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# Glycemic and Biochemical Effects of Polyphenol-Rich Fraction of *Parkia biglobosa* Leaves in Wistar rats Experimentally Induced with Diabetes

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ABSTRACT

This study evaluated the effect of polyphenol-rich fraction of *Parkia biglobosa* leaves (PBF) in rats experimentally induced with diabetes. Thirty albino rats divided into five groups (n=6) were used for this study. Except for group one, the rats were first maintained on high high-calorie diet for eight weeks followed by streptozotocin injection (40 mg/kg). Groups 1 and 2 (normal and diabetic controls respectively) received 1 ml of distilled water throughout the experiment. Group 3 received 50 mg/kg of glibenclamide while groups 4 and 5 received 200 and 400 mg/kg of PBF respectively. After 4 weeks of treatment, after an overnight fast, blood was collected from the rats for haematological and biochemical analyses. Body weight and fasting blood glucose measurements were taken weekly. The *in vitro* antioxidant analysis revealed good ferric reducing power, and DPPH radical-quenching activity ($IC_{50}$ = 0.47mg/ml). The PBF groups had markedly ($p<0.05$) reduced blood glucose and showed better glucose tolerance compared to the untreated group. The PBF groups also had marked ($p<0.05$) diminution of liver enzymes' activities (ALP, ALT, AST) as well as markedly reduced bilirubin levels relative to the diabetic control. Urea, creatinine, and $K^+$ levels were markedly ($p<0.05$) reduced in the PBF groups relative to the untreated, while the PBF groups had markedly high $Na^+$ levels in comparison to the diabetic control. This result justifies the use of the leaves locally for disease management and could serve as a cheaper and low-toxicity alternative in the management of diabetes and associated complications.

***Keywords***: *Parkia biglobosa*, antioxidant, polyphenol-rich, glucose tolerance, Renal function, Liver function

## Introduction

Diabetes mellitus (DM) is a disorder marked by an uncharacteristically high plasma glucose resulting from poor glucose metabolism due to abnormal insulin secretion, insulin deficiency, and/or insulin insensitivity.[1] There are approximately 19 million adults with diabetes in Africa, with a projection of 42 million people by 2045 unless controlled. Diabetes-related deaths are also highest in the continent compared to other regions.[2,3] This is due to socioeconomic constraints which have impacted greatly the fight against the disease with pharmacological interventions relatively insufficient or lacking in most African populations.[4] In 2015, the WHO reported that only 51% of African countries had metformin routinely available and only 40% had insulin, well below the 80% target.[5] The economic impact of diabetes on the already overstretched economy of African countries is enormous. It was reported in 2017 that about 425 billion USD was spent globally in managing diabetes and its associated effects as against 232 billion USD in 2007 and future projections are not encouraging due to increasing population size and prevalence rates.[6]

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Increased reactive oxygen species generation during chronic hyperglycemia and associated oxidative/inflammatory stress are responsible for the various complications of diabetes. Thus, numerous consequences of diabetes may be delayed or prevented with intensive postprandial glycemic control. However, available treatment has proven insufficient and/or less effective for glycemic management [7] leading to the exploration of better alternatives in order to curb the growing disease trend.

Medicinal plants not only offer important therapeutic support for alleviating several health disorders, but they also play important roles in the discovery of novel compounds with great therapeutic potential. Natural compounds are gaining wide acceptance globally due to their better safety profiles relative to synthetic compounds. *Parkia biglobosa* (African locust bean tree) has been widely recognized as an important multipurpose indigenous fruit tree in West Africa.[8,9] The *Parkia biglobosa* plant has been found useful in disease management traditionally and its different effects are backed by both folkloric and scientific evidence.[10,11,12] Present in a variety of plants and plant parts, the therapeutic benefits of polyphenols are well documented in the literature, ranging from basic antioxidant and anti-inflammatory effects to modulation of key cellular enzyme functions.[13,14] We had earlier reported the effectiveness of polyphenol-rich fraction of *P. biglobosa* leaves in attenuating high fructose-induced biochemical aberrations in rats.[15] In this study, the effect of the polyphenol-rich fraction of *P. biglobosa* leaves on the biochemical status of experimentally-induced diabetes in rats was carried out.

## Materials and Methods

*Collection and Preparation of Plant Material*
Plant material collection and preparation were done as earlier reported by.[15]

*In vitro* Antioxidant Activity and α-glucosidase Inhibitory effect of P. biglobosa Leaf Fraction
The method of [16] was used to determine the radical-quenching effect of PBF. 1 ml of 0.135 mM methanol solution of 1,1-diphenyl-2-Picryl Hydrazyl (DPPH) radicals was mixed with 1 ml of PBF solution prepared in methanol containing 0.275-1.375 mg of plant extracts and standards separately (BHT and Rutin). The reaction mixture was vortexed thoroughly and left at dark room temperature for 30 minutes. The absorbance of the mixture was measured using a spectrophotometer at 517 nm. The DPPH radical-quenching ability of PBF was calculated thus:

$$\text{DPPH radical quenching effect} = \frac{(\text{Abs control} - \text{Abs sample})}{(\text{Abs control})} x\ 100$$

Where; $\text{Abs}_{\text{control}}$ is the absorbance of DPPH solution
$\text{Abs}_{\text{sample}}$ is the absorbance of DPPH solution + sample/standard

*Ferric Reducing Antioxidant Power of the Fraction*
The ferric-reducing antioxidant power (FRAP) of the fraction was done according to the method described by.[17] Different volumes (0.275-1.375 mg) of aqueous solution of PBF were added to a solution containing 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanide ($K_2Fe(CN)_6$) (1% w/v). The resulting solution was incubated at 50°C for 20 min, followed by the addition of 2.5 ml of trichloroacetic acid (10% w/v). After incubation, the solution was centrifuged at 3000 rpm for 10 min and the upper layer (2.5 ml) was collected and mixed with 2.5 ml of distilled water and 0.5 ml of ferrous chloride (0.1% w/v). The absorbance was measured at 700 nm against a blank using a spectrophotometer.

*Effect of the Fraction on α-glucosidase Activity in vitro*
Inhibition of pancreatic α-glucosidase activity *in vitro* was determined using dinitrosalicylic acid as described by.[18] The assay mixture containing the solution of the enzyme, α-glucosidase (0.1 ml), acetate buffer (0.1 – 0.5 ml, 0.1 M at pH 5.5) and the *P. biglobosa* fraction 32 ml – 160 ml respectively was added to five test tubes labelled test tube 1-5 and incubated for 15 minutes at room temperature. Afterwards, 0.5 ml of 2 % sucrose and 2 % starch was added to the test tubes as the substrate for α-glucosidase and then incubated in a controlled water bath at 50°C for 30 min. The reaction was stopped with 1 ml of dinitrosalicylic acid (DNSA) and placed in boiling water for 10 minutes. For colour stabilization after heating, 1 ml of 1.4 M sodium potassium tartarate was added to the test tubes immediately and the total volume of the solution was then adjusted to 4 ml with distilled water. After cooling, the absorbance of the solutions was taken at 540 nm using a spectrophotometer. All tests were done in triplicates. The test was repeated for the standard drug (Acarbose) while test tubes used as blank were prepared without any of the plant fraction, standard drug or enzyme solutions. Percentage enzyme inhibition was obtained as follows:

$$\%\ \text{Inhibition} = \frac{(\text{Abs of Ctrl} - \text{Abs of test})}{\text{Abs of Ctrl}} x\ 100$$

*Induction of diabetes*
Diabetes induction was done following the protocol of.[19] Thirty Wistar albino rats were grouped into 5 with 6 rats per group. Apart from group one which served as the normal control, rats in the diabetic group were first maintained on a fructose-supplemented high-fat diet for eight weeks before streptozotocin induction. Fortified diet was prepared by mixing commercially available normal diet feed with Margarine in a ratio of 10:1 w/w. The high-fat diet was fed to the animals with 20% fructose solution *ad libitum*. After eight weeks, rats in the diabetic group were administered a single intraperitoneal injection of streptozotocin (STZ) (40 mg/kg b.w.) prepared in a citrate buffer (pH 4.5). Twenty-four hours post-induction, 5% glucose solution was given to the rats. Diabetes was confirmed 72 hours post-induction with blood sugar testing kits. Blood samples were collected from the tail vein of the rats and those with fasting blood glucose (FBG) higher than 250 mg/dl were regarded diabetic and were selected for the study. After the confirmation of diabetes, the fraction (PBF) and standard drug (Glibenclamide) were orally administered to diabetic animals for four weeks by using a gastric gavage needle as described below.

Group 1: Normal control (No diabetes induction).
Group 2: Diabetic- untreated group control
Group 3 (Std Ctrl): Diabetic + 50 mg/kg b.w. Glibenclamide (Standard control).
Group 4 (PB-200): Diabetic + 200 mg/kg b.w. of *P. biglobosa* fraction.
Group 5 (PB-400): Diabetic + 400 mg/kg b.w. of *P. biglobosa* fraction.
Throughout the experimental period, the body weight and fasting blood glucose of the rats were measured weekly.

*Oral glucose tolerance test of Diabetic Rats Treated with P. biglobosa Leaf Fraction*
On the last day of the study, an oral glucose tolerance test (OGTT) was done to test the response of the rats to glucose loading. After an overnight fast, a single dose of glucose solution (2 g/kg BW) was administered orally to each animal followed by blood glucose measurement at 0 (at fasting state just before the glucose ingestion), 30, 60, 90 and 120 minutes.

*Blood Collection for Biochemical Analyses*
For the haematological studies, 1 ml of blood was collected from each rat into 5 ml EDTA tubes by ocular puncture using capillary tubes. The haematological analyses (packed cell volume (PCV), red blood cell (RBC), haemoglobin concentration (Hb) and total white blood cell (WBC)) were carried out within 24 hours of blood collection using a haematology auto analyzer (Sysmex Kx-21N, USA). For the biochemical assays, about 3 ml of blood was collected from each rat into 5 ml plain tubes (without EDTA). The blood samples were allowed to clot, and then centrifuged at 2500 rpm for 15 minutes and the supernatant (serum) was separated from the pellet and stored at -20°C until when needed.

*Determination of some Biochemical Parameters of Diabetic Rats Treated with P. biglobosa Leaf Fraction*
Glycated haemoglobin (HbA1c) level of the animals was determined using an automated HbA1c analyzer. Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities in whole blood of experimental rats were determined according to the method described by [20] using Randox kits. The determination of total bilirubin was done using the method described by.[21]
The serum urea and creatinine concentrations of experimental rats were determined using the method described by,[22] while serum $Na^+$ and $K^+$ levels were measured according to the method described by. [23]

*Statistical analysis*
The data from the study was analyzed using Statistical Package and Service Solutions (SPSS) version 20.0 and the results were expressed as mean ± standard error of mean (SEM). Significant differences in the result were established by two-way analysis of variance (ANOVA) for the blood glucose and body weight results while one-way ANOVA was used for the other biochemical results. Acceptance level of significance of $p < 0.05$ was set for all the results.

## Results and Discussion

Poor metabolic control, coupled with persistent hyperglycemia are the major contributors to diabetic complications. Early metabolic control is, therefore, important to prevent the development of the so-called "metabolic memory" (persistence of diabetic complications even after achieving glycemic/metabolic control) which potentiates diabetes-associated abnormalities. The antidiabetic effect of the polyphenol-rich fraction of *P. biglobosa* leaves in experimentally-induced diabetes in rats was carried out in this study. Our earlier study had shown the

beneficial effect of this fraction (PBF) in attenuating biochemical aberrations in high fructose-loaded rats as well as its rich polyphenolic profile as detected using Gas chromatography coupled with a flame ionization detector. [15]

1, 1-diphenyl-2-picryl-hydrazyl (DPPH) scavenging assay measures the free radical-quenching capability of various antioxidant compounds while the FRAP assay measures antioxidant molecules' ability to reduce $Fe^{3+}$ to $Fe^{2+}$.[10] The *in vitro* antioxidant result (Table 1) showed that radical-scavenging activity ($IC_{50}$ = 0.47 mg/ml) as well as $Fe^{3+}$ reduction increased with increasing concentration indicating good antioxidant properties of the fraction. Polyphenols, via their antioxidant effect, counteract free radical damage in living systems.[14,24] Thus, the antioxidant effect observed in the *in vitro* study could be a result of the phenolic constituents of the plant sample as earlier reported. [15]

Over-activity of enzymes such as α -amylase and α -glucosidase is one of the contributors to post-prandial hyperglycemia.[25] Thus, mild inhibition of the activities of these enzymes is a therapeutic strategy for managing diabetes. The *in vitro* α-glucosidase assay showed moderate inhibition of α-glucosidase activity by the *P. biglobosa* fraction ($IC_{50}$ = 547.69 µg/ml) with inhibitory activity increasing with increasing concentration of the plant fraction (Table 2). This result indicates the potential of the plant fraction to regulate circulating glucose levels by delaying excessive glucose release into the bloodstream. This result agrees with that of [26] who reported a strong inhibitory effect of the lupeol isolated from *P. biglobosa* leaves on both α-amylase and α–glucosidase activities *in vitro*.

Effective blood glucose control is vital in the prevention of complications associated with diabetes and goes a long way in improving the standard of life of type 2 diabetic patients. The result obtained (Table 3) showed a significant elevation in blood glucose concentration in the diabetic groups immediately after streptozotocin induction, signifying a marked impairment of glucose metabolism in these groups relative to the normal control. This is due to the absence of insulin action possibly resulting from the combined effects of streptozotocin and the high-calorie diet to which the animals were subjected. Streptozotocin destroys pancreatic beta cells, suppressing insulin secretion by these cells, while high calorie (high fat/sugar) diets have been shown to increase ectopic fat deposition (especially in the liver and pancreas), with consequent inhibition of insulin receptor substrate (IRS) 2-associated Glut2 expression and AKT/PI3K pathway activation.[27] This results in impairment of insulin-stimulated glucose uptake and elevated glucose production from the liver via the gluconeogenesis pathway.[28] There was, however, a steady decrease in mean blood glucose concentration in the PBF groups with the highest decline observed with the PBF-400 treatment on week 4.

The 2-hour oral glucose tolerance test (OGTT) (usually done to assess the rate of glucose removal from blood) is useful in predicting diabetes and other issues related to the metabolism of sugars.[29] In this study, a sharp rise in blood glucose was observed after 30 minutes (Table 4), confirming successful oral glucose loading in all the experimental groups.

However, in the PBF groups (especially the PBF-400 group) and the normal control, there was a steady decline in blood glucose concentration with increasing time, indicating improved glucose clearance in these groups. The improvement in glucose metabolism observed in the PBF groups could be linked to the actions of the polyphenols in the fraction.

**Table 1:** *In vitro* Antioxidant Activity of the *Parkia biglobosa* Leaves Fraction

| Concentration (mg/ml) | % Inhibition of DPPH | FRAP |
|---|---|---|
| 0.275 | 28.00 ± 1.00 | 0.17 ± 0.01 |
| 0.550 | 60.10 ± 1.10 | 0.22 ± 0.01 |
| 0.825 | 76.42 ± 0.38 | 0.30 ± 0.03 |
| 1.100 | 77.04 ± 0.07 | 0.56 ± 0.22 |
| 1.375 | 81.84 ± 1.97 | 0.92 ± 0.02 |
| | $IC_{50}$ = 0.47±0.01 mg/ml | |

Results are presented as mean ± SD. (n=3)
DPPH-1, 1-diphenyl-2-picryl-hydrazyl radical, FRAP-Ferric reducing antioxidant power

**Table 2:** Effect of *Parkia biglobosa* Leaves Fraction on α-glucosidase Activity *in vitro*

| Conc. (µg/ml) | % Inhibition (PBF) | % Inhibition (Acarbose) |
|---|---|---|
| 32 | 14.04 ± 0.07 | 32.81 ± 0.18 |
| 64 | 16.41 ± 0.12 | 38.31 ± 0.31 |
| 96 | 17.55 ± 0.09 | 41.09 ± 0.11 |
| 128 | 18.58 ± 0.21 | 41.54 ± 0.19 |
| 160 | 20.53 ± 0.11 | 47.57 ± 0.06 |
| | $IC_{50}$ = 547.69 µg/ml | $IC_{50}$ = 167.06 µg/ml |

Results are presented as mean ± SD. (n=3)

**Table 3:** Effect of the *P. biglobosa* leaves Fraction on Blood Glucose Concentration of Diabetic Rats

| Groups | Day 0 | Week 1 | Week 2 | Week 3 | Week 4 |
|---|---|---|---|---|---|
| Normal | $113.00 \pm 13.05^{a2}$ | $88.00 \pm 3.61^{a1}$ | $90.67 \pm 0.88^{a1}$ | $84.00 \pm 4.73^{a1}$ | $80.00 \pm 2.00^{a1}$ |
| Untreated | $97.00 \pm 8.51^{a1}$ | $371.00 \pm 44.66^{c2}$ | $357.00 \pm 27.19^{d2}$ | $383.00 \pm 43.04^{d2}$ | $312.67 \pm 30.28^{c2}$ |
| Std Ctrl | $103.00 \pm 8.74^{a1}$ | $312.33 \pm 41.53^{bc4}$ | $265.33 \pm 27.45^{c3,4}$ | $212.33 \pm 10.73^{c2,3}$ | $173.33 \pm 6.39^{b1,2}$ |
| PBF-200 | $105.67 \pm 2.33^{a1}$ | $279.67 \pm 4.91^{bc5}$ | $243.67 \pm 8.09^{bc4}$ | $214.33 \pm 8.65^{c3}$ | $194.33 \pm 4.98^{b2}$ |
| PBF-400 | $106.33 \pm 12.91^{a1}$ | $296.67 \pm 28.87^{bc2}$ | $246.00 \pm 19.70^{bc2}$ | $181.00 \pm 2.08^{bc1}$ | $179.33 \pm 10.46^{a1}$ |

Results are presented as mean ± SD. Mean values with different numbers are statistically significant within the groups while those with different alphabets are statistically significant ($p<0.05$) down the groups.

**Table 4:** Effect of the *P. biglobosa* leaves fraction on glucose tolerance of diabetic rats

| | Day 0 | Day 4 | Day 8 | Day 12 | Day 15 |
|---|---|---|---|---|---|
| Normal | $232.00 \pm 19.35^{cd1}$ | $231.67 \pm 17.65^{bc1}$ | $239.33 \pm 17.70^{c1}$ | $239.33 \pm 16.91^{c1}$ | $238.67 \pm 16.02^{c1}$ |
| Untreated | $270.00 \pm 9.17^{de3}$ | $182.00 \pm 9.26^{ab2}$ | $153.00 \pm 11.03^{a1,2}$ | $134.67 \pm 16.80^{a1}$ | $125.67 \pm 8.88^{a1}$ |
| Std Ctrl | $206.00 \pm 7.01^{bc2}$ | $176.67 \pm 5.90^{a1}$ | $180.00 \pm 11.53^{ab1}$ | $191.33 \pm 7.07^{bc1}$ | $193.67 \pm 7.85^{bc1}$ |
| PBF-200 | $299.67 \pm 19.19^{e}$ | $258.33 \pm 14.50^{c}$ | $229.67 \pm 3.33^{bc}$ | $231.00 \pm 13.65^{c}$ | $229.33 \pm 11.10^{c}$ |
| PBF-400 | $167.33 \pm 24.23^{ab1}$ | $161.00 \pm 26.31^{a1}$ | $164.67 \pm 27.23^{a1}$ | $162.33 \pm 25.10^{ab1}$ | $162.33 \pm 28.50^{ab1}$ |

Results are presented as mean ± SD. Mean values with different numbers are statistically significant within the groups while those with different alphabets are statistically significant ($p<0.05$) down the groups

For example, ferulic acid, one of the identified constituents of the fraction, has been previously reported to stimulate increased plasma insulin levels and improve insulin sensitivity and hepatic glycogenesis whilst inhibiting hepatic gluconeogenesis in diabetic rats.[30] In another study, [31] reported that quercetin administration improved glucose metabolism in diabetic rats by upregulating hepatic hexokinase IV expression and facilitating increased activation of Akt (protein kinase B) and its downstream targets.

Persistent weight loss due to muscle wasting is one of the characteristics of poorly managed diabetes mellitus. Insulin insufficiency and/or inaction in diabetes triggers the release of glucagon from pancreatic alpha cells into circulation to compensate for the perceived low glucose concentration in cells. This results in increased protein and fatty acids mobilization to maintain fuel supply to different organs of the body. From the result obtained in this study (Table 5), a significant ($p<0.05$) reduction in the body weight of the diabetic groups after streptozotocin induction was recorded. The reduction in body weight was consistent in the untreated group throughout the experimental period, probably resulting from diabetes-associated hyper-glucagonemia. Upon PBF treatment, marked improvement in body weight was seen in the PBF groups compared to the untreated, suggesting the ability of the fraction to improve glucose utilization in tissues and prevent weight loss. Plant polyphenols have been reported to improve hepatic glucose uptake by upregulating glucokinase activity as well as inhibiting hepatic glucose production (gluconeogenesis) by downregulating key enzymes of the gluconeogenesis pathway such as glucose-6-phosphatase and phosphoenolpyruvate carboxykinase (PEPCK).[32] This observation agrees with the work of [26] who reported improved body weight of diabetic rats treated with butanol fraction of *P. biglobosa* leaves.

Glycated haemoglobin (HbA1c) is an important diagnostic and prognostic marker of diabetes which helps in determining long-term glycemic control in individuals. Its high level is not only an indication of chronic hyperglycemia but is considered a contributor to cardiovascular disease development and other complications even in non-diabetic individuals. [33,34] In the present study, a significant rise in HbA1c level was seen in the untreated group when compared to the non-diabetic group (Table 6). Formation and accumulation of glycation products are accelerated by hyperglycemic and/or oxidative stress conditions in diabetes. These glycation products are slowly degraded and cleared from circulation and thus persist long even after glycemic control has been achieved.[35] It was, however, observed that both doses of the fraction caused significant ($p<0.05$) reductions in HbA1c levels in the treatment groups when compared to the untreated group. This reduction in glycated heamoglobin (HbA1c) levels could be linked to the direct and indirect actions of the polyphenolic constituents of the fraction. The blood glucose lowering effect (indirect effect) of the detected compounds as observed in the improved fasting blood glucose and glucose tolerance results could have contributed to the reduced HbA1c levels. Also, the capability of polyphenolic compounds to inhibit the formation of or neutralize glycation products directly has been reported. Polyphenols alter the deleterious effects of glycation by inhibiting glycation-mediated free radical generation as well as preventing further reactions associated with glycation such as Amadori product formation and glyoxalase system activation. [35]

On the haematological parameters, the result obtained showed significant ($p<0.05$) reductions in red cell indices (PCV, RBC and Hb concentration) of the untreated group relative to the normal control (Table 6). This observation indicates significant haemolytic events mediated by prolonged hyperglycemia. Unlike in other glucose-metabolizing cells where glucose supply and metabolism are impaired due to insulin signalling defect, the glucose transporter on the membrane of red blood cells (glucose permease) is insulin-independent and thus facilitates the accumulation of overly high glucose concentration within and around red blood cells during hyperglycemic states.[34] This results in a cascade of events such as modification of red cell membrane proteins (via spontaneous glycation), heme protein oxidation, increased osmotic fragility of erythrocytes, and increased peroxidation of red cell membrane lipids. [36,37] The concomitant inactivation of the antioxidant systems of the red blood cell results in increased haemolysis and decreased life span of red blood cells.[38] There is also impaired erythropoietin production from the kidneys as a result of glucotoxicity.[38] On treatment with PBF, the result showed that only the highest dose of the fraction caused significant ($p < 0.05$) increases in red cell indices (PCV, RBC, and Hb concentration) relative to the untreated group. This effect is attributable to the antioxidant effects of the constituent polyphenolic compounds in the fraction. Apart from their direct effect on glucose metabolism such as improvement of insulin secretion and sensitivity, glucose absorption inhibition, suppression of glucose production whilst enhancing uptake, etc., polyphenols are also known for their strong antioxidant properties and thus could have contributed to the improvements observed in the red cell parameters. [39]

A significantly ($p<0.05$) high total white blood cell (TWBC), neutrophils and monocyte counts were also seen in the untreated group in comparison to the normal control (Table 6). This observation could be attributed to the combined effects of both hyperglycemia and streptozotocin and is indicative of chronic inflammation in the experimental animals. It has been reported that in hyperglycemic states, there is an abnormal increase in cytokine release and this, coupled with other factors such as angiotensin II release and oxidative stress, triggers the activation of polymorphonuclear and mononuclear white blood cells increasing white blood cell count.[40] The elevated numbers of circulating neutrophils and monocytes could be a result of the hyperglycemia-induced proliferation and expansion of bone marrow myeloid progenitor cells, leading to the expression of inflammatory cytokines. [41] There was also a significant reduction in lymphocyte counts of the diabetic-untreated group relative to the normal control further highlighting the immune-suppressive effect of uncontrolled hyperglycemia. Giese *et al*.[42] reported that chronic hyperglycemia impairs the functionality of lymphocytes in diabetic pigs by suppressing the proliferative capacity of T-cells during infection.

**Table 5:** Effect of the *P. biglobosa* leaf Fraction on Body Weight of Diabetic Rats

| | Day 0 | Week 1 | Week 2 | Week 3 | Week 4 |
|---|---|---|---|---|---|
| Normal | $232.00 \pm 9.35^{cd1}$ | $231.67 \pm 7.65^{bc1}$ | $235.33 \pm 7.70^{c1}$ | $239.33 \pm 6.91^{c1}$ | $238.67 \pm 6.02^{c1}$ |
| Untreated | $270.00 \pm 9.17^{de2}$ | $182.00 \pm 6.26^{ab1}$ | $153.00 \pm 2.03^{a1}$ | $134.67 \pm 6.80^{a1}$ | $125.67 \pm 4.88^{a1}$ |
| Std Ctrl | $206.00 \pm 7.01^{b1c}$ | $176.67 \pm 5.90^{a1}$ | $176.00 \pm 5.53^{ab1}$ | $178.33 \pm 7.07^{bc1}$ | $182.67 \pm 7.85^{bc1}$ |
| PBF-200 | $299.67 \pm 6.19^{e2}$ | $228.33 \pm 4.50^{c1}$ | $229.67 \pm 3.33^{bc1}$ | $231.00 \pm 8.65^{c1}$ | $232.33 \pm 7.10^{c1}$ |
| PBF-400 | $197.33 \pm 4.23^{ab2}$ | $151.00 \pm 6.31^{a1}$ | $154.67 \pm 7.23^{a1}$ | $159.03 \pm 5.10^{ab1}$ | $162.33 \pm 8.50^{ab1}$ |

Results are presented as mean ± SD. Mean values with different numbers are statistically significant within the groups while those with different alphabets are statistically significant($p<0.05$) down the groups.

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**able 6:** Effect of the *P. biglobosa* leaf Fraction on some Biochemical Parameters of Diabetic Rats

| | Normal | Untreated | Std Ctrl | PBF200 | PBF400 |
|---|---|---|---|---|---|
| HbA1c (%) | 5.30 ± 0.19$^a$ | 6.35 ± 0.18$^b$ | 5.25 ± 0.06$^a$ | 5.58 ± 0.17$^a$ | 5.05 ± 0.06$^a$ |
| PCV (%) | 42.75 ± 1.79$^a$ | 38.00 ± 1.58$^b$ | 41.15 ± 0.75$^a$ | 40.30 ± 1.25$^a$ | 42.25 ± 1.25$^a$ |
| Hb (g/dL) | 12.75 ± 0.58$^b$ | 11.08 ± 0.35$^a$ | 11.98 ± 0.13$^a$ | 11.83 ± 0.24$^a$ | 12.43 ± 0.42$^b$ |
| RBC ($10^{12}$/L) | 9.45 ± 0.60$^b$ | 8.23 ± 0.23$^a$ | 8.23 ± 0.23$^a$ | 9.03 ± 0.43$^a$ | 9.28 ± 0.15$^b$ |
| TWBC ($10^9$/L) | 9325.00 ± 151.62$^b$ | 7800.00 ± 109.65$^a$ | 8900.00 ± 137.54$^b$ | 9025.00 ± 149.30$^b$ | 8950.00 ± 117.54$^b$ |
| N ($10^9$/L) | 42.00 ± 1.25$^b$ | 47.75 ± 2.72$^a$ | 47.50 ± 1.04$^a$ | 45.65 ± 0.55$^b$ | 43.75 ± 0.85$^b$ |
| L ($10^9$/L) | 52.25 ± 1.20$^b$ | 49.50 ± 3.57$^a$ | 52.00 ± 1.78$^b$ | 50.25 ± 2.40$^a$ | 52.00 ± 1.08$^b$ |
| M ($10^9$/L) | 4.15 ± 0.85$^a$ | 6.75 ± 0.63$^b$ | 4.45 ± 0.39$^a$ | 4.75 ± 0.48$^a$ | 3.75 ± 0.48$^a$ |
| ALP (U/L) | 20.25 ± 0.63$^a$ | 22.00 ± 0.41$^b$ | 19.00 ± 2.79$^a$ | 20.50 ± 1.85$^{ab}$ | 17.75 ± 1.88$^c$ |
| ALT (U/L) | 9.80 ± 0.49$^a$ | 12.95 ± 0.52$^b$ | 10.25 ± 0.34$^a$ | 10.73 ± 0.49$^a$ | 10.30 ± 0.94$^a$ |
| AST (U/L) | 12.13 ± 2.16$^a$ | 17.53 ± 0.87$^c$ | 15.70 ± 1.15$^{bc}$ | 14.83 ± 0.98$^b$ | 12.83 ± 0.98$^a$ |
| T. Bil. (µmol/L) | 1.63 ± 0.13$^a$ | 2.55 ± 0.09$^b$ | 1.98 ± 0.24$^a$ | 1.83 ± 0.06$^a$ | 1.73 ± 0.15$^a$ |
| Urea (µmol/L) | 36.50 ± 2.50$^a$ | 48.00 ± 2.12$^b$ | 38.00 ± 0.71$^a$ | 39.00 ± 1.68$^a$ | 35.25 ± 2.56$^a$ |
| Crea (µmol/L) | 1.23 ± 0.18$^a$ | 1.93 ± 0.26$^b$ | 1.08 ± 0.13$^a$ | 1.18 ± 0.21$^a$ | 1.05 ± 0.10$^a$ |
| $Na^+$ (µmol/L) | 207.25 ± 6.80$^a$ | 187.50 ± 6.26$^a$ | 213.00 ± 5.73$^b$ | 206.25 ± 3.35$^b$ | 211.25 ± 7.32$^b$ |
| $K^+$ (µmol/L) | 3.30 ± 0.41$^a$ | 4.80 ± 0.18$^b$ | 3.05 ± 0.21$^a$ | 3.08 ± 0.21$^a$ | 3.10 ± 0.18$^a$ |

Results are presented as mean ± SD and mean values with different alphabets are statistically significant ($p<0.05$) down the groups.
HbA1c-glycated haemoglobin, PCV-packed cell volume, Hb-haemoglobin, RBC-red blood cells, TWBC-total white blood cell count, N-neutrophils, L-lymphocytes, M-monocytes, ALP-alkaline phosphatase, ALT-alanine aminotransferase, AST-aspartate aminotransferase, T. Bil-total bilirubin, Crea-creatinine, $Na^+$-sodium ion, $K^+$-potassium ion.

Muller *et al*.[43] also reported the toxic effect of streptozotocin on lymphocytes *in vitro*. Streptozotocin is a glucose analogue, and because lymphocytes depend on glucose for their metabolic needs, it is readily taken up by the lymphocytes where it induces its toxic effects via the induction of apoptosis. The reduction in lymphocyte counts observed in the untreated group could therefore, be a result of either impairment in T-cell proliferative capacity by hyperglycemia or the direct toxic effect of streptozotocin on lymphocytes. Taken together, the results indicate elevated inflammatory processes in the untreated animals compared to the normal control. Elevated low-grade chronic inflammation, characterized by increases in pro-inflammatory markers (IL-17, IFN-$\gamma$, and TNF-$\alpha$), is a common finding in diabetes, especially type 2 diabetes.[44] The result of this study showed that the PBF groups had significantly decreased monocyte and neutrophil counts while only the highest dose (PBF-400) significantly increased lymphocyte count. The reduction in total white blood cell count was, however, non-significant in the treated groups compared to the untreated. These effects observed with the fraction could be attributed to its rich store of polyphenolic compounds. The anti-inflammatory activities of polyphenols have been reported to be mediated through different mechanisms including either direct antioxidant effects, modulation of the immune system, and suppression of the release of various inflammatory mediators. [45,46]

Elevated activities of hepatic enzymes (ALP, ALT and AST) and other biomarkers such as bilirubin signify functional disturbances and/or mitochondrial injury in the liver. Increased gluconeogenesis and ketogenesis, hepatic TAG accumulation, and increased hyperglycemia-associated ROS generation are some of the contributors to hepatic damage in diabetic conditions. [47] In the present study, there were marked increases in serum ALP, ALT, and AST activities as well as a marked increase in bilirubin level in the untreated group in comparison to the normal control (Table 6), indicating compromised integrity of hepatic cells. This observation is suspected to result from the toxic effects of streptozotocin as well as the high-calorie diet on the animals. Earlier studies reported increased inflammation and liver ER stress in high calorie-maintained rats resulting from increased lipotoxicity that is associated with hepatic fat accumulation, along with endoplasmic reticulum (ER) and mitochondrial stress.[48] Treatment with the plant fraction, however, significantly ($p<0.05$) reduced serum ALP, ALT, and AST activities as well as bilirubin concentration in the PBF groups when compared to the untreated group, indicating possible hepato-protective effect of the fraction. This observation could be credited to the bioactivities of the polyphenols in the plant sample. The hepato-protective effect of polyphenols (occurring via direct antioxidant and indirect mechanisms) is well documented. P-coumaric acid is reported to reduce lipid accumulation in the liver by increasing AMPK expression,[49] while quercetin and kaempferol improve lipid metabolism via activation of Akt signalling and enhancement of hepatic antioxidant defence respectively. [31,50]

Functional integrity of the kidney is usually assessed by monitoring changes in serum urea and creatinine levels, and nephropathy is usually suspected when serum levels of these parameters increase. [15] Serum creatinine levels are usually elevated when renal blood flow is impaired [51] while elevated serum urea level is often an indication of renal injury, high protein consumption and/or insufficient water intake. [52] The result obtained showed significantly elevated urea and creatinine concentrations in the untreated group in comparison to the normal control (Table 6), indicating poor renal clearance of these metabolites from circulation. The result also showed significantly ($p<0.05$) reduced $Na^+$ and significantly ($p<0.05$) increased $K^+$ concentrations in the untreated group when compared to the normal control. Chronic hyperkalemia is reportedly higher in diabetic patients compared to normal populations, mainly due to reduced tubular secretion and/or reduced glomerular filtration of $K^+$ resulting from acute kidney injury and chronic kidney disease.[51] Also, uncontrolled diabetes is reported to induce hypovolemic-hyponatremia due to osmotic diuresis as well as ROS-induced glucotoxicity to renal cells [38] and may have been responsible for the anomalies in renal function observed in the untreated group. However, PBF treatment significantly ($p<0.05$) decreased the concentrations of urea, creatinine, and $K^+$ and increased $Na^+$ concentration in the treatment groups relative to the untreated group. This result indicates improved renal function in the PBF groups possibly resulting from improved glycemic control, as improved blood glucose control is positively correlated with improved renal function.[54] Direct antioxidant effect of the polyphenolic constituents of the fraction via scavenging of glucose-mediated free radical production is a possible

mechanism of improved renal function of the plant fraction in these groups.

## Conclusion

Moving on from our earlier study in which we reported improvements in the biochemical status of fructose-loaded rats treated with the polyphenol-rich fraction of *Parkia biglobosa* (PBF) leaves, this study demonstrated positive glycemic and biochemical effects of the PBF in rats experimentally induced with diabetes. This result further justifies the local use of the leaves for health management and could serve as a cheaper and low-toxicity alternative in the management of diabetes and its associated complications. Despite the promising antidiabetic potentials of PBF, further studies might be required to determine the possible mechanism(s) through which the fraction exerts its glucose-lowering effect.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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