., Li, E.T.S., 2005. Reduced adiposity in bitter melon (*Momordica charantia*)–fed rats is associated with increased lipid oxidative enzyme activities and uncoupling protein expression. J. Nutr. 135, 2517–2523.

Dans, A., Villarruz, M., Jimeno, C., Javelosa, M., Chua, J., Bautista, R., Velez, G., 2007. The effect of *Momordica charantia* capsule preparation on glycemic control in Type 2 Diabetes Mellitus needs further studies. J. Clin. Epidemiol. 60, 554–559.

Efird, J.T., Choi, Y.M., Davies, S.W., Mehra, S., Anderson, E.J., Katunga, L.A., 2014. Potential for improved glycemic control with dietary *Momordica charantia* in patients with insulin resistance and pre-diabetes. Int. J. Environ. Res. Public Health 11, 2328–2345.

Faul, F., Erdfelder, E., Lang, A.-G., Buchner, A., 2007. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav. Res. Methods 39, 175–191.

FDA (US. Food and Drug Administration), 2004. Agency Response Letter GRAS Notice No. GRN 000155 [Internet, cited 2016 Jan 1]. Available from: ⟨http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm154385.htm⟩.

FDA (US. Food and Drug Administration), 2001.). Agency Response Letter GRAS Notice No. GRN 000074 [Internet, cited 2016 Jan 1]. Available from: ⟨http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm154182.htm⟩.

Freeman, P.R., 1989. The performance of the two-stage analysis of two-treatment, two-period crossover trials. Stat. Med. 8, 1421–1432.

Fuangchan, A., Sonthisombat, P., Seubnukarn, T., Chanouan, R., Chotchaisuwat, P., Sirigulsatien, V., Ingkaninan, K., Plianbangchang, P., Haines, S.T., 2011. Hypoglycemic effect of bitter melon compared with metformin in newly diagnosed type 2 diabetes patients. J. Ethnopharmacol. 134, 422–428.

Habicht, S.D., Ludwig, C., Yang, R., Krawinkel, M.B., 2014. *Momordica charantia* and type 2 diabetes: from in vitro to human studies. Curr. Diabetes Rev. 10, 48–60.

Hills, M., Armitage, P., 1979. The two-period cross-over clinical trial. Br. J. Clin. Pharmacol. 8, 7–20.

Huang, H.L., Hong, Y.W., Wong, Y.H., Chen, Y.N., Chyuan, J.H., Huang, C.J., Chao, P.M., 2008. Bitter melon (*Momordica charantia* L.) inhibits adipocyte hypertrophy and down regulates lipogenic gene expression in adipose tissue of diet-induced obese rats. Br. J. Nutr. 99, 230–239.

IDF (International Diabetes Federation), 2013. IDF Diabetes Atlas, 6th edn. International Diabetes Federation, Brussels, Belgium (Available from). ⟨http://www.idf.org/diabetesatlas⟩.

IDF (International Diabetes Federation), 2015. IDF Diabetes Atlas, 7th edn. International Diabetes Federation, Brussels, Belgium (Available from). ⟨http://www.diabetesatlas.org⟩.

Jenkins, N.T., Hagberg, J.M., 2011. Aerobic training effects on glucose tolerance in prediabetic and normoglycemic humans. Med. Sci. Sports Exerc. 43, 2231–2240.

John, A.J., Cherian, R., Subhash, H.S., Cherian, A.M., 2003. Evaluation of the efficacy of bitter gourd (*Momordica charantia*) as an oral hypoglycemic agent—a randomized controlled clinical trial. Indian J. Physiol. Pharmacol. 47, 363–365.

Joseph, B., Jini, D., 2013. Antidiabetic effects of *Momordica charantia* (bitter melon) and its medicinal potency. Asian Pac. J. Trop. Dis. 3, 93–102.

Khan, V., Najmi, A.K., Akhtar, M., Aqil, M., Mujeeb, M., Pillai, K.K., 2012. A pharmacological appraisal of medicinal plants with antidiabetic potential. J. Pharm. Bioallied. Sci. 4, 27–42.

Klomann, S.D., Mueller, A.S., Pallauf, J., Krawinkel, M.B., 2010. Antidiabetic effects of bitter gourd extracts in insulin-resistant db/db mice. Br. J. Nutr. 104, 1613–1620.

Kochhar, A., Nagi, M., 2011. Effect of supplementation of traditional medicinal plants on blood glucose in non–insulin-dependent diabetics: a pilot study. J. Med. Food 8, 545–549.

Krawinkel, M.B., Keding, G.B., 2006. Bitter gourd (*Momordica charantia*): a dietary approach to hyperglycemia. Nutr. Rev. 64, 331–337.

Matsumoto, M., Nishimura, T., 1998. Mersenne twister: a 623-dimensionally equidistributed uniform pseudo-random number generator. ACM Trans. Model Comput. Simul. 8, 3–30.

MoHSW (Ministry of Health and Social Welfare, Tanzania.), 2013. Tanzania Service Availability and ReadinessAssessment (2012). Fakara Health Institute, Dar es Salaam (ISBN: 978-9987-9652-6-7).

National Bureau of Statistics, 2013. Tanzania in figrures 2012, Dar es Salaam, Tanzania, Calverton, Maryland, USA. Available at: ⟨http://www.nbs.go.tz/nbs/takwimu/references/Tanzania_in_figures⟩ 2012.pdf.

Nerurkar, P.V., Lee, Y.K., Nerurkar, V.R., 2010. *Momordica charantia* (bitter melon) inhibits primary human adipocyte differentiation by modulating adipogenic genes. BMC Complement. Altern. Med. http://dx.doi.org/10.1186/1472-6882-10-34.

Parikh, N.H., Parikh, P.K., Kothari, C., 2014. Indigenous plant medicines for health care: treatment of Diabetes mellitus and hyperlipidemia. Chin. J. Nat. Med. 12, 335–344.

Peck, R., Mghamba, J., Vanobberghen, F., Kavishe, B., Rugarabamu, V., Smeeth, L., Hayes, R., Grosskurth, H., Kapiga, S., 2014. Preparedness of Tanzanian health facilities for outpatient primary care of hypertension and diabetes: a cross-sectional survey. Lancet Glob. Health 2 (5), e285–e292.

Robertson, J., Mace, C., Forte, G., de Joncheere, K., Beran, D., 2015. Medicines availability for non-communicable diseases: the case for standardized monitoring. Glob. Health. http://dx.doi.org/10.1186/s12992-015-0105-0.

Samjoo, I.A., Safdar, A., Hamadeh, M.J., Raha, S., Tarnopolsky, M.A., 2013. The effect of endurance exercise on both skeletal muscle and systemic oxidative stress in previously sedentary obese men. Nutr. Diabetes. http://dx.doi.org/10.1038/nutd.2013.30.

Senn, S., 2002. Cross-over Trials in Clinical Research. Chichester, England, 2nd ed. J. Wiley, New York, pp. 345.

Shibib, B.A., Khan, L.A., Rahman, R., 1993. Hypoglycaemic activity of *Coccinia indica* and *Momordica charantia* in diabetic rats: depression of the hepatic gluconeogenic enzymes glucose-6-phosphatase and fructose-1, 6-bisphosphatase and elevation of both liver and red-cell shunt enzyme glucose-6-phosphate dehydrogenase. Biochem. J. 292, 267–270.

Singh, J., Cumming, E., Manoharan, G., Kalasz, H., Adeghate, E., 2011. Medicinal CHEMISTRY OF THE ANTI-DIABETIC EFFEcts of *Momordica charantia*: active constituents and modes of actions. Open Med. Chem. J. 5, 70–77.

Srivastava, Y., Venkatakrishna-Bhatt, H., Verma, Y., Venkaiah, K., Raval, B.H., 1993. Antidiabetic and adaptogenic properties of *Momordica charantia* extract: an experimental and clinical evaluation. Phytother. Res. 7, 285–289.

Tag, H., Kalita, P., Dwivedi, P., Das, A.K., Namsa, N.D., 2012. Herbal medicines used in the treatment of diabetes mellitus in Arunachal Himalaya, northeast, India. J. Ethnopharmacol. 141, 786–795.

Tongia, A., Tongia, S.K., Dave, M., 2004. Phytochemical determination and extraction of *Momordica charantia* fruit and its hypoglycemic potentiation of oral hypoglycemic drugs in diabetes mellitus (NIDDM). Indian J. Physiol. Pharmacol. 48, 241–244.

Tsai, C.-H., Chen, E.C.-F., Tsay, H.-S., Huang, C., 2012. Wild bitter gourd improves metabolic syndrome: a preliminary dietary supplementation trial. Nutr. J. http://dx.doi.org/10.1186/1475-2891-11-4.

Welihinda, J., Karunanayake, E.H., Sheriff, M.H.H., Jayasinghe, K.S.A., 1986. Effect of *Momordica charantia* on the glucose tolerance in maturity onset diabetes. J. Ethnopharmacol. 17, 277–282.

Wyne, K., 2008. Information technology for the treatment of diabetes: improving outcomes and controlling costs. J. Manag. Care Pharm. 14 (2), 12–17.

Yakubu, M.T., Sunmonu, T.O., Lewu, F.B., Ashafa, A.O.T., Olorunniji, F.J., Eddouks, M., 2015. Medicinal plants used in the management of diabetes mellitus. Evid-based. complement. Altern. Med. http://dx.doi.org/10.1155/2015/467196.

Zänker, K.S., Mang, B., Wolters, M., Hahn, A., 2012. Personalized diabetes and cancer medicine: a rationale for anti-diabetic nutrition (bitter melon) in a supportive setting. Curr. Cancer Ther. Rev. 8, 66–77.