Chin J Integr Med 2016 Oct;22(10):728-737

ORIGINAL ARTICLE

Bromelain and Cardiovascular Risk Factors in Diabetes: An Exploratory Randomized, Placebo Controlled, Double Blind Clinical Trial

Chit Moy Ley (黎七妹)[1], NI Qing (倪　青)[2], LIAO Xing (廖　星)[3], GAO Huai-lin (高怀林)[4], and Nicola Robinson[1]

ABSTRACT **Objective:** To assess whether the dietary supplement (bromelain) has the potential to reduce plasma fibrinogen and other cardiovascular disease (CVD) risk factors in patients with diabetes. **Methods:** This randomized placebo controlled, double blind, parallel design, efficacy study was carried out in China and investigated the effect of 12 weeks of bromelain (1,050 mg/day) on plasma fibrinogen. This randomized controlled trial (RCT) recruited 68 Chinese diabetic patients [32 males and 36 females; Han origin, mean age of 61.26 years (standard deviation (SD), 12.62 years)] with at least one CVD risk factor. Patients were randomized into either bromelain or placebo group. While bromelain group received bromelain capsule, the placebo group received placebo capsule which consisted inert ingredient and has no treatment effect. Subjects were required to take 1,050 mg (3×350 mg) of either bromelain or starch-filled placebo capsules, two to be taken (2×350 mg) after breakfast and another (350 mg) after dinner, daily for 12 weeks. Plasma fibrinogen, CVD risk factors and anthropometric indicators were determined at baseline and at 12 weeks. **Results:** The change in the fibrinogen level in the bromelain group at the end of the study showed a mean reduction of 0.13 g/L (standard deviation (SD) 0.86g/L) compared with the mean reduction of 0.36 g/L (SD 0.96 g/L) for the placebo group. However, there was no significant difference in the mean change in fibrinogen between the placebo and bromelain groups (mean difference=0.23g/L (SD 0.22 g/L), *P*=0.291). Similarly, the difference in mean change in other CVD risk factors (blood lipids, blood pressure), blood glucose, C-reactive protein and anthropometric measures between the bromelain and placebo groups was also not statistically significant. Statistical differences in fibrinogen between bromelain and placebo groups before the trial despite randomization may have influenced the results of this study. **Conclusion:** This RCT failed to show a beneficial effect in reducing fibrinogen or influencing other selected CVD risk factors but suggests other avenues for subsequent research on bromelain.

KEYWORDS cardiovascular disease, fibrinogen, diabetes, bromelain, randomized control trial

Heart disease and stroke are the leading causes of death and disability among people with type 2 diabetes.[1] Chinese adults with diabetes are 1.7 times more likely to develop cardiovascular disease (CVD) than those with normal fasting glucose, with estimates suggesting that 3.3% of CVD in China is attributable to type 2 diabetes.[2] Heart disease is now known to be the leading cause of death in China.[3] In 2011, China became the global epicentre of the diabetes epidemic with 90 million adults with the disease, representing 9.0% prevalence of diabetes.[4] This suggests that diabetes has already become a major public health problem in China and will significantly contribute to CVD mortality.

People with type 2 diabetes can control their blood glucose by following a careful diet and exercise to lose excess weight, and/or by taking oral diabetes medications. Integrative approaches combined with the correct pharmacological treatment and healthy lifestyles that include optimal nutrition, dietary supplements, exercise, smoking cessation and moderate restriction of alcohol and caffeine may reduce cardiovascular risk factors of stroke, coronary heart disease (CHD), congestive heart failure and renal disease.[5]

1. School of Health and Social Care, London South Bank University, SE1 0AA, London, United Kingdom; 2. Department of Endocrinology, Guang'anmen Hospital, China Academy of Chinese Medical Sciences, Beijing (100730), China; 3. Institute of Basic Research in Clinical Medicine, China Academy of Chinese Medical Sciences, Beijing (100700), China; 4. Department of Endocrinology, Yiling Hospital Affiliated to Hebei Medical University, Shijingzhuang (050091), Hebei Province, China
Correspondence to: Dr. Ley Chit Moy, Tel: 44-0-201815 7940, E-mail: leykara@yahoo.com.my

DOI: 10.1007/s11655-016-2521-2

Conventional therapies as well as dietary management using dietary supplements are being used as measures to prevent the development of complications of CVD in diabetes. Sources of dietary supplements may therefore be worthy of exploration.

Elevated plasma fibrinogen levels have been found to be a strong independent risk factor for developing CVD in humans.[6] The availability of an oral fibrinogen lowering therapy may greatly enhance the management of CVD.[6] One animal study has demonstrated that bromelain had a specific activity to reduce levels of plasma fibrinogen.[7] Providing bromelain as a dietary supplement in humans could potentially provide a therapeutic opportunity which should be explored.

Bromelain is the general name for a family of sulfhydryl proteolytic enzymes obtained from *Ananas comosus*, the pineapple plant.[8] At present, bromelain is marketed as a dietary supplement and is available to the community in health food stores and pharmacies in Europe and the US. In China, it is approved as a registered drug and widely used clinically for reduction of inflammation, specifically thrombophlebitis.[9]

The fibrinolytic activity of bromelain is attributed to its mechanism which appears to enhance the conversion of plasminogen to plasmin, resulting in increased fibrinolysis.[10] Bromelain's specificity is similar to that of the endogenous protease plasmin which degrades fibrin to stimulate the synthesis of anti-inflammatory prostaglandins[7] and subsequently limits the spread of the coagulation process.[11,12] Bromelain may explain the possible mechanism linking impaired fibrinolysis and atherosclerosis associated with CVD in diabetes.[13]

A systematic review suggested that there is more significant evidence from animal studies on the effect of bromelain on cardiovascular systems in animal models.[14] The review also analyzed the small number of human studies on bromelain, CVD related safety and inflammation (carried out in the 1960's and 70's) and highlighted that study design and reporting of clinical outcomes were poor. However, it suggested the need for further research to explore and verify the effects of bromelain.[14] It was decided to carry out a randomized controlled trial (RCT). This aim of the clinical trial was to evaluate the clinical efficacy of bromelain to reduce the risk factors associated with CVD in people with diabetes.

METHODS

This study was an exploratory prospective, randomized placebo controlled double blind, parallel design, efficacy study to assess the effects of 12 weeks of bromelain (1,050 mg/d) supplementation on plasma fibrinogen levels in people with diabetes. The study was conducted in China and was approved by the local ethical committee of Yiling Hospital Affiliated to Hebei Medical University, China, with the ethical approval number: YLLL2011[Y] 003. The trial is reported following the Consolidated Standards of Reporting Trials (CONSORT) statement guidelines[15] and registered with ClinicalTrials.gov at http://www.clinicaltrials.gov (identification number NCT01524159).

Inclusion and Exclusion Criteria

Patients were included if they were: 18 years of age or older; diagnosed with type 2 diabetes, glycosylated haemoglobin (HbA_{1c}) > 6.5% and had one of the following risk parameters: total cholesterol between 5.0–6.2 mmol/L, a body mass index > 25 kg/m^2, waist circumference (WC) > 101.6 cm for men and > 88.9 cm for women, triglycerides (TG) > 1.7 mmol/L, high density lipid cholesterol (HDL-C)< 1 mmol/L for men and < 1.3 mmol/L for women, low density lipid cholesterol (LDL-C) > 2.6 mmol/L or blood pressure > 140/90 mm Hg. Exclusion criteria were: women who were pregnant or trying to conceive or lactating; subjects with severe health problems such as: renal disease, liver disease, cardiovascular disease, and other chronic health conditions; subjects currently taking warfarin, aspirin or statins; subjects already taking bromelain supplements, or other herbal supplements including Chinese medicines which may be effective e.g., cinnamon; subjects with risk factors of CVD and currently treated with any therapeutic methods; a history of allergic reactions to bee stings, olive tree pollen or pineapple; a history of occupational inhalant/skin contact with bromelain; subjects with recent diabetes ketoacidosis (past 2 weeks); unable to provide informed consent, i.e., unable to follow diet control advice or not willing to follow study procedure.

Recruitment of Study Population

Subjects were diabetic inpatients and outpatients, recruited from the Diabetic Unit in Yiling

Hospital Affiliated to Hebei Medical University, China. The investigators identified potential subjects attending at Yiling Hospital by assessing their recent medical reports and blood tests relating to any of the CVD risk parameters. Suitable patients based on the inclusion and exclusion criteria of the study were identified and contacted by phone prior to their hospital appointment by the doctors and were informed about the study. During their hospital appointment, patients were provided with the participant information sheet and consent form. Their contents were explained by the investigators and they were invited to take part in the study. The recruitment was entirely on a voluntary basis and no one was asked to participate against his or her will. The recruited subjects were able to withdraw or dropout from the study at any time.

Randomization of Study Population

After obtaining the signed consent form which indicated the agreement to participate, the patients were randomized into the placebo or treatment group using a computer generated sequence from Statistical Analysis System (SAS) software. In this trial, randomization with allocation concealment by opaque sequentially numbered sealed envelopes was conducted.

Sample Size of Study Population

As there was no previous study on bromelain and fibrinogen on which to base a sample size calculation, this study was initiated as an exploratory trial, not powered to be definitive but to provide the basis for sample size calculation for any future trial. It was decided this study should aim to be a similar size in a review of clinical studies using bromelain as a treatment for osteoarthritis[16] and the recommendation in a pilot study using bromelain on osteoarthritis.[17] Therefore, a total of 72 subjects were recruited which would allow for a 10% dropout.

Intervention Program

Both bromelain [600 Gelatin Dissolving Units (GDU)] and placebo capsules in this study were manufactured by Jilin Province Hongjiu Biotech Co., Ltd., Ping'anchuan Developing Zone, Huinan County, Jilin Province, China, Batch No. 135118. The placebo capsule consisted of inert ingredient (wheat starch) and was indistinguishable in colour and size compared with the bromelain capsule. The capsules therefore appeared identical to study subjects. Each capsule had 350 mg of bromelain or wheat starch (placebo) and was kept in a white plastic bottle and marked A or B. The investigators, clinicians and the patients involved in this trial did not know whether bromelain or placebo capsules were contained in either bottle A or B. This was to achieve double blinding and to ensure that the investigators, clinicians and patients did not know the allocation of the capsules.

Subjects were required to take 1,050 mg (3×350 mg) of either bromelain or starch-filled placebo capsules, two (2×350 mg) after breakfast and another (350 mg) after dinner, daily for 12 weeks. Subjects were instructed to take the capsules 2 h after the meals. This was because bromelain tends to act as a digestive enzyme and its therapeutic benefit may be diminished if taken with food.[8] The 1,050 mg dosage was chosen based on a review by Kelly[8] who suggested that the optimum effect of bromelain occurs starting at a dose of 750–1,000 mg/d for a longer period of time.

Compliance was monitored by the investigators counting the returned capsules at the end of 12 weeks. All investigators called the subjects weekly to ensure that they consumed the capsules as instructed. The compliance was recorded.

Demographic and Lifestyle Measurements

A structured questionnaire (validated and adapted for a Chinese cultural background) was used to collect subjects' information before and after the 12-week intervention. During the study, all subjects were maintained on the standard diet recommended for diabetic patients in the clinic and they were advised not to change their physical activity and lifestyle during the course of the study.

Anthropometric Measurements

Body weight was measured with patients wearing light indoor clothing and using a weighing scale with an accuracy of 0.5 kg. Standing height was recorded without shoes, on a flat surface, with weight distributed evenly on both feet and heels together using a stadiometer to the nearest 0.5 cm. A vertical board with an attached metric rule and a horizontal headboard that could bring into contact with the uppermost point on the head was used to measure height. BMI was calculated by weight in kilograms divided by the square of height in meters (kg/m^2). Waist circumference was taken

midway between the lower margin of the last palpable rib and the top of iliac crest to the nearest 0.5 cm. It was measured with the subject wearing light clothing, standing with feet close together, arms at the side and body weight evenly distributed.

Blood Pressure Measurements

Blood pressure was measured using a calibrated mercury sphygmomanometer and stethoscope. Two measurements were taken from the right arm with cubital fossa supported at heart level after subjects had voided, rested, and been comfortably seated for 10 min. The average of these measurements was used as the blood pressure.

Other Blood Test and Biochemical Data Measurement

All blood tests and other biochemical data were collected and tested before and after 12-week intervention in the standard laboratory in Yiling Hospital. Blood samples were collected in the morning after a 12-h fast. Fasting blood glucose (FBG), postprandial blood glucose (PPBG) and HbA_{1c} were measured using a rapid method NycoCard®HbA_{1c} which is standardized according to the recommendation of the European Reference Laboratory (ERL) at the Diabetes Control and Complications Trial-level and is certified in accordance with the ERL Check-Up Protocol.[18] Fibrinogen level was measured with Clauss-based functional assays using coagulation analyzer (TS4000) from MD Pacific Technology Co., Ltd. Tianjin, China. The operation was based on advanced photo electronic-double magnetic testing system. C-reactive protein (CRP); liver function tests included alanine transaminase (ALT) and aspartate aminotransferase (AST); kidney function tests included blood urea nitrogen (BUN) and creatinine (Cr) were measured using a Biochemistry Automatic Analyzer, model Hitachi 7180 (Hitachi High-Technologies Corporation, Tokyo, Japan). Serum total cholesterol, TG and HDL-C were analyzed on the same Hitachi 7180 Analyzer using commercial reagents. LDL-C concentrations were calculated according to the Friedewald formula.[19]

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) 16.0 for Windows was used for the statistical data analysis. Both descriptive and inferential statistics were employed to describe the quantitative data and to test whether there is a difference in change in plasma fibrinogen and other risk factors for subjects with type 2 diabetes following bromelain intervention compared to placebo group at a predetermined alpha level of 0.05, respectively.

In this study, all studied variables were found to be normally distributed based on the graphical measures using histogram and Q-Q plots ('Q' stands for quartile). Therefore, parametric tests such as independent sample *t*-test and paired sample *t*-test were performed for comparisons of means between groups and within groups respectively for the primary outcome of fibrinogen and secondary outcomes of other CVD risk factors such as blood lipid profiles, blood glucose, CRP, anthropometric indicators and blood pressures.

The treatment effect of bromelain (independent variable) on both primary and secondary outcomes (dependent variables) was further confirmed using an analysis of covariance (ANCOVA). The baseline characteristics of the respondents were used as the covariates to determine their possible influence on the dependent variables after 12-week intervention. For instance, the possible influences of extraneous variables (covariates) such as FBG, PPBG, HbA_{1c}, diabetes duration, CRP and age on the dependent variables, fibrinogen level after 12 weeks of intervention were statistically controlled during the analysis.

The effectiveness of bromelain was determined by comparing the change (from baseline to 12-week measurement) for the bromelain group and the change for the placebo group. This was done using an independent sample *t*-test carried out on the differences (week-12 minus week-0) comparing bromelain and placebo group. Independent sample *t*-tests were also used to compare the mean differences of other parameters (e.g., blood lipid profiles, blood glucose profiles, CRP, anthropometric indicators, blood pressure) between bromelain and placebo groups at baseline and at post-intervention. Intention-to-treat approach which provides unbiased comparisons among treatment groups[20] was used in the analysis of this RCT.

RESULTS

Participant Flow

Of the 149 subjects seen at the hospital in 3-month

period, who were assessed for their eligibility for entry to the trial, 60 did not meet the inclusive criteria. A further 17 were excluded for other reasons (Figure 1). A total of 72 subjects were recruited and randomized to either the bromelain (*n*=37) or the placebo group (*n*=35).

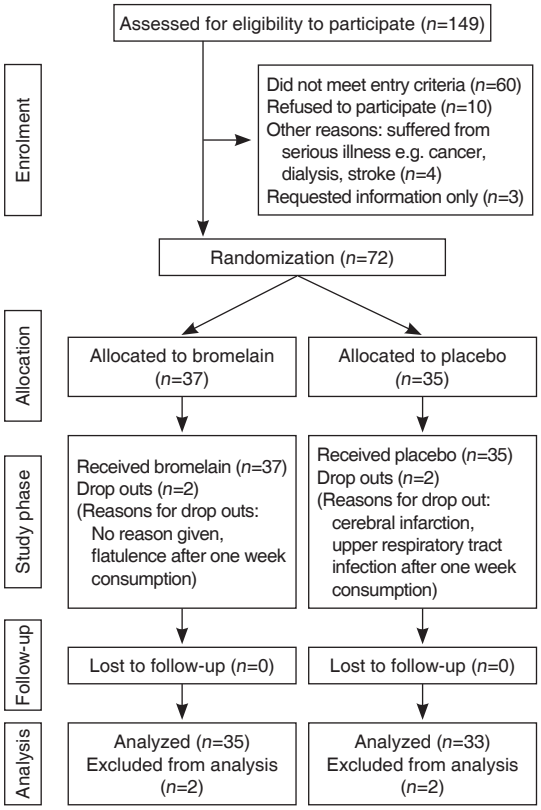

Figure 1. Study Flow Chart Identifying the Number of Diabetes Subjects Enrolled, Allocated to Treatment, Followed up and Analyzed for the RCT (Based on CONSORT Guidelines)

There was a low dropout rate and no adverse events reported during the trial indicating the acceptability and tolerability of the treatment. Four subjects dropped out, two in the bromelain group and two in the placebo group, but this was unrelated to the trial intervention.

Baseline Characteristics of the Study Subjects

The baseline socio-demographic, lifestyle and diabetes characteristics of the study subjects are given in Table 1. There was no difference in all the baseline characteristics among subjects apart for duration of diabetes (*P*>0.05).

Changes in the Study Outcomes

Table 2 gives data at baseline and post-intervention on primary and secondary outcome measures. Despite the randomisation, the difference in mean fibrinogen level was found to be statistically significant between bromelain and placebo groups at the beginning of the study (*P*=0.012). This baseline difference means that demonstrating a significant difference as a result of bromelain intervention was problematic. The independent *t*-test compared the fibrinogen level reductions and indicated there was no significant difference in the mean change in fibrinogen between the placebo and bromelain groups (*P*=0.291). Based on the ANCOVA result, the treatment effect of bromelain was not significant (*P*=0.293) when all the possible covariates (baseline fibrinogen, FBG, PPBG, HbA_{1c}, diabetes duration, CRP and age) were included in the model. Since the value obtained from the statistical analysis exceeds 0.05, there was not enough evidence to show that fibrinogen changed differently in the treatment group after 12 weeks of bromelain intervention when compared with the change in the placebo group (Table 2).

There were also no significant differences in mean change for blood lipids, blood glucose, CRP, anthropometric measures and blood pressure (between the bromelain and placebo groups (Table 2). Using ANCOVA to adjust for the possible influence of baseline anthropometric and biochemical characteristics on these variables at 12 weeks demonstrated that there was no effect of both groups; bromelain was no more effective than placebo with regard to these changing variables.

There were statistically significant for the difference in mean change in LDL-C (*P*=0.019) and PPBG (*P*=0.016) between the bromelain and placebo groups (Table 2). However, the ANCOVA analysis which was used to adjust for the possible influence of baseline anthropometric and biochemical characteristics on these variables at 12 weeks, showed no effect of both groups. Bromelain did not show its effect with regard to changing variables compared with placebo.

There were also no statistically significant differences observed at post-intervention between groups in safety measure indicators of ALT, AST for liver and BUN and Cr for kidney.

DISCUSSION

This RCT failed to show that bromelain could

Table 1. Comparison of Baseline Socio-Demographic Characteristics of Subjects in the Bromelain and Placebo Groups

Socio-demographic characteristic	Bromelain group (37 cases)	Placebo group (35 cases)	*P* value	Socio-demographic characteristic	Bromelain group (37 cases)	Placebo group (35 cases)	*P* value
Age (Years)	61.00 (10.35)	61.54 (14.79)	0.858	Mixed diet with more meat	9 (24.3)	8 (22.9)	0.884
Gender				Physical activity			
Male	16 (43.2)	16 (45.7)		sedentary	10 (27.0)	10 (28.6)	
Female	21 (56.8)	19 (54.3)	0.833	Not sedentary	27 (73.0)	25 (71.4)	0.884
Employment (*N*=71)				In or outdoor exercise for > 15 min			
Retired/no-occupation	25 (67.6)	21 (61.8)		Always (≥3 times/week)	26 (70.3)	24 (68.6)	
General worker	5 (13.5)	7 (20.6)		Sometimes (<3 times/ week) or no exercise	11 (29.7)	11 (31.4)	0.876
Others*	7 (18.9)	6 (17.6)	0.729	Stress			
Marital status				Big	6 (16.2)	5 (14.3)	
Married	35 (94.6)	31 (88.6)		Normal	17 (45.9)	20 (57.1)	
Single	2 (5.4)	4 (11.4)	–	Little or none	14 (37.8)	10 (28.6)	0.623
Education				Diabetes duration (Years)	8.10 (6.95)	12.80 (9.04)	0.016
Primary	7 (18.9)	10 (28.6)		Other diagnosed disease			
Secondary	8 (21.6)	6 (17.1)		High blood pressure	17 (45.9)	15 (42.9)	0.792
Higher secondary	11 (29.7)	10 (28.6)		Heart disease	12 (32.4)	7 (20.0)	0.232
University	11 (29.7)	9 (25.7)		High cholesterol	6 (16.2)	8 (22.9)	0.477
Post-graduate	0	0	0.799	Others**	11 (29.7)	10 (28.6)	0.914
Monthly income				None	11 (29.7)	15 (42.9)	0.246
No income / ≤1,000 ¥	10 (27.0)	5 (14.3)		Family history of diabetes			
1,001–3,000 ¥	22 (59.5)	24 (68.6)		No family history	29 (78.4)	23 (65.7)	
>3,000 ¥	5 (13.5)	6 (17.1)	0.409	Family history with one or > one diabetes	8 (21.6)	12 (34.3)	0.230
Smoking				Type of anti-diabetic medication			
Never	29 (78.4)	30 (85.7)		Oral hypoglycaemic drug (metformin and sulphonylureas) only	14 (37.8)	15 (42.9)	
Current/ex-smoker	8 (21.6)	5 (14.3)	0.419	Insulin only	9 (24.3)	9 (25.7)	
Alcohol				Insulin and oral hypoglycaemic drug	14 (37.8)	11 (31.4)	0.844
Never	32 (86.5)	33 (94.2)		Anti-hypertension medication			
Current/ex-alcohol drinker	5 (13.5)	2 (5.8)	0.264	Yes	4 (10.8)	10 (28.6)	
Diet				No	33 (89.2)	25 (71.4)	0.057
Mixed diet with more vegetable	28 (75.7)	27 (77.1)					

Notes: *others: included farmers, government servant, own business, accountant, engineer and teacher; **others: included cerebral infarction, cataract, hepatic disease, overweight, cerebral haemorrhage, ulcer and prolapsed of lumber intervertebral, kidney disease, hyperthyroidism and cervical osteoarthritis.

reduce fibrinogen levels among diabetic patients at risk of cardiovascular disease compared with those taking a placebo. This may have been because the majority (65.7%, *n*=23) of the diabetic patients at baseline already had low baseline fibrinogen levels ranging from 2.01 to 4.00 g/L. It would therefore have been difficult for fibrinogen levels to reduce any further, particularly for those patients with fibrinogen levels ranging between 2.01 and 3.00 g/L which represented 28.6% (*n*=10) diabetic patients in the bromelain group. At baseline, despite randomization there was a difference in fibrinogen levels between the two groups with those in the placebo group having a fibrinogen levels greater than 4.01 g/L. This difference at baseline may explain why there was a significant drop of fibrinogen in favour of the placebo group over the bromelain group after 12 weeks intervention. Unfortunately this RCT did not screen for high fibrinogen (>4.0 g/L) at the selection or at randomization due to the additional cost required

Table 2. Eeffect of Bromelain on Fibrinogen, Blood Lipid Profile, Blood Glucose Profile, CRP, Anthropometric Measures and Blood Pressure [Mean (SD)]

Variables	Intervention	Bromelain group (35 cases)	Placebo group (33 cases)	Mean difference	*P* value
Fibrinogen (g/L)	Baseline	3.66 (0.99)	4.31 (1.08)	0.65 (0.25)	0.012*
	Post-intervention	3.54 (0.92)	3.95 (0.75)	0.41 (0.20)	0.047*
	Difference	–0.13 (0.86)	–0.36 (0.96)	0.23 (0.22)	0.291
Blood lipid profile					
TC (mmol/L)	Baseline	4.91(0.92)	5.14 (0.83)	0.22 (0.21)	0.294
	Post-intervention	5.04 (0.89)	4.99 (0.98)	0.05 (0.23)	0.834
	Difference	0.13 (0.87)	–0.15 (0.91)	0.27 (0.22)	0.212
HDL-C (mmol/L)	Baseline	1.15 (0.29)	1.23 (0.36)	0.08 (0.08)	0.341
	Post-intervention	1.20 (0.20)	1.21 (0.35)	0.01 (0.07)	0.936
	Difference	0.05 (0.35)	–0.02 (0.25)	0.07 (0.074)	0.964
LDL-C (mmol/L)	Baseline	2.87 (0.78)	3.22 (1.00)	0.35 (0.22)	0.114
	Post-intervention	3.05 (0.70)	2.80 (0.83)	0.25 (0.19)	0.185
	Difference	0.18 (0.94)	–0.42 (1.11)	0.60 (0.25)	0.019*
TG (mmol/L)	Baseline	2.03 (1.03)	1.89 (0.98)	0.13 (0.24)	0.582
	Post-intervention	1.79 (1.04)	1.94 (0.98)	0.14 (0.24)	0.557
	Difference	–0.23 (1.18)	0.05 (1.14)	0.28 (0.28)	0.326
HDL-C/LDL-C ratio	Baseline	0.43 (0.17)	0.41 (0.14)	0.02 (0.04)	0.517
	Post-intervention	0.41 (0.11)	0.45 (0.12)	0.04 (0.03)	0.178
	Difference	–0.02 (0.18)	0.14 (0.11)	–0.06 (0.03)	0.089
Blood glucose profile					
FBG (mmol/L)	Baseline	8.28 (2.56)	7.45 (2.88)	0.83 (0.66)	0.214
	Post-intervention	7.32 (1.28)	7.68 (2.27)	0.36 (0.44)	0.431
	Difference	–0.96 (2.60)	0.23 (2.35)	–1.19 (0.60)	0.053
PPBG (mmol/L)	Baseline	12.38 (3.31)	10.75 (2.81)	1.63 (0.75)	0.032*
	Post-intervention	9.45 (1.28)	9.47 (2.08)	0.02 (0.42)	0.962
	Difference	–2.92 (3.37)	–1.28 (1.94)	–1.65 (0.66)	0.016*
HbA_{1c} (%)	Baseline	11.41 (2.64)	10.95 (2.50)	0.46 (0.62)	0.467
	Post-intervention	9.19 (2.15)	8.58 (1.96)	0.61 (0.50)	0.227
	Difference	–2.22 (2.25)	–2.38 (2.46)	0.15 (0.57)	0.790
CRP (mg/L)	Baseline	4.20 (4.03)	4.56 (5.18)	0.36 (1.12)	0.748
	Post-intervention	2.60 (2.17)	2.28 (0.95)	0.32 (0.41)	0.438
	Difference	–1.60 (4.63)	–2.28 (5.15)	0.68 (1.19)	0.567
Anthropometric measures					
BMI (kg/m^2)	Baseline	25.74 (3.17)	25.52 (2.99)	0.22 (0.75)	0.774
	Post-intervention	25.65 (3.14)	25.38 (2.89)	0.27 (0.73)	0.710
	Difference	–0.09 (0.22)	–0.15 (0.39)	0.06 (0.08)	0.456
WC (cm)	Baseline	90.47 (7.88)	88.74 (8.01)	1.73 (1.93)	0.372
	Post-intervention	90.71 (8.16)	88.74 (7.87)	1.97 (1.95)	0.316
	Difference	0.24 (1.60)	0.01 (1.54)	0.23 (0.38)	0.544
BW (kg)	Baseline	70.46 (10.13)	68.91 (9.66)	1.55 (2.40)	0.708
	Post-intervention	70.21 (10.05)	68.48 (9.57)	1.74 (2.38)	0.636
	Difference	–0.25 (0.62)	–0.43 (0.97)	0.19 (0.20)	0.350

(To Be Continued)

(Continued)

Variables	Intervention	Bromelain group (35 cases)	Placebo group (33 cases)	Mean difference	*P* value
Blood pressure					
SBP	Baseline	132.26 (4.08)	131.21 (2.78)	1.05 (3.27)	0.750
	Post-intervention	127.71 (8.17)	127.06 (10.8)	1.35 (2.56)	0.604
	Difference	–4.54 (9.52)	–4.85 (8.06)	0.31 (2.15)	0.887
DBP	Baseline	81.06 (9.52)	79.55 (8.27)	1.51 (2.17)	0.577
	Post-intervention	76.00 (6.16)	77.27 (6.26)	1.27 (1.51)	0.320
	Difference	–5.05 (8.94)	–2.27 (7.95)	–2.78 (2.05)	0.179

Notes: *shows a significant difference between the bromelain and placebo groups; PPBG=post prandial blood glucose; HbA_{1c}= glycosylated haemoglobin; BMI=body mass index; WC=waist circumference; BW=body weight; SBP: systolic blood pressure; DBP: diastolic blood pressure.

to conduct this screening test. If baseline values of plasma fibrinogen levels had been recorded prior to randomization the possibility of recruiting patients with low or normal fibrinogen level would have been excluded and therefore possibly provided more positive results in favour of bromelain intervention.

There was no significant change or improvement in the blood lipid profile except LDL-C in this RCT. There was also no significant difference on the mean change in HDL-C/LDL-C ratio between groups (*P*=0.089). This RCT showed no improvement on blood lipids using bromelain, which is in contrast with previous studies demonstrating that the fibrinogen reduction is dependent upon lipid and lipoprotein reduction using atorvastatin.[21,22] An animal study evaluating the effects of bromelain supplementation in laying hens on egg production reported that liver cholesterol levels were significantly reduced in the bromelain supplementation group.[23] However, assessment of bromelain in an animal study does not necessarily reflect the exact situation of that in humans which may explain the failure of this RCT to demonstrate the significant result of bromelain on total cholesterol.

Bromelain had no effect on glycaemic control measured by HbA_{1c}. This is consistent with the findings that bromelain did not significantly affect any of the measured clinical parameters in volunteers in a study consisting of several patients who had myocardial infarction or stroke and with high aggregation value.[24] In the present study, for patients with type 2 diabetes, plasma glucose control (FBG and HbA_{1c}) neither deteriorated nor ameliorated using bromelain. The degree of hyperglycaemia in diabetic individuals is correlated with the fibrinogen level but the effect of improving glycaemic control on fibrinogen levels is unclear.[25] This study showed no effect of bromelain on fibrinogen and glycaemic control on HbA_{1c} of the diabetic patients. The proposed association with hyperfibrinogenemia may contribute to the increased risk of atherosclerotic risk in diabetes due to poor glycaemic control could not be determined in this study.[25,26] We observed no treatment effect of bromelain in FBG and HbA_{1c} in this study.

This study demonstrated that bromelain was no more effective than placebo at lowering CRP which in combination with fibrinogen is a signature of inflammatory processes in the body.[27] The results in this RCT contrast with the claims that bromelain has its beneficial effects in its anti-inflammatory properties in a number of clinical trials, particularly in joint inflammation[17,28,29] as well as its importance in finding it effective as an adjunct in the treatment of acute thrombophlebitis due to inflammation of a vein.[30] Since elevated CRP alone has been indicated as a marker for early bacterial infection, active rheumatoid disease, Crohn's disease and acute myocardial infarction and following trauma.[27] Individuals with diabetes tend to be pro-atherogenic which could be due to the inflammation process although the underlying mechanisms have not been fully determined,[13,31,32] other inflammatory indicators such as interleukin (IL)-6 and IL-1 should be considered in future research.

Bromelain did not show any significant effect on the reduction in body weight and BMI. Unlike a previous study which showed that bromelain-pancreatin combinations were effective in digestive insufficiency by increasing fat absorption and reducing faecal weight.[33] Although the association of the benefits of bromelain in blood pressure lowering treatment among diabetic patients was not

evident in our current trial, this result is in support of Gutfreund, et al[34] trial which reported no change in blood pressure in humans at any dosage level, even at the high dosage of 1,840 mg. Blood pressure was measured at 0.5, 2 and 24 h after bromelain administration in this study.[34]

There were a number of limitations in this study. The limitations are the disadvantage by chance of having a substantial imbalance at randomization such as the PPBG and fibrinogen levels which were significantly different at baseline between bromelain and placebo groups; variation of fibrinogen level due to different clinical laboratories practice on using different tests and assays of fibrinogen;[35] insufficient data from the human trials on bromelain and CVD on which to base a pre-study calculation of sample size; small sample size which may reduce the chance of demonstrating significant differences between study groups; possibility of patient compliance in consuming all capsules. In addition, the sample size was too small to reach to any definitive conclusions.

Using the data from this RCT carried out in a diabetic population a retrospective power calculation was conducted which takes into account the difference in mean fibrinogen level for bromelain group, difference in mean fibrinogen level for placebo group (0.36 ± 0.91 g/L), test level (0.05) and total sample size,[72] has a power level of 33.0%, which is far less than the usual minimum power of 80% set when designing RCTs. Indeed if we had known in advance the size of differences that would be found in the RCT and used them in a sample size calculation we would have found based on this difference in mean change and SD, and using a two sided statistical test at 5% test level and 80% power, a sample size of 246 per arm would be required.[36]

In conclusion, the data from this RCT failed to identify efficacy for bromelain (1.05 g/d for 12 weeks) as dietary supplement to reduce plasma fibrinogen or other risk factors associated with CVD in individuals with type 2 diabetes versus placebo. A placebo controlled trial with a larger number of people, with higher fibrinogen levels and/or individuals with a greater number of risk factors of CVD maybe a potential area for further research. The significant results obtained for the reduction of ADP-induced platelet aggregation using orally administered bromelain by Heinicke, et al[24] was not studied in this RCT due to financial constraints. Changes in platelet aggregation may be a potentially useful outcome measure in future research linking bromelain with CVD risk in diabetic patients. However, it is likely that other inflammatory markers such as IL-6, IL-1 and tumour necrosis factor-alpha (TNF-α), a cytokine production, may be more promising and should be considered for inclusion as potential study outcomes in future trials since inflammation may be the underlying mechanism between diabetes and CVD.[13]

Conflict of Interest

There were no conflicts of interest in carrying out this RCT.

Authors Contribution

Main author (Chit Moy Ley) contributed the research design and concept; collection of data; data analysis and interpretation; writing the article. Second author (Ni Q), third author (Liao X) and fourth author (Gao HL) helped to coordinate the execution of the clinical trial in China. Fifth author (Nicola Robinson) was the PhD supervisor for this project, contributed to the design of the project and critically revised the article.

Acknowledgements

The study was sponsored by the Lee Foundation, Malaysia and assisted by Yiling Hospital Affiliated to Hebei Medical University, China. Thank you to London South Bank University for hosting this postgraduate research.

REFERENCES

1. American Heart Association. Cardiovascular disease & diabetes. http://www heart org/HEARTORG/Conditions/Diabetes/WhyDiabetesMatters/Cardiovascular-Disease-Diabetes_UCM_313865_Article jsp 2010; available from: URL: http://www.heart.org/HEARTORG/Conditions/Diabetes/WhyDiabetesMatters/Cardiovascular-Disease-Diabetes_UCM_313865_Article.jsp
2. Chen X, Wang Y. The epidemic of diabetes and its impact on cardiovascular health in contemporary China. CVD Prev Control 2009;4:27-40.
3. China Centers for Disease Control and Prevention, CDC. China facts. http://www chinacdc net cn 2011;Available from: URL: http://www.chinacdc.net.cn
4. International Diabetes Federation. 5th edition of the diabetes atlas released on world diabetes day. http://www idf org/diabetesatlas/news/fifth-edition-release 2011 November 14;Available from: URL: http://www.idf.org/diabetesatlas/news/fifth-edition-release
5. Houston MC. Nutraceuticals, vitamins, antioxidants, and

minerals in the prevention and treatment of hypertension. Progr Cardiovasc Dis 2005;47:396-449.
6. Handley DA, Hughes TE. Pharmacological approaches and strategies for therapeutic modulation of fibrinogen. Thrombosis Res 1997;87:1-36.
7. Livio M, Bertoni MP, deGaetano G, Donati MB. Effect of bromelain on fibrinogen level, prothrombin complex factors and platelet aggregation in the rat. Drugs Expt Clin Res 1978;4:49-53.
8. Kelly GS. Bromelain: a literature review and discussion of its therapeutic applications. Alt Med Rev 1996;1:243-257.
9. China Medicine Centre. Approved Medicine Website. http:// www yaopinnet com/tools/yibao asp 2011 July 26;Available from: URL: http://www.yaopinnet.com/tools/yibao.asp
10. Felton GE. Fibrinolytic and antithrombotic action of bromelain may eliminate thrombosis in heart patients. Med Hypotheses 1980;6:1123-1133.
11. Bhattacharyya BK. Bromelain: an overview. Natl Prod Radiance 2008;7:359-363.
12. Taussig SJ. The mechanism of the physiological action of bromelain. Med Hypotheses 1980;6:99-104.
13. Fonseca V, Desouza C, Asnani S, Jialal I. Nontraditional risk factors for cardiovascular disease in diabetes. Endocrine Rev 2004;25:153-175.
14. Ley CM, Tsiami A, Ni Q, Robinson N. A review of the use of bromelain in cardiovascular diseases. J Chin Integr Med 2011;9:702-710.
15. Schulz KF, Altman DG, Moher D. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. BMJ 2010;24:340.
16. Brien S, Lewith G, Walker A, Hicks SM, Middleton D. Bromelain as a treatment for osteoarthritis: a review of clinical studies. Evid Based Complement Altern Med 2004;1:251-257.
17. Brien S, Lewith G, Walker AF, Middleton R, Prescott P, Bundy R. Bromelain as an adjunctive treatment for moderate-to-severe osteoarthritis of the knee: a randomized placebo-controlled pilot study. Quart J Med 2006;99:841-850.
18. Chinese Diabetes Society. China guideline for type 2 diabetes. Beijing: Peking University Medical Press; 2010.
19. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-501.
20. Chene G, Morlat P, Leport C, Hafner R, Dequae L, Charreau I, et al. Intention-to-treat vs. on-treatment analyses of clinical trial data: experience from a study of pyrimethamine in the primary prophylaxis of toxoplasmosis in HIV-infected patients. Contr Clin Trials 1998;19:233-248.
21. Leibovitz E, Hazanov N, Frieman A, Elly I, Govish D. Atorvastatin reduces fibrinogen levels in patients with severe hypercholesterolemia: additional evidence to support the anti-inflammatory effects of statins. IMAJ 2004;6:456-459.
22. Marais AD, Firth JC, Bateman ME, Byrnes P, Martens C, Mountney J. Atorvastatin: an effective lipid-modifying agent in familial hypercholesterolemia. Arterioscler Thrombosis Vascul Biol 1997;17:1527-1531.
23. Lien TF, Cheng YH, Wu CP. Effects of supplemental bromelain on egg production and quality, serum and liver traits of laying hens. J Anim Sci Adv 2012;2:386-391.
24. Heinicke RM, VanDerWal L, Yokohama M. Effect of Bromelain (ananas) on human platelet aggregation. Experientia 1972;28:844-845.
25. Dunn EJ, Ariens RAS. Fibrinogen and fibrin clot structure in diabetes. Herz 2004;29:470-479.
26. Ajjan R, Grant PJ. Coagulation and atherothrombotic disease. Atherosclerosis 2006;186:240-259.
27. Meletis CD. Cardiovascular disease: phytochemical and nutirional prevention and treatment. Altern Complement Ther 2003;9:158-162.
28. Klein G, Kullich W. Short-term treatment of painful osteoarthritis of the knee with oral enzymes: a randomised, double-blind study versus diclofenac. Clinl Drug Invest 2000;19:15-23.
29. Leipner J, Iten F, Saller R. Therapy with proteolytic enzymes in rheumatic disorders. Bio Drugs 2001;15:779-789.
30. Seligman B. Oral bromelain as adjuncts in the treatmetn of acute thrombophlebitis. Ann Epidemiol 1969;20:22-26.
31. Morrow DA, Ridker PM. C-reactive protein, inflammation, and coronary risk. Med Clin North Am 2000;84:149-161.
32. Standeven KF, Aridns RAS, Grant PJ. The molecular physiology and pathology of fibrin structure/function. Blood Rev 2005;19:275-288.
33. Knill-Jones RP, Pearce H, Batten J, Roger W. Comparative trial of nutrizym in chronic pancreatic insufficiency. Br Med J 1970;3:21-24.
34. Gutfreund AE, Taussig SJ, Morris AD. Effect of oral bromelain on blood pressure and heart rate of hypertensive patients. Hawaii Med J 1978;37:143-146.
35. Mackie IJ, Kitchen S, Machin SJ, Lowe GDO, on behalf of the Haemostasis and Thrombosis Task Force of the British Committee for Standards in Haematology. Guidelines on fibrinogen assays. Br J Haematol 2003;121:396-404.
36. Rosner B, ed. Fundamentals of biostatistics. 7th ed. Canada: Cengage Learning, Inc.; 2010.

(Received May 24, 2014)
Edited by WANG Wei-xia