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Original Article

Modulatory influence of *Parkia biglobosa* protein isolate on testosterone and biomarkers of oxidative stress in brain and testes of streptozotocin-induced diabetic male rats

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Abstract: *Parkia biglobosa seed an important household spice commonly consumed in Nigeria is believed to pos*sess antioxidant activity that may exert modulatory effects in diabetes and diabetic complications. This study investigated the modulatory potential of *Parkia biglobosa* protein isolate (PBPi) on serum testosterone (sTT) level as well as its influence on biomarkers of oxidative stress in brain and testes of streptozotocin-induced diabetic male rats. Animals were made diabetic by single intraperitoneal administration of streptozotocin (STZ; 60 mg/kg body weight). PBPi (200 or 400 mg/kg body weight) was given orally by gavage or insulin (5 U/kg, i.p.) was administered daily to STZ-induced diabetic rats for 28 days. The results revealed a significant elevation in thiobarbituric acid reactive substances (TBARS) levels in the brain and testes of diabetic rats. This was closely associated with a concomitant reduction in levels of sTT and reduced testes weight, a noticeable decline in the glutathione-S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT) as well as total glutathione (Total GSH) level in the brain and testes of diabetic rats. Interestingly, treatment with PBPi efficiently prevented the alterations witnessed in the serum sTT and also ameliorated various alterations in the biomarkers of oxidative stress (TBARS, Total GSH, GST, SOD and CAT) in brain and testes of diabetic rats. These results provide evidence that PBPi could protect the brain and testicular tissues against oxidative stress induced by STZ, via modulation of serum testosterone concentration and also by enhancing antioxidant defence system in STZ-diabetic rats.

Keywords: Antioxidant, brain, oxidative stress, *Parkia biglobosa*, STZ-induced diabetes, testosterone

Introduction

Diabetes mellitus (DM) has been identified as the fourth leading cause of death globally, it ranks high among the common non-communicable chronic metabolic disorder affecting people in both developed and developing countries in recent times [1-3]. The exponential increase in the prevalence of DM has been linked to obesity and increasing sedentary behaviours, urbanization, modernization, genetics and family history as well as nutritional imbalance associated with consumption of high energy, fat and cholesterol rich diets among others [4, 5]. This insidious metabolic disease is characterized by chronic hyperglycaemia (increased blood glucose level) together with impaired carbohydrate, protein and lipid metabolism due to deficiency in insulin secretion or resistance to insulin action or both [6, 7].

Recent documented evidence suggests that prolonged hyperglycaemia promotes the upregulation and overproduction of reactive oxygen species in the mitochondrial, which ultimately leads to oxidative stress [8-10]. This is believed to occur via well established mechanisms, including activation of polyol pathway, increased intracellular formation of advanced glycation end-products, activation of protein kinase C, increased production of superoxide radicals by the mitochondrial electron transport chain and

a general decline in endogenous antioxidant capacity [11-13]. To a great extent, experimental evidence reveals that hyperglycaemia-induced oxidative stress enhances the progression of diabetes and its associated complications [10, 14].

Oxidative stress is known to arise when there is an imbalance between free radical production (especially reactive oxygen species; ROS) and endogenous antioxidant defense system. This shift in balance is associated with oxidative damage to a wide range of biomolecules including lipids, proteins, and nucleic acids, which may eventually impair normal functions of various tissues and organs [15, 16]. Diabetic cerebral disorders have been established at a neurochemical, electrophysiological, structural and cognitive level. Although diabetes is not often considered to have damaging effects on the brain, accumulating evidence in literature reveals that long-term diabetes results in a variety of delicate cerebral disorders, which occur more frequently than is normally believed, even though the pathogenesis is poorly understood [17]. Recurring episodes of hypoglycaemic and hyperglycaemic in diabetes may have profound influence on brain chemistry, particularly on brain neurotransmitters and associated enzymes [18]. Excessive ROS production from the auto-oxidation of elevated intracellular glucose levels has been reported as one of the mechanisms underlying diabetic neuronal injury [19]. It has been established that elevated ROS levels can make the brain susceptible to oxidative stress and damage due to the brain's high oxygen demand as well as its abundant lipid concentration and relative poor antioxidant defense system compared to other tissues [20-22].

Similarly, there is increasing evidence that male reproductive dysfunction in experimental animal model and human with diabetes is one of the consequences of the imbalance between free radical production and antioxidant defense system associated with diabetic conditions [23-27]. Diabetes may affect male reproductive functions in various ways including decreased sperm count and motility, decreased testicular testosterone production, testicular morphological changes and reduced libido [27-31]. The role of medicinal plants in assuaging the devastating effects of hyperglycaemia-induced oxidative stress associated with diabetes in various tissues and organs have been recognized.

Parkia biglobosa popularly known as 'African Locust Beans' is widely consumed in Nigeria and many other West African countries as a spice for flavouring foods [4]. Both aqueous and methanolic extracts of *P. biglobosa* have been reported to possess some beneficial and pharmacological potential such as hepatoprotective, hypoglycaemic, hypolipidaemic, antimicrobial and anti-inflammatory activities in experimental animals [4, 32, 33]. To the best of our knowledge, no study has been carried out to explore the modulatory influence of *Parkia biglobosa* protein isolate (PBPi) on serum testosterone concentration as well as biomarkers of oxidative stress in brain and testes of STZ-induced diabetic rats. We demonstrate here that PBPi at a dose of 200 or 400 mg/kg bw exhibited a significant reversal effect in all biochemical parameters (notably in serum testosterone; sTT and the extent of lipid peroxidation measured by thiobarbituric acid reactive substances; TBARS) measured in serum and tissues (brain and testes) of STZ-challenged rats. Thus, in treatment of neurotoxicity and testicular toxicity, PBPi confer protection against oxidative stress induced by STZ.

Materials and methods

Plant material

Raw and fermented *Parkia biglobosa* seeds were obtained from a local market in Ijebu-Ode, Ogun state, Nigeria. Import permit (P0060156) from the Department of Agriculture, Forestry and Fisheries (DAFF; Republic of South Africa) was obtained. The raw seeds sample were identified and authenticated by the Chief botanist in the Department of Botany, University of Zululand and a voucher specimen (B07) was deposited in the University Herbarium.

Animals

Male Sprague-Dowley rats, weighing about 250-290, were obtained from the animal house of the Department of Biochemistry and Microbiology, University of Zululand. The animals were kept for acclimatization for 7 days prior to the commencement of the study; they were maintained at standard conditions of temperature and relative humidity, with a 12-hour

light/dark cycle. The animals were provided with standard rat pellets and water *ad libitum*. The animal experimental protocols were in accordance with the recommendations of the institutional animal ethical committee (UZREC 171110-030-RA level 02 Dept 2014/74).

Chemicals

All the chemicals used this study were procured from Sigma Chemical Co. (St. Louis, MO, USA), Merck (South Africa) and Sciencell Research Laboratories (Carlsbad, CA, USA).

Preparation of seed extract of Parkia biglobosa

Oven-dried (50°C) seeds of fermented *Parkia biglobosa* were pulverized using an electric blender to obtain a coarse powder. One kilogram of uniform powdered seeds was defatted with hexane to obtain the defatted extract. The defatted extract was air dried and then extracted (1:10 w/v) with butanol to remove possible anti-nutrients. Protein isolate was obtained from the defatted extract using the method described by Nkosi et al. [34]. Briefly, the dry defatted extract was re-suspended in distilled water at pH 10. The resultant suspension was filtered to remove debris and the filtrate adjusted to pH 5, followed by centrifugation at 5000 rpm for 15 minutes at 4°C. The supernatant was discarded while the pellet containing the protein isolate was retained and freeze-dried to yield a brown extract. The lyophilized extract was kept dry until needed.

Induction of diabetes

Following an overnight fasting, diabetes was induced in the selected rats by a single intraperitoneal injection of freshly prepared STZ (Sigma-Aldrich Co.) at a dose of 60 mg/kg body weight; dissolved in 0.1M, pH 4.5 ice cold citrate buffer [35]. Diabetes was confirmed in rats 72 hours after STZ administration by measuring the fasting blood glucose (FBG) levels from blood samples collected from rat-tail vein using a glucometer (Accu Check Roche, Germany). Rats with FBG level above 300 mg/dl were considered diabetic and selected for the study. Treatment commenced on the fourth day and continued for a period of twenty-eight days.

Experimental design

Seventy healthy, male rats (averaging 12 weeks old) were divided into seven groups of ten animals each and treated as follows: Group 1 (control) was given citrate buffer only. Group 2 (PI 200ND), non-diabetic rat, was given citrate buffer and protein isolate (200 mg/kg body weight). Group 3 (PI 400ND), non-diabetic rat, was given citrate buffer and protein isolate (400 mg/kg body weight). STZ-induced diabetic rats were divided in four groups (Groups 4-7). Group 4 (STZ), diabetic control. Group 5 (STZ I), positive control, was given insulin (5 U/kg, i.p.). Group 6 (STZ PI 200) was diabetic and received protein isolate (200 mg/kg body weight). Group 7 (STZ PI 400) diabetic rats that received protein isolate (400 mg/kg body weight). PBPi treatments were given orally by gavage for 28 days.

Collection and processing of blood and tissue samples

At the end of the 28 days of treatment, the rats were fasted overnight and then sacrificed under anesthesia; blood samples were obtained by cardiac puncture in plain tubes without anticoagulants, left for 1 hour to coagulate then centrifuged at 3000 rpm for 15 min at 4°C to obtain serum. The whole brain and testes were collected, washed in saline, blotted dry and weighed. Portions of the rats' brain and testes were homogenized in 56 mM Tris-HCl buffer (pH 7.4) containing 1.15% KCl, and then centrifuged at 10,000 x g for 15 minutes to obtain the supernatants that were stored at -80°C until needed for analysis.

Biochemical and enzyme estimation

The extent of lipid peroxidation in the brain and testes homogenate was determined spectrophotometrically by measuring the formation of thiobarbituric acid reactive substances (TBARS) according to the method described by Varshney and Kale [36], and this was expressed in terms of malondialdehyde (MDA), which is the end product of the reaction. Serum testosterone level was estimated using ELISA kit (Cayman Ltd., USA) according to the manufacturer's instructions while the brain and testes level of total glutathione (Total GSH) as well as the activities of glutathione-S-transferases (GST), superoxide dismutase (SOD) and catalase (CAT), were determined using the corresponding assay kits (ScienCell Research Laboratories, Carlsbad, USA) according to manufacturer's instructions.

Table 1. Effects of PBPi on testes weight of STZ-induced diabetic rats

Testes	Control	PI 200ND	PI 400ND	STZ	STZ I	STZ PI 200	STZ PI 400
Weight (g)	3.29 ± 0.10	3.57 ± 0.25	3.61 ± 0.10	2.60 ± 0.55	3.42 ± 0.08	3.34 ± 0.11	3.40 ± 0.55

Group 1: Control; given citrate buffer only, Group 2: PI 200ND; non-diabetic rat, given citrate buffer + protein isolate (200 mg/kg body weight), Group 3: PI 400ND; non-diabetic rat, given citrate buffer + protein isolate (400 mg/kg body weight), Group 4: STZ; untreated diabetic rats, Group 5: STZ I; diabetic rats treated with insulin (5 U/kg, i.p.), Group 6: STZ PI 200; diabetic rats treated with protein isolate (200 mg/kg body weight). Group 7: STZ PI 400; diabetic rats treated with protein isolate (400 mg/kg body weight).

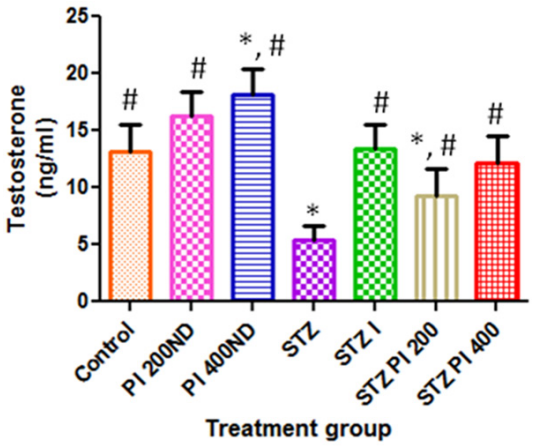

Figure 1. Effects of PBPi on serum testosterone level of STZ-induced diabetic rats. Testosterone (ng/ml). Data are presented as mean ± S.D. (n = 10). Mean differences are significant (P < 0.05) when compared with: *control group, #STZ only.

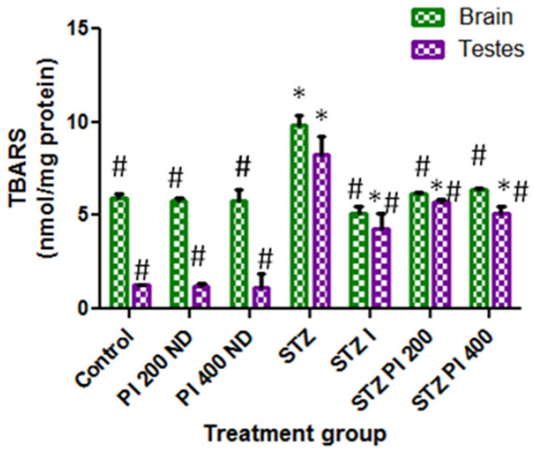

Figure 2. Effects of PBPi on lipid peroxidation levels in brain and testes of STZ-induced diabetic rats. TBARS (nmol/mg protein). Data are presented as mean ± S.D. (n = 10). Mean differences are significant (P < 0.05) when compared with: *control group, #STZ only.

Statistical analysis

All data are presented as the mean ± standard deviation. The data were analyzed by one-way ANOVA followed by Dunnett's test for multiple comparisons using Graphpad Prism (GraphPad Prism version 5.0, GraphPad Software Inc., San Diego, CA). The differences were considered significant at $P < 0.05$.

Results

Effects of PBPi on testes weight and serum testosterone level of STZ-induced diabetic rats

Table 1 and **Figure 1** show the testes weight and serum testosterone level in the experimental rats, respectively. STZ significantly decreased the weight of the testes and serum testosterone level in diabetic animals compared to control rats. Treatment with PBPi (200 or 400 mg/kg bw) for 28 consecutive days caused a significant increase in the testes weight and serum testosterone level as compared to diabetic control.

Effects of PBPi on lipid peroxidation levels in brain and testes of STZ-induced diabetic rats

The extent of lipid peroxidation in brain and testes of experimental rats are shown in **Figure 2**. Induction of diabetes led to a significant increased level of TBARS in brain and testes of diabetic rats. Interestingly, the increased level of TBARS in the brain was reversed near normal when PBPi (200 or 400 mg/kg bw) was administered. In like manner, PBPi (200 or 400 mg/kg bw) supplementation in the treatment groups considerably decreased the TBARS level in the testes of diabetic rats.

Effects of PBPi on antioxidant parameters in brain and testes of STZ-induced diabetic rats

Figure 3 represents the various alterations in the antioxidant status of experimental rats. Induction of diabetes was accompanied by a significant decline in the level of total GSH as well as in the activities of GST, SOD and CAT in the brain and testes of diabetic rats compared

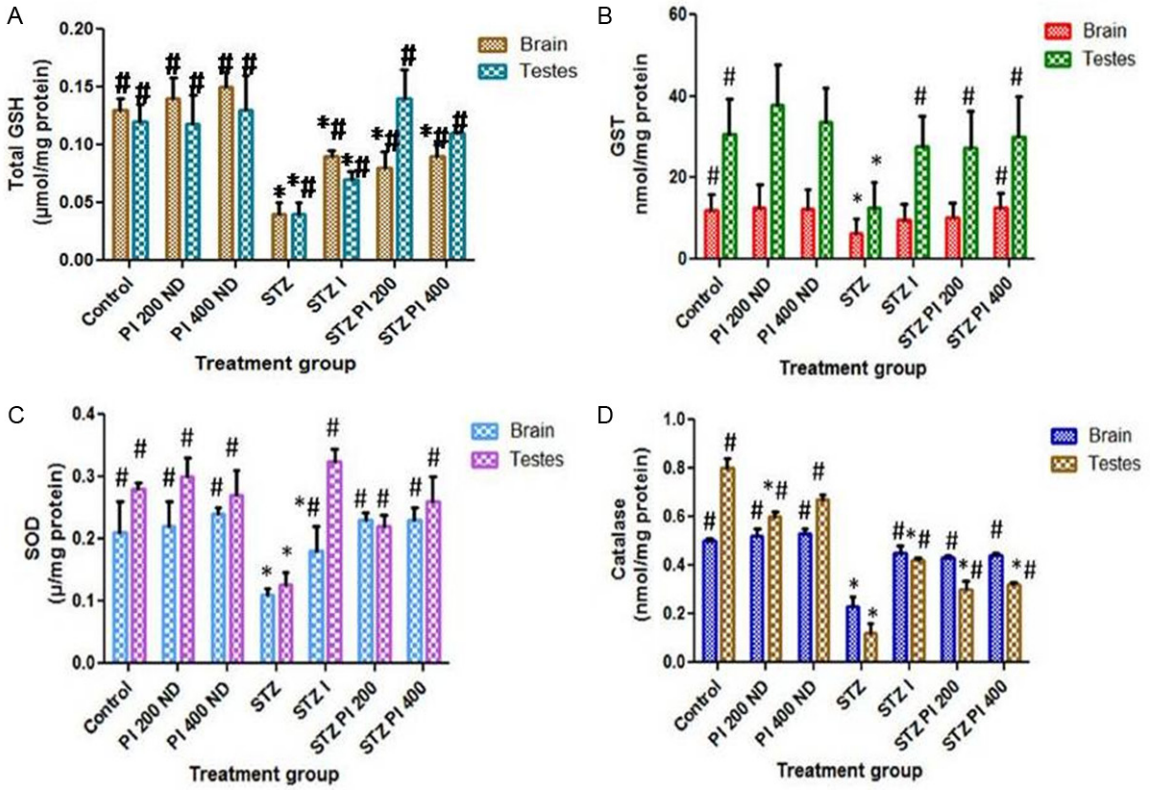

Figure 3. Effects of PBPi on antioxidant parameters in brain and testes of STZ-induced diabetic rats. (A) Total GSH (µmol/mg protein), (B) GST (nmol/mg protein), (C) SOD (µ/mg protein), (D) CAT (nmol/mg protein). Data are presented as mean ± S.D. (n = 10). Mean differences are significant ($P < 0.05$) when compared with: *control group, #STZ only.

to the control. These altered antioxidant status were restored significantly after the administration of PBPi (200 and 400 mg/kg bw).

Discussion

Brain cell injury or impairment as well as reproductive dysfunction are common complications of diabetes, which may occur via several mechanisms [9, 37]. It appears that diabetes, which is characterized by hyperglycaemia-induced oxidative stress, results in multi-organ failure and this account for most common causes of death in people with diabetes [38, 39]. The present work is one of the series of studies showing relationship between STZ-induced free radicals damage and the potentials of nutraceuticals/medicinal plants in reducing oxidative stress while concurrently improving various tissues and organ functions affected by free radicals damage. The results presented in this study demonstrated that PBPi (200 or 400 mg/kg bw) significantly ameliorated all indices of alterations in all biochemical parameters evaluated in serum and tissue homogenates (brain and testes) of STZ-challenged rats. This complemented previously identified protective roles of *P. biglobosa* in human health and disease [4, 33].

Reduction in testes weight and serum testosterone level was observed in diabetic rats. This is in agreement with similar studies reported in literature [40, 41]. Generally, testicular function is controlled via two independent coordinated functions; the biosynthesis of androgens by Leydig cells and the production of spermatozoa in the epithelium of seminiferous tubules. The reduction in testosterone level in this study may arise from oxidative damage in the testes, which may be due to a decrease in the function of both Leydig cells (testosterone producing cell) and Sertoli (supporting cell), which might be due to a reduction in insulin secretion [26, 42].

In the measurement of toxicity in STZ-induced diabetes, enhanced lipid peroxidation plays a

significant role and also serves as one of the most important manifestations of oxidative stress and damage in diabetic complications [43, 44]. Earlier work and sufficient evidence have demonstrated that neuronal cells are sensitive to oxidative insults due to the fact that the brain is susceptible to oxidative stress and damage as a result of its high oxygen demand, abundant lipid content, and relative poor antioxidant defense mechanisms as compared to other tissues [19-21]. Increased TBARS level in brain and testes homogenates in this study was an indication of damage and dysfunction in these organs. This finding is consistent with earlier reports [37, 44-46]. ROS-induced cellular damage due to chronic hyperglycaemia occurs via lipid peroxidation of unsaturated fatty acids, which alters cellular function [47]. The reversal in the elevated levels of TBARS particularly in the brain suggests the protective influence of PBPi against oxidative stress in STZ-induced diabetes.

In addition to the increased level of lipid peroxidation witnessed in the STZ diabetic animals, the endogenous antioxidant defence mechanisms (notably, Total GSH, GST, SOD and CAT) were also altered in the brain and testes. Uncontrolled hyperglycaemia, which promotes free radical generation, may be the underlying causative factor [48]. Our observation is also in agreement with previous documented reports [38, 49-51]. It is interesting to note that in this particular experiment, PBPi rescued total GSH for both brain and testes whereas it did not have any pronounced effect on brain GST activity but did rescue testes GST activity. The possible reason for the observed effect may be as a result of the overwhelming effect of the ROS generated on the antioxidant systems in the brain particularly, GST. GPx and GST work in harmony with GSH and decompose decomposing superoxide and hydrogen peroxide before interacting to form the more reactive hydroxyl radical. It is widely accepted that antioxidant enzymes provide first line defence against ROS in response to oxidative challenges [52]. Enzymatic antioxidants play very important role in protecting the cell against the overwhelming deleterious effect of ROS in hyperglycaemia-induced oxidative stress [53].

SOD and CAT are the two important radical scavenging enzymes. SOD catalyzes the conversion of superoxide radical to H_2O_2, a less reactive intermediate, thereby protecting the cell from the deleterious effect of superoxide radicals while CAT is required to neutralize this reactive specie (H_2O_2) to water (H_2O) and molecular oxygen (O_2) [54-56]. On the other hand, glutathione defends the cells from oxidative damage by reducing disulfide bonds of cytoplasmic proteins to cysteines. The glutathione system includes glutathione, glutathione reductase, glutathione peroxidases and glutathione-S-transferases [57]. The decreased activities of these antioxidants in diabetic conditions might result in reduced protection against free radical damage. This is an indication that PBPi may possess bioactive constituents that are capable of scavenging free radicals, preventing their potential induction of cellular damage.

In conclusion, the observations from this study confirms that STZ (60 mg/kg bw) has an adverse effect on serum testosterone level and on the antioxidant status in the brain and testes of diabetic rats via induction of lipid peroxidation in our animal model. Thus, our study proposes that PBPi showed a modulatory effect by attenuating the above lipid peroxidation in STZ-induced diabetic rats in a dose-dependent manner, with 400 mg/kg bw identified as having particular efficacy with no evidence of toxicity. Taken together, these results provide evidence that PBPi could protect the brain and testicular tissues against oxidative stress induced by STZ, via modulation of serum testosterone concentration and also by enhancing antioxidant defence system in STZ-diabetic rats. Therefore, characterization of the protein isolate from *P. biglobosa* should be further studied in order to elucidate the active component and the exact mechanism by which PBPi interferes with STZ toxicity in animal models.

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Disclosure of conflict of interest

None.

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