Contemporary Clinical Trials Communications 12 (2018) 17–25

Contents lists available at ScienceDirect

Contemporary Clinical Trials Communications

journal homepage: www.elsevier.com/locate/conctc

Preliminary analysis of the effect of Stevia (*Stevia rebaudiana*) in patients with chronic kidney disease (stage I to stage III)

Farhana Rizwan[a,b], Harun Ur Rashid[c], Saquiba Yesmine[d], Forhad Monjur[e], Tapan Kumar Chatterjee[a,f,*]

[a] Department of Pharmaceutical Technology, Jadavpur University, Kolkata, 700032, India
[b] Department of Pharmacy, East West University, Aftabnagar, Dhaka, 1212, Bangladesh
[c] Kidney Foundation Hospital and Research Institute, Mirpur-2, Dhaka, 1216, Bangladesh
[d] Department of Pharmacy, Jahangirnagar University, Savar, Bangladesh
[e] Department of Clinical Pathology, Institute of Child Health and Shishu Sasthya Foundation Hospital, Mirpur-2, Dhaka, 1216, Bangladesh
[f] Department of Pharmaceutical Science and Technology, JIS University, Kolkata, West Bengal, 700109, India

ARTICLE INFO

Keywords:
Stevia
Chronic kidney disease (CKD)
Hypertension
Diabetes mellitus
Angiotensin-II receptor blocker (ARB)
Ca^{2+} channel blocker (CCB)

ABSTRACT

Background: Stevia, *Stevia rebaudiana* (Bertoni), has become an important economic plant for its commercial use as a sweetener. Stevia plays a significant role in the healthcare practice of different cultures and in population. Previous animal and clinical studies demonstrated the efficacy of Stevia against chronic diseases like diabetes and hypertension. This study aimed to investigate the beneficial effect of Stevia in chronic kidney disease (CKD) patients after three (3) months of treatment along with the conventional antihypertensive and anti diabetic medications.

Methods: A prospective, interventional, randomized, single-blind, placebo-controlled trial has been done with 97 participants. Stevia capsule (250 mg) or matching placebo was given to the participants twice daily along with Angiotensin-II Receptor Blocker (ARB) and/or Ca^{2+} Channel Blocker (CCB). First follow up visits were done after 3 months of the interval. Blood and urine samples were collected for the biochemical tests. A structured questionnaire was used for the baseline assessment. Informed consent was taken from each participant.

Results: Both hypertension and diabetes were found to be associated with CKD. Most of the participants (52.3%) of Stevia group were in CKD Stage II. Significant changes were found in Serum creatinine ($p < 0.027$), Serum Uric acid ($p < 0.009$), Fasting blood sugar ($p < 0.041$) and Postprandial blood sugar ($p < 0.013$) and Microalbumin ($p < 0.041$) level in the treatment group.

Conclusion: The initial result demonstrated that Stevia has the potential for a significant improvement of some biochemical parameters in CKD patients. After completion of the nine (9) months clinical trial, the constructive effect of Stevia can be confirmed in this group of patients.

1. Introduction

Stevia, *Stevia rebaudiana* (Bertoni), a sweet herb native to South America, has long been used in the treatment of diabetes and hypertension [1]. Stevioside (5–10%) and rebaudioside-A (2–4%) are the two most abundant glycosides present in the Stevia leaves of the dry matter, respectively and are responsible for the intensely sweet taste [2]. Commercially, the use of stevioside (as a sweetener) has been established in different countries of the world, such as Australia and Japan. Furthermore, the diverse pharmacological effects of stevioside such as antibacterial effects, anticaries effect, antiedema effects, antifungal effects, antihyperglycemic effects on rabbits, hypotensive effects, and hypoglycemic effects in human were confirmed by some animal and clinical trials [3].

Abbreviations used: CKD, Chronic Kidney Disease; ARB, Angiotensin-II Receptor Blocker; CCB, Ca^{2+} Channel Blocker; STV, Stevia; PLC, Placebo; CL, Control (Healthy participant); BMI, Body mass index; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; FBS, Fasting blood sugar; PBS, Postprandial blood sugar; STP, Serum total protein; S uric acid, Serum uric acid; TCO_2, Total CO_2; In. phos., Inorganic phosphate; Se. Cr., Serum creatinine; M. albumin, Microalbumin; UTP, Urinary total protein; ACR, Albumin: Creatinine; PCR, Protein: Creatinine; eGFR, estimated glomerular filtration rate

* Corresponding author. Dept. of Pharmaceutical Science and Technology, JIS University, Kolkata, India.
E-mail addresses: farhanarizwan12@gmail.com, frizwan18@yahoo.com, frezwan@ewubd.edu (F. Rizwan), rashid@bol-online.com (H.U. Rashid), s.yesmine@juniv.edu (S. Yesmine), forhadmonjur@gmail.com, monjur_forhad@yahoo.com (F. Monjur), tkchatterjee_81@rediffmail.com (T.K. Chatterjee).

https://doi.org/10.1016/j.conctc.2018.08.007
Received 4 June 2018; Received in revised form 24 July 2018; Accepted 20 August 2018
Available online 21 August 2018

Numerous studies demonstrated that Stevia extract improves glucose tolerance in both diabetic and non-diabetic human. 35% drop in blood glucose was observed in human volunteers after 8 h of consumption of an extract of Stevia [4]. In rats, extract of Stevia given orally for 40–60 days induced diuresis, natriuresis and an indication of vasodilatation on the kidney, increase renal plasma flow and decrease mean arterial pressure [5].

Initial studies showed Stevia also has antioxidant and anti-inflammatory effects which help to reduce cardiovascular damage and metabolic disorders. Since cardiovascular disease and diabetes are now shown to be correlated with oxidative stress and inflammation, it is therefore of great interest to study the protective effect and influence of *Stevia rebaudiana* on these processes.

Now a day, Chronic Kidney Disease (CKD) becomes a major public health problem worldwide [6]. The prevalence of CKD has reached epidemic status in 10–12% of the populations, and more than 50% of elderly worldwide. Increasing body weight, hypertension and insulin resistance - all contribute to the chance of the increasing the prevalence of CKD with high morbidity and mortality rate [7].

Approximately, more than 450,000 patients in the United States and more than 175,000 patients in Europe are suffering from End-Stage Renal Disease (ESRD) and over one million worldwide currently. But, actually, the overall prevalence of Chronic Kidney Disease (CKD) is expected to be between 30 and 50 fold higher all over the world [8].

The US National Kidney Foundation also strongly recommends that people with diabetes, hypertension or family history of CKD at in higher risk. For the prevention of CKD, screening program and an individualized risk–reduction plan can modify the risk to cause CKD [1]. Again, the NHANES III found diabetes, hypertension, older age, and male sex to be positively associated with increased of elevated Serum creatinine levels to cause CKD [9].

Synthetic antihypertensive medications blocking the renin-angiotensin-aldosterone system (Angiotensin-converting enzyme inhibitors and/or Angiotensin receptor blockers) demonstrated beneficial effects in patients with proteinuria of CKD [10]. Good efficacy and tolerability were found during the long-term use of stevioside in humans. Therefore, the aim of this study was to investigate the effect of Stevia in patients with Chronic Kidney Disease (CKD) stage I to stage III.

2. Materials and methods

2.1. Study design

A total of ninety-seven (97) participants were enrolled in this prospective, interventional, randomized, single-blind study comparing Angiotensin-II Receptor Blocker, Losartan/Valsartan and Ca^{2+} Channel Blocker, Amlodipine with Stevia or placebo. The stevioside capsules 500 mg (250 mg each) twice a day or matching placebo was prescribed to the participants. Three (3) separate groups were taken to evaluate the drug. In study group-1 (STV), the stevioside capsule was given to 44 patients with conventional antihypertensive treatment along with the treatment of chronic kidney disease. In study group-2 (PLC), matching placebo was given to 43 patients with a similar treatment regimen. In study group-3 (CL), only 10 healthy participants were included as a control. Patients were asked to return for follow up visits every three months during active treatment schedule.

2.2. Study subject

The study involved both male and female participants, ranging in age from 31 to 70 years. CKD patients with stage I to stage III were included in the study. The participants who had a myocardial infarction or had undergone coronary-artery bypass grafting or who had a cerebrovascular accident or have undergone coronary angioplasty or who had a transient ischemic attack or who had any history of heart failure before enrolment or morbid obesity and chronic sepsis were excluded.

Table 1
Status of different variables of the participants at Baseline.

Different Variables	Group 1- STV	Group 2- PLC	Group 3- CL
	Mean (± SD)	Mean (± SD)	Mean (± SD)
Age	55 (± 11.75)	53.60 (± 11.27)	47.20 (± 4.87)
Height	5.31 (± .29)	5.26 (± .33)	5.21 (± .30)
Weight	68.82 (± 8.23)	66.52 (± 11.38)	64.20 (± 12.89)
BMI	26.34 (± 3.46)	25.79 (± 3.31)	25.45 (± 4.11)
SBP	133.86 (± 21.37)	133.84 (± 16.83)	111.00 (± 11.005)
DBP	84.77 (± 12.66)	82.67 (± 7.74)	79.00 (± 9.944)
FBS	6.94 (± 2.27)	6.28 (± 1.45)	5.53 (± .48)
PBS	9.58 (± 3.72)	9.32 (± 4.76)	6.34 (± 1.25)
Blood urea	8.3 (± 8.22)	5.47 (± 3.88)	3.2 (± .91)
S. creatinine	87.2 (± 8.22)	105.3 (± 30.7)	70.9 (± 11.04)
STP	71.8 (± 11.76)	74.4 (± 5.37)	71.6 (± 3.66)
S uric acid	361.9 (± 134.6)	303.7 (± 113.1)	368 (± 108.20)
Inorganic phosphate	1.28 (± .67)	1.09 (± .20)	1.14 (± .15)
Ca	2.34 (± .27)	2.24 (± .16)	2.30 (± .09)
Na	138.5 (± 3.05)	138.6 (± 2.67)	139.9 (± 1.2)
K	3.92 (± .51)	4.14 (± .55)	4.24 (± .17)
Cl	102.5 (± 4.27)	103 (± 3.748)	103.7 (± 1.16)
TCO_2	27.41 (± 1.90)	26.86 (± 1.96)	26.10 (± .32)

Values are presented as Mean (± SD). Data were analyzed by Paired sample T-test. Group I- STV = Stevia (n = 44), Group II- PLC = Placebo (n = 43) and Group III- CL = Control (n = 10). Here, BMI = Body mass index, SBP = Systolic blood pressure, DBP = Diastolic blood pressure, FBS = Fasting blood sugar, PBS = Postprandial blood sugar, STP = Serum total protein, S uric acid = Serum uric acid, TCO_2 = Total CO_2.

2.3. Sample size evaluation

In this prospective study, the sample size was evaluated by following formula:

$n = 2[(a+b)^2\sigma^2]/(\mu_1-\mu_2)^2$, where, n = the sample size in each of the groups, μ_1 = population mean in treatment group 1, μ_2 = population mean in treatment group 2, σ^2 = population variance (SD), a = conventional multiplier for alpha = 0.05, b = conventional multiplier for power = 0.80. Study power was considered as 80% (0.80) [11].

2.4. Duration of the study

All treatments were performed for nine (9) months. The whole process was divided into four (4) distinct phases. Such as (i) Baseline study (Initial Stage), (ii) First follow up (after 3 months of Baseline), (iii) Second follow up (after 6 months of Baseline) and (iv) Washout Period (after 9 months of Baseline). But in this article, only the data of first three (3) months (from the baseline period to 1st follow up) were considered.

2.5. Study area

The clinical part of this study was conducted in the Kidney Foundation Hospital and Research Institute, Mirpur-2, Dhaka, Bangladesh. It is a tertiary level of Kidney Hospital and non-profit organization of Dhaka City.

2.6. Patient screening

The study was explained to potential participants and they have the opportunity to ask questions about the study and these participants had as much time as required to decide whether they wish to take part. After taking their consent, the blood test had done, urine sample had been taken and either the stevioside capsule or placebo was given to them and proceed to 1st follow up visit.

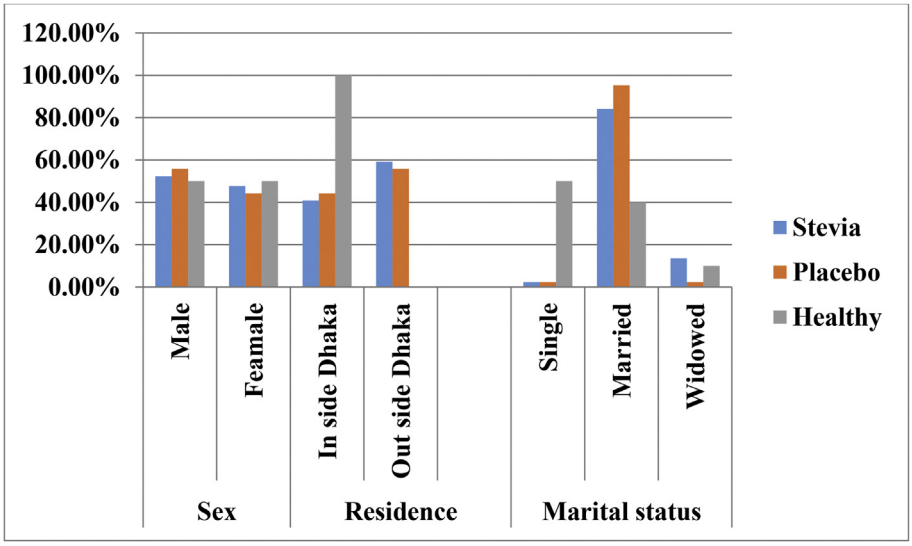

Fig. 1. Distribution (%) of sex, residence and marital status of the Participants.

Table 2
Characteristics of the study population in three (3) separate groups.

Different Variables		Group 1- STV N (%)	Group 2- PLC N (%)	Group 3- CL N (%)	Total Participants N (%)
Body Mass Index (BMI)					
Underweight		1 (2.3%)	1 (2.3%)	0 (0.00%)	2 (2.1%)
Normal		16 (36.4%)	15 (34.9%)	4 (40%)	35 (36.1%)
Overweight		19 (43.2%)	22 (51.2%)	5 (50%)	46 (47.4%)
Obese		8 (18.2%)	5 (11.6%)	1 (10%)	14 (14.4%)
Stages of CKD (GFR: ml/minutes/1.73 m^2)[a]					
Stage-I		5 (11.4%)	4 (9.3%)	0 (0%)	16 (16.5%)
Stage-II		23(52.3%)	13 (30.2%)	0 (0%)	39 (40.2%)
Stage-III		16(36.4%)	26 (60.5%)	0 (0%)	42 (43.3%)
No. of Affected family members					
One (1)		13 (29.5%)	5 (11.6%)	1 (10.0%)	19 (19.6%)
More than one (1)		1 (2.3%)	1 (2.3%)	0 (0.0%)	2 (2.1%)
No		30 (68.2%)	37 (86.0%)	9 (90.0%)	76 (78.4%)
History of Smoking & Alcohol Intake					
Smoking	YES	16 (47.1%)	15 (44.1%)	3 (8.8%)	34 (100%)
	NO	28 (44.4%)	28 (44.4%)	7 (11.1)	63 (100%)
Alcohol	YES	1 (33.3)	2 (66.7%)	0 (0.0%)	3 (100%)
	NO	43 (45.7%)	41 (43.6%)	10 (10.6%)	94 (100%)

Group I- STV = Stevia (n = 44), Group II- PLC = Placebo (n = 43) and Group III- CL = Control (n = 10). Descriptive analysis was performed here. **Range of BMI:** Underweight = BMI (below 18.5), Normal = BMI (18.5–24.9), Overweight = BMI (25–29.9), Obese = BMI (30 +). **Different Stages of CKD:** Stage I = eGFR 90 ml/min/1.73 m^2 or higher, Stage II = eGFR 60–89 ml/min/1.73 m^2, Stage III = eGFR 30–59 ml/min/1.73 m^2.

[a] National Kidney Foundation (NKF) 2002.

2.7. Methodology

A pre-structured and validated questionnaire was used for all participants who were reflected in the present study. Full demographic details of the patients were recorded, including age, sex, educational status, monthly income, clinical history, family history, smoking and alcohol intake status, current medication, physical examination (height and weight) and blood pressure (BP) measurement using the validated device. Stage I, stage II, and stage III CKD patients were considered in this study. Most of the CKD patients having either diabetes or systemic hypertension or both and their clinical condition were stable. Anthropometric parameters were assessed using standardized Techniques. Body weight and height were measured. Body mass index (BMI) was calculated on the basis of weight and height [weight (kg)/ height (m^2).

For Hypertension, the participants who reported current use of antihypertensive medications and those with a Systolic blood pressure (SBP) of 140 mm Hg or greater and Diastolic blood pressure (DBP) of 90 mm Hg or greater was considered [12]. Blood pressure was monitored twice, with an interval of 5 min between each measurement. The mean of these two (2) measurements was recorded. Patients were also encouraged to measure their blood pressure at home in the morning using an automated electronic device.

The blood specimen was obtained for the biochemical investigations, determination of Blood urea, Serum creatinine, Serum electrolytes (Na, K, Cl, TCO_2), Ca, Inorganic phosphate (PO_4), Serum total protein, Blood sugar (fasting and postprandial), Serum uric acid etc. Urine sample for the calculation of the Microalbumin, Urinary total protein (UTP) (spot), urine for ACR (Albumin: Creatinine), and urine for PCR (Protein: Creatinine), estimated glomerular filtration rate (eGFR) etc. Blood specimens were collected for analysis by the biochemistry laboratory. Remaining samples were collected and stored at -40^0c.

Serum electrolytes (Na, K, Cl, TCO_2), Blood urea, Serum creatinine, Blood sugar (fasting and postprandial) and urine for PCR was measured using Beckman Coulter AU480 Auto Analyzer system. Serum Ca, Serum inorganic phosphate, Serum uric acid, Serum total protein, and urine for ACR were measured using Architect c8000 Biochemistry Auto Analyzer system.

Kidney disease was evaluated by the presence or absence of Microalbuminuria, Serum creatinine level, and eGFR. Microalbuminuria was evaluated and greater than 25 mg/L was considered as positive. Elevated Serum creatinine values were defined as greater than 110.5 mmol/L for male and greater than 98.1 mmol/L for female. The simplified Modification of Diet in Renal Disease (MDRD) equation was used to estimate Glomerular Filtration Rate (GFR) and CKD stages, by following the KDOQI guidelines and the criteria of the US National Kidney Foundation. eGFR = 186.3^{*}[Serum $creatinine^{-1.154}$]*[$age^{-0.203}$]. Calculated values were multiplied by 0.742 for the woman. CKD Stage I: ACR < 30 mg/g creatinine and eGFR (90 ml/min/1.73 m^2 or higher); CKD Stage II: ACR 30–299 mg/g creatinine and eGFR(60–89 ml/min/1.73 m^2); CKD Stage III: ACR > 300 mg/g creatinine and eGFR(30–59 ml/min/1.73 m^2) [13].

Diabetic condition was evaluated in two (2) ways: (i) Participants who reported a history of diabetes and those with fasting blood sugar values greater than 5.83 mmol/L or postprandial blood sugar level greater than 8 mmol/L were categorized as diabetic, (ii) Participants with non fasting blood sugar levels greater than 8 mmol/L [14].

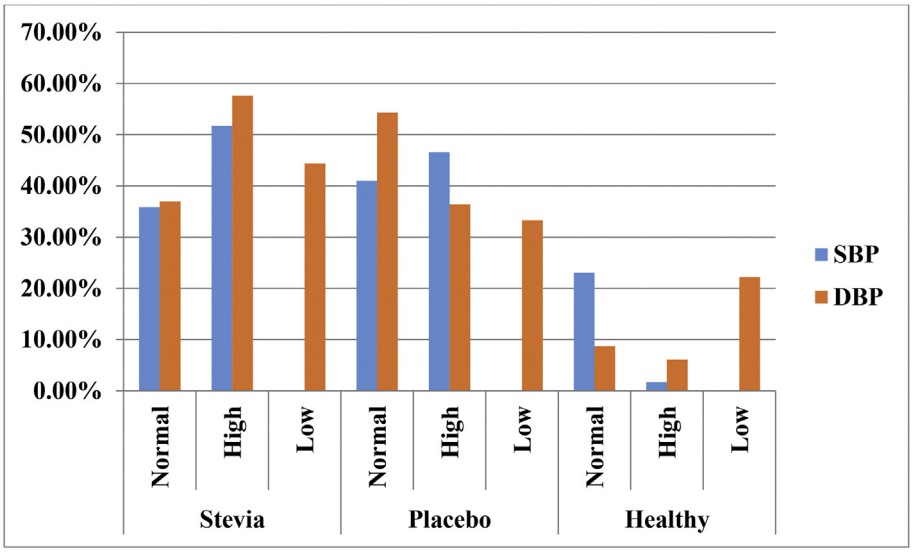

Fig. 2. Distribution (%) of the Systolic and Diastolic blood pressure of different groups of participants. Here, Normal Systolic Blood Pressure (SBP) = > 90–120; High Systolic Blood Pressure (SBP) = > 120–190; Low Systolic Blood Pressure (SBP) = 70–90; Normal Diastolic Blood Pressure (DBP) = > 60–80; High Diastolic Blood Pressure (DBP) = > 80–100; Low Diastolic Blood Pressure (DBP) = 40–60.

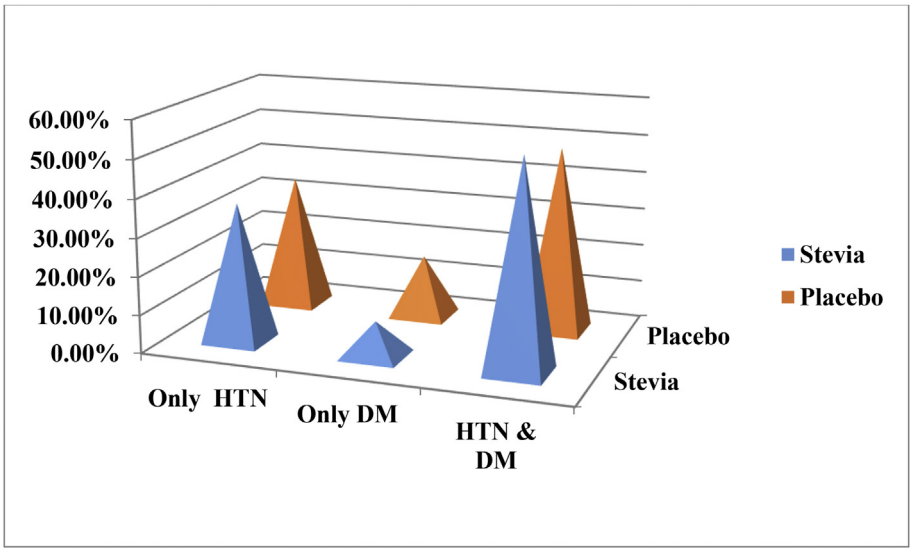

Fig. 3. Distribution (%) of the causes of CKD in the participants of STV and PLC groups. Here, Hypertension = HTN and DM = Diabetes Mellitus.

All the tests were done at the beginning of the treatment and after 3 months of the treatment as well. Approval from the Institutional Review Board (IRB) was collected before starting the research on CKD patients.

2.8. Statistical analysis

Data were checked thoroughly for consistency and completeness after collection and then cleaned, edited and verified to exclude any error or inconsistency. Statistical analysis was performed using the SPSS software for Windows (Version 17.0) (SPSS Inc, Chicago, USA). Descriptive analysis was performed as appropriate. Data of different variables of different (STV, PLC and CL) group of the participants at baseline and after 3 months (1st follow up) of treatment period were analyzed by Paired Sample T-test. Independent Sample T-test was done to do the comparison of different parameters of Group 1 (STV) and Group 2 (PLC) at baseline and 1st follow up study. All the statistical test were considered significant at different levels, (*$p < 0.05$) represents the significant value, (**$p < 0.01$) represents the highly significant values and (***$p < 0.001$) represents the very highly significant value of the parameter. One-way ANOVA followed by Bonferroni's Test were performed to determine the multiple comparisons among the urinary variables of this study. Linear Regression analysis was performed to evaluate the confounding variables.

3. Results

In the Baseline investigation, out of 97 participants, 44 were included in the STV group, 43 were in PLC group and 10 were in the CL group. The Mean (±) age of the participants in STV group was 55 (± 11.75) years, in PLC group was 53.6 (± 11.27) years and in CL group was 47.20 (± 4.87) years, respectively (Table 1).

Fig. 1 shows the percentage of the sex, residence and marital status of the participants of the different study group. In this study, most of the participants were male, 53.61% (STV = 52.3%, PLC = 55.8%, CL = 50%). Fifty participants (51.54%) (STV = 59.1%, PLC = 55.8%, CL = 0.00%) were from outside of Dhaka city and the rest of them resided inside. Married participants were 84.5% (STV = 84.1%, PLC = 95.3%, CL = 40%), widowed were 8.2% (STV = 13.6%, PLC = 2.3%, CL = 10%) and single were 7.2% (STV = 2.3%, PLC = 2.3% CL = 50%). In Table 2, from the Stevia group, maximum (29.5%) CKD patients are having one (1) affected family member and in the placebo group 11.6% having more than one (1) affected family

Table 3
Multiple comparisons among the urinary variables of different study groups of Baseline.

Dependent Variable	Different group	Different group	Std. Error	p value	95% Confidence Interval	
					Lower Bound	Upper Bound
Microalbumin	STV(n = 44)	PLC	55.380	.935	−191.23	78.60
		CL	91.633	.350	−78.23	368.24
	PLC(n = 43)	STV	55.380	.935	−78.60	191.23
		CL	92.004	.093	−22.82	425.46
	CL(n = 10)	STV	91.633	.350	−368.24	78.23
		PLC	92.004	.093	−425.46	22.82
UTP (Spot)	STV(n = 44)	PLC	20.685	1.000	−48.15	52.64
		CL	34.227	.336	−28.47	138.29
	PLC n = 43)	STV	20.685	1.000	−52.64	48.15
		CL	34.365	.386	−31.06	136.38
	CL(n = 10)	STV	34.227	.336	−138.29	28.47
		PLC	34.365	.386	−136.38	31.06
ACR	STV n = 44)	PLC	83.9729	1.000	−223.435	185.716
		CL	138.9435	.352	−118.931	558.059
	PLC n = 43)	STV	83.9729	1.000	−185.716	223.435
		CL	139.5063	.272	−101.442	578.290
	CL(n = 10)	STV	138.9435	.352	−558.059	118.931
		PLC	139.5063	.272	−578.290	101.442
PCR	STV (n = 44)	PLC	.5083	1.000	-.812	1.665
		CL	.8410	.676	−1.022	3.075
	PLC (n = 43)	STV	.5083	1.000	−1.665	.812
		CL	.8444	1.000	−1.457	2.657
	CL(n = 10)	STV	.8410	.676	−3.075	1.022
		PLC	.8444	1.000	−2.657	1.457
eGFR	STV (n = 44)	PLC	4.561	1.000	−7.38	14.84
		CL	7.547	**.000*****	−59.75	−22.98
	PLC (n = 43)	STV	4.561	1.000	−14.84	7.38
		CL	7.577	**.000*****	−63.56	−26.64
	CL(n = 10)	STV	7.547	**.000*****	22.98	59.75
		PLC	7.577	**.000*****	26.64	63.56

Here, (*p < 0.05) = significant, (**p < 0.01) = highly significant, (***p < 0.001) = very highly significant. The data were analyzed by One-way ANOVA followed by Bonferroni's Test. Here, UTP= Urinary total protein, ACR = Albumin: Creatinine; PCR= Protein: Creatinine; eGFR = estimated glomerular filtration rate.

member. Maximum overweight participants (47.4%, 46/97) were found from three different study groups. The major participants have CKD stage II (STV = 52.3%, 23/44) and in CKD stage III (PLC = 60.5%, 26/43). Cigarette smokers were found 47.1% in STV, 44.4% in PLC and 8.8% in CL group. Only one (1) participant from STV and two (2) participants from PLC group were taken alcohol occasionally.

Distribution (%) pattern of the Systolic and Diastolic blood pressure of the participants were presented in Fig. 2. Systolic blood pressure (SBP) was found superior (STV = 51.7% and PLC = 46.6%). Again, high Diastolic blood pressure (DBP) was observed (STV = 57.6%, PLC = 36.4%, CL = 6.1%) and low diastolic blood pressure was observed (STV = 44.4%, PLC = 33.3%, CL = 22.2%). In both study groups, hypertension with diabetes was considered to be the main causes for CKD for these participants (STV = 54.4% and PLC = 48.8%). On the other hand, only hypertension was found in (STV = 36.4% and PLC = 34.9%) and only diabetes was observed for (STV = 9.1% and PLC = 16.3%) participants (Fig. 3).

In Table 3, urinary variables (Microalbumin, UTP, ACR, and PCR) didn't show any significant association among STV, PLC and CL group at the baseline. Again, very highly significant results of eGFR were found between STV and CL (p < 0.000), between PLC and CL (p < 0.000), between CL and STV (p < 0.000), between CL and PLC (p < 0.000) at the baseline.

In Table 4, the comparison of two groups (STV and PLC) was done between Baseline and 1st follow up on data. The Significant difference (p < 0.05) was discovered in Diastolic blood pressure (p < 0.001), Blood urea (p < 0.004), Serum creatinine (p < 0.043), Serum total protein (p < 0.040), Ca (p < 0.008) and Inorganic phosphate (p < 0.043) between STV and PLC group of Baseline data. Whereas, in 1st follow up, a significant difference was found only in Diastolic blood pressure (p < 0.002), urine for PCR (p < 0.050) in both STV and PLC group (Table 4).

In Table 5, the comparison of Baseline and 1st follow up data was made in two groups (STV and PLC). In PLC group significant difference (p < 0.05) was discovered in Systolic blood pressure (p < 0.000), Diastolic blood pressure (p < 0.010), Serum uric acid (p < 0.002), Sodium (Na) (p < 0.010), Chloride (Cl) (p < 0.001), urine for ACR (p < 0.027) and urine for PCR (p < 0.011). Whereas in STV group significant difference (p < 0.05) were found in Systolic blood pressure (p < 0.042), Diastolic blood pressure (p < 0.008), Fasting blood sugar (p < 0.041), Postprandial blood sugar (p < 0.013), Serum creatinine (p < 0.027), Serum uric acid (p < 0.009), Microalbumin (p < 0.041) between Baseline and 1st follow up on study (Table: 5).

In Table 6, The multiple linear regressions model showed surprisingly, gender and age were significantly associated with estimated glomerular filtration rate (eGFR) (p < 0.05) in the baseline stage and 1st follow up (Table 6).

Fig. 4 shows the distribution pattern of Angiotensin-II receptor blocker (ARB), Ca^{2+} channel blocker (CCB) and Antidiabetic drug among the CKD patients. ARB was taken by (STV = 61.2%, PLC = 65.1%) CKD patients and CCB was taken by (STV = 40.9% and PLC = 37.2%) CKD patients, respectively. Only (STV = 2.3% and PLC = 2.3%) CKD patients were taking combined dose. Antidiabetic drugs were given to (STV = 63.6% and PLC = 67.4%) CKD patients.

4. Discussion

The current study demonstrated several insights into the interrelationships between diabetes, hypertension and chronic kidney disease. This study also validated the effect of Stevia in patients with mild to moderate CKD. All the demographic factors like age, gender, educational level, financial condition, affected family members (if); BMI,

Table 4
Comparison of different parameters of Group 1- STV and Group 2- PLC at Baseline and 1st follow up study.

Different Variables	Different Study groups	Baseline Data		1st Follow up Data	
		Mean = (± SD)	p value	Mean = (± SD)	p value
Weight	1	68.82(± 8.23)	.182	68.50(± 8.57)	.259
	2	66.56(± 11.34)		66.71(± 10.90)	
BMI	1	26.341(± 3.46)	.540	26.156(± 3.68)	.094
	2	25.798(± 3.31)		25.690(± 2.88)	
SBP	1	133.86(± 21.37)	.228	122.33(± 13.42)	.509
	2	133.84(± 16.82)		122.00(± 14.53)	
DBP	1	84.77(± 12.66)	**.001*****	79.30(± 5.51)	**.002****
	2	82.67(± 7.74)		78.38(± 10.52)	
FBS	1	6.942(± 2.26)	.217	6.707(± 1.96)	.337
	2	6.286(± 1.42)		6.985(± 2.54)	
PBS	1	9.589(± 3.71)	.160	9.244(± 3.24)	.066
	2	9.324(± 4.75)		10.070(± 4.59)	
Blood urea	1	8.30(± 8.21)	**.004****	5.860(± 4.52)	.233
	2	5.47(± 3.88)		5.298(± 1.44)	
S. creatinine	1	87.226(± 42.58)	**.043***	101.072(± 23.90)	.054
	2	105.258(± 30.69)		110.768(± 30.83)	
STP	1	71.84(± 11.75)	**.040***	71.930(± 10.88)	.102
	2	74.40(± 5.37)		74.225(± 4.90)	
S. uric acid	1	361.98(± 134.66)	.354	341.09(± 107.63)	.895
	2	303.79(± 113.11)		351.25(± 105.00)	
Ca	1	3.209(± 4.08)	**.008****	2.187(± .14)	.876
	2	2.242(± .16)		2.190(± .13)	
PO_4	1	1.287(± .67)	**.043***	1.172(± .20)	.824
	2	1.095(± .20)		1.120(± .20)	
Na	1	138.57(± 3.04)	.323	143.95(± 30.85)	.114
	2	138.63(± 2.67)		140.13(± 2.58)	
K	1	3.921(± .51)	.536	3.988(± .49)	.692
	2	4.149(± .51)		4.108(± .54)	
Cl	1	102.59(± 4.27)	.129	103.53(± 3.29)	.369
	2	103.00(± 3.74)		105.20(± 2.83)	
TCO_2	1	27.41(± 1.89)	.873	27.23(± 2.24)	.972
	2	26.86(± 1.96)		143.95(± 30.85)	
M. albumin	1	133.26(± 261.74)	.420	172.073(± 306.07)	.362
	2	187.78(± 257.30)		172.292(± 253.98)	
UTP (Spot)	1	49.14(± 79.81)	.888	71.040(± 126.40)	.148
	2	56.35(± 76.17)		56.675(± 80.60)	
ACR	1	187.341(± 412.05)	.761	181.404(± 345.22)	.348
	2	226.819(± 351.75)		168.269(± 271.52)	
PCR	1	.650(± .92)	.734	10.960(± 63.65)	**.050***
	2	.679(± .86)		.5195(± .65)	
eGFR	1	65.45(± 21.93)	.719	66.28(± 21.95)	.886
	2	60.51(± 20.26)		62.58(± 20.43)	

Values are presented here as Mean (± SD), where, (*p < 0.05) = significant, (**p < 0.01) = highly significant, (***p < 0.001) = very highly significant as compared to the baseline and 1st follow-up data of different study groups. For study group 1- STV (n = 44) and for study group 2- PLC (n = 43). Data were analyzed by the Independent Sample T-test. Here, BMI = Body mass index, SBP = Systolic blood pressure, DBP = Diastolic blood pressure, FBS = Fasting blood sugar, PBS = Postprandial blood sugar, STP = Serum total protein, S. creatinine = Serum creatinine, S. uric acid = Serum uric acid, TCO_2 = Total CO_2, UTP = Urinary total protein, ACR = Albumin: Creatinine; PCR = Protein: Creatinine; eGFR = estimated glomerular filtration rate.

malnutrition, anemia; causes of the disease like hypertension, diabetes or impaired renal function itself makes overall worsen the condition of the CKD patients [15]. In a different study, it was found that advanced age, female sex, the presence of associated diseases and a low socio-economic status are the relevant factors to decline the condition of the CKD patients [16]. BMI was part of the most important risk factors for CKD. Overweight and obesity had a graded increase in risk for causing CKD. It was a finding from Singapore and India [17]. In another study, 27% and 64% participants were classified as diabetics and hypertensive respectively. Again, 44% of CKD patients were considered obese (BMI ≥ 30.0), including 46% of women and 39% of men [18]. In the current study, 47.4% and 14.4% participants found overweight and obese, respectively. This finding is compatible with the results of other studies.

Previous studies showed that the prevalence of cardiovascular complications among all the patients with CKD. The prevalence of left ventricular hypertrophy is a direct result of the decrease in glomerular filtration rate. Approximately 30% of end-stage renal disease patients demonstrated clinical evidence of ischemic heart disease and heart failure [19]. The present study showed an association between hypertension and diabetes (51.7%) and chronic kidney disease. Another important finding of this study is the improvement of the stage of CKD patients. During 1st follow-up, it was found that out of eighty-three (83) CKD patients, the condition of 72.28% were remaining unchanged and 20.48% patients needed to be improved. However, 7.22% CKD patients were deteriorated. In this regard, treatment with Stevia improved or prevents any further decrease in glomerular filtration rate among CKD patients will be confirmed after finishing the study.

Gislein Elisa et al. found the short duration of action of stevioside. But they didn't find any significant difference in blood glucose levels between placebo and stevioside group (p > 0.05) from 3 months of the treatment period [3]. From another study by Letcia et al. found the significantly decreased (p < 0.05) glucose level during their treatment period for both stevioside and placebo groups [20].

Melis et al. found the significant reduction in blood pressure (p < 0.05) in the active treatment group during the study period. Here, Stevia shows the mechanism of calcium channel antagonism, which is the same as the mechanism of verapamil as antihypertensive agents [21,22]. However, calcium influx in rat smooth muscle cells was

Table 5
Outcome of participants in (STV and PLC) group after 3 months (1st follow up) of treatment period.

Different Variables	Group 2- PLC			Group 1- STV		
	Baseline Data, Mean (± SD)	1st follow up Data, Mean (± SD)	p value	Baseline Data, Mean (± SD)	1st follow up Data, Mean (± SD)	p value
Weight	66.53(± 10.94)	66.17(± 10.48)	.295	68.82 (± 8.23)	68.94 (± 8.97)	.916
BMI	25.79(± 3.16)	25.64 (± 2.90)	.276	26.34 (± 3.46)	26.18 (± 3.64)	.758
SBP	133.46 (± 16.98)	122.05 (± 14.17)	**.000*****	133.86 (± 21.37)	126.14 (± 14.01)	**.042***
DBP	82.56 (± 7.85)	78.33 (± 10.65)	**.010****	84.77 (± 12.66)	79.32 (± 5.45)	**.008*****
FBS	6.34 (± 1.43)	7.04 (± 2.55)	.092	6.94 (± 2.26)	6.19 (± 1.49)	**.041***
PBS	9.45 (± 4.89)	10.14 (± 4.62)	.412	9.58 (± 3.71)	8.11 (± 2.03)	**.013***
Blood urea	5.47 (± 4.06)	5.30 (± 1.46)	0.794	8.30 (± 8.21)	5.84 (± 4.47)	.092
S creatinine	104.52 (± 29.87)	110.19 (± 31.02)	0.182	87.22 (± 42.58)	101.80 (± 24.10)	**.027***
STP	74.41 (± 5.64)	74.10 (± 4.90)	.783	71.84 (± 11.75)	72.09 (± 10.80)	.891
S Uric acid	312.28 (± 107.86)	349.1(± 105.52)	**.002****	361.98 (± 134.66)	303.02 (± 112.38)	**.009*****
Ca	2.23 (± .16)	2.19 (± .13)	.118	3.20 (± 4.08)	2.18 (± .14)	.104
In phos.	1.10 (± .21)	1.11 (± .20)	.635	1.28 (± .67)	1.17 (± .20)	.26
Na	138.77 (± 2.46)	140.18 (± 2.59)	**.010***	138.57 (± 3.04)	139.27 (± 2.39)	.215
K	4.18 (± .50)	4.10 (± .55)	0.286	3.92 (± .51)	3.99 (± .49)	.419
Cl	103.28 (± 3.30)	105.28 (± 2.82)	**.001****	102.59 (± 4.27)	103.50 (± 3.26)	.173
TCO2	26.73 (± 1.81)	26.79 (± 2.41)	.879	27.41 (± 1.89)	27.18 (± 2.24)	.490
M albumin	198.38 (± 267.30)	168.5(± 256.16)	.279	133.26 (± 261.74)	56.20 (± 80.91)	**.041***
UTP(SPOT)	59.82 (± 79.07)	56.20 (± 81.60)	.767	49.14 (± 79.81)	71.13 (± 124.92)	.283
ACR	240.9 (± 366)	160.8 (± 270.93)	**.027***	187.34 (± 412.05)	187.64 (± 343.68)	.997
PCR	.72(± .89)	.50(± .66)	**.011***	.650 (± .92)	10.73 (± 62.92)	.294
e GFR	62.85 (± 18.16)	62.92 (± 20.57)	.964	65.45 (± 21.93)	65.84 (± 21.82)	.918

Values are presented as Mean (± SD), where, (*p < 0.05) = significant, (**p < 0.01) = highly significant, (***p < 0.001) = very highly significant as compared to the baseline and 1st follow up data of different study groups. Data were analyzed by Paired Sample T-test. Here, BMI = Body mass index, SBP = Systolic blood pressure, DBP = Diastolic blood pressure, FBS = Fasting blood sugar, PBS = Postprandial blood sugar, In. phos. = Inorganic phosphate, S. creatinine = Serum creatinine, M. albumin = Microalbumin, STP = Serum total protein, S. uric acid = Serum uric Acid, TCO_2 = Total CO_2, UTP = Urinary total protein, ACR = Albumin: Creatinine; PCR = Protein: Creatinine; eGFR = estimated glomerular filtration rate.

Table 6
Linear Regression analysis of confounding variables considering estimated glomerular filtration rate (eGFR) as dependent variable in the baseline stage and 1st follow up.

Variables	Beta Coefficient (β)	p-value	95% confidence interval for β	
			Lower Bound	Upper Bound
Baseline Data				
(Constant)		0.000	117.699	239.844
Gender	-.223	**0.026***	−20.221	−1.291
Age	-.417	**0.000*****	−1.326	-.479
BMI	.055	0.569	−0.952	1.722
SBP	-.257	**0.023***	−0.585	−0.044
DBP	-.070	0.514	−0.653	0.329
S. uric acid	-.050	0.597	−0.046	0.027
FBS	.010	0.913	−2.338	2.612
1st Follow up				
(Constant)		0.000	50.294	131.088
Gender	-.252	**0.010****	−23.880	−3.389
Age	-.375	**0.000*****	−1.358	-.463
BMI	.264	0.089	−0.181	2.529
SBP	.151	0.438	−0.230	0.526
DBP	.069	0.710	−0.460	0.673
S. uric acid	-.092	0.354	−0.065	0.024
FBS	-.046	0.635	−2.615	1.603

Dependent Variable was eGFR in ml/min/1.73m2 in Baseline and 1st Follow up data. Here, (*p < 0.05) = significant, (**p < 0.01) = highly significant, (***p < 0.001) = very highly significant. Linear regression analysis was performed. Here, BMI = Body mass index, SBP = Systolic blood pressure, DBP = Diastolic blood pressure, FBS = Fasting blood sugar, S. uric acid = Serum uric Acid, eGFR = estimated glomerular filtration rate.

inhibited by the action of Stevia is established already from another study [23].

Ana Rebollo-Rubio et al. mentioned that over 77% of the articles in a review process having a larger number of the male population than the female. However, 100% of the studies using sex as a study variable and showed the poor health condition is perceived by women, compared with men [15]. In the present study, the maximum number of randomly selected CKD patients was found male (53.61%).

John Hopkins University reported from a population-based case-control study that severe renal failure clustered within families independent of high blood pressure and diabetes is very common [24]. From another study, it has been observed that a consistent and high baseline rate of familial clustered directly correlated with CKD throughout the world, such as the United States, Western and Eastern Europe, India, South America, The Middle East and Asia [8]. In a different study, the family history is significant risk factors of CKD associated with renal impairment and representing the genetic influence [8,25]. The current study showed, 19.6% participants having one (1) family member and only 2.1% participants had more than one (1) family member affected by CKD. However, 78.4% did not get a family history of CKD. In this regard, no correlation was found between affected family members with CKD.

In a study in India, only 3.3% of subjects were found with low eGFR and the majority of these had eGFR < 30 ml/min/1.73 m^2. They also observed an inverse association between renal impairment and history of smoking and alcohol intake [17]. In an Australian Aboriginal Community, a cross-sectional study has been done by McDonald et al. documented the similar observations [26]. Another cross-sectional study also suggested an inverse association between current smoking and low eGFR level in Japan [27]. Wendy et al. mentioned that 45% of participants reported a smoking history and among them, 14% were current smokers [18]. Furthermore, some cross-sectional studies have demonstrated a positive association between smoking, alcohol intake and renal impairment [28]. However, no significant relationship was found between CKD and history of smoking and alcohol intake in the present study.

This study represents CKD patients with Diabetes mellitus was 64.4%. However, the majority (51.8%) of diabetes mellitus was found in stage III CKD patients. New et al. revealed that 30% of diabetic patients had CKD stage III in the UK [29]. Narindar et al. reported that Diabetes has recognized as an emerging epidemic for a long time in

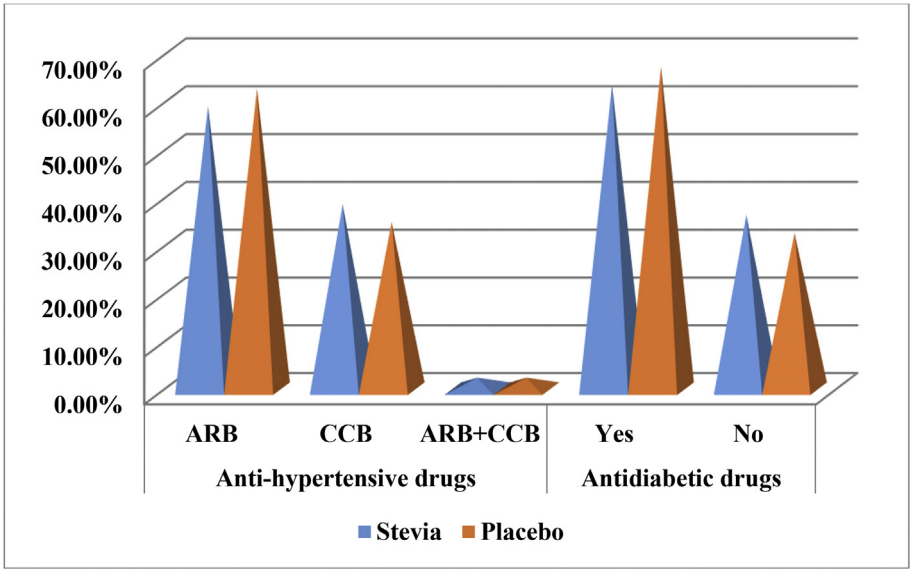

Fig. 4. Distribution (%) of anti-hypertensive and antidiabetic drugs in STV and PLC groups.
Here, ARB = Angiotensin- II Receptor Blocker and CCB = $Calcium^{2+}$ Channel Blocker.

India, where every fifth person is hypertensive also and only 20% of hypertensive and 57% of diabetics knew about their disease [17]. In another study, 27% and 64% participants were categorized as diabetics and hypertensive respectively.

Assessment of Kidney disease was inspired by the presence or absence of Microalbuminuria, Serum creatinine level, and eGFR. Wendy et al. mentioned that 29% participants had Microalbuminuria and of them, 34% had diabetes and 14% having an elevated Serum glucose level [18]. Stevia showed a beneficial effect in CKD patients by improving Microalbuminuria in the current study.

Treatment with stevioside reported a significant reduction in Blood urea level and increasing Serum creatinine level. On the other hand, the significant result was noted in sodium (Na) and chloride (Cl) for placebo treatment in this study. Letcia et al. did not find any changes in different parameters of blood analysis, urine analysis, BMI, Serum creatinine, Blood urea, chloride (Cl), potassium (K) and sodium (Na) [20].

The risks of renal failure associated with a wide range of blood pressure levels were determined by a 16 years a large cohort studies. In a separate study, the patients who received the crude stevioside and placebo, decrease the Diastolic blood pressure ($p < 0.05$). However, several investigations showed that parenteral administration of stevioside decreased blood pressure in rats [20]. But the current study indicated that both the Systolic blood pressure (SBP) and Diastolic blood pressure (DBP) significantly reduced in Stevia and placebo group in human subjects.

Ahmed et al. found the lower prevalence of proteinuria was 2.25% in a cross-sectional study in a rural population in India [30]. While another cross-sectional study from an urban locale estimated a higher prevalence (4.41%) [31]. Kannel et al. suggested the subjects with proteinuria should be worked up adequately because of significant mortality rate [32]. But in this study, placebo group found a significant reduction of Protein: Creatinine (PCR) as well as the Albumin: Creatinine (ACR).

5. Conclusion

In conclusion, our results showed that both diabetes and hypertension are positively associated with the development of CKD. This study revealed the potential benefit of oral Stevia in achieving the reduction in Serum uric acid and Microalbumin. This study highlights the emerging issue of CKD. The rising prevalence of hypertension and diabetes mellitus in CKD patients has the potential to become a future public health problem. Moreover, it is recognized that CKD is an additional cardiac risk factor and this patient is at a higher risk of cardiovascular morbidity. Using oral stevioside with regular drug regimen offers the opportunity for preventing the progression of CKD in human subjects. Benefits and risks of stevioside in CKD patients can be established after completion of the study.

Ethical statement & informed consent

The Chief Clinical Investigator ensures the study was conducted in accordance with the principles of the Declaration of Helsinki and also ensures the study was conducted in full conformity with relevant regulations and with the ICH guidelines for Good Clinical Practice (CPMP/ICH/135/95) July 1996. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki as amended in 1989 and was approved by the Institutional Review Board (IRB) of the Institute of Kidney Foundation Hospital and Research Institute, Mirpur-2, Dhaka. Human Research Ethics Committee Clearance Certificate No. is *KFHRI/ECC-001/2016*.

Written informed consent was collected from each and every patient or participants and ensures to maintain patient confidentiality.

Conflicts of interest

The authors declare that there is no conflict of interest.

Acknowledgment

The authors wish to acknowledge the Kidney Foundation Hospital & Research Institute; Mirpur-2, Dhaka, Bangladesh, for their continued assistance in this study.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.conctc.2018.08.007.

References

[1] National Kidney Foundation, K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification, Am. J. Kidney Dis. 39 (Suppl 1) (2002) S1–S266 ISBN 1-931472-10-6.

[2] R. Abudula, P.B. Jeppesen, S.E.D. Rolfsen, J. Xiao, K. Hermansen, Rebaudioside A potently stimulates insulin secretion from isolated mouse islets: studies on the dose-, glucose-, and calcium-dependency, Metabolism 53 (10) (2004) 1378–1381, https://doi.org/10.1016/j.metabol.2004.04.014.

[3] G.E.C. da Silva, A.H. Assef, C.C. Albino, L.A.F. Ferri, G. Tasin, M.H. Takahashi, W.E. Filho, R.B. Bazotte, Investigation of the tolerability of oral stevioside in brazilian hyperlidemic patients, Braz. Arch. Biol. Technol. 49 (4) (2006) 583–587 https://doi.org/10.1590/S1516-89132006000500007.

[4] A. Doughlas Kinghorn, Stevia, the Genus Stevia, 8 vol 162, Taylor & Francis, 2002 ISBN 0-203-26058-9 (Adobe eReader Format); ISBN 0-415-26830-3 (Print Edition).

[5] M.S. Melis, Chronic administration of aqueous extract of *Stevia rebaudiana* in rats: renal effects, J. Ethnopharmacol. 47 (3) (1995) 129–134 https://doi.org/10.1016/0378-8741(95)01271-E.

[6] A.S. Levey, J. Coresh, E. Balk, A.T. Kausz, A. Levin, M.W. Steffes, R.J. Hogg, R.D. Perrone, J. Lau, G. Eknoyan, National Kidney Foundation practice guideline for chronic kidney disease: evaluation, classification, and stratification, Ann. Intern. Med. 139 (2) (2003) 137–147, https://doi.org/10.7326/0003-4819-139-2-200307150-00013.

[7] M. Pestana, Predicting risk in CKD, port, J. Nephrol. Hypert. 29 (3) (2015) 192–193 http://www.scielo.mec.pt/pdf/nep/v29n3/29n3a02.pdf.

[8] S.G. Satko, J.R. Sedor, S.K. Iyengar, B.I. Freedman, Familial clustering of chronic kidney disease, Semin. Dial. 20 (3) (2007) 229–236, https://doi.org/10.1111/j.1525-139X.2007.00282.x.

[9] J. Coresh, L. Wei, G. McQuillan, Prevalence of high blood pressure and elevated Serum creatinine level in the United States, Arch. Intern. Med. 161 (2001) 1207–1216 https://www.ncbi.nlm.nih.gov/pubmed/11343443.

[10] P. Sharma, R.C. Blackburn, C.L. Parke, Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers for adults with early (stage 1 to 3) non-diabetic chronic kidney diseases, Cochrane Database Syst. Rev. 5 (10) (2011) CD007751, , https://doi.org/10.1002/14651858.CD007751.pub2.

[11] M. Noordzij, G. Tripepi, F.W. Dekker, C. Zoccali, M.W. Tanck, K.J. Jager, Sample size calculations: basic principles and common pitfalls, Nephrol. Dial. Transplant. 25 (2010) 1388–1393, https://doi.org/10.1093/ndt/gfp732.

[12] National High Blood Pressure education program, The Sixth Report of the Joint National Committee Prevention, Detection, Evaluation, and Treatment of High Blood Pressure, National Institute of Health, NIH Publication No., Washington, DC, 1997, pp. 98–4080 http://www.sld.cu/galerias/pdf/servicios/hta/6to._reporte_del_jnc_usa.pdf.

[13] M. Haneda, A joint committee on diabetic nephropathy, a new classification of diabetic nephropathy 2014: a report from the joint committee on diabetic nephropathy, J. Diabetes Investig. 6 (2) (2015) 242–246, https://doi.org/10.1111/jdi.12319 Epub 2015 Mar 1.

[14] Diagnosis and classification of diabetes mellitus, american diabetes association, Diabetes Care 34 (Suppl 1) (2011) S62–S69, https://doi.org/10.2337/dc11-S062.

[15] A. Rebollo-Rubio, J.M. Morales-Asencio, M.E. Pons-Raventos, Review of studies on health-related quality of life in patients with advanced chronic kidney disease in Spain, Nefrologia 35 (1) (2015) 92–109, https://doi.org/10.3265/Nefrologia.pre2014.Jul.12133.

[16] M.J. Ruiz, M. Roman, G. Martin, M.J. Alferez, D. Prieto, Calidad de vida relacionada con la salud en las diferentes terapias sustitutivas de la insuficiencia renal cronica, Rev. Soc. Esp. Enferm. Nefrol. 6 (4) (2003) 6–16 http://www.revistaseden.org/files/art309_1.pdf.

[17] N.P. Singh, G.K. Ingle, V.K. Saini, A. Jami, P. Beniwal, M. Lal, G.S. Meena, Prevalence of low glomerular filtration rate, proteinuria and associated risk factors in North India using Cockcroft-Gault and Modification of Diet in Renal Disease equation: an observational, cross-sectional study, BMC Nephrol. 10 (2009) 4, https://doi.org/10.1186/1471-2369-10-4.

[18] W.W. Brown, R.M. Peters, R.N.S. Ohmit, W.F. Keane, A. Collins, S. Chen, K. King, Early detection of kidney disease in community settings: the kidney early evaluation program (KEEP), Am. J. Kidney Dis. 42 (1) (2003) 22–35 https://doi.org/10.1016/S0272-6386(03)00405-0.

[19] M.K. Khan, H.U. Rashid, S. Yesmine, I.H. Mahmood, S.M.A. Habib, A. Hossain, Assessment of Risk Factors for Cardiovascular Complications in Patients with Chronic Kidney Diseases (CKD) Stage III-V before Dialysis, University Heart Journal, 2013, pp. 25–32 vol. 9 (1) https://www.banglajol.info/index.php/UHJ/article/view/19508/13493.

[20] L.A.F. Ferri, W. Alves-Do-Prado, S.S. Yamada, S. Gazola, M.R. Batista, R.B. Bazotte, Investigation of the antihypertensive effect of oral crude stevioside in patients with mild essential hypertension, Phytother Res. 20 (9) (2006) 732–736 https://doi.org/10.1002/ptr.1944.

[21] M.S. Melis, A.R. Sainati, Effect of Calcium and verapamil on renal function of rats during treatment with stevioside, J. Ethnopharmacol. 33 (3) (1991) 257–262, https://doi.org/10.1016/0378-8741(91)90086-S.

[22] Evaluation of certain food additives, fifty-first report of the joint FAO/WHO expert committee on food additives, world health, Organ Tech. Report Ser. 891 (i-viii) (2000) 1–168 https://www.ncbi.nlm.nih.gov/pubmed/10876377.

[23] C.N. Lee, K.L. Wong, J.C. Liu, Inhibitory effect of stevioside on calcium influx to produce antihypertension, Planta Med. 67 (9) (2001) 796–799, https://doi.org/10.1055/s-2001-18841.

[24] H.H. Lei, T.V. Perneger, M.J. Klag, Familial Aggregation of renal disease in a population-based case-controlled study, J. Am. Soc. Nephrol. l9 (7) (1998) 1270–1276 https://www.ncbi.nlm.nih.gov/pubmed/9644638.

[25] W.W. Brown, R.M. Peters, S.E. Ohmit, W.F. Keane, A. Collins, S.C. Chen, K. King, M.J. Klag, D.A. Molony, J.M. Flack, Early detection of kidney disease in community settings; the kidney early evaluation program (KEEP), Am. J. Kidney Dis. 42 (1) (2003) 22–35 https://doi.org/10.1016/S0272-6386(03)00405-0.

[26] S.P. McDonald, G.P. Maguire, W.E. Hoy, Renal function and cardiovascular risk markers in a remote australian aboriginal community, Nephrol. Dial. Transplant. 18 (8) (2003) 1555–1561 https://doi.org/10.1093/ndt/gfg199.

[27] N. Ishizaka, Y. Ishizaka, E. Toda, H. Shimomura, K. Koike, G. Seki, R. Nagai, M. Yamakado, Association between cigarette smoking and chronic kidney diseases in Japanese men, Hypertens. Res. 31 (3) (2008) 485–492, https://doi.org/10.1291/hypres.31.485.

[28] E.M. Briganti, P. Branley, S.J. Chadban, J.J. McNeil, T.A. Welborn, R.C. Atkins, Smoking is associated with renal impairment and proteinuria in the normal population: the aus diab kidney study. Australian diabetes, obesity and lifestyle study, Am. J. Kidney Dis. 40 (4) (2002) 704–712 https://doi.org/10.1053/ajkd.2002.35677.

[29] J.P. New, R.J. Middleton, B. Klebe, Assessing the prevalence, monitoring, and management of chronic Kidney disease in patients with diabetes compared with those without diabetes in general practice, Diabet. Med. 24 (4) (2007) 364–369 http://europepmc.org/abstract/med/6231149.

[30] I. Ahmed, G.T. John, M.G. Kirubakaram, C.K. Jacob, J. Muliyil, Prevalence of Proteinuria in rural adult population in Tamil Naru, Indian J. Med. Res. 124 (2006) 185–188 https://www.ncbi.nlm.nih.gov/pubmed/17015932.

[31] S.K. Agarwal, S.C. Dash, M. Irshad, S. Raju, R. Singh, R.M. Pandey, Prevalence of Chronic renal failure in adults in Delhi, India, Nephrol. Dial. Transplant. 20 (2005) 1638–1642, https://doi.org/10.1093/ndt/gfh855.

[32] W.B. Kannel, M.J. Stampfer, W.P. Castelli, J. Verter, The prognostic significance of proteinuria: the Framingham study, Am. Heart J. 108 (5) (1984) 1347–1352 https://doi.org/10.1016/0002-8703(84)90763-4.