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Antidiabetic effects of aqueous extract of *Baillonella toxisperma* Pierre (Sapotacae) in streptozotocin-induced diabetic rats

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Abstract
Baillonella toxisperma stem bark is used in Cameroonian traditional medicine to control many ailments such as diabetes mellitus. The present study was designed to evaluate the anti-diabetic potential of *Baillonella toxisperma* stem-barks aqueous extract on STZ-induced type 1 diabetes in rats. Experimental diabetes was induced by intravenous (penile vein) injection of 55 mg/kg of streptozotocin. Animal received distilled water (10 mL/kg), insulin (10 UI/kg) or the aqueous extract of *B. toxisperma* (100 mg/kg, 200 mg/kg and 300 mg/kg). Hypoglycemic effect was assessed by a single oral administration of the extract to normal and diabetic rats and anti-diabetic effect evaluated by administrating daily doses to diabetic rats for 28 days. At the end of experimental period, blood pressure was recorded and serum (blood glucose levels, total cholesterol, triglycerides, LDL-cholesterol, LDL-cholesterol, AST, ALT, creatinine, sodium, calcium and urea), urinary (creatinine, sodium, calcium, urea and protein) biochemical parameters were evaluated. Some oxidative stress parameters were evaluated, and histomorphometry of the pancreas performed.
A single oral administration of the plant extract significantly reduced blood glucose levels in both normal and diabetic rat with a marked effect at higher dose. Injection of streptozotocin induced chronic hyperglycaemia accompanied by polyphagia, polydipsia, polyuria, weight loss, dyslipidemia, oxidative stress, rising on blood pressure, liver, kidney damages and degenerative changes in pancreas. The administration of *Baillonella toxisperma* stem-barks aqueous extract (200 mg/kg and 300 mg/kg) significantly decreased blood glucose levels and blood pressure parameters as compared to diabetic control 28 days post-treatment. Furthermore, the extract improved lipidemia, liver, renal parameters, and countered oxidative stress. Similarly, the plant extract improved morphological changes in pancreas by increased the area of islets cells of Langerhans as compared to diabetic control. These findings suggest that *Baillonella toxisperma* stem-barks aqueous extract exerts its anti-diabetic property by its hypolipidemic and anti-oxidative effects. These results justify the use of this extract in the management of diabetic condition.

Keywords: *Baillonella toxisperma*, streptozotocin, type 1 diabetes, antidiabetic, anti-oxidant effect

1. Introduction
Diabetes mellitus is a syndrome of disordered metabolism, usually due to a combination of hereditary and environmental causes, resulting in abnormally high blood sugar levels (hyperglycaemia) considered as one of the five leading causes of death in the world [1]. This disease is a major global health concern with a projected rise in prevalence from 425 million in 2017 to 629 million in 2045. In Cameroon, the report data from the international diabetes federation (IDF) revealed that more than 680.300 of people were diabetic in which 15.758 dead in 2017 [2]. Poor hyperglycaemia control damages organs, leading to the development of disabling and life-threatening health complications such as cardiovascular disease, neuropathy, nephropathy and retinopathy [2]. Type 1 diabetes is an autoimmune disease characterized by the destruction of pancreatic beta cells causing absolute insulin deficiency [3]. The incidence of type 1 diabetes has considerably risen in the past 30 years resulting from changes in the environment or lifestyle [4]. Type 1 diabetes is diagnosed after onset of over hyperglycaemia [5]; however, evidence is mounting that islet autoimmunity is the first stage of the disease [6].

Islet autoimmunity is defined by the persistent presence of autoantibodies to pancreatic islet antigens. Islet immunity usually starts in early childhood, with incidence peaking in the second year of life [7, 8], and can have a remitting-relapsing course before onset of diabetes [9]. Development of two or more islet autoantibodies marks a point of no return from which 70% of children progress to diabetes over the next 10 years [10]. The conventional control of the type 1 diabetes consists of daily injection of insulin and diet flexibility is encouraged. Due to the inherent side effects such as hypoglycemia, researchers are now intensifying efforts in alternative and complementary medicines [3]. Traditional medicine is used for treatment of diabetes in most African countries where the cost of conventional medicines is burden to the population [11]. Approximately, 80% of rural African communities still use phytotherapy to control or treat diabetes mellitus [12]. Despite the introduction of hypoglycemic agents from natural and synthetic sources, diabetes and its complications continue to be a major medical problem. Therefore, more research is needed to find new suitable drugs for the management of diabetes mellitus and its deleterious effects. *Baillonella toxisperma* (Pierre) is a plant grows in tropical rain forest with hot and humid climates [13-15]. In Cameroon, it is currently found in East and South regions. *B. toxisperma* is used in the treatment of several diseases; it is traditionally used to microbial infections, rheumatism, toothache, hemorrhoids, injury, wound, sexually transmitted infections, diarrhoea and malaria [16]. The decoction of barks or leaves is applied on the skin to prevent and to treat vaginal and oral mycoses, almond oil from *B. toxisperma* fruits is used to treat superficial infections like ringworms [17]. The ethnobotanical informations reported by Dibong *et al.* revealed that *B. toxisperma* may possess anti-diabetic and hypotensive potential [18]. Several studies have already been conducted on *B. toxisperma*, Essama *et al..*, [19] and Saha *et al..*, [20] demonstrated respectively its antifungal and antioxidant activities, but to the best of our knowledge the effect of *Baillonella toxisperma* extract have never been studied before on diabetes and its complications. Hence, the present study was aimed to evaluate the anti-diabetic potential of *Baillonella toxisperma* (Pierre) stem-barks aqueous extract on streptozotocin-induced type 1 diabetes in wistar rat.

2. Materials and Methods

2.1 Plant material and extraction

Baillonella toxisperma (Pierre) (Sapotacae) stem barks were harvested at Oveng (Centre Region, Cameroon). The botanical authentication was done at the National Herbarium of Yaoundé-Cameroon, in comparison with specimen No. 14592/SRF Cam. The plant material was cut in small pieces, dried at room temperature and grounded into powder. 300 g of powder was boiled in 4 L of water (100 °C) for 30 minutes according to the instruction of tradi-practitioner healer. The mixture was cooled at room temperature, filtered, and lyophilized that result to mass with a yield of 9.86% was considered as aqueous extract.

2.2 Phytochemical screening

Phytochemical tests were including triterpenoids, anthocyanins, phenols, tannins, alkaloids, saponins, coumarins, polyosis, reducer compounds and flavonoids, sterols and poly-uronide were carried out at Institute of Medical Research and Medicinal Plant Study (IMPM) of Yaoundé using standard procedures described by Sofowora [21], Harbome [22], Trease and Evans [23] and Edeoga *et al.* [24].

2.3 Animals and experimental induction of type 1 diabetes

Three-months-old male Wistar rats weighing 200-250 g bred in the animal house of the Laboratory of Animal Physiology (Faculty of Science, University of Yaoundé I) were used in the study. They were housed under standard laboratory conditions of temperature (22 ± 5°C) and humidity with 12h light/12h dark cycle. The animals were given standard rat chow and tap water ad libitum. Diabetes was induced by intravenous (penile vein) injection of 55 mg/kg of streptozotocin (Sigma Chemicals) in 0.9% sodium chloride solution after 12-h non fasted diazepam/ketamine (30/10mg/kg) anesthetized rats. Non-diabetic control rats were injected with vehicle. Three days later diabetes induction, fasting blood glucose levels were determined using an Accuchek glucometer (Roche Mannheim, Germany). Animals were then kept under observation for 2 weeks, corresponding to a period during which diabetes develops, stabilizes and induces some physiological impairments [25]. At the end of this period, animals with blood glucose level greater than 250 mg/dL were considered as diabetic, and were used in the present study.

2.4 Evaluation of the effect of single oral administration of *B. toxisperma* aqueous extract on the blood glucose

Normal and diabetic rats were assigned to five different groups of five rats each. Test group was consisted of normal and diabetic animals receiving *B. toxisperma* extract at the dose of 100, 200 and 300 mg/kg body weight, diabetic control received distilled water at 10 mL/kg by oral administration. Glibenclamide at the dose of 5 mg/kg (oral route) or insulin (subcutaneous route) at the dose of 10 UI/kg were respectively given to the normal and diabetic positive control. The base-dose of the extract used in the study was that given by the traditional healer, which, was surrounded by the lower and higher dose. Blood glucose levels from the tail vein were measured using Accuchek active Glucometer and compatible blood glucose test strips at (0 h) 1, 2, 3 and 5 h post extract administration as described our previous protocol [25].
Additionally, the anti-hyperglycemic effect of the extract *B. toxisperma* stem barks was also assessed by the oral glucose tolerance test (OGTT) in normal glycemic rats. The overnight fasted rats for 18 h were fed with the glucose solution (5 g/kg). The blood samples were collected at 0, 0.5, 1 and 2h after administration of glucose and the blood glucose level was estimated as above described.

2.5 Assessment of the effect of cumulated doses of *B. toxisperma* aqueous extract in diabetic rats

Sub-chronic treatment was conducted by a single daily administration of the extract for 28 consecutive days. Diabetic animals were divided into five groups of five rats each: one group served as normal control while another group served as diabetic control; the both groups receiving distilled water (10 mL/kg); three diabetic groups was treated respectively with insulin (10 UI/kg) and the aqueous extract of *B. toxisperma* (200 mg/kg, 300 mg/kg resulting from the results obtained with the effect of a single oral administration dose). Changes in fasting blood glucose levels were monitored at day 7, 14, 21 and 28 through the blood of tail vein. On other hand, body weight, food and water intakes were evaluated before the beginning (day 0) and at the end (day 28) of the study.

2.6 Evaluation of the extract on arterial blood pressure and heart rate

At the end of the treatment period, arterial blood pressure and heart rate of all rats were recorded as described by Bopda *et al.* [26]. Briefly, rats were anesthetized by intraperitoneally injection of carbamate ethyl, 98% (1.5 g/kg). The trachea was

exposed and cannulated to facilitate spontaneous breathing. The arterial blood pressure was measured from right carotid artery via an arterial cannula connected to a pressure transducer coupled with a hemodynamic recorder Biopac Student Lab. (MP35) and computer. After a 20 min stabilization period, systolic pressure, diastolic pressure and heart rate were recorded.

2.7 Determination of some serum biochemical parameters

After blood pressure and heart rate measurement, animals were sacrificed and arterio-venous blood was collected and centrifuged at 1500xg rpm at 4 °C for 15 min. Supernatant (serum) was collected and used for biochemical analysis. Parameters analyzed were consisted to Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), total bilirubin, creatinine, sodium, calcium, protein, urea levels and lipid profile (Total Cholesterol (TC), triglycerides (TG), HDL-cholesterol (HDL), Atherogenic Index (AI) and LDL-cholesterol (LDL)). Atherogenic Index (AI) was calculated according to Wakayashi and Kobaba [27] method using the following formular: AI= (TC - HDL)/TC. The LDL-cholesterol (LDL) was estimated according to the following equation [28]: LDL= TC - (HDL +TG/5). All the parameters analysis was performed according to the instructions described by the Commercial diagnostic kits (Fortress UK).

2.8 Determination of oxidative stress markers in liver and kidney

After blood collection, some organs (liver and kidney in each animal were removed and a section of each (10%) was homogenized using 50 mM HCl-Tris buffer (pH 7.4). Homogenates were centrifuged at 1500xg at 4°C for 15 min. Supernant was collected and used to determine level or activities of some antioxidant species such as Reduced glutathione (GSH) concentration by the method of Ellman [29], Superoxide dismutase (SOD) and catalase (CAT) activities according to the methods of Flohe and Otting [30] and Sinha [31]. Tissue lipid peroxidation expressed in terms of malondialdehyde (MDA) content, according to the method of Wilbur *et al.* [32].

2.9 Histolopathological procedures and histomorphometric assay

Pancreatic tissues were harvested from the animals, and tissue fragments were fixed in 10% buffered formaline solution, dehydrated in gradual concentration of alcohol, embedded in paraffin, and then stained with hematoxylin and eosin (H&E). The slides were examined using light microscope interfaced with Olympus DR10 digital camera system. The area of pancreatic islet was measured using software of area measurement (Image J. version 1.3).

2.10 Statistical analysis

All data were expressed as Mean ± SEM. The statistical analysis were performed, using one-way analysis of variance (ANOVA) followed by Tukey post-test using Graphpad Prism 5.03. A value of $p < 0.05$ was considered as significant.

3. Results

3.1 Phytochemical analysis of the extract

Phytochemical screening of *B. toxisperma* stem-barks aqueous extract revealed the presence of triterpenoids, anthocyanins, phenols, tannins, alkaloids, saponins, coumarins, polyosis, reducer compounds and flavonoids whereas sterols and poly-uronide were absent.

3.2 Hypoglycaemic activity of the extract

Acute effect of *B. toxisperma*

The effect of *B. toxisperma* aqueous extract (BTAE) on blood glucose levels in fasting normal and streptozotocin-diabetic rats is shown in table 1. A single administration of the plant extract to normoglycaemic rats significantly reduced the blood glucose level by 26.12% (p<0.05), 29.81% (p<0.05) and 34.69% (p<0.01) at the respective doses of 100, 200 and 300 mg/kg, 5 hours post-dosing as compared to initial value. The maximum effect was achieved at the dose of 300 mg/kg with the reduction in blood glucose levels of 35.73% (p< 0.01) at 5 h post-dosing as compared to normal control. Glibenclamide (5 mg/kg) exhibits maximal decrease of 52.91%, 5 hours post-dosing as compared to normal control. In diabetic rat, the plant extract significantly (p<0.001) decreased the blood glucose levels 5 hours post-dosing by 36.53%, 38.08% and 56.20% at 100, 200 and 300 mg/kg respectively, as compared to diabetic control. The maximum hypoglycaemic effect of the plant extract was 58.37% (p<0.001) at the dose of 300 mg/kg after 5 hours as compared to initial value. Insulin drastically reduced (p<0.001) blood glucose levels of diabetic rats by 71.03% and 71.92% respectively as compared to diabetic control and initial value 5 hours post-dosing.

Table 1: Effects of a single administration of *Baillonella toxisperma* aqueous extract on blood glucose levels in normoglycaemic and streptozotocin-induced diabetic rats

Treatment	Blood glucose level (mg/dL)				
	0 h	1 h	2 h	3 h	5 h
Normal control	78.28 ± 3.50	84.71 ± 5.21	76.00 ± 7.33	79.71 ± 6.92	78.57 ± 5.56
Glib 5 mg/kg	78.00 ± 2.79	79.50 ± 3.29	63.67 ± 3.12	59.00 ± 4.41	37.00 ± 3.88^{ααγγγ}
BTAE 100 mg/kg	79.86 ± 3.18	68.00 ± 4.94	73.57 ± 4.35	62.86 ± 3.26	59.00 ± 2.47
BTAE 200 mg/kg	78.83 ± 4.80	71.83 ± 4.60	72.00 ± 5.60	56.17 ± 6.54	55.33 ± 5.76^{αγ}
BTAE 300 mg/kg	77.33 ± 5.25	72.50 ± 3.68	53.83 ± 2.46	59.17 ± 3.82	50.50 ± 4.46^{ααγγ}
Normal control	72.6 ± 6.23	76.6 ± 6.02	69.2 ± 4.43	76.4 ± 3.51	75.2 ± 4.35
Diabetic control	408.4 ± 8.19^{ααα}	404.4 ± 7.89^{ααα}	384.8 ± 9.09^{ααα}	398.4 ± 5.36^{ααα}	401.8 ± 7.59^{ααα}
Insulin 10 UI/kg	414.6 ± 7.04^{ααα}	274.2 ± 4.51^{ααα***γγγ}	232.6 ± 6.95^{ααα***γγγ}	212.8 ± 7.30^{ααα***γγγ}	116.4 ± 5.35^{ααα***γγγ}
BTAE 100 mg/kg	418.2 ± 1.65^{ααα}	365.0 ± 3.53^{ααα**γγγ}	325.0 ± 4.18^{ααα***γγγ}	290.0 ± 3.99^{ααα**γγγ}	255.0 ± 3.86^{ααα***γγγ}
BTAE 200 mg/kg	425.4 ± 4.43^{ααα}	362.8 ± 6.12^{ααα**γγγ}	315.2 ± 9.47^{ααα***γγγ}	301.2 ± 4.77^{ααα***γγγ}	248.8 ± 5.5^{ααα***γγγ}
BTAE 300 mg/kg	422.8 ± 8.11^{ααα}	370.4 ± 8.21^{ααα*γγγ}	316.6 ± 10.8^{ααα***γγγ}	279.2 ± 7.93^{ααα***γγγ}	176 ± 6.82^{ααα***γγγ}

Each value represents mean ± ESM, n = 5, α p<0.05, αα p<0.01, ααα p<0.001: compared to normal control; γ p<0.05, γγ p<0.01, γγγ p<0.001: compared with initial value; * p<0.05, ** p<0.01, *** p<0.001: compared to diabetic control. BTAE = *Baillonella toxisperma* aqueous extract, Glib = Glibenclamide

Figure 1 presents the effect of BTAE on postprandial blood glucose levels of normoglycaemic rats. The plant extract failed to reduce the high blood glucose provoked by oral administration of glucose (5 g/kg) whatever the dose. However, Glibenclamide (5 mg/kg) significantly reduced (p<0.001) the blood sugar levels by 45.12% after 2 hours as compared to normal control.

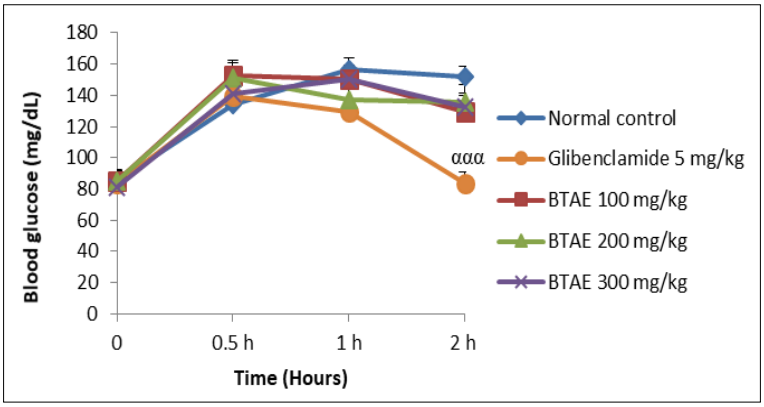


Fig 1: Effects of BTAE on blood glucose levels during oral glucose tolerance test in normoglycaemic rats. Each point represents mean ± ESM, n = 5, ααα p<0.001: compared with normal control.

Effect of repeated dose of *Baillonella toxisperma* bark aqueous extract

The effects of *Baillonella toxisperma* bark aqueous extract on body weight, food and water intakes are summarized in Table 2. The injection of streptozotocin induced significant gradual weight loss, whereas the food and water intakes significantly increased throughout the experimental period. The plant extract administration for 28 days at the dose of 200 and 300 mg/kg significantly increased the body weight (p<0.001), while food and water intakes were decreased (p<0.01) as compared to the streptozotocin-diabetic rats.

Table 2: Body weight, food and water intakes in STZ-induced diabetic rats before the starting and after 28 days of treatment with BTAE

Treatment	Body weight (g)		Food (g/rat/day)		Water (mL/rat/day)	
	Initial	Final	Initial	Final	Initial	Final
Normal control	220.40 ± 1.57	238.20 ± 1.28$^{\gamma\gamma}$	8.20 ± 1.07	9.40 ± 1.03	10.00 ± 1.22	9.60 ± 0.75
Diabetic control	178.80 ± 2.63$^{\alpha\alpha\alpha}$	158.60 ± 2.73$^{\alpha\alpha\alpha\gamma\gamma\gamma}$	17.80 ± 0.86$^{\alpha\alpha\alpha}$	24.20 ± 1.02$^{\alpha\alpha\alpha\gamma\gamma}$	21.40 ± 1.21$^{\alpha\alpha\alpha}$	24.00 ± 1.73$^{\alpha\alpha\alpha}$
Insulin 10UI/kg	175.00 ± 2.28$^{\alpha\alpha\alpha}$	193.40 ± 3.56$^{\alpha\alpha\alpha***\gamma\gamma\gamma}$	18.20 ± 1.46$^{\alpha\alpha\alpha}$	12.20 ± 0.86$^{***\gamma}$	22.20 ± 2.31$^{\alpha\alpha\alpha}$	13.60 ± 1.50$^{***\gamma\gamma}$
BTAE 200 mg/kg	174.40 ± 2.38$^{\alpha\alpha\alpha}$	192.80 ± 3.43$^{\alpha\alpha\alpha***\gamma\gamma\gamma}$	19.60 ± 1.21$^{\alpha\alpha\alpha}$	13.60 ± 1.03$^{***\gamma}$	20.00 ± 1.14$^{\alpha\alpha}$	14.60 ± 1.72**
BTAE 300 mg/kg	177.00 ± 1.05$^{\alpha\alpha\alpha}$	180.60 ± 4.13$^{\alpha\alpha\alpha***}$	20.40 ± 0.87$^{\alpha\alpha\alpha}$	16.00 ± 1.30$^{\alpha\alpha***}$	19.40 ± 1.69$^{\alpha\alpha}$	15.20 ± 1.56**

Value are expressed as mean ± SEM, n = 5. αα p<0.01, ααα p<0.001: compared with normal control; ** p<0.01, *** p<0.001: compared with the diabetic control; γ p<0.05, γγ p<0.01, γγγ p<0.001: compared with initial value. Initial = values after two weeks of diabetes induction (untreated period), final = values after 28 days of treatment.

Anti-diabetic activity of the extract

The evolution of blood glucose in streptozotocin-induced diabetic rat is showed in table 3. Daily administration of the plant extract for 28 days significantly reduced the blood glucose levels (p<0.001) in diabetic rats by 64.98% and 64.54% at the doses of 200 mg/kg and 300 mg/kg respectively, as compared to their initial value; and by 62.03% and 60.97% (p<0.001) as compared to the diabetic control animals. Insulin induced higher blood glucose reduction of 71.85% and 68.4% (p<0.001) respectively, compared to the initial value and to the diabetic control.

Table 3: Effects of repeated administration of BTAE in blood glucose levels in STZ-induced diabetic rats

Treatment	Blood glucose level (mg/dL)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Normal control	72.6 ± 6.23	61.6 ± 5.10	64 ± 2.70	56.6 ± 8.50	64.6 ± 5.39
Diabetic control	425.4 ± 10.59$^{\alpha\alpha\alpha}$	382.2 ± 16.61$^{\alpha\alpha\alpha}$	394.2 ± 15.98$^{\alpha\alpha\alpha}$	380.4 ± 17.18$^{\alpha\alpha\alpha}$	376.6 ± 11.70$^{\alpha\alpha\alpha}$
Insulin 10 UI/kg	422.8 ± 10.75	320.2 ± 15.78$^{\alpha\alpha\alpha*\gamma\gamma\gamma}$	258.8 ± 15.85$^{\alpha\alpha\alpha***\gamma\gamma\gamma}$	204.8 ± 13.34$^{\alpha\alpha\alpha***\gamma\gamma\gamma}$	119 ± 9.79$^{\alpha\alpha***\gamma\gamma\gamma}$
BTAE 200 mg/kg	408.4 ± 11.20	171 ± 8.01$^{\alpha\alpha\alpha***\gamma\gamma\gamma}$	165 ± 10.12$^{\alpha\alpha\alpha***\gamma\gamma\gamma}$	169.4 ± 9.21$^{\alpha\alpha\alpha***\gamma\gamma\gamma}$	143 ± 12.23$^{\alpha\alpha\alpha***\gamma\gamma\gamma}$
BTAE 300 mg/kg	414.6 ± 12.04	187.4 ± 12.81$^{\alpha\alpha\alpha***\gamma\gamma\gamma}$	216 ± 12.09$^{\alpha\alpha\alpha***\gamma\gamma\gamma}$	158.2 ± 12.37$^{\alpha\alpha\alpha***\gamma\gamma\gamma}$	147 ± 10.53$^{\alpha\alpha\alpha***\gamma\gamma\gamma}$

Values represent mean ± SEM, n = 5. αα p<0.01, ααα p<0.001: compared with normal control; * p<0.05, *** p<0.001: compared with the diabetic control; γγγ p<0.001: compared with initial values.

3.3 Effect of *Baillonella toxisperma* stem-bark aqueous extract on blood pressure and heart rate of diabetic rat

Streptozotocin injection induced a significant increase (p<0.001) of systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial blood pressure (MABP) as compared with normal rats (Table 4). The administration of bark aqueous extract from *Baillonella toxisperma* for 28 days at the dose of 200 mg/kg significantly decrease (p 0.001) SBP, DBP, MABP and heart rate respectively by 27.41%, 32.96%, 29.61% and 12.91% as compared to the diabetic control. The plant extract at the dose of 300 mg/kg as well as insulin treatment failed to reduce blood pressure parameters after 28 days as compared to the diabetic control.

Table 4: Effects of repeated administration of BTAE on blood pressure parameters after 28 days of treatment

Treatment	Dynamic parameters			
	SBP (mmHg)	DBP (mmHg)	MABP (mmHg)	Heart rate (BPM)
Normal control	94.40 ± 5.98	77.84 ± 4.65	87.26 ± 5.13	351.13 ± 4.00
Diabetic control	134.45 ± 4.94$^{\alpha\alpha\alpha}$	114.06 ± 2.49$^{\alpha\alpha\alpha}$	125.18 ± 3.52$^{\alpha\alpha\alpha}$	338.16 ± 2.76
Insulin 10 UI/kg	129.01 ± 1.83$^{\alpha\alpha\alpha}$	101.14 ± 6.12$^{\alpha}$	116.80 ± 3.79$^{\alpha\alpha\alpha}$	348.15 ± 3.46
BTAE 200 mg/kg	97.60 ± 4.48***	76.47 ± 3.80***	88.11 ± 3.76***	294.49 ± 3.04$^{\alpha\alpha\alpha***\delta\delta\delta}$
BTAE 300 mg/kg	124.62 ± 3.27$^{\alpha\alpha\alpha}$	106.52 ± 4.95$^{\alpha\alpha}$	116.34 ± 4.00$^{\alpha\alpha\alpha}$	347.42 ± 1.65$^{\rho\rho\rho}$

Each value represents mean ± SEM, n = 5. α p<0.05, αα p<0.01, ααα p<0.001: compared with normal control; *** p<0.001: compared with the diabetic control; δδδ p<0.001: compared with the positive control; ρρρ p<0.001: compared with the dose of 200 mg/kg.
SBP = systolic blood pressure, DBP = diastolic blood pressure, MABP = mean arterial blood pressure and BPM = beats per minute

3.4 Effect of *Baillonella toxisperma* bark aqueous extract on lipid profile, liver and kidney functions

The streptozotocin-induced diabetes in rats significantly elevated (p<0.001) serum total cholesterol, triglycerides, LDL-cholesterol and atherogenic index, whereas HDL-cholesterol significantly reduced (p<0.001) in untreated diabetic rats as compared to control normal rats (Table 4). It was also observed significant increase (p<0.001) in ALT, AST and total bilirubin concentration. At the level of kidney function, STZ injection significantly increased (p<0.01) creatininemia, natremia, uremia, urinary sodium, urinary calcium, proteinuria and urinary volume as compared with normal rats (Table 5). However, a decrease in creatininuria, urinary urea and serum calcium was recorded (p<0.001) as compared to the normal control.

Daily administration of the plant extract significantly reduced (p<0.001) total cholesterol, triglycerides, LDL-cholesterol and atherogenic index. In addition, BTAE has also enhanced HDL-cholesterol level (p<0.001) by 85.61% and 96% at the respective doses of 200 and 300 mg/kg as compared to the diabetic control animals. Likewise, treatment with insulin used as standard drug resulted in a significant decrease of total cholesterol, triglycerides and LDL-cholesterol levels (p<0.001), while no significant changes have been observed with HDL-cholesterol and atherogenic index levels as compared to untreated diabetic rats.

It was also noticed a significant decrease (p<0.001) in the level of ALT, AST and total bilirubin in diabetic animals treated with *Baillonella toxisperma* by comparing to the diabetic control. Treatment with insulin also resulted in significant decrease (p<0.001) of ALT, AST and total bilirubin as compared with diabetic group. The *Baillonella toxisperma*-treated diabetic rats showed a significant decrease (p<0.01) in creatininemia, natremia, urinary sodium, urinary calcium, uremia, proteinuria and urinary volume. Furthermore, *Baillonella toxisperma* treatment significantly increased creatininuria (p<0.05), urinary urea (p<0.001) and serum calcium (p<0.01) as compared to the diabetic control animals. Treatment with insulin resulted in a significant decrease in creatininemia, uremia, proteinuria, natremia, urinary sodium, urinary calcium, and urinary volume. Insulin treatment has also increased creatininuria (p<0.05), urinary urea (p<0.01) and serum calcium (p<0.01) as compared with diabetic group.

Table 5: Effects of repeated administration of BTAE on lipid profile after 28 days of treatment

Parameters	Groups				
	Normal control	Diabetic control	Insulin 10UI/kg	BTAE 200 mg/kg	BTAE 300 mg/kg
Cholesterol (mg/dL)	65.79 ± 1.98	330.94 ± 2.49$^{\alpha\alpha\alpha}$	182.21 ± 1.75$^{\alpha\alpha\alpha***}$	45.46 ± 2.34$^{\alpha\alpha\alpha***}$	52.49 ± 2.12$^{\alpha\alpha***}$
Triglycerides (mg/dL)	25.32 ± 1.99	111.44 ± 1.10$^{\alpha\alpha\alpha}$	65.44 ± 1.82$^{\alpha\alpha\alpha***}$	60.87 ± 1.67$^{\alpha\alpha\alpha***}$	30.29 ± 1.62***
LDL-Cholesterol (mg/dL)	17.80 ± 2.82	297.40 ± 2.28$^{\alpha\alpha\alpha}$	149.44 ± 1.80$^{\alpha\alpha\alpha***}$	12.39 ± 2.25***	24.36 ± 1.86***
HDL-Cholesterol (mg/dL)	42.93 ± 2.77	11.26 ± 1.17$^{\alpha\alpha\alpha}$	19.67 ± 1.86$^{\alpha\alpha\alpha}$	20.90 ± 1.32$^{\alpha\alpha\alpha*}$	22.07 ± 2.48$^{\alpha\alpha\alpha**}$
Atherogenic index	0.35 ± 0.04	0.96 ± 0.00$^{\alpha\alpha\alpha}$	0.89 ± 0.01$^{\alpha\alpha\alpha}$	0.53 ± 0.04$^{\alpha\alpha***\delta\delta\delta}$	0.58 ± 0.04$^{\alpha\alpha\alpha***\delta\delta\delta}$

Each value represents mean ± SEM, n = 5. αα p<0.01, ααα p<0.001: compared with normal control; *p<0.05, **p<0.01, ***p<0.001: compared with the diabetic control; δδδ p<0.001: compared with positive control.

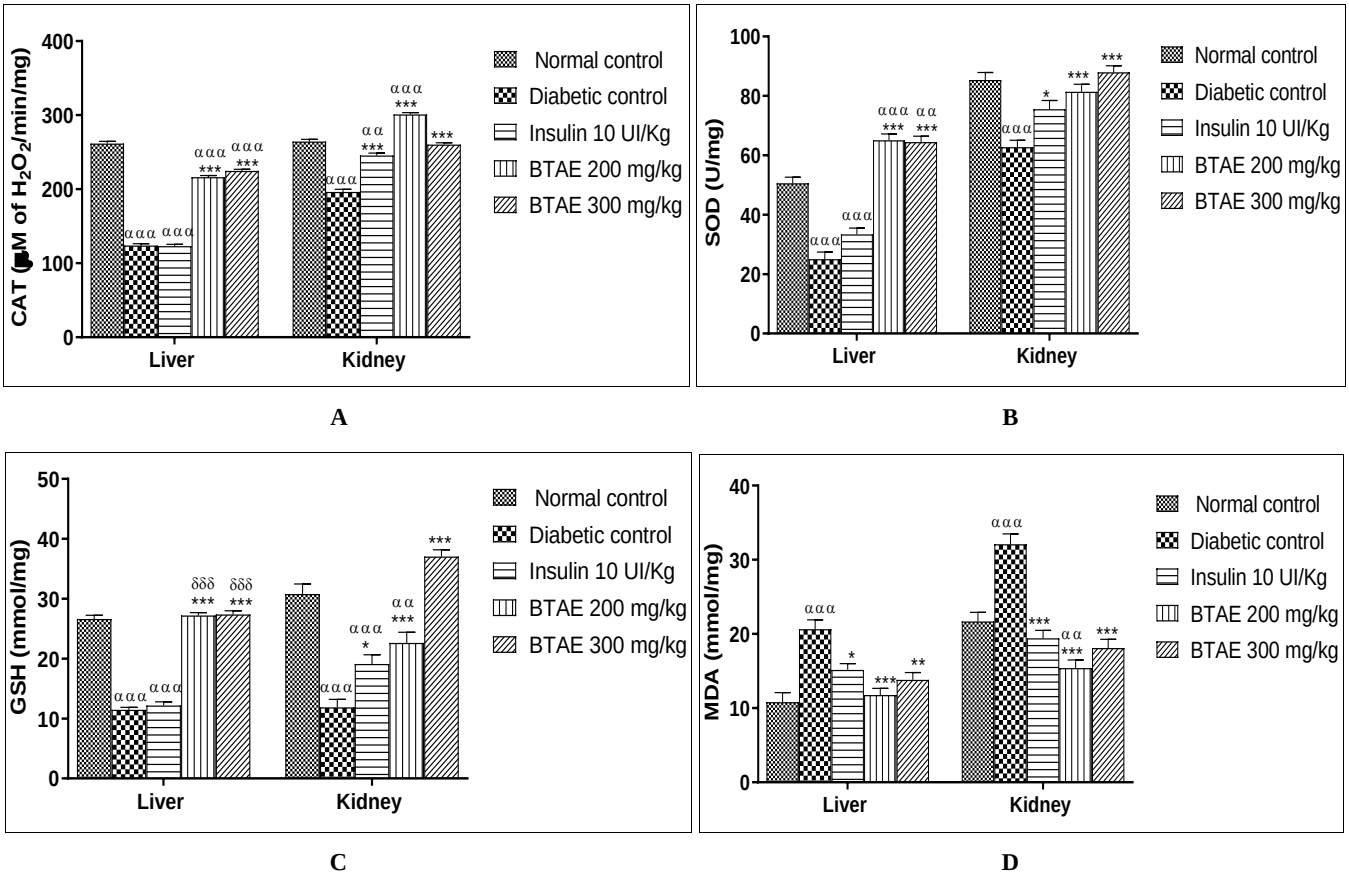
Table 6: Effects of repeated administration of BTAE on some liver and kidney parameters in the streptozotocin-diabetic rats after 28 days of treatment

Parameters	Groups				
	Normal control	Diabetic control	Insulin 10 UI/kg	BTAE 200 mg/kg	BTAE 300 mg/kg
Total bilirubin (mg/dL)	5.25 ± 0.18	8.96 ± 0.11$^{\alpha\alpha\alpha}$	5.55 ± 0.19***	3.65 ± 0.17$^{\alpha\alpha\alpha***}$	4.59 ± 0.18***
ALT (U/L)	55.87 ± 1.61	98.40 ± 1.71$^{\alpha\alpha\alpha}$	64.01 ± 1.40$^{\alpha***}$	38.13 ± 1.38$^{\alpha\alpha\alpha***}$	67.22 ± 1.69$^{\alpha\alpha\alpha***}$
AST (U/L)	112.28 ± 2.80	169.22 ± 2.69$^{\alpha\alpha\alpha}$	117.47 ± 2.20***	91.23 ± 2.88$^{\alpha\alpha\alpha***}$	89.25 ± 2.63$^{\alpha\alpha\alpha***}$
Serum creatinine (mg/dL)	2.05 ± 0.17	12.64 ± 0.38$^{\alpha\alpha\alpha}$	9.78 ± 0.38$^{*\alpha\alpha\alpha}$	4.54 ± 0.37$^{**\alpha}$	6.84 ± 0.21$^{**\alpha\delta}$
Urinary creatinine (mg/dL)	20.72 ± 0.76	3.69 ± 0.27$^{\alpha\alpha\alpha}$	7.84 ± 0.82$^{*\alpha\alpha\alpha}$	10.16 ± 0.48$^{*\alpha\alpha\alpha}$	8.76 ± 0.78$^{*\alpha\alpha\alpha}$
Serum sodium (mg/dL)	7.84 ± 0.28	10.95 ± 0.36$^{\alpha}$	7.62 ± 0.42*	7.87 ± 0.34*	7.41 ± 0.38*
Urinary sodium (mg/dL)	8.74 ± 0.47	15.09 ± 0.44$^{\alpha\alpha}$	9.66 ± 0.43*	5.76 ± 0.28$^{***\alpha}$	7.96 ± 0.38**
Serum calcium (mg/dL)	11.54 ± 0.57	2.96 ± 0.48$^{\alpha\alpha\alpha}$	7.17 ± 0.33$^{**\alpha}$	10.62 ± 0.58***	6.91 ± 0.41$^{**\alpha\alpha}$
Urinary calcium (mg/dL)	6.60 ± 0.69	13.21 ± 0.62$^{\alpha\alpha}$	8.73 ± 0.61*	8.30 ± 0.35*	9.94 ± 0.46$^{\alpha}$
Serum urea (mg/dL)	108.13 ± 1.59	206.34 ± 1.58$^{\alpha\alpha\alpha}$	133.58 ± 1.55**	115.85 ± 1.58***	119.72 ± 1.67***
Urinary urea (mg/dL)	329.31 ± 3.35	125.47 ± 2.36$^{\alpha\alpha\alpha}$	159.19 ± 2.73$^{\alpha\alpha\alpha**}$	234.47 ± 2.53$^{\alpha\alpha\alpha***\delta\delta\delta}$	251.01 ± 2.80$^{\alpha\alpha\alpha***\delta\delta\delta}$
Urinary protein (mg/dL)	1,27 ± 0.06	6,00 ± 0.06$^{\alpha\alpha\alpha}$	2,30 ± 0.07$^{\alpha\alpha\alpha***}$	1,35 ± 0.09$^{***\delta\delta\delta}$	1,36 ± 0.07$^{***\delta\delta\delta}$
Urinary volume (mL/12h)	1.16 ± 0.26	9.20 ± 0.86$^{\alpha\alpha\alpha}$	3.10 ± 0.64***	4.66 ± 0.39$^{\alpha\alpha***}$	5.10 ± 0.71$^{\alpha\alpha**}$

Each value represents mean ± SEM, n = 5. α p<0.05, αα p<0.01, ααα p<0.001: compared with normal control; *p<0.05, **p<0.01, ***p<0.001: compared with the diabetic control; δδδ p<0.001, δ p<0.05: compared with positive control.

3.5 Effect of barks aqueous extract on some oxidative stress parameters

The effects of BTAE on oxidative stress parameters are shown in figure 2. Streptozotocin-induced type 1 diabetes resulted in a significant decrease (p<0.001) of GSH concentration (Fig 2C), SOD (Fig 2B) and CAT activities (Fig 2A) and significant increase in MDA concentration (Fig 2D) as compared with normal rats. The administration of BTAE for 28 days significantly enhanced (p<0.001) GSH concentration, SOD and catalase activities in liver and kidney. However, the concentration of MDA was lowered (p<0.001) as compared to the diabetic control animals. Treatment with the insulin showed a significant increase of SOD, CAT activities and GSH level in kidney, while no significant change has been observed in the liver. Furthermore, insulin treatment significantly lowered MDA level in liver and kidney as compared to the diabetic control animals.

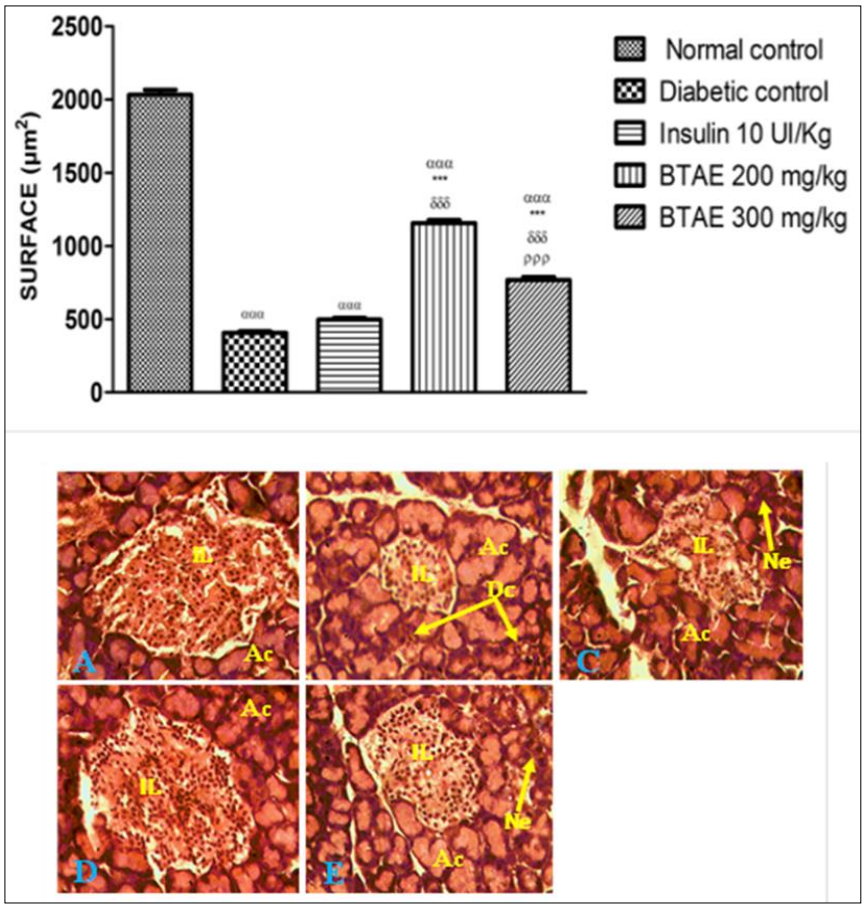


Each bar represents mean ± SEM, n = 5. αα p<0.01, ααα p<0.001: compared with normal control; *p<0.05, **p<0.01, ***p<0.001: compared with the diabetic control; δδδ p<0.001: compared with the positive control

Fig 2: Effects of repeated administration of BTAE on catalase (A), SOD (B) activities, GSH (C) and MDA concentration (D) after 28 days of treatment

3.6 Effects of the plant on the histomorphometry of pancreas

Histomorphometric examination of pancreas is shown in figure 3. In diabetic control rats as well as insulin-treated diabetic rats, the histological section of pancreatic tissues presents degenerative and necrotic changes and shrinkage in the islets of Langerhans. The pancreas of *Baillonella toxisperma*-treated diabetic groups showed islets of Langerhans with distinct β-cell mass; nevertheless, it remained few cells with some degeneration, degranulation and necrosis. Furthermore, STZ injection significantly decreased the area of islets of Langerhans by 79.96% as compared to normal control. BTAE given orally for 28 days at the dose of 200 mg/kg and 300 mg/kg significantly reversed this necrosis by enhanced the area of islets cells of as compared to diabetic control. No significant change has been obtained with insulin treatment on islets cells area as compared to diabetic control.



Each bar represents mean ± SEM (n = 14). ααα $p<0.001$: compared with normal control; ***$p<0.001$: compared with the diabetic control; δδδ $p<0.001$: compared with the positive control; ρρρ $p<0.001$: compared with the dose of 200 mg/kg.

Fig 3: Effects of repeated administration of BTAE on histomorphometry of islets cells of Langerhans. A: Normal control, showing normal cells in the islet of Langerhans (HE×400). B: Diabetic control. Shrunken islets of Langerhans displaying degenerative and necrotic changes (HE×400). C: Insulin-treated group. Degeneration and necrosis changes of pancreatic β-cells (HE×400). D and E: BTAE-treated groups. The pancreases showed viable cells in the islet of Langerhans; nevertheless, it remained few cells with some degeneration, degranulation and necrosis HE×400). IL: Islet of Langerhans; Ac: Acini cell; Dc: Degenerative changes; Ne: Necrosis

4. Discussion

The aim of the present study was to evaluate the anti-diabetic effect of *Baillonella toxisperma* Pierre stem-barks aqueous extract on the streptozotocin-induced type 1 diabetes in rats. The current findings revealed that single oral administration of aqueous extract of *Baillonella toxisperma* significantly reduced the blood glucose levels by dose dependent manner in normal rats and in STZ-induced type 1 diabetic rats. Similarly, in sub-chronic treatment, the doses of 200 and 300 mg/kg of *Baillonella toxisperma* aqueous extract given orally for 28 consecutive days to type 1 diabetic model rats as well as insulin, significantly reduced hyperglycaemia ($p<0.001$) by the end of the treatment as compared to the untreated diabetic animals. It is important to notice that the plant extract did not bring back the glycaemia toward normal value but its progressive decrease attesting the cumulative effect of the extract. This hypoglycaemic activity may be resulted from the presence of some chemical compounds in the plant extract. The phytochemical analysis of BTAE revealed the presence of phenols, saponins, alkaloids, flavonoids, tannins and triterpenoids which potent anti-diabetic activity have been reported [33-35]. Terpenoids are known to reduce glycemia through many mechanisms including peripheral glucose consumption [36], insulin-like activity, inhibition of gluconeogenesis and glycogenolysis [37]. Flavonoids and polyphenols are capable to lower blood glucose by enhancing GLUT-2 expression in pancreatic β-cells and increasing expression and promoting translocation of GLUT-4 [38, 39]. Saponins mediate their hypoglycemic effect through inhibition of intestinal glucose uptake, increase hepatic glucose storage, enhanced proliferation/regeneration of β-cells of the pancreas resulting to increase insulin secretion [40] and peripheral glucose uptake [41]. Previous studies showed that, tannins extracted from different plant materials exhibited α-amylase and α-glucosidase inhibitory activities [42], thus decreasing glucose transport through the intestinal epithelium. Similarly flavonoids [43] and alkaloids [44] have been reported to inhibit α-glucosidase, hence their remarkable additive effect in reducing blood glucose level in the current study. These different natural compounds could act synergically to induce the global hypoglycaemic effect as observed in the present study. The possible mechanism by which aqueous extract of *Baillonella toxisperma* lowered the blood glucose levels may involves potentialisation of the insulin effect, enhancing glycogen level in liver by an increase in glycogenesis and/or a decrease in glycogenolysis, either by increasing the pancreatic secretion of insulin from the cells of islet of Langerhans or probably by stimulating peripheral glucose cells uptake. In other hand, the hypoglycaemic activity could be also mediated by enhancing proliferation/regeneration and revitalisation of remaining β-cells of the pancreas resulting in increase of insulin secretion. This result is in agree with the degenerative and necrotic changes, shrinkage reversed in the islets of Langerhans and

enhanced the area of islets cells of Langerhans induced by the extract. However, further investigations are needed to determine the exact mechanism of this extract.

Diabetes induced by streptozotocin provoked hyperglycemia accompanied by symptoms like loss of weight, polydipsia and polyphagia [45]. Body weight loss in STZ-induced diabetic rats is due to the increase of protein and fats catabolism secondary to insulin deficiency [46, 47].

Polyphagia may due to defect in neuropeptide Y system and leptin effect which are response respectively to food intake and food deprivation [48]. Increase water intake is associated with loss of body weight and cells dehydration produced through mechanisms like increasing in protein catabolism, lipolysis and cells permeability. This polydipsia may reflect the increase in urine output needed to excrete the urea resulting from the additional dietary protein metabolism [49]. Daily administration of BTAE significantly increased body weight and reduced polydipsia and polyphagia as compared to the diabetic untreated rats. These improvements suggest that the plant extract might have beneficial effects on glucose metabolism avoiding protein catabolism and muscle wasting possibly due to the enhancement of insulin secretion and/or action [50].

In diabetes, chronic hyperglycemia and hyperlipidemia are implicated in the increase generation of reactive oxygen species (ROS) by promote the pro-inflammatory effects of interleukin-6 (IL-6), tumor necrosis factor (TNF) and angiotensin II which is an inflammatory adipokine [51, 52]. Inflammation is a source of oxidative stress, which is also involved in the development of atherosclerosis and high blood pressure [53]. The increase in the production of reactive oxygen species such as superoxide anions is implicated in the pathogenesis of elevated blood pressure through lipid peroxidation which is a response to endothelial dysfunction and vascular damage [54]. Moreover, the excessive ROS production induced by pro-inflammatory agents occurred in oxidative damage in several organs, such as the pancreas, liver and kidney [52].

Hence, untreated diabetic rats in this study exhibited significant increase (p<0.01) of lipid profile (TC, TG, LDL), liver parameters (ALT, AST, total bilirubin) and kidney parameters (serumcreatinine, serum and urinary sodium, urinary calcium, blood urea, blood protein and urinary volume) as well as significant decrease (p<0.001) of HDL, urinary creatinine and blood calcium. Similarly diabetic state in this study were related to significant increase (p<0.001) of blood pressure as compared with normal rats. This reflects the oxidative damage related with chronic hyperglycaemia.

BTAE given orally to diabetic rats for 28 days significantly reduced (p<0.01) TC, TG, LDL, ALT, AST, total bilirubin, blood creatinine, blood and urinary sodium, urinary calcium, blood urea, blood protein and urinary volume, whereas HDL, urinary creatinine and blood calcium increased. Additionally, the plant extract significantly decrease blood pressure. This suggests that the plant extract may have a direct effect on lipid absorption and metabolism that can lead to the improvement of diabetic lipid profile, blood pressure and reversion of liver and renal damages. Epidemiological, in vitro, and animal studies have indicated that flavonoids have beneficial impact on cardiovascular disease, involving lipoprotein oxidation, blood platelet aggregation, and vascular reactivity [55]. The plant extract could exert its effect on blood pressure via multi-action pathway involving anti-oxidant, anti-thrombogenic, anti-inflammatory, and vaso-relaxant activities of flavonoids within the extract [56-58].

The repeated administration of BTAE for 28 days exhibited antioxidant effect by increasing (p<0.001) SOD, CAT, GSH activities in liver and kidney. Moreover, daily administration of the plant extract significantly lowered MDA level (p<0.001). This suggests the ability of *Baillonella toxisperma* to counteract the deleterious effect of ROS on lipids. This hypothesis is correlated with significant recovering in pancreas architecture revealed by histological staining and histomorphometrical assay and improvement of liver and kidney parameters of diabetic rats treated with *Baillonella toxisperma* aqueous extract for 28 days. Thus, the antioxidant activities of *Baillonella toxisperma* aqueous extract are probably due to the presence of polyphenols like flavonoids, phenolic compounds, triterpenes and tannins which are known to have antioxidant properties [59]. Flavonoids exhibit potent anti-oxidative and free radical scavenging activities [60]. Furthermore, phenolic compounds in plants have the ability to scavenge free radicals and inhibit lipid peroxidation, so it can prevent cell damage [61, 62].

Therefore, the extract possesses anti-diabetic and antioxidant activities which may explain the traditional use of this plant for the management of diabetes and its complications. The present results enriched information on biological activities of medicinal plants. However, further investigations are needed to elucidate the exact mechanisms of the extract.

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