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Kalanchoe crenata decoction and infusion improve insulin resistance and fasting glycemia of diabetic obese rats MACAPOS 2

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ABSTRACT

Objective: Kalanchoe crenata is a medicinal plant traditionally used in diabetes management. Type 2 diabetes is a metabolic disorder characterized by abnormally high glycemia and insulin resistance. We investigated the effects of *Kalanchoe crenata* aqueous extracts on diabetic rats.

Methods: Six-week-old normal rats were fed a high-fat diet for 16 weeks. Then, selected obese rats intravenously received a unique dose of streptozotocin (22.5 mg/kg b.w.). One week later, the obtained diabetic rats received *K. crenata* decoction (DKc) or infusion (IKc) once daily, respectively, at 37.5, 75.0, or 150.0 mg/kg b.w., or Metformin(70 mg/kg) once daily for 42 days, during which the fasting glycemia, urine volume, and water intake were recorded. At the end of the treatment, animals were subjected to oral glucose tolerance and insulin tolerance tests. They were then sacrificed; serum and pancreas were collected for insulinemia evaluation and histological analysis, respectively.

Results: Obese diabetic animals had fasting glycemia > 200 mg/dL. The DKc, IKc, and Metformin reduced ($P < 0.05$) glycemia with the maximum effect ($P < 0.01$) in the 6th week and improved glucose tolerance and insulin resistance. The extracts decreased 24-h urine volume as well as water intake and reduced insulinemia ($P < 0.01$): IKc75 (−66.4%) and IKc150 (−59.3%). This result was associated with decreased results of Homeostatic Model Assessment for Insulin Resistance (HOMA-IR). The extracts improved the size of Langerhans islets. Infusion improved diabetes parameters to a greater extent than decoction.

Conclusion: Kalanchoe crenata aqueous extracts improved glucose tolerance and insulin resistance in diabetic rats. This study contributed to justifying the empirical use of this plant for diabetes management.

1. Introduction

Type 2 diabetes mellitus is a metabolic disorder of carbohydrate metabolism in which glucose is underutilized as an energy source and overproduced due to insufficient insulin action, leading to a chronic high glycemic condition.[1,2] Inadequate secretion of insulin and/or increased insulin resistance are the typical pathophysiological characteristics of diabetes. Type 2 diabetes constitutes 90%–95% of diabetes mellitus cases.[2] In addition to inadequate secretion of insulin and insulin resistance, type 2 diabetes can also be caused by a combination of genetic factors and environmental factors such as obesity, lack of exercise, and stress.[3] In 2019, it was estimated that about 463 million people were living with diabetes worldwide, with a global prevalence of 9.3%[4]; and by 2021, this number had increased to 536 million, with a global prevalence of 10.5%.[5] This reflects an ascendancy of the pandemy.[5] Despite there being several oral hypoglycemic drugs as well as insulin therapy, a

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complete and successful cure for diabetes mellitus remains untouched because of several intrinsic deficiencies and adverse effects of the used molecules.[6] In finding new therapies against diabetes mellitus, medicinal plants appear as a potential source of new and effective molecules.

Kalanchoe crenata (Andrews) Haw. (Crassulaceae) is a common plant found in several African regions, where it is used to treat many ailments, particularly inflammatory diseases. *K. crenata* has been used in the western region of Cameroon for decades to treat diabetes mellitus. In our previous research, organic extracts of this plant exhibited anti-hyperglycemic activities[7]. Here we demonstrated that at 135 and 200 mg/kg, its water/ethanol extract significantly reduced the blood glucose levels in normal rats; and in diabetic rats, it maintained the glycemia (antihyperglycemic activity) at the normal levels.[7] The thin layer chromatography (TLC) revealed the presence of sterols, 3β-sitosterol glycoside, phenolic compounds, flavonoids, and saponins in the CH_2CH_2/CH_3OH extract of *K. crenata* fresh leaves.[8] Furthermore, alkaloids, saponins, terpenoids, and flavonoids were identified in the leaves of *K. crenata* extract. Some compounds of flavonoids and alkaloids can activate the synthesis and translocation of glucose transporter 4 (GLUT-4), potentiate insulin release, and increase expression of skeletal muscle GLUT-4.[9] To produce a phytomedicine, which may be cheaper and available for poor rural populations for diabetes mellitus management, this study was undertaken to determine the effects of *K. crenata* aqueous extracts on diabetic rats' glycemia.

2. Materials and methods

2.1. K. crenata aqueous extracts

The whole plant of *K. crenata* (Andrews) Haw. was harvested in Bamendjou in the west region of Cameroon and was identified by Pr. Jean Lagarde BETTI at the National Herbarium in Yaoundé-Cameroon, where a voucher specimen was deposited under the serial number 50103/YA; the plant name was checked and confirmed on the 11th of March 2024, via the link http://www.worldfloraonline.org. The plants were sliced into small pieces, shade-dried, and then ground. The obtained powder was used to prepare two aqueous extracts: decoction (DKc) and infusion (IKc). For decoction: the powder of the plant was introduced in water and boiled for 15 min then filtered. For infusion: the powder of the plant was introduced in boiled water and left until cooled then filtered. According to the previous work,[7] three doses (37.5, 75.0, 150.0 mg/kg) of each extract were prepared. Based on the dose administered to humans by traditional healers the equivalent dose was calculated using the NOAEL calculation method.[10]

2.2. Phytochemical screening

Qualitative phytochemistry was performed using standard assays found in the literature.

2.2.1. Determination of the total polyphenols

The total phenolic content of the extracts was evaluated according to the following method.[11] An aliquot of 0.1 mL of extract (4 mg/mL) was mixed with 0.75 mL of Folin-Ciocalteu reagent (10-fold diluted). Then, the mixture was kept at room temperature (5 min); 0.75 mL of sodium carbonate (Na_2CO_3, 6% w/v) was added. The mixture was homogenized and incubated at room temperature in darkness for 90 min, the absorbance was read at 725 nm against the blank reagent using a Spectrophotometer (Biobase; 2015 version). Gallic acid (0–1000 μg/mL) was considered as standard. The total polyphenol contents of each extract were calculated from the standard curve. The results were expressed as micrograms of gallic acid equivalent per gram of dry matter (μg GAE/g DM). Each test was performed in triplicate.

2.2.2. Determination of the total flavonoids

According to the method described by Aiyegoro and Okoh, the total flavonoid content of the extracts was evaluated as follows[12]: An aliquot of 0.5 mL of extract (4 mg/ mL) was added into a tube containing 1.5 mL of methanol; then, 0.1 mL of aluminum chloride ($AlCl_3$, 10% w/v), 1 mL of potassium acetate (CH_3COOK, 1 M) and 2.8 mL of distilled water was added successively. The mixture was homogenized, incubated at room temperature for 30 min and the absorbance was read at 415 nm against blank reagent. Quercetin (0–1000 μg/mL) was used as standard. The total flavonoid content of each extract was calculated from the standard curve and expressed as micrograms of quercetin equivalent per gram of dry matter (μg QE/g DM). Each test was performed in triplicate.

2.2.3. Determination of the total tannins

The total tannin content of the extracts was evaluated according to the following method.[13] 1 mL of the extract (4 mg/mL) was added to 5 mL of working solution (50 g of vanillin +4 mL of HCl (1 N in 100 mL of distilled water)), and the mixture was incubated at 30 °C for 20 min. The absorbance was read at 500 nm against the blank reagent. Tannic acid (0–1000 μg/mL) was used as standard. The tannin content of each extract was expressed as a microgram of tannic acid equivalent per gram of dry matter (μg TAE/g DM) from the standard curve. Each test was performed in triplicate.

2.3. Animals

Wistar rats were raised in the animal house of the Laboratory of Human Metabolism and Non-Communicable Diseases of the Institute of Medical Research and Medicinal Plants Studies (IMPM), Yaoundé-Cameroon. They were maintained under environmental conditions and fed with a standard local diet (3400 kcal: carbohydrates 50%–55%, fats 15%–20%, proteins 25%–30%),[14] with free access to water. *In vivo* experiments were conducted following the institutional committee of the Cameroonian Ministry of Scientific Research and Innovation guidelines and regulations adopted from the European Union on Animal Care (CEE Council 86/609)[15] and were approved by the University of Buea Institutional Animal Care and Use Committee (UB-IACUC), authorization number: 14/2022. The ARRIVE (Animal Research Reporting of *In Vivo* Experiments) guidelines were also observed.

2.4. Induction of diabetic obese rats

Before diabetes induction, male albino *Wistar* rats (six weeks old) were kept for two weeks of acclimatization in the animal house with *ad libitum* access to a normal standard diet (ND) and water. After the baseline period, rats were randomly divided into two groups: the normal group (ND subjected to the standard diet) and the high-fat diet group (HFD receiving a high-fat diet, 4730 kcal: carbohydrates 65%–70%, fats 25%–30%, and proteins 10%–15%).[14] After 4 months under the respective feeding regimen, obese rats were selected considering their Lee index and body weight gain.[16] The selected obese animals were then subjected to oral glucose tolerance and insulin tolerance tests. All the rats that were intolerant to glucose and resistant to insulin were selected. To induce permanent hyperglycemia, the glucose intolerant and insulin resistant rats received (iv) a unique dose (22.5 mg/kg) of streptozotocin[17] (Sigma Aldrich, 18883-66-4). One week later, those with fasting glycemia >126 mg/dL were considered diabetic.[18]

2.5. Assessment of DKc and IKc's effects on diabetic rats

Diabetic rats were divided into eight groups of five animals each and received the following substances: the diabetic control group (HFDZ), distilled water; six test groups, DKc or IKc respectively at 37.5, 75.0, and 150.0 mg/kg b.w.; positive control group (Met), the reference drug, Metformin (70.0 mg/kg). The animals were treated once daily for 6 weeks. During treatment, the fasting glycemia was evaluated at the beginning, then every two weeks; and the volume of the water intake was recorded every week. At the end of the treatment, the 24-h urine volume

of each animal was measured; also, the oral glucose tolerance test (OGTT) as well as the insulin tolerance test (ITT) were performed. Following these tests, the rats were sacrificed by cervical dislocation under light ether anesthesia (cotton impregnated with two drops of ether in a closed 2 L-container for direct inhalation). Blood was then collected in dried tubes and centrifuged; the serum was used to determine the blood insulin level; the pancreas was collected for histological analysis.

2.6. Glycemic reactivity

2.6.1. OGTT

The OGTT was performed according to the following protocol: after a 12-h fasting period, all rats were given glucose (D-glucose, Central Drug House, 2.5 mg/kg b.w.). Blood glucose was estimated just before administration and at 30, 60, and 120 min after oral glucose administration.

2.6.2. ITT

The ITT was carried out according to the protocol described by Mvongo and collaborators with slight modifications.[18] After a 12-h fasting period, each animal's blood glucose level was measured, and then the animal received a subcutaneous injection of insulin (Actrapid Human HM, 2 UI/kg b.w.). The glycemia was then estimated at 15, 30, and 60 min after insulin administration.

2.6.3. Insulinemia and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR)

The serum insulin level was evaluated using ELISA kits (Elabscience, Rat Insulin, ELISA kit, E-EL-R3034), and following the manufacturer's instructions. The HOMA-IR was calculated using the following formula: fasting insulin (μUI/mL) × fasting glycemia ((mM/L)/22.5).[19]

2.7. Histological analysis of the pancreas

After fixation, a small slice of the pancreas tissue was removed and placed in histological cassettes for dehydration before being embedded in paraffin. Subsequently, sections with a thickness of 5 μm were made and recovered on slides. The slides were stained in hematoxylin, and then in eosin. After staining, the slides were observed and photographed using a microscope (Scientico STM-50) equipped with a camera (Celestron 44421).

2.8. Statistical analysis

The results were expressed as Mean ($\overline{X}$) ± Standard Error of Mean (SEM). The statistical analyses were performed by one-way analysis of variance (ANOVA) associated with Tukey's test followed by the Dunnett test, using GraphPad Prism 8.0.1. The difference between and within various groups was significant at $P < 0.05$.

3. Results

3.1. Phytochemical screening

Following the phytochemical screening, active compounds identified in *K. crenata* aqueous extracts (both in DKc and IKc) were alkaloids, phenolic compounds, flavonoids, tannins, glycosides, anthraquinones, coumarins, anthocyanins, and saponins, and terpenoids and sterols were not detected by the test.

3.2. Total polyphenol, flavonoid, and tannin contents

K. crenata decoction (DKc) and infusion (IKc) were shown to contain almost the same concentrations of polyphenol, flavonoid, and tannin (Table 1).

Table 1
Quantitative phytochemistry.

Extracts	DKc	IKc
Polyphenols (Concentration, μg GAE/g DM)	891.11 ± 13.97	896.11 ± 11.89
Flavonoids (Concentration, μg QE/g DM)	207.71 ± 10.72	194.17 ± 5.97
Tannins (Concentration, μg TAE/g DM)	4455.56 ± 41.57	4494.44 ± 217.87

GAE: Gallic Acid Equivalent; **QE:** Quercetin Equivalent; **TAE**: Tannic Acid Equivalent; **DM:** Dry Matter. $n = 3$.

3.3. Effects of DKc and IKc on fasting glycemia, water intake, urine volume

3.3.1. Effects of DKc and IKc on fasting glycemia

The high-fat diet in diabetic control rats significantly increased glycemia compared to the normal control rats (NC). The treatment with *K. crenata* aqueous extracts at 37.5, 75.0, and 150.0 mg/kg b.w. (decoction DKc and infusion IKc) or Met (70.0 mg/kg b.w.), from the 14th day, significantly decreased the fasting glycemia respectively ($P < 0.05$): 14.4% (DKc37.5), −23.9% (DKc75), −27.0% (DKc150); −10.3% (IKc37.5), −33.5% (IKc75), −40.8% (IKc150) and −23.3% (Met), compared to the diabetic control group (HFDZ) (Table 2). The treatment of DKc and IKc, or the Met, has progressively and dose-dependently decreased the blood glucose level. The reduction of the glycemia at day 42 was 42.0% (DKc150), 49.0% (IKc150), and 41.0% (Met) ($P < 0.01$) compared to the initial glycemia value (Table 2).

3.3.2. Effects of DKc and IKc on water intake

Water intake significantly increased in diabetic control compared to the NC rats. Both infusion and decoction of *K. crenata,* as well as Metformin, significantly decreased diabetic rats' water intake (Table 3). On day 42, treatment of IKc at 150 mg/kg showed the biggest reduction in water intake (−55.84%, $P < 0.01$).

Table 2
Fasting glycemia (mg/dL) of rats during the treatment.

Groups	0 days	14 days	28 days	42 days	
NC	84.4 ± 3.3	84.6 ± 1.8	84.0 ± 2.3	82.0 ± 1.0	(-3%)
HFDZ	238.9 ± 9.9**	249.8 ± 7.2**	254.1 ± 4.6	237.4 ± 10.9	(-1%)
Met	236.0 ± 6.3**	192.0 ± 12.8**$^{\beta a}$ [-23%]	186.4 ± 13.8**$^{\beta a}$ [-26.6%]	139.2 ± 1.8**$^{\beta b}$	(-41%)
DKc37.5	233.1 ± 6.2**	210.9 ± 8.0**^{a} [-14.4%]	197.9 ± 6.3**$^{\alpha a}$ [-22.1%]	157.9 ± 9.3**$^{\beta b}$	(-32%)
IKc37.5	231.7 ± 12.0**	218.6 ± 15.7**^{a} [-10.3%]	202.7 ± 14.1**$^{\alpha a}$ [-20.22%]	161.1 ± 3.1**$^{\beta b}$	(-30%)
DKc75	234.7 ± 7.8**	189.9 ± 6.3**$^{\beta a}$ [-23.9%]	177.1 ± 4.5**$^{\beta a}$ [-30.3%]	122.9 ± 2.5**$^{\beta b}$	(-47%)
IKc75	232.8 ± 11.0**	165.0 ± 11.1**^{a} [-33.5%]	138.2 ± 6.1**$^{\beta b}$ [-45.6%]	124.3 ± 9.3**$^{\beta b}$	(-46%)
DKc150	219.4 ± 8.8**	170.2 ± 10.2**$^{\beta a}$ [-27.0%]	149.4 ± 12.7**$^{\beta a}$ [-40.2%]	129.0 ± 11.3**$^{\beta b}$	(-42%)
IKc150	235.8 ± 14.3**	150.1 ± 15.2**$^{\beta b}$ [-40.8%]	141.8 ± 6.2**$^{\beta b}$ [-44.2%]	120.5 ± 7.7**$^{\beta b}$	(-49%)

NC: normal control; HFDZ: Diabetic control rats. Diabetic rats treated for 42 days once daily with 37.5, 75.0, and 150.0 mg/kg b.w. *K. crenata* decoction (DKc37.5, DKc75, DKc150) or infusion (IKc37.5, IKc75, IKc150), or Metformin 70 mg/kg b.w. (Met). ( ): % of glycemia variation compared to the initial glycemia value; [ ]: % of glycemia variation compared to HFDZ. $\overline{X}$ ± SEM; $n = 5$. Significant difference: **$P < 0.01$ compared to NC; $^{a}P < 0.05$, $^{b}P < 0.01$ compared to HFDZ; $^{\alpha}P < 0.05$, $^{\beta}P < 0.01$ compared to initial value.

Table 3
Water intake volume (as % of initial value = 100) of rats during the treatment.

Groups	0 day	7 days	14 days	21 days	28 days	35 days	42 days
NC	100.0	93.3 ± 1.7	98.3 ± 3.3	89.4 ± 2.6	95.1 ± 3.1	90.5 ± 2.9	95.3 ± 3.0
HFDZ	100.0	120.2 ± 3.9$^{**\beta}$	121.9 ± 6.8$^{**\beta}$	133.5 ± 4.4$^{**\beta}$	128.7 ± 4.0$^{**\beta}$	104.4 ± 3.1*	112.6 ± 8.1*
Met	100.0	124.8 ± 0.2$^{**\beta}$	56.0 ± 0.7$^{**\beta b}$	53.9 ± 0.9$^{**\beta b}$	76.6 ± 1.3$^{*\beta b}$	56.8 ± 1.2$^{**\beta b}$	58.9 ± 0.8$^{**\beta b}$
DKc37.5	100.0	108.2 ± 1.9^{a}	79.8 ± 2.1$^{*\beta b}$	75.8 ± 1.9$^{\beta b}$	64.2 ± 1.7$^{**\beta b}$	74.4 ± 1.9$^{*\beta b}$	67.7 ± 2.0$^{*\beta b}$
IKc37.5	100.0	107.4 ± 3.2^{*a}	78.5 ± 0.8$^{**\beta b}$	51.7 ± 1.1$^{**\beta b}$	65.1 ± 0.8$^{**\beta b}$	53.0 ± 1.1$^{**\beta b}$	57.7 ± 0.7$^{**\beta b}$
DKc75	100.0	131.6 ± 9.8$^{**\beta}$	102.4 ± 9.8^{b}	86.8 ± 6.3^{b}	80.2 ± 4.3$^{*\alpha b}$	69.4 ± 5.2$^{**\beta b}$	59.5 ± 3.1$^{**\beta b}$
IKc75	100.0	104.1 ± 3.5^{b}	69.0 ± 0.1$^{**\beta b}$	63.5 ± 0.7$^{**\beta b}$	70.4 ± 1.2$^{**\beta b}$	66.2 ± 1.2$^{**\beta}$	53.1 ± 0.6$^{**\beta b}$
DKc150	100.0	100.0 ± 1.5^{b}	78.4 ± 1.2$^{**\beta b}$	61.5 ± 1.1^{*b}	60.2 ± 1.8$^{**\beta b}$	54.1 ± 1.1$^{**\beta b}$	48.7 ± 1.0$^{**\beta b}$
IKc150	100.0	98.6 ± 8.0^{b}	58.7 ± 1.5$^{**\beta b}$	58.7 ± 1.3$^{**\beta b}$	55.2 ± 1.2$^{**\beta b}$	45.5 ± 0.8$^{**\beta b}$	44.2 ± 0.9$^{**\beta b}$

NC: normal control; HFDZ: Diabetic control rats. Diabetic rats during the 42 days treatment once daily with 37.5, 75, 150 mg/kg b.w. *K. crenata* decoction (DKc37.5, DKc75, DKc150) or infusion (IKc37.5, IKc75, IKc150) or Metformin 70 mg/kg b.w. (Met). $\overline{X} \pm$ SEM; $n = 5$. Significant difference: $^{*}P < 0.05$, $^{**}P < 0.01$ compared to NC; $^{a}P < 0.05$, $^{b}P < 0.01$ compared to HFDZ; $^{\alpha}P < 0.05$, $^{\beta}P < 0.01$ compared to initial value.

3.3.3. *Effects of DKc and IKc on urine volume*

Compared to normal animals, diabetic control rats showed a significant increase in urine volume ($P < 0.01$). Treatment of diabetic animals with DKc and IKc at different doses, or Metformin significantly ($P < 0.01$) reduced the 24-h urine volume. The most potential activity was observed in the treatment group of IKc at 150 mg/kg (−53.8%) (Fig. 1).

3.4. *Effects of DKc and IKc on glycemia reactivity in diabetic obese rats*

3.4.1. *OGTT*

After glucose administration, glycemia increased at 30, 60, and 120 min in the HFDZ group rats (+57.62%, +76.23%, and +61.18%) compared to normal rats. The area under the curve (AUC) was notably high: 164 in HFDZ vs 100 in NC (Table 4). *K. crenata* decoction, infusion at all doses, and Metformin significantly decreased the glycemia of diabetic rats 60 and 120 min after oral glucose administration ($P < 0.01$, Fig. 2). The maximum anti-hyperglycemic effects on glucose tolerance were observed in the DKc75 and IKc75 groups (Fig. 2A—B), with respective AUCs of 92.2 and 94.3 compared to NC with 100.4 (Table 4).

3.4.2. *Insulin sensitivity*

The glycemia level of diabetic rats treated with decoction at 75 mg/kg (Fig. 3A) or infusion at 150 mg/kg (Fig. 3B), or Metformin 70 mg/kg, was slightly similar to that of the NC animals. The AUCs were shown to be low in different treated test groups, compared to diabetic control: respectively 202.0, 205.1, and 208.9 for NC, Met, and IKc150, vs 270.0 for HFDZ (Table 4).

3.5. *Effects of DKc and IKc on insulinemia and HOMA-IR*

At the end of the 42-day treatment, the HFDZ rat's blood insulin level remained very high (67 µUI/mL) compared to NC rats (32 µUI/mL). Treatment of DKc at 37.5 and 150 mg/kg decreased blood insulin level, while the 75 mg/kg dose raised it (+24 µUI/mL) compared to HFDZ (Fig. 4A). Treatment of IKc at 75 and 150 mg/kg decreased the blood insulin level by 66.4% and −59.3%, respectively (Fig. 4A). Compared to the HFDZ group, treatment of IKc as well as Metformin significantly decreased the results of HOMA-IR ($P < 0.01$): 82.5% (IKc75), −80.0% (IKc150), −80.0% (Met), with a greater decrease than that of DKc: 30.38% (DKc75) and −65.00% (DKc150), as shown in Fig. 4B.

3.6. *Effects of the extracts on the pancreas morphology of diabetic obese rats*

Histologic analysis of the pancreas of rats showed well-organized islets with normal sizes in the NC rats (Fig. 5A). In diabetic animals, a reduction in the size of the islets was observed as well as an abnormal appearance of their borders (Fig. 5B). Compared to the normal rats, Metformin (Fig. 5C) and the extracts (decoction: Fig. 5D—F; infusion: Fig. 5G—I) considerably reduced the damages.

4. Discussion

In the previous studies, diabetic obese rats obtained after 16 weeks of a high-fat diet were intolerant to glucose and resistant to insulin.[7,9,15] Streptozotocin at a low dose (22.5 mg/kg) induced in obese rats MACAPOS 2 a fasting hyperglycemia.[17] This result might be due to a favorable environment for the hyperglycemia onset created by the high-fat diet.[20]

Significant fasting blood glucose reduction was observed from the second week of treatment of diabetic rats with *K. crenata* decoction (DKc), infusion (IKc), or Metformin (Met). The extract dose-dependently decreased the glycemia of diabetic rats to a greater extent than that of Metformin, and the best hypoglycemic activity was observed for IKc. The extracts, therefore, may act via mechanisms different from Metformin. It was reported that *K. crenata* extract could inhibit the mitochondrial fat synthesis pathway and reduce hepatic fat storage, through the activation of the AMPK (AMP-activated protein kinase) pathway, improving insulin sensitivity.[21] *K. crenata* decoction and infusion contain alkaloids,

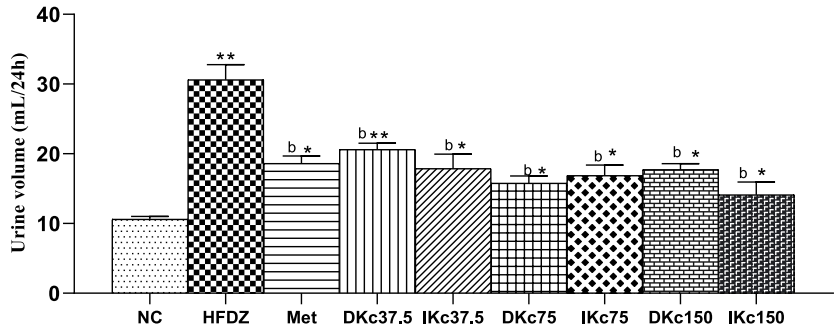

Fig. 1. Rat urine volume (in 24 h) after 42 days of treatment with DKc, IKc or Met. NC: normal control; HFDZ: Diabetic control rats (High-fat diet). Diabetic rats treated with *K. crenata* decoction respectively at 37.5, 75.0, or 150.0 mg/kg b.w. (DKc37.5, DKc75, DKc150) or with *K. crenata* infusion respectively at 37.5, 75.0, or 150.0 mg/kg b.w (IKc37.5, IKc75, IKc150) or with Metformin 70 mg/kg b.w (Met). $\overline{X} \pm$ SEM; $n = 5$. Significant difference: $^{*}P < 0.05$, $^{**}P < 0.01$ compared to NC; $^{b}P < 0.01$ compared to HFDZ.

Table 4
AUC of OGTT and ITT in rats after treatment.

Groups	NC	HFDZ	Met	DKc37.5	IKc37.5	DKc75	IKc75	DKc150	IKc150
OGTT	100.5	164.4	125.9	117.6	143.8	92.2	94.3	132.6	116.3
ITT	202.0	270.0	205.1	243.8	231.6	221.2	216.4	210.0	208.9

NC: normal control; HFDZ: Diabetic control rats; DKc37.5, DKc75, DKc150: Diabetic rats treated with *K. crenata* decoction respectively at 37.5, 75, and 150 mg/kg b.w.; IKc37.5, IKc75, IKc150: Diabetic rats treated with *K. crenata* infusion decoction respectively at 37.5, 75, and 150 mg/kg b.w.; Met: Metformin 70 mg/kg b.w.

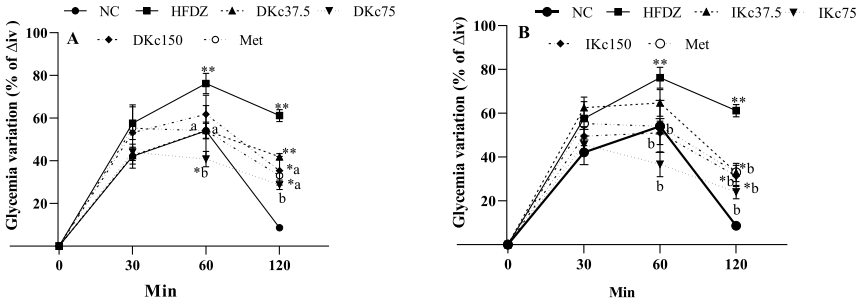

Fig. 2. Glycemia variation (expressed as % of initial value variation (Δiv)) in rats during OGTT after 42 days of treatment with *K. crenata* decoction, infusion, or Metformin. NC: normal control; HFDZ: Diabetic control rats. Diabetic rats treated with (A) *K. crenata* decoction respectively at 37.50, 75.0, or 150.0 mg/kg b.w. (DKc37.5, DKc75, DKc150) or (B) *K. crenata* infusion respectively at 37.50, 75.0, or 150.0 mg/kg b.w. (IKc37.5, IKc75, IKc150), or Metformin 70 mg/kg b.w. (Met). $\overline{X} \pm$ SEM; $n = 5$. Significant difference: $^{*}P < 0.05$, $^{**}P < 0.01$ compared to NC; $^{a}P < 0.05$, $^{b}P < 0.01$ compared to HFDZ.

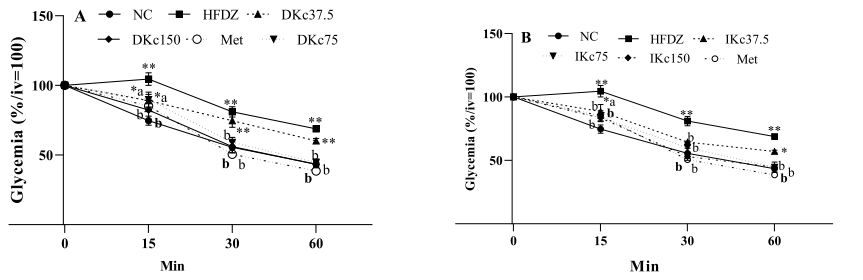

Fig. 3. Glycemia (expressed as % of initial value) in rats during ITT after 42 days of treatment with *K. crenata* decoction, infusion, or Metformin. NC: normal control; HFDZ: Diabetic control rats. Diabetic rats treated with (A) *K. crenata* decoction respectively at 37.5, 75.0, or 150.0 mg/kg b.w. (DKc37.5, DKc75, DKc150) or (B) *K. crenata* infusion respectively at 37.5, 75.0, or 150.0 mg/kg b.w. (IKc37.5, IKc75, IKc150), or Metformin 70 mg/kg b.w. (Met). $\overline{X} \pm$ SEM; $n = 5$. Significant difference: $^{*}P < 0.05$, $^{**}P < 0.01$ compared to NC; $^{a}P < 0.05$, $^{b}P < 0.01$ compared to HFDZ.

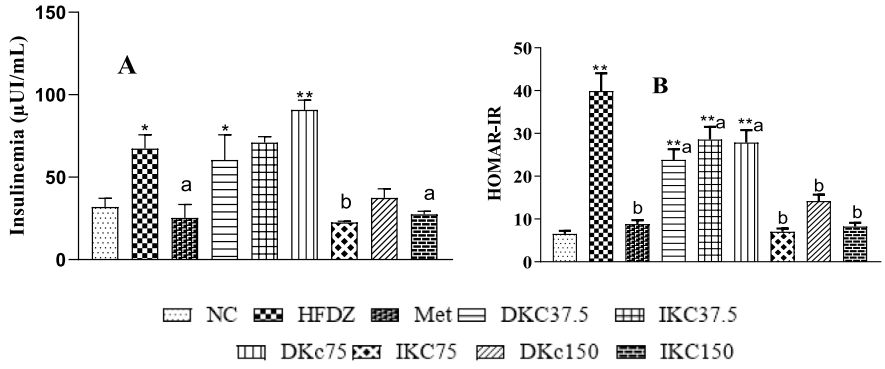

Fig. 4. (A) Insulinemia and (B) HOMA-IR of rats after 42 days of treatment with *K. crenata* decoction, infusion, or Metformin. NC: normal control; HFDZ: Diabetic control rats; DKc37.5, DKc75, DKc150: Diabetic rats treated with K. crenata decoction respectively at 37.5, 75, and 150 mg/kg b.w.; IKc37.5, IKc75, IKc150: Diabetic rats treated with K. crenata infusion decoction respectively at 37.5, 75, and 150 mg/kg b.w.; Met: Metformin at 70 mg/kg b.w. $\overline{X} \pm$ SEM; $n = 5$. Significant difference: $^{*}P < 0.05$, $^{**}P < 0.01$ compared to NC; $^{a}P < 0.05$, $^{b}P < 0.01$ compared to HFDZ.

saponins, tannins, and flavonoids. Flavonoids and alkaloids activate the synthesis and translocation of glucose transporter 4 (GLUT-4), potentiate insulin release, and increase the expression of skeletal muscle GLUT-4.[9]

Blood glucose decrease went along with a reduction in water intake and 24-h urine volume. Indeed, any drop in glycemia leads to a decrease in blood osmolarity, leading to a reduction in water needs and therefore lowering the water intake. Also, the drop of glucose level reduces renal glucose filtration and hence, decreases the urine osmolarity and consequently, the urine volume.[22] A decrease in the blood glucose level, water intake, and 24-h urinary volume clearly shows an improvement in the diabetes conditions of the rats. During the OGTT in diabetic obese rats, both *K. crenata* aqueous extracts and Metformin showed remarkable antihyperglycemic activities. OGTT and ITT are the major indicators of diabetes control. OGTT sheds light on the peripheral clearance of a glucose load, orally administered, while ITT monitors the whole-body insulin action.[23] The antihyperglycemic activity of the extracts could result from several mechanisms, such as the inhibition of hepatic glucose production, the insulin-like and/or insulin-secreting effects, and the improvement in the peripheral use of glucose in the muscles and adipocytes, by regulating the activity and expression of key enzymes and glucose transporters.[24] The extracts also improved the sensitivity of peripheral tissues to insulin. During the ITT at the end of the six-week

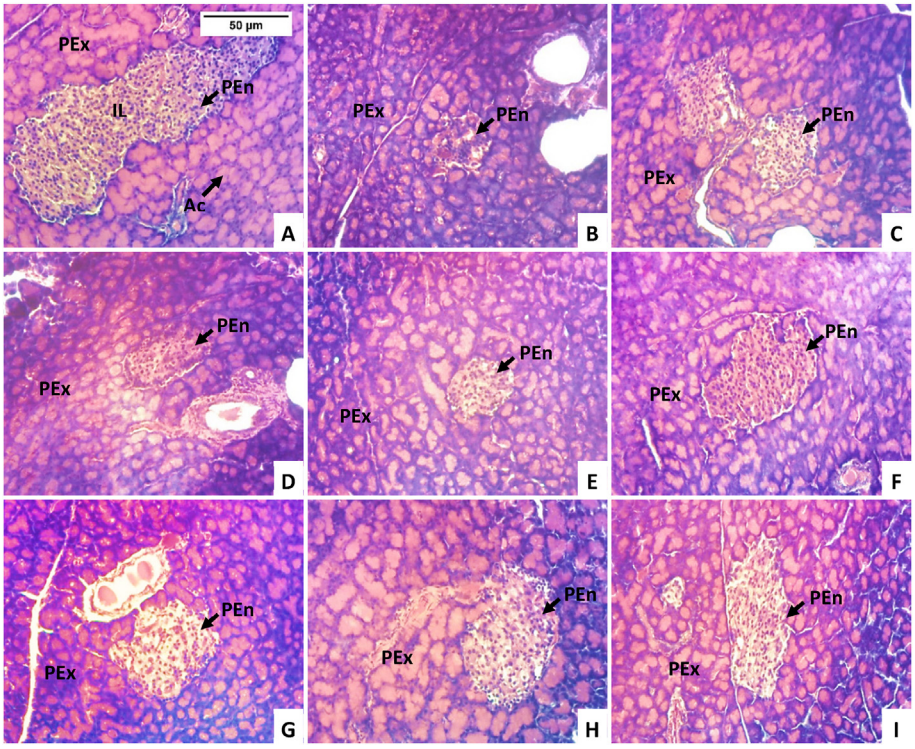

Fig. 5. Microphotography of pancreas of diabetic obese rats after 42 days of treatment with *K. crenata* decoction, infusion, or Metformin. NC: normal control (A); HFDZ: Diabetic control rats (B); Met: Metformin at 70 mg/kg b.w. (C); DKc37.5, DKc75, DKc150: *K. crenata* decoction 37.5, 75 and150 mg/kg b.w. (D, E, F respectively); IKc37.5, IKc75, IKc150: *K. crenata* infusion 37.5, 75 and 150 mg/kg b.w. (G, H, I respectively). PEx: exocrine pancreas; PEn: endocrine pancreas; IL: islets; Ac: acini. (× 100; hematoxylin and eosin, HE).

treatment, the DKc and IKc improved insulin tolerance, especially in 75 mg/kg groups. Several studies have shown that medicinal plants improve the sensitivity of peripheral tissues to insulin by activating the known sequence of insulin signaling pathways, namely the expression levels of InsR-α, IRS-1, PI3K, tyrosine-induced phosphorylation of insulin receptor, AMPK/ACC, and MAPK phosphorylation.[25] Fasting glycemia and insulinemia allowed us to determine HOMA-IR which is the mean to assess the insulin resistance level.[26] As Metformin, *K. crenata* infusion and decoction, significantly reduced HOMA-IR, and the infusion exhibited the best activity. Also, DKc and IKc significantly reduced glycemia of diabetic rats during OGTT and ITT tests. These results sufficiently show that *K. crenata* aqueous extracts improved the peripheral utilization of insulin. View the increased insulin level and size of the Langerhans islets in rats of DKc75 group, as seen on pancreas morphology, the extract activity could primarily be mediated through the reconstitution of pancreatic beta cells affected by streptozotocin, and might not only have peripheral action but also may promote the production of insulin by the beta cells of the pancreas.

In the current investigation, the result showed that aqueous extracts of *K. crenata* (decoction and infusion) at the tested doses, improved insulin and glucose tolerance, and reduced fasting blood glucose in diabetic obese rats MACAPOS 2. However, it lacks the HPLC profile of the respective extracts due to the current condition limit, which may contribute to the quality control for the extracts. The molecular mechanism of the antihyperglycemic activity of *K. crenata* extracts has not been elucidated. The further *in vitro* study of the activities of the extracts on pancreatic cells may be beneficial to uncover the mechanism of action of the antihyperglycemic activity of the extracts.

5. Conclusion

This study provides evidence that aqueous extracts of *K. crenata* (decoction and infusion) at the tested doses, improved insulin and glucose tolerance, and reduced fasting blood glucose in diabetic rats. Therefore, the aqueous extracts of *K. crenata* have potential antihyperglycemic activity, with a slight advantage for the infusion although the difference was not significant for some parameters. This may justify the use of the plant in the treatment of diabetes. These extracts could therefore be potential candidates for the development of an improved traditional medicine for the treatment of diabetes with a preference for infusion.

CRediT authorship contribution statement

Ngakou Mukam Joseph: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mvongo Clémence:** Methodology, Investigation, Formal analysis, Data curation. **Mfopa Adamou:** Methodology, Investigation, Data curation. **Kamgang Tchawou Armel Georges:** Investigation, Formal analysis, Data curation. **Nkoubat Tchoundjwen Sandrine:** Methodology, Investigation. **Fankem Gaëtan Olivier:** Software, Methodology, Data curation, Conceptualization. **Noubissi Paul Aimé:** Writing – review & editing, Validation, Supervision, Project administration, Investigation, Conceptualization. **Fokam Tagne Michel Archange:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization. **Kamgang René:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Essame Oyono Jean-Louis:** Writing – review & editing, Validation, Supervision, Project administration.

Ethics approval and consent to participate

In vivo experiments were conducted with the approval of the institutional committee of the Cameroonian Ministry of Scientific Research and Innovation which has adopted the guidelines and regulations of the European Union on Animal Care (CEE Council 86/609). The study is reported following ARRIVE guidelines.

Availability of data and material (ADM)

All data generated or analyzed during this study are included in this manuscript. These data are available from the corresponding author upon request.

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Declaration of interest

The authors declare that they have no conflict of interest to disclose.

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Abbreviations

AMPK: Adenosine 5' monophosphate-activated protein kinase; AUC: Area Under the Curve; DKc: Decoction; HFD: High Fat Diet; HFDZ: High Fat Diet plus streptozotocin; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; IKc: Infusion; IRS: Insulin Receptor Substrate; ITT: Insulin Tolerance Test; MACAPOS: Maize, Cassava, Palm Oil, and Sugar; MAPK: Mitogen-activated protein kinase; Met: Metformin; NC: Normal Control; OGTT: Oral Glucose Tolerance Test; PI3K: Phosphatidylinositol 3-kinase; STZ: Streptozotocin; NOAEL: No Observed Adverse Effect Level.

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