

Original Research

Therapeutic Efficacy of Polyphenol-Rich Fraction of *Boesenbergia rotunda* in Diabetic Rats: A Focus on Hypoglycemic, Antihyperlipidemic, Carbohydrate Metabolism, Antioxidant, Anti-Inflammatory and Pancreato-Protective Activities

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Abstract

Introduction: *Boesenbergia rotunda* is a famous culinary/medicinal herb native to Southeast Asia region and it is traditionally used in the treatment of several diseases. This study investigated the anti-diabetic properties of *Boesenbergia rotunda* polyphenol extract (BRE) in high fructose/streptozotocin-induced diabetic rats. **Method:** The *in vitro* antioxidant activity was evaluated using DPPH and ABST colorimetric assays, while the Folin-Ciocalteu method was used for the total phenolic content of BRE. For diabetes induction, a combination of high fructose solution and streptozotocin was administered to the rats and diabetic rats were orally administrated with BRE (100 and 400 mg/kg) for 5 weeks. The fasting blood glucose, body weight gain, food and water consumption were determined during the treatment period. **Results:** BRE showed excellent *in vitro* DPPH and ABTS scavenging activity with high phenolic content. BRE significantly lowered fasting blood glucose level, HbA1c, lipid profile, hepatorenal biochemical parameters and ameliorated the IPGTT in diabetic rats. Additionally, BRE reversed body weight loss, attenuated food and water intake, serum insulin level, pancreatic β -cell function and pancreatic cell morphology. Furthermore, fructose 1,6 biphosphatase, glucose-6-phosphatase, malondialdehyde (MDA) and proinflammatory cytokines levels were also ameliorated in the BRE-treated diabetic rats, while pancreatic antioxidant enzymes activities (GSH, SOD and CAT) were significantly increased in the treated rats. **Conclusions:** In conclusion, the results showed that BRE effectively displayed antidiabetic effects and has possible value for antidiabetic oral medication.

Keywords: *Boesenbergia rotunda*; diabetes mellitus; polyphenols; hypoglycemic activity; oxidative stress

1. Introduction

Diabetes mellitus is a chronic metabolic disease which has evolved over the years as a major global public health issue [1]. The increase in the prevalence of the disease has been largely attributed to globalization, environmental factors as well as changes in lifestyle and behaviours [2]. Typically, diabetes mellitus is principally depicted by excessive blood glucose concentration (hyperglycemia), together with alterations in lipid, carbohydrate and protein metabolism [3,4]. Out of the three different types of diabetes, type II diabetes mellitus is the most rampant accounting for approximately 90–95% of all global cases [4]. Insulin resistance, pancreatic beta cell dysfunction and insufficient insulin production are the characteristics hallmarks of type II diabetes mellitus [5,6]. Hyperglycaemia is the major clinical feature in diabetes and it is responsible for the multiple tissue and organ damages associated with the disease. Hyperglycemia through oxidative-inflammatory outburst forms the pathological and physiological basis for all diabetic complications including nephropathy, neuropathy,

cardiovascular diseases, retinopathy and Alzheimer's disease [6,7].

While significant progress has been made in the development of synthetic antidiabetic drugs, unfortunately adverse side effects as well as the inability of these drugs to impede or slow down the development of diabetic associated comorbidity have necessitated the search for effective antidiabetic alternatives [8,9]. Medicinal plants have been enormously explored for their health promoting roles and low toxicity especially in the treatment of diabetes and its associated complications. Numerous bioactive components from medical plants have been found as excellent antidiabetic agents through their inhibitory effects on several diabetic related pathways [3,10]. Besides, medicinal plants possesses excellent antioxidant and anti-inflammatory properties which makes them significantly relevant in tackling key components of diabetes and its complications [3,11]. For instance, *Tiliacora triandra* was reported to showed significant attenuative effect against diabetic nephropathy and testicular dysfunction via im-



proving antioxidant defense and alleviating oxidative stress [12]. In addition, several other medicinal plants including *Solanum macrocarpon* Linn, *Bridelia ferruginea* Benth and *Alchemilla mollis* have been reported to show protective effects against diabetic induced complication via modulation of oxidative stress and enhancement of antioxidant activities [13–15].

Boesenbergia rotunda is a medicinal and culinary herb that originated from Southeast Asia and China. It is called “Krachai” or finger roots and famously used as a culinary herb in Thai dishes [16]. Traditionally, *B. rotunda* is used as an aphrodisiac (Thai ginseng), as well as in the treatment of ulcer, rheumatic diseases, wound, bacteria infections, muscle pain and gastrointestinal disorders [17–19]. Aside the ethno-pharmacological properties of *B. rotunda*, scientific investigations have shown that the extracts/bioactive compounds from the plant have displayed significant antimicrobial, wound healing, antioxidant, anti-inflammatory, neuroprotective, *in vitro* antidiabetic, antiobesity and anticancer properties [16,20,21]. *B. rotunda* is rich in flavonoids and polyphenolic compounds, notably flavones, and these compounds have been extensively implicated in the bioactivity of the plant including its effect on diabetes, diabetic neuropathic pain, inflammation and obesity [22,23]. Our earlier studies showed that *B. rotunda* ameliorated diabetic peripheral neuropathy [22], additionally, it has been shown that *B. rotunda* showed anti-glycation, α -glucosidase and pancreatic lipase inhibitory effects, suggesting its potentials as an antidiabetic agent [18,20]. However, despite numerous studies on the therapeutic action of *B. rotunda*, its *in vivo* antidiabetic properties is yet to be explored. As such, this study examined the antidiabetic effects of *B. rotunda* in fructose/streptozotocin-induced diabetes in rats.

2. Materials and Methods

2.1 Preparation of *B. rotunda* Extract

B. rotunda roots were purchased from Saburi Medicinal Herbs Store, Hat Yai, Songkla, Thailand in March 2021. After taxonomic authentication (voucher specimen: BR-TT-050), the specimen was kept at the herbarium of Faculty of Thai Medicine, Prince of Songkla University. The dried roots of *B. rotunda* were pulverized to fine powder and exhaustively macerated with 95% ethanol. The extract was dried using a rotary evaporator and the dried dark brown extract was suspended in distilled water successively extracted with n-hexane and ethyl acetate. The ethyl acetate fraction was subsequently evaporated to dryness and stored in tightly closed glass bottles at 4 °C until further use.

2.2 Total Phenolic Content

To assess the total phenolic content (TPC) of BRE, 20 mg of BRE was solubilized in 10 mL of DMSO and 25 μ L of the aliquot of the mixture was added to 75 μ L of 10% Na_2CO_3 followed by 100 μ L Folin-Ciocalteu reagent in methanol (1:1 v/v). The solution was incubated in the

dark for 30 min and the absorbance was read at 750 nm. TPC concentration was calculated using gallic acid as standard and the results was expressed as milligrams gallic acid equivalents per gram extract (mg GAE/g).

2.3 DPPH Assay

The DPPH working solution was prepared by diluting the DPPH stock solution (50 μ M) in methanol to obtain an absorption value of 1 ± 0.02 at 515 nm. BRE (30 μ L) was added to 170 μ L of DPPH working solution and absorbance read at 515 nm. Ascorbic acid was used as positive controls. The percentage inhibition of DPPH of BRE was calculated using:

$$\% \text{ DPPH inhibition} = \frac{(\text{Ac}-\text{As})}{\text{Ac}} \times 100$$

Where: Ac = absorbance of control

As = absorbance of sample solution

The DPPH radical scavenging capacity of BrE was expressed as IC_{50} value (μ g/mL).

2.4 ABTS Assay

ABTS (2,2-azino-di-3-ethylbenzothiazoline-sulphonic acid) was employed for the determination of ABTS radical scavenging assay. ABTS stock solution (7 mM) was prepared through the reaction of 7mM ABTS and 2.45 mM of potassium persulphate as the oxidant agent (1:1 v/v). The working solution of ABTS⁺ was obtained by diluting the stock solution in ethanol to obtain an absorption value of 0.70 at 734 nm. BRE (30 μ L) was added to 195 μ L of ABTS⁺ solution and the absorbance measured at 734 nm. The percentage inhibition of ABTS⁺ was calculated. The ABST radical scavenging capacity of BrE was expressed as IC_{50} value (μ g/mL).

2.5 Animals Husbandry

The male Sprague-Dawley rats used in this study were six weeks old with body weight between 140–180 g. The animals were accommodated in stainless steel cages (6 rats/cage) in a temperature, relative humidity and light/dark cycle controlled room (22 ± 2 °C, 65–70% and 12-h light/dark cycle, respectively). Standard rat food and water were provided *ad libitum* and the animals were acclimatized to the environmental conditions for seven days prior to the start of the experiment. All the rats were in good health conditions before the commencement of the experiment.

2.6 Experimental Design and Diabetes Induction

After acclimatization, the rats were equally allotted into four groups: normal control (NOR-C) and diabetic model group (DIAB-C), both groups were treated with 5% DMSO, and two BRE intervention groups at 100 and 400 mg/kg (DIAB-BRE-100 and DIAB-BRE-400, respectively). Prior to the induction of diabetes, the rats in the diabetic model and BRE intervention groups were given 30% fructose solution for 4 weeks, while the counterparts

in the normal control group received normal water. Thereafter, all the rats were fasted overnight and the rats fed with 30% fructose received an intraperitoneal injection of streptozotocin (STZ, 35 mg/kg, solubilized in 0.1 M citrate buffer; pH 4.5). The normal rats were also injected with citrate buffer solution. The fasting blood glucose concentration of all the rats was determined three days after administration of STZ from the blood taken from the tail end of the rats. All rats with fasting glucose values greater than 250 mg/dL were adjudged as diabetic. After confirmation of diabetes, the rats were treated as stated above for 5 weeks, during this period, daily water and food intake was evaluated, while the blood glucose concentration was measured weekly.

2.7 Intraperitoneal Glucose Tolerance Test (IPGTT)

At the end of the treatment, intraperitoneal glucose tolerance test was performed by injecting 2 g/kg of glucose solution to all the rats and the blood glucose concentration of each of the rats was periodically evaluated over a time course of 2 hours (0, 30, 60, 90 and 120 mins).

2.8 Animal Sacrifice and Biochemical Analysis

The rats were fasted overnight, euthanized with 150 mg/kg of sodium thiopental and peripheral blood samples were taken through cardiac puncture. Thereafter, all the rats were sacrificed by cervical dislocation. The pancreas and liver were immediately excised, cleaned with physiological saline.

After high speed centrifugation of the blood samples collected, the serum obtained was used for analysing blood chemistry parameters including liver function parameters (AST, ALT and ALP), kidney function (BUN, creatinine, uric acid), lipid profile (HDL, LDL-c, total cholesterol and triglyceride) and glycated haemoglobin (HbA1c) using automated chemistry analyzer. Fasting serum insulin levels was also evaluated using ELISA kits. Homeostasis model assessment insulin resistance (HOMA-IR) was calculated as $\text{HOMA-IR} = \text{FBG (mmol/L)} \times \text{FINS (mIU/L)} / 22.5$ [7].

The pancreas specimens were homogenized (10% w/v) in 0.1 M PBS (pH 7.4), centrifuged (3000 rpm for 10 min at 4 °C) and the supernatant collected was evaluated for the levels of malondialdehyde (MDA), tumor necrosis factor (TNF- α), interleukin 1 beta (IL-1 β) and the activities of SOD, GSH and CAT were determined using kits from Abcam (Cambridge, UK).

The liver tissues were also homogenized in 0.1 M Tris-HCl buffer (pH 7.5), centrifuged (3000 rpm for 10 min at 4 °C) and the supernatant obtained was used for the determination of hepatic hexokinase (HK), glucose 6-phosphatase (G6Pase) and fructose 1,6-bisphosphatase (FBP1) content using previous reported methods [24–28].

2.9 Histopathology

A portion of the pancreas harvested from the rats was fixed in 10% neutral buffered formalin solution, embedded using paraffin and sliced into sections. The sections were deparaffinized, mounted on microscope slides, routinely stained with haematoxylin and eosin and visualized under light microscope.

2.10 Insulin Immunohistochemistry

The immunostaining of anti-insulin in the pancreatic tissues was performed on deparaffinized tissue sections. In brief, the pancreatic tissues were incubated in antigen retrieval buffer for 15 min at 95 °C. Thereafter, the sections were incubated with primary anti-insulin overnight at 4 °C. The sections were further incubated with biotinylated secondary antibody for 1 h at room temperature. All tissue sections were visualized under light microscope.

2.11 Data Analysis

Data were compared using one-way ANOVA followed Tukey multiple comparison post hoc test. All data analyses were performed with GraphPad Prism version 5.0 software (San Diego, CA, USA). *p* values < 0.05 were considered statistically significant.

3. Results

3.1 Total Phenols Content and Antioxidant Property of BRE

The total phenolic content and *in vitro* antioxidant activity of BRE are shown in Table 1. BRE showed high content of total phenols of 23.50 ± 4.22 mg GAE/g. In addition, the antioxidant capacity of BRE against DDPH and ABTS radicals were 205.97 ± 0.01 and 34.97 ± 0.01 $\mu\text{g/mL}$, respectively when compared to the positive control ascorbic acid (6.61 ± 0.01 and $2.83 \pm \mu\text{g/mL}$, respectively).

3.2 Effect of BRE on Body Weight, Food and Water Intake in Diabetic Rats

According to the body weight measurement after the 8th week of treatment, the average body weight of the animals in the NOR-C group was 435 ± 13.0 g, while the average body weight of rats in the DIAB-C group was 285.8 ± 28.5 g, indicating a 35% decrease in the body weight compared to the NOR-C rats (Fig. 1a). Nevertheless, the body weight loss observed in the diabetic model rats was significantly attenuated in the rats administered with BRE (359.1 ± 17.4 and 383.3 ± 19.1 g for 100 and 400 mg/kg, respectively; Fig. 1a).

As indicated in Fig. 1b,c, the results showed that there were significant differences in the food and water consumption of the DIAB-C rats compared to the NOR-C rats. Nevertheless, the increase in water and food intake observed in the DIAB-C group were significantly improved in the rats administered with BRE (Fig. 1b,c).

Table 1. Total phenolic content and antioxidant activity of BRE.

Sample	Total phenolic content (mg GAE/g)	DPPH (IC ₅₀ µg/mL)	ABTS (IC ₅₀ µg/mL)
BRE	23.50 ± 4.20	205.97 ± 0.01	34.97 ± 0.01
Ascorbic acid	-	6.61 ± 0.01	2.83 ± 0.02

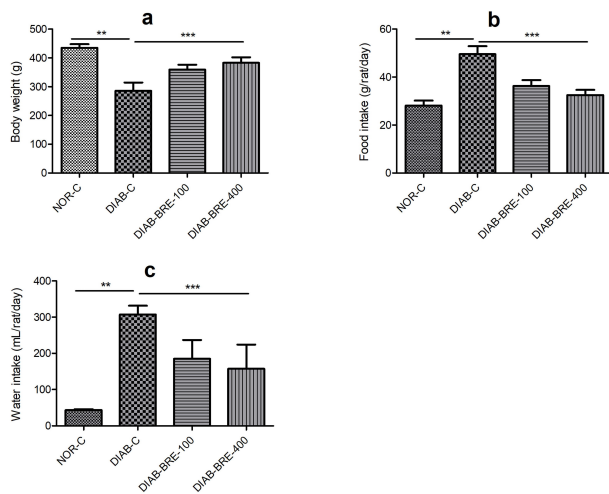


Fig. 1. Effect of BRE on (a) body weight (b) food intake (c) water intake in diabetic rats. Data are displayed as the mean ± SD. ** $p < 0.05$ compared to NOR-C group; *** $p < 0.05$ compared to DIAB-C group.

3.3 Effect of BRE on Blood Glucose and Insulin Alterations in Diabetic Rats

Compared with NOR-C group, the rats in the DIAB-C group developed severe hyperglycemia throughout the duration of the study. There was marked increase in fasting blood glucose concentration of the DIAB-C group 72 h after STZ injection to the end of the 8th week in comparison to NOR-C rats ($p < 0.05$; Fig. 2a). However, the significant increase in the blood glucose concentration observed in the DIAB-C group was significantly and dose dependently abrogated in diabetic rats administrated with BRE ($p < 0.05$; Fig. 2a).

Additionally, in the IPGTT, it was observed that the DIAB-C group had significantly higher blood glucose level at all the measured time point compared to the corresponding values from the NOR-C rats, while BRE treated rats showed significantly reduced blood glucose concentration from 0–120 mins post glucose administration when compared to the DIAB-C rats (Fig. 2b). The fasting insulin levels (Fig. 2c) in the DIAB-C rats was significantly decreased, while HOMA-IR (Fig. 2d) was notably increased compared to the NOR-C rats. However, the fasting insulin levels of were markedly increased, while HOMA-IR was down-regulated in BRE intervention groups compared to the DIAB-C group (Fig. 2c,d).

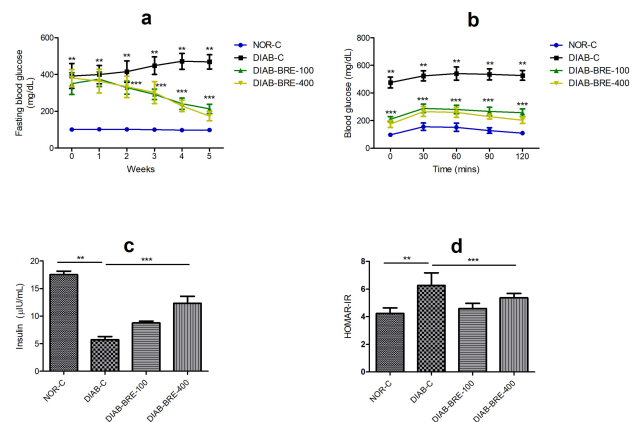


Fig. 2. Effect of BRE on (a) fasting blood glucose (b) intraperitoneal glucose tolerance (c) insulin (d) HOMA-IR in diabetic rats. Data are displayed as the mean ± SD. ** $p < 0.05$ compared to NOR-C group; *** $p < 0.05$ compared to DIAB-C group.

3.4 Effect of BRE on Serum Biochemical Markers in Diabetic Rats

The serum levels of biomarkers related to kidney and liver function including BUN, creatinine, uric acid, AST, ALP and ALT were observably increased in the DIAB-C rats compared with NOR-C rats ($p < 0.05$; Table 2). After 8 weeks of treatment, BRE significantly decreased creatinine, BUN and uric acid levels in the treated diabetic rats groups ($p < 0.05$; Table 2). AST, ALT and ALP levels in the treated rats were also significantly reduced in the BRE treated groups compared to the DIAB-C group ($p < 0.05$; Table 2).

3.5 Effect of BRE on Serum Lipid Profiles in Diabetic Rats

In the DIAB-C group, serum total cholesterol, triglycerides and LDL-c were significantly increased, while HDL was reduced compared with the NOR-C rats (Table 2). Whereas, after treatment with BRE, the total cholesterol, triglycerides and LDL-c were notably reduced while HDL-c was significantly increased in all the treated groups compared to the DIAB-C group (Table 2).

3.6 Effect of BRE on Pancreas Histological in Diabetic Rats

The results shown in Fig. 3 showed histological appearances of the pancreas of the normal, diabetic and diabetic treated rats. The histopathological appearances of pancreas of the normal rats appeared normal without any tissue degeneration. Whereas, the severity of pancreatic degeneration in the DIAB-C group was highlighted as char-

Table 2. Effect of BRE on serum lipids, kidney and liver biochemical markers in diabetic rats.

Groups/parameters	NOR-C	DIAB-C	DIAB-BRE-100	DIAB-BRE-400
Creatinine (mg/dL)	0.32 ± 0.04	0.93 ± 0.19	0.59 ± 0.08	0.41 ± 0.04
Uric acid (mg/dL)	1.91 ± 0.19	4.05 ± 0.41	2.93 ± 0.67	2.33 ± 0.37
BUN (mg/dL)	21.0 ± 3.89	59.80 ± 9.10	42.0 ± 4.00	31.50 ± 4.37
ALP (U/L)	89.0 ± 8.80	177.80 ± 14.56	121.67 ± 11.69	110.16 ± 27.00
ALT (U/L)	53.83 ± 5.98	88.50 ± 10.89	63.0 ± 6.54	59.5 ± 7.76
AST (U/L)	117.90 ± 12.38	211.66 ± 18.45	161.0 ± 14.76	147.5 ± 26.11
Cholesterol (mg/dL)	78.83 ± 7.41	162.50 ± 19.70**	110.33 ± 8.26***	89.16 ± 13.16***
Triglyceride (mg/dL)	110.0 ± 9.81	202.60 ± 20.24**	140.50 ± 8.91***	128.30 ± 22.22***
LDL-c (mg/dL)	32.67 ± 4.45	60.0 ± 5.93**	43.17 ± 5.67***	32.67 ± 5.24***
HDL (mg/dL)	24.00 ± 4.33	9.50 ± 1.51**	15.60 ± 2.88***	18.50 ± 1.87***

Data are shown as mean ± SD. **Indicates significant difference compared to NOR-C group ($p < 0.05$). ***Indicates significant difference compared to DIAB-C group ($p < 0.05$).

acterized by loss of acini, inflamed β -cells and well as decrease in the mass of islets of Langerhans. Treatment with the BRE significantly restored the pancreatic histomorphology to almost normal intactness compared to the DIAB-C group (Fig. 3).

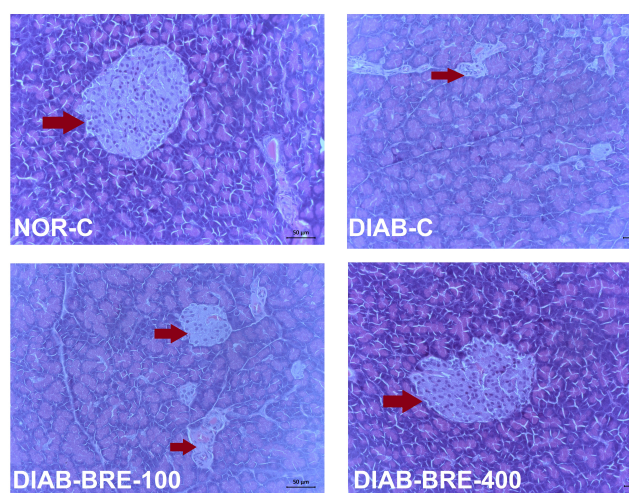


Fig. 3. Effect of BRE on pancreatic histology in hematoxylin and eosin staining of diabetic rats. Brown arrow: beta cells. Magnification 200 \times .

3.7 Effect of BRE on Insulin Immunohistochemical Analysis

Regarding the immunohistochemical staining of insulin-secreting islet beta cells, the results indicated that diabetic rats showed significantly reduced insulin positive stain when compared to the normal rats that displayed large insulin positive β -cell (Fig. 4). Whereas, treatment of diabetic rats with BRE showed significant increase in insulin positive response (Fig. 4).

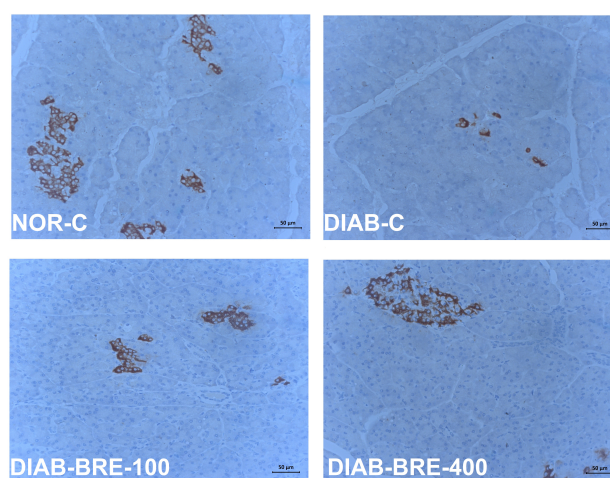


Fig. 4. Effect of BRE on insulin immunostaining (brown colour) in diabetic rats $\times 200$ (brown colour: insulin-positive β cells).

3.8 Effect of BRE on Carbohydrate Metabolizing Enzyme Activities

As shown in Fig. 5, the activities of HK was significantly decreased, while the activities of FBP1 and G6Pase were markedly increased in the DIAB-C (Fig. 5a–c). Whereas, the treatment of diabetic rats with BRE significantly attenuated the activities of these enzymes compared to the DIAB-C rats.

3.9 Effects of BRE on Pancreas Antioxidants and Lipid Peroxidation

According to the data shown Fig. 6, the DIAB-C rats displayed marked decrease in pancreatic levels of antioxidants enzymes (SOD, GSH and CAT) compared to the NOR-C rats (Fig. 6a–c). After 5 weeks of BRE supplementation, the levels of these antioxidant enzymes were significantly enhanced compared to the DIAB-C group (Fig. 6a–c). A similar but opposite trend was observed for the lipid peroxidation (MDA), the DIAB-C group showed signifi-

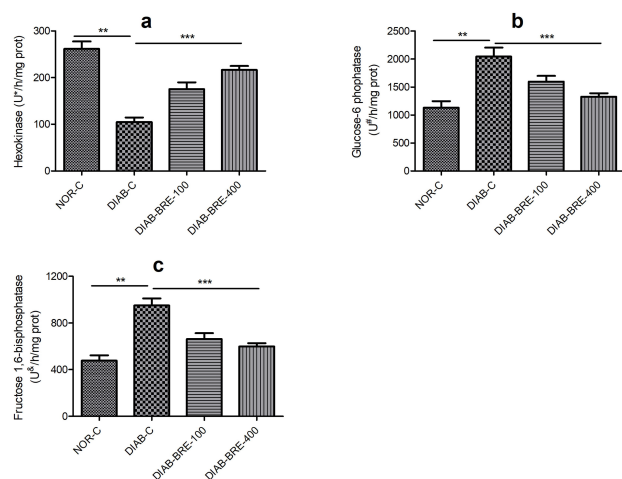


Fig. 5. Effect of BRE on (a) hexokinase (b) glucose-6 phosphatase (c) fructose 1,6-bisphosphatase in diabetic rats. Data are displayed as the mean \pm SD. ** $p < 0.05$ compared to NOR-C group; *** $p < 0.05$ compared to DIAB-C group. U*: μ mol of glucose phosphorylated/h/mg protein; U^h: μ mol of phosphate liberated/h/mg protein; U[&]: μ mol of phosphate liberated/h/mg protein.

cantly elevated MDA level when juxtaposed to the NOR-C rats, while treatment with BRE markedly reduced pancreatic MDA level (Fig. 6d).

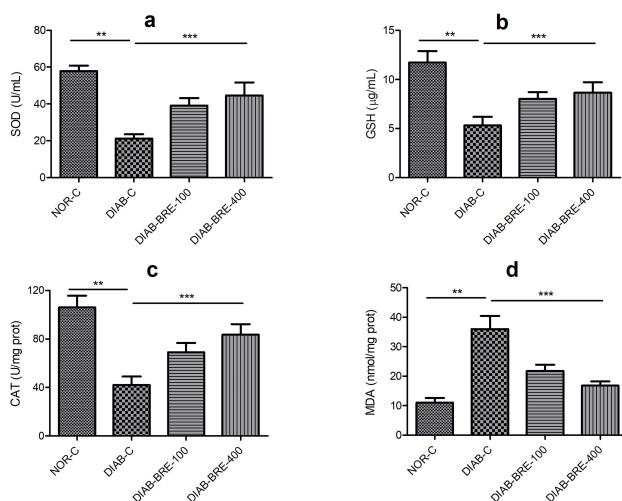


Fig. 6. Effect of BRE on pancreatic (a) superoxide dismutase (b) glutathione (c) catalase (d) malonaldehyde in diabetic rats. Data are displayed as the mean \pm SD. ** $p < 0.05$ compared to NOR-C group; *** $p < 0.05$ compared to DIAB-C group.

3.10 Effects of BRE on Pancreas Proinflammatory Cytokines

There were significant increases in pancreatic TNF- α and IL-1 β levels in the DIAB-C rats compared to NOR-

C rats (Fig. 7a,b). Oral administration of BRE to the diabetic animals markedly reversed the levels of these aforementioned cytokines when compared to the DIAB-C rats (Fig. 7a,b).

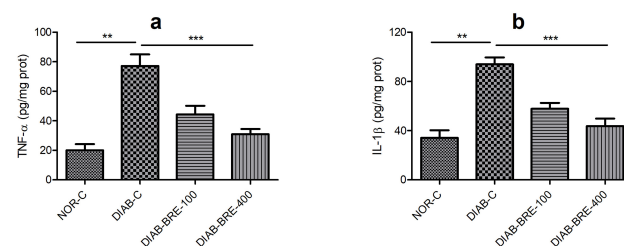


Fig. 7. Effect of BRE on pancreatic (a) tumor necrosis factor alpha (b) interleukin 1 beta in diabetic rats. Data are displayed as the mean \pm SD. ** $p < 0.05$ compared to NOR-C group; *** $p < 0.05$ compared to DIAB-C group.

4. Discussion

In recent years, the use of natural products for the treatment of diabetes has attracted a lot of attention, due to the huge financial burden associated with the use of synthetic antidiabetic drugs as well as the increasing number of complications associated with diabetes among several other reasons [12,29]. Thus, this study investigated the antidiabetic effects of the polyphenol rich fraction of *B. rotunda* in high fructose/streptozotocin induced diabetic rats.

High sugar/fat and streptozotocin have been extensively used as one of the most preferred choice for inducing type 2 diabetes mellitus due to similarities to the pathogenesis of type 2 diabetes in humans. High fat/sugar diet can promote the development of insulin resistance and hyperinsulinemia, while a low-dose of STZ results in modest impairment in insulin secretion, which bears close resemblance to human type 2 diabetes. Thus the combination of these two approaches mimics the phenotype and pathogenesis of type 2 diabetes in humans [30,31]. STZ, an antibiotic from *Streptomyces achromogenes* causes β -cell damage through alkylation of DNA strands leading to loss of β -cell activity, hyperglycemia, reduced pancreatic insulin secretion, polyuria and glycosuria [31,32]. The administration of high fructose and low dose STZ to the rats led to significant increase in blood glucose level, polyuria and polyphagia. Furthermore, insulin resistance and impaired insulin secretion are vital parameters contributing to the onset of hyperglycemia [33]. Whereas, glucose tolerance test and HbA1c are two of the most frequently used approach for the detection of glucose disposal abnormalities and glycaemic control, respectively [34–36]. HbA1c is clinically used for the diagnosis of prolonged diabetes. The oxidation of excessive glucose in the blood during diabetes leads to generation of free radicals, protein glycosylation and oxidative breakdown of glycosylated proteins,

leading to increased HbA1c level [37,38]. In this study, the fasting blood glucose of diabetic rats treated with BRE was significantly reduced, while insulin secretion, impaired glucose tolerance and HbA1c were ameliorated in BRE treated diabetic rats, suggesting that BRE attenuated physiological parameters via the regulation of blood glucose homeostasis in the diabetic rats. In addition, treatment of diabetic rats with BRE led to significant weight increase. Decrease in insulin secretion in diabetes can alter carbohydrate metabolism, leading to the breakdown of structural protein and fats in diabetic animals. The increased in body weight gain in treated diabetic rats can be attributed to the increase in insulin secretion which prohibits protein catabolism in the muscles [39,40].

Diabetes has been shown to be a major contributor to the high prevalence of cardiovascular disease. In fact, cardiovascular disease is one of the main cause of mortality and morbidity in diabetic patients, as diabetic patients have higher risk of cardiovascular disease than non-diabetic people [41]. Diabetic dyslipidemia characterised by increased lipids profiles including triglyceride, total cholesterol, low density lipoprotein cholesterol and low HDL are markers of endothelial dysfunction and cardiovascular risk in diabetes [4,5,42,43]. Dyslipidemia in diabetes occurs due to hyperglycemia and insulin impairment resulting in reduced efficiency of lipase enzyme culminating into lipolysis, lipotoxicity and hyperlipidemia [3,4]. The elevated lipid profiles (TC, TG and LDL-C) in this study was remarkably reduced by BRE in the treated diabetic rats, which is consistent with the previous reports that *Boesenbergia rotunda* regulated the lipid metabolism [23].

Diabetes has been extensively reported to cause hepatorenal toxicity characterized by increased serum levels of liver and kidney function enzymes including ALT, AST, ALP, BUN, creatinine and uric acid [4,44]. Hepatorenal cell necrosis induced by systemic inflammation leads to the release of these enzymes into the blood stream [40]. Treatment of diabetic rats with BRE significant reduced hepatorenal toxicity, as evidenced by attenuated serum levels of ALP, ALT, AST, BUN, creatinine and uric acid in the treated rats. These results suggested that BRE possesses hepatorenal protective effect in diabetes.

The results from this study indicated that BRE improved chronic inflammation and oxidative stress. Insulin resistance and persistent hyperglycemia results in excessive production of reactive radicals (ROS and RNS) which promotes oxidative stress as well as alterations in the lipids and protein. The effects of these oxidative cytotoxic events can initiate glycoxidation, lipid peroxidation, inflammation and oxidative damages [33,45]. The vicious cycle between oxidative stress triggered increase in inflammatory cytokines and vice versa in diabetes is majorly responsible for most secondary complications associated with diabetes [3,33]. Accumulating studies have shown that diabetes is associated with reduced antioxidant enzyme ca-

pacities, increased lipid peroxidation and proinflammatory cytokines [5,12,44]. In line with these previous studies, treatment with BRE significantly restored pancreatic SOD, GSH, CAT and reduced MDA, TNF- α and IL-1 β levels in the diabetic rats, suggesting that BRE has antioxidant and anti-inflammatory properties.

Previous studies have suggested that insulin deficiency is associated with increased HOMA-IR, which correlated with altered pancreatic histopathological observation including reduced islet mass and number, hypocellular cells and congested blood capillaries which could be an explanation for the significant reduction in serum insulin level of the untreated diabetic rats [4,46,47]. Furthermore, immunohistochemical images showed that the untreated diabetic rats had significantly reduced area of insulin immunoreactivity, which further suggests impaired insulin secretion and beta-cell function owing to loss of beta-cell mass and function in the untreated diabetic animals. BRE treated animals showed significant pancreatic tissue regeneration and protective effects on pancreatic islet function.

Diabetes have been shown to significantly increase glycogenolysis, gluconeogenesis and glycolysis due to excessive hepatic glucose production. The liver plays a vital role in maintaining glucose homeostasis and the activation of key carbohydrate metabolising enzymes involved in these pathways, resulting in continuous glucose production, thus aggravating hyperglycemia [48,49]. Furthermore, insulin insufficiency/resistance stimulates these enzymes, resulting in carbohydrate metabolism dysfunction and hyperglycemia, since insulin physiologically impedes gluconeogenesis and glycogenolysis via the alteration of post translational changes in the gene expression of these enzymes [50,51]. In addition, the bye-products of glycolysis including protein kinase C and AGEs have been associated to several diabetic complications [52]. Hyperglycemia is mainly driven by excessive glucose generation through gluconeogenesis and glycogenolysis. Glucose-6-phosphatase is the main enzyme responsible for controlling gluconeogenesis, thus limiting glucose outflow while fructose-1,6-bisphosphatase regulates gluconeogenesis by catalysing the irreversible stage of gluconeogenesis [53,54]. The untreated diabetic rats displayed increased levels of glucose-6-phosphatase and fructose-1,6-bisphosphatase in the hepatic tissues, suggesting glycogenolysis and gluconeogenesis, which corresponds with high blood glucose level and low insulin secretion in the untreated diabetic rats. Treatment with BRE significantly reduced the activities of these enzymes which also corresponds with decrease in blood glucose and increase in insulin levels in the treated rats.

Phenolic compounds have been extensively reported as excellent antioxidant, antidiabetic, antiglycation and anti-inflammatory secondary metabolites that are abundantly present in medicinal plants [10,55,56]. Previous reports have indicated that BRE is a remarkable reservoir of polyphenolic compounds, primarily flavanones, flavones,

prenylated flavonoids and chalcones [16,57]. In agreement with these previous studies, we identified and isolated six flavonoids including pinocembrin, boesenbergin A, panduratin A, pinostrobin, cardamonin and alpinetin from BRE. The details regarding the isolation of these compounds was extensively reported in our previous study [22]. Regarding the potentials of BRE polyphenols in the treatment of diabetes, Chatsumpun et al. reported the ability of panduratin A, isopanduratin A and hydroxypanduratin A to inhibit α -glucosidase and pancreatic lipase enzyme [18]. In addition, pinocembrin, pinostrobin, alpinetin, cardamomin, boesenbergin B, panduratin A and isopanduratin A displayed significant anti-glycation properties, thus indicating their ability to treat diabetes [20]. As such, these compounds may be linked to the remarkable ability of BRE against diabetes in this study.

5. Conclusions

In conclusion, the study revealed that *Boesenbergia rotunda* extract (BRE) displayed antidiabetic, antihyperlipidemic, antioxidant and anti-inflammatory effects in fructose-streptozotocin induced diabetic rats. Treatment with BrE led to significant reduction in blood glucose level, serum lipid profiles (LDL-c, TC and TG), serum hepatorenal parameters (BUN, creatinine, uric acid, AST, ALP and ALT), enzymes involved in carbohydrate metabolism (glucose 6-phosphatase and fructose 1,6 biphosphatase), and increased serum insulin levels. Furthermore, BrE administration increased pancreatic antioxidant enzyme activity (SOD, GSH and CAT), decreased pancreatic inflammatory markers (TNF- α and IL-1 β), malondialdehyde levels and improved histopathological architecture of the pancreas. Hence these result for the first time validates the potency of *Boesenbergia rotunda* as an antidiabetic agent and suggests it could serve as an alternative remedy in ameliorating or protecting against type-2 diabetes and its complications.

Author Contributions

TW and OJO conceived and designed this study; TW, CL and OJO performed the experiments; SS and QZ analyzed the data; TW, CL, SS and QZ interpreted the results of the experiments; OJO wrote and revised the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

The animal experiment received approval from the Ethics Committee of Anhui Medical University (AHYKDX-LLWYH-2021-1008).

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Conflict of Interest

The authors declare no conflict of interest.

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