RETRACTED ARTICLE: Antihyperglycemic and anti-inflammatory activities of polyphenolic-rich extract of Artocarpus heterophyllus lam stem bark in streptozotocin-induced diabetic rats

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Abstract: Context: Artocarpus heterophyllus Lam (Moraceae) stem bark has been used locally in managing diabetes mellitus with scanty scientific information. Objectives: This study investigates the in vitro antioxidants of polyphenolic-rich extract of Artocarpus heterophyllus stem bark as well as its antidiabetic activity in streptozotocin-induced diabetic rats. Materials and methods: Fifty male Wistar rats were used with induction of diabetes by a single intraperitoneal injection of streptozotocin (45 mg/kg body weight) and were orally administered with 400 mg/kg free and bound phenols of Artocarpus heterophyllus stem bark. Hence, cervical dislocation method was used to sacrifice the animals on 28th day of the experiment; antihyperglycemia and anti-inflammatory parameters were assessed. Results: The polyphenolic extracts demonstrate antioxidant potential (IC50 hydrogen peroxide = 112.49 and 128.50 µg/mL for bound and free phenols, respectively), and DPPH (IC50 = 98.19 and 114.81 µg/mL for bound and free phenols, respectively) and strong inhibitory activity against amylase (IC50 = 95.82 and 110.01 µg/mL for bound and free phenols, respectively) and glucosidase (IC50 of 83.33 and 91.05 µg/mL for bound and free phenols, respectively). There was a significant (p < 0.05) increase in glycogen, insulin concentration, pancreatic β-cell scores (HOMA-β), antioxidant enzymes and hexokinase activities; and glucose transporter concentration in diabetic animals administered with the extracts and metformin. Also, a significant (p < 0.05) reduction in fasting blood glucose, lipid peroxidation, glucose-6-phosphatase and in all anti-inflammatory parameters were observed in diabetic rats administered with the extracts and metformin.

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PUBLIC INTEREST STATEMENT
In this study, antihyperglycemic and anti-inflammatory activities of Artocarpus heterophyllus stem bark polyphenolic extract was assessed on streptozotocin-induced diabetic rats and it was discovered that the extract has antidiabetic potentials. This may be due to the present of gallic acid, catechin, caffeic acid, rutin and quercetin in the extract.
Discussion and conclusion: The extracts demonstrate antidiabetic potential which may be useful in managing diabetes mellitus.

Subjects: Bioscience; Food Science & Technology; Health and Social Care; Medicine, Dentistry, Nursing & Allied Health

Keywords: Antioxidant; hexokinase; glycogen; pancreatic β-cell scores; lipid peroxidation

1. Introduction
Diabetes mellitus is a metabolic disease characterized by hyperglycemia and impaired glucose, lipids and protein metabolism (Ahmed, Sharma, Kumar, Bajaj, & Verma, 2015a; Edrees, Elbehiry, & Elmosaad, 2017). This is due to insulin deficiency leading to abnormal high blood glucose levels called hyperglycemia (Singh, Rai, Jaiswal, & Watal, 2008). The resulting hyperglycemia from defects in insulin action or insulin production may lead to a number of complications which may include changes in biochemical parameters such as the formation of advanced glycation end-products (AGEs) enhanced expression of pro-inflammatory cytokine genes (Ceriello & Testa, 2009; Renard, Costalat, Chevassus, & Bringer, 2006). Excessive reactive oxygen species (ROS) production in diabetes mellitus patients due to reduction in antioxidant enzymes could lead to tissue injury or apoptosis (Shi, Liao, & Pan, 2011). Both oxidative stress and inflammation play chief roles in the development of insulin resistance, dyslipidemia, β-cell dysfunction, liver malfunction and nephropathy amongst other ailments (Omodanisi, Aboua, & Oloyintebu, 2012; Shi et al., 2011).

Persistently high glucose levels could lead to a reduction in liver glycogen concentration and glycolysis enzyme activities (Naik, 2010). In addition, hyperglycemia increases inflammatory markers such as tumor necrosis factor (TNF)-α, interleukins (e.g. IL-1, IL-6, etc.) and nuclear factor-κB amongst others (McCune & Johns, 2002; Sharma, Jha, Dubey, & Pessarakli, 2012). Overproduction of pro-inflammatory cytokines enhances inflammatory stress in diabetes mellitus patients leading to various complications. According to the World Health Organization (WHO, 2016) more than 422 million adults worldwide are suffering from this complex multi-factorial disease. Several conventional drugs like metformin and glimepiride amongst others have been used to manage diabetes mellitus worldwide but they are characterized with severe side effects such as vomiting, nausea, hypoglycemia, abnormal weight gain and renal impairments. Additionally, lack of accessibility (especially in the rural areas) and affordability particularly with the current economic meltdown globally (Palleria et al., 2016), have all necessitated the study and use of unconventional sources of anti-diabetic drugs, without or with reduced adverse effects (Sarker et al., 2015). Functional foods such as the stem, bark, leaves, roots and fruits of Artocarpus heterophyllus have been documented as effective in the management of diabetes mellitus with no side effects (Ajiboye et al., 2016).

Ajiboye et al. (2016) reported that Artocarpus heterophyllus Lam (jack fruit) belongs to the Moraceae family, which grown in tropical climates. It is a rich source of carbohydrates, minerals, dietary fiber and vitamins (Omar, El-Beshbishy, Moussa, Taha, & Singab, 2011). Locally, this plant has been used in the management of not only diabetes mellitus, but also for hypertension, hepatitis infections and other ailments in some parts of Nigeria and other African countries (Ajiboye et al., 2016). Some reports have been documented on the ethanol extract of this plant in the management of diabetes mellitus by Ajiboye et al. (2017) & Ajiboye et al. (2016) but with scanty or no information on the anti-hyperglycemic and anti-inflammatory effects of the polyphenolic extract of plant. Hence, the focus of this study determines the antihyperglycemic and anti-inflammatory effects of a polyphenolic-rich extract of Artocarpus heterophyllus Lam.
2. Materials and methods

2.1. Sample collection and authentication
The fresh peeled stem bark of Artocarpus heterophyllus was obtained on the 10 September 2015 at a farm in Ibadan, Oyo State, Nigeria. The bark was identified and authenticated by a senior taxonomist (Mr. Omotayo) at the Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria, with voucher specimen number UHAE 119.

2.2. Sample preparation
The stem bark of Artocarpus heterophyllus was air-dried at room temperature (25°C) for 4 weeks to constant weight and then grounded into a fine powder using an electric blender. This was then stored at room temperature in an air-tight container.

2.3. Chemicals
All chemicals such as acetone, sodium hydroxide, hydrochloric acid, ethylacetate, sodium phosphate, potassium ferricyanide, 1,1-diphenyl-2-picrylhydrazyl, gallic acid, hydrogen peroxide, ascorbic acid, dinitrosalicylic acid and p-nitrophenylglucopyranoside were incurred from Sigma-Aldrich Inc. (St. Louis, MO, USA) while all the assay kits used were procured from Randox Laboratories Ltd. (Antrim, UK).

2.4. Extraction of free phenol
The ground sample was soaked in acetone for 72 h. The mixture was thereafter sieved with Whatman No. 1 filter paper and the filtrate was evaporated to dryness using a freeze dryer according to the method described by Chu, Sun, Wu, and Liu (2002). This extract was then stored at −4°C.

2.5. Extraction of bound phenol
The obtained residue from the free phenol extraction was dried and hydrolyzed with NaOH. The pH of the mixture was lowered by concentrated HCl to 2 and the bound phenol was extracted using ethylacetate (Chu et al., 2002). Both the free and bound phenols were then re-dissolved in distilled water and used for various analyses.

2.6. Experimental animals
A total of 50 male Wistar rats (aged 6 to 8 weeks) weighing between 150 and 170 g obtained from the Animal Holding Units of Afe Babalola University, Ado-Ekiti (ABUAD) were used for this study. The animals were kept in clean plastic cages and positioned in a well-ventilated house. All animals were allowed free access to Afe Babalola University Animal feed (commercial feed) and water for a week before commencement of the experiment. Also, the animals were fed with pelleted diet (Afe Babalola University Animal feed) and water ad libitum throughout the experimental period. This experiment was approved by the ABUAD Animal Ethical Committee (ABUAD/SCI/101).

2.7. Determination of ferric reducing antioxidant potential (FRAP)
The method described by Pulido, Bravo, and Saura-Calixto (2000) was used in this determination. Briefly, 2.5 mL of the extract was mixed with 2.5 mL 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min and 2.5 mL of 10% TCA was added to the mixture. Thereafter, it was centrifuged at 650 g for 10 min, and 5 mL of the supernatant was mixed with equal volumes of distilled water and 1 mL 0.1% ferric chloride and the absorbance was read at 700 nm. The FRAP was calculated and expressed as gallic acid equivalent.

2.8. Determination of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging ability
A solution of DPPH (0.135 mmol/L) in methanol was prepared and 1 mL of the solution was added to 3 mL of the extract suspension in water at different concentrations. The mixture was incubated for 30 min and absorbance was measured at 517 nm using an AJ-1C03 spectrophotometer. Gallic acid was used as reference (Blois, 1958).
2.9. Determination of hydrogen peroxide scavenging
The phenolic extract was dissolved in 0.1 M phosphate buffer (pH 7.4) at various concentrations and mixed with 600 μL of hydrogen peroxide solution. Ascorbic acid was used as the reference compound. The absorbance values of the reaction mixture were read at 230 nm after 10 min (Ruch, Cheng, & Klaunig, 1989).

2.10. Determination of α-amylase inhibitory activity
Different concentrations of 250 µL volumes of the extract were incubated at 25°C for 10 min with 500 µL of hog pancreatic amylase (2 U/mL) in 100 mmol/L phosphate buffer (pH 6.8). After this, 250 µL of 1% starch dissolved in 100 mmol/L phosphate buffer (pH 6.8) was added to the mixture and incubated at 25°C for 10 min, followed by the addition of 1% dinitrosalicylic acid (DNS) (color reagent) which was then boiled for 10 min. The absorbance was measured at 540 nm. The inhibitory activity was expressed as a percentage of the control sample without inhibitors (Shai et al., 2010).

2.11. Determination of α-glucosidase inhibitory activity
Substrate solution of p-nitrophenylglucopyranoside (pNPG) was prepared in 20 mM phosphate buffer (pH 6.9). A 100 µL sample of α-glucosidase was pre-incubated with 50 µL of the different concentrations of the extracts for 10 min. Afterward, 50 µL of 3.0 mM (pNPG) as a substrate, dissolved in 20 mM phosphate buffer (pH 6.9) was added to start the reaction. The reaction mixture was incubated at 37°C for 20 min and stopped by adding 2 mL of 0.1 M Na₂CO₃. The α-glucosidase activity was determined by measuring the yellow-colored para-nitrophenol released from pNPG at 405 nm (Kim, Jeong, Wang, Lee, & Rhee, 2005).

2.12. HPLC-DAD (high-performance liquid chromatography-diode-array detector)
The *Artocarpus heterophyllus* stem bark phenolic sample, at a 10 mg/mL concentration, was injected by means of a model SIL-20A Shimadzu Auto sampler. Separations were carried out using Phenomenex C₁₈ column (4.6 mm x 250 mm x 5 μm particle size). The mobile phase was water with 1% phosphoric acid (v/v) (solvent A) and HPLC grade methanol (solvent B) at a flow rate of 0.6 mL/min and injection volume of 40 μL. The composition gradient was: 5% solvent B reaching 15% at 10 min; 30% solvent B at 35 min, 65% solvent B at 50 min and 98% solvent B at 65 min, followed by 70 min of isocratic elution until 75 min. At 80 min the gradient reached the initial conditions again, following the method described by Adefegha et al. (2016) with slight modifications. The sample and mobile phases were filtered through a 0.45 μm membrane filter (Millipore) and then degassed by an ultrasonic bath prior to use. Stock solutions of standard references were prepared in the methanol at a concentration range of 0.030–0.500 mg/mL. Quantifications were carried out by integration of the peaks using the external standard method, at 254 nm for gallic acid; 280 nm for catechin; 327 nm for caffeic acid; and 366 for quercetin and rutin. The chromatography peaks were confirmed by comparing its retention time with those of the reference standards and by DAD spectra (200 to 600 nm). All chromatography operations were carried out at ambient temperature and in triplicates.

2.13. Induction of diabetes mellitus
Single intraperitoneal injection of freshly prepared streptozotocin of 45 mg/kg body weight in citrate buffer was used to induce type II diabetes mellitus in the Wistar rats. Seventy-two hours after induction, blood samples were obtained from the tips of the rat’s tail and the fasting blood glucose levels were determined using OneTouch Ultra glucometer (LifeScan, U.S.A.) to confirm diabetes. Rats with fasting blood glucose levels ≥200 mg/dl (Ajiboye et al., 2016) were used for the experiment.

2.14. Animal grouping
The rats were divided into five (5) groups of 10 animals per group and treated as follows:

- **Group A:** non-diabetic control rats received distilled water (Normal control)
Group B: untreated diabetic rats received distilled water (Diabetic control)
Group C: diabetic rats received 400 mg/kg body weight of free phenol of Artocarpus heterophyllus
Group D: diabetic rats received 400 mg/kg body weight of bound phenol of Artocarpus heterophyllus
Group E: diabetic control rats received 5 mg/kg body weight of Metformin

The 400 mg/kg body weight of free and bound phenols were used based on oral glucose tolerance test carried out by the authors.

2.15. Collection of blood samples
The animals were sacrificed on day 28 of the treatment. The rats were sacrificed using cervical dislocation method and blood was collected from the jugular vein.

2.16. Preparation of serum and tissue homogenates
Blood samples for serum were collected in plain bottles and allowed to stand at 25°C (room temperature) for 30 min to form clots. These were then centrifuged at 3000 g (gravity) for 5 min and the supernatant (serum) was collected with the aid of Pasteur pipettes. The obtained serum was labeled accordingly and stored until further use for various analyses. Additionally, organs of interest such as the liver and pancreas were excised and placed in sterile containers having cold Tris-HCl buffer. Paper towel was used to dry the organs which were weighed separately. Thereafter, the organs were homogenized in cold Tris-HCl buffer of 1:10 w/v and centrifuged for 15 min at 3000 g to obtain a clear supernatant.

2.17. Determination of fasting blood glucose
Accu-chek Advantage II Clinical Glucose meter was used as described by Ahmad, Pischetsrieder, and Ahmed (2007) in determining fasting blood glucose level.

2.18. Determination of liver glycogen
Phenol-sulphuric acid method described by Lo, Russel, and Taylor (1970) was employed in determining liver glycogen concentration.

2.19. Determination of serum insulin, anti-inflammatory, glucose transporter 2 and homeostatic model assessment score
This was assayed by Enzyme-linked immunosorbent assay (ELISA). The serum insulin, pancreatic Interlukin-1 alpha (IL-1α), Interlukin-6 (IL-6), Tumour Necrosis Factor-α (TNF-α), Nuclear Factor-B (NF-kB) and hepatic GLUT 2 concentrations were measured by an enzyme-linked immunosorbent assay (ELISA) method using an ultrasensitive rat insulin ELISA kit in a multiple reader (Voller, Bartlett, & Bidwell, 1978). Homeostatic model assessment (HOMA-IR and HOMA-β) scores were calculated at the end of the intervention according to the following formulas:

\[ \text{HOMA-IR} = \left( \frac{\text{Fasting serum insulin in U/L} \times \text{Fasting blood glucose in mmol/L}}{22.5} \right) \]

\[ \text{HOMA-β} = \left( \frac{\text{Fasting serum insulin in U/L} \times 20}{\text{Fasting blood glucose in mmol/L}} - 3.5 \right) \]

Note: Conversion factor: insulin (1 U/L = 7.174 pmol/L).

2.20. Determination of lipid peroxidation
The method described by Varshney and Kale (1990) was used in this assay. Briefly, a 0.4 mL aliquot of the liver homogenate was mixed with 1.6 mL of Tris-KCl buffer and 0.5 mL of 30% TCA. Thereafter, 0.5 mL of 0.75% TBA was added to the mixture and placed in a water bath for 45 min at 80°C. This was then cooled and centrifuged at 3000 g for 5 min. The clear supernatant was collected and the absorbance was measured against a distilled water blank reference at 532 nm.

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2.21. Determination of antioxidant enzyme activities
The activities of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were determined as described in commercial kits (Randox Laboratories Ltd., Antrim, UK).

2.22. Determination of hexokinase
The test tubes were appropriately labeled as blank and test. Two milliliters of 0.2 M Tris buffer, 0.2 mL of 0.09 g/mL glucose, 0.1 mL of 10 mM ATP and 0.3 mL of 10 mM MgCl₂ were added to the blank and test, after which 0.1 mL of the sample was added to the test and 0.1 mL of distilled water was added to the blank. The mixture was thoroughly mixed and incubated at 30°C for 15 min. Thereafter, 0.5 mL of 5% TCA was added to both blank and test, and the absorbance was read using a spectrophotometer at 340 nm (Akinyosoye, Fawole, & Akinyanju, 1987).

2.23. Determination of glucose-6-phosphatase
This was determined as described in commercial kits (Randox Laboratories Ltd., Antrim, UK).

2.24. Data analysis
All data in this study were expressed as the mean ± SEM of 10 replicates unless stated otherwise. Analysis of variance (ANOVA) followed by Tukey-Kramer tests for differences between means and was used to detect any significant differences between the treatment groups in the study. This was performed using SPSS version 20.0 and the differences were considered statistically significant at p < 0.05.

3. Results
FRAP scavenging ability of the polyphenolic-rich extract of Artocarpus heterophyllus is depicted in Figure 1. Both free (180.10 ± 2.34) and bound (220.21 ± 3.20) phenolics demonstrated good ferric-reducing antioxidant potential (FRAP) with bound phenol having better FRAP scavenging ability than free phenol. Both samples competed favorably with the standard (gallic acid) (239.00 ± 3.42).

As the concentration of the polyphenolic-rich extract of Artocarpus heterophyllus stem bark increases so does the DPPH radical scavenging ability of the extract increases (Figure 2). The scavenging ability of bound phenol (for example, at concentration of 20 μg/mL: 20.14 ± 0.08) is significantly (p < 0.05) higher than that of free phenol (at concentration of 20 μg/mL: 16.05 ± 0.34). Likewise, the gallic acid standard (at concentration of 20 μg/mL: 29.01 ± 0.05) shows significant (p < 0.05) increase in a dose-dependent manner, more than bound phenol.

The hydrogen peroxide scavenging ability of the polyphenolic-rich extract of Artocarpus heterophyllus stem bark also increases in dose-dependent manner, with bound phenol (at concentration of 20 μg/mL: 26.12 ± 0.13) (demonstrated a significant (p < 0.05)) increase in scavenging ability.
than free phenol (at concentration of 20 μg/mL: 18.03 ± 0.09). Although vitamin C (the standard) (at concentration of 20 μg/mL: 28.02 ± 0.08) used was able to scavenging hydrogen peroxide radical more than bound and free phenolics (Figure 3).

Figures 4 and 5 show the inhibitory effects of the polyphenolic-rich extract of Artocarpus heterophyllus stem bark against in vitro alpha-amylase and alpha-glucosidase. There were significant (p < 0.05) increase in the inhibitory effects of free (at concentration of 100 μg/mL: 37.00 vs 45.01) and bound phenol (at concentration of 100 μg/mL: 42.10 vs 50) against α-amylase and α—glucosidase in a concentration-dependent manner. In addition, bound phenol had significantly (p < 0.05) higher inhibitory activities against both α-amylase and α—glucosidase when compared to free phenol. Furthermore, free and bound phenol (at concentration of 100 μg/mL: 42 vs 40 and 50 vs 47) demonstrated high inhibitory effects against α-amylase and α—glucosidase than the standard acarbose.

The HPLC profile of the Artocarpus heterophyllus extract was also acquired as shown in Figure 6. The extract contains the following compounds: gallic acid (retention time-t_R = 9.71 min; peak 1; 2.83 mg/g), catechin (t_R = 19.05 min; peak 2; 0.26 mg/g), caffeic acid (t_R = 24.93 min; peak 3; 1.57 mg/g), rutin (t_R = 40.68 min; peak 4; 4.27 mg/g) and quercetin (t_R = 50.12 min; peak 5; 1.69 mg/g).

As shown in Figure 7, 72 h after diabetes induction, fasting blood glucose levels in all the induced groups significantly (p < 0.05) increased compared to the normal control. At day 14 of the
In the present study, the diabetic rats administered both free (120.00 ± 1.78) and bound (100.01 ± 0.89) Artocarpus heterophyllus stem bark demonstrated significant (p < 0.05) decrease in fasting blood glucose level compared to diabetic-untreated rats (260.78 ± 2.09). Similarly, on day 28 of the treatment, there was significant (p < 0.05) reduction in fasting blood glucose levels of diabetic rats administered 400 mg/kg free phenol (92.01 ± 3.01) and 400 mg/kg bound phenol (86.03 ± 1.36), respectively, as well as those administered 5 mg/kg metformin (94.01 ± 1.56). However, there was no significant (p > 0.05) increase in fasting blood glucose levels of diabetic rats administered 400 mg/kg bound phenol and normal control rats, as well as in diabetic rats administered 400 mg/kg free phenol and 5 mg/kg metformin.
Glycogen concentration significantly (p < 0.05) decreased in diabetic control (12.02 ± 2.10) rats compared to the normal control (48.23 ± 2.45), as well as in diabetic rats administered both free (42.32 ± 3.21) and bound (46.78 ± 2.65) phenolic extracts and in diabetic rats administered metformin (Figure 8). Nevertheless, at day 28 of the experiment, there was no significant (p < 0.05) increase between diabetic rats administered 400 mg/kg bound phenol and normal control rats while there was a significant (p < 0.05) increase in liver glycogen concentration of diabetic rats administered 400 mg/kg free phenol compared to diabetic rats administered metformin.
Table 1. Administration of polyphenols from Artocarpus heterophyllus stem bark on the body weight (g) of normal and streptozotocin-diabetic rats

| Groups                                      | Initial body weight | Final body weight |
|---------------------------------------------|---------------------|-------------------|
| Normal control                              | 159.17 ± 5.04<sup>a</sup> | 195.44 ± 4.21<sup>a</sup> |
| Diabetic Control                            | 167.48 ± 4.11<sup>a</sup> | 120.67 ± 6.10<sup>a</sup> |
| Diabetic rats administered 400 mg/kg free phenol | 169.92 ± 4.41<sup>a</sup> | 192.97 ± 5.24<sup>a</sup> |
| Diabetic rats administered 400 mg/kg bound phenol | 163.16 ± 5.22<sup>a</sup> | 194.27 ± 4.65<sup>a</sup> |
| Diabetic rats administered 5 mg/kg metformin | 169.39 ± 4.52<sup>a</sup> | 176.1 ± 3.23<sup>b</sup> |

Each value is a mean of 10 determination ± Standard Error of Mean (SEM). Values with different superscripts across the column are significantly different (p < 0.05).

Table 2. Administration of polyphenols from Artocarpus heterophyllus stem bark on insulin concentration, HOMA-IR and HOMA-β of normal and streptozotocin-diabetic rats

| Groups                                      | Insulin (pmol/L) | HOMA-IR | HOMA-β   |
|---------------------------------------------|------------------|---------|----------|
| Normal control                              | 100.21 ± 1.01<sup>a</sup> | 2.64 ± 0.11<sup>a</sup> | 240.56 ± 2.31<sup>a</sup> |
| Diabetic Control                            | 26.14 ± 2.11<sup>c</sup> | 3.13 ± 0.21<sup>a</sup> | 5.53 ± 1.15<sup>e</sup> |
| Diabetic rats administered 400 mg/kg free phenol | 94.12 ± 4.11<sup>a</sup> | 2.77 ± 0.20<sup>d</sup> | 151.55 ± 3.01<sup>c</sup> |
| Diabetic rats administered 400 mg/kg bound phenol | 98.46 ± 3.12<sup>a</sup> | 2.69 ± 0.31<sup>c</sup> | 200.01 ± 4.01<sup>b</sup> |
| Diabetic rats administered 5 mg/kg metformin | 92.31 ± 2.01<sup>b</sup> | 2.77 ± 0.01<sup>b</sup> | 139.88 ± 2.45<sup>d</sup> |

Each value is a mean of 10 determination ± Standard Error of Mean (SEM). Values with different superscripts across the column are significantly different (p < 0.05).
diabetic control (5.21 ± 1.10) rats compared to diabetic rats administered phenolic extracts (both bound and free) (13.21 ± 0.34 vs 14.26 ± 0.67) and metformin (10.21 ± 0.29) was significantly (p < 0.05) reduced. Diabetic rats administered bound and free phenols showed more significant (p < 0.05) or greater hexokinase activity than diabetic rats administered metformin. Whereas the activity of glucose-6-phosphatase was significantly (p < 0.05) increased in diabetic control rats (84.20 ± 2.01) compared to diabetic rats administered phenolic extracts (30.04 ± 1.06 vs 25.06 ± 0.80) and metformin (38.01 ± 0.45), diabetic rats administered 400 mg/kg bound phenol and normal control rats demonstrated no significant (p > 0.05) increase.

Figure 11 depicts the concentration of glucose transporter 2 (GLUT 2) which was significantly (p < 0.05) decreased in the diabetic control rats (20.92 ± 2.10) than in the diabetic rats administered phenolic extracts (64.21 ± 2.78 vs 72.19 ± 2.11) and metformin (58.12 ± 1.89). Additionally, the
diabetic rats administered 400 mg/kg free and bound phenol demonstrated significant (p < 0.05) increase in GLUT 2 concentration than in the diabetic rats administered metformin.

There was a significant (p < 0.05) increase in anti-inflammatory [Interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and nuclear factor-kappa B (NF-κB)] (20.21 ± 1.20, 52.21 ± 2.10 and 62.49 ± 1.00, respectively) levels of pancreatic diabetic control rats compared to diabetic rats administered the polyphenolic-rich extract of *Artocarpus heterophyllus* stem bark (6.46 ± 0.45 vs 5.21 ± 1.00, 10.21 ± 1.24 vs 8.29 ± 1.03 and 13.21 ± 0.87 vs 11.98 ± 0.34, respectively) on streptozotocin-induced diabetic rats (Figure 12–14). Diabetic rats administered phenolic extracts demonstrated a significant (p < 0.05) decrease in all the anti-inflammatory studied compared to the diabetic rats administered with metformin.
4. Discussion

In the present study polyphenolic-rich extract of *Artocarpus heterophyllus* stem bark demonstrated strong antidiabetic agent. This is in accordance with earlier report on medicinal plants use in the management of diabetes mellitus as documented by Koehn and Carter (2005). Medicinal plants with free radical scavenging abilities, α-amylase and α-glucosidase activities have especially been reported to be useful in managing diabetes mellitus. In this study, a polyphenol-rich extract of *Artocarpus heterophyllus* stem bark demonstrated abilities to scavenge free radicals that easily accept electrons or hydrogen radicals to become stable diamagnetic molecules (Figures 1–3), and this finding is in accordance with a report by Ajiboye et al. (2016). This therefore hints that this

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**Figure 13. Administration of polyphenols from *Artocarpus heterophyllus* stem bark on pancreatic TNF-α concentration of normal and streptozotocin-diabetic rats.**

Each value is a mean of 10 determination ± Standard Error of Mean (SEM) Values with different superscripts are significantly different (p < 0.05).

**Legend:** NC: Normal control, DC: Diabetic control, D+ FP: Diabetic rats administered 400 mg/kg free phenol, D+ BP: Diabetic rats administered 400 mg/kg bound phenol and D+ Met: Diabetic rats administered 5 mg/kg metformin.

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**Figure 14. Administration of polyphenols from *Artocarpus heterophyllus* stem bark on pancreatic NF-κB concentration of normal and streptozotocin-diabetic rats.**

Each value is a mean of 10 determination ± Standard Error of Mean (SEM) Values with different superscripts are significantly different (p < 0.05).

**Legend:** NC: Normal control, DC: Diabetic control, D+ FP: Diabetic rats administered 400 mg/kg free phenol, D+ BP: Diabetic rats administered 400 mg/kg bound phenol and D+ Met: Diabetic rats administered 5 mg/kg metformin.
extract may be useful in managing diseases that result from the accumulation of oxidative stress, with diabetes mellitus being an example of such.

Inhibiting carbohydrate hydrolyzing enzymes, particularly \( \alpha \)-amylase and \( \alpha \)-glucosidase (Figures 4 and 5) in the gastrointestinal tract, plays an important role in minimizing postprandial hyperglycemia (Adefegha & Oboh, 2016). In this study, the extract demonstrates the ability to inhibit these enzymes in a concentration-dependent manner, probably due to gallic acid, catechin, caffeic acid, rutin and quercetin (Figure 6), which are all present in the extract. These compounds are well admitted as potential antioxidants, free radical scavengers, and inhibitors of lipid peroxidation amongst others (Ojo et al., 2017; Pereira, Fachinetto, & De Souza, 2009). The polyphenolic-rich extract of Artocarpus heterophyllus stem bark shows a significant increase in the inhibitory properties of \( \alpha \)-amylase and \( \alpha \)-glucosidase than acarbose, suggesting that it will be useful in curtailing the side effects related to synthetic drugs.

Insulin deficiency in diabetes mellitus patients, encourage gluconeogenesis (Naik, 2010) which may be attributed to reduction in body weight of diabetic rats as observed in this study (Table 1). However, administration of polyphenolic-rich extract of Artocarpus heterophyllus stem bark was able to revere this abnormal decrease in body weight of the diabetic rats. This may be linked to the ability of the extract to increase insulin concentration associated to the phenolic compounds in the extract (Figure 6).

The balance between insulin and glucagon to maintain a stable blood glucose level called glucose homeostasis, is crucial for the utilization of glucose by the liver, muscles and adipose tissues. In this study, streptozotocin was used to induce hyperglycemia in rats because is a methylating agent for DNA and acts as a nitric oxide donor to damage the \( \beta \)-cells of the pancreas (Bhuvaneswari & Krishnakumari, 2012). One of the main features of diabetes mellitus is excessive glucose concentration (hyperglycemia) cause by insulin deficiency. Persistent hyperglycemia over time may affect almost all the organs in the body system, especially the brain, retina, kidney and liver (Malini, Kanchana, & Rajadurai, 2011). Insulin concentration was significantly decreased in diabetic control rats (Table 2), due to selective destruction of \( \beta \)-cells of the pancreas. But at the end of 28 days of oral administration, the
polyphenolic-rich extract of *Artocarpus heterophyllus* stem bark to diabetic rats demonstrated significant increase in insulin concentrations. This may be attributed to the bioactive compounds present in the extract as indicated in Figure 6. These compounds have the ability to regenerate the damaged β-cells of the pancreas and boost insulin secretion. This was supported by the significant increase in HOMA-β (β-cell function) in both polyphenolic-rich extract and metformin-treated groups compared to the diabetic control rat group (Table 2). Additionally, a significant decrease in HOMA-IR index in diabetic rats administered polyphenolic-rich extract and metformin compared to the diabetic control rats support the improvement in insulin sensitivity and secretion as well as in stimulation of peripheral glucose absorption in those groups. Furthermore, this claim may be attributed to anti-oxidative ability of the bioactive compounds found in the extract (Figure 6) which is in accordance with the report of IIic et al. (2014) on *Aframomum melegueta* Schum.

Patel, Kumar, Laloo, and Hemalatha (2012) reported that increase in reactive oxygen species (ROS) production may trigger damage to fundamental macromolecules like proteins, lipids, carbohydrates and DNA, thereby leading to the incapability of the body’s defense mechanism in protecting cellular integrity. Free radical accumulation causes lipid peroxidation which is a process by which the lipids of the cell membrane undergo catabolism, leading to tissue damage. The polyunsaturated fatty acids of the liver are compromised by the broken down cell membrane structure, leading to disruption in its functionality (Manohar, Jayasree, Kishore, Rupa, & Dixit, 2012). In this study, MDA levels (marker for lipid peroxidation) increased significantly in the liver of diabetic rats when compared to the normal control (Table 3). This increase in MDA levels observed in the diabetic rats suggests damage to cell membranes that could lead to increase in ROS generation (Farombi, Abolaji, Adedara, Maduako, & Omodanisi, 2015). Administration of diabetic rats with polyphenolic-rich extract from *Artocarpus heterophyllus* stem bark led to a significant decrease in the levels of MDA by reducing lipid peroxidation. This may be attributed to the anti-lipid peroxidation bioactive compounds present in the extract.

In another vein, the activities of antioxidant enzymes (SOD, CAT and GPx) in diabetic mellitus patients normally reduce due to amelioration of ROS-induced oxidative stress as reported by Naugler and Karin (2008). The first line of defense against ROS is SOD, because it is responsible for the dismutation of superoxide radicals to water, while catalase eliminates hydrogen peroxide and GPx uses glutathione (GSH) as a substrate to detoxify hydrogen and lipid peroxides (Kumar et al., 2013; McCabe & Johns, 2002). There was an observed reduction in the above-mentioned antioxidant enzyme activities in the diabetic control rats when compared to other diabetic-treated rats.

| Groups                        | SOD (nmol/min/mg protein) | CAT (nmol/min/mg protein) | GPx (nmol/min/mg protein) | MDA (nmol/mg protein) |
|-------------------------------|---------------------------|---------------------------|---------------------------|-----------------------|
| Normal control                | 86.21 ± 2.06<sup>a</sup>  | 64.27 ± 2.11<sup>a</sup>  | 96.23 ± 3.26<sup>a</sup>  | 4.09 ± 2.18<sup>a</sup> |
| Diabetic control              | 20.46 ± 1.06<sup>a</sup>  | 15.69 ± 1.10<sup>a</sup>  | 27.87 ± 2.13<sup>a</sup>  | 12.19 ± 1.16<sup>c</sup> |
| Diabetic rats administered 400 mg/kg free phenol | 76.21 ± 4.10<sup>c</sup>  | 52.46 ± 2.18<sup>c</sup>  | 82.34 ± 3.12<sup>c</sup>  | 5.02 ± 2.04<sup>a</sup>  |
| Diabetic rats administered 400 mg/kg bound phenol | 79.21 ± 3.14<sup>b</sup>  | 59.68 ± 3.10<sup>b</sup>  | 86.12 ± 1.29<sup>b</sup>  | 4.60 ± 1.24<sup>a</sup>  |
| Diabetic rats administered 5mg/kg metformin | 60.96 ± 2.32<sup>d</sup>  | 46.47 ± 3.20<sup>d</sup>  | 73.21 ± 3.16<sup>d</sup>  | 4.56 ± 1.36<sup>a</sup>  |

Each value is a mean of 10 determination ± Standard Error of Mean (SEM).
Values with different superscripts across the column are significantly different (p < 0.05).
However, at the end of the experiment, diabetic rats administered polyhenolic-rich extract demonstrated a significant increase in these enzyme activities, which may be attributed to the anti-oxidative nature of the bioactive compounds present in the extract.

Insulin insufficiency in diabetic mellitus patients may actually be the main reason responsible for significant reduction in the activities of liver hexokinase, because its activity depends on insulin. Hexokinase is an important regulatory enzyme in the oxidation of glucose in the liver. In this study, the hexokinase activity of diabetic control rats (Figure 9) was impaired, which triggered a reduction in glucose oxidation (via glycolysis) and led to hyperglycemia (Kumar et al., 2014; Naik, 2010) as observed in Figure 7. However, there was a significant increase in the liver hexokinase activities of diabetic rats that were administered the polyphenolic-rich extract of *Artocarpus heterophyllus* stem bark, probably due to regeneration of damaged pancreatic β-cells by the extract which encouraged an increase in insulin concentration (Table 2). A decrease in insulin concentration and increase in glucagon concentration in diabetic mellitus patients is responsible for significant increases in glucose-6-phosphatase activity, an important enzyme in gluconeogenesis and glycogenolysis (Nelson & Cox, 2010). This was observed in the current study (Figure 10), but it was, however, ameliorated after administering the polyphenolic-rich extract to diabetic rats probably due to increase in insulin concentration as shown in Table 2.

Glucose transporter 2 (GLUT 2) or solute carrier family 2, facilitates the transport of glucose out of the mucosal cells, thereby allowing its entry into portal circulation and its transportation to the liver, pancreas, small intestine and kidney. GLUT 2 functions mainly in the rapid uptake and release of glucose (Murray et al., 2009). Maughana (2009) reported that glucose transport is the rate-limiting step in carbohydrate metabolism, which is facilitated by glucose transporters (GLUT 2). In diabetes mellitus patients, the hepatic concentration of GLUT 2 normally decreases (Al-Shaqha, Khan, Salam, Azzi, & Chaudhary, 2015), and this is consistent with the results observed in this study (Figure 11). However, after administration of diabetic rats with the *Artocarpus heterophyllus* stem bark polyphenolic-rich extracts, there were significant increases in GLUT 2 concentration. This may be a pathway to reverse the glucose uptake in liver cells coupled with increase in insulin secretion as demonstrated by the extract. This could be a turning point in the management of diabetes mellitus.

Persistent hyperglycemia in diabetes mellitus patients may trigger increased inflammation in tissues (especially in pancreatic cells) due to responses to harmful stimuli or damage to cells (Omodanisi et al., 2017). Pancreatic IL-6, TNF-α and NF-κB cytokines play a crucial role in hyperglycemia-induced diabetic rats (Gaur, Rana, Nema, Kori, & Sharma, 2009; Navarro-González & Maizo-Fernández, 2008; Sermugapandian, Rubini, & Martina, 2018). Diabetic control rats (Figures 12 to 14, respectively) showed an increased level of IL-6, TNF-α and NF-κB. Conversely, these were ameliorated after administration of diabetic rats with polyphenolic-rich extracts of *Artocarpus heterophyllus* stem bark. This demonstrated the anti-inflammatory properties of the bioactive compounds present in the extract.

5. Conclusion

From this study, it can be deduced that free and bound phenolic extracts of the *Artocarpus heterophyllus* stem bark demonstrated high antioxidant potentials, inhibited both α-amylase and α-glucosidase; and posed gallic acid, catechin, caffeic acid, rutin and quercetin as bioactive compounds in the extracts. These extracts ameliorate fasting blood glucose levels, increase liver glycogen, improve insulin concentration, enhance pancreatic β-cell and their functions; improve antioxidants enzymes, liver hexokinase activities and GLUT 2; reduce glucose-6-phosphatase activity and improve the concentrations of all the anti-inflammatory parameters determined. The brilliant performance of the extracts may be attributed to its bioactive compounds.
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Contributions
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Availability of data and materials
The datasets supporting the conclusions of this article are included within the article.

Authors’ Contributions
All authors participated in research design, data analysis and written of the manuscript.

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This experiment was approved by the ABLAUD Animal Ethical Committee (ABJUD/SC/J/101).

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