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

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A B S T R A C T

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 antidiabetic properties [4]. Polyphenols and especially flavonoids are known for

Abbreviations

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
OGTT	Oral glucose tolerance test
sdLDL-c	small density c-LDL
TC	Total cholesterol
TG	Triglycerides
VLDL	Very Low-Density Lipoprotein

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 arteriosclerosis [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 *Baillonella 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 *Baillonella 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 for 48 h with 800 mL of solvent (water/ethanol (1:1, v/v)). The resulting supernatant was filtered using Whatman N°. 1 filter paper (Whatman International Limited, Kent, England) and concentrated to about 10% of the original volume by a rotavapor before drying in an oven at 50 °C to obtain hydroethanolic extracts.

2.5. Postprandial glucose-lowering potential in normoglycemic rats

The inhibitory effects of the extract on starch digestion was performed on twenty (20) male albinos 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 (t_0). 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],

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 Glibenclamide (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 logTG/HDL-c [26,27]. Non-HDL cholesterol was calculated as TC - HDL-c [28].

2.8.4. Measurement of hepatoxicity 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 Bonferonni 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 min of −14.5% which stabilized for up to 300 min, while no reduction 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	t_0 (mg/dL)	t_{60} (mg/dL)	t_{90} (mg/dL)	t_{120} (mg/dL)	t_{180} (mg/dL)
Negative control (rats + water)	80.00 ± 2.00[a] (0.00°%)	76.27 ± 3.05[a] (−4.66%)	75.75 ± 6.65[a] (−5.31%)	73.15 ± 5.18[a] (−8.56%)	70.27 ± 4.38[a] (−12.16%)
Positive control (rats + 1 g/kg of starch)	82.33 ± 3.21[a] (0.00°%)	108.33 ± 4.04[b#] (24%)	114.33 ± 4.04[b#] (27.88%)	108.33 ± 8.08[b#] (24.00%)	100.66 ± 7.02[b#] (18.21%)
Test group (rats + 1 g/kg of starch + 400 mg/kg of *B. toxisperma*)	81.67 ± 3.79[a] (0.00°%)	101.66 ± 6.66[c#] (19.67%)	102.33 ± 3.51[c#] (20.19%)	97.66 ± 1.53[c#] (16,38%)	99.33 ± 1.53[b#] (17.76%)
Reference group (rats + 1 g/kg of starch + 3 mg/kg of Acarbose)	84.15 ± 3.04[a] (0.00°%)	92.52 ± 5.54[d#] (9.96%)	93.19 ± 5.23[d#] (10.74%)	83.58 ± 2.06[d#] (−0.68%)	83.54 ± 5.18[c#] (−0.72%)

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 t_0.

Table 2
Effect of extract on sucrose digestion.

Groups	t_0 (mg/dL)	t_{30} (mg/dL)	t_{60} (mg/dL)	t_{120} (mg/dL)	t_{180} (mg/dL)
Negative control (rats + water)	72.00 ± 12.60^a (0.00°%)	70.45 ± 4.76^a (−2.15%)	68.33 ± 2.45^a (−5.09%)	65.23 ± 6.18^a (−9.40%)	64.02 ± 5.18^a (−11.08%)
Positive control (rats + 2 g/kg of sucrose)	69.25 ± 5.85^a (0.00°%)	$122.75 \pm 10.92^{b\#}$ (43.47%)	$123.75 \pm 6.39^{b\#}$ (43,97%)	$86.75 \pm 6.34^{b\#}$ (20.13%)	$85.00 \pm 4.69^{b\#}$ (18.58%)
Test group (rats + 2 g/kg of sucrose + 400 mg/kg of *B. toxisperma*)	70.20 ± 12.46^a (0.00°%)	$109.20 \pm 5.26^{b\#}$ (35.96%)	$105.80 \pm 9.26^{c\#}$ (33.94%)	$88.00 \pm 7.18^{b\#}$ (20.26%)	$84.00 \pm 4.36^{b\#}$ (15.34%)
Reference group (rats + 2 g/kg of sucrose + 3 mg/kg of Acarbose)	69.00 ± 1473^a (0.00°%)	$77.20 \pm 6.86^{a\#}$ (10.51%)	$76.00 \pm 10.98^{a\#}$ (9.75%)	$65.40 \pm 5.76^{a\#}$ (−5.20%)	$65.00 \pm 15.23^{a\#}$ (−6.62%)

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 t_0.

Table 3
Effect of extract on the glucose levels (mg/dL) after 5 h of treatment.

Groups	t_0 (mg/dL)	t_{30} (mg/dL)	t_{120} (mg/dL)	t_{300} (mg/dL)
Normal Control (non diabetic rats)	74.00 ± 3.64^a (0.00%)	73.50 ± 4.67^a (−0.67%)	72.70 ± 4.80^a (−1.76%)	70.54 ± 2.30^a (−4.67%)
Diabetic Control (diabetic rats + water)	$\geq 600.00^b$ (0.00%)	$\geq 600.00^b$ (0.00%)	$\geq 600.00^b$ (0.00%)	$\geq 600.00^b$ (0.00%)
Test group (diabetic rats + 400 mg/kg of *B. toxisperma*)	$\geq 600.00^b$ (0.00%)	$513.00 \pm 15.36^{c\#}$ (−14.50%)	$520.40 \pm 12.70^{c\#}$ (−13.26%)	$524.80 \pm 17.63^{c\#}$ (−12.53%)
Reference group (diabetic rats + 4 mg/kg of Glibenclamide)	$\geq 600.00^b$ (0.00%)	$555.75 \pm 6.55^{d\#}$ (−7.37%)	$481.75 \pm 16.26^{d\#}$ (−19.71%)	$468.50 \pm 27.15^{d\#}$ (−21.92%)

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 t_0.

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.

Table 4
Effect of extract on the glucose levels (mg/dL) after 14 days of treatment.

Groups	t_0 (mg/dL)	$t_{7\ days}$ (mg/dL)	$t_{14\ days}$ (mg/dL)
Normal Control (non diabetic rats)	74.00 ± 3.64^a (0.00%)	73.25 ± 1.71^a (−0.83%)	73.00 ± 2.58^a (−1.22%)
Diabetic Control (diabetic rats + water)	$\geq 600.00^b$ (0.00%)	$511.60 \pm 18.51^{b\#}$ (−14.73)	$498.00 \pm 29.49^{b\#}$ (−17.00%)
Test group (diabetic rats + 400 mg/kg of *B. toxisperma*)	$\geq 600.00^b$ (0.00%)	$274.60 \pm 20.60^{c\#}$ (−54.23%)	$282.20 \pm 20.90^{c\#}$ (−52.97%)
Reference group (diabetic rats + 4 mg/kg of Glibenclamide)	$\geq 600.00^b$ (0.00%)	$218.00 \pm 37.42^{d\#}$ (−63.67%)	$214.75 \pm 23.13^{d\#}$ (−64.21)

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 t_0.

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/dL versus 6.07 mg/dL). 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 5
Effect of the extract on glycogen content.

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

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.

Table 6
Effect of the extract on lipid profile.

Groups	Lipid profile			
	TG (mg/dL)	CT (mg/dL)	HDL-c (mg/dL)	LDL-c (mg/dL)
Normal Control (non diabetic rats)	84.19 ± 2.18[a]	153.13 ± 2.10[a]	49.29 ± 2.18[a]	108.39 ± 12.28[a]
Diabetic Control (diabetic rats + water)	152.61 ± 1.47[b]	177.80 ± 2.62[b]	32.61 ± 6.47[b]	115.54 ± 19.47[a]
Test group (diabetic rats + 400 mg/kg of *B. toxisperma*)	128.31 ± 2.40[c]	129.13 ± 2.28[c]	36.31 ± 2.10[b]	110.43 ± 9.50[a]
Reference group (diabetic rats + 4 mg/kg of Glibenclamide)	112.62 ± 2.44[d]	189.62 ± 3.53[b]	35.62 ± 2.55[b]	108.22 ± 12.34[a]

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
Effect of the extract on cardiovascular risk.

Groups	Cardiovascular risk indices			
	CRR	AC	AIP	non HDL-c
Normal Control (non diabetic rats)	3,11 ± 0.19[a]	2.11 ± 0.45[a]	0.04 ± 0.001[a]	103.84 ± 15.17[a]
Diabetic Control (diabetic rats + water)	5.45 ± 1.01[b]	4.45 ± 0.65[b]	0.07 ± 0.001[a]	145.19 ± 12.17[b]
Test group (diabetic rats + 400 mg/kg of *B. toxisperma*)	3.56 ± 0.90[a]	2.55 ± 0.31[a]	0.06 ± 0.001[a]	92.82 ± 08.12[a]
Reference group (diabetic rats + 4 mg/kg of Glibenclamide)	5.32 ± 1.04[b]	4.32 ± 0.93[b]	0.06 ± 0.001[a]	154.00 ± 21.22[b]

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
Effect of the extract on hepatotoxicity and nephrotoxicity markers.

Groups	ALAT (UI/mL)	ASAT (UI/mL)	Urea (g/L)	Creatinine (mg/dL)
Normal Control (non diabetic rats)	63.98 ± 1.44[a]	50.40 ± 2.00[a]	11.30 ± 0.01[a]	1.02 ± 0.004[a]
Diabetic Control (diabetic rats + water)	66.14 ± 2.29[a]	51.68 ± 1.88[a]	32.25 ± 1.12[b]	4.56 ± 0.09[b]
Test group (diabetic rats + 400 mg/kg of *B. toxisperma*)	71.36 ± 5.15[b]	59.09 ± 4.33[b]	29.63 ± 1.39[b]	1.05 ± 0.07[a]
Reference group (diabetic rats + 4 mg/kg of Glibenclamide)	66.70 ± 1.55[a]	41.42 ± 2.82[c]	17.26 ± 1.44[c]	1.81 ± 0.46[a]

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 antihyperlipidemic 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 consequence of reducing 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: glyconeogenesis 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 insulin sensitivity would activate phosphatase with consequent dephosphorylation of glycogen synthase. This phosphatase is also involved in the dephosphorylation of glycogen phosphorylase 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 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 Hydroxy 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, 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; 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 antihyperlipidemic 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.

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Declaration of competing interest

The authors declare that they have no competing interests.

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