

Effect of Plantain Bulb's Extract-Beverage Blend on Blood Glucose Levels, Antioxidant Status, and Carbohydrate Hydrolysing Enzymes in Streptozotocin-Induced Diabetic Rats

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ABSTRACT: The pharmacological properties of various parts of plantain trees have directed its use in folkloric management of diabetes and other human ailments. However, little is known about plantain bulb extract (PBE) and their mechanisms of action. This study evaluated the effect of PBE-beverage blends (including 1% and 2 % cocoa powder) sweetened with honey on blood glucose levels, antioxidant status, and carbohydrate hydrolysing enzyme activities in streptozotocin (STZ)-induced diabetic rats. Animals were selected at random and distributed into 7 groups (n=7), as follows: normal control (NC), untreated diabetic rats, diabetic rats treated with acarbose (STZ-ACA), diabetic rats administered PBE (STZ-PBE), diabetic rats administered honey and PBE (STZ-HPBE), diabetic rats administered 1% cocoa powder-with HPBE blend (STZ-CHPBE-1), and diabetic rats administered 2% cocoa powder with HPBE blend (STZ-CHPBE-2). Compared with the controls, untreated diabetic rats exhibited increased blood glucose levels and hydrolysing enzyme activities, and significant decreases in the activities of antioxidant (catalase, superoxide dismutase, glutathione-S-transferase, and glutathione peroxidase) enzyme and non-enzymatic (glutathione) antioxidants. However, changes in activities were comparatively reversed in all rats administered plantain bulb formulations. CHPBE-2 was slightly more effective than CHPBE-1. Overall, both blends could serve as nutraceutical and/or functional drinks in the management of diabetes.

Keywords: cocoa powder, hyperglycaemia, oxidative stress, plantain bulb, therapeutic agents

INTRODUCTION

Diabetes mellitus (DM) is a prevalent endocrine malady. DM currently affects 425 million individuals, and the prevalence is predicted to affect 629 million individuals by 2045 (International Diabetes Federation, 2017; Sodipo et al., 2020). Recent data show that the worldwide epidemiological burden of DM is on the increase, including the incidence of long-term complications and the side effects of synthetic antidiabetic drugs. Moreover, the high cost of DM treatment drugs causes a financial burden on national economies and living standards (Chinsebu, 2019; Oboh et al., 2020). These and other factors have caused a shift towards the use of herbal products for DM management (Özkum et al., 2013), which are widely embraced owing to their perceived cultural acceptability, safety, availability, and affordability (Mensah et al., 2019). Recently, the role of oxidative stress in the progression of DM and its associated complexities has been explored

and well documented (Asmat et al., 2016; Dos Santos et al., 2019). Therefore, plant-based materials containing rich antioxidant compounds are being seriously considered to salvage the situation (Söhretoğlu et al., 2012; Ademiluyi et al., 2014; Oboh et al., 2018).

The veracity of extracts from different parts of plants (including roots, stems, leaves, and fruits) as anti-hyperglycaemic agents has been established (Hussein, 2008; Singab et al., 2014; Emordi et al., 2018). For example, banana root and shoot extracts, plantain root and stem extracts have been adjudged by folklore to possess medicinal properties such as antidiabetic and antilipidemic potentials (Omobuwajo et al., 2011; Kalita et al., 2016). In addition, parts (e.g. roots, stems, leaves, and fruits) of *Musa* spp. (especially bananas) have been shown to have health promoting properties (Ibegbu et al., 2012; Lakshmi et al., 2014; Nguyen et al., 2017).

Plantains and banana belong to the *Musa* genus [plantain: *Musa* AAB (*Musa parasidiaca*); banana: *Musa* AAA].

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Plantain plants produce larger and more angular starchy fruits of hybrid triploid cultivars than other species in the *Musa* family, and are generally used for cooking (Robinson, 1996). There has been much research on several parts of banana plants, however, the amount of research on plantain is limited despite the roots and stems of plantain being adjudged by folklore to possess medicinal properties, including antidiabetic and antilipidemic potentials (Omobuwajo et al., 2011; Kalita et al., 2016).

Cocoa powder is a by-product of cocoa (*Theobroma cacao*). Cocoa is an important ingredient in the beverage industry and is known to contain polyphenols including flavonols, (–)-epicatechin, (+)-catechin, and quercetin, a type of flavonoid with antioxidant properties (Sanbongi et al., 1997). Several studies have reported nutraceutical potential of cocoa powder extracts for modulating/controlling blood glucose levels and promoting antioxidant enzymes (Amin et al., 2004; Tomaru et al., 2007; Jalil et al., 2008). Indeed, a recent study corroborated the veracity of cocoa powder in the management of DM (Olasope et al., 2016).

The blend of plantain bulb extracts (PBEs) with cocoa powder has an unpleasant after taste, which requires sweetening before consumption. Natural honey is the most ancient sweetener and comprises of several components (including vitamins and mineral elements), including fructose and glucose as the main substances (Saha, 2015). Honey has been used in folklore medicine and comes with a myth that it is unsuitable for diabetic patients. However, studies have reported honey is safe for consumption by diabetic patients, considering its low glycaemic index and overall effect on glycaemic control (Kadirvelu and Gurtu, 2013; Bobiş et al., 2018). The capabilities of honey to regulate intestinal food metabolism, antioxidant and anti-inflammatory activities, and to reduce blood glucose levels have been reported (Nemoseck et al., 2011; Sahlan et al., 2019; Shafira et al., 2019; Sahlan et al., 2020). However, information is lacking on the use of plantain bulb or corm extracts, either in their pure or blended forms, with beverages. Therefore, this study was designed to evaluate the antihyperglycemic, hepatic-protective, and nephro-protective potentials of PBE beverage blends (with 1% or 2% cocoa powder). We assessed blood glucose levels, antioxidant status, and modulatory activities of α -amylase and α -glucosidase enzymes after periodic administration of PBE blends to streptozotocin (STZ)-induced diabetic rats.

MATERIALS AND METHODS

Drugs, chemicals, and reagents

STZ was procured from Sigma-Aldrich Co. (St. Louis, MO, USA). Acarbose was purchased from Glenmark Pharma-

ceuticals Ltd. (Watford, UK). Auto-analyser glucometers were obtained from Infopia Co., Ltd. (Gyeonggi, Korea). All reagents were of analytical grade and water was glass distilled. Natural honey was sourced from the bee farm of the Department of Crop, Soil, and Pest management, Federal University of Technology, Akure, Nigeria. Standardized natural cocoa powder (non-alkalized) was purchased from Oba market, Akure, Nigeria.

Preparation of PBE and beverage blends

Fresh bulbs (corm) of mature plantain plants was harvested from a private plantation in Ibadan, Oyo State, Nigeria. The outer layers of the bulbs were removed to expunge adhering soil matters, washed, cut into small pieces, and crushed mechanically using a sugar cane crushing machine at room temperature. The mash (pulp) was then filtered through a muslin cloth to extract the juices. Cocoa powder [1% (3 g) or 2% (6 g) cocoa powder] was added to 300 g of plantain corm juice with an equal amount (30 g) of honey, and the mixture was uniformly mixed together with an electric blender. The blend was freeze-dried and the lyophilized blend was stored in glass vials in a freezer ($-18\pm 2^\circ\text{C}$) until analysis.

Animal handling and experimental design

Male Wistar albino rats (190.6~205.7 g) were procured from the Animal House, Biochemistry Department, Federal University of Technology, Akure, Nigeria. Rats were kept under laboratory conditions (temperature $28\pm 1^\circ\text{C}$, relative humidity $11\pm 1\%$, and 12-h light/dark cycle for 2 weeks acclimatization and throughout the experimental period. Rats had free access to food and water *ad libitum*. Animal handling and use received the Institutional Animal Ethics Approval Committee with ethics approval (FUTA/SAAT/2018/013).

Induction of DM by STZ

Type 1 DM was induced by fasting rats overnight prior to intraperitoneal (i.p.) injection with a fresh solution of STZ [50 mg/kg body weight (bwt) in 0.1 M citrate buffer (pH 4.5)]. Blood glucose levels were assessed after 72 h by pricking rats' tail to extract blood, which was assessed using a glucometer. Rats with a blood glucose level exceeding 200 mg/dL were considered diabetic and selected for experimentation (Qinna and Badwan, 2015). In this study, rats exhibited blood glucose values between 433 and 594 mg/dL.

Rats were grouped (seven rats in each group) as follows:

- NC: normal control rats administered 1 mL/kg bwt (i.p.) of 0.1 M citrate buffer (pH 4.5)
- STZ-IND: STZ-induced diabetic rats
- STZ+ACA: diabetic rats treated with 25 mg of acarbose/kg bwt

- STZ+PBE: diabetic rats orally treated with 200 mg/kg bwt of PBE
- STZ+HPBE: diabetic rats treated with 200 mg/kg bwt PBE and 10% honey
- STZ+CHPBE-1: diabetic rats treated with HPBE and 1% cocoa powder
- STZ+CHPBE-2: diabetic rats treated with HPBE and 2% cocoa powder

Rats were fed with normal rat chow and water *ad libitum* throughout the experimental period, which lasted for 14 days (Ademiluyi et al., 2014). Samples were administered orally once daily for the 14 days in a constant volume of the chosen dosage. The dose of PBE administered was pre-determined following the results of a toxicological assessment (200~2,000 mg/kg bwt). The results showed that at all concentrations evaluated, there was no apathy, behavioural changes, damage to organs, or death (data not published); therefore, in the present study we used the lowest effective concentration of PBE (200 mg/kg bwt). Furthermore, acarbose was chosen as the anti-diabetic drug for treatment of DM based on results from Oboh et al. (2018). The animals were fasted overnight and were sacrificed via cervical decapitation. Blood was obtained via cardiac puncture and poured into plain bottles for serum preparation. The liver, kidney, pancreas, and intestine tissues were quickly removed, washed with cold saline solution, and blotted individually with ash-free filter paper.

Evaluation of feeding behaviour

Throughout the experimental period, rats had *ad libitum* access to food and drinking water. Food consumption was monitored daily, and body weight of individual rats was calculated every four days during the morning maintenance schedule.

Tissue preparation

Blood was centrifuged at 3,000 g for 10 min at 4°C to obtain clear sera, which was stored at -20°C prior to biochemical analysis. Tissues were homogenized separately in phosphate buffer (0.1 M, pH 7.4) using a Teflon homogenizer. The homogenate was centrifuged and the supernatant used in biochemical assays. Pancreas was cut into small pieces with the aid of stainless scissors and put into 3 volumes of 200 mmol/L sodium phosphate buffer (pH 6.9 with 6 mmol/L NaCl) and homogenized. The homogenates were then centrifuged at 12,000 g for 15 min at 4°C to obtain the post-mitochondrial fractions, which were stored at 4°C until use in biochemical assays.

Determination of catalase (CAT) activity

CAT activity was evaluated using the method of Beers and Sizer (1952) with modification. Briefly, 50 µL of tissue homogenate was added to a mixture containing 500

µL of 59 mM H₂O₂ and 950 µL of 50 mM phosphate buffer (pH 7.0). The reaction was carried out at 25°C and absorbance was monitored at 570 nm for 3 min at 150 s interval. One unit of enzyme activity was defined as the amount of enzyme catalyzing decomposition of 1 µmol H₂O₂ per min at 25°C and pH 7.0.

Determination of superoxide dismutase (SOD) activity

SOD activity was determined according to the method of Misra and Fridovich (1972). Approximately 40 µL aliquots of supernatant were pipetted into clean-sterilized test tubes containing 160 µL of distilled water to make a 1:4 (5 fold) dilution, and 2.5 mL of 0.05 M carbonate buffer (pH 10.2) was added. The reaction was initiated by addition of 300 µL freshly prepared 0.3 mM adrenaline, mixed by inversion. The absorbance was measured at 480 nm for 150 s (at 30 s interval) against the reagent blank (prepared by replacing the homogenate with distilled water).

Determination of glutathione-S-transferase (GST) activity

GST activity was carried out as described by Mannervik and Guthenberg (1981). Aliquots (30 µL) of tissue homogenate were added to reaction mixtures containing 150 µL of 1 mM 1-chloro-2,4-dinitrobenzene (CDNB) and 0.1 M phosphate buffer (pH 6.5), and incubated at 28°C. The absorbance was monitored for 3 min (every 15 s) at 340 nm. One unit of enzyme activity was defined as the amount of enzyme catalyzing the formation of 1 unit of S-2,4-dinitrophenyl glutathione from CDNB and glutathione (GSH) per min at 28°C pH 6.5.

Determination of glutathione peroxidase (GPx) activity

Evaluation of GPx activity in tissue homogenate was carried out following the method of Rotruck et al. (1973). Exactly 500 µL of tissue homogenate was added to 250 µL of 0.2 M phosphate buffer (pH 8.0), 50 µL sodium azide (10 mM), 100 µL GSH (4.0 mM), 50 µL H₂O₂ (2.5 mM), and 500 µL of distilled water. Reaction mixtures were incubated for 3 min at room temperature; 50 µL of 10% trichloroacetic acid was then added, mixed, and the mixture was centrifuged for 10 min at 3,000 rpm. The supernatant (50 µL) was then added 450 µL 0.3 M disodium hydrogen phosphate buffer (pH 7.4) and 500 µL of freshly prepared 0.6 mM dithiobisnitro-benzoate in 0.2 M sodium phosphate buffer, pH 8.0. The absorbance was read at 412 nm against blank (prepared by replacing homogenates with distilled water).

Determination of GSH levels

Reductions in GSH content were determined by the method described by Ellman (1959). Supernatants (1 mL) were added to 500 µL of Ellman's reagent [19.8 mg of 5,5'-dithiobis(2-nitrobenzoic acid) in 100 mL of 0.1% so-

dium citrate] and 3.0 mL of 0.2 M phosphate buffer (pH 8.0), and the absorbance was read at 412 nm using a spectrophotometer.

Determination of nitric oxide (NO) levels

NO levels in the liver and kidney tissues were determined using the protocol described by Miranda et al. (2001) in a medium containing 400 μ L of 2% VCl_3 in 5% HCl, 200 μ L of 0.1% *N*-(1-naphthyl) ethylenediamine dihydrochloride and 200 μ L of 2% sulfanilamide (in 5% HCl). The reaction mixture was incubated at 37°C for 60 min, and nitrite levels (which correspond to estimated levels of NO) were determined spectrophotometrically at 540 nm, based on the reduction of nitrate to nitrite by VCl_3 . NO levels were expressed in nanomoles of NO per milligram of protein.

Determination of lipid peroxidation

Oxidative degradation of lipids in the liver and kidneys was determined by measuring the concentrations of thiobarbituric acid reactive substances (TBARS) in tissue homogenates by following the method of Ohkawa et al. (1979). Tissue homogenate (100 μ L) was mixed with 200 μ L of 8.1% sodium dodecyl sulphate, 500 μ L of 0.8% thiobarbituric acid, and 500 μ L of acetic acid solution (2.5 M HCl, pH 3.4), and heated at 100°C for 1 h. The absorbance was read at 532 nm using a spectrophotometer.

Determination of α -amylase and α -glucosidase inhibitory activities

α -Amylase and α -glucosidase activities were evaluated following the methods of Worthington (1993) and Apostolidis et al. (2007), respectively, as described in the report of Ademiluyi et al. (2014) with slight modifications. Fifty microliters of pancreas homogenates were prepared in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl), and added to 50 μ L of 1% starch solution. Reaction mixtures were incubated (25°C/10 min), and 200 μ L of dinitrosalicylic acid was added for colour development. Mixtures were then boiled in a water bath (5 min) to stop the reaction, cooled to room temperature, and then diluted with 2 mL of distilled water. The absorbance was measured at 540 nm in a ultraviolet-visible spectrophotometer. α -Amylase activity was expressed in units/mg protein.

α -Glucosidase activity was evaluated in the small intestine. Small intestine homogenates (15 μ L) were prepared in 0.1 M phosphate buffer (pH 6.9), and added to 15 μ L of 3 mM GSH and 445 μ L of 0.1 M phosphate buffer (pH 6.9), and incubated (37°C/10 min). Then, 40 μ L of 5 mM *p*-nitrophenyl- α -D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to the mixture and the mixture was incubated at 37°C for 10 min. Sodium carbonate (2 mL) was then added and the absorbance

was measured at 405 nm. Total protein content of the enzyme solution was determined according to Lowry et al. (1951).

Data analysis

GraphPad Prism (version 5.0, GraphPad Software, San Diego, CA, USA) was used for statistical analysis of experimental results. Data were expressed as mean \pm standard error of the mean (SEM). Mean values were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Levels of significance were accepted at $P < 0.05$, $P < 0.01$, and $P < 0.001$.

RESULTS AND DISCUSSION

Average food intake and changes in average body weight of rats

The average food intake of the rats ranged from 20.05 g to 32.61 g. The NC group had the highest food intake, whereas the negative control group (STZ-induced and untreated) had the lowest (Table 1). The low food intake observed for the negative control group may be related to their disease state, which may have caused a reduction in appetite. Among the different treated groups, there was no significant difference ($P > 0.05$) in food consumption compared with the STZ-IND group. Body weight of the NC group was increased (by 9.48 g) at the end of the experimental period (weight gain of 4.84%), while average body weight of diabetic-induced/untreated group decreased by 4.15%.

Effect of PBE and formulated herbal beverages on blood glucose levels in diabetic rats

Blood glucose levels were measured daily over the 14 day study period (Fig. 1). Initial (day 1) blood glucose readings ranged from 433 to 594 mg/dL, showing that the rats were diabetic, whereas the NC rats had an average pre-prandial reading of 118 mg/dL. However, daily treatment with extract significantly decreased blood glucose levels of the diabetic rats, with final blood glucose levels ranging from 164 to 210 mg/dL after 14 days of treatment. The blood glucose levels of diabetic (untreated) and NC groups was maintained at high (545 to 579 mg/dL) and low (97 to 162 mg/mL) concentrations, respectively. In addition, there was no significant difference ($P > 0.05$) in blood glucose level for NC and STZ-IND groups during the 14 day study period.

PBE treatment decreased blood glucose levels from 560 to 210 mg/dL. Although there were no significant differences ($P > 0.05$) in blood glucose levels during the first eight days (543 to 553 mg/dL), readings on day 12 (428 mg/dL) and day 14 (210 mg/dL) significantly differed ($P < 0.001$) from each other and from those of the first

Table 1. Average food intake and changes in body weight of rats administered plantain bulb extract and beverage blends

Treatment group	Average food intake (g/rat/d)	Initial body weight (g)	Final body weight (g)	Change in weight (g)	Weight gain or loss (%)
NC	32.61±1.66 ^a	195.63±5.38 ^{ns}	205.11±6.91 ^a	9.48 ^a	4.85 ^a
STZ-IND	20.05±0.56 ^c	200.54±6.13	192.21±12.35 ^c	-8.33 ^g	-4.15 ^d
STZ + ACA	28.83±1.22 ^b	202.63±8.31	206.88±10.11 ^{bc}	4.25 ^e	2.10 ^c
STZ + PBE	28.54±0.47 ^b	202.31±5.42	206.53±8.33 ^{bc}	4.22 ^f	2.09 ^c
STZ + HPBE	28.33±0.80 ^b	204.74±6.73	209.00±7.15 ^{ab}	4.26 ^d	2.08 ^c
STZ + CHPBE-1	29.61±0.52 ^b	210.88±4.49	215.39±6.03 ^{ab}	4.51 ^b	2.14 ^b
STZ + CHPBE-2	30.15±0.49 ^{ab}	209.67±6.15	214.15±9.13 ^{ab}	4.48 ^c	2.14 ^b

Values are mean±SD (n=7).

Different letters (a-g) are significantly different at $P<0.05$.

^{ns}Not significant.

NC, normal control; STZ IND, STZ-induced without treatment; STZ + ACA, diabetic rats treated with acarbose; STZ + PBE, diabetic rats treated with plantain bulb extract; STZ + HPBE, diabetic rats treated with plantain bulb extract + honey; STZ + CHPBE-1, diabetic rats treated with HPBE+1% cocoa powder; STZ + CHPBE-2, diabetic rats treated with HPBE + 2% cocoa powder.

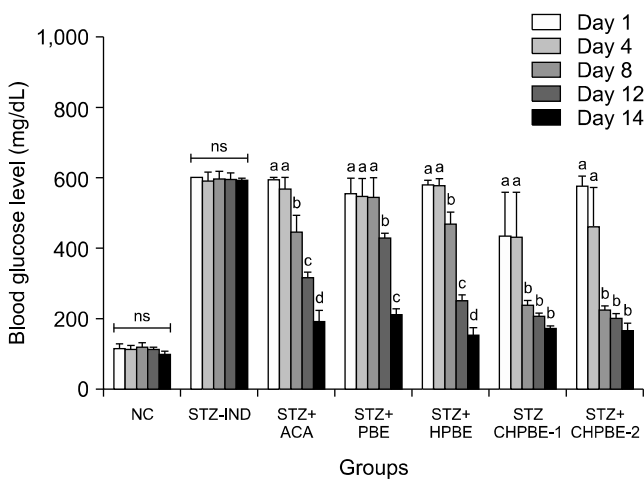


Fig. 1. Effect of plantain bulb extract-beverage blend on blood glucose level of streptozotocin (STZ)-induced diabetic rats. Bars represent mean±SD (n=7). Different letters (a-d) are significantly different at $P<0.05$. ns, not significant. Group abbreviations are the same as Table 1.

eight days. Groups treated with extracts containing 1% and 2% cocoa powder showed reduced postprandial responses (585 to 152 mg/dL and 433 to 171 mg/dL, respectively). In addition, for both the STZ+CHPBE-1 and STZ+CHPBE-2 groups, blood glucose levels for days 8 to 14 did not significantly differ ($P>0.05$) from one other, but significantly differed ($P<0.001$) from levels at day 1 and day 4.

Overall, the STZ+CHPBE-2 group showed the most significant decline in blood glucose levels (greater than group treated with acarbose), with the value at day 14 similar to that of the NC group [118 mg/dL (day 1) to 97 mg/dL (day 14)]. These results suggest that the extract possesses hypoglycaemic effect, with incorporation of cocoa powder promoting this property in the herbal beverage. Although, the bioactive compounds responsible for these action(s) could not be ascertained in the present study, a previous report showed that *Musa sp.* are a rich source of functional oligo and polysaccharides, which

have been reported to possess antihyperglycaemic effects (Kim et al., 2005; Samanta et al., 2015; Wobiwo et al., 2019; Zhu et al., 2019). In addition, cocoa extracts exhibit hypoglycaemic potentials, credited to their rich contents of polyphenols such as tannins and (-)-epicatechin (Chee et al., 2011). We concluded that the observed blood glucose lowering effect of PBE and its blended products (particularly CHPBE-2) could be as a result of synergistic and/or additive effect of these compounds.

The result of this study agree with those of Olasope et al. (2016), which reported that fasting blood glucose levels of alloxan-induced diabetic rats significantly decreased upon administration of cocoa powder. In addition, plantain root and stem juices have been reported to lower blood glucose levels (Omobuwajo et al., 2011). Similar results were also shown in a study by Adedayo et al. (2016), which published the antihyperglycemic properties of certain *Musa sp.* [*Musa sapientum* (Latundan banana), *Musa acuminata* (Cavendish banana), and *Musa acuminata* (red Dacca)]. Other plant extracts such as *Sargassum hystrix* extract, have also been reported to exert significant blood glucose lowering potential (Gotama et al., 2018). The significant reduction in blood glucose levels in diabetic rats upon daily administration with the formulated beverages may arise from synergistic contribution of both plantain bulb juice and cocoa powder.

Effects of PBE beverage blends on antioxidant enzymes in liver and kidney homogenates from diabetic rats

Pathological conditions such as diabetes are characterized by increased production of free radicals and oxidative stress. Aerobic organisms have an integrated oxidative system to block the harmful effects of reactive species, however diabetes may undermine compensatory responses of endogenous antioxidant defences (Betteridge, 2000). The activities of antioxidant enzymes such as CAT, SOD, and GPx are the first line of defence against oxidative stress. The integrity of these antioxidant enzymes de-

termine the potency of endogenous antioxidants in scavenging free radicals and preventing the development of oxidative stress-induced complexities such as diabetes. In this study, we evaluated enzymatic and non-enzymatic antioxidant molecules in organs important for metabolism (liver and kidney).

CAT and SOD are key scavenging enzymes that terminate free radicals (Sellamuthu et al., 2013). CAT is an endogenous antioxidant enzyme abundantly present in biological tissues, including liver and kidney, and is the most effective defence against hydrogen peroxide (an important reactive oxygen species). Whereas SOD protects the body against oxidative stress by catalysing reactions of superoxide radicals and eventually converting radicals to water and oxygen (Raj et al., 2016).

Significant ($P < 0.01$) decreases in CAT and SOD activities were observed in both liver (95% and 92%, respectively) and kidney (95% and 90%, respectively) homogenates from untreated diabetic rats (STZ-IND) compared with NC rats (Fig. 2). Diabetes decreases antioxidant enzyme activities as a consequence of overwhelming oxida-

tive stress that results from glucose autoxidation, protein glycation, and lipid peroxidation; this oxidative stress may also depress the activities of the endogenous antioxidant protection system (Giugliano et al., 1996). Decreased CAT and SOD activities can cause deleterious effects, including free-radical induced cellular damage (Sözmen et al., 2001; Merghem et al., 2019). Moreso, earlier report showed that diabetes induces increased production of radicals such as superoxide anions, which decrease inherent antioxidant defence mechanisms in human (Ceretta et al., 2012).

Upon treatment with PBE beverage blends, there was significant ($P < 0.05$) upregulation in CAT and SOD activities. Treatment of diabetic rats with plantain bulb beverage (CHPBE-1; CHPBE-2) blends augmented the activity of CAT. Samples containing PBE promoted endogenous enzyme activity in the organs evaluated, with significant increases in CAT activity observed in liver (11 to 18-fold) and kidney (11 to 13-fold) homogenates (Fig. 2A and 2B). Enhanced activity of CAT enzyme in tissues suggests an improved antioxidant status of the organs

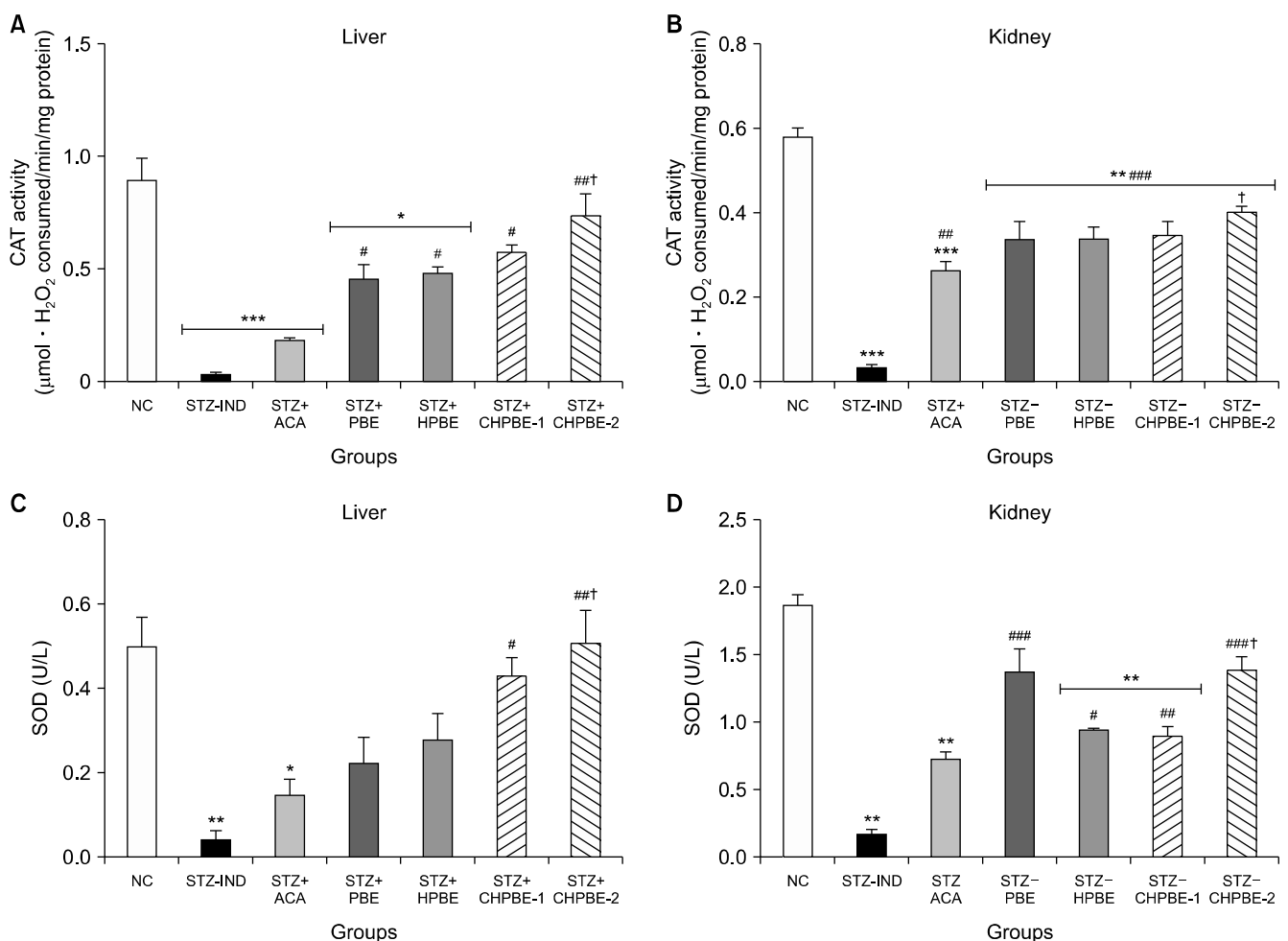


Fig. 2. Effect of plantain bulb extract-beverage-blend on (A and B) catalase (CAT) and (C and D) superoxide dismutase (SOD) activities in the liver and kidney homogenates of streptozotocin (STZ)-induced diabetic rats. Bars represent mean \pm SEM ($n=7$). Values are statistically different at * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ vs NC; # $P < 0.05$, ## $P < 0.01$, and ### $P < 0.001$ vs STZ-IND; † $P < 0.05$ vs STZ+ACA. Group abbreviations are the same as Table 1.

and a reduction in diabetes-induced oxidative damage.

In addition, SOD activity was significantly affected by diabetic status (Fig. 2C and 2D); significantly lower levels of SOD activity was observed in liver and kidney homogenates of STZ rats (1.86 to 0.18 U/L) compared with NC rats (0.50 to 0.04 U/L). Reduced SOD activity might be due to increased utilization of SOD in scavenging free radicals. Conversely, administration of the PBE beverage blends over the 14 day treatment period restored SOD activity in diabetic rats to levels similar to those of NC rats, especially those administered CHPBE-2 (0.51 and 1.38 U/L in liver and kidneys, respectively). In a previous study, *Musa balbisiana* shoot extracts significantly improved liver SOD activity in STZ-induced diabetic rats compared with treatment with root extracts (Kalita et al., 2016). This result is in line with a study by Shodehinde et al. (2015), which showed that plantain based diets improve antioxidant status of STZ-induced diabetic rats. The effect was credited to the rich polyphenolic compounds, which exhibit antioxidant and antidiabetic properties.

Breakdown of hydrogen peroxide or other organic hydroperoxides into non-toxic products is achieved by GST and GPx alongside GSH (Agrawal et al., 2015). GST level in STZ-IND rats were lowered in the liver (86.1%) and kidney (77.7%) homogenates compared with the NC group (Fig. 3A and 3B). However, PBEs and beverage blends exhibited potent antioxidant activity, since they increased GST activity in the treated groups. Notably, CHPBE-2 exhibited a significant antioxidant effect, observed by improved GST activity in the livers (518%) and kidneys (351%) of treated diabetic rats. These values were significantly higher than those of the synthetic drug treated group (STZ+ACA).

Similarly, there was a decrease in GPx activity in both liver and kidney homogenates of untreated diabetic rats (0.27 and 0.26 U/L, respectively) compared to the NC group (3.18 and 1.83 U/L, respectively) (Fig. 3C and 3D). However, administration of PBE beverage blends for 14 days restored GPx activity in the livers (1.26 to 3.13 U/L) and kidneys (1.07 to 1.80 U/L) of treated rats.

This study showed that the activities of endogenous antioxidant enzymes (CAT, SOD, GST, and GPx) were significantly ($P < 0.05$) decreased in the livers and kidneys of untreated diabetic rats (STZ-IND). Decreased activities of CAT and SOD may be attributed to elevated endogenous production of superoxide anions and hydrogen peroxide, respectively, by the auto-oxidation of excess glucose. This reduced antioxidant status may promote cell disruption and cell damage and, eventually, induce complications. However, daily administration of PBE or beverage blends improved antioxidant status of hepatic tissues by significantly ($P < 0.05$) increasing the activities of antioxidant enzymes, as compared with untreated or

acarbose treated diabetic rats.

There was a progressive increase in CAT and SOD activities with addition of cocoa powder (1% and 2%), and addition of CHPBE-2 increased activities of these enzymes to levels similar to that observed for NC rats. Thus, the PBE and beverage blends helped restore antioxidant defence in tissues. These results indicate that the PBE and beverage blends possess antioxidative potential. Similarly, in a previous study, Prakasam et al. (2005) reported elevated activities of SOD, CAT, and GPx in the livers and kidneys of diabetic rats following treatment with *Casearia esculenta* root extracts.

Effect of PBE beverage blends on non-enzymatic antioxidants in the liver and kidney homogenates of diabetic rats

GSH is a direct scavenger of free radicals. GSH plays a significant role in the endogenous non-enzymatic antioxidant system as it acts as a reducing agent and detoxifies hydrogen peroxide in the presence of GPx (Winterbourn, 1995). As expected, NC rats possessed high levels of GSH (0.85 and 0.83 mg/mL in livers and kidneys, respectively) whereas untreated diabetic rats exhibited significantly ($P < 0.05$) lower level of GSH (0.15 and 0.11 mg/mL, respectively; 83% reduction in both liver and kidney tissues, Fig. 4A and 4B). This reduction may be a consequence of decreased synthesis or increased degradation of GSH, induced by oxidative stress, which is detrimental to cell integrity.

Administration of PBE or beverage blends, particularly those with cocoa powder incorporation (CHPBE-1 and CHPBE-2), induced a potent protective effect on both livers and kidneys. Indeed, significant ($P < 0.05$) increases in GSH was observed in the livers (0.65 mg/mL for CHPBE-1 and 0.63 mg/mL for CHPBE-2) and kidneys (0.75 mg/mL for CHPBE-1 and 0.79 mg/mL for CHPBE-2) of treated rats. These results suggest that plantain bulb based beverages have potential to increase biosynthesis of GSH or reduce oxidative stress.

STZ-inducement increases free radicals and reactive oxygen species (ROS), coupled with increased levels of the oxidative stress marker (TBARS), which interrupt the antioxidant system (Konda et al., 2019). Excessive production of ROS and decreased level of GSH promote lipid peroxidation. TBARS is an important end product of lipid peroxidation and is therefore an important biomarker for oxidative stress, a major characteristic of DM. Moreover, TBARS levels can be used to indicate the extent of oxidation in tissues or organs. Reduced activities of CAT and SOD (as observed in STZ-IND rats) promotes initiation and propagation of lipid peroxide (Krishnasamy et al., 2016). As stated earlier, increased blood glucose levels (hyperglycaemia) premeditates the generation of free radicals during auto-oxidation (Malini et al., 2011), which could attack membranes through peroxidation,

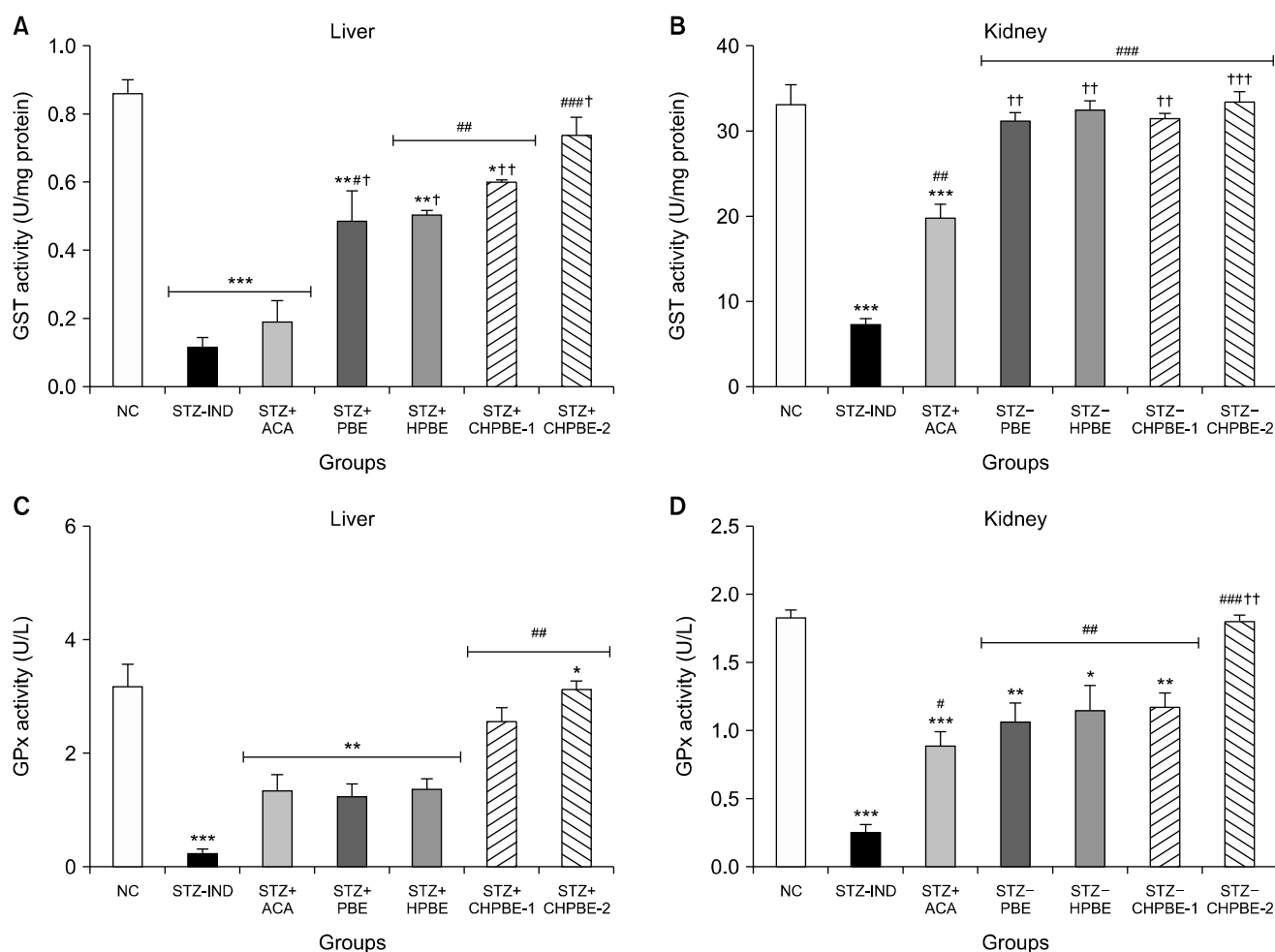


Fig. 3. Effect of plantain bulb extract-beverage-blend on (A and B) glutathione-S-transferase (GST) and (C and D) glutathione peroxidase (GPx) in the liver and kidney homogenates of streptozotocin (STZ)-induced diabetic rats. Bars represent mean \pm SEM (n=7). Values are statistically different at * P <0.05, ** P <0.01, and *** P <0.001 vs NC; # P <0.05, ## P <0.01, and ### P <0.001 vs STZ-IND; † P <0.05, †† P <0.01, and ††† P <0.001 vs STZ+ACA. Group abbreviations are the same as Table 1.

causing tissue damage. Increased lipid peroxidation decreases membrane fluidity and alters membrane-bound enzyme activity, which impairs membrane function (Arulselvan and Subramanian, 2007). Low levels of lipid peroxide may invigorate insulin secretion, whereas increased endogenous peroxide concentrations induce uncontrolled lipid peroxidation, resulting in cellular infiltration and damage to islet cells common to type 1 diabetes (Metz, 1984).

The untreated diabetic group showed a greater increase in lipid peroxidation, as shown by increased levels of TBARS in liver (32.91 mmol/mg protein; Fig. 4C) and kidney (31.57 mmol/mg protein; Fig. 4D) homogenates compared with the NC group (liver: 1.62 mmol/mg protein; kidney: 11.94 mmol/mg protein). These results indicate occurrence of a higher rate of lipid peroxidation in the tissues as a consequence of hyperglycaemia. In addition, diminished enzymatic and non-enzymatic antioxidant defence systems may be responsible for increased lipid peroxidation. Diabetic rats have been reported to show increased levels of TBARS in the liver (Manoharan

et al., 2009; Harini and Pugalendi, 2010). However, treatment with PBE and beverage blends decreased levels of TBARS in liver and kidney homogenates to near normal levels (liver: 2.05 to 5.39 mmol/mg protein; kidney: 11.75 to 14.45 mmol/mg protein). This indicates PBE and beverage can effectively scavenge free radicals and detoxify ROS. Treatment with PBE beverage blends was effective in depleting TBARS levels, thus, exhibited anti-lipid peroxidative activity. Similar to the current finding, Manzari-Tavakoli et al. (2013) and Pouraboli et al. (2016) reported a remarkable decrease in lipid peroxidation levels in STZ-induced diabetic animals after treatment with *Otostegia persica* and *Dracocephalum polychaetum* shoot extracts, respectively.

Effect of PBE beverage blends on NO levels

Reduction in NO bioavailability in DM is associated with increased free radical production (Obboh et al., 2018; Oyeleye et al., 2020). In this study, low levels of NO (7.55 μ mol NOx/mg protein) was observed in the liver and kidney homogenates of untreated STZ-induced diabetic

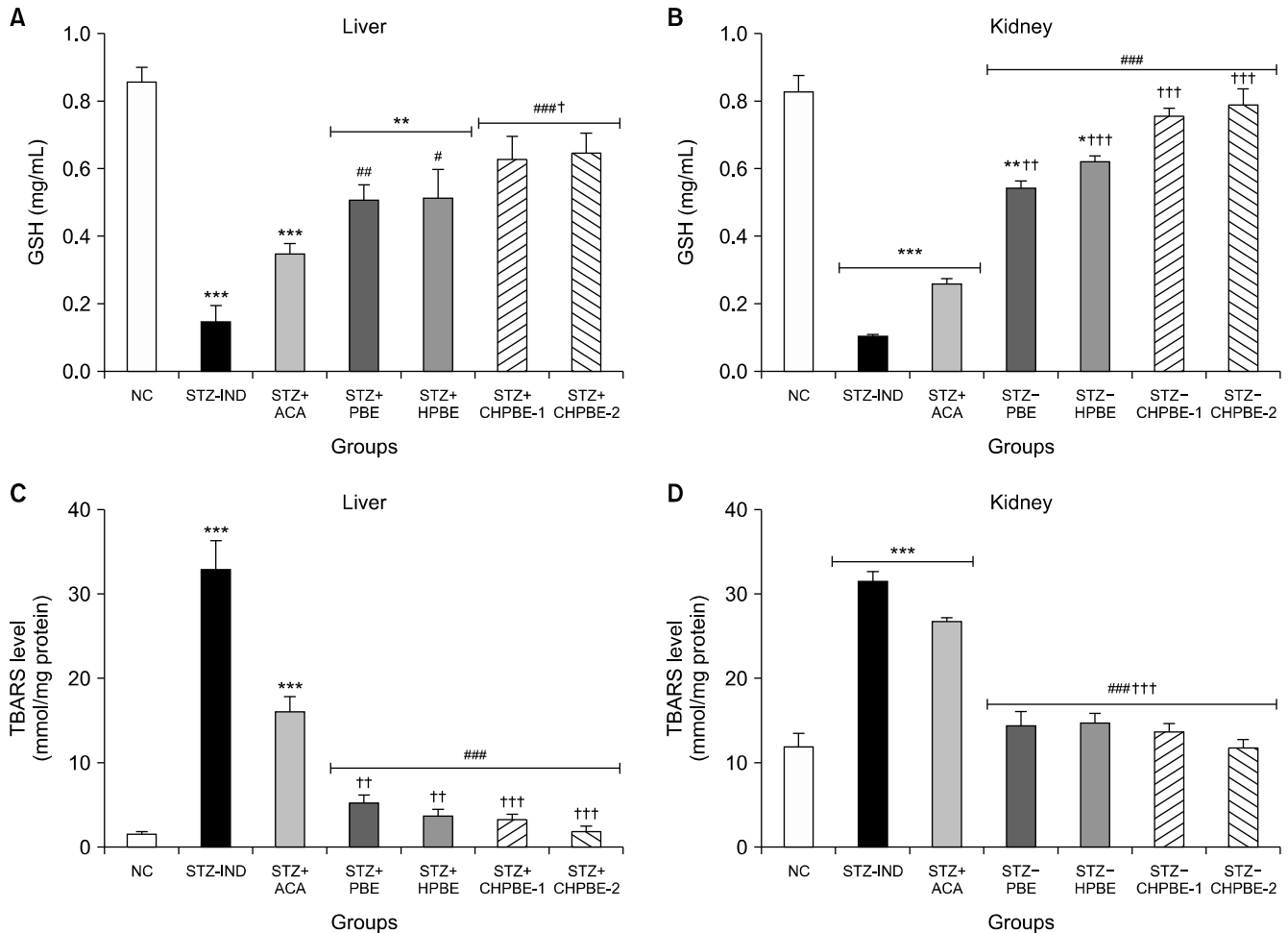


Fig. 4. Effect of plantain bulb extract-beverage-blend on (A and B) glutathione (GSH) and (C and D) thiobarbituric acid reactive species (TBARS) levels in the liver and kidney homogenates of streptozotocin (STZ)-induced diabetic rats. Bars represent mean \pm SEM ($n=7$). Values are statistically different at $*P<0.05$, $**P<0.01$, and $***P<0.001$ vs NC; $#P<0.05$, $##P<0.01$, and $###P<0.001$ vs STZ-IND; $^1P<0.05$, $^{11}P<0.01$, and $^{111}P<0.001$ vs STZ+ACA. Group abbreviations are the same as Table 1.

rats. These contents represent an approximately 80% reduction in NO levels compared with homogenates from NC rats (Fig. 5A and 5B), which may reflect increased utilization/conversion of NO to highly reactive peroxynitrite radicals as a result of oxidative stress (Suarez-Pinzon et al., 2001). STZ-ACA, STZ-PBE, and STZ-HPBE groups exhibited increased NO levels (15.59, 18.15, and 20.48 $\mu\text{mol NO}_x/\text{mg}$ protein, respectively) in liver homogenate; however, these values did not significantly differ among groups but STZ+CHPBE-2 group significantly differed from STZ-IND group at $P<0.001$. In a similar fashion, the kidney homogenates of STZ+PBE, STZ+HPBE, and STZ+CHPBE-1 groups showed increased NO levels of 19.15, 21.14, and 22.08 $\mu\text{mol NO}_x/\text{mg}$ protein, respectively with no significant difference from one another but significantly differed from NC at $P<0.05$. However, diabetic rats treated with CHPBE-1 and CHPBE-2 exhibited significantly high NO levels relative to NC rats (23.21 and 31.57 $\mu\text{mol NO}_x/\text{mg}$ protein, respectively). These results confirmed that inclusion of cocoa powder promoted NO upregulation, possibly via increased production/bio-

availability of endothelial NO within the normal threshold or physiological level, to improve vascular function (Andújar et al., 2012).

Effect of PBE beverage blends on carbohydrate hydrolysing enzymes

Carbohydrate hydrolysing enzymes play pivotal roles in modulating carbohydrate hydrolysis. Pancreatic α -amylase is an important enzyme responsible for the breakdown of dietary carbohydrates (starch to monosaccharides) in the digestive system. Monosaccharides are further degraded to glucose by α -glucosidase, which is absorbed into the blood stream and elevates blood glucose levels (Alqahtani et al., 2019). Inhibition of α -amylase and α -glucosidase activities is used in the treatment of diabetes as it promoted lowered postprandial glucose levels (Tundis et al., 2010).

In this study, we observed elevated activities of α -amylase (1.54 units/g protein) and α -glucosidase (1.31 units/g protein) in the untreated diabetic rats compared with NC rats, representing increases of approximately 8-fold

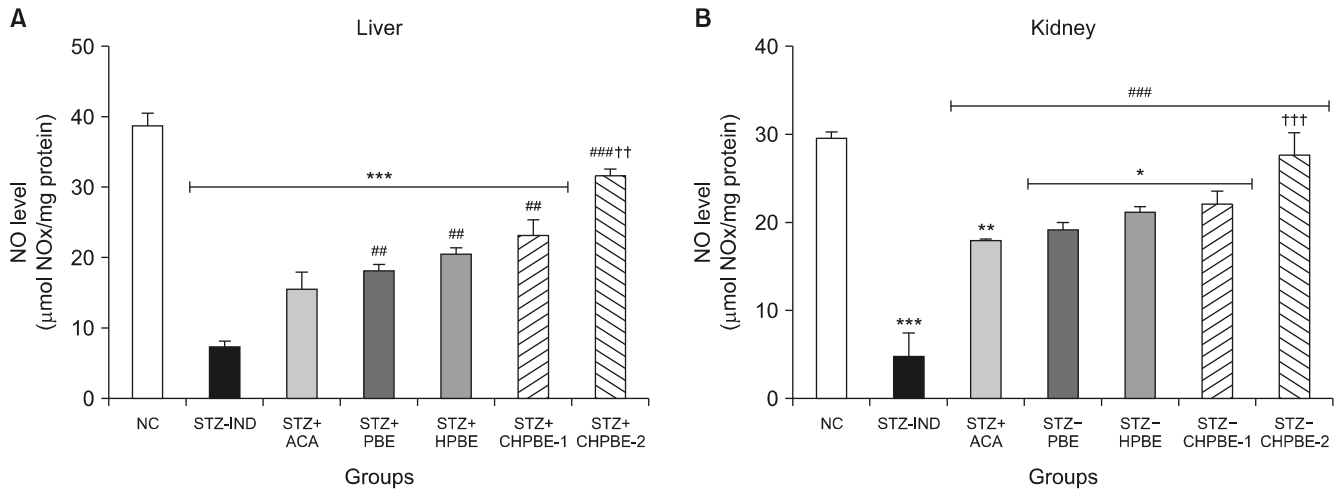


Fig. 5. Effect of plantain bulb extract-beverage-blend on nitric oxide level in the liver and kidney homogenates of streptozotocin (STZ)-induced diabetic rats. Bars represent mean \pm SEM (n=7). Values are statistically different at * P <0.05, ** P <0.01, and *** P <0.001 vs NC; # P <0.05, ## P <0.01, and ### P <0.001 vs STZ-IND; ††† P <0.001 vs STZ+ACA. Group abbreviations are the same as Table 1.

and 3-fold, respectively (Fig. 6). These results imply that the increased glucose production and absorption into the blood stream were beyond the threshold level. However, upon administration of the PBE and beverage blends, the activities of these hydrolysing enzymes were remarkably reduced. Interestingly, α -amylase activity in rats treated with CHPBE-1 and CHPBE-2 did not significantly differ (P >0.05). The lowered enzyme activity shows there must have been downregulated activity of carbohydrate hydrolysing enzymes in the digestive tract, which increased glucose absorption and decreased blood glucose levels (Fig. 1).

To conclude, this study showed that PBE and beverage blends possess significant blood glucose lowering potential, which may be mediated through α -amylase and α -glucosidase inhibitory activities inducing hypoglycaemic properties. Furthermore, the blends are protective against STZ-induced liver and kidney damage by promoting in-

creased levels of endogenous enzymes and non-enzymatic antioxidant enzyme activity. The beverages showed more pronounced effects in restoring levels of oxidative stress markers in livers and kidneys. Reduced lipid peroxidation (low TBARS levels) alongside improved antioxidant status (elevated endogenous antioxidant systems) may be important mechanisms for preventing diabetic complications. Our findings suggest that plantain bulb beverage blends, particularly CHPBE-2, could serve as functional herbal beverage for controlling hyperglycaemia and associated complications.

AUTHORS' CONTRIBUTIONS

Olagunju AI conceptualized, designed the research, and wrote the first draft, Oluwajuyitan TD carried out the laboratory experiments, and Oyeleye SI carried out the

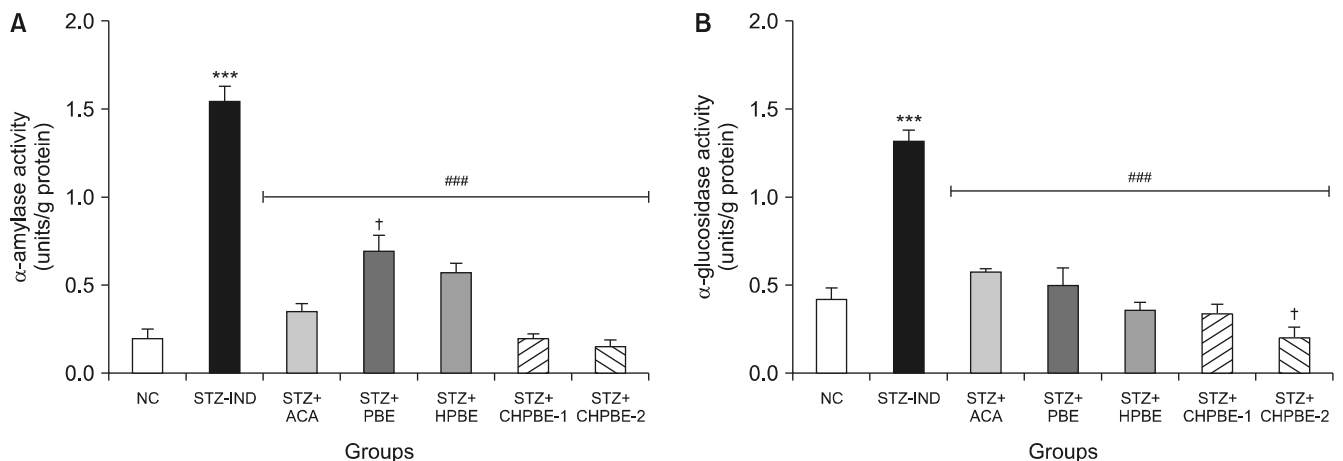


Fig. 6. Effect of plantain bulb extract-beverage-blend on pancreatic α -amylase and intestinal α -glucosidase activities of streptozotocin (STZ)-induced diabetic rats. Bars represent mean \pm SEM (n=7). Values are statistically different at *** P <0.001 vs NC; ### P <0.001 vs STZ-IND; † P <0.05 vs STZ+ACA. Group abbreviations are the same as Table 1.

statistical analysis and revised the draft.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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