

Ameliorative Activity of Ethanol Extract of *Artocarpus heterophyllus* Stem Bark on Pancreatic β -Cell Dysfunction in Alloxan-Induced Diabetic Rats

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Abstract

This study sought to investigate the ameliorative effects of ethanol extract *Artocarpus heterophyllus* (EAH) in alloxan-induced diabetic rats. The rats were divided into 6 groups, with groups 1 and 2 serving as nondiabetic and diabetic control, respectively; group 3 serving as diabetic rats treated with 5 mg/kg glibenclamide; and groups 4 to 6 were diabetic rats treated with 50, 100, and 150 mg/kg of EAH, respectively. Assays determined were serum insulin, lipid peroxidation, and antioxidant enzyme activities. EAH stem bark reduced fasting blood glucose and lipid peroxidation levels and increased serum insulin levels and activities of antioxidant enzymes. Data obtained demonstrated the ability of EAH stem bark to ameliorate pancreatic β -cell dysfunction in alloxan-induced diabetic rats.

Keywords

Artocarpus heterophyllus, alloxan, diabetes

Received June 27, 2016. Received revised November 12, 2016. Accepted for publication November 23, 2016.

Diabetes mellitus is a chronic metabolic disease that affects more than 7.5% of the world population.¹ It is caused by an inherited or acquired deficiency of insulin secretion that results in an increased blood glucose level, which in turn produces adverse effects on different body systems.² There are limitations to currently available drugs, which merit the consideration of new agents, especially in the form of herbal medicine with the potential for greater efficacy or fewer side effects.³

The practice of traditional medicine is as old as the origin of man.⁴ The use of plants in traditional medicine falls outside the mainstream of Western or Orthodox medicine. It has been shown that two thirds of the world population (mainly in developing countries) rely on traditional medicine for their health care.⁵ Some plants have been used in the treatment and management of diabetes such as *Ricinus communis* among others.⁴ Also, the use of *Artocarpus heterophyllus* leaf has been documented.⁶

In addition, several reports have cited the antidiabetic effects of the root and fruit of *Artocarpus heterophyllus*, which could be attributed to its high antioxidant contents.⁶ Furthermore, it has been reported locally that the stem of *Artocarpus heterophyllus* is useful in the management of

diabetes. In this context, the present study aimed to ascertain the acclaimed reports.

Materials and Methods

Plant Material, Authentication, and Processing

The *Artocarpus heterophyllus* stem bark was obtained from a nearby farm in the outskirts of Ibadan. It was identified and authenticated at the herbarium of the Department of Botany, University of Ibadan, Oyo State, Nigeria.

Thereafter, the air-dried *Artocarpus heterophyllus* stem bark was powdered in an electric blender and soaked in 500 mL 70% ethanol until exhaustion, followed by filtration to yield the ethanol extract, which was evaporated until dryness under reduced pressure and called EAH (ie, ethanol extract of *Artocarpus heterophyllus*).

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Experimental Animals

Thirty male Wistar rats weighing 150 to 200 g were used in this study. These animals were obtained from the Animal House, Afe Babalola University, and were maintained under standard conditions of temperature ($24 \pm 5^{\circ}\text{C}$). All animals were allowed free access to standard laboratory food and water 7 days before starting the experiment and during the period of the experiment. All animals were fed with pelletized diet and water ad libitum.

Chemicals

Chemicals and reagents were procured from Sigma-Aldrich, Inc (St Louis, MO). All enzyme assay kits were products of Randox Laboratories Ltd, Antrim, UK. All other chemicals were of analytical grade and prepared in all-glass apparatus using sterilized distilled water.

Induction of Diabetes

Diabetes was induced in the rats by a single intraperitoneal injection of freshly prepared alloxan of 150 mg/kg body weight in normal saline. Two days after alloxan administration, 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, USA) to confirm diabetes. The diabetic rats exhibiting blood glucose levels greater than or equal to 200 mg/mL were included in this study as reported earlier.^{7,8}

Doses Selected

The dose selection for the EAH stem bark was founded on the acute toxicity study, which did not show any adverse effect following oral administration of doses up to 3500 mg/kg. According to modified method of Ojo et al,⁹ experimental oral doses of 50, 100, and 150 mg/kg of the maximum possible dose of the extract was selected based on the acute toxicity study.

Animal Grouping

Rats were divided randomly into 6 groups with 5 animals in each and treated as follows:

- ND: Control rats received distilled water.
- D: Alloxan-induced diabetic rats received distilled water.
- GLI5: Diabetic rats received glibenclamide (5 mg/kg body weight).
- EHA50: Diabetic rats received 50 mg/kg body weight EHA.
- EHA100: Diabetic rats received 100 mg/kg body weight EHA.
- EHA150: Diabetic rats received 150 mg/kg body weight EHA.

Collection and Treatment of Blood Samples

The animals were sacrificed on the 21st day, after oral administration of EAH for 20 days. Blood samples were collected into plain sample tubes for serum analysis by cervical dislocation of the animals.

Preparation of Serum

The blood samples were allowed to stand at room temperature for 30 minutes to form clot after which it was centrifuged at 3000g for 15 minutes. After centrifugation, the clot formed a sediment at the bottom of the centrifuge tube, and the supernatant, which is the serum, was

collected using a Pasteur's pipette. The serum thus obtained was appropriately labelled and stored until use for further analysis.

Preparation of Tissue Homogenates

The rats were decapitated using cervical dislocation and the liver was rapidly excised and placed on ice and weighed. The liver tissues were subsequently homogenized in cold Tris-HCl buffer (1:10 w/v) with about 10 strokes at approximately 1200 rev/min in a centrifuge. The homogenate was centrifuged for 10 minutes at 3000g to yield a pellet that was discarded, and a low-speed supernatant, which was kept for lipid peroxidation assay.¹⁰

Determination of Fasting Blood Sugar

Fasting blood sugar was determined using the Accu-check Advantage II Clinical Glucose meter.¹¹ In this method, blood was collected from the tips of the rats' tails and a drop placed on the indicator portion of the Accu-check strip to determine the fasting blood glucose level of each rat.

Determination of Serum Insulin and Homeostatic Model Assessment Scores

This was assayed by enzyme-linked immunosorbant assay (ELISA).¹² The serum insulin concentrations were measured by an ELISA method using an ultrasensitive rat insulin ELISA kit (Mercodia, Uppsala, Sweden) in a multiplate reader. The absorbance for the calibrators (except for calibrator 0) was plotted against their concentration by using an appropriate computer program (MS Excel) with a display of the equation of the curve. The equation was then used to calculate the concentration of insulin in the samples.

Also, homeostatic model assessment (HOMA-IR and HOMA- β) scores were calculated at the end of the intervention according to the following formula:

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

$$\text{HOMA-}\beta = \left(\frac{\text{Fasting serum insulin in U/L}}{20} \right) \div \left(\frac{\text{Fasting blood glucose in mmol/L} - 3.5}{10} \right)$$

$$\text{Conversion factor: insulin (1 U/L} = 7.174 \text{ pmol/L)}$$

Determination of Lipid Peroxidation

Lipid peroxidation was determined according to the method described by Adam-Vizi and Seregi.¹³ An aliquot of 0.4 mL of the sample was mixed with 1.6 mL of Tris-KCl buffer to which 0.5 mL of 30% TCA was added. Then 0.5 mL of 0.75% TBA was added and placed in a water bath for 45 minutes at 80°C . This was then cooled in ice and centrifuged at 3000g for 5 minutes. The clear supernatant was collected and the absorbance was measured against a reference blank of distilled water at 532 nm. The malondialdehyde level was expressed as units/mg protein.

Determination of Catalase (CAT) Activity

The catalase activity of the homogenates was determined according to the method described by Sinha.¹⁴ One millimeter of the supernatant fraction of the homogenate was mixed with 19 mL distilled water to give a 1:20 dilution. The assay mixture contained 4 mL of hydrogen peroxide solution (800 μ mol) and 5 mL of phosphate buffer at pH 7.0 in a 10-mL flat bottom flask. Thereafter, 1 mL of the properly diluted sample was rapidly mixed with the reaction mixture by a gentle swirling motion at room temperature. Then 1-mL portion of the reaction mixture was withdrawn and blown into 2 mL dichromate/acetic acid reagent at 60-second intervals. The hydrogen peroxide contents of the withdrawn sample were determined by reading the absorbance at 570 nm.

Determination of Superoxide Dismutase (SOD) Activity

The SOD activity in the liver was determined according to the method described by Misra and Fridovich.¹⁵ An aliquot of the sample was added to 2.5 mL of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer. The reactions were initiated by the addition of 0.3 mL freshly prepared 0.3 mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5 mL buffer, 0.3 mL of adrenaline, and 0.2 mL of water. The increase in absorbance at 480 nm every 30 seconds was monitored.

Glutathione Peroxidase (GPx) Assay

The method described by Rotruck et al¹⁶ was employed for this determination. The reaction mixture containing 500 μ L phosphate buffer, 100 μ L sodium azide, 200 μ L of GSH, and 100 μ L of H₂O₂ was added to 500 μ L of the sample, after which 600 μ L of the sample was added and thoroughly mixed. The whole reaction mixture was incubated at 37°C for 3 minutes after which 0.5 mL TCA was added and thereafter centrifuged at 3000g for 5 minutes. To 1 mL of each of the supernatants, 2 mL of K₂HPO₄ and 1 mL DTNB was added and the absorbance was read at 412 nm against a blank.

Histopathological Studies

The tissues were fixed in formalin. They were dehydrated in ascending grades of ethanol, cleared in xylene, and processed to paraffin blocks, sectioned (5 μ m thick), and stained with hematoxylin and eosin stain. They were examined using light microscopy for demonstration of pathological changes in tissues, which included atrophy, cell destruction, and necrosis.¹⁷

Data analysis

All data are expressed as the mean of 5 replicates $\pm$ standard error of mean. Statistical evaluation of data was performed by SPSS version 16. Using one-way analysis of variance, followed by Dunett's post hoc test for multiple comparisons, values were considered statistically significant at $P < .05$ (confidence level = 95%).¹⁸

Results

The effect of EAH stem bark on fasting blood glucose is shown in Table 1. After 48 hours of diabetes induction, fasting blood glucose levels in the 5 experimental groups D, GLI5, EHA50, EHA100, and EHA150 were significantly higher when

Table 1. Effect of Ethanol Extract of *Artocarpus heterophyllus* on Fasting Blood Glucose Levels of Alloxan-Induced Diabetic Rats*.

Groups	Initial Fasting Blood Glucose Level (mg/dL)	Fasting Blood Glucose Level (mg/dL) at 48 Hours of Alloxan Induction	Fasting Blood Glucose Level (mg/dL) at 3 Weeks of Treatment
ND	83.60 $\pm$ 1.20 ^a	83.46 $\pm$ 0.10 ^a	84.40 $\pm$ 1.10 ^a
D	84.10 $\pm$ 0.08 ^a	238.00 $\pm$ 0.10 ^b	308.12 $\pm$ 1.10 ^e
GLI5	82.40 $\pm$ 0.10 ^a	313.00 $\pm$ 2.10 ^c	98.10 $\pm$ 1.00 ^d
EHA50	82.89 $\pm$ 0.16 ^a	324.00 $\pm$ 1.20 ^d	94.30 $\pm$ 1.10 ^c
EHA100	83.20 $\pm$ 0.09 ^a	335.00 $\pm$ 2.10 ^d	88.40 $\pm$ 1.12 ^b
EHA150	83.40 $\pm$ 0.06 ^a	346.00 $\pm$ 3.40 ^e	84.20 $\pm$ 1.46 ^a

Abbreviations: ND, control rats received distilled water; D, alloxan-induced diabetic rats received distilled water; GLI5, Diabetic rats received glibenclamide (5 mg/kg body weight); EHA50, diabetic rats received 50 mg/kg body weight EHA; EHA100, diabetic rats received 100 mg/kg body weight EHA; EHA150, diabetic rats received 150 mg/kg body weight EHA.

*Values are means $\pm$ standard error of mean of 5 animals per group. Values with different superscripts along the column are significantly different ($P < .05$).

Table 2. Effect of Ethanol Extract of *Artocarpus heterophyllus* on Serum Insulin, HOMA-IR, and HOMA- β *.

Group	Insulin (pmol/L)	HOMA-IR	HOMA- β
ND	143.44 $\pm$ 1.50 ^a	4.16 $\pm$ 0.01 ^a	81.93 $\pm$ 2.20 ^a
D	50.48 $\pm$ 0.28 ^e	5.36 $\pm$ 0.03 ^d	4.71 $\pm$ 0.16 ^e
GLI5	100.28 $\pm$ 0.60 ^d	3.38 $\pm$ 0.41 ^c	47.80 $\pm$ 1.46 ^d
EHA50	110.24 $\pm$ 0.05 ^c	3.58 $\pm$ 0.33 ^b	55.16 $\pm$ 1.56 ^c
EHA100	126.41 $\pm$ 0.18 ^b	3.85 $\pm$ 0.22 ^b	68.27 $\pm$ 0.72 ^b
EHA150	141.94 $\pm$ 1.24 ^a	4.11 $\pm$ 0.25 ^a	81.13 $\pm$ 1.29 ^a

Abbreviations: ND, control rats received distilled water; D, alloxan-induced diabetic rats received distilled water; GLI5, Diabetic rats received glibenclamide (5 mg/kg body weight); EHA50, diabetic rats received 50 mg/kg body weight EHA; EHA100, diabetic rats received 100 mg/kg body weight EHA; EHA150, diabetic rats received 150 mg/kg body weight EHA; HOMA-IR, Homeostatic Model Assessment–Insulin Resistance; HOMA- β , Homeostatic Model Assessment β -cell.

*Values are means $\pm$ standard error of the mean of 5 animals per group. Values with different superscripts along the column are significantly different ($P < .05$).

compared to the control group (ND). After 3 weeks of treatment, a significant reduction in fasting blood glucose levels was observed in diabetic rats treated with glibenclamide and with the 3 doses of EHA in comparison with the untreated diabetic animals (group D).

Table 2 shows the effect of the EAH stem bark on serum insulin, HOMA-IR, and HOMA- β in alloxan-induced diabetic rats. Serum insulin and HOMA- β levels were statistically reduced, and HOMA-IR increased in alloxan-induced diabetic rats (group D) when compared to control animals (Table 2). The comparison of the diabetic groups treated with glibenclamide or EHA revealed significant increase of the parameters serum insulin and HOMA- β and decrease of HOMA-IR.

Furthermore, a HOMA-IR level was increased on induction of diabetes, as shown in Table 2 (diabetes control group). A

Table 3. Effects of Ethanol Extract of *Artocarpus heterophyllus* on Liver Antioxidant Enzyme Activities and MDA Levels in Alloxan-Induced Diabetic Rats*.

Groups	SOD (U/mg protein)	GPx (nm/min/mg)	CAT (U/mg protein)	MDA ($\times 10^{-9}$ mmol/mL)
ND	10.2 $\pm$ 1.10 ^a	86.209 $\pm$ 0.10 ^a	7.42 $\pm$ 0.19 ^a	1.02 $\pm$ 0.01 ^a
D	2.10 $\pm$ 0.01 ^e	24.62 $\pm$ 1.10 ^e	1.26 $\pm$ 0.13 ^e	4.92 $\pm$ 0.20 ^e
GL15	4.20 $\pm$ 1.10 ^d	38.49 $\pm$ 2.10 ^d	3.42 $\pm$ 1.19 ^d	2.88 $\pm$ 1.22 ^d
EHA50	6.32 $\pm$ 1.42 ^c	62.10 $\pm$ 1.10 ^c	5.20 $\pm$ 0.10 ^c	1.89 $\pm$ 0.12 ^c
EHA100	9.04 $\pm$ 0.01 ^b	64.78 $\pm$ 2.10 ^b	6.42 $\pm$ 0.09 ^b	1.22 $\pm$ 0.14 ^b
EHA150	9.89 $\pm$ 1.01 ^a	84.98 $\pm$ 2.04 ^a	7.56 $\pm$ 0.15 ^a	1.08 $\pm$ 1.14 ^a

Abbreviations: SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; MDA, malondialdehyde; ND, control rats received distilled water; D, alloxan-induced diabetic rats received distilled water; GL15, Diabetic rats received glibenclamide (5 mg/kg body weight); EHA50, diabetic rats received 50 mg/kg body weight EHA; EHA100, diabetic rats received 100 mg/kg body weight EHA; EHA150, diabetic rats received 150 mg/kg body weight EHA.

*Values are means $\pm$ standard error of mean of 5 animals per group. Values with different superscripts along the column are significantly different ($P < .05$).

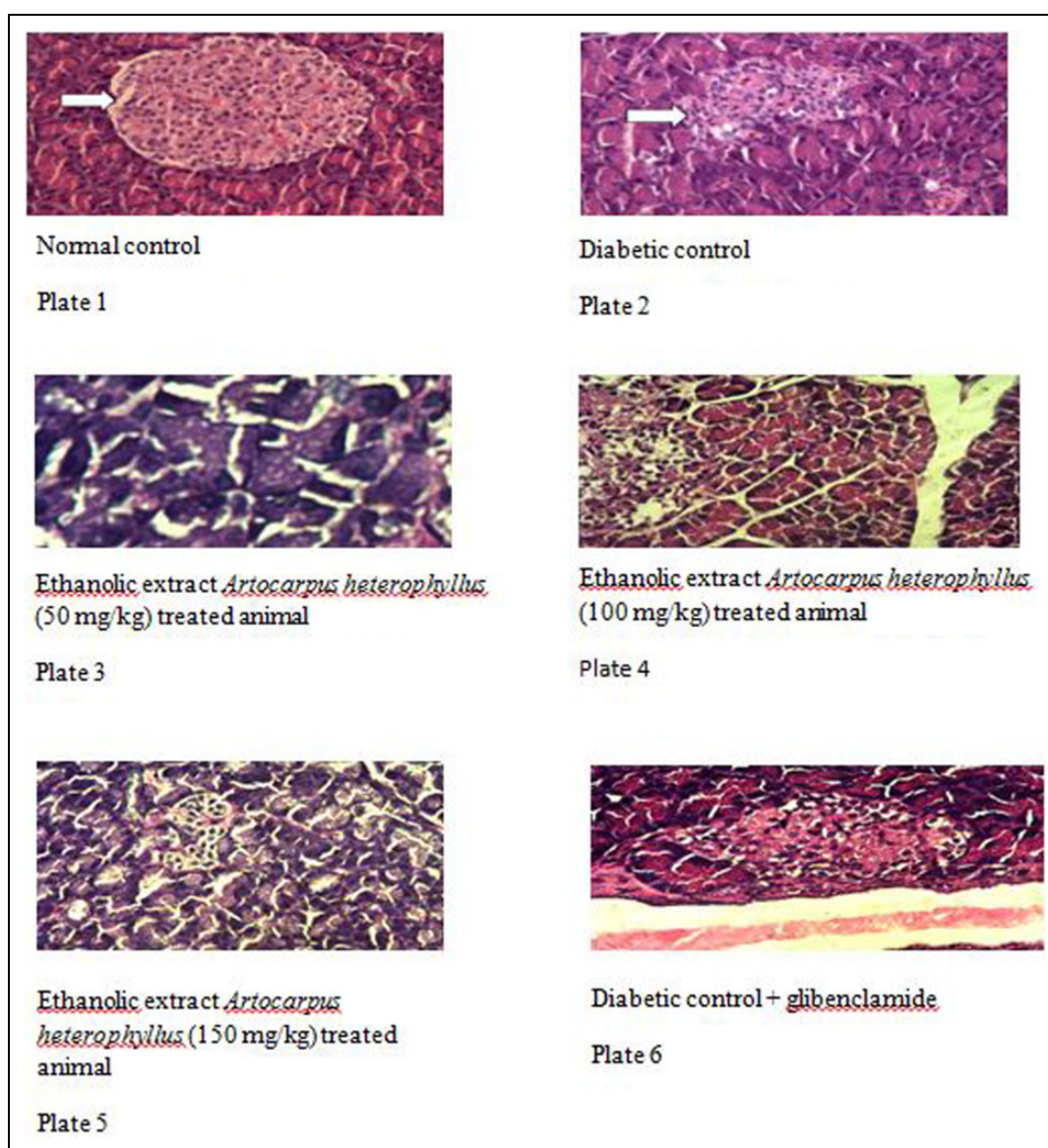


Figure 1. Histopathological studies of pancreas. (Plate 1) Pancreas section of normal control rat parenchymal cells and islet cells. (Plate 2) Pancreas section of diabetic control rats showing mild hyperplasia of islet cells with mild infiltration of inflammatory cells, reduction in β islets cells with dilated and atrophic islets. (Plate 3) Pancreas section of diabetic + 50 mg/kg *Artocarpus heterophyllus* treated rats showing absence of ballooning, inflammatory cells, and regeneration of pancreas toward near normal architecture. (Plate 4) Pancreas section of diabetic + 100 mg/kg *Artocarpus heterophyllus* treated rats showing pancreatic islets indicating normal pancreas. (Plate 5) Pancreas section of diabetic + 150 mg/kg *Artocarpus heterophyllus* treated rats pancreatic islets of normal pancreas. (Plate 6) Pancreas section of diabetic + glibenclamide treated rats showed almost normal pancreas histology.

significant ($P < .05$) reduction in HOMA-IR levels was noticed in diabetes + 5 mg/kg glibenclamide, diabetes + 50 mg/kg EAH, diabetes + 100 mg/kg EAH, and diabetes + 150 mg/kg + EAH groups when compared to the level of insulin in the diabetes control group.

Oxidative stress was assessed by determining the levels of antioxidant enzymes (SOD, GPx, and CAT) as well as malondialdehyde (MDA), a measure of lipid peroxidation in the liver of alloxan-induced diabetic rats, as shown in Table 3. The untreated diabetic rats (diabetes control) showed significantly ($P < .05$) reduced liver SOD, GPx, and CAT ($P < .05$) activities compared to the nondiabetic rats (normal control) and treated groups. Treatment with EAH (at various concentrations) significantly ($P < .05$) increased the activities of SOD, GPx, and CAT. The diabetic control group showed a significant ($P < .05$) increase in liver MDA levels, compared to the normal control, whereas diabetic rats treated with EAH reduced the MDA levels in a concentration-dependent manner of EAH.

Moreover, the histopathological studies of the pancreas of nondiabetic rats (control rats) showed normal histology (Figure 1, Plate 1). Diabetic untreated rat shows degeneration of pancreatic β -cell (Figure 1, Plate 2). The diabetic group treated with 5 mg/kg glibenclamide showed almost normal pancreas histology (Figure 1, Plate 6), while the diabetic rats treated with various doses of EAH stem bark showed significant improvement in the regeneration of damaged β -cells of pancreas in concentration-dependent manners (Figure 1, Plates 3-5).

Discussion

In this study, the effects of oral administration of various doses of EAH stem bark in alloxan-induced diabetes rats was investigated through a 3-week posttreatment period. The reduction in fasting blood glucose levels of diabetic rats treated with varied doses of the EAH stem bark showed that the extract possessed antihyperglycemic properties, which may be probably due to the antioxidative nature of the plant.¹⁹

Diabetes is a heterogeneous disorder characterized by decrease in insulin secretion due to pancreatic β -cell dysfunction in response to hyperglycemia.²⁰ In this study, decreased insulin levels and loss of β -cell integrity were observed in the alloxan-induced diabetic rats. However, at the end of 21 days of treatment, these were ameliorated in both EAH stem bark and glibenclamide rats with restoration of the pancreatic morphology to normal. This increased serum insulin levels could be due to regeneration of pancreatic β islets observed in the histological studies of the pancreas, which has been additionally supported by the significantly higher HOMA- β index (β -cell function) in the treated groups compared to the diabetes control group. The significant reduction in HOMA-IR index in diabetic rats treated with various doses of EAH stem bark confirmed the improvement of insulin sensitivity as well as the stimulation of peripheral glucose absorption in these groups. Therefore, the antihyperglycemic effect of EAH stem bark could be attributed

to the presence of phenol in the extract, which possesses antioxidative potential.¹⁹

Lipid peroxidation is one of the characteristic features of chronic diabetes.^{21,22} The increased lipid peroxidation during diabetes, as found in the present study, may be due to the inefficient antioxidant system prevalent in diabetes. The status of lipid peroxidation as well as altered levels of certain endogenous radical scavenger was taken as evidence for oxidative stress.²³ Free radical scavenging enzymes like SOD, GPx, and CAT protect the biological system from oxidative stress.²⁴ The decrease in activity of the enzymes in the present study could be attributed to the excessive utilization of these enzymes in attenuating the free radicals generated during diabetes mellitus. Previous reports have shown an elevation in the status of lipid peroxidation in the liver after alloxan induction,²⁵ which was in accordance with the findings of this study. The activities of antioxidant enzymes observed in diabetic rats treated with EAH stem bark could have prevented reactive oxygen species from causing further damage to membrane lipids.

Conclusion

Various doses of *Artocarpus heterophyllus* stem bark possess strong antidiabetic activity via reducing fasting blood glucose, improved insulin sensitivity, amelioration of pancreatic β -cell and β -cell functions, reduced lipid peroxidation, and improved antioxidant enzymes activities in diabetic rats. This may be probably ascribed to the antioxidative nature of the plant.

Acknowledgments

The authors would like to thank all the laboratory technologists in the Department of Chemical Sciences, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria.

Author Contributions

All the authors were involved in every aspect of the research and preparation of this article.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Ethical Approval

The uses of animals in this study was approved by ABUAD Ethical Committee (ABUAD/SCI/004).

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