

## Assessment of Hepatoprotective and Antioxidant Effect of *Acioa barteri* Extract (ABE) in Alloxan-Induced Diabetic Rats

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### ABSTRACT

This study aimed to investigate the effects of *Acioa barteri* extract (ABE) on hepatocellular enzyme activity, hepatic function, and antioxidant stress indices in diabetic rats induced with alloxan. The antidiabetic effect of ABE was evaluated in six experimental groups: normal controls, diabetics untreated, diabetics treated with 200mg/kg, 400mg/kg, or 800 mg/kg ABE, and diabetics treated with 3 mg/kg Glibenclamide. ABE was orally administered to induce diabetes, and alloxan-monohydrate was intraperitoneally administered. Diabetic untreated rats exhibited significantly elevated levels of alkaline phosphatase, aspartate, and alanine transaminase activities, as well as higher concentrations of total bilirubin, conjugated bilirubin, and malondialdehyde. They also showed decreased levels of total protein, albumin, globulin, and protein-bound iodine, along with reduced antioxidant enzyme activity. In contrast, diabetic rats administered ABE demonstrated reduced hepatocellular enzyme activity and improved hepatic function. These rats exhibited increased levels of total protein, globulin, and albumin, as well as higher levels of glutathione, superoxide dismutase, glutathione peroxidase, and catalase activities, compared to diabetic untreated rats. The findings suggest that ABE may help prevent oxidative stress and improve hepatic functions in diabetic rats. ABE treatment led to decreased hepatocellular enzyme activity and improved hepatic function, along with increased antioxidant enzyme activities. These results highlight the potential of ABE as a therapeutic option for diabetes-induced liver dysfunction. Further research is warranted to explore its mechanisms of action and potential clinical applications.

**Keywords:** *Acioa barteri*; Antioxidant enzymes; Diabetes mellitus; Hepatic enzymes; Hepatic functions; Oxidative stress; Protein-bound iodine

### INTRODUCTION

Diabetes mellitus is the most prevalent medical condition among the aging population in both males and females aside from high blood pressure but could develop at any age. The persistent rise in the number of diabetic patients globally is a serious health concern due to the high number of mortality associated with its pathogenesis if not properly managed (Nasri et al., 2015). There are lower incidences of diabetes mellitus in developing countries relative to developed countries which have been attributed to the increased consumption of vegetables, foods rich in dietary fiber, complex carbohydrates, and lesser amounts of highly processed food coupled with less pollution from harmful industrial wastes unlike in developed countries.

When insulin secretion by the Langerhans cells is impaired or the receptors are not

responding to circulating insulin, the body experiences glucose dysregulation and associated health problems. (Nasri et al., 2015). Several medicinal plants possess antidiabetic properties and have become very useful in the management of diabetes but there is no available cure for diabetes to date. Medicinal plant extracts with antidiabetogenic properties have been reported to mediate antidiabetic activities by stimulating the regeneration of pancreatic  $\beta$ -cells in type I diabetes and improving insulin sensitivity in type II diabetes (Mihailović et al., 2021). They also mediate antioxidant activities that reduce oxidative stress linked to insulin insensitivity and prevention of severe complications associated with untreated diabetes (Mihailović et al., 2021).

Medicinal plant extracts are viable sources of therapeutic agents against diabetes and oxidative stress-linked diseases due to the high contents of bioactive phytochemicals and antioxidants present in them (Ogbonna et al., 2020; Rathor, 2021). The intake of adequate amounts of

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foods including herbal extracts containing sufficient antioxidants could prevent renal and hepatic injuries and other undesirable effects on various organs in the body common in unabated diabetes (Dahiya et al., 2022). Diabetes if poorly managed may result in various health complications including injury to vital organs, ketoacidosis, heart failure, diabetic wound and amputation, neuropathy, and finally death (Craeto et al., 2021; Matoori, 2022).

*Acioa barteri* Engl. generally known as monkey fruit is a member of the *Chrysobalanaceae* family (Ogechi Ozioma, 2020). It is scattered across West Africa and Asia but is found in large populations in Nigeria and China, respectively. It contains numerous bioactive phytochemicals with alkaloids, flavonoids, phenols, terpenes, and tannins in high concentrations (Ogechi Ozioma, 2020). Vitamins B<sub>12</sub>, B<sub>6</sub>, B<sub>3</sub>, and vitamins C and E have also been detected in *Acioa barteri* extract in significant quantities. In traditional medicine, *Acioa barteri* extract is therapeutically effective for male infertility, menstrual pain, gastrointestinal tract infection, dyslipidemia, and hepatic disorders (Aloh et al., 2015; Anyanwu et al., 2023; Igbe et al., 2018). We evaluated the effects of *Acioa barteri* ethanol extract (ABE) on hepatocellular enzyme activities, hepatic function indices, and antioxidative profile in alloxan monohydrate-induced diabetic rats.

## MATERIALS AND METHODS

### Medicinal plant source

*Acioa barteri* leaves are a medicinal plant employed in this study. The *Acioa barteri* leaves (ABE) were sourced from farms and forests within Olokoro in Umuahia South, Abia State, and were authenticated by a taxonomist at the Herbarium Unit in our Institution. The *Acioa barteri* leaves were carefully handpicked, washed in clean running water, and allowed to dry at room temperature until there was no change in their dry weight. The dried *Acioa barteri* leaves were pulverized into a coarse powder, carefully transferred into a sterile clean polythene bag, and stored in a desiccator.

### Laboratory animals

In this study, thirty-six mature albino male rats were the experimental animals employed in this study. The rats were purchased from Abia State University, Umuahia Campus, and acclimatized for 14 days before the study commenced. All albino rats received finisher feed and water throughout the research study.

### Chemicals and drugs

Analytical-grade chemicals and drugs including commercial assay kits were used in this study. The solvent (ethanol), and diabetogenic agent (alloxan monohydrate) were obtained from a well-known manufacturer (Sigma Aldrich, USA).

### Preparation of medicinal plant extract

A quantity (500 g) of coarsely ground *Acioa barteri* leaves was weighed into a clean contain and cold macerated with 1.5 L undiluted ethanol for three days using an automatic shaker to enhance the extraction of the bioactive constituents. It was filtered after three days with a Whatman number one filter paper, and the solvent evaporated using a rotary evaporator. After solvent evaporation, 11.05% corresponding to 55.25 g of ABE was obtained as its percentage yield.

### Experimental design

In this research, a total of thirty (36) male albino rats were selected and divided into six distinct groups, with each group consisting of six rats (n = 6 per group). In the first group, rats were administered a solution of normal saline at a dosage of 1 ml/kg. Meanwhile, groups 2 to 6 were subjected to diabetes induction through the administration of alloxan-monohydrate. The experimental groups 2 – 6 consist of the following: a group of untreated diabetic subjects, a group of diabetic subjects treated with 200 mg/kg of ABE, a group of diabetic subjects treated with 400 mg/kg of ABE, a group of diabetic subjects treated with 800 mg/kg of ABE, and a group of diabetic subjects treated with 3 mg/kg of Glibenclamide. Following 72 hours of alloxan-monohydrate induction, the blood glucose concentration of the animals was assessed using a glucometer. Rats with blood glucose levels exceeding 200 mg/dl were classified as diabetic. The diabetic rats were orally administered appropriate doses of ABE and Glibenclamide, respectively for 28 days. After the administration of ABE and Glibenclamide to relevant groups on day 28, the rats were made to fast overnight and anesthetized on day 29 with an intraperitoneal injection of 25 mg/kg pentobarbital. A sufficient volume of blood was withdrawn from the rats after a few minutes of the anesthetization via cardiac puncture for laboratory analyses.

### Induction of diabetes

Alloxan monohydrate solubilized in normal saline (150 g/kg) was administered intraperitoneally to rats fasted overnight and after 3 days their blood glucose levels were determined.

In the present investigation, rats that exhibited blood glucose levels exceeding 200 mg/dl as a result of alloxan monohydrate induction were classified as having diabetes.

### Laboratory analysis

The levels of aspartate transaminase (AST) and alanine transaminase (ALT) in the serum were quantified using Randox assay kits, as described in reference (Reitman and Frankel, 1957). The activity of alkaline phosphatase was determined using the method developed by Englehardt et al. in 1970 (Englehardt et al., 1971). The total serum protein and albumin were determined using the Lowry method (Rizvi et al., 2022) and Dumas and Biggs (1972) respectively. In addition, the methods described by Jendrassik and Grof (1938) were used to assess the levels of total bilirubin, as well as conjugated and unconjugated bilirubin.

The serum globulin concentration was measured by taking the difference between albumin and total protein concentration. The Hallman et al., (1951) method was used to measure the amount of protein-bound iodine (Hallman, 1951). The enzymatic activities of reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) were assessed using the methods of Jollow et al., (1972); Awad *et al.* (2016), Misra and Fridovich, 1972 and Rotruck *et al.* (1973) respectively (Awad et al., 2016; Jollow et al., 1974; Misra and Fridovich, 1972; Rotruck et al., 1973). The malondialdehyde (MDA) concentration was evaluated using the Ohkawa method (Ohkawa et al., 1979)

### Statistical analysis

SPSS version 22 and Graph Pad version 9 were used for the statistical analysis of the data. To determine the amount of statistical difference, ANOVA was used to test for statistical difference, and the means of the various groups were compared using Tukey's posthoc procedure using a 95 percent confidence interval (P 0.05).

## RESULTS

### Hepatoprotective effects of ABE and glibenclamide on liver function enzymes in diabetic rats

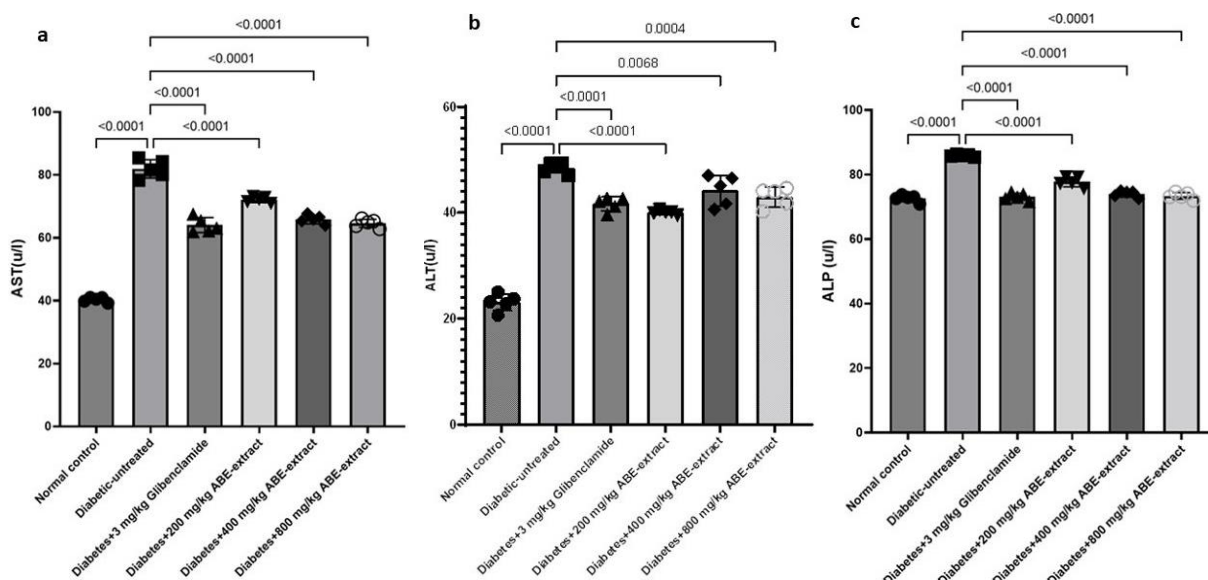
The study aimed to investigate the effects of ABE and glibenclamide on aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) activity in alloxan-induced diabetic rats. AST and ALT are involved in amino acid metabolism and are used to assess hepatic function. While ALP is also implicated in hepatic function, the enzyme is also involved in bone mineralization and bile acid metabolism. Some

studies have shown that elevation of ALP levels in serum can indicate a reduction or obstruction of bile flow within the liver.

In Figure 1 a, the level of AST was significantly increased in all diabetic rats upon alloxan administration when compared to the normal control groups, indicating altered liver. However, diabetic rats treated with various doses (200 mg/kg, 400/800 mg/kg) of ABE showed a reduction in AST levels against the untreated group. Notably, an extract dose of 800mg/kg showed the most reduction in AST levels and this was relative to the observed effects of 3 mg/kg of glibenclamide, suggesting potential dose-dependent hepatoprotective effects of ABE. Similarly, diabetic rats exhibited elevated ALT and ALP levels (Figure 1 b and c). The observed effects may be due to the impaired liver function induced by diabetes upon alloxan induction. Increased beta cell destruction leads to dysregulated glucose metabolism and generation of reactive oxygen species which can cause liver tissue damage. When the liver is damaged or undergoes necrosis, AST, ALT, and ALP are released into the bloodstream, leading to elevated levels of these enzymes in the serum. The results from our study have shown that treatment with the ABE resulted in a decline in the elevated ALT and ALP levels. Overall, these results suggest that ABE intervention may alleviate liver dysfunction and modulate abnormal enzyme levels associated with diabetes.

### Effect of ABE on total serum protein, albumin, and globulin concentrations in alloxan-induced diabetic rats

We next aimed to examine the impact of ABE on the levels of total serum protein, albumin, and globulin in rats with diabetes. These are important markers used in assessing the integrity of liver function and overall protein status. The total protein levels were significantly altered due to diabetes as the untreated diabetic group showed a reduced protein level when compared to the control. Interestingly, the diabetic rats administered 200 mg/kg of ABE displayed the most significant increase in serum total protein across all extract groups, indicating a dose-dependent effect of the ABE (Table I). While the extract groups did show improvements in total protein levels, there were no significant differences in the total protein levels in the diabetic rats that received a dosage of 400 mg/kg of ABE and 3 mg/kg of glibenclamide. With the concentrations of albumin, it was observed that rats administered with a dosage of 3 mg/kg of glibenclamide, and various doses of ABE (200 mg/kg, 400 mg/kg, and 800 mg/kg) exhibited an elevation in albumin



**Figure 1. Effects of ABE and glibenclamide on liver function parameters.** The bars show the mean and standard deviation (n = 5) of the data set. The statistical analyses employed in this study consisted of a one-way analysis of variance (ANOVA) followed by a post hoc Tukey test. The mean value and the standard deviation (SD) are depicted in bar graphs accompanied by a significance cut-off.

**Table I. Total serum proteins, albumin, and globulin concentrations of alloxan-induced rats administered ABE.**

| Treatment groups                 | Total protein (g/dl)     | Albumin (g/dl)         | Globulin (g/dl)          |
|----------------------------------|--------------------------|------------------------|--------------------------|
| Normal control                   | 5.68±0.30 <sup>c</sup>   | 3.43±0.34 <sup>c</sup> | 2.25±0.26 <sup>b</sup>   |
| Diabetic untreated               | 4.19±0.03 <sup>a</sup>   | 2.27±0.12 <sup>a</sup> | 1.92±0.13 <sup>a</sup>   |
| Diabetes + 200 mg/kg ABE         | 6.04±0.14 <sup>d</sup>   | 3.11±0.11 <sup>b</sup> | 2.93±0.12 <sup>e</sup>   |
| Diabetes + 400 mg/kg ABE         | 5.79±0.14 <sup>c</sup>   | 3.07±0.06 <sup>b</sup> | 2.71±0.15 <sup>d,e</sup> |
| Diabetes + 800 mg/kg ABE         | 5.44±0.07 <sup>b</sup>   | 2.99±0.15 <sup>b</sup> | 2.45±0.17 <sup>b,c</sup> |
| Diabetes + 3 mg/kg glibenclamide | 5.58±0.04 <sup>b,c</sup> | 3.07±0.08 <sup>b</sup> | 2.51±0.05 <sup>b,c</sup> |

Values are presented as mean ± standard deviation (n = 4). Mean are on the same row with different letter superscripts are significantly different (P < 0.05).

concentrations when compared to the untreated diabetic rats. An elevation in globulin concentration was noted in diabetic rats administered with ABE at doses of 200 mg/kg and 400 mg/kg, in comparison to the control group of non-diabetic rats. Similarly, administration of glibenclamide at a dosage of 3 mg/kg, along with ABE at a dose of 800 mg/kg, resulted in a notable elevation in globulin levels when compared to rats with untreated diabetes. The results suggest that ABE administration can impact serum protein parameters in diabetic rats, with dose-dependent effects observed for total protein and albumin concentrations (Table I). These findings have implications for liver function and protein status as diabetes can disrupt protein synthesis and turnover, leading to imbalances in total protein concentrations.

**Effect of ABE on total, conjugated, and unconjugated bilirubin concentrations in alloxan-induced diabetic rats**

Bilirubin is a yellow pigment derived from the breakdown of heme and plays a crucial role in evaluating liver function (Méndez-Sánchez et al., 2016). In this study, we aimed to investigate the effect of ABE on the concentrations of total bilirubin, conjugated bilirubin, and unconjugated bilirubin in alloxan-induced diabetic rats. Total bilirubin represents the collective measurement of both conjugated and unconjugated forms. In Table II, significant (P<0.05) elevations in total bilirubin concentrations were observed in the diabetic-untreated rats and those administered 800 mg/kg ABE, indicating impaired liver function in these animals. However, there were no significant changes in total bilirubin concentrations in rats

treated with 3 mg/kg glibenclamide, 200 mg/kg ABE, and 400 mg/kg ABE compared to the normal control group. Treatment with the extract at these doses (200mg/kg, and 400mg/kg) may have led to a reduction in total bilirubin concentrations compared to the diabetic-untreated rats, suggesting a potential beneficial effect on liver function. Conjugated bilirubin represents the fraction that has undergone glucuronidation in the liver and is excreted in bile. The results presented in Table II demonstrated a significant increase in conjugated bilirubin concentrations in the diabetic-untreated rats which is suggestive of impaired hepatic excretory function. In contrast, there was a significant reduction at 400mg/kg of ABE when compared to the diabetic untreated group. The levels showed more trending but no statistical against the glibenclamide. Unconjugated bilirubin represents the fraction that has not undergone glucuronidation and is mostly bound to albumin. Unconjugated bilirubin concentrations in rats treated with glibenclamide and different doses of ABE exhibited slight decreases compared to the diabetic-untreated rats, although these changes were not statistically significant. The observed reductions in total bilirubin and conjugated bilirubin concentrations suggest an improvement in hepatic function, potentially contributing to the amelioration of liver dysfunction associated with diabetes.

#### **Effect of ABE on the protein-bound iodine concentrations of alloxan-induced diabetic rats.**

Protein-bound iodine concentration serves as a measure of thyroid function and can reflect alterations in protein metabolism. We investigated the effect of ABE on protein-bound iodine concentrations in alloxan-induced diabetic rats. Figure 2 illustrates the results, indicating a significant reduction in protein-bound iodine concentration in the diabetic-untreated rats and those administered 3 mg/kg glibenclamide, 200 mg/kg ABE, 400 mg/kg ABE, and 800 mg/kg ABE when compared to the normal control. Conversely, diabetic rats administered 3 mg/kg glibenclamide exhibited a significant reduction in protein-bound iodine concentration compared to the diabetic-untreated rats, implying a potential influence of glibenclamide on thyroid function in the context of diabetes. The results of this study highlight the impact of ABE and glibenclamide on protein-bound iodine concentrations in alloxan-induced diabetic rats. The observed reductions in protein-bound iodine concentrations suggest a potential modulation of thyroid function by glibenclamide,

while the effects of ABE on thyroid function require further investigation.

#### **Effect of ABE on antioxidant enzyme activities in alloxan-induced diabetic rats**

The present study aimed to investigate the effect of ABE on the activities of key antioxidant enzymes, including reduced glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT), in alloxan-induced diabetic rats. The significance of antioxidant enzymes in alloxan-induced diabetic rats lies in their essential role in combating oxidative stress and maintaining redox homeostasis. These enzymes protect cells from the damaging effects of reactive oxygen species (ROS) by scavenging free radicals, detoxifying peroxides, and converting harmful molecules into harmless byproducts. The evaluation of their activities provides valuable insights into the function of the antioxidant defense and the effectiveness of potential therapeutic interventions aimed at mitigating oxidative damage in diabetes. There were observed reductions in GSH and GPx activity in the diabetic-untreated rats and 200 mg/kg ABE-treated rats (Table II). However, an increase in dose administration at 400 mg/kg ABE, and 800 mg/kg ABE, significantly elevated GSH levels. Furthermore, levels of SOD and CAT were improved significantly at an extract dose of 800mg/kg when compared to the normal control and other extract doses. These findings suggest a potential influence of high ABE doses on antioxidant activity in alloxan-induced diabetes (Table II).

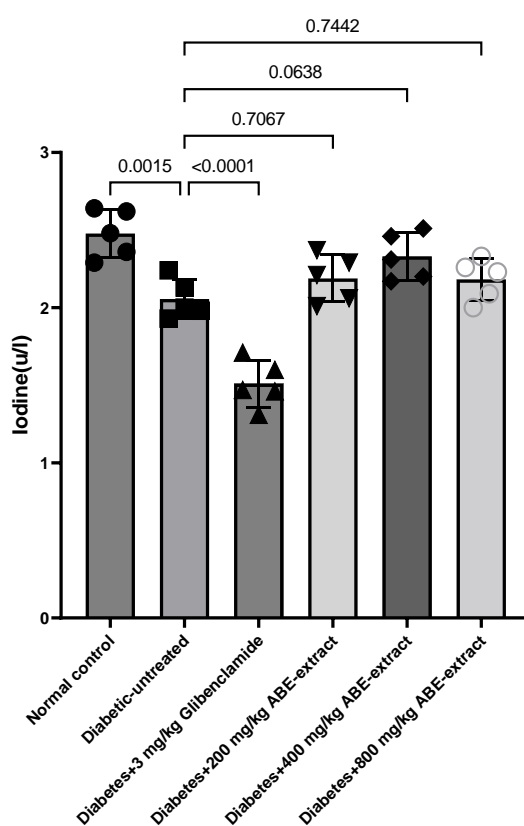
#### **Effect of ABE on the malondialdehyde (MDA) concentrations of alloxan-induced diabetic rats.**

Malondialdehyde is a widely recognized marker of lipid peroxidation and oxidative stress (Ito et al., 2019). The findings revealed a significant reduction in MDA levels in all extract doses and rats treated with glibenclamide (Figure 3). The significance of MDA measurement in this study lies in its ability to reflect the extent of lipid peroxidation, a key pathway involved in cellular damage induced by ROS. Elevated MDA levels indicate an imbalance between ROS generation and the antioxidant defense system, leading to oxidative stress. Therefore, the observed reduction in MDA concentrations following treatment with ABE and Glibenclamide suggests their potential as therapeutic agents for combating oxidative stress-associated complications in diabetes.

**Table II. Total serum bilirubin, conjugated bilirubin, and unconjugated bilirubin concentrations of alloxan-induced diabetic rats administered ABE.**

| Treatment groups                 | Total bilirubin (mg/dl)  | Conjugated bilirubin (mg/dl) | Unconjugated bilirubin (mg/dl) |
|----------------------------------|--------------------------|------------------------------|--------------------------------|
| Normal control                   | 0.68±0.04 <sup>a</sup>   | 0.51±0.03 <sup>a</sup>       | 0.19±0.05 <sup>a</sup>         |
| Diabetic untreated               | 0.84±0.07 <sup>c</sup>   | 0.67±0.09 <sup>c</sup>       | 0.17±0.08 <sup>a</sup>         |
| Diabetes + 200 mg/kg ABE         | 0.71±0.02 <sup>a,b</sup> | 0.59±0.01 <sup>b</sup>       | 0.12±0.02 <sup>a</sup>         |
| Diabetes + 400 mg/kg ABE         | 0.65±0.04 <sup>a</sup>   | 0.52±0.02 <sup>a</sup>       | 0.13±0.02 <sup>a</sup>         |
| Diabetes + 800 mg/kg ABE         | 0.77±0.04 <sup>b</sup>   | 0.61±0.02 <sup>b,c</sup>     | 0.16±0.05 <sup>a</sup>         |
| Diabetes + 3 mg/kg glibenclamide | 0.71±0.05 <sup>a,b</sup> | 0.56±0.03 <sup>a,b</sup>     | 0.16±0.03 <sup>a</sup>         |

Values are presented as mean ± standard deviation (n = 4). Means on the same row with different letter superscripts are significantly different (P < 0.05).



**Figure 2. Protein-bound iodine concentrations of alloxan-induced rats administered ABE. All statistical analyses were carried out using one-way ANOVA with post hoc Tukey test. Bar graphs with dots indicate mean ± SD.**

## DISCUSSION

Diabetes mellitus has consistently posed global health challenges due to the critical role of glucose in human metabolism and the inevitability of glucose in the human diet. Diabetes mellitus is one of the major causes of impaired vision, multiple organ failure, and metabolic disorders in the ageing population of humans including both sexes but could occur in earlier ages (Nawaz et al.,

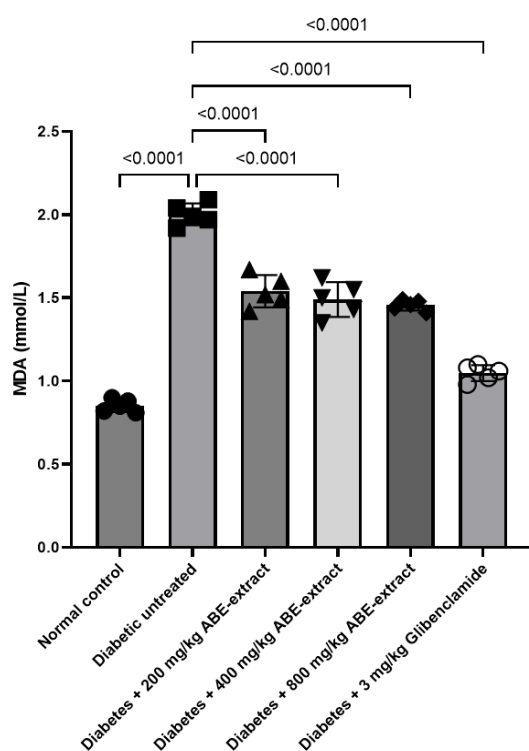
2017). This study evaluated hepatocellular enzyme activities, hepatic function parameters and antioxidative activity of alloxan-induced diabetic rats administered *Acioa barteri* extract (ABE). Early detection of diabetes mellitus, lifestyle changes, dietary restriction, and monitoring of blood glucose levels and organ functions including medical interventions remain the major steps towards successful management of diabetes mellitus (Ibeh et al., 2020). While some conventional therapies may not be harmful to the kidneys or liver, studies have shown the beneficial effect of medicinal plants in reducing kidney and liver damage, either alone or in combination with conventional therapy (Nwankpa et al., 2020).

The notable increase in ALT, AST, and ALP activities in diabetic-untreated rats, in comparison to normal controls, suggests hepatocellular injury and the outflow of these hepatic enzymes into hepatic tissues. The significant rise in ALT and AST activities in diabetic-untreated rats serves as a strong indicator of hepatic injury and compromised hepatic integrity, leading to increased leakage of these enzymes from the hepatic membranes. These results correspond with the research conducted by Chen et al. (2017), Islam et al. (2020), and Noroozi et al. (2022), as they also observed an elevation in AST, ALT, and ALP activities in diabetic-untreated rats (Chen et al., 2017; Islam et al., 2020; Noroozi Karimabad et al., 2022). These enzymes are highly concentrated in the hepatocytes but damage to the hepatic membrane histo-architecture could be responsible for their elevated activities in the extra-hepatic tissues due to their increased concentration. Despite the kidney, and heart being suggested to also contribute some of these enzymes, increased AST, ALP, and ALP activities are good markers of liver injury than any other organs. Conversely, the significant reductions in the ALT, AST, and ALP activities of diabetic rats administered 3 mg/kg Glibenclamide, 200, 400, and 800 mg/kg ABE respectively relative to diabetic-untreated rats

**Table III. Antioxidant profile of alloxan-induced diabetic rats administered ABE.**

| Treatment groups                 | GSH (U/L)               | GPx (U/L)                 | SOD (U/L)               | CAT (U/L)               |
|----------------------------------|-------------------------|---------------------------|-------------------------|-------------------------|
| Normal control                   | 61.03±1.62 <sup>c</sup> | 46.41±1.03 <sup>c</sup>   | 38.87±0.59 <sup>d</sup> | 32.91±0.77 <sup>d</sup> |
| Diabetic untreated               | 50.12±1.60 <sup>a</sup> | 35.77±0.51 <sup>a</sup>   | 30.58±0.84 <sup>a</sup> | 26.57±0.71 <sup>a</sup> |
| Diabetes+200 mg/kg ABE           | 51.41±1.12 <sup>a</sup> | 36.55±0.58 <sup>a</sup>   | 31.64±0.78 <sup>a</sup> | 28.50±0.87 <sup>b</sup> |
| Diabetes+400 mg/kg ABE           | 56.27±2.62 <sup>b</sup> | 37.03±1.33 <sup>a,b</sup> | 32.82±1.12 <sup>b</sup> | 29.74±0.61 <sup>c</sup> |
| Diabetes+800 mg/kg ABE           | 55.36±1.38 <sup>b</sup> | 38.34±0.41 <sup>b</sup>   | 34.28±0.63 <sup>c</sup> | 29.88±0.99 <sup>c</sup> |
| Diabetes + 3 mg/kg Glibenclamide | 55.12±1.54 <sup>b</sup> | 38.34±1.02 <sup>b</sup>   | 35.30±0.43 <sup>c</sup> | 30.63±0.60 <sup>c</sup> |

Values are presented as mean ± standard deviation (n = 4). The means on the same column with different letter superscripts are significantly different (P < 0.05).



**Figure 3. Malondialdehyde (MDA) concentration of alloxan-induced diabetic rats administered ABE.**

showed that their hepatic membrane integrity was restored by the therapeutic potency of each of the treatments in line with the findings of Uroko *et al.* (2022a). The significantly reduced hepatic enzyme activities in the ABE-treated diabetic rats compared to the diabetic-untreated rats suggest that aside from the antidiabetic property of ABE, its use against diabetes could protect or reverse multiple organ failure commonly associated with diabetic mellitus pathogenesis. The hepatoprotection conferred on diabetic rats administered different doses of ABE respectively was comparable to the hepatoprotective effect of glibenclamide-treated diabetic rats. Thus, ABE could serve as a viable alternative to Glibenclamide

for the management and prevention of hepatic disorder in diabetic conditions.

Similarly, the significantly reduced levels of serum proteins and substantially raised levels of conjugated and unconjugated bilirubin in the diabetic untreated rats compared with the normal control indicated a substantial decline in the hepatic function of the diabetic untreated rats which agrees with the findings of Abou-Seif *et al.* (2019). The adverse effects of untreated diabetes on the hepatic integrity and functions could have adversely impaired the ability of the hepatic cells to synthesize sufficient proteins including albumin required for various biochemical functions. The very low albumin levels in the diabetic untreated rats could predispose the rats to increased risk of atherosclerosis and cardiovascular disorders due to the inability of insufficient circulating albumin concentration to transport fatty acids including low-density lipoprotein from extra-hepatic tissues to the liver for metabolism as reported by Uroko *et al.* (2015) (Ikechukwu *et al.*, 2015). Contrarily, the substantially raised serum total protein, and albumin and significantly declined levels of conjugated and unconjugated bilirubin in the diabetic rats administered different doses of ABE are attributed to the hepatoprotective property of the extract that protected the liver from adverse hepatic effects of diabetes and maintained improved hepatic functions in the rats. These findings agree with the reports by Abou-Seif *et al.* (2019) and Uroko *et al.* (2022a).

Serum protein-bound iodine has been described as the portion of the total serum iodine that can be precipitated with the serum proteins but cannot be removed by dialysis and used to indirectly evaluate the thyroid function. The significant reduction in the protein-bound albumin iodine concentrations in the diabetic untreated rats and alloxan-induced diabetic rats administered ABE and glibenclamide respectively, relative to the normal control indicated that the alloxan induction caused impaired thyroid functions in the diabetic rats. The significantly

elevated protein-bound iodine concentrations in the diabetic rats administered 400 mg/kg ABE compared to the diabetic-untreated rats and diabetic rats administered 3 mg/kg Glibenclamide respectively suggested that ABE has much more protective effects on the thyroid gland than every other dose of ABE tested. Besides, the significantly reduced protein-bound iodine concentration in the Glibenclamide treated diabetic rats relative to the normal control, diabetic untreated, and diabetic rats administered ABE respectively, showed that glibenclamide has an adverse effect on protein-bound iodine and thyroid functions in line with the reports of Acland (1971)(Acland, 1971).

The significant decrease in antioxidant enzyme activities, such as GSH, GPx, SOD, and CAT activities, in both untreated diabetic rats and diabetic rats administered 200 mg/kg ABE when in comparison to normal controls and diabetic rats given 400 and 800 mg/kg ABE and 3 mg/kg glibenclamide, indicates that diabetes depletes antioxidant enzyme activities in rats. These results relate to the findings of Ashor et al. (2016) and Balbi et al. (2018) (6,32), who reported that hyperglycemia in diabetes promotes increased free radical generation and oxidative stress, reduces antioxidant status, and elevates lipid peroxidation (Ashor et al., 2016; Balbi et al., 2018). The significantly reduced antioxidant enzyme activities in diabetic rats administered 200 mg/kg ABE suggest that ABE exerted negligible stimulatory effects on the antioxidant enzyme activities at low doses and is likely not confer sufficient antioxidative effect on the diabetic rats. Thus, reduced antioxidant enzyme activities in the diabetic untreated and diabetic rats administered 200 mg/kg ABE respectively, indicated that diabetic rats were predisposed to oxidative stress which induced diabetic various complications linked to oxidative attack by free radicals. The substantially elevated GSH, GPx, SOD, and CAT enzyme activities in the diabetic rats administered 400, and 800 mg/kg ABE respectively compared to the diabetic untreated rats but similar to the rats administered glibenclamide could be due to the antioxidant properties of ABE in line with Uroko et al. (2021) and Kim et al. (2022) (Kim et al., 2022; Uroko et al., 2021). These findings suggest that raised doses of ABE might have induced synthesis of these antioxidant enzymes or stabilized the amount of the circulating enzymes which could have prevented oxidative stress-induced damage and complications in the diabetes-induced rats administered 400, and 800 mg/kg ABE respectively. The significant rise in the GSH, CAT, GPx, and SOD activities in the diabetic rats administered ABE is consistent with the findings of

Sarfraz et al., (2017)(Sarfraz et al., 2017) and Uroko et al. (2022b) (Uroko et al., 2023) that medicinal plant extract with antioxidant property prevents oxidative stress and lipid peroxidation by promoting the induction of antioxidant enzymes and stabilization of the circulating antioxidants.

The significantly higher levels of malondialdehyde (MDA) in the diabetic rats that were untreated in comparison with the normal control indicated increased levels of lipid peroxidation and oxidative stress associated the diabetes progression in the diabetic untreated rats which align with the findings of Ashor et al. (2016) (Ashor et al., 2016). Contrarily, the significant decline in the diabetic rats administered 200mg/kg, 400mg/kg, and 800 mg/kg ABE respectively relative to the diabetic untreated rats indicated that treatment of diabetic rats with ABE could substantially prevent lipid peroxidation and its associated adverse health consequences in line the findings of Uroko et al. (2022b) (Uroko et al., 2023).

## CONCLUSION

The results of this study showed that ABE could confer hepatocellular protection in diabetic rats, improve hepatic functions, and prevent oxidative stress and leakage of hepatocellular enzymes to extra-hepatic tissues. However, further studies are required to isolate and characterize the phytoconstituents of ABE responsible for its hepatoprotection and antioxidative properties.

## DECLARATIONS: ETHICAL CLEARANCE

The research was done following the recommendations for the treatment and use of laboratory animals, and a letter of ethical approval with the reference number MOUAU/VPP/EC/21/005 was duly obtained for the study from the Research Ethics Committee College of Veterinary Medicine, Michael Okpara University of Agriculture Umudike.

## CONSENT FOR PUBLICATION

Not Applicable

## AVAILABILITY OF DATA AND MATERIAL

All generated data during this study are available upon request.

## COMPETING INTERESTS

No competing interests

## FUNDING

No funding was received for this study.



## AUTHORS' CONTRIBUTIONS

RIU and HNO designed the experiments and Literature search. RIU, HNO, CA, PCN, and BCU carried out experimental analyses, data collection, interpretation, and discussions. The paper for publication was drafted by RIU and HNO. All authors approved the final version of the manuscript for publication.

## ACKNOWLEDGMENTS

We knowledge all the staff at the Department of Biochemistry for the discussions and support during this study. All the members of the Uroko lab also offered wonderful discussions and insight into this paper.

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