Antihyperglycemic and antihyperlipidemic activities of hydroethanolic extract of the fruit of *Baillonella toxisperma* in streptozotocin-induced diabetic rats

Takuissu Nguemto Guy Roussel a, Fonkoua Martin b, Youovop Fotso Janvier Aime c, Edoun Ebouel Ferdinand Lanvin a, Dibacto Kemadjou Ruth Edwige a, Azantsa Kingue Boris b, Ngondi Judith Laure b, *, Oben Julius Enyong b

**a** Institute of Medical Research and Medicinal Plants Studies, Ministry of Scientific Research and Innovation, Cameroon
**b** Department of Biochemistry, Faculty of Science, University of Yaounde 1, Yaounde, Cameroon

**ARTICLE INFO**

**Keywords:**
- *B. toxisperma* fruits
- Antihyperglycemic and antihyperlipidemic activities
- Diabetes

**ABSTRACT**

**Objective:** This work evaluated the antihyperglycemic and antihyperlipidemic activities of pulp extracts of *B. toxisperma* fruits in rats.

**Methods:** The regulatory ability of the extract on the secretory capacity of pancreatic beta cells (oral glucose tolerance test), and digestion and/or absorption of carbohydrates (starch and sucrose) were evaluated on normal Wistar rats. Diabetes was induced in Wistar rats by intravenous administration of streptozotocin (50 mg/kg), and the animals were treated by the administration of a single daily dose of 400 mg/kg BW of extract. The effect of the extract on blood glucose levels of diabetic rats was monitored 30 min, 2 h, and 5 h after administration, and on the 7th and 14th days of treatment. After 2 weeks of treatment, the rats were sacrificed, liver was preserved for the determination of glycogen content. The serum was prepared and markers for nephro and hepatotoxicity were assessed, as well as cardiovascular risk.

**Results:** The hydroethanolic extract of *B. toxisperma* fruits significantly reduced glucose concentrations after administration of starch and sucrose in normoglycemic rats by limiting the glycemic peak (increasing of 19.67% vs 27.88% for positive control and 35.96% vs 43.97% for positive control, for starch and sucrose respectively). No effect was observed after glucose administration. *B. toxisperma* fruits significantly decreased glucose levels by 14.5% and 54.23% respectively 30 min after administration and 7th days of treatment respectively. The extract decreased plasma triglycerides and total cholesterol levels in diabetic rats; it also decreased the cardiovascular risk through the reduction of Cardiac Risk Ratio, Atherogenic Coefficient, and non-High-Density Lipoprotein Cholesterol. The extract also promoted renal function but not structural integrity of the liver.

**Conclusions:** This study suggests that the hydroethanolic pulp extracts of *B. toxisperma* fruits are good antihyperglycemic and antihyperlipidemic properties agents and could be a potential source of compounds for diabetes management.

1. Introduction

*Baillonella toxisperma* (Sapotaceae) has its wood as the principal part mostly used, its bark is used for medicinal properties while its fleshy fruits are instead used for cooking and cosmetics purposes, and its pulp is directly consumed [1]. Nutritionally, fruits are rich in macro and micronutrients. In particular, there are carbohydrate contents of 89.6%, potassium of 27.5 mg/100 g, and calcium of 37.5 mg/100 g. The content of polyphenols, flavonoids, and proanthocyanins was 686.7, 141.1, and 28 μg/mg dry matter respectively [2]. This fruit, because of its nutritional composition, could therefore be highly prized in terms of food, but not only because its richness in secondary metabolites could also direct interest towards potential medicinal virtues. Our previous study already shown the antioxidant and antihyperglycemic properties in vitro of the *Baillonella toxisperma* fruits [3]. In the same way, many fruits of the family *Sapotaceae* like *Chrysophyllum cainito* have shown antioxidant properties [4]. Polyphenols and especially flavonoids are known for
their antihyperglycemic, antioxidant and antihyperlipidemic properties [5]. Several studies have shown that polyphenols-rich foods significantly correlated to a lower risk of diabetes and its complications [6,7].

Diabetes is an endocrine and metabolic disorder characterized by chronic hyperglycemia resulting from a deficiency in the action and/or secretion of insulin [8]. The major component of diabetic hyperglycemia is postprandial hyperglycemia, which is controlled by many factors: the carbohydrate content of meals, the rate of digestion and intestinal absorption of carbohydrates [9], and the cellular capture of glucose [10]. Diabetes is also generally associated with a state of obesity resulting from a high absorption of lipids, increased lipogenesis and decreased lipolysis which affect adipocyte function by a decrease in adiponectin secretion and stimulation of leptin secretion and adipocyte differentiation [11]. In addition, low activation of Peroxisome Proliferator-Activated Receptor gamma is noted in type 2 diabetic subjects; they control the oxidation of fatty acids, lipoprotein metabolism and are involved in carbohydrate metabolism by stimulating insulin sensitivity [12]. Diabetic hyperglycemia can exacerbate macrovascular complications such as cardiovascular diseases through hyperlipidemia [13]. Diabetic hyperglycemia is responsible for a decrease in lipoprotein lipase activity responsible for triglycerides catabolism. High levels of triglycerides stimulate the enrichment of c-HDL and of c-LDL with triglycerides and their cholesterol depletion through Cholesterol Ester Transfer Protein. This is followed by hydrolysis of the triglycerides portion mediated by hepatic lipase, resulting in the production of low c-HDL (which are catabolized and eliminated from the circulation) and small and dense LDL particles that invade the arterial wall and contribute to atherosclerosis [14].

Hyperglycaemia and hyperlipidemia are thus highly involved in the development of diabetes and its complications, such as hepatotoxicity and nephrotoxicity [15,16].

The management of this disease could be achieved through the search for natural compounds with hypoglycaemic and hypolipidemic properties, as alternatives or complements for the synthetic antidiabetic drugs which have shown side effects such as gastrointestinal disturbances, hepatic disorders and renal tumors [17]. Moreover, the search for compounds with several mechanisms of action can help solve the problem of plurimedication, which complicates the tolerance to certain drugs in patients, thus their efficacy. Many antidiabetic mechanisms were attributed to polyphenols: inhibition of digestion and absorption of carbohydrates, stimulation of insulin secretion and glucose uptake, and inhibition of hepatic production of glucose [18,19]. Polyphenols are also responsible for many antihyperlipidemic mechanisms, including decreased triglycerides and hepatic cholesterol, decreased c-LDL, increased c-HDL, inhibition of Acyl-CoA: cholesterol acyltransferase, and inhibition of Hydroxy Methyl Glutaryl-CoA reductase [20,21].

The present study was conducted to evaluate the antihyperglycemic and antihyperlipidemic activities of the hydroethanolic extract of Bal lonella toxisperma’s fruit, a plant of the Sapotaceae family.

2. Methods

2.1. Chemicals and reagents

Acarbose, Citrate, Ethanol, Glibenclamide, Starch, Streptozotocin, Sucrose, Total Cholesterol kit, Triglyceride kit, HDL-cholesterol kit, Phosphate buffer, L-Alanine, L-Aspartate, α-ketoglutarate, Chlorhydric acid, Sodium Hydroxide, Dinitrophenylhydrazine, Pyruvic acid, Creatinine, Iodine, Potassium Iodate, EDTA, Sodium Salicylate, Sodium Nitroprussiate, Sodium Hypochlorite, Urea, Urease.

2.2. Plant material

Fruits of Bal lonella toxisperma were harvested in Ondodo (East-Cameroon). They were identified at the Cameroon National Herbarium (N°. 54060/HNC) in Yaounde, where voucher specimens have been deposited under the reference number.

2.3. Experimental animals

Adult male Wistar albino rats weighing 180–230 g were obtained from the animal house of the Department of Biochemistry, University of Yaoundé I, Cameroon. The animals were acclimatized in the experimental animal room for 7 days with a 12 h light and 12 h dark cycle before the start of experimentation. Standard feed and water was provided ad libitum to all experimental animals.

2.4. Extraction

After drying in an oven at 50 °C for 3 days, the pulp was separated from the kernel. The pulp (100 g) was ground and extracted by maceration overnight for 18 h but had access to water and their glycemia was taken into 4 groups with each group consisting of five rats. The rats were fasted before the start of experimentation. Standard feed and water was provided ad libitum to all experimental animals.

2.5. Postprandial glucose-lowering potential in normoglycemic rats

The inhibitory effects of the extract on starch digestion was performed on twenty (20) male albino Wistar rats. The rats were divided into 4 groups with each group consisting of five rats. The rats were fasted overnight for 18 h but had access to water and their glycemia was taken at the start of the test (t0). Half an hour before the administration of the starch solution (1 g/kg of body weight (BW)), each group of rats received either water, a inhibitor of carbohydrate digestion drug (Acarbose 3 mg/kg BW), or the extract (400 mg/kg BW), except for the negative control group, which received water. The treatment lasted for 3 h and their glycemia was measured at 30 min time intervals (60, 90, 120 and 180 min) using a glucometer [22].

The inhibitory effects of the extract on sucrose digestion was performed as described with starch digestion above with slight modifications. Modifications included 2 g/kg BW of sucrose, used to replace starch. Blood glucose was measured at 30, 60, 120, and 180 min.

2.6. Experimental induction of diabetes

The schedules and procedures were performed in the experimental animal house of the Laboratory of Biochemistry of the University of Yaoundé I, Cameroon. The study was approved by the institutional animal ethical committee. In accordance to Al-Shamaony et al. [23],

**Abbreviations**

| Abbreviation | Description |
|--------------|-------------|
| AC | Atherogenic Coefficient |
| AIP | Atherogenic Index of Plasma |
| ALAT | Alanine Amino Transferase |
| apo B | Apolipoprotein B |
| ASAT | Aspartate Amino Transferase |
| ATP | Adenosine Tri Phosphate |
| BW | Body Weight |
| c-HDL | High-Density Lipoprotein Cholesterol |
| c-LDL | Low-Density Lipoprotein Cholesterol |
| EDTA | Ethylene Diamine Tetra Acetic |
| IDL | Intermediary Density Lipoprotein |
| OGGT | Oral glucose tolerance test |
| sdLDL-c | small density c-LDL |
| TC | Total cholesterol |
| TG | Triglycerides |
| VLDL | Very Low-Density Lipoprotein |
diabetes was induced by intraperitoneal administration of streptozotocin (50 mg/kg BW) dissolved in freshly prepared 0.01 M citrate buffer pH 4.5. After 48 h, rats with marked hyperglycemia (fasting blood glucose = 200 mg/dL) were selected and used for the study.

2.7. Experimental design on diabetic rats

In the experiment, a total of 20 rats (15 diabetic rats, 5 normal rats) were used. The rats were randomly assigned into 4 groups of 5 rats each after the induction of diabetes. One non-diabetic control group (receiving only water); one diabetic control group (receiving only water) and two other groups receiving respectively Gilbenclamide (4 mg/kg in water), hydroethanolic extract of fruit (400 mg/kg) in water of *B. toxisperma*. The blood glucose level of each rat was recorded at the beginning, 30 min, 120 min, 300 min, 7th day, and 14th day. The Trinder glucose activity test [22], using glucose oxidase was used to monitor the blood glucose using a test strip in tail vein blood. After 14 days of treatment, the 12 h fasted animals were sacrificed by cervical decapitation under anesthesia. Blood was withdrawn with EDTA tubes and centrifuged at 3000 rpm for 10 min to obtain the plasma which was stored at −20 °C for the measurement of parameters of lipid profile. Liver was collected, washed with saline solution and stored in the freezer at −20 °C for measurement of glycogen content.

2.8. Biochemical analysis

2.8.1. Measurement of glycogen content

The glycogen content was measured to evaluate the effect of the extract on the glycogenogenesis by the method of Suzuki et al. [24].

2.8.2. Measurement of serum lipid profile

Total cholesterol (TC), triglycerides (TG), and High-Density Lipoprotein Cholesterol (c-HDL) were estimated using standard kits of Chronolab. Low Density Lipoprotein Cholesterol (c-LDL) concentration was calculated using the formula of Friedwald et al. [25] using the following equation: LDL-c = TC - (HDL-c + TG)/5.

2.8.3. Measurement of cardiovascular risk indices

Four cardiovascular risk indices were evaluated: CRR (Cardiac Risk Ratio), AC (Atherogenic Coefficient), AIP (Atherogenic Index of Plasma) and non-HDL-c. CRR was calculated as TC/HDL-c, AC was calculated as (TC-HDL-c)/HDL-c, and AIP as log(TG/HDL-c) [26,27]. Non-HDL cholesterol was calculated as TC - HDL-c [28].

2.8.4. Measurement of hepatotoxicity and nephrotoxicity markers

The hepatotoxicity was evaluated by determining the markers of hepatic cytolysis: ALAT and ASAT activities [29]. The nephrotoxicity was evaluated by determining the markers of glomerular filtration: creatinine and urea plasma levels [30].

2.9. Statistical analysis

All data were expressed as mean values ± standard deviation. Significant differences among the groups were determined by the Kruskal Wallis test, following the post hoc of Bonferroni using the SPSS statistical analysis program. Statistical significance was considered at p < 0.05.

3. Results

3.1. Effect of extract on starch digestion

The variation in glycemia following the administration of starch is presented in Table 1. The positive control expressed the highest glycemic values compared to the negative group throughout the experiment. The extract limited the observed peak blood glucose level to 102.33 mg/dL compared to 114.33 mg/dL in the positive control. Subsequently, a significant and progressive decrease in blood glucose level was observed in both groups until 180 min, with no significant difference observed in the final blood glucose levels of either group. Acarbose more significantly (p < 0.05) limited the peak blood sugar level (93.19 mg/dL) compared to the extract. Acarbose caused a greater decrease in blood glucose levels throughout the experiment.

3.2. Effect of extract on sucrose digestion

The variation in glycemia following the administration of sucrose is presented in Table 2. The positive control expressed the highest glycemic values compared to the negative group throughout the experiment. The extract limited the observed peak blood glucose level to 109.2 mg/dL compared to 123.75 mg/dL in the positive control. Subsequently, a significant and progressive decrease in blood glucose level was observed in both groups until 180 min, with no significant difference observed. Acarbose more significantly (p < 0.05) limited the peak blood sugar level (77.2 mg/dL) compared to the extract. Acarbose equally brought about a greater decrease in blood glucose level throughout the experiment.

3.3. Acute hypoglycemic activity

The acute hypoglycemic activity of the extract is presented in Table 3. After streptozotocin administration, we noted an induced glycemic level of 600 mg/dL at least (detection limit of the glucometer) against 74.00 mg/dL in normal control. Any variation of glycemia was observed in diabetic control groups during the experimentation. In the extract group, there was a significant decrease in blood glucose level after 30 mins compared to the reference group (−7.3%). However, after 120 min−300 min, the decrease was significantly greater in the reference group −19.71% and −21.92%, respectively compared to −13.26% and −12.53% in the extract group.

| Table 1 | Effect of extract on starch digestion. |
|---------|--------------------------------------|
| Groups  | t₀ (mg/dL) | t₀₀ (mg/dL) | t₁₀ (mg/dL) | t₂₀ (mg/dL) | t₃₀ (mg/dL) |
| Negative control (rats + water) | 80.00 ± 2.00a | 76.27 ± 3.05a | 75.75 ± 6.65a | 73.15 ± 5.18a | 70.27 ± 4.38a |
| Positive control (rats + 1 g/kg of starch) | 82.33 ± 3.21a | 108.33 ± 4.04a | 114.33 ± 4.04a | 108.33 ± 8.08a | 100.66 ± 7.02a |
| Test group (rats + 1 g/kg of starch + 400 mg/kg of B. toxisperma) | 81.67 ± 3.79a | 101.66 ± 6.66a | 102.33 ± 3.51a | 97.66 ± 1.53a | 99.33 ± 1.53a |
| Reference group (rats + 1 g/kg of starch + 3 mg/kg of Acarbose) | 84.15 ± 3.04a | 92.52 ± 5.54a | 93.19 ± 5.23a | 83.58 ± 2.06a | 83.94 ± 5.18a |

The values in parentheses are the percentage variation; results are expressed as mean in glycemia ± standard deviation. The values assigned different letters are significantly different at p<0.05 between the different groups on the same time. # (p<0.05): significant difference in the same group compared to t₀.
The values in parentheses are the percentage variation; results are expressed as mean in glycemia ± standard deviation. The values assigned different letters are significantly different at p<0.05 between the different groups on the same time. # (p<0.05): significant difference in the same group compared to t₀.

The values in parentheses are the percentage variation; results are expressed as mean in glycemia ± standard deviation. The values assigned different letters are significantly different at p<0.05 between the different groups on the same time. # (p<0.05): significant difference in the same group compared to t₀.

### 3.4. Sub-acute hypoglycemic activity

The sub-acute hypoglycemic activity of the extract is presented in Table 4. While a slight decrease in blood glucose level was noted in the diabetic control group, the extract resulted in a more significant decrease in blood sugar level of −54.23% after one week, which stabilized after two weeks compared to −17% for the diabetic group. The extract was less effective than the reference which resulted in a −62% reduction in blood glucose at the end of treatment.

### 3.5. Effect of extract on glycogen content

The effect of the extract on glycogen content is presented in Table 5. The positive control expressed the lowest glycogen content compared to the negative control. However, in the group treated with the extract, glycogen levels were significantly higher compared to the positive control group (6.51 mg/mL versus 6.07 mg/mL). No significant difference was noted between the extract and reference groups.

### 3.6. Effect of extract on serum lipid profile

The effect of the extract on lipid profile is presented in Table 6. The triglyceride and total cholesterol levels are higher in the diabetic control group compared to the negative control. However, significantly lower triglyceride and total cholesterol levels were noted in the extract group (128.31 mg/dL and 129.13 mg/dL respectively) compared to the positive control group (152.61 mg/dL and 177.8 mg/dL respectively). Triglyceride levels in the extract group were significantly higher than those of the reference group while total cholesterol levels were lower.

With regard to cholesterol sub-fractions, the c-HDL of the positive control was significantly lower than that of the negative control. No significant difference was observed between the extract group and the positive control group as well as between the extract group and the reference group. For c-LDL, no significant difference was observed between the different groups.

### 3.7. Effect of extract on cardiovascular risk

The effect of the extract on cardiovascular risk is presented in Table 7. Except with AIP, with which no significant differences were noted between the different groups, significantly higher values were noted with the other indices (CRR, AC, and non-HDL-c) in the positive control group compared to the negative control. These different indices were significantly lower in the test group compared to the positive control (3.56 vs 5.45, 2.55 vs 4.45, and 92.82 vs 145.19 respectively for CRR, AC, and non-HDL-c). The same observation was made in comparison to the reference group.

Table 2

| Groups                      | t₀ (mg/dL) | t₁₀ (mg/dL) | t₂₀ (mg/dL) | t₃₀ (mg/dL) | t₄₀ (mg/dL) | t₅₀ (mg/dL) | t₆₀ (mg/dL) | t₇₀ (mg/dL) |
|-----------------------------|------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Negative control (rats + water) | 72.00 ± 12.60a | 70.45 ± 4.76ab | 68.33 ± 2.45ab | 65.23 ± 6.18ab | 64.02 ± 5.18ab | 63.80 ± 5.18ab | 64.65 ± 5.18ab | 65.40 ± 5.18ab |
| Positive control (rats + 2 g/kg of sucrose) | 69.25 ± 5.85ab | 122.75 ± 10.92ab | 123.75 ± 6.39ab | 126.75 ± 6.39ab | 129.75 ± 6.39ab | 132.75 ± 6.39ab | 135.75 ± 6.39ab | 138.75 ± 6.39ab |
| Test group (rats + 2 g/kg of sucrose + 400 mg/kg of B. toxisperma) | 70.20 ± 12.46ab | 109.20 ± 5.26ab | 105.80 ± 9.26ab | 88.00 ± 7.18ab | 84.00 ± 4.36ab | 80.00 ± 4.36ab | 76.00 ± 4.36ab | 72.00 ± 4.36ab |
| Reference group (rats + 2 g/kg of sucrose + 3 mg/kg of Acraborne) | 69.00 ± 14.73ab | 77.20 ± 6.86ab | 76.00 ± 10.98ab | 65.40 ± 5.76ab | 65.00 ± 15.23ab | 65.00 ± 15.23ab | 65.00 ± 15.23ab | 65.00 ± 15.23ab |

The values in parentheses are the percentage variation; results are expressed as mean in glycemia ± standard deviation. The values assigned different letters are significantly different at p<0.05 between the different groups on the same time. # (p<0.05): significant difference in the same group compared to t₀.

Table 3

Effect of extract on the glucose levels (mg/dL) after 14 days of treatment.

| Groups                      | t₀ (mg/dL) | t₁₀ (mg/dL) | t₂₀ (mg/dL) | t₃₀ (mg/dL) | t₄₀ (mg/dL) | t₅₀ (mg/dL) | t₆₀ (mg/dL) | t₇₀ (mg/dL) |
|-----------------------------|------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Normal Control (non diabetic rats) | 74.00 ± 7.30a | 72.00 ± 7.30a | 70.54 ± 7.30a | 70.00 ± 7.30a | 67.50 ± 7.30a | 65.00 ± 7.30a | 62.50 ± 7.30a | 59.50 ± 7.30a |
| Diabetic Control (diabetic rats + water) | 600.00 ± 15.36ab | 513.00 ± 15.36ab | 520.40 ± 15.36ab | 524.80 ± 15.36ab | 528.20 ± 15.36ab | 531.60 ± 15.36ab | 535.00 ± 15.36ab | 538.40 ± 15.36ab |
| Reference group (diabetic rats + 4 mg/kg of Acraborne) | 600.00 ± 555.75ab | 481.75 ± 555.75ab | 468.50 ± 555.75ab | 452.25 ± 555.75ab | 436.00 ± 555.75ab | 420.75 ± 555.75ab | 405.50 ± 555.75ab | 390.25 ± 555.75ab |

The values in parentheses are the percentage variation; results are expressed as mean in glycemia ± standard deviation. The values assigned different letters are significantly different at p<0.05 between the different groups on the same time. # (p<0.05): significant difference in the same group compared to t₀.

Table 4

Effect of extract on the glucose levels (mg/dL) after 14 days of treatment.

| Groups                      | t₀ (mg/dL) | t₁₀ (mg/dL) | t₂₀ (mg/dL) | t₃₀ (mg/dL) | t₄₀ (mg/dL) | t₅₀ (mg/dL) | t₆₀ (mg/dL) | t₇₀ (mg/dL) |
|-----------------------------|------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Normal Control (non diabetic rats) | 74.00 ± 7.30a | 73.25 ± 1.71b | 73.00 ± 2.58b | 72.75 ± 3.46b | 72.50 ± 4.36b | 72.25 ± 5.26b | 72.00 ± 6.16b | 71.75 ± 7.06b |
| Diabetic Control (diabetic rats + water) | 600.00 ± 511.60b | 498.00 ± 18.51b | 495.25 ± 29.49b | 492.50 ± 30.62b | 490.75 ± 31.83b | 489.00 ± 33.04b | 487.25 ± 34.25b | 485.50 ± 35.46b |
| Reference group (diabetic rats + 4 mg/kg of Acraborne) | 600.00 ± 218.00b | 214.75 ± 37.42b | 213.50 ± 23.15b | 212.25 ± 19.71b | 211.00 ± 16.42b | 209.75 ± 13.13b | 208.50 ± 10.84b | 207.25 ± 8.55b |

The values in parentheses are the percentage variation; results are expressed as mean in glycemia ± standard deviation. The values assigned different letters are significantly different at p<0.05 between the different groups on the same time. # (p<0.05): significant difference in the same group compared to t₀.

Table 5

Effect of the extract on glycogen content.

| Groups                      | Glycogen content (g/L) |
|-----------------------------|------------------------|
| Normal Control (non diabetic rats) | 6.89a |
| Diabetic Control (diabetic rats + water) | 6.07b |
| Test group (diabetic rats + 400 mg/kg of B. toxisperma) | 6.51a |
| Reference group (diabetic rats + 4 mg/kg of Glibenclamide) | 6.67a |

The values in parentheses are the percentage variation; results are expressed as mean ± standard deviation. The values assigned different letters are significantly different at p<0.05.
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The values in parentheses are the percentage variation; results are expressed as mean ± standard deviation. The values assigned different letters on the same column are significantly different at p<0.05.

Table 7

| Groups                  | Cardiac risk indices | CRR | AC | AIP | non HDL-c |
|-------------------------|----------------------|-----|----|-----|-----------|
| Normal Control (non diabetic rats) | 3.11 ± 2.11 ± 0.04 ± 103.84 ± | 0.19² | 0.45² | 0.001² | 15.17² |
| Diabetic Control (diabetic rats + water) | 5.45 ± 4.45 ± 0.07 ± 145.19 ± | 1.01² | 0.65² | 0.001² | 12.17² |
| Test group (diabetic rats + 400 mg/kg of B. toxisperma) | 3.56 ± 2.55 ± 0.06 ± 92.82 ± | 0.90³ | 0.31³ | 0.004³ | 08.12³ |
| Reference group (diabetic rats + 4 mg/kg of Glibenclamide) | 5.32 ± 4.32 ± 0.06 ± 154.00 ± | 1.04³ | 0.59³ | 0.001³ | 21.22³ |

CRR: Cardiac Risk Ratio; AC: Atherogenic Coefficient; AIP: Atherogenic Index of Plasma. The values in parentheses are the percentage variation; results are expressed as mean ± standard deviation. The values assigned different letters on the same column are significantly different at p<0.05.

3.8. Effect of extract on hepatotoxicity and nephrotoxicity markers

The effect of the extract on markers of hepatotoxicity and nephrotoxicity is presented in Table 8. No significant difference was noted between the positive control and the negative control groups for the hepatotoxicity markers. ALAT and plasma ASAT activities were significantly higher in the extract group (71.36 UI/mL and 59.09 UI/mL respectively) compared to the positive control (66.14 UI/mL and 51.68 UI/mL respectively). The same observation was made in comparison to the reference group.

Plasma urea and creatinine levels in the positive control were significantly higher than those in the negative control. In the extract group, creatinine levels were lower (p < 0.05) than in positive control.

Table 8

| Groups                  | ALAT (UI/mL) | ASAT (UI/mL) | Urea (g/ L) | Creatinine (mg/dL) |
|-------------------------|-------------|--------------|-------------|-------------------|
| Normal Control (non diabetic rats) | 63.98 ± 50.40 ± 11.30 ± 1.02 ± | 1.44² | 2.00² | 0.01² | 0.004² |
| Diabetic Control (diabetic rats + water) | 66.14 ± 51.68 ± 32.25 ± 4.56 ± 0.09³ | 2.29² | 1.88² | 1.12³ | 145.19³ |
| Test group (diabetic rats + 400 mg/kg of B. toxisperma) | 71.36 ± 59.09 ± 29.63 ± 1.05 ± 0.07³ | 5.15³ | 4.33³ | 1.39³ | 2.10³ |
| Reference group (diabetic rats + 4 mg/kg of Glibenclamide) | 66.70 ± 41.42 ± 17.26 ± 1.81 ± 0.46³ | 1.55³ | 2.82³ | 1.44³ | 1.39³ |

The values in parentheses are the percentage variation; results are expressed as mean ± standard deviation. The values assigned different letters on the same column are significantly different at p<0.05. 1.05 mg/dL versus 4.06 mg/dL; while no difference was noted for uremia. The uremia of the reference group was lower (p < 0.05) than that of the extract group, while no difference was noted for creatinine.

4. Discussion

Diabetes is a metabolic disorder characterized by chronic hyperglycemia. The high blood glucose level can induce hyperlipidemia through insulin resistance [31]. Hyperglycaemia and hyperlipidemia are therapeutic targets in the management of diabetes and its complications. The present study aimed to evaluate the antihyperglycemic and anti-hyperlipidemic properties of hydroethanolic extract of B. toxisperma’s fruit.

As for the antihyperglycemic power of the extract, it was evaluated in normoglycemic rats through the inhibition of the digestion of carbohydrates. While in diabetic rats, we evaluated the hypoglycemic potential in the short and medium-term, as well as the effects on glucose storage through neoglucogenesis.

It is known that to reduce postprandial hyperglycemia, which is a component of diabetic hyperglycemia, it would be interesting to reduce the activity of α-glucosidases after food intake and in particular α-amylase and invertase, with the subsequent reduction of glucose production and therefore its absorption [32]. The extract acts essentially by limiting the glycemic peak whether following the administration of starch, or sucrose, which reflects the fact that the extract acts mainly by inhibiting the activities of α-amylase and invertase and therefore by inhibiting the digestion of starch and invertase. The inhibition of intestinal digestion of starch and sucrose by the extract could be due to the presence of alkaloids in this extract which are known for their ability to inhibit α-glucosidases, thus decreasing carbohydrate digestion [33].

Streptozotocin is a chemical substance used as an experimental model to induce hyperglycemia. Indeed, by selectively destroying the beta cells of the pancreas, it decreases the synthesis of insulin resulting in hyperglycemia [34]. After the induced hyperglycemia in rats by streptozotocin, the hydroethanolic extract and B. toxisperma fruits brought about a decrease in acute (120 min after administration) and sub-acute (14 days of treatment), effects that were comparable to that of Tolbutamide. This extract could act through the same mechanism as Glibenclamide which is an antidiabetic drug from the class of sulfonylurea. The sulfonylureas block the ATP-dependent potassium pump thereby enabling calcium entry and subsequently the release of insulin, which will then stimulate the binding and utilization of glucose by cells [35], which could explain the drop in glycemia in the test group. This hypoglycaemic effect could as well be explained by the presence of polyphenols in these extracts which could simultaneously inhibit the digestion and absorption of carbohydrates. Indeed, polyphenols are known to be powerful inhibitors of digestive enzymes or intestinal glucose transporters [36,37]. Nikkila and Kekki [38] had also demonstrated the hypoglycaemic properties of certain fruits belonging to the Sapotaceae family but on an experimental model of hyperglycemia-induced by alloxan.

In order to determine whether this hypoglycemic effect was associated with a possible effect of the extract on certain pathways of carbohydrate metabolism: glycogenoegenesis and/or glycogenolysis, we quantified hepatic glycogen. The extract brought about high glycogen levels. These results would be due to the ability of the extract to stimulate following glucose capture as shown above, its storage in the form of glycogen by activation of glycogen synthase, an active enzyme in dephosphorylated form, dephosphorylation induced by insulin-dependent phosphatases [39]. The extract in stimulating glycogen synthase and therefore its inactivation [40], which could also reflect the possible effect of the extract on glycogenolysis inhibition hence high glycogen levels are observed.

Diabetic hyperglycemia is strongly associated with the development of
of hyperlipidemia and dyslipidemia which are known to be the main causes of cardiovascular diseases. This justified the choice of the hydroethanolic extract of the fruits of B. toxisperma on the lipid profile markers. The extract lowered triglyceride and total cholesterol levels while no effect was observed on cholesterol sub-fractions (c-HDL and c-LDL). The hypotriglyceridemic effect is believed to result from the ability of the extract to stimulate insulin sensitivity and therefore inhibit hepatic triglyceride production. Indeed, insulin resistance leads to the activation of hormone-sensitive intracellular lipase which releases large quantities of non-esterified fatty acids into the blood, which further serve as a substrate for the synthesis of triglycerides in the liver [41, 42]. The hypocholesterolemic effect could also be a consequence of the beneficial effect of the extract on insulin sensitivity and therefore on the inhibition of Hydroxyl Methyl Glutaryl CoA reductase, which is the key enzyme in endogenous cholesterol synthesis. On the other hand, this effect may also be due to the ability of the extract to inhibit cholesterol esterase, which is responsible for the hydrolysis of cholesterol esters and consequently for increasing the absorption of dietary cholesterol [43, 44]. The absence of effects on the levels of HDL-c and LDL-c would be due to the fact that the extract would act much more on the key enzymes of lipid synthesis and not on the transport forms of these lipids. Dyslipidemias associated with diabetes increase the risk of cardiovascular disease, which can be assessed by several indices: CRR, AG, AIP, and non-HDL-c [28, 45]. The extract decreased cardiovascular risk through the reduction of CRR, AC, and non-c-HDL. This effect would reflect the beneficial effect of the extract on the lipid profile. These different indices predict cardiovascular risk better than LDL-c or HDL-c alone. The non-c-HDL for example includes apo B, lipoprotein a, VLDL, IDL, and LDL-c; and is strongly correlated to sdLDL-c [46, 47].

Diabetes is associated with hepatotoxicity and nephrotoxicity. ALAT and ASAT transaminases are indicators of liver structural integrity [48], while urea and creatinine are biomarkers of renal damage [49]. The extract resulted in a significant increase in plasma activity of ALAT and ASAT, which would indicate a possible hepatotoxic effect of the extract. This could be due to its high alkaloid content, as high-dose alkaloids are known to be toxic [50]. However, the extract resulted in a significant decrease in creatinine levels. This effect is due to the presence of phenolic compounds which, because of their role as antioxidants, limit the effects of pro-oxidants at the renal level, with the consequence that glomerular filtration is maintained in the treated groups [49]. had already linked the increased clearance of these wastes to improved glomerular filtration.

5. Conclusion

The present study showed that the hydroethanolic pulp extracts of the B. toxisperma fruits possess antihyperglycemic and anti-hyperlipidemic activities. It is encouraging enough to warrant further studies on the constituents responsible for these activities, their mechanism of action and to establish their therapeutic in the management of diabetes and its complications.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of competing interest

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Acknowledgment

The authors would like to express their thanks to the Department of Biochemistry of the University of Yaounde, for providing all the necessary lab facilities.

References

[1] Doucet J, Roudot Y, Le moali, une espèce « phare » de l’exploitation forestière en Afrique centrale. Parcs et Reserves 2007;6(2):25–31.
[2] Fungo R, Muyonya J, Kasya A, Okia C, Juwas C, Baida-Fusor J. Nutrients and bioactive compounds content of Ballonella toxisperma, Trichocysta abut and Pentaclethra macrophylla from Cameroon. Food Sci Nutr 2015;3(4):292–301.
[3] Tabuasi N, Ngondi J, Oben J. Antioxidant and glucose lowering effects of hydroethanolic extract of Ballonella toxisperma pulp. J Food Res 2020;9(2):20–29.
[4] Hegde K, Arathi A, Anupama M. Evaluation of antidiabetic activity of hydroalcoholic extract of Chrysophyllum quandu fruits. DJSR 2017;8(1):316–22.
[5] Umema A, Horie M, Murotomi K, Nakajima Y, Yoshida Y. Antioxidative and antidiabetic effects of natural polyphenols and isoflavones. Molecules 2016;21(6):708.
[6] Yoona K, Jennifer B, Peter M. Polyphenols and glycemic control. Nutrients 2016;8 (17):27
[7] Huang P, Chi C, Liu T. Areca nut cyanamidines ameliorate streptozotocin-induced hyperglycaemia by regulating glucogenesis. Food Chem Toxicol 2013;55: 57–67.
[8] Maulik N, Reenu S, Rita M. Assessment of oxidative stress and lipid status in patients of type 2 diabetes mellitus with and without complications. Int J Bioc hem Res 2016;13(1):1–10.
[9] Holz J, Gribble F, Horowitz M, Rayner C. Roles of the gut in glucose homeostasis. Diabetes Care 2016;39:884–92.
[10] Vadder F. Detection portale des nutriments et controle de l’homeostasie energetique par l’axe nerveux intestin-cerveau. Universite de Lyon: These de Doctorat; 2014. p. 1–15.
[11] Yeo C-R, Yang C, Wong T-Y, Popovich D. A quantified ginseng (Panax ginseng C.A. Meyer) extract influences lipid absorption and increases adiponectin expression in 3T3-L1 cells. Molecules 2011;16(1):477–92.
[12] Michalik L, Awerus J, Berger J, Chatterjee V, Glass C, Gonzalez FJ, et al. International union of pharmacology. LXX. Peroxisome proliferator-activated receptors. Pharmaco 2006;58:726–41.
[13] Pisciochi A, Pop A. The role of antioxidants in the chemistry of oxidative stress: a Review. Eur J Med Chem 2015;97:55–74.
[14] Schofield J, Liu Y, Rao-Balakrishna P, Malik R, Soran H. Diabetes dyslipidemia. Diabetes Ther 2016:7:203–19.
[15] Palsamy P, Sivakumar S, Subramanian S. Resveratrol attenuates hyperglycemia-mediated oxidative stress, proinflammatory cytokines and protects hepatic microsome ultrastructure in streptozotocin-nicotinamide-induced experimental diabetic rats. Chem Biol Interact 2010;186:200–10.
[16] Hakim P, Pfaffer A. Role of oxidative stress in diabetic kidney disease. Med Sci Mon Int Med J Exp Med 2015:21:55–74.
[17] American Diabetes Association. Standards of medical care in diabetes-2017. Diabetes Care 2017;40(1):1–80.
[18] Xiao J, Hogger P. Dietary polyphenols and type 2 diabetes: current insights and future perspectives. Curr Med Chem 2015;22(1):23–38.
[19] Hanhineva K, Torronen R, Bondia-Pons I, Pekkinen J, Kolehmainen M, Melanson E, et al. Resolution of obesity and cardiovascular disease, which can be assessed by several indices: CRR, AC, AIP, and non-HDL-c [28, 45]. The extract decreased cardiovascular risk through the reduction of CRR, AC, and non-c-HDL. This effect would reflect the beneficial effect of the extract on the lipid profile. These different indices predict cardiovascular risk better than LDL-c or HDL-c alone. The non-c-HDL for example includes apo B, lipoprotein a, VLDL, IDL, and LDL-c; and is strongly correlated to sdLDL-c [46, 47].

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[34] Sajeeth C, Manna P, Manavalan R, Jolly C. Antidiabetic activity of a polyherbal formulation, Ed/aý/500 in streptozotocin induced diabetic male Albino rats: a research. IJDFR 2010;1(1):311–22.

[35] Sola D, Rossi L, Schianca G, Maffioli P, Bigliocca M, Mella R, et al. Sulfonylureas and their use in clinical practice. Arch Med Sci 2015;11(4):840–8.

[36] Tsuchita T, Shintani T, Sato H. Amylase inhibitory activity from nut seed skin polyphenols. J Agric Food Chem 2013;61:4570–6.

[37] Boath A, Stewart D, McDougall G. Berry components inhibit glucosidase in vitro: synergies between acarbose and polyphenols from black currant and rowanberry. Food Chem 2012;135:929–36.

[38] Omoboyowa D, Igar E, Olu G, Olugu K. Anti-diabetic activity of methanolic extract of seed cotyledon of Chrysophyllum albidum in alloxan-induced diabetic rats. Biokemistri 2016;28(2):88–95.

[39] Duparc T. Communication inter-organes dans le controle du metabolisme glucidique: mise en evidence de l’implication du monoxyde d’azote et de l’apeline dans l’hypothalamus. Thèse de Doctorat. 2012. p. 1–93. Université de Toulouse.

[40] Nakamura K. Human metabolism. Lect. Notes 2014:30–123.

[41] Raulo M, Hukku M. Plasma triglyceride transport kinetics in diabetes mellitus. Metabolism 1973;22:1–22.

[42] Warraich H, Wong N, Rana J. Role for combination therapy in diabetic dyslipidemia. Curr Cardiol Rep 2015;17:32.

[43] Cerqueira N, Oliveira E, Gest D, Santos-Martins D, Moreira C, Moorthy H, et al. Cholesterol biosynthesis: a mechanistic overview. Biochemistry 2016;55:5483–506.

[44] Heidrich J, Contos L, Hunsaker L, Deck L, Vander D. Inhibition of pancreatic cholesterol esterase reduces cholesterol absorption in the hamster. BMC Pharmacol 2004;4:5.

[45] National Cholesterol Education Program. Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (ATP 111): third report of the. NCEP 2002:106. 3143–3421.

[46] Srisawasdi P, Chaloeysup S, Teerajetyal P, Pokulikorn A, Nakasem C, Varavanant S, et al. Estimation of plasma small dense LDL cholesterol from classic lipid measures. Am J Clin Pathol 2011;136:20–9.

[47] Brehm A, Pfeifer G, Pacini G, Vierhapper H, Roden M. Relationship between serum lipoprotein ratios and insulin resistance in obesity. Clin Chem 2004;50:2316–22.

[48] Pradke P. Renal function in diabetic nephropathy. World J Diabetes 2010;1(2):48–56.

[49] Li Y, Zhang Y, Zhang L, Li X, Yu J, Zhang H, et al. Protective effect of tea polyphenols on renal ischemia/reperfusion injury via suppressing the activation of TLR4/NF-kappaB p65 signal pathway. Gene 2014;542:46–51.

[50] Kuldau G, Bacon C. Clavicipitaceous endophytes: their ability to enhance resistance of grasses to multiple stresses. Biol Control 2008;46:57–71.