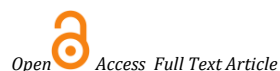


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Research Article

## Ethanollic Stem-Bark Extract of *Blighia unijugata* Possesses Anti-Hyperglycemic and Anti-Hyperlipidemic Activity in a Streptozotocin-Induced Diabetes Model

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### Abstract

**Introduction:** The global rise in the incidence of diabetes mellitus, particularly type 2 diabetes mellitus (T2DM), and its associated complications have become a public health threat. Besides hyperglycemia, hyperlipidemia has been associated with diabetes due to the defect in insulin secretion and/or action. Medicinal plants are being investigated to discover drug alternatives with better efficacies, lesser adverse effects, and cost-effectiveness. This work investigated the anti-hyperglycemic and anti-hyperlipidemic activity of ethanollic stem bark extract of *Blighia unijugata* (EBU) in streptozotocin (STZ)-induced diabetic Sprague-Dawley (SD) rats.

**Method:** T2DM was induced in male SD rats by a single intraperitoneal injection of STZ (50 mg/kg in 0.1 M citrate buffer, pH 4.5) and confirmed 72 hours later. EBU (100 and 200 mg/kg) and glibenclamide (5 mg/kg) were administered orally to the diabetic rats (n = 5) for 28 days. The effect of the treatments on fasting blood glucose (FBG), lipid profile, atherogenic predictor indices, and body weight were assessed.

**Results:** EBU treatments significantly reduced ( $p \leq 0.001$ ) elevated blood glucose, total cholesterol, triglycerides, and Very Low-Density Lipoprotein cholesterol (VLDL-c,  $p \leq 0.001$ ) but increased High-Density Lipoprotein cholesterol (HDL,  $p \leq 0.05$ ) compared to the diabetic control. Also, all the atherogenic risk predictor indices were significantly reduced ( $p \leq 0.001$ ). In addition, EBU treatment mitigated the significant weight loss ( $p \leq 0.01$ ) associated with the diabetic state when compared to the normal control.

**Conclusion:** These findings first report the anti-hyperglycemic, anti-hyperlipidemic, and anti-atherogenic properties of the stem bark of ethanollic extract of *Blighia unijugata*, and can be further studied and used as an anti-diabetic and anti-hyperlipidemic agent.

**Keywords:** *Blighia unijugata*; hyperglycemia; atherogenic index; hyperlipidemia; diabetes

## INTRODUCTION

Diabetes is a major cause of blindness, kidney failure, heart attacks, stroke, and lower limb amputation. In 2021, 537 million adults were reported to be living with diabetes. This number is predicted to increase to 643 million by 2030, and 783 million by 2045. Its prevalence has been rising more rapidly in low- and middle-income countries than in high-income countries<sup>1,2</sup>. With the predicted exponential increase in occurrence, diabetes poses a high patient, social, and economic burden globally<sup>3-5</sup>.

Type 2 diabetes mellitus (T2DM), a predominant type of diabetes mellitus, is a metabolic disease characterized by hyperglycemia, caused mainly by insulin resistance or inadequate insulin production<sup>2,6</sup>. Besides hyperglycemia associated with T2DM, abnormal lipid metabolism has also been reported, hence collectively named diabetic dyslipidemia<sup>7-9</sup>. Diabetic dyslipidemia is characterized by elevated triglycerides (TG), high very-low-density lipoprotein cholesterol (VLDL-c), postprandial hyperlipidemia, high low-density lipoprotein cholesterol (LDL-c), and reduced high-

density lipoprotein cholesterol (HDL-c). The main association between diabetes and the higher risk of atherosclerosis and cardiovascular diseases is reflected by these lipid alterations which are more atherogenic than general dyslipidemia<sup>10-13</sup>. This means that patients with diabetic dyslipidemia are at an increased risk of developing the associated microvascular and macrovascular complications, morbidities, and mortalities<sup>14,15</sup>.

The global rise in the incidences of T2DM, and its associated cardiovascular mortalities has called for improved preventive, diagnostic, and therapeutic measures to tackle this public health threat. Although several therapeutic interventions exist for managing T2DM, these existing pharmacological agents such as sulfonylureas, metformin, and thiazolidinediones cannot adequately improve the consequence of insulin resistance namely hyperglycemia and diabetic dyslipidemia. Furthermore, high rates of adverse effects and the cost of these drugs have limited their use. Therefore, research into viable drug alternatives, including herbal medicinal plants, to address these needs is at the core of several diabetes-related research pursuits.<sup>16-19</sup> Research into medicinal plants will

also serve the developing countries which are seeing greater increases in cases of diabetes and are more likely to face the issue of not being able to afford or readily access conventional medications.

*Blighia unijugata* Baker (Sapindaceae) is an evergreen shade-producing tree. It is common in tropical Africa and thrives best in sunny regions with moderate amounts of rainfall<sup>20-22</sup>. Locally, it is used as a purgative, sedative, analgesic, anti-emetic, anthelmintic, and for treating post-partum hemorrhage, vertigo, and boils<sup>23-25</sup>. The antimicrobial properties<sup>26-29</sup>, anti-oxidant activities<sup>29-31</sup>, cytotoxicity<sup>28, 30</sup>, molluscicidal activity<sup>32, 33</sup>, hypotensive effect<sup>34, 35</sup>, anti-inflammatory effect<sup>36</sup>, and anti-depressant properties<sup>37</sup> of *Blighia unijugata* have been studied in pre-clinical models. Its effect on total body weight<sup>24, 38</sup>, estrogen, total protein, and total cholesterol<sup>38</sup>. The effect of its seed oil on weight gain, and lipid profile<sup>39</sup> has been studied. Also, its ability to increase hemoglobin, white blood cells, and packed cell volume has been studied<sup>24</sup>.

Previous studies done in our lab on the ethanolic stem bark extract of *Blighia unijugata* showed its ability to reduce serum total cholesterol in SD rats<sup>38</sup>. However, the activity of the stem bark extract on lipid profile, T2DM, diabetic dyslipidemia, and atherogenic indices have not been investigated. Also, *Blighia sapida*, a sister species, has been reported to have anti-hyperglycemic and anti-hyperlipidemic effects<sup>40</sup> suggesting a possible family-wise property between the two species. This work studied the anti-hyperlipidemic and anti-hyperglycemic activities of the ethanolic extract of *Blighia unijugata* (EBU) in diabetic SD rats.

## METHOD

### Plant collection

The fresh stem bark of *Blighia unijugata* was harvested from Kwahu Asakraka in the Eastern Region of Ghana in the month of August 2016. It was authenticated by a Botanist of the Department of Herbal Medicine of the Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi, Ghana. A sample with voucher number KNUST/HM1/2016/SB 17 was prepared and kept at the herbarium of the department.

### Preparation of *Blighia unijugata* stem bark extract

The collected plant material was air-dried and powdered using a hammer mill (Liming®, China). An 800 g quantity was weighed, extracted with 70 % (v/v) ethanol by cold maceration for 3 days, and concentrated in a rotary evaporator (Rotavapor R-210, Buchi, Switzerland) at 60 °C. It was finally oven dried (Gallenkamp®, UK) at 50 °C to a constant weight. A dark slurry residue of 31.3 g was obtained and refrigerated until reconstituted into a solution for administration. This extract was labeled as EBU and stored for use in this study.

### Phytochemical screening

Screening of EBU for the presence of alkaloids, coumarins, flavonoids, glycosides, saponins, steroids, tannins, and triterpenoids was done using various tests previously described by Sofowora<sup>41</sup>, Evans<sup>42</sup>, and Shaikh & Patil<sup>43</sup>. These tests are described below:

#### Alkaloids (Dragendorff's Test)

A 0.5 g quantity of EBU was extracted with 20 ml of ammoniacal alcohol and filtered. The residue obtained after evaporating the filtrate was shaken with 1 % H<sub>2</sub>SO<sub>4</sub> and filtered. The filtrate was made alkaline with dilute ammonia solution and shaken with chloroform; the chloroform layer was separated and evaporated to dryness. The residue was

dissolved in 1 % H<sub>2</sub>SO<sub>4</sub> and one drop of Dragendorff's reagent (Sigma Aldrich Co. Ltd. Irvine, UK) was added. An orange-red precipitate shows alkaloids are present<sup>41-43</sup>.

#### Coumarins (NaOH paper test)

0.5 g of moistened EBU is taken in a test tube. The mouth of the test tube is then covered with filter paper that has been treated with 1 M NaOH. This is heated for a few minutes in a water bath. The presence of a yellow fluorescence from the paper under the UV light indicates the presence of coumarins<sup>43</sup>.

#### Flavonoids (Kumar test)

A 0.5 g quantity of EBU was dissolved in water and filtered; to this 2 ml of 10 % NaOH (BDH Laboratories, England) solution was added to produce a yellow coloration. A change in color from yellow to colorless on the addition of dilute HCl (BDH Laboratories, England) indicates the presence of flavonoids<sup>41-43</sup>.

#### Saponins (Frothing test)

A 1 g quantity of EBU was dissolved in distilled water and filtered. The filtrate was vigorously shaken and left to stand for 5 minutes. The presence of froth which persisted on standing indicated the presence of saponins in this extract<sup>41-43</sup>.

#### Phytosterols (Lieberman Burchard's test)

0.5 g of EBU was extracted with 5 ml chloroform and filtered into a test tube. Several drops of acetic anhydride (Sigma Aldrich Co. Ltd. Irvine, UK) were added and mixed carefully. Two (2) drops of concentrated H<sub>2</sub>SO<sub>4</sub> (BDH Laboratories, England) were gently added to the test tube through the side. The formation of violet to blue colored ring at the junction of two liquids shows the presence of steroid moiety<sup>41-43</sup>.

#### Terpenoids (Salkowski test)

0.5 g of EBU was extracted with 5 ml chloroform, and filtered into a test tube. The careful addition of concentrated H<sub>2</sub>SO<sub>4</sub> (BDH Laboratories, England) to the test tube wall produced a violet coloration showing the presence of terpenoids<sup>41-43</sup>.

#### Glycosides (General test)

A 0.5 g quantity of EBU was extracted by warming with 5 ml of dilute H<sub>2</sub>SO<sub>4</sub> in a water bath for 2 minutes and filtered. The filtrate was turned alkaline by adding 2-5 drops of 20 % NaOH (BDH Laboratories, England). 1 ml of Fehling's solution A and B (Sigma Aldrich Co. Ltd. Irvine, UK) was added to the filtrate and dried in a water bath for 2 minutes. The presence of a brick-red precipitate indicates the presence of glycosides<sup>41-43</sup>.

#### Tannins (Ferric Chloride test)

A 0.5 g quantity of EBU was boiled with distilled water for 5 minutes. The boiled extract was cooled, filtered, and made up to 25 ml. To 1 ml of the extract, 10 ml of distilled water was added followed by 2-10 drops of 1 % Ferric chloride solution (Sigma Aldrich Co. Ltd. Irvine, UK). A blue-black or blue-green coloration shows a positive test for tannins<sup>41-43</sup>.

### Experimental animals and husbandry

The processes and procedures employed during the experiment stayed in agreement with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH, Department of Health Services Publication). The procedures for the research work were approved by the Department of Pharmacology, KNUST, Ghana with an ethical approval number (FPPS/PCOL/0043/2015).

Male SD rats (140-180 g) were procured from the Centre for Plant Medicine Research, Mampong, Ghana, and acclimatized for one week prior to study initiation. Rats were housed in sets of five (5) under controlled colony conditions and fed *ad libitum*.

### Induction of hyperglycemia

The procedure for induction was adopted from the works of Sridevi *et al.*, (2011) and Ramachandra *et al.*, (2012) with some modifications<sup>44,45</sup>. Male rats were fasted overnight and given a single intraperitoneal injection of freshly prepared solution of STZ (50 mg/kg in 0.1 M citrate buffer, PH 4.5). Rats were given 20 % glucose for 24 hours thereafter to control for any STZ-induced hypoglycemia. Blood glucose was checked daily from tail vein bleeds using a glucometer (Acu-Chek®, Roche, Mannheim, Germany). Rats with consistent fasting blood glucose (FBG) greater than 250 mg/dl (13.8 mmol/L) after 7 days of induction were considered to be diabetic and included in the study.

### Drug preparation, animal groupings, and drug administration

EBU and glibenclamide (Daonil® 5 mg tablet, Sanofi-Aventis, Guildford, UK) were dissolved or suspended in measured quantities of distilled water and administered orally by gavage in volumes not greater 5 ml/dose/day. The diabetic rats were randomly divided into five groups (n=5) and treated with 2 ml/kg distilled water, 100 and 200 mg/kg of EBU, or 5 mg/kg glibenclamide respectively for 28 days (to mimic the commonly used drug course). Glibenclamide was selected as a positive control because we hypothesized that EBU similarly increases the secretion of insulin as glibenclamide. EBU doses were selected from an earlier experiment in the study of EBU on uterine fibroids<sup>38</sup> and those for Glibenclamide were from what was commonly used in literature<sup>46-48</sup>. A normal control group (n=5), treated with distilled water was kept under similar experimental conditions. The rats in each group were weighed at the beginning and end of the experiment.

On the 28th day after drug administration, blood samples (3 ml) were collected from the severed jugular vein of rats using serum-separating gel tubes (BD Vacutainer® blood collection Tube Product, USA). Blood was allowed to clot at room temperature (27 °C) after which the gel tubes were centrifuged (Heraeus Labofuge 300, UK) at 3000 rpm for 20 minutes. The clear supernatant was pipetted in plain tubes to conduct biochemical analysis. Serum lipid profiles (TC, TG,

HDL-c, LDL-c, VLDL-c) and fasting blood glucose (FBG) was determined using an automated clinical chemistry analyzer (ABX Pentra C200, Horiba Medical, USA). Values of the atherogenic index (LDL-c / HDL-c), coronary risk index (TC/HDL-c), and log (TG /HDL-c) were calculated<sup>49-51</sup>. From the initial and final weights, the percentage changes in the body weight of each group were determined.

### Statistical analysis

The data obtained were analyzed with GraphPad Prism (version 6) and values were expressed as mean ± standard error of the mean (SEM). Data were analyzed with one-way analysis of variance (ANOVA) and suitable *post hoc* tests. P values ≤ 0.05 was considered statistically significant.

## RESULTS

### Phytochemical test

Phytochemical tests are the preliminary tests that identify the presence of biologically active secondary metabolites. These secondary metabolites are considered to be responsible for the pharmacological action of various plant extracts. EBU stem-bark extract tested positive for tannins, saponins, terpenoids, glycosides, and flavonoids (Table 1.0).

### Treatment with EBU reduces fasting blood glucose in STZ-induced diabetes mellitus rats

Administration of STZ caused a significant rise ( $p \leq 0.0001$ ) in blood glucose as indicated by the increased fasting blood glucose in the diabetic rats when compared to the normal control group (Figure 1. a). Treatment of the hyperglycemic rats with EBU caused a significant reduction ( $p \leq 0.001$ ) in fasting blood glucose in a manner like glibenclamide treatment ( $p \leq 0.001$ ) when both treatment groups when compared with the diabetic control groups (Figure 1. b).

Constituent	Result
Alkaloids	-
Coumarins	-
Flavonoids	+
Glycosides	+
Saponins	+
Steroids	-
Tannins	+
Terpenoids	+

+, present; -, absent; EBU, ethanolic stem bark extract of *Blighia unijugata*.

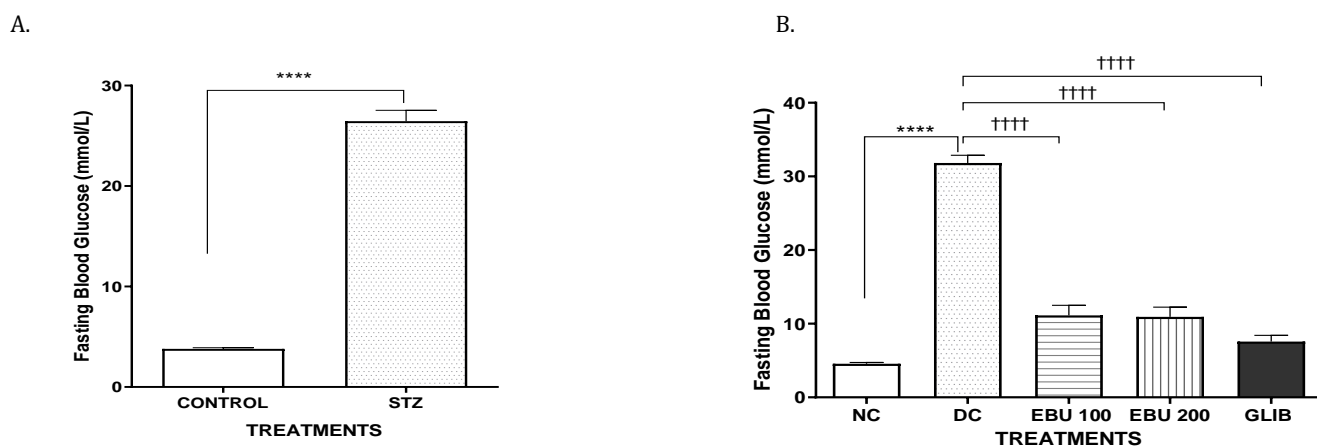


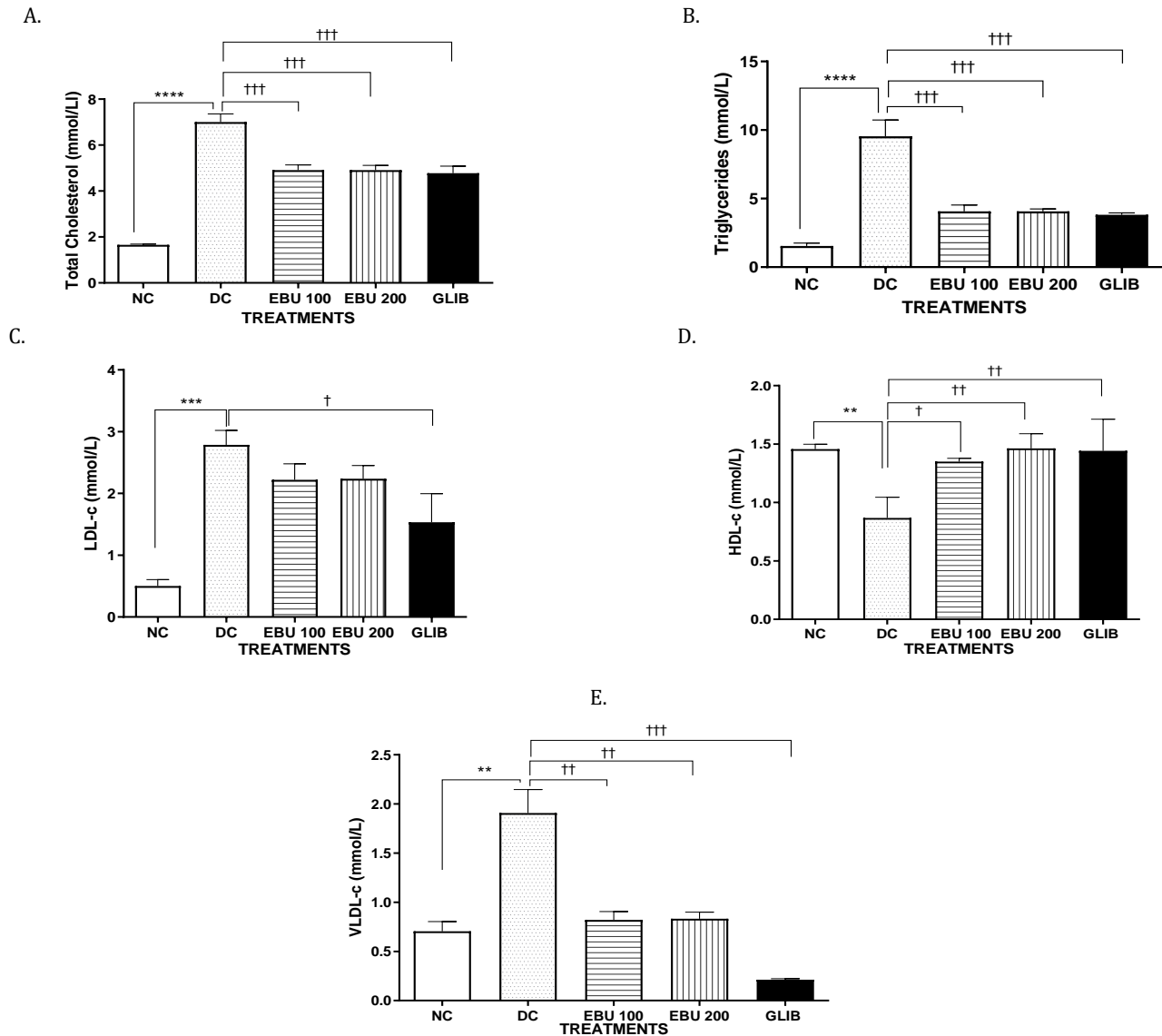
Figure 1: Fasting blood glucose in control and STZ-treated rats.

- Administration of STZ caused a significant rise in fasting blood glucose compared to the normal control (NC: no STZ, 2 ml/kg distilled water; \*\*\*\* $p \leq 0.0001$ )
- Administration of EBU (100 & 200 mg/kg) to hyperglycemic rats caused a significant reduction (\*\*\*\* $p \leq 0.0001$ ) in fasting blood glucose compared to the diabetic control group (DC: 2 ml/kg distilled water). This reduction by EBU was similar to that of the glibenclamide-treated group (GLIB, 5 mg/kg, \*\*\*\* $p \leq 0.0001$ ).

### Treatment with EBU mitigates dyslipidemia associated with diabetes rats

The diabetic rats had significant elevation (all  $p$ -values  $\leq 0.01$ ) of TC, TG, LDL-c, and VLDL-c and a significant decrease ( $p \leq 0.01$ ) in HDL-c when compared with the normal control group (Figure 2 a-e). Treatment of the diabetic rats with EBU caused a significant reduction (all  $p$ -values  $\leq 0.01$ ) in TC, TG,

and VLDL-c, and a significant increase (all  $p$ -values  $p \leq 0.05$ ) in HDL-c. LDL-c levels were not significantly ( $p > 0.05$ ) reduced when EBU treated group was compared with the diabetic control group. Similarly, glibenclamide caused a significant reduction ( $p \leq 0.001$ -  $p \leq 0.05$ ) in serum TC, TG, VLDL-c, and LDL-c and a rise in HDL-c ( $p \leq 0.01$ ) when compared with the diabetic control group.



**Figure 2: Lipid profile in normal control, diabetic control, EBU, and glibenclamide-treated groups of Sprague-Dawley rats after 28 days of treatment.**

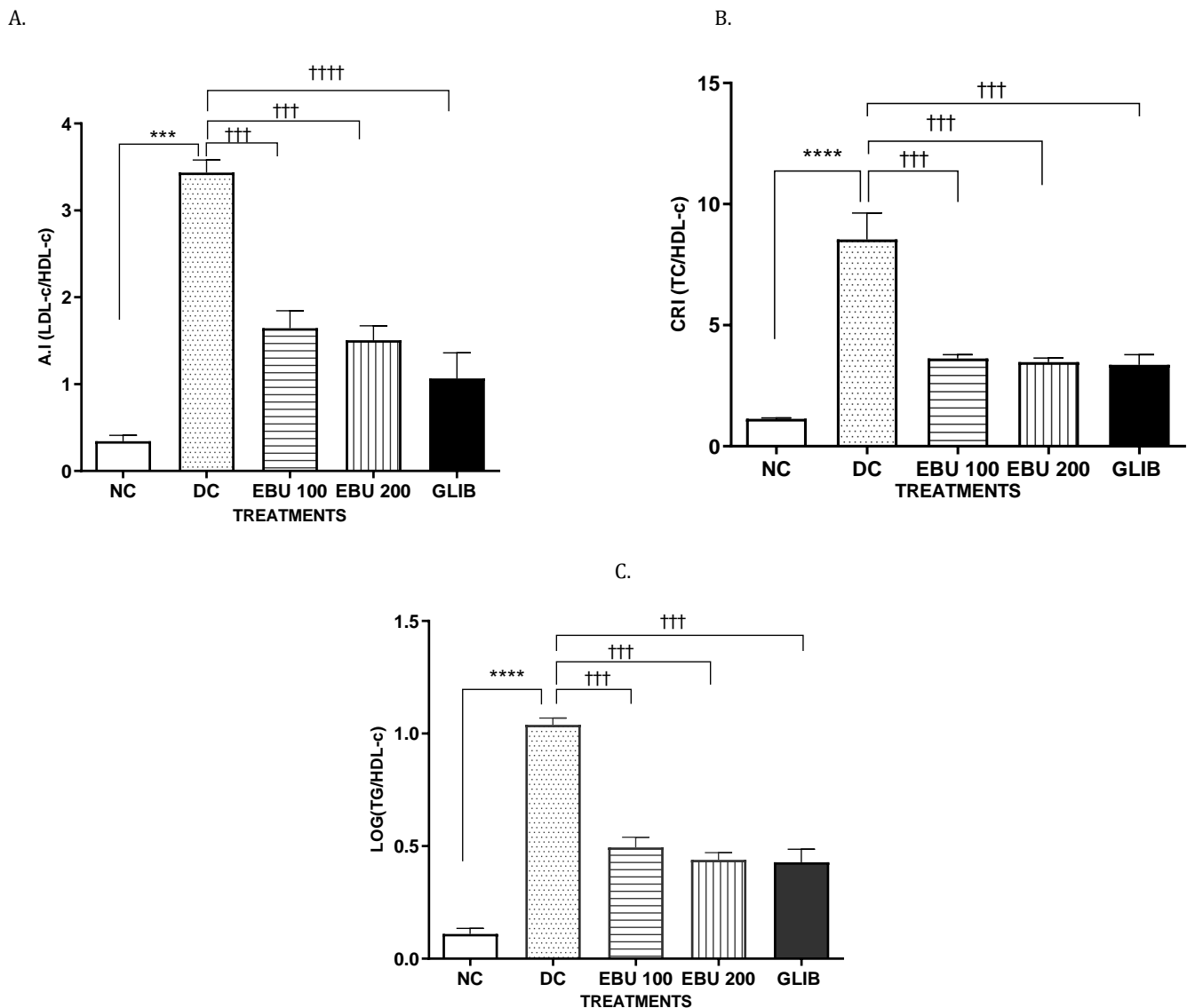
- A significant increase in total cholesterol (TC) was in the diabetic control group ( $****p \leq 0.0001$ ) when compared with NC. There was a significant reduction in TC when the diabetic rats were treated with EBU (100 & 200 mg/kg;  $+++p \leq 0.001$ ) and glibenclamide (GLIB, 5 mg/kg;  $+++p \leq 0.001$ ). as compared to the diabetic control group).
- A significant rise in triglycerides (TG) was observed in the diabetic control group ( $****p \leq 0.0001$ ) when compared with the NC group. There was a significant reduction in TG when the diabetic rats were treated with EBU (100 & 200 mg/kg) and glibenclamide (GLIB, 5 mg/kg). ( $+++p \leq 0.001$ - EBU & GLIB treated groups were compared with the diabetic control group using one-way ANOVA followed by Tukey's multiple comparisons test).
- Low-Density Lipoproteins:** A significant rise in Low-Density Lipoprotein cholesterol (LDL-c) was observed in the diabetic control group ( $***p \leq 0.001$ ) when compared with the NC group. Treatment with EBU (100 & 200 mg/kg) caused a reduction in LDL-c but it was not significant when compared with the diabetic control. In the glibenclamide (GLIB, 5 mg/kg) group versus the DC group, a significant reduction ( $\dagger p \leq 0.05$ ) in LDL-c was observed.
- High-Density Lipoproteins:** A significant decrease in HDL-c was observed in the diabetic control group ( $**p \leq 0.01$ ) when compared with the normal control group. There was a significant increase in HDL-c when the diabetic rats were treated with EBU (100 & 200 mg/kg) and glibenclamide (GLIB, 5 mg/kg). ( $\dagger p \leq 0.05$ ,  $\dagger\dagger p \leq 0.01$ - EBU & GLIB treated groups were compared with the diabetic control group using one-way ANOVA followed by Tukey's multiple comparisons test).
- Very Low-Density Lipoproteins:** A significant rise in Very Low-Density Lipoprotein cholesterol (VLDL-c) was observed in the diabetic control group ( $**p \leq 0.01$ ) when compared with the NC group. There was a significant reduction in VLDL-c when the diabetic rats were treated with EBU (100 & 200 mg/kg,  $\dagger\dagger p \leq 0.01$  for both doses) and glibenclamide (GLIB, 5 mg/kg,  $+++p \leq 0.001$ ). (EBU & GLIB treated groups were compared with the diabetic control group using one-way ANOVA followed by Tukey's multiple comparisons test).



### Treatment with EBU improves the poor atherogenic predictor indices associated with diabetes rats

The risk of atherogenesis and coronary artery disease development in the diabetic rats was high as seen in the significant increase ( $p \leq 0.001$ ) of the atherogenic index (AI),

coronary risk index (CRI), and log (TG/HDL-c) when compared with the normal control group (Figure 3. a-c). EBU and glibenclamide treatment resulted in a significant reduction ( $p \leq 0.001$ ) in AI, CRI, and log (TG/HDL-c) when compared to the diabetic control.



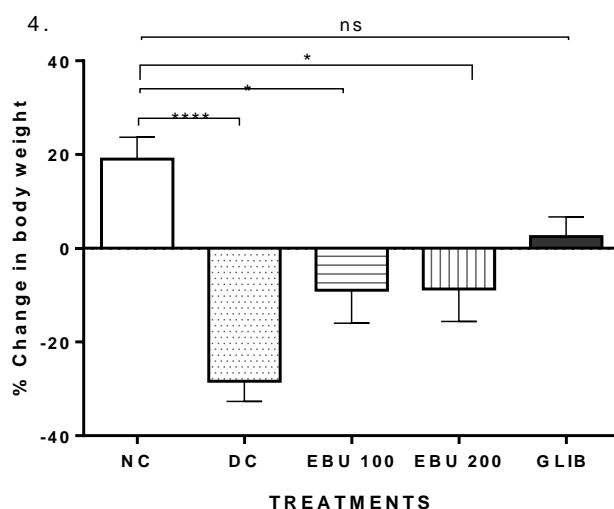
**Figure 3: Atherogenic predictor indices in normal control, diabetic control, EBU, and glibenclamide-treated groups of Sprague-Dawley rats after 28 days of treatment.**

- Atherogenic Index:** A significant increase in Atherogenic Index (AI) was observed in the diabetic control group ( $***p \leq 0.001$ ) when compared with the normal control group (NC). A significant reduction in AI was observed when the diabetic rats were treated with EBU (100 & 200 mg/kg) and glibenclamide (GLIB, 5 mg/kg). ( $+++p \leq 0.001$  for EBU &  $++++p \leq 0.0001$  for GLIB treated groups when compared with the diabetic control group using one-way ANOVA followed by Tukey's multiple comparisons test).
- Coronary Risk Index:** A significant increase in coronary risk index (CRI) was observed in the diabetic control group ( $****p \leq 0.0001$ ) when compared with the NC group. A significant reduction in CRI was observed when the diabetic rats were treated with EBU (100 & 200 mg/kg) and glibenclamide (GLIB, 5 mg/kg). ( $+++p \leq 0.001$  - EBU & GLIB treated groups were compared with the diabetic control group using one-way ANOVA followed by Tukey's multiple comparisons tests).
- Log (TG/HDL-c):** A significant increase in Log (TG/HDL-c) was observed in the diabetic control group ( $****p \leq 0.0001$ ) when compared with the normal control group (NC). A significant reduction in Log (TG/HDL-c) was observed when the diabetic rats were treated with EBU (100 & 200 mg/kg) and glibenclamide (GLIB, 5 mg/kg). ( $+++p \leq 0.001$  - EBU & GLIB treated groups were compared with the diabetic control group using one-way ANOVA followed by Tukey's multiple comparisons test).

### Treatment with EBU mitigates the weight loss associated with diabetes rats

The diabetic control rats had a significant loss ( $p \leq 0.0001$ ) in body weight (Figure 4). The loss in body weight was

significantly lower ( $p \leq 0.01$ ) after treatment of the diabetic rats with EBU when compared with the normal control group. Rats treated with glibenclamide had a non-significant ( $p > 0.05$ ) change in body weight when compared to the normal control group.



**Figure 4: Percentage Changes in body weights in normal control, diabetic control, EBU, and glibenclamide-treated groups of SD rats.**

Rats in the Diabetic control group had a significant loss of body weight when compared to the normal control group. This loss in body weight was significantly improved after treatment with EBU (100 and 200 mg/kg) and glibenclamide (GLIB, 5 mg/kg). (Values are presented as means  $\pm$  SEM,  $n = 5$ , ns: non-significant, \*\*\*\* $p < 0.0001$ , \* $p < 0.01$ -Diabetic control group and drug treatment groups were compared to the normal control group using one-way ANOVA followed by Tukey's multiple comparisons test).

## DISCUSSION

We studied the anti-hyperglycemic and anti-hyperlipidemic activities of the ethanolic stem bark extract of *Blighia unijugata* (EBU) in streptozotocin (STZ) - induced diabetic SD rats. Treatment of the hyperglycemic rats with EBU and glibenclamide reduced the elevated blood glucose levels. Glibenclamide stimulates high insulin release from the remaining beta cells and hence improves insulin function to cause the reduction of elevated blood glucose.

In diabetic rats, impaired insulin secretion leads to uncontrolled action of lipolytic hormones in the adipose tissue to release free fatty acids which are eventually converted by the liver. Triglycerides and cholesterol levels are elevated due to the inhibition of lipoprotein lipases and metabolic abnormalities respectively <sup>52</sup>. An ideal therapeutic agent should be one that in addition to establishing a proper glycemic control, must have a favorable activity on plasma lipids, particularly LDL-c. In addition to the anti-hyperglycemic action, EBU also affected key markers of the lipid profile. EBU and glibenclamide showed a reduction in TG, TC, and VLDL-c and an elevation in HDL-c levels. Glibenclamide is a hypoglycemic agent and its' effect on lipid profile could suggest this activity was achieved by resolving the primary diabetic condition. With EBU, this anti-hyperlipidemic activity seen could be from resolving the underlying diabetic condition and/or by a direct anti-hyperlipidemic effect. The ability of EBU to decrease total cholesterol in SD rats has been reported <sup>38</sup>, and unpublished data from our lab suggests an anti-hyperlipidemic activity of EBU in hyperlipidemic rats. EBU's direct action on hyperlipidemia could account for its effect on the lipid profile. The elevated level of HDL-c shown by treatment with EBU is highly desirable as it decreases the risk of cardiovascular

complications. The findings from the reduction in the atherogenic risk predictor indices [atherogenic index, coronary risk index, and log (TG/HDL-c)] further corroborate the reduced risk of atherosclerosis and cardiovascular diseases.

Administering EBU caused a reduction in weight loss in diabetic rats. The loss in weight in T2DM can be attributed to protein wasting because carbohydrate is unavailable as an energy source <sup>53</sup> and impaired insulin secretion and function decreases glycogen synthesis <sup>44, 45</sup>. The reduction in weight loss seen after treatment with EBU and glibenclamide may be due to increased insulin secretion.

Mohammed et al reported similar anti-hyperglycemic and weight gain activity using the chloroform fraction of *B. unijugata* leaves. When administered, this fraction reduced glucose levels and caused a reversed the weight loss in diabetes albino rats <sup>54</sup>. This indicated the anti-diabetic agents found in the leaves, may also be present in the stem-bark. Also considering previous reports on *Blighia sapida* <sup>40</sup>, the similar findings with *Blighia unijugata* suggest the anti-hyperglycemic and anti-hyperlipidemic effects observed may be common among the members of the genus.

The bioactive agents present in various medicinal plants have reduced blood glucose levels by mechanisms such as exerting insulin-like effects, protecting beta cells, acting as antioxidants, increasing glucose oxidation, hepatic glycogen, and glucose uptake, decreasing intestinal glucose absorption and gluconeogenesis among others <sup>55</sup>. EBU contained tannins, saponins, terpenoids, glycosides, and flavonoids. Glycosides, alkaloids, terpenoids, and flavonoids have been reported to have anti-diabetic effects by increasing the release of insulin and regeneration of the pancreatic beta cells <sup>56</sup>. Saponins

stimulate insulin secretion hence lower blood glucose levels<sup>55</sup>. Flavonoids and terpenoids also have antioxidant properties and hence may mitigate oxidative stress and the destruction of the remnant beta cells<sup>40, 56-61</sup>. These actions may account for the anti-hyperglycemic effect of EBU. Alkaloids, flavonoids, saponins, triterpenoids, and polyphenols among others, have been reported to exert anti-hyperlipidemic effects by inhibiting lipid biosynthesis, increasing lipid metabolism, redistribution, transport, and excretion, and mitigating insulin resistance<sup>62, 63</sup>. This may explain the anti-hyperlipidemic action of EBU which also contained saponins, terpenoids, glycosides, and flavonoids.

In conclusion, our work reveals that EBU possesses anti-hyperglycemic and anti-hyperlipidemic properties as shown in the STZ-induced diabetes mellitus model. This study, therefore, adds to the growing body of work which gives scientific credence to the medicinal value of *Blighia unijugata*. Further studies to isolate, purify and characterize the bioactive compounds, and to elucidate the exact mechanism will be required in future studies.

### Disclosures:

The authors have declared that no competing interests exist.

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