+-Original Research Article

Antidiabetic effects of *Crassocephalum crepidioides* (Benth) (*Asteraceae*) aqueous extract in streptozotocin-induced diabetic rat

ABSTRACT

Background and Aim: Diabetes mellitus is an increasing disease empirically controlled with medicinal plants, whose many virtues of some are still unknown even to the peoples who eat them as food. The study aimed to evaluate antihyperglycemic and antidiabetic effects of *Crassocephalum crepidioides* aqueous aerial parts extract in normal and diabetic rats.
Place and Duration of study: Laboratory of Animal Biology and Physiology (University of Douala), July - November 2016.
Experimental procedure: Normal, glucose-overloaded normal, and Streptozotocin (STZ)-induced diabetic Wistar rats received the *Crassocephalum crepidioides* aqueous extract at various doses (13.5–300 mg/kg) in single administration and their fasting blood glucose was followed for over 5h. In prolonged treatment, streptozotocine-induced diabetic rats received daily administration of the plant extract for 21 days and, their blood glucose level, body weight, food and water intakes, were followed weekly, while their serum biochemical parameters were evaluated after 21 days treatment. Type 1 diabetes was induced by an intravenous administration of a single dose of streptozotocin (55 mg/kg). Glibenclamide (10 mg/kg) was used as standard treatment for comparison with the plant extract.
Results and Conclusion: The acute administration of *Crassocephalum crepidioides* extract did not reduce blood glucose levels of normal and diabetic rats, but significantly reduced ($P<0.05$ – $P<0.01$) the thirtieth minute increase of glycemia in glucose-overloaded rats. Moreover, the 21-days treatment with the extract induced significant decreases ($P<0.05$ – $P<0.001$) in serum glucose, creatinine, triglycerides, total cholesterol, LDL-cholesterol and ALAT/ASAT levels or activities, and significant increases ($P<0.001$) in serum HDL-cholesterol and body weight of diabetic rats. The *C. crepidioides* aqueous extract has potential antidiabetic effects, justifying its traditional use towards diabetes mellitus.

***Key words**: Streptozotocin, Diabetes mellitus, Crassocephalum crepidioides, hypoglycemic, hypolipidemic, rats.*

1. INTRODUCTION

Diabetes mellitus is a growing, chronic and multifactor disease with lethal complications, known as a worldwide major public health problem. The number of diabetics increased by almost 62.46% from 2009 to 2019, with 19 million cases in Africa. Approximately 4.2 million adults died in 2019 due to diabetes mellitus and its complications[1]. Type 1 diabetes is the second increasing and devastating form after type 2 diabetes mellitus (90%), affecting about 1.1 million children and adolescents[1]. It is characterized by the most pancreatic beta cells destruction due to an auto-immune reaction or not [1,2], leading to body weight loss, hyperphagia, hyperdipsia, hyperglycemia and several metabolic disorders.

Nowadays, considerable progresses made in conventional antidiabetic drugs still remain unsatisfactory for the large mass of the world's population [3] due to their limited efficacy, undesirable side effects, their exorbitant cost, and sometimes to their unavailability and the purchasing power, especially in underdeveloped and developing countries, leading increasing of interest and demand for traditional herbal medicines [4]. Several plants have been studied for their safety and antidiabetic efficiency [5], but therapeutic properties of some as *Crassocephalum crepidioides* Benth (S. Moore), are still unexplored. It is a tall flowering and branching grass plant from Crassocephalum genus and Asteraceae family[6], growing annually in tropical and subtropical forest and savanna, widely distributed worldwide [7-8]. Commonly called as "red flower regleaf" or "firewood" (English), "Eleuleu" (Bakossi, South-West Cameroon), "njo'o fula'e" (Bafang, West Cameroon), "Efo Ebolo" or "Ebire"

(Yoruba, South-West Nigeria), Gbolo (Benin) [7], it is used as a vegetable, green fodder for poultry and livestock [8], medicines to treat diseases (indigestion, headache, stomach pain, cough, epilepsy [9], etc.), or is also known for its reported antibiotic, anti-inflammatory, anti-helminthic, antidiabetic, anti-malarial [10,11], anti-tumor [9], anti-oxidant and anti-hepatotoxic [12] properties. Strongly aromatic, bitter or tasteless [13], phytochemical studies revealed the presence of a lot of water, flavonoids, tannins, saponins, steroids, alkaloids, phytates, oxalate, ascorbic acid, mucilages, coumarins, reducing compounds, antracenic derivatives [10,14]. In order to scientifically prove the traditional and empirical knowledge, the present work aimed to study the anti-hyperglycemic and antidiabetic effects of *Crassocephalum crepidioides* Benth (S. Moore).

2. MATERIAL AND METHODS

2.1 Chemicals

Streptozotocin (STZ) was purchased from Sigma Chemical Co. (Saint Louis, MO, USA), Glibenclamide (GB) obtained from Mylan Laboratory, Accu-chek Plus blood glucose test strips and glucometter from Roche Diagnostics (Mannheim, Germany) and all other reagents and chemicals (Extra pure analytical grade) from common commercial suppliers were used in the study.

2.2 Plant material

Fresh leaves and stems (aerial parts) of *C. crepidioides* were harvested in July 2016 in Banguem, a locality of Koupé-Manengouba Department (South–West Region, Cameroon). Botanical identification of the plant was made at the National Herbarium of Yaoundé in comparison to the voucher specimen No. 24250 / SFR Cam.

2.3 Preparation of *Crassocephalum crepidioides* aqueous extract

Fresh leaves and stems (aerial parts) of the plant were cut, dried at room temperature and ground into powder using a grinder. Dried powder (400g) was macerated in 4 L of boiling distilled water for 10 min and then kept 12 h at room temperature before filtering. The filtrate was concentrated by freeze-drying, yielding 56.73 g (W/W 14.2%) well-dried aqueous residue, and stored at –20 °C until use.
For administration to rats in each experiment, the dried aqueous extract was weighed and dissolved in distilled water to obtain 30 mg/ml stock solutions every 3 days. Fixation of plant dosing was based on usual dosage by traditional healer (around 13.5 mg/kg dried extract / body weight (BW)) and after a pharmacological screening.

2.4 Animals

Adult male albino Wistar rats (3-month-old weighing 200 – 250 g) were used. They were raised in the animal core facility of the Faculty of Science, University of Douala. They were housed in colony cages (5 rats per cage), at controlled room temperature (28 ± 2º C) and humidity (80 – 85%), on a 12 h light/dark cycle and allowed free access to tap water and standard rat diet. Before testing for blood glucose level, the rats were fasted overnight for 12 or 16 h according to the experiment, with free access to water.

2.5 Induction of diabetes mellitus

Overnight–fasted rats were first anesthetized by short inhalation of Isoflurane via a small mask to avoid pain and stress using modified methods of Flintoff (2014) and Miller *et al* (2016). Briefly, 2 ml of isoflurane solution was soaked in cotton placed at the bottom of a suitable mask and, the apparatus was placed at the animal's mouth end so as to cover its nostrils for a maximum of 1 min. The soaked cotton was used to anesthetize by inhalation about 4 rats and replaced until the end of the work.
Diabetes was induced by a single intravenous injection (caudal vein) of STZ (55 mg/kg freshly prepared in ice cold 0.9% saline solution)[5] in overnight–fasted rats anesthetized. The procedure was performed in darkness to avoid degradation of STZ. Control rats received the vehicle alone. After STZ injection, a 5% glucose solution was given to rats for 24 h, allowing them to withstand the deep hypoglycemia that usually occurs later after STZ injection[17]. Three days after STZ injection, rats with a fasting blood glucose level of at least 250 mg/dL were considered diabetic and used in the experiments.

2.6 Measurement of fasting blood glucose level

Blood drop sample was collected from overnight-fasted rats and determination of blood glucose was carried out by glucose-peroxidase method using test strips (Accu-chek Aviva) and an appropriate glucose meter (Accu-chek Aviva Connect, Roche Diagnostics, Germany). More precisely, for fasting blood glucose determination (at 0, 0.5, 1, 2, 3 and 5 h for acute experiment, and at 0, 8, 15 and 21 days for subacute experiment), the rat was covered with a clean cloth, the tail tip was slightly injured, and the released blood drop deposited on the reactive zone of a strip connected to the glucometer. Repeated bleeding was feasible in the short term by removing the clot[5,18].

2.7 Assessment of acute effects of *Crassocephalum crepidioides* aqueous extract in normal rats

A total of 30 normal rats were randomly divided into six groups (five rats each) as follow:
Group 1: Normal control (NC) rats received distilled water (10 mL/kg)
Group 2: Normal treated rats received the standard drug, glibenclamide (10 mg/kg)
Groups 3, 4, 5 and 6 consisted of normal treated rats receiving *C. crepidioides* aqueous extract at different doses (13.5, 75, 150 and 300 mg/kg respectively).
All groups of rats received a single oral administration of the treatments by gavage. Blood glucose levels were measured before the administration of treatments (0 h), and at 1, 2, 3 and 5 h after.

2.8 Assessment of Glucose Tolerance Test in normal rats

Only doses of the plant extract of 75, 150 and 300 mg/kg were tested. A total of thirty (30) overnight fasted (16h) normal rats were randomly divided into six groups (5 rats each):
Group 1: normal control rats (NC) received distilled water (10 mL/kg)
Group 2: normal rats received distilled water (10 mL/kg) with D-glucose solution (5 mg/kg)
Group 3: normal rats received the glibenclamide (GB, 10 mg/kg) with D-glucose solution (5 mg/kg)
Groups 4, 5 and 6 constituted of normal rats receiving the plant extract at doses of 75, 150 and 300 mg/kg respectively, each with D-glucose solution (5 mg/kg).
All groups of rats received a single oral administration of the treatment by gavage. The D-glucose solution was administered by gavage, thirty minutes after treatments administration. Blood glucose levels were measured before the administration of treatments (-30 minutes), before the D-glucose administration (0 h), and at 30, 60, 120, and 150 minutes after.

2.9 Assessment of acute effects of *Crassocephalum crepidioides* aqueous extract in Diabetic rats

Only doses of 75, 150 and 300 mg/kg of the plant extract were tested. A total of twenty-five (25) diabetic and 5 normal rats were randomly divided into six groups (five rats each) as follow:
Group 1: Normal control (NC) rats received distilled water (10 mL/kg)
Group 2: Diabetic control (DC) rats received distilled water (10 mL/kg)
Group 3: Diabetic treated rats received the standard drug, glibenclamide (10 mg/kg)
Groups 4, 5 and 6 consisted of diabetic treated rats receiving *C. crepidioides* aqueous extract at doses of 75, 150 and 300 mg/kg respectively.
All groups of rats received a single oral administration of the treatments by gavage. Blood glucose levels were measured before the administration of treatments (0 h), and at 1, 2, 3 and 5 h after.

2.10 Experimental design for evaluating sub-acute effects of *Crassocephalum crepidioides* aqueous extract in diabetes rats

A total of 30 rats were used, twenty-five diabetic rats randomly divided into five diabetic groups (5 rats in each) and a group of five non diabetic rats. Rats were daily treated by gavage for 21 days starting 3 days after STZ injection with the respective drug or vehicle as follows:
Group 1: NC rats received 10 mL/kg of distilled water
Group 2: DC rats received 10 mL/kg of distilled water
Group 3: diabetic rats received the standard drug (GB, 10 mg/kg)
Goups 4, 5 and 6: diabetic rats administered with the aqueous *C. crepidioides* extract at doses of 75, 150 and 300 mg/kg respectively.
Blood glucose level was measured in 12 h fasted rats before the first treatment administration (day 0) and weekly (days 8, 15 and 22 respectively called W1, W2 and W3). Body weight and food and water intakes were monitored daily. At the end of the experimental period (day 22), the rats were anesthetized (by Isoflurane inhalation) [15,16] after fasting blood glucose determination, and the

anesthetized rats were then euthanized by decapitation. Blood samples were then collected from the abdominal aorta accessed via laparotomy [5]. The serum obtained after blood centrifugation (3000 g/ 10 min) was stored at –20 °C until analysis.

2.11 Biochemical analysis of serum

The serum was analyzed using diagnostic kits from commerce (SGM ITALIA, Rome, ITALY) for total proteins (Biuret), creatinine (colorimetric), Triglycerides (GPO–PAD method), total cholesterol (CHOD–PAD Method), HDL-cholesterol (colorimetric), ALAT (colorimetric), ASAT (colorimetric). Serum LDL-cholesterol[19] and atherogenic risk index (ARI)[20] were determined by calculation.

2.12 Statistical analysis

Data are presented as mean ± standard error of mean. One-way and two-way analysis of variance with Turkey's multiple comparison post test was performed to assess differences between groups (GraphPad PRISM Software, Version 5.03, San Diego, California, USA). *P*<0.05 was considered statistically significant.

3. RESULTS

3.1 Effect of single doses of *Crassocephalum crepidioïdes* aqueous extract on blood glucose of normal and diabetic rats

Single administration of *C. crepidioïdes* aqueous extract (13.5, 75, 150 and 300 mg/kg) produced non effects on blood glucose of normal and diabetic rats (Figure 1A and 1B). The glibenclamide (GB, 10 mg/kg) induced a significant and time-dependent reduction of glycemia only in normal rats, with a maximum fall at 5h (*P*<0.001), compared with NC or to 0h (figure 1A).

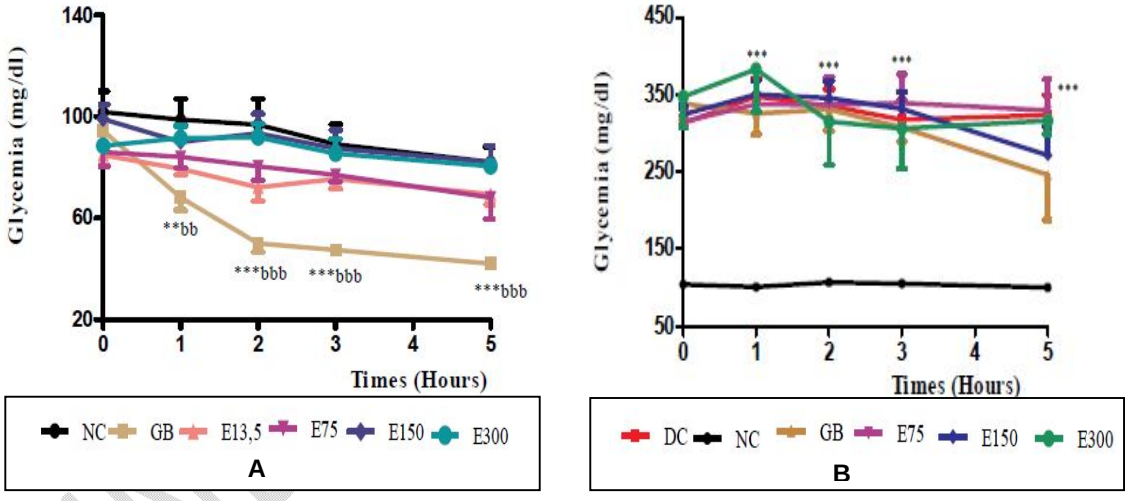

Fig. 1. Blood glucose level changes in normal (A) and Streptozotocin-diabetic (B) rats treated with single doses of *C. crepidioides* aqueous aerial parts extract.

*Test drugs significant from NC or DC, *P<0.05; **P<0.01; ***P<0.001;*
Blood glucose at different Time interval significant from initial value (0h), ^{a}P<0.05, ^{aa}p<0.01, ^{aaa}P<0.001
Mean ± SEM=Mean values ± Standard error of means of 5 rats
AE= Aqueous extract at indicated doses in mg/kg; GB= Glibenclamide (10 mg/kg); NC or DC = Normal control or Diabetic control (Distilled H2O, 10 mL/kg)

3.2 Effects of single doses of *Crassocephalum crepidioïdes* aqueous extract on oral glucose tolerance in normal rats

The blood glucose levels of all glucose-fed rats significantly increased (*P*<0.001) at the thirtieth minute following glucose overloading, compared with Normal Control (NC) or to T0 (Figure 2). The Hyperglycemic Control (HGC) rats had the maximal increase (91%). Interestingly, the thirtieth minute blood glucose increase was significantly and dose-dependently (*P*<0.001) reduced by 56.77%, 43% and 22% at respective doses of 75, 150 and 300 mg/kg of the plant extract, compared to the HGC group at T30. Glibenclamide induced the highest inhibition of 82.7% (*P*<0.001) at the same time.

Then, the blood glucose gradually decreased in each group from T30 to T150, where it greatly decreased in glibenclamide-treated rats (44.51%) and normalized in the plant extract doses (150 and 300 mg/kg)-treated ones (P<0.05), all compared to the HGC rats in which blood glucose level was still higher (P<0.001) than NC rats or the T0 (Figure 2).

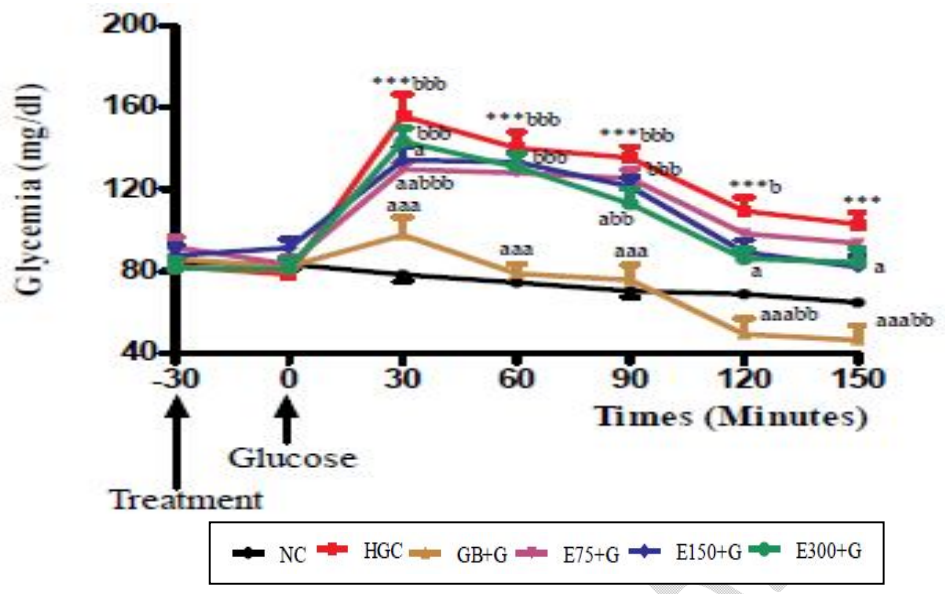

Fig. 2. Effects of single doses of aqueous aerial parts extract of *C. crepidioides* on oral glucose tolerance test in normal rat.

*Test drugs significant from NC (*P<0.05; **P<0.01; ***P<0.001), or HGC (^{a}P<0.05, ^{aa}p<0.01, ^{aaa}P<0.001)*
Blood glucose at different Time interval significant from value at 0 min, ^{b}P<0.05; ^{bb}P<0.01; ^{bbb}P<0.001
Mean ± SEM=Mean values ± Standard error of means of 5 rats
AE= Aqueous extract at indicated doses in mg/kg; GB= Glibenclamide (10 mg/kg); NC or HGC= Normal control or Hyperglycemic control (Distilled H2O, 10 mL/kg)

3.3 Subacute effects of *Crassocephalum crepidioides* aqueous extract on blood glucose level of streptozotocin-induced diabetic rats

In diabetic control (DC) rats, blood glucose increased significantly (P<0.001) by 30.11% at week 3 compared to W0 (Figure 3). The *C. crepidioides* aqueous extract (75, 150 and 300 mg/kg) has significantly and dose-dependently decreased the blood glucose levels of diabetic rats (P<0.001) until the third week (W3), where it decreased by 33.71%, 50% and 59.60% respectively, all compared to the DC rats. But, the maximal blood glucose decrease (60.48%; P<0.001) occurred at W2 with the dose of 300 mg/kg. Glibenclamide (10 mg/kg) induced a time-dependent blood glucose decrease, with a maximum drop (70.57%; P<0.001) at W3, compared with DC rats (Figure 3).

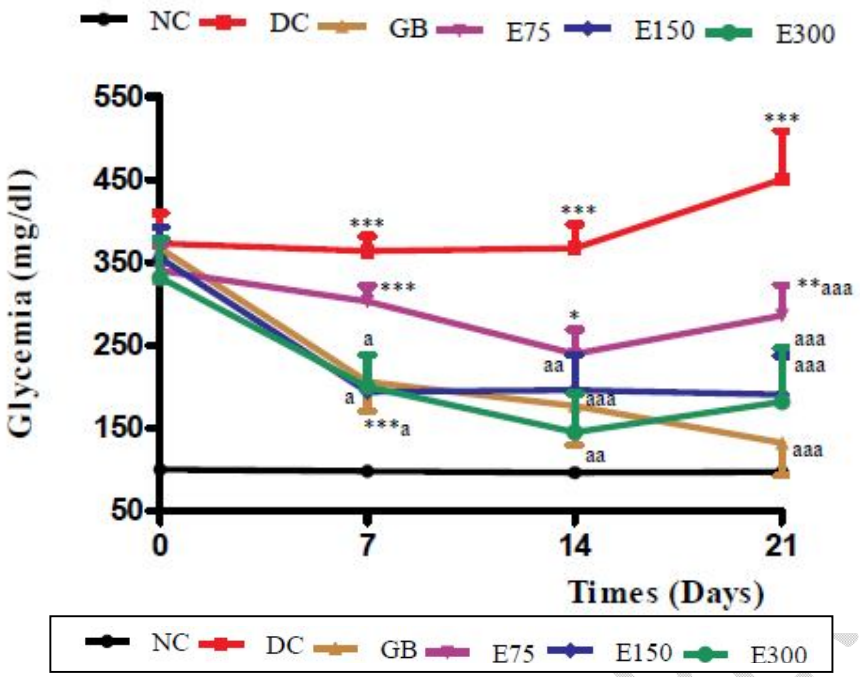

Fig. 3. Changes in blood glucose level of Streptozotocin-diabetic rats after sub-acute treatment with *C. crepidioides* aqueous aerial parts extract.

*Test drugs significant from NC (*P<0.05; **P<0.01; ***P<0.001) or DC (^{a}P<0.05, ^{aa}p<0.01, ^{aaa}P<0.001)*
Mean ± SEM=Mean values ± Standard error of means of 5 rats
AE= Aqueous extract at indicated doses in mg/kg; GB= Glibenclamide (10 mg/kg); NC or DC= Normal control or Diabetic control (Distilled H2O, 10 mL/kg)

3.4 Subacute effects of *C. crepidioides* aqueous extract on body weight and, food and water intakes of streptozotocin-induced diabetic rats

Body weight and, daily food and water consumptions did not change in normal control rats (NC). Body weight of diabetic control rats (DC) significantly decreased (*P*<0.001) at week 3 (W3), compared to NC rats and the initial weight (W1) (Table 1). The *C. crepidioides* aqueous extract at doses of 75 and 150 mg/kg induced a significant increase (*P*<0.001) in body weight of diabetic rats at W3, compared to DC rats. The high dose (300 mg/kg) stabilized the body weight throughout the experimental period. The body weight of glibenclamide–treated diabetic rats significantly increased (*P*<0.001) at W3, compared to DC rats (Table 1).

Food consumption of DC rats significantly increased by 101% (*P*<0.001) at W3, compared with NC and to W1. Diabetic rats treated with plant extract (75 - 300 mg/kg) slightly increased their food intake at W1, but significantly lowered it at W3, in comparison to DC rats (*P*<0.01 – *P*<0.001). Dietary intake of Glibenclamide-treated rats at W3 was significantly increased compared to W1 (*P*<0.05), but lower than the DC at W3 (*P*<0.001) (Table 1).

The water intake of all diabetic groups at W1 was significantly greater (*P*<0.001) than NC rats. At W3, DC rats increased their water consumption, compared to W1 (77.60%; *P*<0.001) and to the NC (3.56 times; *P*<0.001). The plant extract at all doses and Glibenclamide decreased their water consumption at W3, compared to DC rats (*P*<0.05 - *P*<0.01) (Table 1).

Table 1. Effects of sub-acute administration of *C. crepidioides* aqueous extract on body weight, food and water intakes of STZ-diabetic rats.

	Body weight gain (%)	
	W1	W3
NC (10 mL/kg)	-0.62±0 .77	+0.54±0.59

DBC (10 mL/kg)	-2.04±2.59	-10.18±0.81 ***^{bbb}
GB (10 mg/kg)	-8.48±3.23***	-1.93±2.59 ^{aaabb}
AE75 (75 mg/kg)	+1.36±0 .96	+ 7.42±0.36 ^{aaa}
AE150 (150 mg/kg)	+0.11±0.26	+5.46±0.69 ^{aa}
AE300 (300 mg/kg)	+0.42±0.92	+1.87±0.80 ^{a}

Table 1. continued

	Food intake (g/rat/day)		Water intake (mL/rat/day)	
	W1	W3	W1	W3
NC (10 mL/kg)	15.02±1.50	17.91±1.12	12.46±0.80	13.70±0.83
DBC (10 mL/kg)	17.07±0.73	34.60±1.63 ***^{bbb}	27.50±1.35 ***	48.84±1.83 ***^{bbb}
GB (10 mg/kg)	17.43±1.58	21.67±0.80 ^{aaa}	21.11±2.18**	29.70±0.97 ***^{aaabb}
AE75 (75 mg/kg)	20.10±1.50	21.41±0.95 ^{aaa}	29.50±2.18***	38.25±0.90 ***^{aaabb}
AE150 (150 mg/kg)	21.20±1.25	22.05±0.58 ^{aaa}	41,13±1.40***^{aaa}	37.06±1.01 ***^{aaa}
AE300 (300 mg/kg)	29.73±1.63 ***^{aaa}	24.90±0.49 **^{aaa}	33.16±0.96***	28.41±1.00***^{aaa}

*Test drugs significant from NC (*P<0.05; **P<0.01; ***P<0.001) or DC (^{a}P<0.05, ^{aa}p<0.01, ^{aaa}P<0.001) at the same time point;*
Week 3 (W3) value significant from Week 1 (W1) value, ^{b}P<0.05; ^{bb}P<0.01; ^{bbb}P<0.001
Mean ± SEM=Mean values ± Standard error of means of 5 rats
AE= Aqueous extract at indicated doses; GB= Glibenclamide; NC or DC= Normal control or Diabetic control (Distilled H2O, 10 mL/kg)

3.5 Subacute effects of *Crassocephalum crepidioides* aqueous extract on serum biochemical parameters of streptozotocin-induced diabetic rats

3.5.1 Effects on serum protein and creatinine

Twenty-four days after STZ injection, serum protein significantly decreased (*P*<0.01) and serum creatinine increased (*P*<0.001) in DC rats, compared to NC ones. The *C. crepidioides* aqueous extract at all doses administered did not improve the serum protein decrease, but significantly and dose-dependently decreased the serum creatinine levels (*P*<0.001) in diabetic treated rats, with a normalization at the dose of 300 mg/kg, compared to DC rats. Glibenclamide (10 mg/kg) had no effect on these parameters (Table 2).

3.5.2 Effects on serum ALAT/ASAT activities

The serum ALAT and ASAT activities of diabetic control rats significantly increased (*P*<0.001) by 76.82% and 79.69%, respectively, compared with NC rats. Only the high dose of 300 mg/kg plant extract significantly reduced the serum ALAT activity's increase (46.78%; *P*<0.01), while all the extract doses significantly reduced the increase in serum ASAT activity (*P*<0.01), all compared to DC rats. Glibenclamide (10 mg/kg) did not significantly reduce these parameters in diabetic rats (Table 2).

3.5.3 Effects on lipid profile

Untreated diabetic rats (DC) showed significantly elevated serum triglyceride (42%; *P*<0.05), total cholesterol (52.66%; *P*<0.001), LDL–cholesterol (72%; *P*<0.001), and significantly reduced serum HDL–cholesterol (67%; *P*<0.001) levels, 24 days after STZ-injection, compared with NC rats (Table 2). The 21-days treatment with *C. crepidioides* aqueous extract reduced the elevated serum triglyceride levels at all doses, with a maximum and significant decrease at the dose of 300 mg/kg of BW (52.21%; *P*< 0.01), compared with DC rats. In the other hand, the plant extract significantly reduced the elevated total cholesterol at doses of 150 mg/kg (28.49%; *P*<0.01) and 300 mg/kg (34.90%; *P*<0.001), and dose-dependently decreased the LDL-cholesterol and increased the HDL-cholesterol levels of

diabetic rats, with maximum effects at the dose of 300 mg/Kg (60.53%; *P*<0.01 for LDL-cholesterol and 61.63%; *P*<0.001 for HDL-cholesterol), compared with DC rats. After 21 days of treatment, the glibenclamide induced significant reductions in serum total cholesterol (24.37%; *P*<0.01), LDL-cholesterol (45.99%; *P*<0.05), and a significant increase in serum HDL-cholesterol (61.73%; *P*<0.001) levels, compared to DC rats. The calculated atherogenic risk index (ARI) linked to the particle size of lipoproteins, showed a high risk of 61% for DC rats to develop cardiovascular diseases than glibenclamide-treated diabetic rats (13%) and the extract-treated ones (–12% for the dose of 300 mg/kg) (Table 2).

Table 2. Effects of sub-acute administration of *C. crepidioides* aqueous extract on serum biochemical parameters of STZ-diabetic rats.

Treatments	**Total Protein** (g/dl)	**Creatinine** (mg/dl)	**ALAT** (IU)	**ASAT** (IU)
NC (10 mL/kg)	6.60±0.18	0.12±0.01	41.90±4.31	38.27±12.00
DBC (10 mL/kg)	5.47±0.23 **	0.36±0.03 ***	180.81±16.62 ***	188.42±26.07 ***
GB (10 mg/kg)	5.97± 0.317	0.31± 0.02 ***	127.36±23.55 **	142.01±4.52 ***
AE75 (75 mg/kg)	5.65±0,07 *	0.25±0.03 **aa	140.65±13.84 ***	93.31±8.60 aaa
AE150 (150mg/kg)	5.79±0,03	0.21±0.01 aaa	122.80±17.79 **	95.16±14.32 aa
AE300 (300 mg/kg)	6.03±0,03	0.12±0.01 aaa	96.22±4.78 aa	111.16±6.19 *aa

Table 2. continued

Treatments	**Triglyceride** (mg/dl)	**Total Cholest.** (mg/dl)	**HDL-Cholest.** (mg/dl)	**LDL-Cholest.** (mg/dl)	**ARI** ----
NC (10 mL/kg)	64.43±2.35	102.35±3.16	82.87±2.36	24.76±4.22	0.89±0.02
DBC (10 mL/kg)	111.69±6.81*	156.25±15.12***	27.58±2.27***	87.40±12.92***	1.61±0.02***
GB (10 mg/kg)	95.92±12.38	118.17 ±2.64aa	72.08±9.38 aaa	47.20±6.46 a	1.13±0.04aa
AE75 (75 mg/kg)	98.39±7.21	137.04±3.53*	71.90± 2.49 aaa	53.27±11.73	1.13±0.03aa
AE150 (150 mg/kg)	87.18±14.13	111.73 ±2.19 aa	53.79±6.85*	44.34±5.54 a	1.20±0.11aa
AE300 (300 mg/kg)	53.37±5.71 aa	101.72±3.91aaa	71.90±8.50 aaa	34.50±5.35 aa	0.88±0.10 aaa

*Test drugs significant from NC (*P<0.05; **P<0.01; ***P<0.001) or DC (aP<0.05, aap<0.01, aaaP<0.001)*
Mean ± SEM=Mean values ± Standard error of means of 5 rats
AE= Aqueous extract at indicated doses; GB= Glibenclamide; NC or DC= Normal control or Diabetic control (Distilled H2O, 10 mL/kg)

4. DISCUSSION

In view of worldwide diabetes increase and the side effects of modern anti-diabetic drugs, and following many other studies assessing pharmacological properties of plants used to treat diabetes mellitus [5,21], the present work assessed the anti-diabetic effects of *Crassocephalum. crepidioides* (Benth.) S. Moore aqueous extract (Asteraceae) in rat using streptozotocin (STZ)–induced experimental diabetes model, and compared to those of Glibenclamide.

Acute administration of *C. crepidioides* aqueous extract (13.5 – 300 mg/kg of body weight) did not change blood glucose levels of normal and diabetic rats. Unfortunately, administered 30 minutes before glucose overloading in normal rats, the plant extract reduced the blood glucose increase at the thirtieth minute following the glucose administration compared to the hyperglycemic control rats, with the most efficiency at the dose of 300 mg/kg. Acute treatment with Glibenclamide (10 mg/kg) decreased the blood glucose levels of normal rats and mostly prevented the blood glucose increase in glucose tolerance test, compared to each control group. These results suggest that the plant extract would have probably act on peripheral tissues, inhibiting intestinal glucose absorption and/or increasing peripheral glucose uptake in other organs, then reducing blood glucose increase after glucose overloading as suggested by Kebieche (2009) for *Ranunculus repens* L. flavonoidic extracts. The plant extract compounds would have also stimulate incretins secretion from digestive tract as reported for berbery roots [23], bitter melon [24] and soybean roots [25], or have mimic incretins, as reported for medicinal plants miming incretins effects [26], for enhancing the insulin secretion.

Incretins as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) released after meal, help to increase the insulin secretion and to inhibit glucagon release from pancreatic β-cells and α-cells respectively, and reduce the nutrients absorption rate from gut into blood [26,27].

Sub-acute administration of *C. crepidioides* aqueous extract to diabetic rats induced progressive and dose-dependent fall in blood glucose levels, compared to diabetic control rats. Baroni *et al.* (2008) have reported that *Smallanthus sonchiafolus* hydro-ethanolic extract hypoglycemic effect in diabetic rats would be due to its flavonoïds compounds. These effects of *C. crepidioides* aqueous extract would also be due to its flavonoid's compounds [10] since it has been reported that flavonoids would reduce the glycemia by inhibiting intestinal glucose transporters, reducing genes expression controlling the neoglucogenese, increasing hepatic glucose stockage, and/or by reducing glycogen hydrolyze [29,30].

The 21-days treatment with *C. crepidioides* aqueous extract increased or stabilized the body weight of diabetic rats, thus preventing the body weight loss observed in DC rats, although their proteinemia did not significantly increase compared to DC rats. Rajiv and Sasikumar (2012) also showed that, low doses of *Merremia emerginata* Burm. F. methanoïc extract did not significantly improve protein decrease in diabetic rats, despite the body weight loss reduction observed. However, it has been reported that *C. crepidioides* aqueous extract would increase the proteinemia in normal rats after 21 days of treatment [32]. Thus, the stabilization and/or increase in body weight associated with the smalless protein increase observed in *C. crepidioides* aqueous extract-treated diabetic rats would probably be due to the fact that the steroids probably contained therein [10] would have stimulated the synthesis of proteins, part of which would have been integrated into the tissues to compensate for the degradation of tissue proteins due to diabetes. Moreover, the significant reduced serum creatinine observed in *C. crepidioides* aqueous extract-treated diabetic rats, confirms the reducing or inhibiting effects of the plant extract on muscle proteins degradation, thus which would prevent protein depletion and muscle atrophy, preserve the kidney against diabetic renal toxicity, and also promote the stabilized and/or increased body weight as above observed in extract-treated rats. In DC rats, body weight loss was correlated to elevated food and water consumption. However, extract–treated diabetic rats would have reduced this polyphagia and polydipsia by inhibiting intestinal nutrients absorption, and/or inhibiting satiety.

The significant decrease in ALAT and ASAT serum activities of *C. crepidioides* aqueous extract-treated animals and sometimes more than glibenclamide compared to DC rats, would suggest protective effect of the plant extract (thanks to it flavonoids contain) against liver dysfunction and, cardiac, renal and/or muscle toxicity. These effects of the plant extract are similar to those of *Camomile Recutita* flowers ethanoic extract [33].

The *C. crepidioides* aqueous extract at all doses and glibenclamide significantly improved the lipid profile by decreasing serum triglyceride, total cholesterol, LDL-cholesterol and increasing serum HDL-cholesterol, thus reducing the atherogenic risk in diabetic rats, compared to DC rats. Interestingly, the plant extract dose of 300 mg/kg normalized these different lipid parameters, sometimes more than (triglycerides decrease) or similarly (HDL-cholesterol increase) to glibenclamide. Many studies have reported that multiple flavonoids, terpenes, saponins and other phenolic compounds contained in plant extracts would exert their cholesterol-lowering effects by inhibiting the activity of Acyl-Coenzyme A cholesterol acyl transferase (ACAT), by inhibiting the intestinal cholesterol absorption by binding to bile acids in the intestine, and/or by increasing biliary excretion[34,35]. It has been reported that the *C. crepidioides* aqueous extract contains in addition to flavonoids and steroids several other biological molecules like terpenes, saponins, etc... Thus, because of these multiple compositions, the beneficial effects of the plant on diabetic dyslipidemia would be mediated by one or more of these compounds[10]. Bahar *et. al.* (2016) had also reported beneficial effects of the methanolic extract of *C. crepidioides* on dyslipidemia induced by a high lipid diet in rats, and suggested the involvement of flavoinoids contained in plant.

5. CONCLUSION

The singlely administration of *C. crepidioides* S. (Moore) aerial parts aqueous extract did not change the blood glucose of normal and diabetic rats, but induced moderate antihyperglycemic impact. Its prolonged administration to STZ-diabetic rats induced potent antidiabetic effects, with efficiency at the dose of 300 mg/kg better than glibenclamide. These effects might be due to the combined action of all or at least some of its metabolites. This plant extract may be useful for alternative oral treatment of

type 1 diabetes and its metabolic alterations. However, it would be important to assess the effects of this plant on the release of intestinal incretins to better explain its mechanism of action.

ETHICAL APPROOVAL

All authors hereby declare that "All Guidelines for Care and Use of Laboratory Animals as described in the European Community Guidelines (EEC Directive 2010 / 63 / EU of the September 22, 2010)" were followed, as well as specific national's ethical committee lows were applicable. All experiments have been examined and approved by the Institutional Ethical Committee of the University of Douala (Ref N° CEI – 2015/01954).

SOURCE OF FUNDING – Please declare the source of funding for the purchase of all the things you used including the rats.

CONFLICT OF INTEREST – Provide conflict of interest statement please

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