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# Evaluation of antidiabetic potential and protective effects of *Acioa barteri* against biochemical changes in alloxan-induced diabetic rats

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**ABSTRACT:** *Acioa barteri* is a medicinal plant commonly known as Monkey fruit that grows in the tropical rain forests in many countries in West Africa. The plant extracts are useful in traditional medicine against diverse diseases and medical conditions. This study evaluated the antidiabetic and protective effect of *Acioa barteri* extract (EEABL) against biochemical changes in alloxan-induced diabetic rats. The lethal dose of EEABL was evaluated using standard procedure. Antidiabetic and protective properties of EEABL were evaluated using sixty mature male albino rats selected into six groups containing ten rats each. Group 1 was the normal control rats without alloxan-induction but received 2mL/kg of normal saline. Groups 2 – 6 were rats induced diabetes by the intraperitoneal administration of 150 mg/kg alloxan-monohydrate dissolved in normal saline. Group 2 was the diabetic control, and group 3 was treated with 3 mg/kg Glibenclamide. Groups 4, 5, and 6 were treated with 200, 400, and 800 mg/kg EEABL, respectively for 28 days. The results of the acute toxicity study of EEABL showed that it has a lethal dose ($LD_{50}$) value above 5000 mg/kg. The phytochemical study showed that EEABL contains alkaloids (96.89±0.42 mg/100g), flavonoids (105.98±1.73 mg/kg), and phenols (77.58±0.29 mg/kg) in high concentrations. While tannins (13.13±0.43 mg/100g) and terpenes (18.24±0.78 mg/100g) were detected in moderate concentration in EEABL, cardiac glycoside (10.39±0.20 mg/100g), steroids (8.28±0.19 mg/100g) and saponins (6.34±0.19 mg/100g) were found to be present in low concentrations. Treatment with EEABL lowered blood glucose, urea, and creatinine significantly in the alloxan-induced diabetic rats compared to the diabetic control. Treatment with EEABL also improved haematological parameters, antioxidant vitamins ($B_{12}$, $B_6$, $B_2$, C, and E), serum electrolytes, and lipid profile in the alloxan-induced diabetic rats compared to the diabetic control. This study revealed that EEABL has antidiabetic effects and confers protection against biochemical changes in alloxan-induced diabetic rats.

**KEYWORDS**: *Acioa barteri;* antioxidant vitamins; hyperglycaemia; lipid profile; phytochemicals; renal functions.

## 1. INTRODUCTION

Diabetes mellitus is a medical disorder associated with metabolic syndrome, physical inactivity, unhealthy diets, and lifestyle. However, it has been linked to genetic polymorphism, making it more common in some families and races than others [1, 2]. It is a non-infectious disease usually caused by either defective pancreatic beta cells and a decline in insulin secretion or a drastic reduction in the sensitivity of the insulin receptors to available insulin concentration, sometimes both. Previous studies have reported hyperglycaemia,

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hyperlipidaemia, and ketoacidosis as significant features of diabetes. Besides, there are increased risk of cardiovascular disorder, impaired kidney functions, and hepatic injury in diabetic conditions if not properly managed. The population of diabetic patients in the world presently is estimated to be around 200 million people. Still, it has been projected by the World Health Organization to rise to more than 300 million people in the year 2025. A significant percentage of diabetic cases are insulin-dependent [3]. Due to the adverse health complications of persistent hyperglycaemia, most therapeutic agents used in managing diabetic patients are aimed toward increasing insulin secretion, the sensitivity of insulin receptors to insulin secretion, preventing the release of glucose glycogen storage, and gluconeogenesis to bring blood glucose levels under control [4]. Many diabetic patients are unaware of their diabetic status because of the asymptomatic nature of the early phase of diabetes, especially insulin-dependent diabetes [5]. However, there are numerous available antidiabetic drugs, including sulfonylureas, Glibenclamide, and metformin none of the available antidiabetic agents could effectively cure diabetes. The use of synthetic antidiabetic agents has been shown from a literature survey to cause serious adverse effects, hepatic malfunction, gain in body weight, and impaired reproductive functions. Medicinal plants with proven antidiabetic activities are now a viable treatment option for managing diabetic conditions due to their high therapeutic efficacy, availability, low cost, and negligible side effects [6].

*Acioabarteri* (Hoof. f. ex. Oliv.) Engl. is an average-sized medicinal plant from the Chrysobalanaceae family, commonly known as a Monkey fruit. It is distributed across riverine areas, tropical rainforests, and savannah forests, especially in West Africa. It could exist as a climbing shrub or smaller tree with an average height of about 12 meters. The *barteri*species possess pharmacological activity against numerous diseases and medical conditions. Plant extracts from various parts of *barteri*species are effective in managing neurological diseases, rheumatism, menstrual cramps, epilepsy, gastrointestinal tract disorders, and impaired reproductive functions in males [7, 8]. Methanol extract of *Acioabarteri*has been shown to confer hepatoprotection and prevents alteration of lipid profile in experimental rats [9]. It is used as alternative medicine in south-eastern Nigeria to enhance male libido and sperm quality and to improve male reproductive health [10]. The extract of *Acioabarteri* has been reported to be rich in numerous phytochemicals, including alkaloids, flavonoids, terpenoids, saponins, phenols, cardiac glycosides, tannins, and steroids, and relatively safe for consumption [11]. The promising results from research on medicinal plants suggest the future of therapeutic agents against many diseases and health challenges. This study evaluated the antidiabetic potential and protective effects of ethanol extract*Acioa barteri* (EEABL) leaves against biochemical changes in alloxan-induced diabetic rats.

## 2. RESULTS

### 2.1. Phytochemical profile of EEABL

Table 1 and 2 show the qualitative and quantitative phytochemical compositions in the EEABL, respectively. The qualitative phytochemical screening showed that EEABL contains alkaloids, flavonoids, and phenols in relatively high concentrations, with tannins and terpenes present in EEABL in moderate concentrations. The EEABL contains cardiac glycoside, saponins, and steroids in low concentrations. The quantitative phytochemical results of the EEABL analysis indicated that alkaloids, flavonoids, and phenols were the most abundant phytochemical contents in the EEABL. Contrarily, saponins, cardiac glycoside, and steroids were the least available phytochemicals in the EEABL.

**Table 1.** Phytochemical composition of EEABL

| Phytochemicals | Bioavailability | Content (mg/100g) |
|---|---|---|
| Alkaloids | +++ | 96.89±0.42 |
| Flavonoids | +++ | 105.98±1.73 |
| Tannins | ++ | 13.13±0.43 |
| Saponins | + | 6.34±0.19 |
| Terpenes | ++ | 18.24±0.78 |
| Cardiac glycoside | + | 10.39±0.20 |
| Phenols | +++ | 77.58±0.29 |
| Steroids | + | 8.28±0.19 |

Keys: + = low concentration; ++ = moderate concentration, +++ = high concentration. The results of the qualitative analysis were displayed as mean ± standard deviation of the triplicate determination.

**Table 2.** Acute toxicity study result of EEABL

| Groups | Dose (mg/kg) | Number of deaths | % Mortality |
|---|---|---|---|
| 1 | 10 | 0/3 | 0.00 |
| 2 | 100 | 0/3 | 0.00 |
| 3 | 1000 | 0/3 | 0.00 |
| Phase II | | | |
| 1 | 1600 | 0/3 | 0.00 |
| 2 | 2900 | 0/3 | 0.00 |
| 3 | 5000 | 0/3 | 0.00 |

### 2.2. Acute toxicity effects of EEABL in Wistar albino rats

The acute toxicity study of the EEABL showed that all the rats administered with various doses of EEABL (10 – 5000 mg/kg) displayed no signs and symptoms of toxicity after the administration. All the rats in phases I and II were physically stable, and no death was recorded more than 24 hrs after the acute toxicity study.

### 2.3. Acute effects of EEABL on the blood glucose levels of diabetic rats

The pre-induction blood glucose levels in Figure 1 demonstrated insignificant variations in the blood glucose concentrations in the diabetic control, EEABL, and Glibenclamide groups compared to the normal control.

The post-diabetes induction blood glucose concentrations in Figure 1 indicated a significant rise in the diabetic control, EEABL, and Glibenclamide groups, respectively, compared to the normal control. The post-induction blood glucose levels in the diabetic rats treated with 200, 400, and 800 mg/kg of EEABL and diabetic rats treated with 3 mg/kg of Glibenclamide decreased significantly relative to the diabetic control. At the same time, the post-induction blood glucose level in the diabetic rats treated with 3 mg/kg of Glibenclamide was considerably reduced compared to the post-induction blood glucose levels in the diabetic rats treated with different doses of EEABL respectively.

The results in Figure 1 showed substantially elevated blood glucose levels after 2 hours of induction of diabetes in the diabetic control and diabetic rats treated with 3 mg/kg Glibenclamide, 200, 400, and 800 mg/kg of EEABL, respectively, in comparison to the normal control. Contrarily, 2 hours after the induction of diabetes, all the diabetic rats treated with different doses of EEABL and 3 mg/kg Glibenclamide, respectively, exhibited a significant reduction in the blood glucose levels relative to the diabetic control.

There were considerably increased blood glucose levels in the diabetic control and diabetic rats treated with 200, 400, and 800 mg/kg EEABL, respectively, compared to the normal control after 4 hours of the diabetes induction. However, the diabetic rats treated with 3 mg/kg Glibenclamide exhibited no significant increase in the blood glucose level after 4 hours of the diabetes induction compared with the normal control. In contrast, the diabetic rats treated with 3 mg/kg of Glibenclamide, 200, 400, and 800 mg/kg of EEABL, respectively, showed a significant decrease in the blood glucose levels after 4 hours of diabetes induction compared with the diabetic control (Figure 1).

### 2.4. Effects of EEABL on the percentage blood glucose fall in diabetic rats

The was no significant increase in the percentage fall in the blood glucose level of the diabetic control relative to the normal control (Figure 2). Conversely, the diabetic rats treated with Glibenclamide, 200, 400, and 800 mg/kg of EEABL showed a significant percentage fall in the blood glucose level after 4 hours of the diabetes induction compared to the normal and diabetic controls, respectively.

### 2.5. Effects of EEABL on the haematological parameters of diabetic rats

The results in Table 3 indicated a significant reduction in the RBC counts of the diabetic control and diabetic rats treated with 400 and 800 mg/kg EEABL, respectively, compared with the normal control. The diabetic rats treated with 200 mg/kg EEABL and 3 mg/kg Glibenclamide showed no significant decline in the RBC counts relative to the normal control. In contrast, the diabetic rats treated with Glibenclamide and a varying dose of EEABL indicated a considerable increase in the RBC counts compared to the diabetic control.

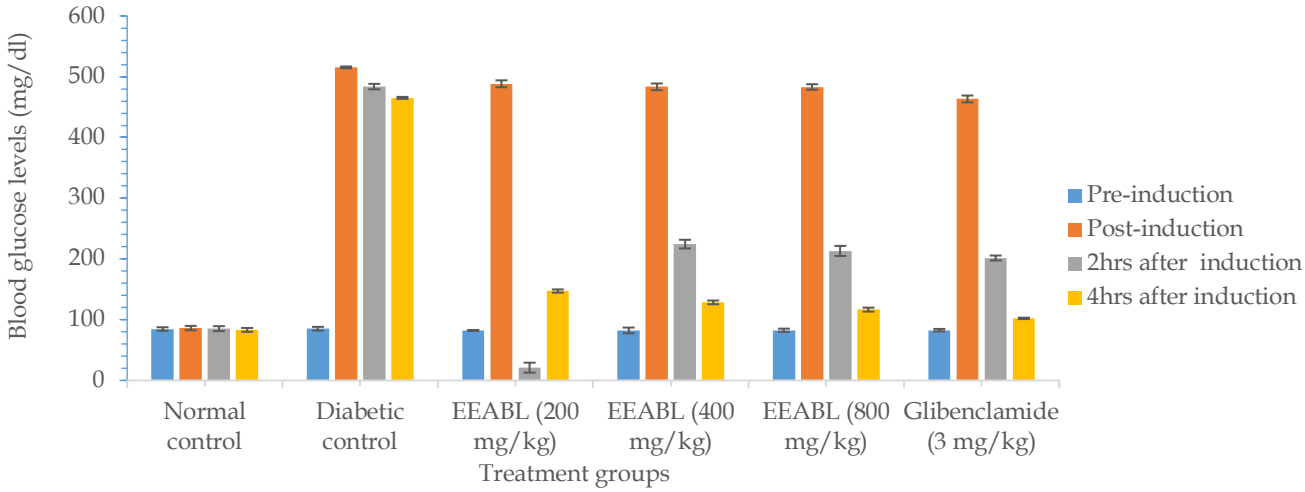


**Figure 1**. Acute changes in the blood glucose levels of diabetic rats treated with EEABL

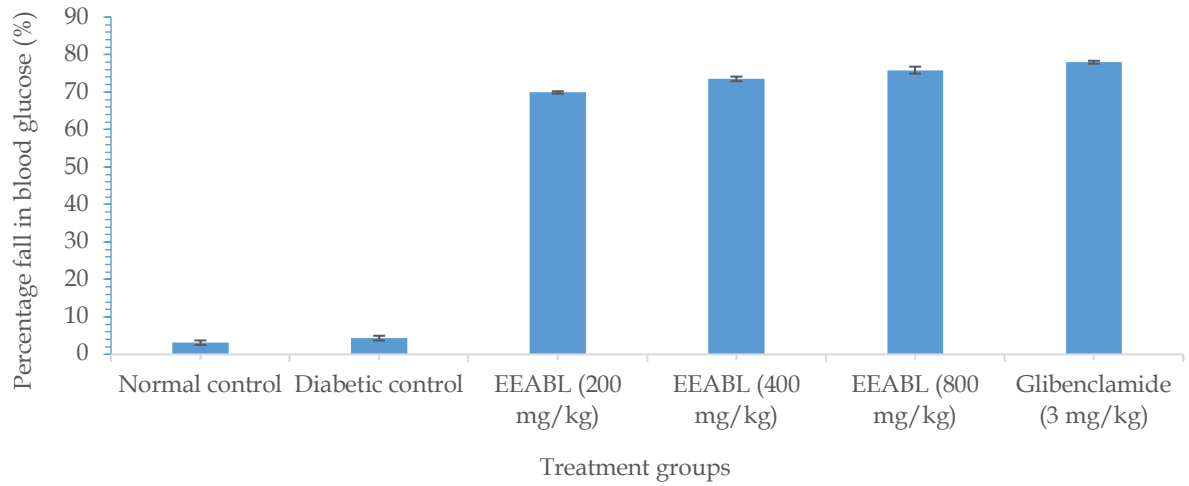


**Figure 2.** Percentage fall in blood glucose levels of diabetic rats treated with EEABL

The PCV counts in Table 3 decreased significantly in the diabetic control and diabetic rats treated with 200, 400, and 400 mg/kg EEABL, respectively, compared with the normal control. However, the diabetic rats treated with 3 mg/kg Glibenclamide showed no significant decrease in the PCV counts relative to the normal control. Conversely, the PCV counts were significantly elevated in the diabetic rats treated with different doses of EEABL and Glibenclamide, respectively, compared with the diabetic control.

The results in Table 3 displayed a significant decline in the Hb concentrations of the diabetic control and diabetic rats treated with 400 and 800 mg/kg, respectively, compared to the normal control. Besides, the diabetic rats treated with 200 mg/kg EEABL and Glibenclamide showed no significant reduction in their Hb concentration relative to normal. Contrarily, all the diabetic rats treated with EEABL and Glibenclamide showed significantly elevated Hb concentration compared with the diabetic control.

The results in Table 3 showed significantly elevated WBC counts in the diabetic control and diabetic rats, which received 400 and 800 mg/kg EEABL compared to the normal control. Contrarily, the diabetic rats treated with 200 mg/kg EEABL and Glibenclamide showed no significant increase in WBC counts compared with the normal control. Similarly, the WBC counts of all the diabetic rats treated with different doses of EEABL and Glibenclamide decreased significantly compared to the diabetic control.

**Table 3**. Haematological parameters of alloxan-induced diabetic rats treated with EEABL

| Treatment groups | Normal control | Diabetic control | EEABL (200 mg/kg) | EEABL (400 mg/kg) | EEABL (800 mg/kg) | Glibenclamide (3 mg/kg) |
|---|---|---|---|---|---|---|
| RBC x106/mm3 | 7.80±0.37$^{d}$ | 6.42±0.13$^{a}$ | 7.40±0.38$^{b,c,d}$ | 7.16±0.46$^{b}$ | 7.27±0.05$^{b,c}$ | 7.68±0.17$^{c,d}$ |
| PCV (%) | 49.25±2.22$^{d}$ | 40.00±1.63$^{a}$ | 46.25±2.22$^{b,c}$ | 44.75±2.75$^{b}$ | 45.50±0.58$^{b}$ | 48.50±1.29$^{c,d}$ |
| Hb (g/dl) | 19.70±1.55$^{c}$ | 14.60±0.78$^{a}$ | 18.28±1.45$^{b,c}$ | 16.83±0.89$^{b}$ | 17.20±1.09$^{b}$ | 19.08±0.83$^{c}$ |
| WBC x10$^{3}$/mm3 | 9.10±0.48$^{a}$ | 15.15±0.95$^{c}$ | 10.11±1.05$^{a}$ | 13.40±0.61$^{b}$ | 12.63±1.73$^{b}$ | 10.19±0.50$^{a}$ |
| PLT x10$^{3}$/mm$^{3}$ | 113.75±9.25$^{a}$ | 149.30±6.80$^{d}$ | 131.22±6.99$^{c}$ | 130.75±2.50$^{c}$ | 126.00±7.35$^{b,c}$ | 118.50±3.42$^{a,b}$ |
| MCV (fl) | 63.11±0.74$^{a}$ | 62.31±1.36$^{a}$ | 62.51±0.86$^{a}$ | 62.56±0.88$^{a}$ | 62.61±0.71$^{a}$ | 63.13±0.56$^{a}$ |
| MCH (fl) | 25.24±1.62$^{b}$ | 22.74±0.81$^{a}$ | 24.69±1/14$^{b}$ | 23.53±0.58$^{a,b}$ | 23.67±1.48$^{a,b}$ | 24.83±0.66$^{b}$ |
| MCHC (g/dl) | 39.98±2.13$^{b}$ | 36.49±0.75$^{a}$ | 39.48±1.60$^{b}$ | 37.62±0.50$^{a,b}$ | 37.79±2.09$^{a,b}$ | 39.32±0.72$^{b}$ |

The results in the table indicated mean ± standard deviation (n = 10), and the values in the same row with different letter superscripts are significantly different (P < 0.05).

The PLT counts in Table 3 indicated a significant increase in the diabetic control and diabetic rats, which received 200, 400, and 800 mg/kg EEABL, respectively, compared with the normal control. Asides, the diabetic rats treated with 3 mg/kg of Glibenclamide showed no significant rise in the PLT counts relative to the normal control. However, the EEABL and Glibenclamide-treated diabetic rats exhibited a significant decline in the PLT counts compared to the diabetic control.

The results in Table 3 showed that the diabetic control and diabetic rats treated with different doses of EEABL and Glibenclamide exhibited no significant changes in the MCV levels compared to the normal control. Similarly, there were no significant variations in the MCV levels of the diabetic rats treated with EEABL and Glibenclamide, respectively, relative to the diabetic control.

The diabetic control exhibited a significant decline in the MCH level compared to the normal control (Table 3). Also, all the diabetic rats treated with different doses of EEABL and Glibenclamide showed no significant decrease in the MCH level relative to the normal control.

The significantly reduced MCHC concentrations in the diabetic control compared to the normal control (Table 3). In contrast, there was no significant decline in the MCHC concentrations of the diabetic rats treated with 3 mg/kg Glibenclamide, 200, 400, and 800 mg/kg EEABL relative to the normal control, respectively. The diabetic rats treated with 200 mg/kg EEABL and 3 mg/kg Glibenclamide exhibited significantly elevated MCHC concentrations compared to the diabetic control.

### 2.6. Effects of EEABL on the serum vitamins concentrations of diabetic rats

Table 4 showed a significant decline in the serum vitamin $B_{12}$ levels in the diabetic control, Glibenclamide, and diabetic rats treated with varying doses of EEABL, respectively, relative to the normal control. Conversely, the serum vitamin $B_{12}$ increased significantly in the diabetic rats treated with Glibenclamide, 400 and 800 mg/kg EEABL, respectively, compared with the diabetic control.

Table 4 indicated a substantial decline in the serum vitamin $B_6$ concentrations of the diabetic control and diabetic rats that received 200 and 400 mg/kg EEABL and Glibenclamide, respectively, compared to the normal control. The diabetic rats treated with 800 mg/kg EEABL showed a substantial increase in the serum vitamin $B_6$ concentration relative to the normal control. Conversely, all the diabetic rats treated with different doses of EEABL and Glibenclamide displayed a significant increase in their serum vitamin $B_6$ concentration compared with the diabetic control.

It was evidenced in Table 4 that the diabetic control and diabetic rats treated with 200 and 400 mg/kg EEABL, respectively, showed a significant decrease in the serum vitamin C concentrations relative to the normal control. Still, there was a significant elevation in the serum vitamin C concentration of diabetic rats treated with 800 mg/kg EEABL compared with the normal control. In contrast, there was a significant decline in the serum vitamin C concentration of the diabetic rats treated with 3 mg/kg Glibenclamide compared with the normal control. Contrarily, the diabetic rats treated with 800 mg/kg EEABL and 3 mg/kg Glibenclamide respectively, had significantly elevated serum vitamin C concentration relative to the diabetic control rats.

The diabetic control and all the diabetic rats treated with different doses of EEABL and Glibenclamide showed a significant reduction in serum vitamin E levels compared to the normal control (Table 4). The treatment of the diabetic rats with 3 mg/kg Glibenclamide and different doses of EEABL caused a significant increase in the serum vitamin E concentrations relative to the diabetic control.

**Table 4.** Serum vitamin concentrations of EEABL-treated diabetic rats

| Treatment groups | Normal control | Diabetic control | EEABL (200 mg/kg) | EEABL (400 mg/kg) | EEABL (800 mg/kg) | Glibenclamide (3 mg/kg) |
|---|---|---|---|---|---|---|
| Vitamin $B_{12}$ (mg/dl) | 1.59±0.08$^{d}$ | 0.83±0.07$^{a}$ | 0.95±0.03$^{a}$ | 1.22±0.05$^{b,c}$ | 1.17±0.16$^{b}$ | 1.32±0.05$^{c}$ |
| Vitamin $B_6$ (mg/dl) | 1.08±0.02$^{c}$ | 0.88±0.03$^{a}$ | 0.96±0.03$^{b}$ | 0.95±0.04$^{b}$ | 1.10±0.04$^{c}$ | 0.96±0.04$^{b}$ |
| Vitamin $B_2$ (mg/dl) | 2.08±0.02$^{d}$ | 1.24±0.04$^{a}$ | 1.65±0.12$^{c}$ | 1.52±0.03$^{b}$ | 1.61±0.02$^{c}$ | 1.61±0.07$^{c}$ |
| Vitamin C (mg/dl) | 63.85±1.95$^{c}$ | 57.88±1.69$^{a}$ | 59.96±1.31$^{a,b}$ | 58.79±1.82$^{a}$ | 71.12±0.80$^{d}$ | 61.83±0.69$^{b,c}$ |
| Vitamin E (mg/dl) | 13.40±0.57$^{c}$ | 10.37±0.59$^{a}$ | 12.07±0.54$^{b}$ | 12.05±0.25$^{b}$ | 11.86±0.38$^{b}$ | 10.80±0.21$^{b}$ |

The results in the table indicated mean ± standard deviation (n = 10), and the values in the same row with different letter superscripts are significantly different (P < 0.05).

### 2.7. Effect of EEABL on serum electrolyte concentrations of diabetic rats

The results in Table 5 indicated a significant rise in the serum sodium ($Na^+$) levels of the diabetic control and diabetic rats treated with 200, 400, and 800 mg/kg EEABL, respectively, compared with the normal control. In contrast, the diabetic rats treated with Glibenclamide showed no significant increase in the serum $Na^+$ level relative to the normal control. The diabetic rats treated with EEABL and Glibenclamide showed a considerable decline in the serum $Na^+$ level compared with the diabetic control.

The serum potassium ($K^+$) levels of the diabetic control, and all the diabetic rats treated with different doses of EEABL and Glibenclamide, respectively, displayed significant increases in the serum $K^+$ levels compared with the normal control (Table 5). Treatment of the diabetic rats with 400 mg/kg EEABL and 3 mg/kg Glibenclamide drastically reduced the serum $K^+$ levels relative to the diabetic control.

The serum calcium ($Ca^{2+}$) level in the diabetic control decreased significantly, unlike the diabetic rats treated with 400 and 800 mg/kg EEABL and Glibenclamide, which increased considerably compared to the normal control (Table 5). All the EEABL and Glibenclamide treated diabetic rats had significantly elevated serum $Ca^{2+}$ levels relative to the diabetic control.

The serum inorganic phosphate ion ($PO_4^{2-}$) concentrations in Table 5 displayed a significant increase in the diabetic rats treated with 400 and 800 mg/kg EEABL, respectively, relative to the normal control. The diabetic rats treated with Glibenclamide showed no significant rise in the serum $PO_4^{2-}$ concentrations compared to the normal control. The diabetic rats administered 200 mg/kg EEABL had significantly reduced serum $PO_4^{2-}$ levels compared to the diabetic control. Still, the diabetic rats treated with 3 mg/kg of Glibenclamide showed a significant decrease in the serum $PO_4^{2-}$ level relative to the diabetic control.

**Table 5.** Serum electrolyte concentrations of diabetic rats treated with EEABL

| Treatment groups | Normal control | Diabetic control | EEABL (200 mg/kg) | EEABL (400 mg/kg) | EEABL 800 mg/kg) | Glibenclamide (3 mg/kg) |
|---|---|---|---|---|---|---|
| $Na^+$ (mEq/L) | 131.29±2.23$^{a}$ | 142.97±2.47$^{c}$ | 139.99±0.79$^{b}$ | 138.72±2.04$^{b}$ | 139.38±2.03$^{b}$ | 134.04±1.52$^{a}$ |
| $K^+$ (mEq/L) | 7.92±0.26$^{a}$ | 10.11±0.27$^{c,d}$ | 9.76±0.28$^{c}$ | 9.14±0.18$^{b}$ | 10.22±0.32$^{d}$ | 8.84±0.38$^{b}$ |
| $Ca^{2+}$ (mg/dl) | 7.74±0.15$^{b}$ | 7.22±0.27$^{a}$ | 7.66±0.29$^{b}$ | 8.13±0.27$^{c}$ | 8.35±0.14$^{c,d}$ | 8.64±0.29$^{d}$ |
| $PO_4^{2-}$ (mmol/L) | 2.60±0.28$^{a,b}$ | 4.51±0.51$^{d}$ | 2.27±0.06$^{a}$ | 3.08±0.27$^{c}$ | 3.12±0.12$^{c}$ | 2.83±0.11$^{b,c}$ |
| $Cl^-$ (mEq/L) | 91.41±0.94$^{a}$ | 94.95±0.21$^{b}$ | 93.48±0.99$^{b}$ | 94.37±1.44$^{b}$ | 90.21±1.52$^{a}$ | 89.33±0.97$^{a}$ |
| $Mg^{2+}$ (mg/dl) | 2.20±0.09$^{b}$ | 2.05±0.05$^{a}$ | 2.35±0.05$^{c}$ | 2.62±0.09$^{d}$ | 2.79±0.14$^{e}$ | 2.58±0.11$^{d}$ |
| $HCO_3^-$ (mmol/L) | 18.58±0.34$^{b,c}$ | 19.69±0.10$^{e}$ | 17.68±0.55$^{a}$ | 19.22±0.32$^{d,e}$ | 18.98±0.11$^{c,d}$ | 18.20±0.62$^{a,b}$ |

The results in the table indicated mean ± standard deviation (n = 10), and the values in the same row with different letter superscripts are significantly different (P < 0.05).

The results in Table 5 indicated a significant rise in the serum chloride ion ($Cl^-$) level in the diabetic control and diabetic rats treated with 200 and 400 mg/kg EEABL, respectively, compared to the normal control. While the diabetic rats treated with 800 mg/kg EEABL and 3 mg/kg Glibenclamide respectively, indicated no significant decline in the serum $Cl^-$ levels relative to the normal control. There was a substantial decline in the serum $Cl^-$ concentration in the diabetic rats treated with 800 mg/kg EEABL and Glibenclamide, respectively, compared to the diabetic control.

The serum $Mg^{2+}$ concentration of the diabetic control declined significantly relative to the normal control (Table 5). Conversely, the serum $Mg^{2+}$ concentrations in the Glibenclamide-treated diabetic rats and diabetic rats treated with 200, 400, and 400 mg/kg of EEABL were significantly increased compared to the normal control and diabetic control, respectively.

The significantly elevated serum $HCO_3^-$ level in the diabetic control and diabetic rats treated with 400 mg/kg EEABL compared with the normal control (Table 5). In contrast, there were no significant changes in the serum $HCO_3^-$ levels of the diabetic rats treated with 800 mg/kg EEABL and Glibenclamide, respectively, compared to the normal control. The diabetic rats treated with Glibenclamide, 200 and 800 mg/kg EEABL, respectively, had significantly reduced $HCO_3^-$ levels relative to the diabetic control.

### 2.8. Effects of EEABL on the serum urea and creatinine concentrations of diabetic rats

The results in Figures 3 and 4 demonstrated significantly increased serum urea and creatinine concentrations,respectively, in the diabetic control and all the diabetic rats treated with EEABL compared with the normal control. In contrast, the diabetic rats treated with Glibenclamide showed no significant variations in the serum urea and creatinine compared with the normal control. Conversely, all the diabetic rats treated with EEABL and Glibenclamide showed a substantial decrease in serum urea and creatinine concentrations relative to the diabetic control.

### 2.9. Effects of EEABL on the serum total cholesterol levels in diabetic rats

There was a significant elevation in the serum T. CHOL concentrations in the diabetic control and diabetic rats treated with 3 mg/kg Glibenclamide, 200, 400, and 800 mg/kg EEABL, respectively, compared with the normal control (Figure 5). Treatments of the diabetic rats with different doses of EEABL and Glibenclamide caused a significant decline in the serum T. CHOL relative to the diabetic control.

### 2.10. Effects of EEABL on the HDL-cholesterol levels in diabetic rats

Figure 6 showed a significantly reduced serum HDL-C concentration in the diabetic control relative to the normal control. Conversely, the diabetic rats treated with 3 mg/kg Glibenclamide, 200, 400, and 800 mg/kg EEABL displayed a significant increase in the serum HDL-C concentrations compared with the normal control and diabetic control, respectively.

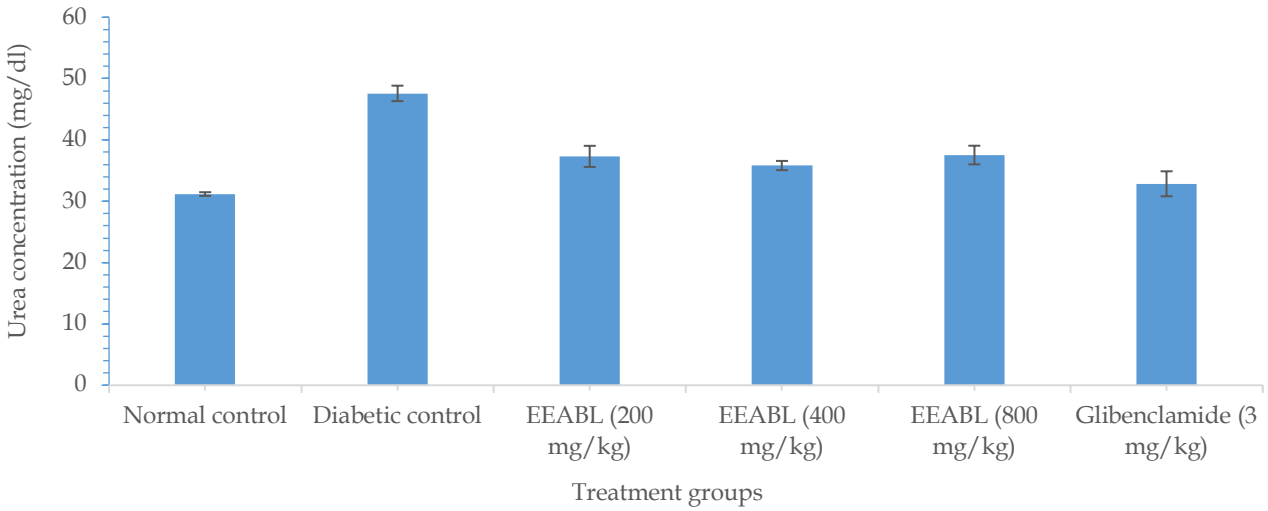


**Figure 3**. Serum urea concentrations of diabetic rats treated with EEABL

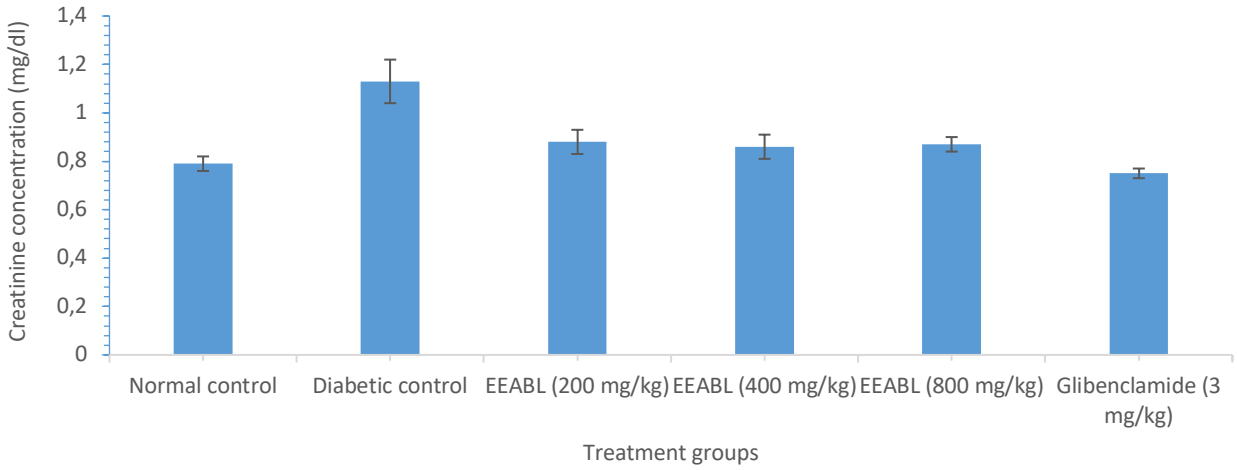


**Figure 4.** Serum creatinine concentrations of diabetic rats treated with EEABL

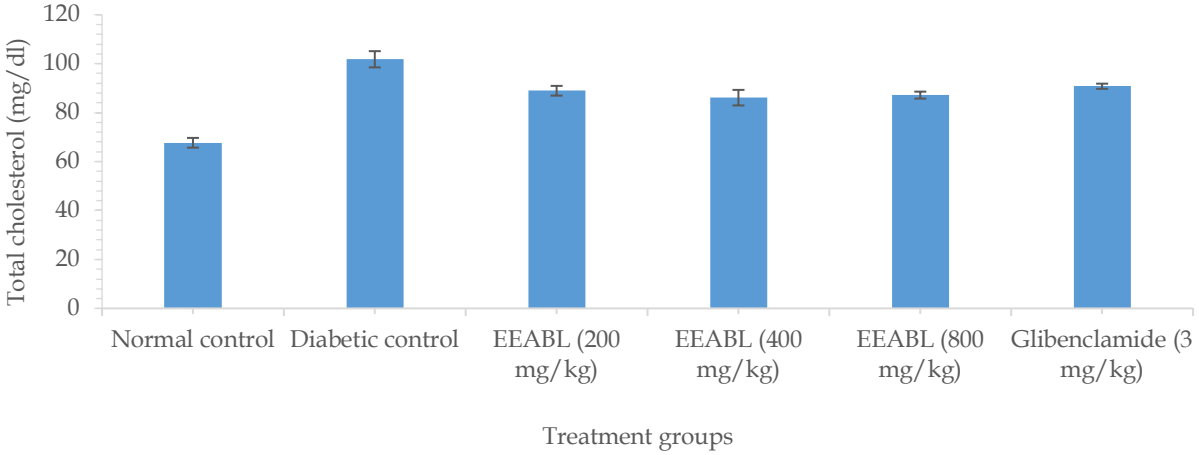


**Figure 5.** Total serum cholesterol concentrations of diabetic rats treated with EEABL

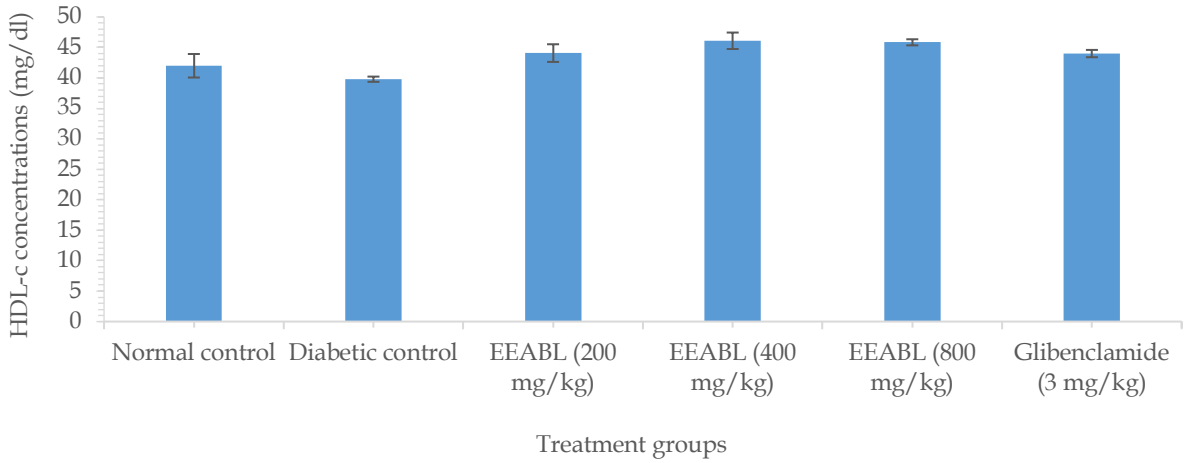


**Figure 6**. Serum HDL-cholesterol concentrations of diabetic rats treated with EEABL

### 2.11. Effects of EEABL on the TAGand LDL-cholesterol levels in diabetic rats

The diabetic control and diabetic rats treated with 800 mg/kg EEABL and 3 mg/kg Glibenclamide respectively, showed significant elevation in the serum TAG (Figure 7) and LDL-C(Figure 8) concentrations relative to the normal control. Contrarily, in the diabetic rats treated with 200 and 400 mg/kg, respectively, EEABL showed a significant decline in the serum TAG and LDL-C concentrations compared to the normal

control. In contrast, all the diabetic rats treated with different doses of the EEABL and Glibenclamide displayed a significant decline in the serum TAG and LDL-C concentrations relative to the diabetic control.

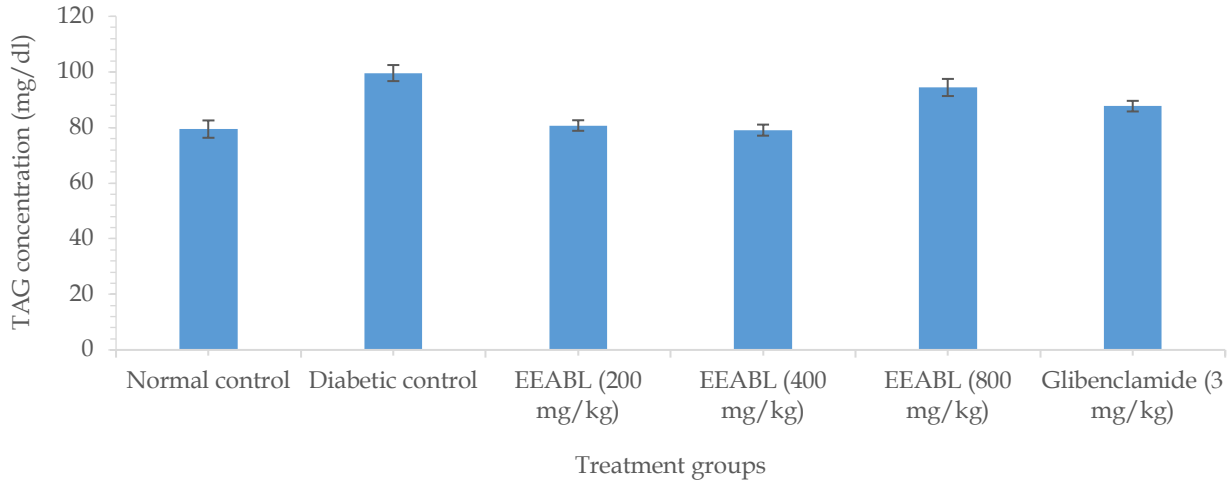


**Figure 7.** Serum TAG concentrations of diabetic rats treated with EEABL

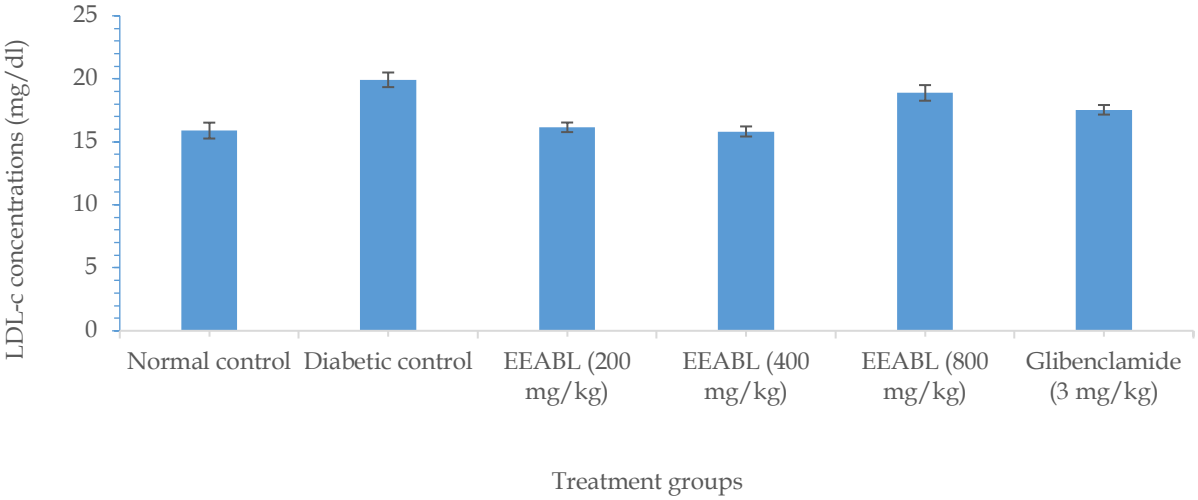


**Figure 8.** LDL-c concentrations of diabetic rats treated with EEABL

### 2.12. Effects of EEABL on the VLDL-cholesterol levels in diabetic rats

The results in Figure 9 further showed significantly elevated VLDL-C concentrations in the diabetic control and diabetic rats treated with 3 mg/kg Glibenclamide, 200, 400, and 800 mg/kg EEABL, respectively, with the normal control. Conversely, the diabetic rats treated with Glibenclamide and different doses of EEABL exhibited a significant decline in the serum VLDL-C concentrations relative to the diabetic control.

## 3. DISCUSSION

Diabetes mellitus is a global health challenge that results from excessive accumulation of blood glucose levels triggered by either lack of or insufficient insulin secretion by the beta cells or insensitivity of the insulin receptors to the action of the circulating insulin concentrations. Aside from the efforts to restore the circulating blood glucose level to normal, deliberate steps are taken via lifestyle changes or the administration of therapeutic agents that could prevent severe complications associated with unmitigated diabetes. Experimental studies commonly employ alloxan, scientifically known as 5,5-dihydroxyl pyrimidine-2,4,6-trione, for the induction of diabetes in experimental animals for the investigation of the pathogenesis of diabetes and the potency of therapeutic agents against diabetes [12]. There is a need to ascertain the physiological and biochemical functional status of tissues and organ functions in diabetic rats because of the adverse effects of toxic reactive free radicals generated from the metabolic transformation of alloxan which induces diabetes via its destructive impact on the beta cells. This study evaluated the effects of EEABL on the

blood glucose levels of diabetic rats, haematological parameters, serum vitamins, electrolytes, lipid profile, urea, and creatinine concentrations of alloxan-induced diabetic rats.

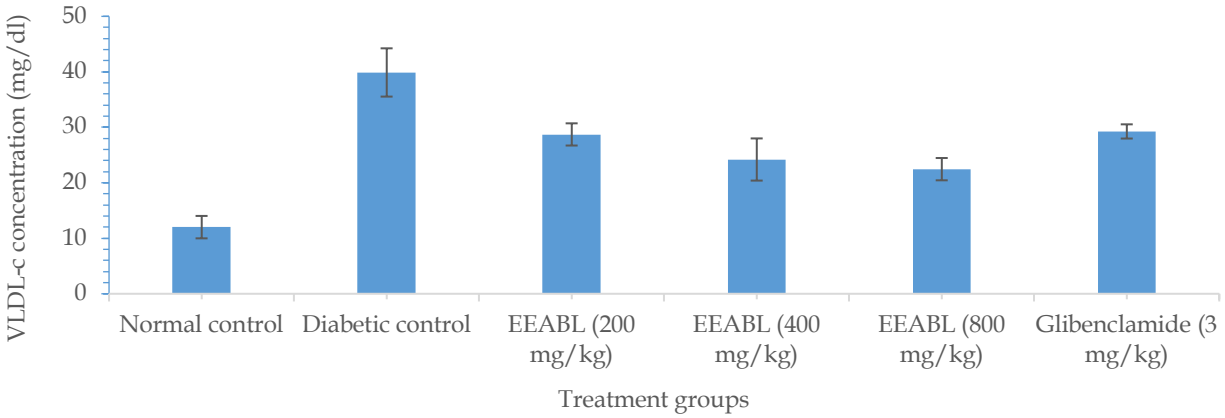


**Figure 9.** Serum VLDL-c concentrations of diabetic rats treated with EEABL

The high amounts of phytochemicals, including alkaloids, flavonoids, phenols, tannins, and terpenes present in the EEABL, showed that it is rich in bioactive compounds with potent pharmacological properties. Medicinal plant extracts containing bioactive phytochemicals like alkaloids, flavonoids, and phenols have been therapeutically effective in managing common health challenges like diabetes, hyperlipidaemia, and oxidative stress. The anti-hyperlipidaemia, renal, and hepatoprotection exhibited in the diabetic rats treated with EEABL could be attributed to the therapeutic potential of the phytochemicals like flavonoids, phenol, terpenes, and glycoside, in line with Xiao *et al*. [13]. The numerous phytochemicals detected in the EEABL suggest that it could help manage various health challenges, diseases, and metabolic syndrome-related diseases like diabetes and its associated complications, which agree with previous findings [14, 15, 16].

The absence of signs or symptoms of toxicity and death of the rats administered 10 – 5000 mg/kg EEABL after 24 h indicated that EEABL is relatively safe for consumption. The absence of toxicity signs in the rats administered with EEABL showed that EEABL is not capable of causing substantially immediately after consumption of even its increased dose. However, there is a need to evaluate the chronic toxicity effects of EEABL to avert any toxicity associated with its regular consumption. Some drugs and medicinal plant extracts that have shown to be safe for consumption after acute toxicity study has demonstrated chronic toxicity. The absence of adverse reactions and death even when 5000 mg/kg of EEABL was administered to rats suggests that EEABL has a high safety margin in line with Ezembu *et al.* [17].

The normal blood glucose levels in the rats in all the groups used for the study before the induction of diabetes showed that they were healthy and free from any pre-existing diabetic condition. Conversely, the high blood glucose levels obtained in the diabetic control and all the EEABL and Glibenclamide treated showed that the rats were made diabetic by the administration of the induction of the diabetic agents. The persistently elevated blood glucose levels in the diabetic control after 2- and 4-hours post-induction of diabetes and the sight decrease in the percentage fall in the blood glucose indicated persistent hyperglycaemia in the diabetic control, which aligns with Kifle *et al*. [18]. The persistent hyperglycaemia in the diabetic control suggests that the diabetic control rats could suffer severe hyperglycaemia and complications that could massively affect their chance of survival and even cause death if unabated. In contrast, the time and dose-dependent significant reduction in blood glucose and high percentage fall in percentage fall in blood glucose levels showed the antihyperglycemic effects of EEABL on diabetic rats. The antidiabetic activities exhibited by EEABL in diabetic rats are comparable to that of Glibenclamide. However, much time was required for the EEABL fully restore hyperglycaemia to the average level relative to the normal control, similar to the findings of Kifle *et al*. [18]. The EEABL administration might have lowered the elevated blood glucose levels in the diabetic rats via its stimulatory effects on the pancreatic beta responsible for the synthesis and secretion of insulin which could have increased the concentration of the circulating insulin and the sensitivity of the insulin receptors to the insulin. Alternatively, the bioactive constituents of the EEABL might have achieved hypoglycaemic effects on diabetic rats by causing a decline in insulin resistance and inducing increased expression of genes that produce gene products that control carbohydrates, proteins, and lipid metabolism. There are possibilities that EEABL administration caused a decline in blood glucose levels by preventing the hepatic generation and release of glucose into the blood. The substantial dose-dependent hypoglycaemic effects of EEABL, which restored the considerably elevated blood glucose levels in the diabetic rats to a near

normal after 4 hours, suggest that EEABL is a potent antidiabetic agent that could help manage diabetes in line with Khana *et al*. [2]. The high antihyperglycemic effect exhibited by EEABL in diabetic rats could be attributed to the high phytochemical contents, including flavonoids, terpenes, phenols, and alkaloids which have been linked to the antidiabetic activities of medicinal plants, which aligns with previous reports [2, 19].

The significant reduction in the RBC, PCV, Hb, MCH, and MCHC of the diabetic control compared with the normal control could be attributed to the toxic effects of the reactive free radicals emanating from hyperglycaemia and alloxan including their metabolites, on the haematological parameters which align with findings of Milosevic and Panin [20]. The adverse effects of hyperglycaemia on the rats could have caused the haemolysis of the red blood cells, thereby causing their depletion. The decline in erythropoietic activity of the diabetic control occasioned by the adverse effects of hyperglycaemia could have impaired the RBC synthesis and reduced PCV and Hb levels in the rats. The significantly increased WBC and PLT in the diabetic control rats compared to the normal control are attributed to the pathogenesis of diabetes. The diabetic control rats could have increased the synthesis of WBC as an immunological response to the adverse effects of hyperglycaemia. Also, the increased PLT in diabetic rats indicated diabetic complications such as hypertension in the rats, which could have adversely affected the blood vessels and blood clotting [21].

Conversely, treatment of the diabetic rats with different doses of EEABL caused substantially increased RBC, PCV, Hb, WBC, MCH, and MCHC and drastically lowered WBC and PLT count compared, which showed that EEABL was effective in restoring average concentrations of the haematological parameters in diabetic rats [22, 23]. The ability of the EEABL to replenish haematological parameters in diabetic rats showed that it could be helpful in the prevention of anaemia in diabetic conditions. Contrarily, the no significant alterations of the MCV concentration in the diabetic control and diabetic rats treated with Glibenclamide and EEABL, respectively, compared to the normal control, suggest that diabetes has no significant effects on the MCV counts of diabetic rats.

The significant decline in the serum concentrations of vitamins $B_{12}$, $B_6$, $B_2$, C, and E in the diabetic control compared with the normal control suggests that deficiency of vitamins in diabetic conditions could contribute to diabetes pathogenesis and complications. Vitamin $B_{12}$ is needed in the body to enhance optimal biochemical and physiological functions, including replenishing RBC counts and neurological and brain functions. The substantial decline in the serum vitamin $B_{12}$ in diabetic control suggests that deficiency in vitamin $B_{12}$ in untreated diabetes could be responsible for the neurological disorders associated with severe diabetic conditions. The substantially reduced Vitamin B6 in the diabetic control could have impacted negatively on the hyperglycaemic index of the diabetic rats and caused a decline in the Hb synthesis. Vitamin B6 is required to enhance glucose uptake, metabolism, Hb synthesis, and neurological functions. Vitamin $B_2$, as a cofactor, plays a critical role in glucose metabolism and is required in an adequate amount to enhance the synthesis of sufficient RBC needed for optimal body functioning. The significantly low vitamin $B_2$ in the diabetic control could have contributed to the decline in the RBC count of the diabetic control and probably aggravated hyperglycaemia in the rats. Despite numerous functions of vitamin C in enhancing growth, wounding healing, immune system, and promoting healthy teeth and bones, vitamin C plays a critical therapeutic role in managing diabetes via its established antioxidant activities that could scavenge or quench reactive free radicals released from the metabolism of alloxan and adverse effects of hyperglycaemia in the body. The significantly diminished serum vitamin C concentration in the rat with diabetes suggested reduced antioxidant levels to counter free radical attacks in the diabetic rats. Vitamin E is a potent antioxidant that scavenges reactive free radicals and prevents oxidative stress, lipid peroxidation, and dyslipidaemia. It also promotes effective glucose utilization in the body, and its decreased concentration in the serum of the diabetic control rats could be attributed to hyperglycaemia in the rats. The increased serum vitamin $B_{12}$, $B_6$, $B_2$, C, and E concentrations in the diabetic rats treated with EEABL and Glibenclamide compared to the diabetic control could be attributed to the therapeutic effects of each treatment. The treatment with EEABL might have improved the absorption of these vitamins from dietary sources, which enhanced their serum availability, unlike the diabetic control, which might have lost much of them via malabsorption. The improved levels of the serum vitamins in EEABL-treated diabetic rats could have improved their haematological indices and reduced complications associated with severe diabetes. The effect of EEABL on the serum antioxidant vitamins, including vitamins C and E, aligns with the finding of Aloh*et al*. [9].

The assessment of the concentrations of serum electrolytes in diabetes is a vital medical tool employed in evaluating the prognosis of a diabetic patient aside from the monitoring of the blood glucose level to ascertain the impact of diabetes on renal functions as a guide for early interventions. The diabetic control rats in this study exhibited significantly elevated serum concentrations of the $Na^+$, $K^+$, $Cl^-$, $PO_4^{2-}$, and $HCO_3^-$, which suggested impaired renal functions due to the reduced ability of the glomerular to filter these ions in excess from the blood for excretion via urine with a potential cause of electrolyte imbalance with attendant health consequences [2, 24]. The alterations in the serum electrolyte levels in the diabetic control align with

Khanduker et al. [25]. Metabolic acidosis associated with persistent hyperglycaemia in the diabetic control could have caused the accumulated serum $HCO_3^-$ ions in the diabetic control compared to the normal control [24]. Treatment of the diabetic rats with different doses of EEABL caused a decline in the serum $Na^+$, $K^+$, $Cl^-$, $PO_4^{2-}$, and $HCO_3^-$ ions with some EEABL, effectively restoring their serum concentrations to the level comparable with the normal control. The decline in the serum levels of $Na^+$, $K^+$, $Cl^-$, $PO_4^{2-}$, and $HCO_3^-$ ions in the diabetic rats treated with EEABL showed that the treatment of the diabetic rats with different doses of EEABL attenuated the adverse effects of diabetes on renal functions of the rats to a greater extent contrary to the diabetic control in line with previous findings [24. 26]. In addition, the significantly reduced serum $PO_4^{2-}$ concentration in the diabetic rats treated with 200 mg/kg EEABL compared with diabetic control showed that lower doses of EEABL could reduce the risk of vascular disease in diabetes, which aligns with Toussaint *et al*., that reduction in the serum $PO_4^{2-}$ concentration decreases the chances of vascular disease in chronic renal disease [27]. Contrarily, the significantly depleted serum $Ca^{2+}$, and $Mg^{2+}$ concentrations in the diabetic control, relative to the normal control, were significantly increased in the diabetic rats treated with EEABL, similar to the diabetic rats treated with Glibenclamide. The considerably increased serum $Ca^{2+}$ and $Mg^{2+}$ concentrations in the diabetic rats treated with EEABL relative to diabetic control showed the improved capacity of the glomerular and proximal tubules to filter and reabsorb excess serum $Ca^{2+}$ and $Mg^{2+,}$ respectively, contrary to possible excretion in the diabetic control.

The serum urea and creatinine concentrations are tightly regulated by the glomerular of the kidney, which filters excess circulating urea and creatinine from the blood for excretion out of the body via urine. It has become a more reliable and viable procedure to evaluate both the serum urea and creatinine concentrations to ascertain the renal function status than using the serum concentration of either urea and creatinine alone, as other variables could alter their serum concentrations under normal renal function. The significantly elevated serum urea and creatinine concentrations in the diabetic control compared to the normal control could be attributed to the impaired renal functions in the untreated diabetic rats and agree with Amartey *et al*. [28]. The reactive free radicals associated with hyperglycaemia in untreated diabetes might have adversely affected the renal integrity and the ability of the renal glomerular to filter urea and creatinine from the blood effectively for elimination from the body through urinary excretion in line with Kumsa *et al*. [29]. The significant decline in the serum urea and creatinine concentrations of the diabetic rats treated with EEABL and Glibenclamide, respectively, compared with diabetic control, showed the renal protective effects of each therapeutic agent, which improved the renal functions of the treated diabetic rats in line with Korrapati *et al*. [30]. These decline in the serum urea and creatinine concentrations in the diabetic rats treated with EEABL indicated that EEABL significantly attenuated the adverse effects of diabetic progression on renal function and increased the glomerular filtration of urea and creatinine from the blood and their subsequent elimination from the blood in with Amartey *et al*. [28]. Still, the glomerular filtration and elimination of urea and creatinine in the diabetic rats treated with EEABL were substantially reduced compared to the Glibenclamide treated diabetic rats and the normal control, respectively, suggesting the treatment with EEABL was not able to restore the renal functions to the optimal level [30]. The decline in serum urea and creatinine concentrations in the EEABL-treated diabetic rats indicated that treatment with Glibenclamide confers higher renal protection in diabetic rats than in EEABL.

The assessment of the serum lipid profile is usually performed in patients with poor health conditions, especially those involving metabolic syndrome like diabetes mellitus, to monitor and reduce the risk of developing cardiovascular disease. The significantly elevated serum T. CHOL, TAG, LDL-C, and VLDL-C coupled with the reduced HDL-C concentrations in the diabetic control compared to the normal control showed that severe diabetes increases the risk of cardiovascular disease and various metabolic syndrome complications in line with Fox *et al.* [31]. The substantially increased serum T. CHOL, TAG, LDL-C, and VLDL-C and decreased serum HDL-C concentrations in the diabetic control rats could be attributed to the adverse effects of diabetes on carbohydrate and lipid metabolism in line with previous findings [27, 32]. The abnormally elevated serum T. CHOL, TAG, LDL-C, and VLDL-C concentrations in diabetic control could get quickly deposited on the wall of the arteries, thereby reducing the diameters of the blood vessels and increasing the blood pressure, which could lead to heart failure, stroke, and other related complications which agree with Duraipandiyan *et al*., and Azam *et al.* [33, 34]. The substantially decreased serum HDL-C concentration is needed to maintain healthy blood vessels required to transport lipid materials out of the blood vessels to the liver for metabolism. The low serum HDL-C concentrations in the diabetic control predisposed it to develop arterial clogs by the lipid droplets, especially the high LDL-C that ought to be eliminated [33].

Conversely, the significantly reduced serum T. CHOL, TAG, LDL-C, and VLDL-C and elevated serum HDL-C concentration in the diabetic rats treated with EEABL and Glibenclamide, respectively, relative to the diabetic control showed the therapeutic effects of the treatments in preventing dyslipidaemia associated with diabetes in line with Uroko *et al*. [35]. The positive impact of EEABL on the treated diabetic rats could be

attributed to the improved carbohydrate and lipid metabolism in the diabetic rats due to the antihyperglycemic and antihyperlipidemic effects of EEABL, which are similar to the findings of Azam *et al*. [34]. The high serum HDL-C concentration in diabetic rats could transport excess LDL-C and other lipid materials from the arterial walls to the hepatocyte for breakdown, thereby reducing the risk of narrowing the blood vessels and preventing the adverse health associated health consequences dyslipidaemia and diabetes complications [34].

## 4. CONCLUSION

The data obtained from this study showed that EEABL possesses substantial antihyperglycemic activity that could restore blood glucose levels of diabetic rats to near normal contrary to the significantly elevated blood glucose levels in the diabetic control rats. The study also showed that untreated diabetes mellitus could lead to abnormal levels of haematological parameters and serum vitamins, impaired renal functions, and dyslipidaemia in diabetic rats, in addition to hyperglycaemia. Treatment of diabetic rats with EEABL effectively attenuated the adverse effects of diabetes on the haematological parameters, serum vitamins, serum electrolytes, urea, creatinine, and lipid profile. These findings suggest that treatment with EEABL could improve the biochemical and physiological functions in a diabetic condition which could be vital in managing diabetes.

## 5. MATERIALS AND METHODS

### 5.1. Plant material

The *Acioabarteri* leaves were used in the study. The fresh leaves of *A. barteri* were collected from a forest at AgbamaOlokoro, Umuahia South. A taxonomist identified the plant leaves at our institution's Herbarium unit, and the voucher number was adequately filled at the Herbarium. The fresh leaves of *A. barteri* were selected from the debris of plant materials and other possible contaminants and rinsed in clean tap water to remove specks of dirt. The leaves were then cut to smaller sizes, spread on a clean, sterile dry surface, and allowed to dry under a shade until a constant dry weight was attained. The dried plant leaves were ground into a coarse powder using a mechanized grinder. The ground plant was weighed and poured into a dry clean container for extraction.

### 5.2. Experimental animals

Seventy-eight (78) albino rats were purchased from the Animal House, Physiology Unit, College of Natural Sciences and acclimatized in our animal house for two weeks before the complete study. They were fed standard feed for laboratory animals and given adequate access to clean drinking water.

### 5.3. Chemicals and drugs

The chemicals and drugs employed in this study were all analytical grades obtained from reputable chemical manufacturers and stores. The ethanol solvent, Alloxan monohydrate, and Glibenclamide were purchased from Sigma Aldrich, USA.

### 5.4. Extraction of the plant material

A quantity of 1.2 kg of the coarsely ground plant sample was weighed into sterile, clean, dry calibrated containers, and 3.5 L of ethanol was added to it and macerated for 48 h under regular agitation to ensure even distribution of the solvent and proper extraction of the phytoconstituents. The extraction mixture was filtered after 48 h with a Whatman No one filter paper, and the solvent was eliminated via a rotary evaporator to obtain a concentrated extract. The filtrate was weighed its percentage yield was calculated as 9.98 %, corresponding to 99.8 g of the extract.

### 5.5. Experimental design

The antidiabetic study of EEABL involved sixty male Wistar rats selected into six groups containing ten rats each. The groups are the normal control, diabetic control, diabetes + 200 mg/kg of EEABL, diabetes + 400 mg/kg of EEABL, diabetes + 800 mg/kg of EEABL, and diabetes + 3 mg/kg of Glibenclamide. All the groups except the normal control (administered 1 ml/kg of normal saline only) were alloxan monohydrate-induced diabetic rats. The diabetic control rats were the alloxan-induced untreated diabetic rats, and the Glibenclamide treated diabetic rats were the standard control. The EEABL-treated groups were the alloxan-induced diabetic rats used to evaluate the antidiabetic effects of different doses of EEABL. Treatment of the diabetic rats with EEABL and Glibenclamide commenced after the rats had been confirmed diabetic and lasted for 28

consecutive days. The changes in the blood glucose levels of the normal control and alloxan monohydrate-induced diabetic rats were evaluated after pre-induction, post-induction, 2 hrs post-induction, and 4 hrs post-induction using a commercial blood glucose assay kit, and blood samples were obtained for the analyses by making a minute tail puncture in the rats. The rats were subjected to an overnight fast on the 28th day. On the 29th day, they were anesthetized with intraperitoneal administration of 25 mg/kg pentobarbital. After 10 mins, blood samples were collected from the rats by cardiac puncture for biochemical and haematological analyses.

### 5.6. Diabetes mellitus induction in rats

Wistar albino rats subjected to an overnight fast were administered 150 mg/kg of alloxan monohydrate dissolved in normal saline intraperitoneally and allowed to stay for 72 hrs for the manifestation of diabetes. Rats with elevated blood glucose levels above 300 mg/dl were considered diabetic and used to evaluate the antidiabetic effects of EEABL.

### 5.7. Phytochemical analyses

The qualitative phytochemical screening and quantitative determination of the phytochemical contents in the EEABL were conducted according to the methods of Harbone [36].

### 5.8. Acute toxicity study of EEABL

The acute toxicity of EABL was evaluated using eighteen male rats according to the method of Lorke [37].

### 5.9. Biochemical analyses

The lipid profile includes total serum cholesterol (T. Chol.), high-density lipoprotein cholesterol (HDL-C), triacylglycerol (TAG), Low-density lipoprotein cholesterol (LDL-C), and very-low-density-lipoprotein cholesterol (VLDL-C) concentrations were estimated using the procedures in the Randox commercial kits for lipid profile assay. Radox assay kits were used to evaluate the creatinine and urea levels. The serum sodium ($Na^+$), calcium ($Ca^{2+}$), chloride ($Cl^-$), potassium ($K^+$), bicarbonate ions, and magnesium ($Mg^{2+}$) were determined by the methods of Tietz [38]. The vitamins, including $B_{12}$ (cyanocobalamin), $B_6$ (pyridoxine), $B_2$ (riboflavin), C (ascorbic acid), and E (α-tocopherol), were estimated with a High-Pressure Liquid Chromatography according to the procedures outlined by Parveen *et al*., and Petteysa and Frankb [39, 40].

### 5.10. Determination of haematological parameters

The erythrocyte (RBC), platelet (PLT), and total white blood cell (WBC) counts were determined by the hemocytometry method as described by Ochei and Kolhatkar, and the evaluation of the packed cell volume (PCV) was carried out by a microhematocrit centrifuge (Jouan A13 model) [41]. The haemoglobin (Hb) concentrations, mean cell volume (MCV), mean cell haemoglobin (MCH), and mean cell haemoglobinconcentrations (MCHC) were measured spectrophotometrically with the cyanmethemoglobin procedures.

### 5.11. Statistical analysis

A one-way analysis of variance (ANOVA) and a Duncan multiple range comparison tests was carried out on the data generated with a statistical significance difference attained at a 95 % confidence level ($P < 0.05$). Statistical Products and Service Solutions (SPSS) version 22 was used for the data analysis.

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**Author contributions:** Concept – C.A.., R.I.U.; Design –R.I.U., C.A., E.U.O.; Supervision –R.I.U., E.L.M.; Resources – R.I.U., C.F.A., P.C.N., M.E.U.; Materials – B.C.U.; P.C.N., C.A.; Data Collection and/or Processing – R.I.U., E.L.M.; M.E.U.; Analysis and/or Interpretation – R.I.U., E.U.O., C.F.A..; Literature Search – R.I.U., C.A., E.L.M; Writing – R.I.U.; Critical Reviews – E.L.M., B.C.U., M.E.U., E.U.O.; C.F.A., C.A.

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