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ORIGINAL ARTICLE

# Leaf Extracts of *Glyphaea brevis* Attenuate High Blood Glucose and Lipids in Diabetic Rats Induced with Streptozotocin

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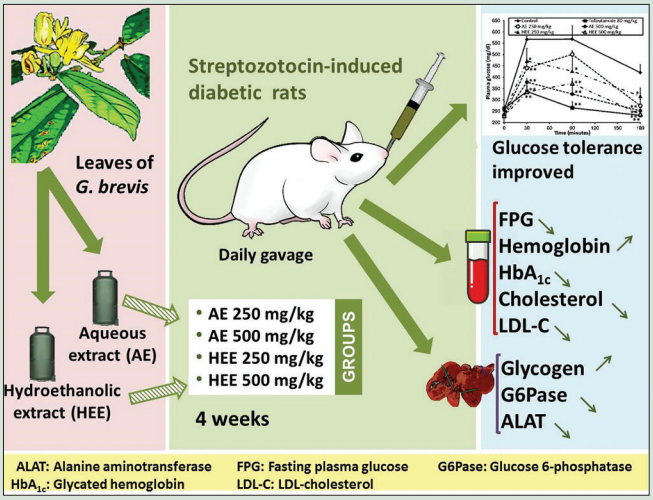
## ABSTRACT

**Background:** Diabetes is a non-communicable disease causing impairment of carbohydrate and lipid metabolisms. Previous ethnobotanical surveys showed that leaves of *Glyphaea brevis* (*G. brevis*) are used conventionally in the treatment of diabetes with limited scientific evidence. **Objective:** We aimed to determine the effects of aqueous (AE) and hydroethanolic extracts (HEE) of *G. brevis* leaves in attenuating diabetes-linked hyperglycemia and hyperlipidemia in an animal model. **Materials and Methods:** Thirty streptozotocin-induced diabetic male rats were divided into six groups receiving the following daily treatments for 4 weeks orally: control (distilled water), reference (tolbutamide 80 mg/kg) and 4 tests (AE 250 mg/kg, AE 500 mg/kg, HEE 250 mg/kg, and HEE 500 mg/kg). The effects of each treatment on postprandial hyperglycemia were assessed using oral glucose tolerance and oral starch tolerance tests. Blood was collected to assess the effects of treatments on fasting plasma glucose, glycated hemoglobin ($HbA_{1c}$), and lipid profile. Liver glycogen and gluconeogenic enzyme activity were also measured. **Results:** AE- and HEE-treated rats had 36%–64% lower fasting blood glucose levels, 34%–73% lower postprandial glycemia, and 15%–75% lower $HbA_{1c}$ than rats from control group ($P < 0.01$). AE and HEE treatments also brought about a significant increase in liver glycogen levels and lower gluconeogenic enzyme activity ($P < 0.01$). Extract-treated groups also had lower plasma total cholesterol and low-density lipoprotein-cholesterol concentrations ($P < 0.01$). **Conclusion:** These results suggest that treatment with leaf extracts of *G. brevis* is effective in attenuating hyperglycemia and hyperlipidemia in streptozotocin-induced diabetic rats. Future studies will determine the active compounds accountable for these beneficial effects.

**Key words:** Diabetes, *Glyphaea brevis*, hyperglycemia, hyperlipidemia

## SUMMARY

- Aqueous and hydroethanolic extracts of *Glyphaea brevis* were evaluated for their potential effects on some metabolic disturbances such as high blood glucose and lipids in an experimental model of diabetes induced by streptozotocin. The treatment of diabetic rats with *G. brevis* extracts for 28 days significantly reduced blood glucose (fasting and postprandial), glycated hemoglobin, lipids (total cholesterol, low-density lipoproteins-cholesterol and triglycerides) and hepatic glyconeogenesis enzymes activity. We conclude that *G. brevis* extract possess antihyperglycemic and antiyperlipidemic effects that may find applications in the management of diabetes.




**Abbreviations Used:** AE: Aqueous extract; ALAT: Alanine aminotransferase; ANOVA: Analysis of variance; cAMP/PKA: Cyclic adenosine monophosphate/protein kinase A; DMRT: Duncan's Multiple Range Test; EDTA: Ethylenediaminetetraacetic acid; FPG: Fasting plasma glucose; G6Pase: Glucose-6-posphatase; GI: Glycemic index; $HbA_{1c}$: Glycated hemoglobin; HDL: High-density lipoproteins; HEE: Hydroethanolic extract; HMGCoA: Hydroxyl-methyl glutaryl-CoA; LDL: Low-density lipoproteins; TIG: Total incremental glucose.

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## INTRODUCTION

The term "diabetes" is commonly used to describe a metabolic disorder of multiple etiologies characterized by persisting hyperglycemia with disturbances in the metabolism of carbohydrates, fats, and proteins and such metabolic disturbances are consequences of the impairment of insulin secretion and/or insulin action.[1] The world prevalence of diabetes was estimated to 285 million people affected and is supposed to increase to 439 million by 2030.[2] At the same time, diabetes was reported to have caused the death of 3.96 million adult people worldwide, with 6% of deaths in African adults, and 15.7% in North America.[3] Thus, diabetes is viewed as one of the leading causes of premature mortality and situation is expected to aggravate, especially



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in developing countries since diabetes prevalence is increasing along with subsequent hyperlipidemia and cardiovascular risk.[4] All these epidemiological observations highlight the emergency of investing in primary and secondary prevention to tackle the progression of this public health threat. Unfortunately, current strategies for prevention and treatment have failed to reverse the progression of diabetes and the search for innovative treatments remains imperative.

Medicinal plants have been in use in the management of various diseases and their symptoms in various civilizations throughout the ages. The World Health Organization has encouraged such initiatives, especially in low-and middle-income countries where access to modern medicine is limited.[5] In the area of diabetes research, several studies have been aimed at evaluating the antidiabetic and antihyperlipidemic properties of various plants used in local pharmacopeia and in some cases, have brought about a scientific validation of the knowledge (sometimes ancestral) of many populations around the globe. Due to the huge diversity of molecules occurring naturally in plants, mechanisms of action are diversified, including stimulation of insulin secretion,[6] reduction of the gluconeogenetic pathway,[7] stimulation of hepatic glycogenesis,[8] and reduction of intestinal absorption of glucose.[9] These facts suggest the opportunity to investigate further plant sources of antidiabetic agents to obtain low-cost and easily-available therapies for diabetes.

*Glyphaea brevis*, a Tiliaceae, is a medicinal plant with various uses in Africa and South America. For instance, the leaves are used in a decoction for the treatment of palpitations, hepatitis and poisoning in Cameroon,[10] for the treatment of fever and female sterility in Ivory Coast,[11] and for the relief of sleepiness, bacterial infections, convulsions, sexual impotency, and some age-related brain disorders in Nigeria.[12-14] Burned seeds or fruits are ground and mixed with salt to treat cough as well as heart and tooth diseases in the Democratic Republic of Congo.[15] Despite this variety of therapeutic uses, it is only during the last decade that a growing number of serious scientific studies focused on the possible health benefits of *G. brevis*. Such studies revealed its anticonvulsant,[14] antioxidant,[16,17] α-amylase-inhibiting,[18] anti-inflammatory,[17] and antimicrobial[17,19] properties. The decoction of the leaves of *G. brevis* is also in use in the treatment of diabetes in Lagos State, Nigeria, as revealed in a recent ethnobotanical survey.[20] However, diabetes is a complex and multifactorial disease with only a few observable symptoms (fatigue, polyuria, and soars on feet) that can easily be ascribed to other illnesses. As a result, traditional systems of medicine are not always equipped with suitable tools to diagnose diabetes. Therefore, a scientific study of the potential ability of *G. brevis* to alleviate hyperglycemia and hyperlipidemia characterizing diabetes would provide scientific justification or rebuttal to its use in the traditional system of medicine.

This study therefore aimed to assess the possible lowering effects of extracts of *G. brevis* leaves on blood glucose and lipids *in vivo* in diabetic rats induced with streptozotocin.

## MATERIALS AND METHODS

### Animals

Male Albino rats from the Wistar strain (*Rattus norvegicus*), aged 12 weeks and weighing 180–200 g were procured from our laboratory. They were maintained in polypropylene cages with husk renewed every 24 h under controlled conditions (room temperature of 25°C, 12/12-h dark/light cycle) and were free to access food and water. All the investigations using experimental animals in this work were approved by the Institutional Ethics Committee of the University of Douala.

### Plant material

Green leaves from the aerial part of several plants of *G. brevis* were collected between March and May in the city of Douala, Cameroon. Taxonomy was confirmed at the Cameroon National Herbarium, Yaounde, Cameroon (Voucher specimen no. 10781/SRF/Cam). The leaves were dried in an oven at 40°C till constant weight and ground into a fine powder using an FFC-37 mill (Agro-Mac, Douala, Cameroon). For aqueous extraction, 150 g of dry leaf powder was introduced in 2 L of distilled water. The mixture was boiled for 15 min and filtered after cooling at room temperature. The resulting filtrate was evaporated at 50°C and the remaining powder (aqueous extract or AE) was kept in a sealed flask at 2°C with a yield of 18.8% (w/w). Hydroethanolic extraction was performed as follows: 150 g of dry leaf powder was introduced in 2 L of a hydroethanolic solvent (1:1) and the mixture was hermetically covered. Forty-eight hours later, the mixture was filtered at room temperature and the resulting filtrate (Filtrate 1) was collected and kept at 2°C. The remaining wet powder was once again extracted for 48 h under the same conditions as for the first extraction. This yielded a second filtrate (Filtrate 2). Both filtrates (1 and 2) were mixed and evaporated at 50°C. This process led to hydroethanolic extract (HEE) that was also kept in a sealed flask at 2°C with a yield of 21% (w/w).

### Reference drug

Tolbutamide (Sigma-Aldrich, MO, USA), a pharmaceutical antidiabetic agent, was used as a reference to evaluate the possible metabolic effects of *G. brevis*.

### Diabetes experimental induction

Diabetes was induced in overnight-fasted rats were rendered diabetic by intravenous injection of a single dose of streptozotocin (50 mg/kg bw) solubilized in a citrate buffer (0.1 M; pH 4.5). Diabetic status was assessed 5 days later by measurement of fasting blood glucose, urinary volume and qualitative detection of sugar in the urine. Animals presenting a fasting glycemia >235 mg/dl were retained for the study.[21]

### Starch cooking

Cooked starch was prepared by dissolving 10 g of cornstarch (Sigma-Aldrich) in 100 ml of distilled water. The resulting mixture was boiled for 15 min with constant stirring and cooled at room temperature.

### Experimental design

Thirty diabetic male rats were divided into six groups of five animals each in metabolic cages (1 animal per cage) according to the following:

- Group I (Control): Control group (Diabetic + distilled water)
- Group II (Tolbutamide): Reference group (Diabetic + Tolbutamide 80 mg/kg)
- Group III (AE250): Aqueous extract (AE) treated group (Diabetic + AE 250 mg/kg)
- Group IV (AE500): Aqueous extract (AE) treated group (Diabetic + AE 500 mg/kg)
- Group V (HEE250): HEE treated group (Diabetic + HEE 250 mg/kg)
- Group VI (HEE500): HEE treated group (Diabetic + HEE 500 mg/kg).

All treatments were administered by daily gastric intubation over 28 days. The rats had free access to food and drinking water. Body weight, food intake as well as water intake were measured every 2 days while 24-h urine collections and measurements were performed every week.

On day 2, the effects of *G. brevis* on oral glucose tolerance were assessed by monitoring the changes in glycemia after single intubation of glucose. Rats were made fasting for 12 h before the test. 15 min after

assigned treatment (water, tolbutamide, AE, or HEE), a glucose solution (2.22 M; 5 ml/kg bw) was given to all rats by the oral route. Blood was collected from each rat at the before the glucose load as well as 30, 90 and 180 min thereafter.

On day 5, changes in glycemia following cooked starch intubation were monitored to assess the effects of *G. brevis* on oral starch tolerance. Rats were fasted for 12 h before the test. 15 min after assigned treatment (water, tolbutamide, AE, or HEE), cooked starch (0.2 g/ml; 5 ml/kg bw) was administered orally to all rats. Blood samples were collected from each rat at the beginning of the test and 30, 60, 120, and 240 min after starch intubation.

On the final day ($28^{th}$), all rats were sacrificed under anesthesia (α-ketoglutarate-ketamine) after a 12-h fast.

## Blood collection and processing

Blood was collected from the tail tip of each rat in sodium fluoride tubes for the oral glucose and starch tolerance tests. After sacrifice, jugular vein-derived blood samples were collected in three tubes: Ethylenediaminetetraacetic acid tube for hemoglobin quantification, sodium fluoride tube for glucose assessment and heparin tube for the assessment of other biochemical parameters such as lipid profile. All blood collections undergone 10-min centrifugation at 2000 g. The resulting plasmas were stored at −20°C in Eppendorf tubes for biochemical analysis.

## Liver collection and processing

The liver of each rat was taken after dissection, rinsed in a 0.9% NaCl solution and dried on filter paper. A total of 1.5 g of the liver was kept at-20°C for glycogen quantification while the remaining part of the liver was mashed in a buffer (0.15 M KCl/10 mM $KH_2PO_4$; pH 7.4) on ice. The mixture undergone a 10-min centrifugation at 2000 g. and supernatant was collected in Eppendorf tubes and stored at −20°C for the assessment of gluconeogenic enzymes alanine aminotransferase (ALAT) and glucose-6-phosphatase.

## Biochemical analysis of carbohydrate metabolism

Plasma and urinary glucose were measured using the glucose oxidase method.[22] Plasma total incremental glucose (TIG) for each rat was calculated by summing the variations in blood glucose after correction for time zero following starch intubation.[23] The glycemic index (GI) was calculated using the formula:. $GI = \frac{TIG_x}{TIG_{Control}} \times 100$ [23] Liver glycogen was quantified through the tissue digestion method using potassium hydroxide.[24] Liver ALAT activity was measured by the 2,4-dinitrophenylhydrazine method.[25] The phosphomolybdic reaction method was used to measure the liver glucose-6-phosphatase activity.[26]

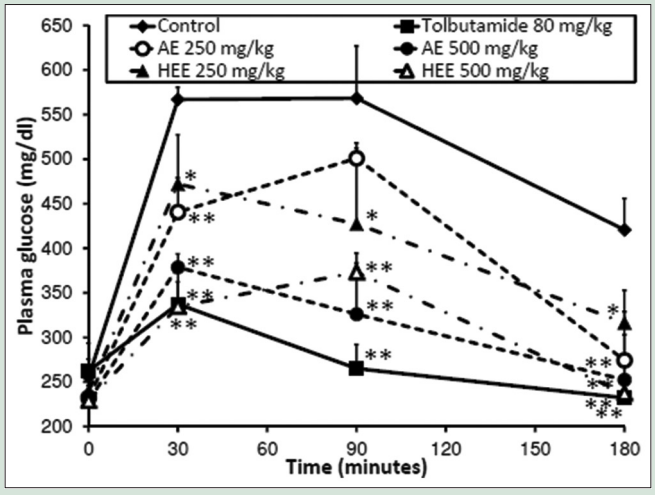


**Figure 1:** Changes in postprandial plasma glucose in streptozotocin-induced diabetic rats treated with aqueous or hydroethanolic extracts of *Glyphaea brevis* during oral glucose tolerance test (means ± standard deviation, $n = 5$). *$P < 0.05$; **$P < 0.005$ as compared with control group

## Biochemical analysis of lipid metabolism

Plasma total cholesterol (TC) was assessed by the cholesterol oxidase method[27] while high-density lipoprotein (HDL) cholesterol was assessed directly after an antibody-antigen reaction.[28] Triglycerides were measured by the method described earlier by Bucolo and David.[29] Low-density lipoprotein cholesterol (LDL-cholesterol) concentrations in the plasma were mathematically estimated using the equation derived by Friedewald *et al*.[30] Atherogenic risk indices were determined by calculating the TC/HDL-cholesterol and LDL-cholesterol/HDL-cholesterol ratios.

## Biochemical analysis of protein metabolism

Plasma protein concentration was measured by the enzymatic-colorimteric method. Hemoglobin was measured by reaction with potassium ferricyanide,[31] while glycated hemoglobin ($HbA_{1c}$) was assessed by the sulfuric acid/phenol method.[32]

## Statistical analysis

Statistical analysis of data was performed using the SPSS for Windows software version 23.0 (SPSS Inc., Chicago, Illinois, USA). Data normality was evaluated with the Levene's test. The one-way analysis of variance and Duncan's Multiple Range Test as *post hoc* test were used to compare means for each variable among the different groups within a 95% confidence interval. Results are presented as means ± standard deviation.

# RESULTS

## Glucose tolerance test

Oral administration of glucose to rats triggered a significant increase ($P < 0.01$) in their blood glucose concentrations 30 min later on [Figure 1]. However, postprandial plasma glucose was reduced significantly ($P < 0.05$) for extract-treated rats than those from the control group, resulting in lower values ($P < 0.005$) of TIG as well as GI [Table 1].

## Starch tolerance test

The starch load led to a significant increase in plasma glucose concentration only in the control (+79.20%) and HEE 250 (+41.07%)

**Table 1:** Total incremental glucose and glycemic index in streptozotocin-induced diabetic rats treated with aqueous or hydroethanolic extracts of *Glyphaea brevis* during oral glucose tolerance test

| Group | TIG (mg/dl) | GI (%) | Inhibition (%) |
|---|---|---|---|
| Control | 790.00±25.16$^a$ | 100±0.00$^a$ | - |
| Tolbutamide | 92.00±41.78$^b$ | 11.64±5.28$^b$ | 88.36±5.28$^a$ |
| AE 250 | 519.00±68.69$^c$ | 65.69±8.70$^c$ | 34.31±8.70$^b$ |
| AE 500 | 239.67±48.81$^d$ | 30.34±6.18$^d$ | 69.66±6.18$^c$ |
| HEE 250 | 496.67±81.08$^c$ | 62.86±10.27$^c$ | 37.13±10.27$^b$ |
| HEE 500 | 263.00±59.19$^d$ | 33.29±7.49$^d$ | 66.71±7.49$^c$ |

In each column, values not sharing the same superscript letter are significantly different at $P<0.05$ (DMRT). Mean±SD ($n=5$). TIG: Total incremental glucose; GI: Glycemic index; AE: Aqueous extract; HEE: Hydroethanolic extract; DMRT: Duncan's multiple range test; SD: Standard deviation

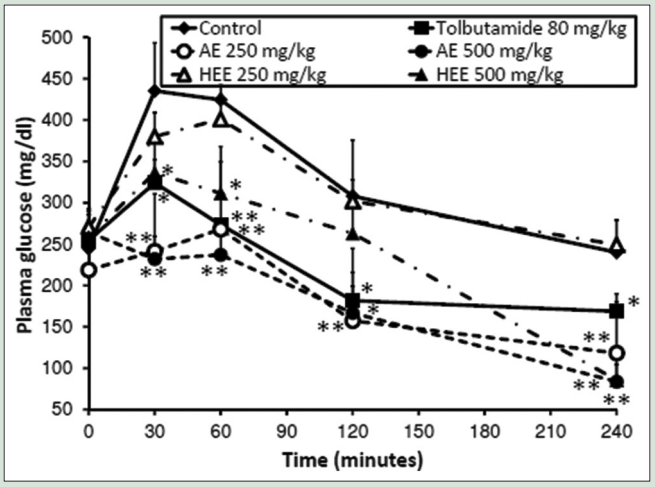


**Figure 2:** Changes in postprandial plasma glucose in streptozotocin-induced diabetic rats treated with aqueous or hydroethanolic extracts of *Glyphaea brevis* during oral starch tolerance test (means ± standard deviation, $n = 5$). *$P < 0.05$; **$P < 0.005$ as compared with control group

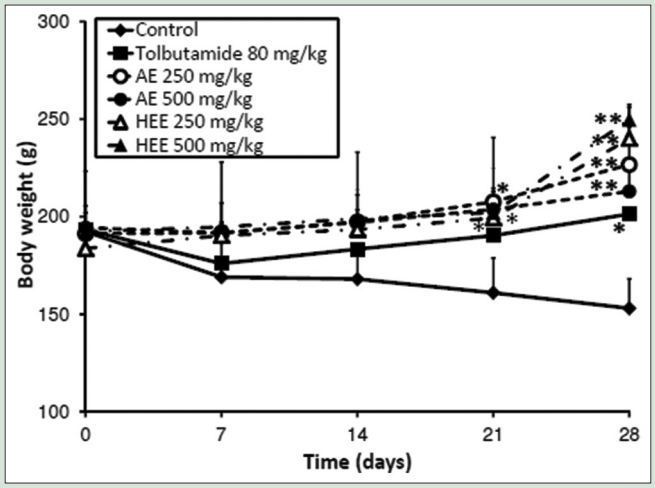


**Figure 3:** Changes in body weight of streptozotocin-induced diabetic rats treated with aqueous or hydroethanolic extracts of *Glyphaea brevis* (means ± standard deviation, $n = 5$). *$P < 0.05$; **$P < 0.005$ as compared with control group

**Table 2:** Total incremental glucose and glycemic index inf streptozotocin-induced diabetic rats treated with aqueous or hydroethanolic extracts of *Glyphaea brevis* during oral starch tolerance test

| Group | TIG (mg/dl) | GI (%) | Inhibition (%) |
|---|---|---|---|
| Control | 467.01±81.41$^{a}$ | 59.11±10.30$^{a}$ | - |
| Tolbutamide | 62.67±28.11$^{b}$ | 7.93±3.56$^{b, c}$ | 86.58±6.02$^{ab}$ |
| AE 250 | 125.33±32.14$^{c}$ | 15.86±4.07$^{b}$ | 73.16±6.88$^{a}$ |
| AE 500 | 62.37±13.80$^{d}$ | 7.89±1.75$^{c}$ | 86.65±2.95$^{b}$ |
| HEE 250 | 271.02±75.34$^{c}$ | 34.30±9.54$^{d}$ | 41.97±16.13$^{c}$ |
| HEE 500 | 149.32±90.69$^{d}$ | 18.90±11.48$^{b-d}$ | 68.02±19.42$^{a-c}$ |

In each column, values not sharing the same superscript letter are significantly different at $P<0.05$ (DMRT). Mean±SD ($n=5$). TIG: Total incremental glucose; GI: Glycemic index; AE: Aqueous extract; HEE: Hydroethanolic extract; DMRT: Duncan's multiple range test; SD: Standard deviation

groups, as depicted in Figure 2. At the end of the test, final glycemia was significantly lower than initial one in rats from extract-treated groups (AE 250: −47.45%; AE 500: −68.18%; HEE 500: −69.96%; $P < 0.05$), resulting in reduced values of TIG and GI of cornstarch [Table 2].

## Fasting plasma glucose, liver glycogen and gluconeogenic enzymes

Four-week treatment of diabetic rats with tolbutamide and both *G. brevis* extracts brought about significant decreases in fasting plasma glucose [Table 3]. Most important reductions were found in groups TOLB 80 (−58.63%, $P < 0.001$) and AE 500 (−58.35%, $P < 0.001$). Activities of hepatic glucose-6-phosphatase as well as ALAT were significantly reduced in extract-treated groups with the highest reduction found in group AE 500 ($P < 0.01$). Extract treatment significantly increased liver glycogen concentrations with the highest increases obtained with aqueous extract. Both urinary glucose and urinary volume were significantly lower in those groups in comparison with the control group ($P < 0.005$). From a comparative point of view on *G. brevis* extracts, the aqueous extract was the most efficient in improving glucose metabolism in diabetic rats.

## Lipid metabolism

Biochemical parameters characterizing lipid metabolism are presented in Table 4. The 28-day treatment with extracts triggered a decrease in plasma cholesterol (TC and LDL-cholesterol) concentrations. The most important antihypercholesterolemic effects were observed with the HEE. Extract treatment did not alter HDL-cholesterol levels in comparison with the control group. *G. brevis* extracts did not bring about significant changes in plasma triglycerides levels except in the HEE 250 group. Calculation of atherogenicity indices TC/HDL and LDL/HDL revealed significantly lower ($P < 0.01$) values predicting a lower atherogenic exposure for the rats in the extract-treated groups than for those in the control group.

## Protein metabolism

Table 5 presents the concentrations of blood proteins, total hemoglobin and $HbA_{1c}$ of rats at the end of the study. Treatment with extracts led to higher values of total protein concentrations than in the diabetic control group. The same trend was noticed for the total hemoglobin concentrations ($P < 0.01$). $HbA_{1c}$ percentages in rats treated with *G. brevis* extracts or tolbutamide were significantly reduced in comparison to those occurring in the untreated control group ($P < 0.001$).

## Body weight

The results from the monitoring of the rats ponderal changes during the study period are represented in Figure 3. Diabetes caused a drastic loss in body weight in the control group throughout the study (−20.31%, $P < 0.01$) while body weight was not markedly affected during the first 2 weeks in rats treated with extracts. Yet, an increase in body weight was noticed in the treated groups during the last 2 weeks (AE 500 mg/kg: +9.80%, $P < 0.05$; HEE 500 mg/kg: +29.81%, $P < 0.005$).

# DISCUSSION

Our study aimed to assess the possible antihyperglycemic and antihyperlipidemic effects of extracts of *G. brevis* leaves *in vivo* in a streptozotocin model of diabetes. Streptozotocin is known to induce type-1 diabetes in experimental animals since it triggers β-cells destruction as proposed by several authors.[33,34]

Treatment with *G. brevis* extracts caused a significant decrease of hyperglycemia in diabetic rats [Table 3]. In many plants, flavonoids, chalcones, tannins, xanthones, organic acids, cinnamic acid and derivatives, sugars, curcuminoids, and alkaloids have been identified as

**Table 3:** Biochemical parameters of carbohydrate metabolism in streptozotocin-induced diabetic rats treated with aqueous or hydroethanolic extracts of *Glyphaea brevis*

| Parameter | Control | Tolbutamide (80 mg/kg) | AE | | HEE | |
|---|---|---|---|---|---|---|
| | | | 250 mg/kg | 500 mg/kg | 250 mg/kg | 500 mg/kg |
| Plasma | | | | | | |
| Initial glucose (mg/dl) | 278.66±18.72$^{a}$ | 272.33±12.58$^{a}$ | 278.33±19.55$^{a}$ | 283.33±18.61$^{a}$ | 267.33±8.14$^{a}$ | 293.33±11.93$^{a}$ |
| Final glucose (mg/dl) | 317.33±9.29$^{a}$ | 112.66±7.37$^{b}$ | 185.33±7.09$^{c}$ | 118.01±11.53$^{b}$ | 202.66±8.62$^{c}$ | 149.66±19.03$^{d}$ |
| Liver | | | | | | |
| G6Pase* | 3.53±0.74$^{a}$ | 1.03±0.33$^{b}$ | 1.72±0.34$^{c}$ | 0.90±0.07$^{b}$ | 2.62±0.40$^{a}$ | 1.84±0.60$^{b, c}$ |
| ALAT** | 3.14±1.10$^{a}$ | 0.38±0.14$^{b}$ | 1.57±0.10$^{c}$ | 1.43±0.45$^{c}$ | 3.06±1.98$^{a}$ | 2.28±0.33$^{a}$ |
| Glycogen (mg/100 g tissue) | 14.04±0.99$^{a}$ | 28.88±1.53$^{b}$ | 25.20±4.42$^{b}$ | 39.95±4.83$^{c}$ | 25.75±2.23$^{b}$ | 30.20±3.87$^{b}$ |
| Urine | | | | | | |
| Glucose (g/L) | 61.05±7.52$^{a}$ | 33.11±4.83$^{b}$ | 15.89±4.32$^{c}$ | 10.96±4.15$^{c}$ | 40.47±3.75$^{b}$ | 32.45±3.93$^{b}$ |
| Volume (ml) | 75.00±8.19$^{a}$ | 54.33±5.51$^{b}$ | 34.33±4.04$^{c}$ | 25.33±3.51$^{d}$ | 53.00±3.61$^{b}$ | 25.37±2.52$^{d}$ |

*Glucose-6-phosphatase activity (μmol of inorganic phosphate released/min/mg protein); **Alanine aminotransferase activity (U/mg protein). In each line, values not sharing the same superscript letter are significantly different at $P<0.05$ (DMRT). Mean±SD ($n=5$). AE: Aqueous extract; HEE: Hydroethanolic extract; DMRT: Duncan's multiple range test; SD: Standard deviation

**Table 4:** Blood lipid profile and atherogenicity indices of streptozotocin-induced diabetic rats treated with aqueous or hydroethanolic extracts of *Glyphaea brevis*

| Parameter | Control | Tolbutamide (80 mg/kg) | AE | | HEE | |
|---|---|---|---|---|---|---|
| | | | 250 mg/kg | 500 mg/kg | 250 mg/kg | 500 mg/kg |
| Total cholesterol (mg/dl) | 173.89±7.13$^{a}$ | 121.68±8.95$^{b}$ | 142.46±28.72$^{a}$ | 135.50±12.91$^{b}$ | 138.93±12.34$^{b}$ | 127.73±5.26$^{b}$ |
| HDL-cholesterol (mg/dl) | 48.15±5.12$^{a}$ | 50.68±5.53$^{a}$ | 54.65±13.96$^{a}$ | 56.09±9.77$^{a}$ | 45.17±16.87$^{a}$ | 55.46±13.07$^{a}$ |
| LDL-cholesterol (mg/dl) | 113.52±11.21$^{a}$ | 64.14±7.48$^{b, c}$ | 77.68±13.39$^{b, c}$ | 70.21±7.37$^{b}$ | 70.67±13.56$^{b, c}$ | 56.36±5.79$^{c}$ |
| Triglycerides (mg/dl) | 61.11±5.35$^{a}$ | 34.28±4.28$^{b}$ | 50.63±7.07$^{a}$ | 45.95±11.97$^{a, b}$ | 115.48±1.67$^{c}$ | 79.52±17.86$^{a}$ |
| TC/HDL (ratio) | 3.65±0.51$^{a}$ | 2.42±0.27$^{b}$ | 2.63±0.15$^{b}$ | 2.44±0.23$^{b}$ | 3.35±0.52$^{a-c}$ | 2.39±0.54$^{b, c}$ |
| LDL/HDL (ratio) | 2.39±0.47$^{a}$ | 1.28±0.24$^{b}$ | 1.44±0.13$^{b}$ | 1.27±0.17$^{b}$ | 1.79±0.11$^{a, c}$ | 1.08±0.41$^{b}$ |

In each line, values not sharing the same superscript letter are significantly different at $P<0.05$ (DMRT). Mean±SD ($n=5$). AE: Aqueous extract; HEE: Hydroethanolic extract; DMRT: Duncan's multiple range test; SD: Standard deviation; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; TC: Total cholesterol

**Table 5:** Biochemical parameters of protein metabolism in streptozotocin-induced diabetic rats treated with aqueous or hydroethanolic extracts of *Glyphaea brevis*

| Group | Proteins (g/L) | Hemoglobin (g/dl) | $HbA_{1c}$ (%) |
|---|---|---|---|
| Control | 29.47±4.39$^{a}$ | 8.70±1.20$^{a}$ | 12.35±0.55$^{a}$ |
| Tolbutamide | 41.42±2.39$^{b}$ | 14.30±1.40$^{b}$ | 4.50±0.48$^{b}$ |
| AE 250 | 35.25±8.85$^{a, b}$ | 12.70±1.30$^{b}$ | 10.40±1.79$^{a}$ |
| AE 500 | 38.05±7.99$^{a, b}$ | 14.40±0.50$^{b}$ | 4.27±0.60$^{b}$ |
| HEE 250 | 33.58±6.51$^{a, b}$ | 13.50±0.30$^{b}$ | 4.81±1.71$^{b}$ |
| HEE 500 | 37.84±3.55$^{a, b}$ | 13.90±0.40$^{b}$ | 3.03±0.61$^{b}$ |

In each column, values not sharing the same superscript letter are significantly different at $P<0.05$ (DMRT). Mean±SD ($n=5$). AE: Aqueous extract; HEE: Hydroethanolic extract; DMRT: Duncan's multiple range test; SD: Standard deviation; $HbA_{1c}$: Glycated hemoglobin

antidiabetic plant molecules throughout the years.[35]

Previous phytochemical investigation of *G. brevis* extracts showed that they contained the following secondary metabolites: saponins, tannins, steroids, anthraquinones, flavonoids, terpenoids, iminosugars and cinnamic acid-derived glucoside.[36,37] Many studies highlighted the antidiabetic effects of flavonoids as well as the mechanisms of their action.[6] As an example, catechin, a flavan-3-ol, was identified as a modulator of insulin secretion.[35] Isoflavones, commonly known as phytoestrogens may also act as potential antidiabetics. Genistein is known to increase glucose-stimulated insulin secretion in cell lines and mouse pancreatic islets.[35] Flavonoids antidiabetic activity is mediated by activation of the cyclic adenosine monophosphate/protein kinase A signaling cascade which leads to an increase in intracellular concentration of $Ca^{2+}$ ions. As a result, insulin secretion gets stimulated.[35] Many tannins such as ellagitannins exert their antidiabetic action by inhibiting α-glucosidase enzymes.[35] Sugar-like structures such as kotalanol and salacinol are known to have α-glucosidase inhibitory effects that are sometimes comparable to that of acarbose and voglibose which are commonly prescribed.[35] Such observations lead us to hypothesize that flavonoids (found in great amount in aqueous and HEE)[16] as well as glycosides and sugar-like molecules may be responsible for the antidiabetic effect of *G. brevis*.

Hyperglycemia characterizing diabetes is known to result from increased glucose production in the liver coupled to reduced uptake of blood glucose by peripheral cells due to a lack of insulin. Therefore, stimulation of insulin secretion by the pancreas would be a probable mechanism explaining the antihyperglycemic action of *G. brevis*.[6,8] However, the antidiabetic effects of our extracts may also involve other mechanisms. Such extrapancreatic actions may include: (i) stimulation of peripheral use of glucose;[38] (ii) increase in both glycolytic and glycogenic processes;[8] and/or (iii) reduction of dietary glucose availability through inhibition of α-glucosidases.[39] This last hypothesis tends to be supported by a previous *in vitro* study where we found *G. brevis* leaf extracts to have α-amylase inhibition properties.[18] Indeed, α-amylase inhibitors have been reported to reduce and delay peak postprandial blood glucose in other *in vivo* studies.[40] Lower postprandial plasma glucose observed in extract-treated groups during the oral starch tolerance test would be linked to amylase inhibition which results in the reduction of starch's GI [Table 1]. Lowering the GI of food corresponds to limiting glucose bioavailability and is of interest for diabetic patients. This gives patients a choice as well as reduces the size of the healthcare budget.[41]

Insulin is known to stimulate the synthesis of proteins and to delay proteolysis.[42] In streptozotocin-induced diabetic rats, protein degradation may result in a decrease of blood hemoglobin and glycation processes affect a wide array of proteins.[43] Hemoglobin glycation leads to $HbA_{1c}$ which accounts for 3.4%–5.8% of erythrocytic hemoglobin in normoglycemic patients and can rise up to 16% in patients living

with diabetes.[44] Therefore, $HbA_{1c}$ is viewed as a good indicator of the mean blood glucose concentration in a diabetic patient over the past months.[21] Treatment of diabetic rats with *G. brevis* extracts caused total hemoglobin and $HbA_{1c}$ levels to be in the normal range [Table 5]. Insulin plays an important role in liver glycogenesis by activating glycogen synthase. Streptozotocin-induced diabetes impairs the ability of the liver to perform glycogenogenesis because of the defective activation of glycogen synthase.[45] In our study, extract-treated diabetic rats had hepatic glycogen values reverted in the normal range and this obsevation may be the consequence of increased insulin secretion [Table 3].[9,46]

Glucose-6-phosphatase is a liver enzyme catalyzing the breakdown glucose-6-phosphate into phosphate and glucose, the latter being exported to the bloodstream at the end of gluconeogenetic or glycogenolytic processes. In normal physiological conditions, insulin has an inhibitory effect on the hepatic production of glucose through enzyme inhibition of glucose-6-phosphatase and fructose-1,6-bisphosphatase; this explains why the activity of these enzymes is increased during diabetes.[47] Catalyzing the conversion of alanine into pyruvate which may be converted into glucose, ALAT is also viewed as a hepatic enzyme of gluconeogenesis. Extract treatments of rats reduced the activity of all the above-mentioned gluconeogenic enzymes, highlighting a reduction in hepatic gluconeogenesis and suggesting once more a stimulation of insulin secretion.[7,21]

It has been evidenced by several studies that experimental diabetes induced by streptozotocin injection causes a drastic reduction in body weight.[21,48] Weight loss in diabetic rats is an illustration of the degradation of structural proteins consecutive to the unavailability of carbohydrates as metabolic fuel.[49] Weight loss was counterbalanced in extract-treated diabetic rats [Figure 3] while no significant difference in food intakes was found among the different groups (data not shown). This finding is once more evidencing the ability *G. brevis* to reduce hyperglycemia through a possible stimulation of insulin secretion because of its anabolic effect on protein metabolism.[42]

Hyperlipidemia is one of the complications linked to diabetes.[4] The most frequent model of dyslipidemia in people living with diabetes involves higher concentrations of LDL-cholesterol as well as triglycerides, and reduced concentrations of HDL-cholesterol.[50] Treatment with extracts caused a decrease in blood LDL-cholesterol and atherogenicity indices in comparison with the untreated group. Various factors may be accountable of the hypolipidemic effect of *G. brevis* in this study. Since we did not compare our extracts with a reference hypolipidemic drug, the decrease in blood lipids noticed in the extract-treated groups may be linked to an increased secretion of insulin which has a stimulatory action on lipoprotein lipase activity[51] and inhibits lipid peroxidation.[52] From this point of view, the improvement of the blood lipids concentrations in the animals treated either with tolbutamide (which is not a hypolipidemic drug by itself) or the extracts would be merely the consequence of their pancreatic-based antihyperglycemic effects. However, previous studies showed that phenolic compounds such as flavonoids were present in *G. brevis* extracts.[11,16,19] These compounds are known to have a hypolipidemic effect by either inhibiting the enzymes involved in cholesterol biosynthesis like hydroxyl-methyl glutaryl-CoA reductase or reducing the intestinal absorption of dietary cholesterol.[51]

## CONCLUSION

Our study shows that *G. brevis* is effective in controlling blood glucose in fasted and postprandial states, liver gluconeogenesis as well as blood lipids in experimental diabetes induced by streptozotocin. These effects could be achieved through stimulation of insulin secretion and/or inhibition of α-glucosidases. This supports the traditional use of *G. brevis* and may find applications in the development of alternative therapies for human diabetes. Isolation of active compounds is in progress in our laboratory to elucidate their molecular mechanism of action.

## Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

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## Conflicts of interest

There are no conflicts of interest.

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