Journal of Complementary and Alternative Medical Research

19(2): 43-55, 2022; Article no.JOCAMR.92736
ISSN: 2456-6276

Antidiabetic Effects of *Crassocephalum crepidioides* (Benth) (*Asteraceae*) Aqueous Extract in Streptozotocin-Induced Diabetic Rat

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Authors' contributions

This work was carried out in collaboration among all authors. Author TMC contributed to the design and supervision of the study, performed the statistical analysis and wrote the first draft of the manuscript. Author EP wrote the protocol, conducted the experiment and managed the literature searches. Author BZC managed the serum assays for the study. Authors DDPD and DAB provided guidance during the design of the study and corrected the manuscript. Author CSP designed the study and supervised all the work. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JOCAMR/2022/v19i2389

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/92736

Original Research Article

Received 27 August 2022
Accepted 29 October 2022
Published 04 November 2022

ABSTRACT

Background and Aim: Diabetes mellitus is an increasing disease empirically controlled with medicinal plants, whose many virtues are still unknown even by people who eat them as food. The study aimed to evaluate the antihyperglycemic and antidiabetic effects of *Crassocephalum crepidioides* aerial parts aqueous extract in normal and diabetic rats.
Place and Duration of Study: Laboratory of Animal Biology and Physiology (University of Douala), July - November 2016.

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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 a single administration, and their fasting blood glucose was followed for over 5h. In prolonged treatment, Streptozotocin-induced diabetic rats received daily administration of the plant extract for 21 days and, blood glucose level, body weight, food, and water intake were followed weekly, while serum biochemical parameters were evaluated after 21 days of 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-day 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 poten antidiabetic effects, justifying its traditional use for diabetes mellitus.

Keywords: 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 people with diabetes 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 and its complications [1]. Type 1 diabetes is the second-increasing and most devastating form after type 2 diabetes (90%), affecting about 1.1 million children and adolescents [1]. It is characterized by most pancreatic beta cell 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 progress made in conventional antidiabetic drugs remains unsatisfactory for the large mass of world's population [3] due to their limited efficacy, undesirable side effects, their exorbitant cost, and sometimes to their unavailability and 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 the therapeutic properties of some as *Crassocephalum crepidioides* Benth (S. Moore), are still unexplored. It is a tall flowering and branching grass plant from the Crassocephalum genus and Asteraceae family [6], growing annually in tropical and subtropical forests 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, anti-diabetic, anti-malarial [10,11], anti-tumor [9], anti-oxidant and anti-hepatotoxic [12] properties. Strongly aromatic, bitter, or tasteless [13], phytochemical studies revealed in its aerial parts the presence of a lot of water, flavonoids, tannins, steroids, mucilages, coumarins, reducing compounds, combined, anthracene derivatives C-heterosides, saponins, phytates, oxalate, ascorbic acid, protein, lipid, and also minerals such as calcium, sodium, potassium, iron, magnesium, manganese, zinc etc. [10,14]. 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. MATERIALS AND METHODS

2.1 Chemicals

Streptozotocin (STZ) was purchased from Sigma Chemical Co. (Saint Louis, MO, USA), Glibenclamide (GB) was obtained from Mylan Laboratory, Accu-chek Plus blood glucose test strips and glucometers 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 Materials

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 first made by Professor BETTI Jean Lagarde from Department of Biology of Plant Organisms of the Faculty of Science, the University of Douala, and subsequently 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 (400 g) was macerated in 4 L of boiling distilled water for 10 min and then kept for 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 extract dosing was based on the usual dosage by a traditional healer (around 13.5 mg/kg dried extract/body weight (BW)), the therapeutic dose calculated after acute toxicity study [15] and pharmacological screening. Glibenclamide was used as standard drug (10 mg/kg bw) [5].

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 ± 2ºC) 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 levels, 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. Procedure was performed in darkness to avoid STZ degradation. Control rats received the vehicle alone. After the 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” [16]. Three days after STZ injection, rats with 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, 1, 2, 3, and 5 h or -30, 0, 30, 60, 90, 120 and 150 min for the acute experiment, and at 0, 8, 15, and 21 days for the subacute experiments), the rat was covered with a clean cloth then, the tail tip was slightly injured, and the released drop of blood was deposited on the reactive zone of a strip connected to the glucometer. Repeated bleeding was feasible in the short term by removing the clot” [5,17].

2.7 Assessment of Acute Effects of *Crassocephalum crepidioides* Aqueous Extract in Normal Rats

A total of 30 normal rats fasted for 12h 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 appropriate treatment by gavage. Blood glucose levels were measured before treatments administration (0 h), and at 1, 2, 3 and 5 h after.

2.8 Assessment of Glucose Tolerance Test in normal rats

Only plant extract doses 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).

Each rat received a single administration of appropriate treatment by gavage. The D-glucose solution was administered by gavage, thirty minutes after treatments administration. Blood glucose levels were measured before treatments administration (-30 minutes), before D-glucose administration (0 minute), and at 30, 60, 120, and 150 minutes after.

2.9 Assessment of Acute Effects of *Crassocephalum crepidioides* Aqueous Extract in Diabetic rats

Only plant extract doses of 75, 150 and 300 mg/kg 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 different drugs by gavage. Blood glucose levels were measured before drugs administration (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 *C. crepidioides* aqueous 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 7, 14 and 21 respectively called W1, W2 and W3). Body weight and food and water intakes were monitored daily. At the end of the experimental period (day 21), rats were anesthetized (by Isoflurane inhalation) [18,19] after fasting blood glucose determination, and anesthetized rats were then euthanized by decapitation. Blood samples were then collected from abdominal aorta accessed via laparotomy [5]. Serum obtained after blood centrifugation (3000 g/ 10 min) was stored at –20 °C until analysis.

2.11 Serum Biochemical Analyzes

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 [20] and atherogenic risk index (ARI) [21] were determined by calculation.

2.12 Statistical Analyzes

Data are presented as mean ± standard error of mean. One-way and two-way analyzes of variance with Turkey's multiple comparison post-test were performed to assess differences between groups (GraphPad PRISM Software, Version 5.03, San Diego, California, USA). $P<0.05$ was considered 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 (Fig. 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 5 h ($P<0.001$), compared with NC or to 0h (Fig. 1A).

3.2 Effects of Single Doses of *Crassocephalum crepidioïdes* Aqueous Extract on Oral Glucose Tolerance in Normal Rats

Blood glucose 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 (Fig. 2). Hyperglycemic Control (HGC) rats had the maximal increase (91%). Interestingly, the thirtieth minute blood glucose increase was significantly ($P<0.001$) reduced and in an inversely dose-dependent manner by 56.77%, 43% and 22% at respective plant extract doses of 75, 150 and 300 mg/kg, compared to HGC group at T30. Glibenclamide induced the highest inhibition of 82.7% ($P<0.001$) at the same time. Then, blood glucose gradually decreased in each group from T30 to T150, where it greatly decreased in glibenclamide-treated rats (54.95%; $P<0.001$) and normalized in plant extract doses (150 and 300 mg/kg)-treated ones (20.19% and 18.06% respectively; $P<0.05$), all compared to HGC rats in which blood glucose was still higher ($P<0.001$) than NC rats or at T0 (Fig. 2). In sum, only the dose extract of 300 mg/kg significantly reduced the mean blood glucose ($P<0.05$) after glibenclamide ($P<0.001$), compared to HGC (AUC from T0 to T150).

3.3 Effects of Subacute Administration of *Crassocephalum crepidioides* Aqueous Extract on Blood Glucose Level of Streptozotocin-Induced Diabetic Rats

Blood glucose of diabetic control (DC) rats increased significantly ($P<0.001$) by 30.11% at day 21 (D21) compared to D0 (Fig. 3). *C. crepidioides* aqueous extract (75, 150 and 300 mg/kg) significantly and dose-dependently decreased blood glucose levels of diabetic rats ($P<0.001$) until the 21st day where it decreased by 33.71%, 50% and 59.60% respectively, all compared to DC rats. But, the maximal blood glucose decrease (60.48%; $P<0.001$) occurred at D14 with the dose extract of 300 mg/kg. Glibenclamide (10 mg/kg) induced a time-dependent blood glucose decrease, with a maximal drop (70.57%; $P<0.001$) at D21, compared to DC rats (Fig. 3).

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). *C. crepidioides* aqueous extract at doses of 75 and 150 mg/kg induced significant increases ($P<0.001$) 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. Body weight of glibenclamide–treated diabetic rats increased significantly ($P<0.001$) at W3, compared to DC rats (Table 1).

Food consumption of DC rats increased significantly by 101% ($P<0.001$) at W3, compared to NC and W1. Diabetic rats treated with the plant extract (75 - 300 mg/kg) slightly increased their food intake at W1, but lowered it significantly 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 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).

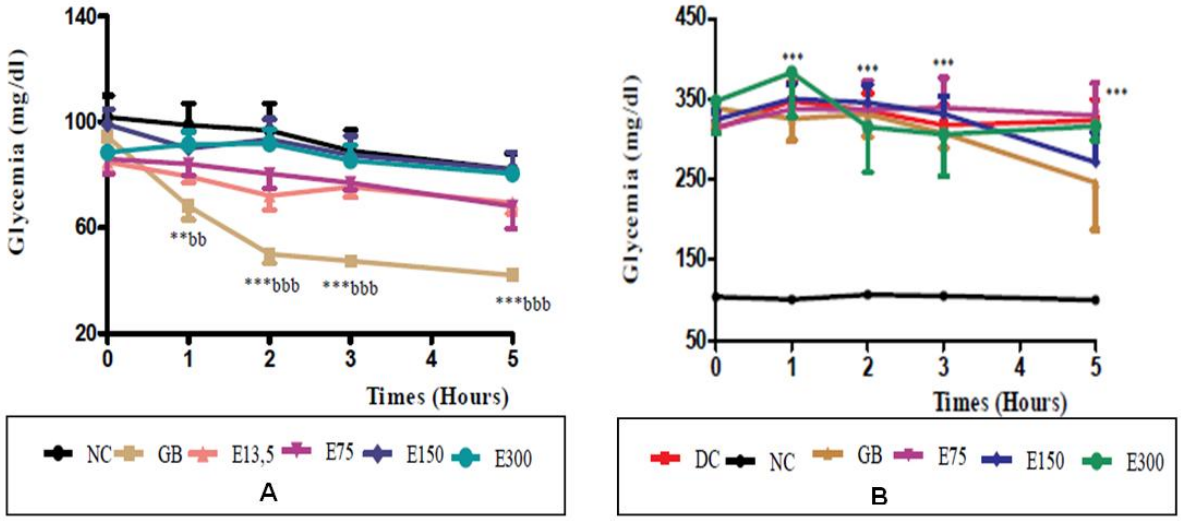

Fig. 1. Blood glucose level changes in normal (A) and streptozotocin-diabetic (B) rats treated with single doses of *C. crepidioides* aqueous aerial parts extract

*Test drugs significant from NC or DC, *P<0.05; **P<0.01; ***P<0.001;*
Blood glucose at different Time intervals significant from the 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
E= 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)

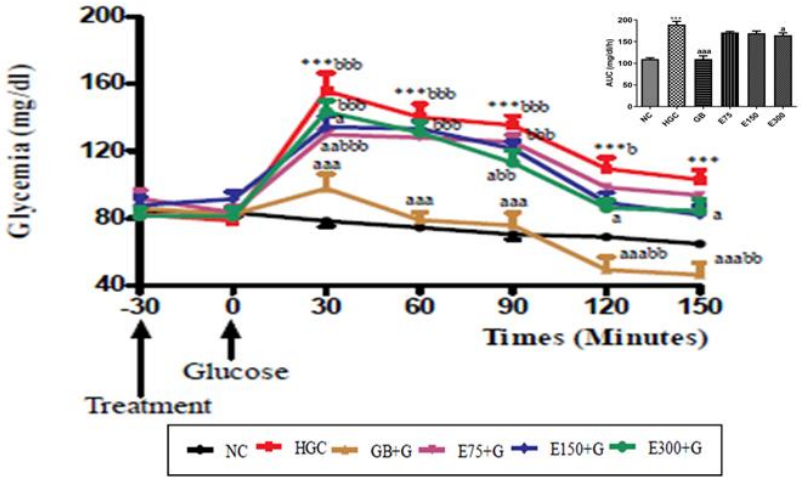

Fig. 2. Effects of single doses of aqueous aerial parts extract of *C. crepidioides* on oral glucose tolerance test in normal rats

*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 intervals significant from the 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
E= 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)

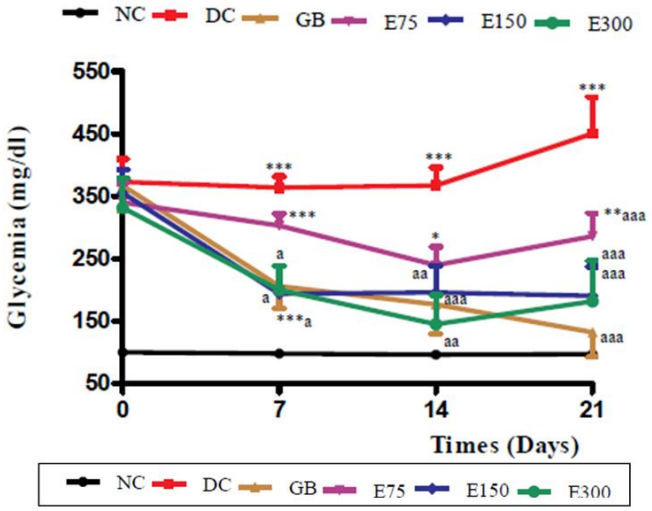

Fig. 3. Changes in blood glucose level of Streptozotocin-diabetic rats after sub-acute treatment with *C. crepidioides* aqueous aerial parts extract

*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
E= 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)

3.5 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. *C. crepidioides* aqueous extract at all doses did not improve the decrease of serum protein, but significantly and dose-dependently decreased serum creatinine levels (*P*<0.001) of 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

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 extract doses significantly reduced the increase of 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 elevated serum triglyceride levels at all doses, with a maximum effect at dose of 300 mg/kg bw (52.21%; *P*< 0.01), compared to DC rats. In the other hand, the plant extract significantly reduced elevated total cholesterol levels at doses of 150 mg/kg (28.49%; *P*<0.01) and 300 mg/kg (34.90%; *P*<0.001); Additionally, the decrease in LDL-cholesterol and increase in HDL-cholesterol observed in plant extract-treated diabetic rats were dose-dependent, 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 to DC rats. After 21 days of treatment, 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 extract-treated ones (–12% for the dose of 300 mg/kg) (Table 2).

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

Treatments	Body weight gain (%)		Food intake (g/rat/day)		Water intake (mL/rat/day)	
	W1	W3	W1	W3	W1	W3
NC (10 mL/kg)	-0.62±0 .77	+0.54±0.59	15.02±1.50	17.91±1.12	12.46±0.80	13.70±0.83
DBC (10 mL/kg)	-2.04±2.59	-10.18±0.81 ***^{bbb}	17.07±0.73	34.60±1.63 ***^{bbb}	27.50±1.35 ***	48.84±1.83 ***^{bbb}
GB (10 mg/kg)	-8.48±3.23 ***	-1.93±2.59 ^{aaabb}	17.43±1.58	21.67±0.80 ^{aaa}	21.11±2.18 **	29.70±0.97 ***^{aaabb}
E75 (75 mg/kg)	+1.36±0 .96	+ 7.42±0.36 ^{aaa}	20.10±1.50	21.41±0.95 ^{aaa}	29.50±2.18 ***	38.25±0.90 ***^{aaabb}
E150 (150 mg/kg)	+0.11±0.26	+5.46±0.69 ^{aa}	21.20±1.25	22.05±0.58 ^{aaa}	41,13±1.40 ***^{aaa}	37.06±1.01 ***^{aaa}
E300 (300 mg/kg)	+0.42±0.92	+1.87±0.80 ^{a}	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
E= Plant aqueous extract at indicated doses; GB= Glibenclamide; NC or DC= Normal control or Diabetic control (Distilled H2O, 10 mL/kg)

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)	Triglyceride (mg/dl)	Total Cholest. (mg/dl)	HDL-Cholest. (mg/dl)	LDL-Cholest. (mg/dl)	ARI ----
NC (10 mL/kg)	6.60±0.18	0.12±0.01	41.90±4.31	38.27±12.00	64.43±2.35	102.35±3.16	82.87±2.36	24.76±4.22	0.89±0.02
DBC (10 mL/kg)	5.47±0.23 **	0.36±0.03 ***	180.81±16.62 ***	188.42±26.07 ***	111.69±6.81 *	156.25±15.12 ***	27.58±2.27 ***	87.40±12.92 ***	1.61±0.02 ***
GB (10 mg/kg)	5.97± 0.317	0.31±0.02 ***	127.36±23.55 **	142.01±4.52 ***	95.92±12.38	118.17±2.64 ^{aa}	72.08±9.38 ^{aaa}	47.20±6.46 ^{a}	1.13±0.04 ^{aa}
E75 (75 mg/kg)	5.65±0,07 *	0.25±0.03 **^{aa}	140.65±13.84 ***	93.31±8.60 ^{aaa}	98.39±7.21	137.04±3.53 *	71.90± 2.49 ^{aaa}	53.27±11.73	1.13±0.03 ^{aa}
E150 (150mg/kg)	5.79±0,03	0.21±0.01 ^{aaa}	122.80±17.79 **	95.16±14.32 ^{aa}	87.18±14.13	111.73±2.19 ^{aa}	53.79±6.85*	44.34±5.54 ^{a}	1.20±0.11 ^{aa}
E300 (300 mg/kg)	6.03±0,03	0.12±0.01 ^{aaa}	96.22±4.78 ^{aa}	111.16±6.19 *^{aa}	53.37±5.71 ^{aa}	101.72±3.91 ^{aaa}	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 (^{a}P<0.05, ^{aa}p<0.01, ^{aaa}P<0.001)*
Mean ± SEM=Mean values ± Standard error of means of 5 rats
E= Plant 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 side effects of modern anti-diabetic drugs, and following many other studies assessing pharmacological properties of plants used to treat diabetes [5,22], 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 body weight) did not change blood glucose levels in 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 hyperglycemic control rats, The dose extract of 300 mg/kg was more efficient in glucose tolerance test (GTT), but less than glibenclamide (10 mg/kg) which decreased the blood glucose in normal rats and highly prevented its increase in GTT, compared to each control. These first results suggest that the plant extract would probably have acted on peripheral tissues (digestive tract and others), inhibiting intestinal glucose absorption and/or increasing cells glucose uptake, therefore reducing the blood glucose increase after glucose overloading as suggested by Kebieche and Meraihi [23] for *Ranunculus repens* L. flavonoidic extracts. On the other hand, the plant extract would also probably have stimulated the intestinal incretins secretion during passage through the digestive tract (administered orally) as reported for berbery roots [24], bitter melon [25] and soybean roots [26], and/or would, through its numerous bioactive metabolites, have mimicked the incretins insulin-secretory effect as reported for medicinal plants miming incretins effects [27], leading thus to a slight insulinaemia increase which would has been the cause of the observed slight hypoglycaemia. Incretins like glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) released after meal, help ~~to~~ increasing β-cells insulin secretion and inhibiting α-cells glucagon release, and reduce the gut absorption of nutrients [27,28]. Various studies have shown that polyphenols (notably flavonoids or even their secondary metabolites) could modulate carbohydrate metabolism and exhibit antidiabetic activities by inhibiting sugars degradation and intestinal absorption, improving liver and muscles glucose uptake, protecting the pancreatic β cell and having direct insulin-secretory effects or through incretins. Mucilages compounds from plants such as fenugreek (*Trigonella graenum*) regulate postprandial glycemia playing an important role in the treatment of hyperglycemia [29]. These different compounds already identified in *C. crepidioides* extracts [10,14] would be involved in its observed antihyperglycaemic effects, giving it a preventive and curative powers against diabetes and its complications.

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. [30] reported that *Smallanthus sonchiafolus* hydro-ethanolic extract hypoglycemic effect in diabetic rats would be due to its flavonoïds compounds. The flavonoid compounds probably contained in the *C. crepidioides* aqueous extract (10) would also be involved in the hypoglycaemic effects of the plant observed in diabetic rats. Indeed, as reported, these metabolites would have reduced blood glucose by inhibiting intestinal sodium/glucose co-transporters, reducing the expression of genes involved in the control of gluconeogenesis, increasing hepatic glucose storage, and/or by reducing glycogen hydrolysis [31,32].

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 [33] also showed that, low doses of *Merremia emerginata* Burm. F. methanoïc extract did not significantly improve protein decrease in diabetic rats, despite the observed body weight loss reduction. However, *C. crepidioides* aqueous extract would has increased the proteinemia in normal rats after 21 days of treatment [15]. 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 steroids probably contained therein [10] would have stimulated proteins synthesis, 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. Body weight loss in DC rats was correlated to elevated food and water consumption. However, extract–treated diabetic rats would have reduced these polyphagia and polydipsia by inhibiting intestinal nutrients absorption, and/or satiety.

The significant decrease in serum ALAT and ASAT activities of *C. crepidioides* aqueous extract-treated animals and sometimes more than glibenclamide compared to DC rats, would suggest a protective effect of the plant extract against liver dysfunction and, cardiac, renal and/or muscle toxicity. These plant extract effects are similar to those of *Chamomile Recutita* flowers ethanoic extract [34], and would result on effects of some or all of its bioactive metabolites like flavonoids.

C. crepidioides aqueous extract at all doses and glibenclamide significantly improved 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 to (HDL-cholesterol increase) glibenclamide. Many studies have reported that multiple flavonoids, terpenes, saponins and other phenolic compounds contained in plants extracts would exert their cholesterol-lowering effects by inhibiting the Acyl-Coenzyme A cholesterol acyl transferase (ACAT) activity, inhibiting the intestinal cholesterol absorption by binding to bile acids in the intestine, and/or increasing biliary excretion [35,36]. *C. crepidioides* aerial parts aqueous extract has been reported containing 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) also reported beneficial effects of the methanolic extract of *C. crepidioides* on high lipid diet-induced dyslipidemia in rats, and suggested the involvement of flavoinoids contained in plant.

Oxidative stress is the cause of diabetes and plays a major role in the onset of complications and in insulin responses. However, although the antioxidant effects of the plant extract have not been evaluated in these diabetic rats, it is likely that the association of the biometabolites of this plant extract (ascorbic acid, flavonoids, polyphenol, phytate, zinc...) whose antioxidant properties are widely reported, would have effectively fought synergistically against oxidative stress and its deleterious effects, thus contributing to the improvement of glucose, lipid and protein metabolism. For example, the ascorbic acid would allow the regeneration of vitamin E and glutathione. The zinc would protect insulin from free radical attack, allowing it to maintain its activity, maintained membrane fluidity, insulin stability and insulin receptor synthesis [29].

5. CONCLUSION

Single 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 intestinal incretins release and oxidative stress, to better explain its action mechanism.

CONSENT

It is not applicable.

ETHICAL APPROVAL

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 September 22, 2010)" were followed, as well as specific national's ethical committee laws were applicable. All experiments have been examined and approved by the Institutional Ethical Committee of the University of Douala (Ref N° CEI – 2015/01954).

ACKNOWLEDGEMENTS

We wish to express our sincere thanks to the Alexander von Humboldt Foundation for its

award of the equipment grant to one of the authors, and to Professor BETTI Jean Lagarde from Department of Biology of Plant Organisms of the Faculty of Science, the University of Douala for his help to the plant's identification.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
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