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Original article

Cathinone: An alkaloid of *Catha edulis* (Khat) exacerbated hyperglycemia in diabetes-induced rats

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ABSTRACT

Cathinone, the main bioactive alkaloid of *Catha edulis* (khat), slightly increased the blood sugar levels of healthy animals, while its effect on blood sugar levels of diabetic animals has not yet been reported. This study investigated the *in vitro* inhibition of cathinone on α-amylase and α-glucosidase as well as its *in vivo* glycemic effects in diabetes-induced rats. Rats were fed on a high fat diet for five weeks, which then intraperitoneally injected with streptozotocin (30 mg/kg). Diabetic rats were distributed randomly into diabetic control (DC, n = 5), 10 mg/kg glibenclamide-treated group (DG, n = 5), and 1.6 mg/kg cathinone-treated group (CAD, n = 5). Additional healthy untreated rats (n = 5) served as a nondiabetic negative control group. Throughout the experiment, fasting blood sugar (FBS), caloric intake and body weight were recorded weekly. By the 28th day of treatment, rats were euthanized to obtain blood samples and pancreases. The results demonstrated that cathinone exerted a significantly less potent *in vitro* inhibition than α-acarbose against α-amylase and α-glucosidase. As compared to diabetic control group, cathinone significantly increased FBS of diabetic rats, while insulin levels of diabetic rats significantly decreased. In conclusion, cathinone was unable to induce a substantial *in vitro* inhibition on α-amylase and α-glucosidase, while it exacerbated the hyperglycemia of diabetes-induced rats.

1. Introduction

Cathinone is the main active alkaloid that is responsible for the psychostimulant effect of khat (*Catha edulis*) (Jenčič et al., 2017). Additionally, cathinone is a natural alkaloid belongs to the phenylalkylamine alkaloids (Majchrzak et al., 2018) constituting 70% of the total content of phenylalkylamines in fresh leaves of khat (Crombie et al., 1990). Khat consumers believe that khat has the ability to reduce elevated blood sugar (Nwoye et al., 2010), which attracts more consumers, particularly diabetics.

Several studies investigated the glycemic activity of khat, however; the findings are still conflicting (Alsalahi et al., 2016), which could be due to the fact that khat contains several alkaloids (cathinone, cathine, norephedrine, merucathinone, merucathine and 62 cathedulins) (Crombie et al., 1990) and flavonoids (myricetin, quercetin and kaempferol) (Alsalahi et al., 2016). Cathinone in khat leaves is unstable alkaloid decomposes within 2–3 days after harvesting (Ben-Shabat et al., 2014), hence it is irrational to interpret the biological activities of khat extracts in terms of its cathinone content. Additionally, the processes of dryness, extraction and storage of the fresh leaves of khat are not cathinone-conservative (Al-Obaid et al., 1998). Consequently, investigation of the biological activities of cathinone should be investigated using pure cathinone to obtain relevant findings to that of fresh khat leaves.

The sympathomimetic activity of khat in terms of cathinone has been suggested to increase the blood sugar levels of nondiabetic animals, antagonize insulin action in type 2 diabetic consumers and delay the intestinal absorption of glucose in healthy consumers (Alsalahi et al., 2016). However, all the former explanations have been still suggestions, which lack an evidence-based support.

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Interestingly, the sympathomimetic activity of cathinone leads to a delay in the intestinal absorption of glucose drew the attention to investigate the ex-pancreatic inhibitory effect of cathinone against intestinal α-amylase and α-glucosidase enzymes that catalyze the hydrolysis of intestinal carbohydrates to glucose (Chiasson et al., 1996), an aspect which has not yet been investigated.

Based on the literature, two studies have been investigated the glycemic activity of the crude fractions of khat alkaloids and flavonoids in diabetic (Betrie and Engidawork, 2016) and nondiabetic animal models (Al-Qirim et al., 2002). However, there is only one study that evaluated the effects of pure cathinone on plasma amino acids and blood sugar levels in healthy rats, and the findings indicated that cathinone slightly increased blood sugar in rats (Al-Meshal, 1988). Nonetheless, there are no other studies on the glycemic activity of cathinone in Type 2 diabetes-induced animals. To the best of our knowledge, this is the first preclinical animal based-study that was conducted to evaluate the *in vitro* inhibitory activity of cathinone against α-amylase and α-glucosidase enzymes and to further confirm the *in vivo* glycemic effects of cathinone in Type 2 diabetes-induced male *Sprague-Dawley* rats through measuring the blood metabolic parameters and morphophysiological changes in the pancreatic sections.

2. Materials and methods

2.1. Chemicals and reagents

α-Acarbose (Acros organic, USA), porcine pancreatic α-amylase, potato starch solution, α-glucosidase, 4-Nitrophenyl 3-D-glucopyranoside (PNPG), D-glucose, glibenclamide, formalin, Harris' hematoxylin working solution and eosin working solution were purchased from Sigma-Aldrich, St. Louis, MO, USA. Streptozotocin, DPPIV inhibitor and MILLIPLEX MAP Rat Metabolic Hormone Magnetic Bead Panel (Metabolism Multiplex Assay, CAT# RMHMAG-84K) were purchased from EMD Millipore Corporation, USA. The one-touch Accu-Check® Performa glucometer was purchased from Roche Diagnostics GmbH, Mannheim, Germany. The 60% high-fat diet (TD.06414) was obtained from Harlen, USA, while the normal rodent diet (Altromin 1324) was purchased from Altromin, Lage, Germany. S (-)-Cathinone HCl was obtained from Lipomed, USA under license of the Ministry of Health, Malaysia (KKM-55/BPF/213/005/12 Jid. 3 (38)) on 6 November 2015.

2.2. Animals

Totally, 20 healthy male *Sprague-Dawley* rats (7–8 weeks, 180–200 g) were purchased from the AEU (Animal Experiment Unit), Faculty of Medicine, University of Malaya. The animals were kept in the AAALAC-accredited animal facility for one week to adapt housing conditions of a temperature of 20 ± 4 °C, relative humidity (30 – 70%), light cycle (12 h light and 12 h dark) with free access to normal rodent diet and tap water *ad libitum*.

2.3. In vitro inhibitory effects of cathinone against α-amylase and α-glucosidase

According to Qasem et al. (2018), 20 μl of 250, 125, 62.5, 31.25 or 15.625 μg/ml of cathinone and α-acarbose were pipetted separately in triplicates into their specified wells of 96-wells plate, which was followed by pipetting 50 μl of the porcine pancreatic α-amylase into each well. After that, the plate was incubated at 28 °C for 10 min. Then 100 μl of potato starch solution was pipetted into each well of the plate and re-incubated at 28 °C for 10 min. Finally, 100 μl of DNSA (3,5-dinitrosalicylic acid) were added to each well to stop the reaction and the plate was incubated in a water bath at 88 °C for 15 min. The absorption was measured spectrophotometry at 540 nm.

The cathinone inhibitory effect against α-glucosidase was performed by pipetting 100 μl of α-glucosidase enzyme and 50 μl of each serial dilution (250, 125, 62.5, 31.25 and 15.625 μg/ml) of cathinone or α-acarbose standard into a 96-wells plate in triplicates and incubated at 25 °C for 10 min. Then, 50 μl of P-nitrophenyl-alpha-D-glucopyranoside (PNPG) was transferred to the reacting mixture and re-incubated at 25 °C for five minutes. After that, the catalytic reaction was stopped by adding 80 μl of 0.1 M sodium carbonate reagent. Finally, the 96-well plate was measured spectrophotometry at 405 nm.

Each assay was repeated three times to estimate the IC_{50}, while the inhibitory effects of cathinone or α-acarbose against α-amylase and α-glucosidase enzymes were expressed as the percentage of inhibition according to the following equation:

$$Percentage\ \ of\ \ inhibition = \left(\frac{Ac - (As - Ab)}{Ac}\right) \times 100$$

where Ac, Ab and As indicate the absorbance of negative control, absorbance of blank and absorbance of cathinone, respectively.

2.4. In vivo glycemic activity of cathinone in animal model

2.4.1. Ethics and grouping of animals

This animal based-study was approved by FOM IACUC (Faculty of Medicine Institutional Animal Care and Use Committee), University of Malay (Ethic ID.: 2015–180505/PHAR/R/ASAM). This study maintained the animals according to the guidelines of Care and Use of Laboratory Animals (Care and Animals, 1986), and the internationally guidelines of EEC directive of 1986; 86/609/EEC (Directive, 1986). The rats were distributed randomly into four groups (n = 5 /group); namely, negative nondiabetic control (NC), negative diabetic control (DC), glibenclamide-treated positive diabetic control (GD), and cathinone-treated diabetic group (CAD).

2.4.2. Induction of Type 2 diabetes mellitus to rats

Out of the 20 healthy rats, 15 rats were selected randomly and fed on 60% high-fat diet (HFD) and tap water *ad libitum* for five weeks. Simultaneously, another five rats were fed on normal rodent diet and tap water *ad libitum* for five weeks. By the end of the 5th week of diet induction, all rats were deprived of their specified diet overnight. Then, the 15 rats that fed on HFD were injected intraperitoneally (IP) with a single dose of 30 mg/kg of streptozotocin (STZ), which was freshly prepared in 2 ml/kg of 0.1 mM citrate buffer (pH = 4.5). Simultaneously, the 5 rats that fed on normal rodent diet, were IP-injected with 2 ml/kg of freshly prepared 0.1 mM citrate buffer (pH = 4.5). After one week of the chemical induction, STZ-injected rats that developed fasting blood sugar levels between 7.8 and 15.6 mmol/L and postprandial blood sugar levels $\geq$ 11.1 mmol/L were considered Type 2 diabetes and then distributed randomly into three groups; namely, DC, GD and CAD (n = 5), while other five rats were allocated simultaneously in the NC group, and the treatment was started immediately (Barrière et al., 2018; Wang et al., 2011; Zhang et al., 2008). During the treatment period, all rats in the DC, GD, CAD and NC were reallocated on a daily calculated normal rodent diet with free access to tap water *ad libitum*.

2.4.3. Dose selection of cathinone

In this study, we used pure cathinone, while the dose of cathinone was selected based on the stimulatory effect of fresh leaves of khat that was equivalent to 0.5 mg/kg cathinone (Patel, 2000). The corresponding dose of cathinone for rats was calculated using the equation by Shin et al. (2010): Animal equivalent dose (mg/kg)

is equal to the human dose (mg/kg) multiplied by the conversion factor, where the conversion factor of the equivalent dose of rats is 6.17. Accordingly, the calculated dose of cathinone to be given to rats is 3.1 mg/kg, however, the dose of cathinone in this study was selected to be half (1.6 mg/kg) of the dose given to monkeys (Nyongesa et al., 2014).

2.4.4. Treatment of rats

Over four weeks, rats in the DC, GD, CAD and NC groups received a single oral daily dose of 5 ml/kg deionized water, 10 mg/kg glibenclamide (Pandit et al., 2010), 1.6 mg/kg cathinone (Nyongesa et al., 2014) and 5 ml/kg deionized water, respectively. By the end of treatment, all rats were diet-deprived overnight and anesthetized with ketamine/xylazine (60:7.5 mg/kg, I.P.) to withdraw blood samples via cardiac puncture. Finally, rats were given an overdose of ketamine/xylazine to be dissected for harvesting pancreas tissues, which were kept in 10% buffered formalin.

2.4.5. Metabolic measurements

2.4.5.1. Blood sugar levels. Rats were diet-deprived overnight but allowed free access to tap water to measure FBS before and after HFD induction, baseline FBS at the first day of treatment and weekly throughout the treatment period using a one-touch glucometer.

After induction of diabetes and on the day of starting the treatment, post-prandial blood sugar (two hours after food) was measured from the tip of the tail to provide extra criteria for identifying the induction of T2DM (Zhang et al., 2008).

2.4.5.2. Oral glucose tolerance test (OGTT). In the fourth week of treatment, rats were diet-deprived overnight but allowed free access to tap water and exposed to 2 g/kg of an oral glucose. The blood glucose level was measured from the tip of the tails by using a one-touch glucometer at 0 time and then at every 30 min up to two hours (0, 30, 60, 90 and 120 min) after glucose administration (Pandit et al., 2010).

2.4.5.3. Body weights and caloric intake. Body weights (g) were recorded at the day of starting and ending of 60% HFD feeding. After STZ-induction, body weights were also weekly recorded starting from the first day of treatment (baseline body weight) and then weekly during the treatment period (at the end of the first, second, third and fourth weeks).

During the treatment period, a specified fixed quantity of normal rodent diet was offered to rats (F1), and the remaining quantity of diet was weighed (F2) to calculate the consumed quantity F= (F1-F2) (Aziz et al., 2011) at the end of first, second, third and fourth weeks. Then caloric intake was calculated by multiplying F (g) by 3279 kcal/kg (the metabolic energy of normal rodent diet (Altromin 1324) and divided by 1000 (Kcal/g/day) (Nirwane and Majumdar, 2016).

2.4.5.4. Biochemical markers. Blood samples were collected via cardiac puncture into the free EDTA-containing tubes for quantification of serum amylase, lipase, total protein, triglycerides, total cholesterol, low-density lipoprotein and high-density lipoprotein. After labeling samples with random codes, the samples were analyzed blindly by another assessor in the Biochemistry Laboratory, Faculty of Veterinary, Universiti Putra Malaysia.

2.4.5.5. Metabolic endocrine hormones. Extra blood samples were withdrawn into EDTA-containing tubes that contain 10 μl of dipeptidyl peptidase 4 inhibitor (DPP IV) for quantification of plasma C-peptide 2, insulin, leptin, and total glucose-dependent insulinotropic polypeptide (GIP) using a Multiplex kit. After labeling samples with random codes, the assay was performed blindly by another assessor in the Medical Molecular Biology Institute, Universiti Kebangsaan Malaysia.

2.4.5.6. Homeostatic model assessment (HOMA 2 model). The index of HOMA was applied for estimating pancreatic β-cells function (HOMA-B), insulin resistance (HOMA-IR) and insulin sensitivity (HOMA-S) (Antunes et al., 2016). Fasting insulin (μU/ml) and FBS (mmol/L) were used to calculate HOMA utilizing HOMA 2 calculator software (Unit, 2020).

2.4.6. Morphohistological measurements

2.4.6.1. Qualitative evaluation of pancreatic sections. The processed pancreas tissues were embedded in paraffin wax, and 5 μm sections were stained with hematoxylin and eosin for a microscopical histology examination (100× magnification) and quantitative morphometric evaluation.

2.4.6.2. Quantitative evaluation of pancreatic sections.

2.4.6.2.1. Morphometric evaluation. From four animals (n = 4) per group, the randomly selected stained pancreas sections were evaluated blindly under Leica microscope for the volume density of islets expressed as a percentage of islets per 50 microscopic fields, the number of islets of Langerhans per 50 microscopic fields at 40× magnification, the number of β- and α-cells per 10 islets at 100× magnification, the volume density of β-cells expressed as a percentage of β-cells to the total number of α- and β-cells per 10 islets and the size distribution of 10 islets expressed as the area (μm^2) and was assessed by measuring major axis and minor axis coordinates of each islet (Jelodar et al., 2007).

$$\text{Area of islets} = \frac{\text{major axis} \times \text{minor axis} \times 1000000}{4 \times \text{linear magnification}}$$

where the linear magnification is equal to the eyepiece magnification (100×) multiplied by the used magnification power (40×) (Findlay and Thomas, 1980).

2.4.6.2.2. Double immunofluorescence staining of pancreatic β- and α-cells. Pancreatic β-cell mass, α-cell mass and β-/α-cells ratio were investigated using insulin and glucagon immunofluorescence assay. Blocks of pancreatic tissues from each group was selected randomly and examined blindly. The pancreatic tissues were sectioned into 5 μm, then placed on poly-L-lysine-coated glass slides and then incubated at 37 °C for 48 hr in an incubator. The slides were deparaffinized with three changes of xylene, 5 min each and two changes of 100% ethanol for 5 min each, 95% and 80% ethanol for 5 min each. After rinsing with distilled water, the sections were subjected to antigen unmasking with a fresh citrate buffer (10 mmol/l sodium citrate containing 0.05% Tween 20, pH 6). Slides were immersed and incubated for 40 min in pressure cooker. After cooling, the slides were rinsed with distilled water for 5 min each. Then the sections were incubated with 2.5% normal horse serum for 1 h to suppress the non-specific binding of IgG. After that slides were incubated with mouse monoclonal [K79bB10] to glucagon (ab10988, Abcam, Cambridge, UK, diluted 1:1,000) for overnight at 4 °C. Then, the slides were rinsed with PBS for 3×, 5 min and incubated with rabbit polyclonal antibody (H-86) to insulin (sc-9168; Santa Cruz Biotechnology, CA, USA, diluted 1:1,00) for 2 h at 4 °C. The slides were washed for 3×, 5 min with PBS. Then the slides were incubated for 30 min with VectaFluor Duet Reagent (VecaFluorTM Duet Double Labeling Kit; DyLight® 594 anti-Rabbit IgG, DyLight® 488 Anti-Mouse IgG kit; DK-8828; 30 Ingold Road, Burlingame, CA 94010, USA). After that, the slides were rinsed for 3× with PBS, 5 min each. Finally, the slides were mounted in Ultra-Cruz mounting medium (Santa Cruz Biotechnology, CA, USA) and gently covered with the coverslip. Immunofluorescence images examination were carried out using a Fluorescence microscope

attached with camera (Nikon H600L, Tokyo, Japan). Quantification of the percentage positive for insulin and glucagon cells was preformed using ImageJ software (NIH, Bethesda, MD, USA). Insulin and glucagon-positive cell mass was determined using the formula:

$$\text{Insulin cell mass(mg)} = \frac{\text{Insulin positive area}}{\text{total tissue area}} \times \text{total pancreatic weight (mg)}$$

$$\text{Glucagon cell mass(mg)} = \frac{\text{Glucagon positive area}}{\text{total tissue area}} \times \text{total pancreatic weight(mg)}$$

2.5. Statistical analysis

The statistical analysis of data was performed using IBM SPSS software package (version 20). One-way ANOVA was used for the normally distributed data, followed by a post hoc Tukey test, while the Games-Howell post hoc test was applied if homogeneity was not assumed. To compare means difference of pairs of variables, independent samples *t*-test was applied (Freudenberg et al., 2016). $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ indicated a significant difference, a highly significant difference, and a very highly significant difference, respectively. Means differences were expressed as the mean ± standard deviation or mean ± standard error.

3. Results

3.1. In vitro inhbitoin of cathinone on α-amyalse and α-glucosidase

Cathinone exhibited a descending inhibition effects on α-amylase so that the higher the concentration of cathinone, the lower the inhibition on α-amylase, while α-acarbose exhibited ascending inhibition effects on α-amylase so that the higher the concentration of α-acarbose, the higher the inhibition on α-amylase. The cathinone IC_{50} (92.60 ± 3.29 μg/ml) against α-amylase was significantly ($P \leq 0.001$) four times higher than that of α-acarbose (21.58 ± 0.82 μg/ml). Both cathinone and α-acarbose exhibited an ascending inhibition on α-glucosidase, however, the IC_{50} of cathinone (194.21 ± 0.89 μg/ml) on α-glucosidase was significantly ($P \leq 0.001$) four times higher than that of α-acarbose (51.59 ± 0.98 μg/ml).

3.2. In vivo glycemic activity of cathinone in the diabetic-induced animal model

3.2.1. Blood sugar levels

The mean initial levels of FBS of rats at baseline was not significantly different among groups. After five weeks of feeding on allocated diets, the level of FBS of rats fed on high fat diet (6.05 ± 0.35 mmol/L) was significantly ($P \leq 0.001$) higher than rats fed on normal rodent diet (4.88 ± 0.38 mmol/L).

By the end of diet allocation, after injecting rats with streptozotocin (STZ), the levels of FBS of the STZ-injected rats (9.05 ± 0.95 mmol/L) was significantly ($P \leq 0.001$) higher than rats in the NC group (4.62 ± 0.22 mmol/L). Similarly, the levels of postprandial blood sugar of the STZ-injected rats (16.73 ± 2.10 mmol/L) was significantly ($P \leq 0.001$) higher than the NC group (6.42 ± 0.40 mmol/L).

By the end of the first, second, third and fourth weeks of treatment, Table 1 shows that the levels of FBS of the DC group were not significantly different from those of the CAD group. Upon comparing the weekly changes in the levels of FBS of the different groups against their baseline (W0), the levels of FBS of the NC group remained unchanged, while those of the GD group significantly ($P \leq 0.01$) decreased from week to another. Conversely, the levels of FBS of the DC group continued to increase from one week to another and were significantly increased at the end of the second ($P \leq 0.01$), third ($P \leq 0.001$) and fourth ($P \leq 0.001$) weeks. Similarly, the levels of FBS of the CAD group continued to increase weekly, however, reached a significant ($P \leq 0.001$) level at the end of the fourth week.

3.2.2. Oral glucose tolerance test

Table 2 shows that the levels of blood glucose of the CAD group were not significantly different from those of the DC group at 0, 30, 60, 90 and 120 min of exposure to oral glucose.

Upon comparing the changes in the levels of blood sugar of the different groups against baseline (0 min), the level of the blood sugar of the NC group peaked significantly ($P \leq 0.01$) at 30 min and declined gradually to be around the baseline level, while the level of the blood sugar of the GD group significantly ($P \leq 0.001$) peaked at 60 min and declined gradually to be around the baseline level. However, the levels of the blood sugar of the CAD and DC groups continued to increase significantly and reached a maximum significant ($P \leq 0.001$) peak at 120 min.

3.2.3. Body weight changes

The mean initial body weight of rats at baseline was not significantly different among groups. After five weeks of feeding on allocated diet, body weights of rats fed on the high-fat diet (486.67 ± 29.13 g) was significantly ($P \leq 0.001$) higher than rats fed on normal rodent diet (399.00 ± 34.35 g).

By the end of the first, second, third and fourth weeks of treatment, Table 3 shows that the body weights of the CAD group did not altered significantly as compared to DC group.

Upon comparing the weekly changes in body weights of different groups against baseline (W0), Table 3 shows that NC group significantly gained weight at the end of the third and fourth weeks ($P \leq 0.05$ and $P \leq 0.01$, respectively), while GD group showed significant changes, whereas both DC and CAD groups continued to lose weight, and significantly ($P \leq 0.05$) showed less weight at the end of the fourth week as compared to baseline.

3.2.4. Caloric intake changes

By the end of first, second, third and fourth weeks of treatment, Table 4 shows that the CAD group significantly ($P \leq 0.001$) consumed lower calories than those of the DC group. Upon comparing the weekly changes in caloric intake of the different groups against baseline (W1), Table 4 shows that the caloric intake of the CAD group declined markedly from one week to another and reached significant ($P \leq 0.01$ and $P \leq 0.001$, respectively) decreases at the end of the 3rd and 4th weeks.

3.2.5. Biochemical markers

Serum levels of total protein of the NC (82.60 ± 6.14 g/L), DC (84.72 ± 4.04 g/L), GD (87.56 ± 2.94 g/L) and CAD (72.60 ± 13.96 g/L) groups were not significantly different. On the other hand, the serum levels of amylase of the NC (2461.20 ± 163.79 U/L), DC (2294.40 ± 278.00 U/L), GD (2982.40 ± 208.05 U/L) and CAD (1956.00 ± 417.15 U/L) groups demonstrated that the serum level of amylase of GD group was significantly higher than those of the DC and CAD groups ($P \leq 0.01$ and $P \leq 0.001$, respectively). Conversely, the serum levels of lipase of the NC (10.28 ± 1.33 U/L), DC (11.12 ± 3.61 U/L), GD (12.12 ± 1.93 U/L) and CAD (11.56 ± 1.77 U/L) were not significantly different.

The serum levels of triglycerides of NC (1.30 ± 0.45 mmol/L), DC (0.89 ± 0.29 mmol/L), GD (2.07 ± 0.88 mmol/L) and CAD (0.94 ± 0.17 mmol/L) group were not significantly different, while comparing the serum levels of total cholesterol of the NC (2.26 ± 0.42 m

Table 1
Weekly fasting blood sugar levels.

Week	NC	DC	GD	CAD
W0	4.62 ± 0.22	8.88 ± 0.45	8.76 ± 0.81	9.52 ± 1.38
W1	4.54 ± 0.29	10.54 ± 0.81	8.03 ± 1.10	9.96 ± 1.47
W2	4.50 ± 0.34	12.82 ± 1.61$^{\#\#}$	7.84 ± 0.78**	10.00 ± 1.95
W3	4.54 ± 0.21	13.78 ± 1.52$^{\#\#\#}$	7.46 ± 0.90**	11.72 ± 1.90
W4	4.16 ± 0.21	15.58 ± 1.43$^{\#\#\#}$	7.40 ± 1.21***	17.66 ± 1.65$^{\#\#\#}$

Data are presented as the mean ± standard deviation (n = 5). $^{\#\#}P \leq 0.01$ and $^{\#\#\#}P \leq 0.001$ indicate significant differences in fasting blood sugar levels of groups against the fasting blood sugar at baseline (W0). $^{**}P \leq 0.01$ and $^{***}P \leq 0.001$ indicate significant differences in the fasting blood sugar levels of GD and CAD groups against that of DC group. W0, week 0 (Baseline at the first day of starting treatment); W1, 2, 3 and 4, the time at the end of the 1st, 2nd, 3rd and 4th weeks of treatment, respectively. NC, nondiabetic control group; DC, diabetic control group; GD, glibenclamide-treated diabetic group; CAD, cathinone-treated diabetic group.

Table 2
Glucose levels of oral glucose tolerance test.

Time (min)	NC	DC	GD	CAD
0	4.54 ± 0.21	13.78 ± 1.52	7.46 ± 0.90**	11.72 ± 1.90
30	7.70 ± 1.01$^{\#\#}$	17.36 ± 1.36	10.30 ± 1.70***	15.30 ± 1.44$^{\#}$
60	7.48 ± 1.50	19.24 ± 2.63$^{\#\#}$	14.42 ± 2.51$^{*,\ \#\#\#}$	17.36 ± 1.61$^{\#\#}$
90	6.20 ± 1.08	20.62 ± 2.19$^{\#\#\#}$	11.66 ± 2.63$^{***,\ \#}$	19.22 ± 1.24$^{\#\#\#}$
120	5.44 ± 0.34$^{\#}$	22.20 ± 1.42$^{\#\#\#}$	9.44 ± 2.19***	23.58 ± 2.50$^{\#\#\#}$

Data are presented as the mean ± standard deviation (n = 5). $^{\#}P \leq 0.05$, $^{\#\#}P \leq 0.01$ and $^{\#\#\#}P \leq 0.001$ indicate significant differences in blood sugar of different groups against the blood sugar levels at baseline (0 min). $^{**}P \leq 0.01$ and $^{***}P \leq 0.001$ indicate significant differences in the levels of the blood sugar of the GD and CAD groups compared to DC group; 0, time at 0 min; 30, 60, 90 and 120 min, the times in minutes at which the levels of the blood sugar were measured. NC, nondiabetic control group; DC, diabetic control group; GD, glibenclamide-treated diabetic group; CAD, cathinone-treated diabetic group.

Table 3
Weekly body weight changes.

Week	NC	DC	GD	CAD
W0	418.00 ± 20.80	466.00 ± 21.04	474.00 ± 29.66	466.00 ± 37.98
W1	445.00 ± 18.37	453.00 ± 24.90	463.00 ± 31.54	447.00 ± 40.25
W2	453.00 ± 17.54	442.00 ± 20.80	453.00 ± 28.64	429.00 ± 36.47
W3	463.00 ± 18.91$^{\#}$	420.00 ± 27.39	434.00 ± 21.62	407.00 ± 43.82
W4	481.00 ± 22.47$^{\#\#}$	409.00 ± 29.03$^{\#}$	435.00 ± 26.93	377.00 ± 39.62$^{\#}$

Data are presented as the mean ± standard deviation (n = 5). $^{\#}P \leq 0.05$ and $^{\#\#}P \leq 0.01$, indicate a significant difference in the body weights of different groups against the body weights at baseline (W0). W0, week 0 (Baseline at the first day of starting treatment); W1, 2, 3 and 4, the time at the end of the 1st, 2nd, 3rd and 4th weeks of treatment, respectively. NC, nondiabetic control group; DC, diabetic control group; GD, glibenclamide-treated diabetic group; CAD, cathinone-treated diabetic group.

Table 4
Weekly caloric intake.

Week	NC	DC	GD	CAD
W1	272.40 ± 33.97	373.80 ± 31.43	400.00 ± 54.89	282.00 ± 31.58**
W2	308.40 ± 39.31	380.40 ± 42.46	373.60 ± 39.02	229.40 ± 41.87***
W3	360.60 ± 25.77$^{\#\#}$	393.40 ± 25.77	341.00 ± 42.21	199.80 ± 31.87$^{***,\#\#}$
W4	380.40 ± 39.02$^{\#\#}$	426.20 ± 26.09	318.40 ± 29.75$^{***,\#}$	164.00 ± 25.93$^{***,\#\#\#}$

Data are presented as the mean ± standard deviation (n = 5). $^{\#}P \leq 0.05$, $^{\#\#}P \leq 0.01$and $^{\#\#\#}P \leq 0.001$, indicate significant differences in the caloric intake of different groups against the caloric intake at baseline (W1). $^{**}P \leq 0.01$and $^{***}P \leq 0.001$, indicate significant differences in caloric intake of GD and CAD groups against that of DC group. W1, week 1 (Baseline at the end of the first week of starting treatment); W 2, 3 and 4, the time at the end of 2nd, 3rd and 4th weeks of treatment, respectively. NC, nondiabetic control group; DC, diabetic control group; GD, glibenclamide-treated diabetic group; CAD, cathinone-treated diabetic group.

mol/L), DC (2.14 ± 0.42 mmol/L), GD (3.66 ± 0.78 mmol/L) and CAD (2.24 ± 0.38 mmol/L) groups demonstrated that only the serum level of total cholesterol of GD group was significantly ($P \leq 0.01$) higher than that of DC group. The serum levels of low-density lipoprotein of the NC (0.40 ± 0.06 mmol/L), DC (0.42 ± 0.07 mmo l/L), GD (0.64 ± 0.11 mmol/L) and CAD (0.35 ± 0.07 mmol/L) groups demonstrated that the serum level of the low-density lipoprotein of the GD group was significantly ($P \leq 0.01$) higher than that of the DC group. Similarly, comparing the serum level of high-density lipoprotein of the NC (1.56 ± 0.36 mmol/L), DC (1.43 ± 0. 20 mmol/L), GD (2.52 ± 0.52 mmol/L) and CAD (1.70 ± 0.29 mmo l/L) groups demonstrated that the serum level of the high-density lipoprotein of the GD group was significantly ($P \leq 0.01$) higher than that of DC group.

3.2.6. Metabolic endocrine hormones

The levels of GIP of NC (6.76 ± 1.55 pg/ml), DC (44.85 ± 24.19 pg/ml), GD (22.55 ± 4.41 pg/ml) and CAD (15.88 ± 7.37 pg/ml) groups were not significantly different. Similarly, the leptin levels of the NC (781.74 ± 358.72 pg/ml), DC (1278.20 ± 497.37 pg/ml), GD (1159.50 ± 606.04 pg/ml) and CAD (743.31 ± 398.72 pg/ml) groups were not significantly different, however; the leptin levels of CAD group was markedly lower than that of DC by 2 times. Likewise, the levels of C-peptide 2 of the NC (630.77 ± 175.45 pg/ml), DC (800.19 ± 109.85 pg/ml), GD (760.95 ± 203.66 pg/ml) and CAD (588.07 ± 68.83 pg/ml) were not significantly different. Nonetheless, comparing the insulin levels of the NC (7.35 ± 4.92 μU/ml), DC (11.60 ± 7.05 μU/ml), GD (14.52 ± 5.33 μU/ml) and CAD (5.57 ± 2.07 μU/ml) groups indicated that the insulin level

of the CAD group was significantly ($P \leq 0.05$) lower than that of the DC group by 2 times.

3.2.7. Homeostatic model assessment (HOMA 2 model)

The values of HOMA-B of the NC (119.66 ± 77.63), DC (18.44 ± 1. 61), GD (68.98 ± 7.86) and CAD (7.28 ± 3.27) groups demonstrated that the value of HOMA-B of the DC group was markedly lower than that of NC group by 6.5 times. In addition, the value of HOMA-B of CAD group was significantly ($P \leq 0.01$) lower than that of DC group by 2.5 times, while the value of HOMA-B of GD group was significantly ($P \leq 0.001$) higher than that of DC group by 4 times. Likewise, the values of HOMA-S of the NC (94.12 ± 25.69), DC (40.06 ± 11.90), GD (53.82 ± 16.45) and CAD (81.94 ± 17.82) groups demonstrated that the value of HOMA-S of the DC group was significantly lower than those of the NC and CAD groups ($P \leq 0.01$ and $P \leq 0.05$, respectively) by 2 times . However, the values of HOMA-IR of the NC (1.04 ± 0.85), DC (1.92 ± 1.21), GD (1.7 7 ± 1.22) and CAD (1.27 ± 0.26) groups were not significantly different.

3.2.8. Qualitative histological evaluation

For all the histological sections, islets of Langerhans were scattered throughout the acinar exocrine pool. The acinar cells and islets of Langerhans of the NC group were compact in shape with well-demarcated outlines. The islets of Langerhans of the DC, GD, and CAD groups were vacuolated and somewhat demarcated in outlines. It appeared that pancreatic sections of the DC, and CAD groups showed a damage to the architecture of islets of Langerhans which became disorganized and more vacuolated with a lower density of β-cells than those of the nondiabetic NC group (Fig. 1).

3.2.9. Quantitative morphometric histological evaluation

Fig. 2a shows that the area of islets of Langerhans of the DC group was significantly ($P \leq 0.01$) smaller than that of the NC group. Conversely, the area of the GD group was significantly ($P \leq 0.05$) larger than that of the DC group, while that of the CAD group was significantly ($P \leq 0.05$) smaller than that of DC group. Fig. 2b shows that the number and the density volume of islets of Langerhans of the DC group were significantly ($P \leq 0.001$) smaller than that of NC group. Fig. 3c shows that the number of β-cells, the number of α-cells and the density volume of β-cells of the DC group were significantly ($P \leq 0.001$) lower than those of the NC group, while the number of α-cells of the GD and CAD groups were significantly ($P \leq 0.05$) greater that of the DC group.

3.2.10. Double immunofluorescence staining of pancreatic β- and α-cells

Fig. 3B shows that the percentage of positive insulin cells (β-cells) of DC group was significantly ($P \leq 0.001$) lower than that of NC group. The percentages of positive insulin cells (β-cells) of GD group was significantly ($P \leq 0.001$) higher than that of DC group, while that of CAD group was significantly ($P \leq 0.05$) lower than DC group.

Fig. 3C shows that the positive glucagon cells (α-cells) of the CAD group was significantly ($P \leq 0.05$) higher than that of DC group.

Fig. 3D showed that the percentage of the positive insulin cells to glucagon cells of DC group was significantly ($P \leq 0.001$) lower than that of NC group. On the other hand, the percentages of the positive insulin cells to glucagon cells of GD group was significantly ($P \leq 0.001$) higher than those of DC group, while the percentages of the positive insulin cells to glucagon cells of the CAD group was significantly ($P \leq 0.001$) lower than that of DC group.

4. Discussion

The *in vitro* inhibitory effects of cathinone against the catalytic activities of both pancreatic α-amylase and intestinal α-glucosidase enzymes were investigated since they are key digestive enzymes in the conversion of intestinal carbohydrates into

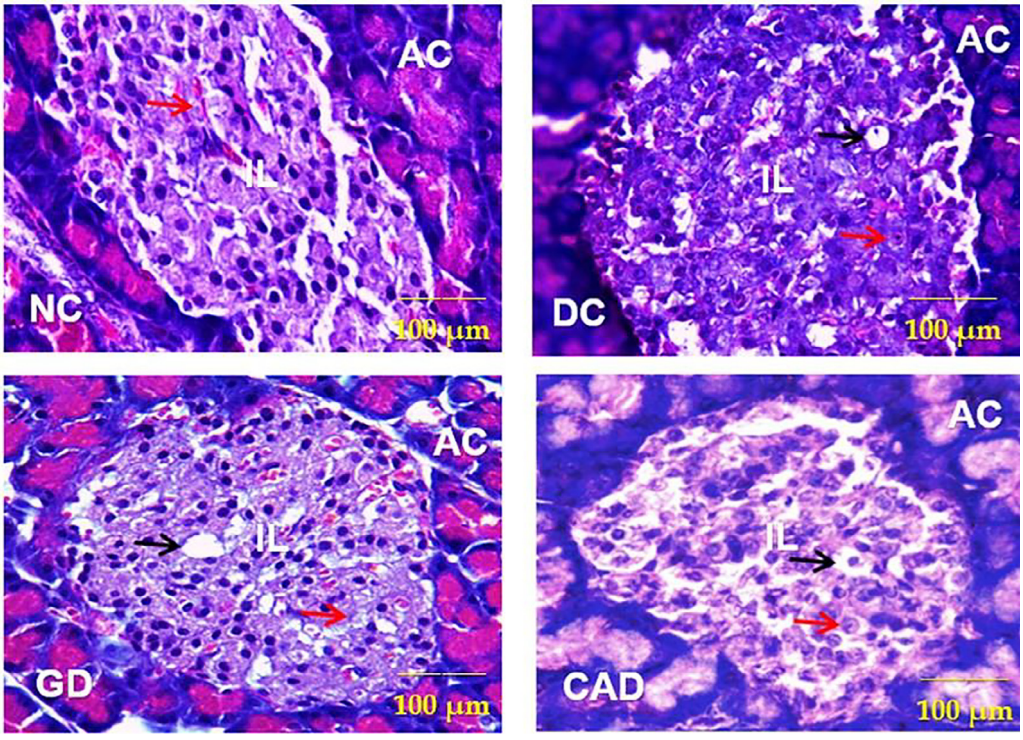

Fig. 1. Histopathological changes in pancreatic sections. Black arrows point to vacuolated areas. Red arrows point to β-cells, AC, Acinar cells; IL, the islet of Langerhans; NC, nondiabetic control group; DC, diabetic control group; DG, glibenclamide-treated diabetic group; CAD, cathinone-treated diabetic group. H&E stain; 100×.

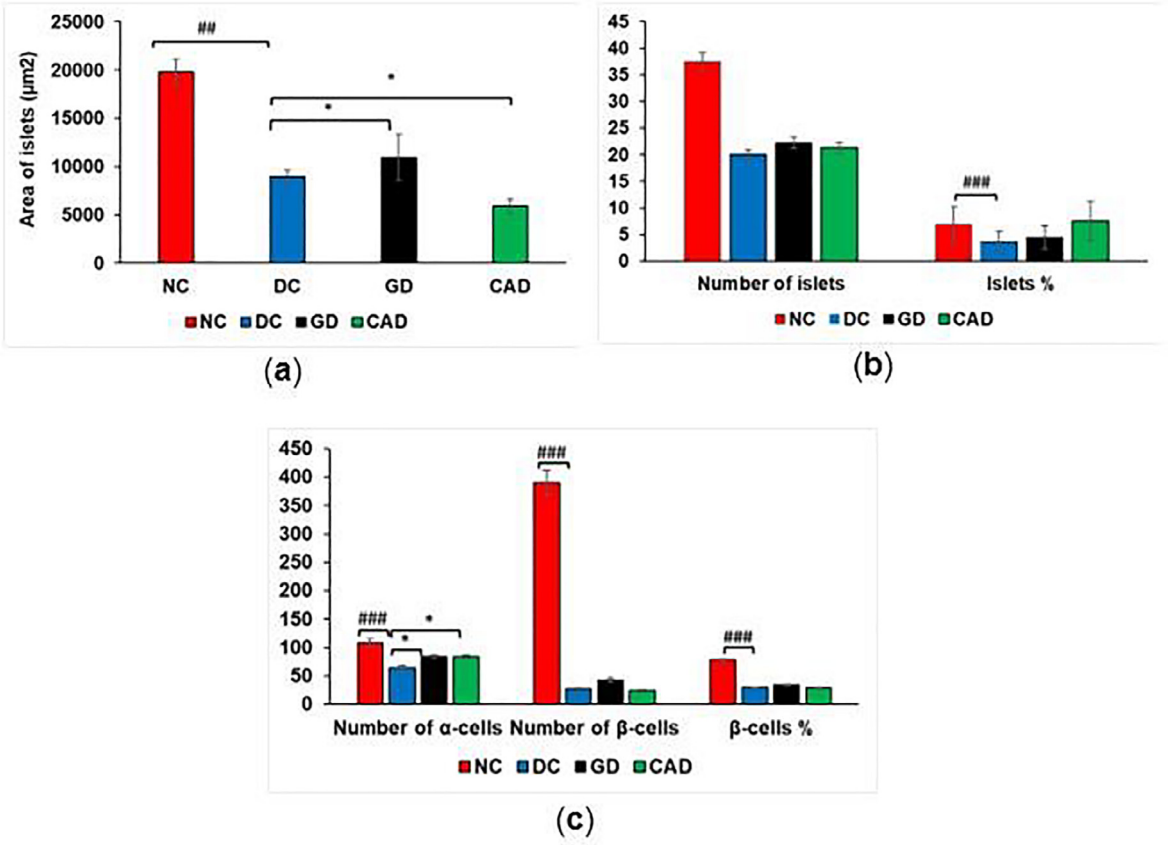

Fig. 2. Morphometric evaluation of the pancreatic sections, (**a**) The size distribution of islets of Langerhans per 50 microscopic field was expressed as area in μm^2. $^{\#\#}P \leq 0.01$, area of islets of the DC group was significantly smaller than that of NC group. $^{*}P \leq 0.05$, the area of the GD group was significantly larger than that of DC group, while that of CAD group was smaller than DC group; (**b**) Number of islets of Langerhans per 50 microscopic fields was counted using 40× magnification. The density volume of islets of Langerhans was expressed as a percentage of islets per 50 microscopic fields. $^{\#\#\#}P \leq 0.001$, the number and density volume of islets of Langerhans of the DC group were significantly smaller than that of NC group; (**c**) Number of β- and α-cells per 10 islets was counted using 100× magnification. The density volume of β-cells per 10 islets of Langerhans was expressed as a percentage of β-cells to the total number of α- and β-cells. $^{\#\#\#}P \leq 0.001$, the number of β-cells, the number of α-cells and the density volume of β-cells of the DC group was significantly greater than that of NC group; $^{*}P \leq 0.05$, the number of α-cells of GD and CAD groups were significantly greater than that of DC group. Data are graphically presented as the mean ± SE. NC, nondiabetic control group; DC, diabetic control group; DG, glibenclamide-treated diabetic group; CAD, cathinone-treated diabetic group.

glucose (Chiasson et al., 1996). In addition, α-acarbose was used as a standard reference since its efficacy in inhibiting the catalytic activities of pancreatic α-amylase and α-glucosidase enzymes has been established (Chiasson et al., 1996). Unlike α-acarbose, our *in vitro* findings indicated that cathinone exhibited a descending inhibition effects against α-amylase, which was opposite to the ascending inhibitory effect of α-acarbose, while the inhibitory effect of cathinone against α-glucosidase enzymes behaved similarly to α-acarbose, which could be due to the nature of the binding of cathinone to the molecule of α-amylase and α-glucosidase (Qian et al., 1995). However, the 4-times higher values of IC_{50} of cathinone against α-glucosidase enzyme than α-acarbose indicating that the inhibitory effects of cathinone would be not so effective as α-acarbose in controlling postprandial hyperglycemia of T2DM (Chiasson et al., 1996).

Investigating the *in vivo* glycemic activity of cathinone in T2DM suggested the recruitment of diabetes-induced animal model to avoid the possible hazards arising during evaluating therapeutic agents in humans (Ganeshpurkar and Saluja, 2017). Although the extrapolation of the findings from the animal model to human has been still a disputable issue, a high fat diet- STZ T2DM-induced animal model was reported to hold two important traits of human T2DM, namely; insulin deficiency and insulin resistance (Furman, 2015; Zhang et al., 2008). Rats were selected because they are more sensitive to a lower dose of STZ than mice (Sakata et al., 2012), particularly male rats (Furman, 2015). In addition; rats are preferred rather than mice in case of diabetes-STZ induced rodents' model because the dose of STZ in mice has a narrow safety margin (Acharjee et al., 2013). Accordingly, our study recruited male *Sprague-Dawley* rats, which are widely used to investigate the glycemic effect of medicinal plants (Furman, 2015), and such strain is more susceptible to develop diabetes mellitus with small doses of STZ (Wang-Fischer and Garyantes, 2018). In our study, the rats fed on 60% HFD *ad libitum* for five weeks (Zhang et al., 2008) gained higher body weights (2 times) and developed higher levels of FBS (FBS was more than 5.6 mmol/L) than rats fed on normal rodent diet *ad libitum*. The former findings indicated that HFD-fed rats became pre-diabetic according to Rydén et al. (2007), which might be due to the accumulation of fats (Srinivasan et al., 2005). In addition, the subsequent injection of HFD-fed rats with an intraperitoneal single dose of STZ (30 mg/kg) drove the rats to develop FBS in a range between 7.8 and 15.6 mmol/L and postprandial blood sugar levels more than 11.1 mmol/L, which might be due to that STZ mediated β-cells destruction making those rats insulin deficient (Furman, 2015; Zhang et al., 2008), which was also evident by the findings of their pancreatic histological sections.

STZ was selected to induce a diabetic metabolic state rather than alloxan, which induced diabetes for studying ROS-mediated β-cell death (Radenković et al., 2016). In addition, STZ as a diabetogenic agent provides more advantages over alloxan, such as the

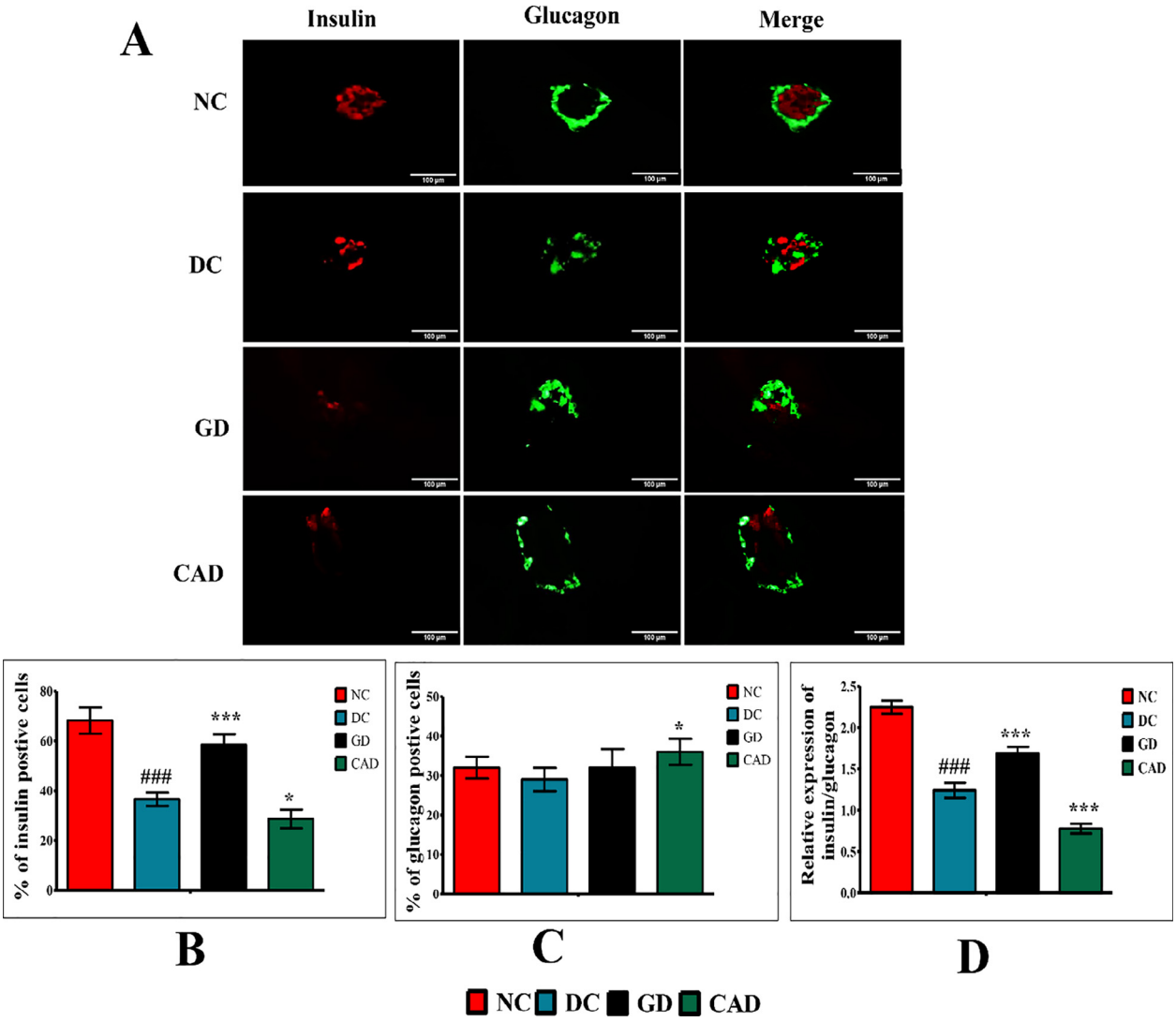

Fig. 3. Immunofluorescence of insulin, glucagon and insulin to glucagon ratio, (A): Double immunofluorescence labeling using glucagon (green) and insulin (red) in pancreatic sections (scale bar = 100 μm), (B): Percentage of insulin positive cells, (C): Percentage of glucagon positive cells, (D): Insulin to glucagon ratio. $^{\#\#\#}P \leq 0.001$ vs. NC group, while $^{*}P \leq 0.001$ and $^{***}P \leq 0.05$ vs. DC group. NC: nondiabetic control, GD: glibenclamide-treated diabetic group, CAD: cathinone-treated diabetic rats.

lower incidence of diabetic ketosis (Srinivasan et al., 2005) and the wide range of doses (King and Bowe, 2016). Even though both agents can be used to induce short duration diabetes, STZ remains the inducer of choice for the longer study of diabetes (4 weeks and more) (Radenković et al., 2016). In general, STZ could induce different degrees of hyperglycemia (mild to severe) according to the injected dose, route of injection, the species of experimental animals or even the strain within the same species (Qinna and Badwan, 2015). On the other hand, STZ was intraperitoneally injected because intraperitoneal injection is more convenient to rodents than intravenous, it is easy applicable (Sakata et al., 2012) and it does not need anesthesia during injection (Sakata et al., 2012).

The stability of the T2DM-induced animal model during the treatment period was assessed as some T2DM-induced animal models may be sometimes reversible owing to the spontaneous regeneration ability of pancreatic β-cells of rodents (Srinivasan et al., 2005), which could be interpreted wrongly as evidence of a possible hypoglycemic activity of the therapeutic agent. For such reason, T2DM-induced rats underwent oral glucose challenge three weeks after starting the treatment in addition to the weekly monitoring of FBS (King, 2012). Interestingly, our findings of OGTT and weekly FBS of T2DM-induced rats indicated an equivalent gradual increase in blood glucose across the timeline, which means that the T2DM-induced model was stable during the period of treatment.

In the diabetic control rats, the loss in body weights progressed from one week to another, which was due to the sustained uncontrolled hyperglycemia and their levels of FBS continued to increase progressively. In addition, the levels of blood sugar of the diabetic control rats after oral glucose challenge continued to increase until reaching a maximum peak at 120 min indicating a decline in the functions of pancreatic β-cells (King, 2012), particularly that the value of HOMA-B of the diabetic control rats [as a surrogate of the pancreatic β-cell activity (Wallace et al., 2004)] was 6.5 and 4 times lower than those of the nondiabetic control and glibenclamide-treated diabetic rats, respectively. The former findings could be resulted from the destructive effect of STZ on pancreatic β-cells (Furman, 2015; Zhang et al., 2008), particularly that the number, density volume and area of islets of *Langerhans* as well as the number of α- and β-cells and the density volume of β-cells of the diabetic control rats were lower than those of the nondiabetic control rats. The latter finding was also consistent with findings of the double immunofluorescence of pancreatic β-cells, where the percentage of β-cells were less than those of the nondiabetic control.

Our findings indicated that the glibenclamide-treated diabetic rats conserved their body weight from a week to another which could be due to that glibenclamide is associated with an increase in body weights of T2DM patients (Rains et al., 1988) because of the hypoglycemic effect of glibenclamide (Rambiritch et al., 2014) that stopped the diabetes-induced muscles wasting (Rains et al., 1988). On the other hand, the serum level of amylase of the glibenclamide-treated diabetic rats was significantly higher than that of the diabetic control and cathinone-treated diabetic rats due to that glibenclamide acts through releasing insulin (Rambiritch et al., 2014) of which secretion is interrelated to the secretion of amylase (Patel et al., 2006). The pancreatic β-cells of the glibenclamide-treated diabetic rats were active in secreting insulin since the value of HOMA-B of these rats, as a surrogate of the pancreatic β-cell activity (Wallace et al., 2004) was higher than that of the diabetic control rats. The later finding was also consistent with the findings of the double immunofluorescence of pancreatic β-cells, where the percentage of B-cells of the glibenclamide-treated diabetic rats were higher than that of the diabetic control rats. Regarding the lipid profile of the glibenclamide-treated diabetic rats, our findings indicated that the serum levels of total cholesterol, low-density lipoprotein and high density lipoprotein were higher than those of the diabetic control rats, which could be due to that glibenclamide changed the metabolism of lipoproteins (Mughal et al., 1999).

The cathinone-treated diabetic rats continued to lose body weights from one week to another, which could be a misleading sign of an effective hypoglycemia of cathinone (King, 2012). Since measuring blood sugar levels is an endpoint for testing therapeutic-induced hypoglycemia in the diabetes-induced animal models (King, 2012), our findings indicated that levels of FBS of the cathinone-treated diabetic rats continued to increase from one week to another, which could explain that the continued loss in body weight of rats was due to the catabolic effect of the uncontrolled hyperglycemia. In addition, cathinone-treated diabetic rats consumed lower calories than the diabetic control rats indicating the progressive loss in the body weight and the decline in caloric intake of those rats were due to a synergistic effect of the uncontrolled hyperglycemia and the potent anorectic effect of cathinone, respectively (Zelger and Carlini, 1980). The latter anorectic effect of cathinone could be independent of leptin regulation because the cathinone-treated diabetic rats had a two times decrease in leptin level than the diabetic control, which could be an attempt to enhance the appetite of those rats to compensate the resulted loss in their body weight and the decline in their caloric intake (Klok et al., 2007). The former findings could be emphasized by the findings of the reduction in the insulin level of cathinone-treated diabetic rats as compared to diabetic control rats indicating that the activity of pancreatic β-cells of those rats was not as active as those of diabetic control rats (Wallace et al., 2004) since the value of HOMA-B of the cathinone-treated diabetic rats was lower than that of the diabetic control. The latter finding is also consistent with the findings of the double immunofluorescence labelling of pancreatic β-cells, where the percentage of β-cells of the cathinone-treated diabetic rats were lower than that of diabetic control. In addition, the value of HOMA-S of the cathinone treated rats was higher than that of diabetic control indicating the need of rats for insulin to normalize the sustained hyperglycemia (Wallace et al., 2004). Accordingly, the former findings could indicate that the peripheral sympathomimetic activity of cathinone (Patel, 2000) blocked the release of insulin by inhibiting the α-adrenoreceptors on the pancreatic β-cells (Fyles et al., 1986), particularly that α2-adrenergic receptors are linked to T2DM (Prates et al., 2018). On the other hand, cathinone exacerbates the hyperglycemic situation of T2DM-induced rats through its hepatic sympathomimetic-glycogenolytic activity (Al-Habori and Al-Mamary, 2004), taking into consideration that cathinone could increase slightly the FBS of nondiabetic rats after acute exposure (Al Meshal, 1988), which could have been more pronounced if the duration of exposure had been prolonged. Regarding lipid profile, our findings indicated that cathinone has no significant effect on serum levels of triglycerides, total cholesterol, low density lipoprotein and high density lipoprotein of T2DM-induced rats which were in line with those findings in T2DM khat chewers (Atef et al., 2017).

Collectively, the lack of hypoglycemic activity of cathinone in T2DM-induced rats and our earlier *in vivo* findings of glycemic effects of khat (Alsalahi et al., 2016) could provide us with indictors that chewing of fresh leaves of khat by type 2 diabetics in the claim of its ability to reduce their elevated blood sugar should be regarded as catastrophic because such claim would lead to poor glycemic control due to the bad compliance to the prescribed hypoglycemic agents or even interfering with the hypoglycemic activity of such prescribed agents. Finally, extrapolation of our findings of *in vitro* glycemic activity indicated that cathinone has no hypoglycemic activity, while our findings of the *in vivo* glycemic activity indicated that cathinone exacerbates the hyperglycemic situation of the T2DM-induced rats. In fact, further *in vivo* investigation in nondiabetic and diabetic rats for amylase and α-glucosidase inhibitory assay should be elucidated to confirm the mechanism of the glycemic effects of cathinone. In addition, further studies should focus on the finding of the underlying mechanisms of the hyperglycemic activity of cathinone.

5. Conclusions

Cathinone has no substantial *in vitro* inhibition effects against both α-amylase and α-glucosidase enzymes, which indicates that cathinone is not effective in controlling post prandial glucose through blocking the intestinal conversion of carbohydrates into glucose. Conversely, cathinone exacerbated the hyperglycemia of T2DM-induced rats. Accordingly, such findings supported our earlier suggestion of the association between chewing khat with the progression and poor prognosis of T2DM, which has still needed further clinical investigation in T2DM khat chewers.

CRediT authorship contribution statement

Abdulsamad Alsalahi: Writing - original draft, Data curation, Formal analysis, Conceptualization, Methodology, Investigation. **Zamri Chik:** Supervision, Writing - review & editing, Data curation, Conceptualization, Resources. **Zahurin Mohamed:** Supervision, Writing - review & editing, Data curation, Methodology, Resources. **Nelli Giribabu:** Investigation, Methodology, Formal analysis. **Mohammed Abdullah Alshawsh:** Project administration, Supervision, Writing - review & editing, Data curation, Conceptualization, Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Acharjee, S., Ghosh, B., Al-Dhubiab, B.E., Nair, A.B., 2013. Understanding type 1 diabetes: etiology and models. Can. J. Diabetes 37, 269–276. https://doi.org/10.1016/j.jcjd.2013.05.001.

Al-Habori, M., Al-Mamary, M., 2004. Long-term feeding effects of Catha edulis leaves on blood constituents in animals. Phytomedicine 11, 639–644. https://doi.org/10.1016/j.phymed.2003.06.004.

Al-Meshal, I.A., 1988. Effect of (−)-cathinone, an active principle of Catha edulis forssk. (Khat) on plasma amino acid levels and other biochemical parameters in male wistar rats. Phytother. Res. 2, 63–66. https://doi.org/10.1002/ptr.2650020203.

Al-Obaid, A.M., Al-Tamrah, S.A., Aly, F.A., Alwarthan, A.A., 1998. Determination of (S)(−)-cathinone by spectrophotometric detection. J. Pharm. Biomed. Anal. 17, 321–326. https://doi.org/10.1016/S0731-7085(97)00203-3.

Al-Qirim, T.M., Shahwan, M., Zaidi, K.R., Uddin, Q., Banu, N., 2002. Effect of khat, its constituents and restraint stress on free radical metabolism of rats. J. Ethnopharmacol. 83, 245–250. https://doi.org/10.1016/S0378-8741(02)00251-9.

Al Meshal, I., 1988. Effect of (−)-Cathinone, an Active Principle of *Catha edulis Forssk.* (Khat) on Plasma Amino Acid Levels and Other Biochemical Parameters in Male *Wistar* Rats. Phytother. Res. 2, 63–66.

Alsalahi, A., Alshawsh, M.A., Mohamed, R., Alyousefi, N.A., Alshagga, M.A., Shwter, A. N., Mohamed, Z., 2016. Conflicting reports on the role of the glycemic effect of Catha edulis (Khat): A systematic review and meta-analysis. J. Ethnopharmacol. 186, 30–43. https://doi.org/10.1016/j.jep.2016.03.045.

Antunes, L.C., Elkfury, J.L., Jornada, M.N., Foletto, K.C., Bertoluci, M.C., 2016. Validation of HOMA-IR in a model of insulin-resistance induced by a high-fat diet in Wistar rats. Arch. Endocrin. Metab. 60, 138–142. https://doi.org/10.1590/2359-3997000000169.

Atef, Z.A., Bamashmos, M.A., Alghazali, G., 2017. Effect of Qat on the level of blood glucose and lipids among Yemeni patients with type 2 diabetes. Egypt. J. Obes. Diabetes Endocrinol. 3, 100.

Aziz, H.A., Peh, K.K., Tan, Y.T.F., 2011. Herbal delivery system for treatment of obesity administration of encapsulated khat-extracts on body weight of rats. Obes. Res. Clin. Pract. 5, e305–e312. https://doi.org/10.1016/j.orcp.2011.03.008.

Barrière, D.A., Noll, C., Roussy, G., Lizotte, F., Kessai, A., Kirby, K., Sarret, P., 2018. Combination of high-fat/high-fructose diet and low-dose streptozotocin to model long-term type-2 diabetes complications. Sci. Rep. 8, 424. https://doi.org/10.1038/s41598-017-18896-5.

Ben-Shabat, S., Goloubinoff, P., Dudai, N., Lewinsohn, E., 2014. Farming Amphetamines: Khat (Catha edulis Forsk.) a Traditional Plant with Mild Stimulating Psychoactive and Medicinal Properties. In: Yaniv, Z., Dudai, N. (Eds.), Medicinal and Aromatic Plants of the Middle-East. Springer, Netherlands, Dordrecht, pp. 181–197.

Betrie, A.H., Engidawork, E., 2016. Effect of extracts of khat (Catha edulis F.) on glucose handling in normoglycaemic, diabetic, and glucose loaded rodents. Eth. Pharm. J. 32, 9–22. https://doi.org/10.4314/epj.v32i1.2.

Institute of Laboratory Animal Resources (US). Committee on Care, & Use of Laboratory Animals, 1986. Guide for the care and use of laboratory animals (No. 86). US Department of Health and Human Services, Public Health Service, National Institutes of Health.

Chiasson, J.-L., Josse, R.G., Leiter, L.A., Mihic, M., Nathan, D.M., Palmason, C., Wolever, T.M., 1996. The effect of acarbose on insulin sensitivity in subjects with impaired glucose tolerance. Diabetes Care 19, 1190–1193. https://doi.org/10.2337/diacare.19.11.1190.

Crombie, L., Crombie, W.M.L., Whiting, D.A., 1990. Chapter 4 Alkaloids of Khat (Catha Edulis). In: Brossi, A. (Ed.), The Alkaloids: Chemistry and Pharmacology, vol. 39. Academic Press, Nottingham, UK, pp. 139–164.

Directive, C., 1986. 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. Off. J. Eur. Commun 29, L358.

Findlay, J.A., Thomas, N.W., 1980. Histology and cytology of the islets of Langerhans in the Mongolian gerbil (Meriones unguiculatus). Cells Tissues Organs 108, 446–462. https://doi.org/10.1159/000145344.

Freudenberg, F., Resnik, E., Kolleker, A., Celikel, T., Sprengel, R., Seeburg, P.H., 2016. Hippocampal GluA1 expression in Gria1−/− mice only partially restores spatial memory performance deficits. Neurobiol. Learn. Mem. 135, 83–90. https://doi.org/10.1016/j.nlm.2016.07.005.

Furman, B.L., 2015. Streptozotocin-Induced Diabetic Models in Mice and Rats. Curr. Protoc. Pharmacol., 70, 5.47.41–45.47.20. http://dx.doi.10.1002/0471141755.ph0547s70.

Fyles, J., Cawthorne, M., Howell, S., 1986. The characteristics of β-adrenergic binding sites on pancreatic islets of Langerhans. J. Endocrinol 111, 263–270. https://doi.org/10.1677/joe.0.1110263.

Ganeshpurkar, A., Saluja, A.K., 2017. Experimental animal models used for evaluation of potential immunomodulators: A mini review. Bull. Fac. Pharm. Cairo Univ. 55, 211–216. https://doi.org/10.1016/j.bfopcu.2017.08.002.

Jelodar, G., Maleki, M., Sirus, S., 2007. Effect of Fumitory, Celery and Lemon on Blood Glucose and Histopathology of Pancreas of Alloxan Diabetic Rats. J. Appl. Anim. Res. 31, 101–104. https://doi.org/10.1080/09712119.2007.9706640.

Jenčič, B., Jeromel, L., Ogrinc Potočnik, N., Vogel-Mikuš, K., Vavpetič, P., Rupnik, Z., Pelicon, P., 2017. Molecular imaging of alkaloids in khat (Catha edulis) leaves with MeV-SIMS. Nucl. Instrum. Methods Phys. Res. B. 404, 140–145. https://doi.org/10.1016/j.nimb.2017.01.063.

King, A., Bowe, J., 2016. Animal models for diabetes: Understanding the pathogenesis and finding new treatments. Biochem. Pharmacol. 99, 1–10. https://doi.org/10.1016/j.bcp.2015.08.108.

King, A.J.F., 2012. The use of animal models in diabetes research. Br. J. Pharmacol. 166, 877–894. https://doi.org/10.1111/j.1476-5381.2012.01911.x.

Klok, M.D., Jakobsdottir, S., Drent, M.L., 2007. The role of leptin and ghrelin in the regulation of food intake and body weight in humans: a review. Obes. Rev. 8, 21–34. https://doi.org/10.1111/j.1467-789X.2006.00270.x.

Majchrzak, M., Celiński, R., Kuś, P., Kowalska, T., Sajewicz, M., 2018. The newest cathinone derivatives as designer drugs: an analytical and toxicological review. Forensic Toxicol. 36, 33–50. https://doi.org/10.1007/s11419-017-0385-6.

Mughal, M.A., Maheri, W.M., Aamir, K., Jan, M., Ali, M., 1999. The effects of glibenclamide on serum lipids and lipoproteins in type II non-insulin dependent diabetes mellitus. J. Pak. Med. Assoc. 49, 89–91.

Nirwane, A., Majumdar, A., 2016. Resveratrol and pterostilbene ameliorate the metabolic derangements associated with smokeless tobacco in estrogen deficient female rats. J. Funct. Foods 23, 261–277. https://doi.org/10.1016/j.jff.2015.12.030.

Nwoye, L.O., Shatoor, A.S., Soliman, H.S., Al-Hashem, F.H., 2010. In vivo acute effects of orally administered hydro-ethanol extract of Catha edulis on blood glucose levels in normal, glucose-fed hyperglycemic, and alloxan-induced diabetic rats. Saudi Med. J. 31, 627–633.

Nyongesa, A. W., Oduma, J.A., Nakajima, M., Odongo, H.O., Adoyo, P.A., al'Absi, M., 2014. Acute and sub-chronic effects of purified cathinone from khat (Catha edulis) on behavioural profiles in vervet monkeys (Chlorocebus aethiops). Metab. Brain Dis., 29, 441–449. http://dx.doi.10.1007/s11011-013-9441-z.

Pandit, R., Phadke, A., Jagtap, A., 2010. Antidiabetic effect of Ficus religiosa extract in streptozotocin-induced diabetic rats. J. Ethnopharmacol. 128, 462–466. https://doi.org/10.1016/j.jep.2010.01.025.

Patel, N., 2000. Mechanism of action of cathinone: the active ingredient of Khat (Catha Edulis. East Afr. M. J. 77. https://doi.org/10.4314/eamj.v77i6.46651.

Patel, R., Pariente, J.A., Martinez, M.A., Salido, G.M., Singh, J., 2006. Effect of Insulin on Acetylcholine-Evoked Amylase Release and Calcium Mobilization in Streptozotocin-Induced Diabetic Rat Pancreatic Acinar Cells. Ann. N. Y. Acad. Sci. 1084, 58–70. https://doi.org/10.1196/annals.1372.027.

Prates, K.V., de Oliveira, J.C., Malta, A., Matiusso, C.C.I., Miranda, R.A., Ribeiro, T.A., Barella, L.F., 2018. Sympathetic innervation is essential for metabolic homeostasis and pancreatic beta cell function in adult rats. Mol. Cell Endocrinol. 462, 119–126. https://doi.org/10.1016/j.mce.2017.09.031.

Qasem, M.A., Noordin, M.I., Arya, A., Alsalahi, A., Jayash, S.N., 2018. Evaluation of the glycemic effect of Ceratonia siliqua pods (Carob) on a streptozotocin-nicotinamide induced diabetic rat model. PeerJ 6,. https://doi.org/10.7717/peerj.4788 e4788.

Qian, M., Haser, R., Payan, F., 1995. Carbohydrate binding sites in a pancreatic α-amylase-substrate complex, derived from X-ray structure analysis at 2.1 Å resolution. Protein Sci. 4, 747–755. https://doi.org/10.1002/pro.5560040414.

Qinna, N.A., Badwan, A.A., 2015. Impact of streptozotocin on altering normal glucose homeostasis during insulin testing in diabetic rats compared to normoglycemic rats. Drug Des. Dev. Ther 9, 2515–2525. https://doi.org/10.2147/DDDT.S79885.

Radenković, M., Stojanović, M., Prostran, M., 2016. Experimental diabetes induced by alloxan and streptozotocin: The current state of the art. J. Pharmacol. Toxicol. Methods 78, 13–31. https://doi.org/10.1016/j.vascn.2015.11.004.

Rains, S.G.H., Wilson, G.A., Richmond, W., Elkeles, R.S., 1988. The Effect of Glibenclamide and Metformin on Serum Lipoproteins in Type 2 Diabetes. Diabet. Med. 5, 653–658. https://doi.org/10.1111/j.1464-5491.1988.tb01074.x.

Rambiritch, V., Maharaj, B., Naidoo, P., 2014. Glibenclamide in patients with poorly controlled type 2 diabetes: a 12-week, prospective, single-center, open-label, dose-escalation study. Clin. Pharmacol. 6, 63–69. https://doi.org/10.2147/CPAA.S54809.

Rydén, L., Standl, E., Bartnik, M., Van den Berghe, G., Betteridge, J., de Boer, M.-J., et al., 2007. Guidelines on diabetes, pre-diabetes, and cardiovascular diseases: executive summary: The Task Force on Diabetes and Cardiovascular Diseases of the European Society of Cardiology (ESC) and of the European Association for the Study of Diabetes (EASD). Eur. Heart J., 28, 88–136. http://dx.doi.10.1093/eurheartj/ehl260.

Sakata, N., Yoshimatsu, G., Tsuchiya, H., Egawa, S., Unno, M., 2012. Animal Models of Diabetes Mellitus for Islet Transplantation. Exp. Diabetes Res. 2012,. https://doi.org/10.1155/2012/256707 256707.

Shin, J.-W., Seol, I.-C., Son, C.-G., 2010. Interpretation of animal dose and human equivalent dose for drug development. J. Korean Orient. Med. 31, 1–7.

Srinivasan, K., Viswanad, B., Asrat, L., Kaul, C.L., Ramarao, P., 2005. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: A model for type 2

diabetes and pharmacological screening. Pharmacol. Res. 52, 313–320. https://doi.org/10.1016/j.phrs.2005.05.004.
Unit, D.T., 2020. Model 2 homeostatic model assessment software calculator.
Wallace, T.M., Levy, J.C., Matthews, D.R., 2004. Use and Abuse of HOMA Modeling. Diabetes Care 27, 1487. https://doi.org/10.2337/diacare.27.6.1487.
Wang-Fischer, Y., Garyantes, T., 2018. Improving the Reliability and Utility of Streptozotocin-Induced Rat Diabetic Model 8054073 J. Diabetes Res. 2018, 8054073. https://doi.org/10.1155/2018/8054073.
Wang, Y., Campbell, T., Perry, B., Beaurepaire, C., Qin, L., 2011. Hypoglycemic and insulin-sensitizing effects of berberine in high-fat diet- and streptozotocin-induced diabetic rats. Metabolism 60, 298–305. https://doi.org/10.1016/j.metabol.2010.02.005.
Zelger, J.L., Carlini, E.A., 1980. Anorexigenic effects of two amines obtained from Catha edulis Forsk. (Khat) in rats. Pharmacol. Biochem. Behav. 12, 701–705. https://doi.org/10.1016/0091-3057(80)90152-5.
Zhang, M., Lv, X.Y., Li, J., Xu, Z.G., Chen, L., 2008. The characterization of high-fat diet and multiple low-dose streptozotocin induced type 2 diabetes rat model. Exp. Diabetes Res. 2008,. https://doi.org/10.1155/2008/704045 704045.