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Effect of *Catha edulis* (khat) on pancreatic functions in streptozotocin-induced diabetes in male Sprague-Dawley rats

Abdulsamad ALSALAH, Mohammed A. ALSHAWSH, Zamri CHIK, and Zahurin MOHAMED

Department of Pharmacology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

Abstract: People consume *Catha edulis* (khat) for its euphoric effect, and type 1 diabetics have claimed that khat could reduce elevated levels of blood sugar. However, khat has been suggested to provoke diabetes mellitus through destruction of pancreatic β -cells. This study investigated the effect of an ethanolic khat extract on pancreatic functions in type 1 diabetes (T1DM)-induced male Sprague-Dawley rats and to assess its *in vitro* cytotoxicity in rat pancreatic β -cells (RIN-14B). T1DM was induced in a total of 20 rats with a single intraperitoneal injection of 75 mg/kg of streptozotocin. The rats were distributed into four groups (n=5): the diabetic control, 8 IU insulin-treated, 200 mg/kg khat-treated, and 400 mg/kg khat-treated groups. Another 5 rats were included as a nondiabetic control. Body weight, fasting blood sugar, and caloric intake were recorded weekly. Four weeks after treatment, the rats were sacrificed, and blood was collected for insulin, lipid profile, total protein, amylase, and lipase analysis, while pancreases were harvested for histopathology. *In vitro*, khat exerted moderate cytotoxicity against RIN-14B cells after 24 and 48 h but demonstrated greater inhibition against RIN-14B cells after 72 h. Neither 200 mg/kg nor 400 mg/kg of khat produced any significant reduction in blood sugar; however, 200 mg/kg khat extract provoked more destruction of pancreatic β -cells as compared with the diabetic control. Ultimately, neither 200 mg/kg nor 400 mg/kg of khat extract could produce a hypoglycemic effect in T1DM-induced rats. However, 200 mg/kg of khat caused greater destruction of pancreatic β -cells, implying that khat may cause a direct cytotoxic effect on pancreatic β -cells *in vitro*.

Key words: *Catha edulis*, diabetes mellitus, khat, streptozotocin-induced diabetes rats

Introduction

Traditionally, *Catha edulis* Forskal (khat) is a plant that is consumed by at least 20 million people worldwide because of its recreational effect [16] and also of the claim that it could reduce elevated blood sugar of healthy and diabetic chewers [5].

Although several studies have been carried out on the glycemic activity of khat using different *in vivo* models, the glycemic effect of khat remains conflicting [9]. These studies were conducted either in non-diabetic animal models [3, 6], an animal model with metabolic syndrome [31], a model with acute pancreatitis [15], or in alloxan-induced type 2 diabetes model [19]. However, the gly-

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Addresses corresponding: M. A. Alshawsh, Department of Pharmacology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

Z. Mohamed, Department of Pharmacology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia



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cemic activity of khat in type 1 diabetes animal model has not been reported in the literature.

Type 1 diabetes mellitus (T1DM) is a metabolic disorder that is characterized by absolute insulin deficiency due to an autoimmune destruction of pancreatic β -cells [11, 30]. It was reported that khat may produce a destructive effect on pancreatic β -cells of chronic chewers rendering khat as a prospective risk factor in the development of diabetes mellitus (DM) [21]. In fact, the direct toxic effects of khat on the heart, liver and kidney of experimental animals have been evoked [1, 8]. Nonetheless, the associated toxicity of khat on pancreatic β -cells has not yet been evaluated. Accordingly, this study aimed to examine if the ethanolic extract of khat could produce an *in vitro* cytotoxic effect on pancreatic β -cells (RIN-14B) and to further examine the *in vivo* effect of khat on blood sugar and pancreas in T1DM induced male Sprague-Dawley rats.

Materials and Methods

Plant materials

An amount of three kilograms of pesticide-free fresh shoots of *Catha edulis* (Hammadani cultivar, Yemen) were collected from Hammadan region, Sana'a, Yemen in January 2015. The plant materials were identified by a taxonomist and given a voucher specimen (CAED/2015/110), which was then deposited in the Laboratory of Pharmacognosy, Faculty of Pharmacy, Sana'a University, Yemen. The fresh shoots were rinsed in distilled water to wash out debris and dust particles. Next, fresh leaves were cut off and allowed to dry in a dark cabinet for two weeks. After grinding, the dried leaves produced 500 g of fine powder.

Chemicals and reagents

Streptozotocin was obtained from (Sigma Aldrich, USA), NovoMix 30 FlexPen insulin was purchased from (Novo Nordex, Denmark), ELISA Rat/mouse insulin kit was obtained from (Millipore Merck, USA). Eagle's Minimum Essential Medium and RPMI-1640 media were obtained from (Sigma-Aldrich, USA).

Cells line

Rat pancreatic islets cell tumor (RIN-14B) and human normal liver (WRL 68) cell lines were obtained from (ATCC, USA).

Animals

A total of 25 healthy, 7–8 weeks male Sprague-Dawley rats were obtained from the Animal Experiment Unit, Faculty of Medicine, University of Malaya. The animals were housed in AAALAC-accredited animal facility and allowed to acclimatize for one week under temperature of $20 \pm 4^\circ\text{C}$, relative humidity (30–70%) and with 12 h light and 12 h dark cycle. Rats were allowed free access to a rodent diet (1324, Altromin, Lage, Germany) and tap water *ad libitum*. The rats were maintained according to Guidelines for Proper Conduct of Animal Experiments [29].

Preparation of an ethanolic extract of khat

The dry powder of khat was macerated in 85% ethanol (100 g/l each maceration) for 72 h with frequent shaking [14]. The liquid extract was finally filtered, and the filtrate was concentrated using a rotary evaporator at 40°C and allowed to evaporate until a dry paste of extract of khat was obtained, which was kept at -20°C for further work.

In vivo effect of the ethanolic khat extract on blood sugar of T1DM-induced rats

Animal ethics: This animal study was approved by the Institutional Animal Care and Use Committee (IACUC), Faculty of Medicine, University of Malaya (ethics approval no. 2015-180505/PHAR/R/ASAM).

Induction of T1DM in rats: Induction of T1DM in rats was carried out according to the protocol of Furman [24] with a slight modification. Rats were deprived of food overnight, and tail-tip fasting blood sugar (FBS) was measured using a one-touch Accu-Check[®] Performa glucometer (Roche Diagnostics GmbH, Mannheim, Germany). Rats were rendered diabetic by a single intraperitoneal injection of 75 mg/kg of streptozotocin (STZ), freshly prepared in 0.1 mM citrate buffer (pH=4.5), while rats in the nondiabetic normal control group were intraperitoneally injected with 2 ml/kg of 0.1 mM citrate buffer. Three days post-induction, FBS was measured, and rats with FBS values between 13.9 and 33.3 mmol/l were considered to have T1DM, and enrolled into the study [24].

Study design: Rats were distributed randomly into five groups (n=5): the nondiabetic control (NC), diabetic control (DC), diabetic insulin-treated (DI), diabetic low dose khat-treated (DK200), and diabetic high dose khat-treated (DK400) groups. The rats were treated over four

weeks with a single daily dose of 5 ml/kg deionized water (DW) orally, 5 ml/kg DW orally, 8 IU/day insulin (NovoMix30 FlexPen) subcutaneously [39], 200 mg/kg khat extract orally, and 400 mg/kg khat extract orally, respectively [34]. Body weight (g), FBS (mmol/l), and caloric intake (kcal/g) were recorded at the 1st, 7th, 14th, 21st, and 28th days of treatment. Caloric intake was calculated by multiplying food consumption (g) by 3,279 kcal/kg (provided by Altromin 1324) divided by 1,000 (Kcal/g/day) [13, 33]. On the 28th day of the experiment, rats were deprived of diet overnight, and blood samples were collected via cardiac puncture under anesthesia with ketamine/xylazine (60:7.5 mg/kg, i.p.) [38]. Blood samples were centrifuged, and serum was kept at -20°C for biochemistry analysis. Amylase (U/l), lipase (U/l), total protein (g/l), triglycerides (mmol/l), total cholesterol (mmol/l), high-density lipoprotein (mmol/l) and low-density lipoprotein (mmol/l) were analyzed in the Biochemistry Laboratory, Faculty of Veterinary, Universiti Putra Malaysia. Insulin ($\mu\text{U/ml}$) was measured using a Rat/Mouse Insulin ELISA kit (Merck Millipore, Burlington, MA, USA). The pancreas was harvested from all animals and kept in 10% buffered formalin. The processed pancreas tissues were embedded in Paraffin wax, and 5 μm sections were stained with hematoxylin and eosin for histology examination and quantitative morphometric evaluation.

Quantitative morphometric evaluation: Stained pancreas sections from four animals ($n=4$) per group were quantitatively evaluated under light microscope (Leica microscope) for the number of islets per 50 microscopic fields at $40\times$ magnification [28], for the volume density of islets expressed as a percentage of islets per 50 microscopic fields [28], for the number of β - and α -cells per 10 islets at $100\times$ magnification [28], for 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 [28], and for 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.

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

where the linear magnification is equal to the eyepiece magnification ($100\times$) multiplied by the used magnification power ($40\times$) [22].

In vitro cytotoxicity of the ethanolic extract of khat
Cell culture: RIN-14B (rat pancreatic islets cell tumor)

and WRL 68 (human normal liver) cells were sub-cultured in Eagle's Minimum Essential Medium (EMEM, Sigma-Aldrich, St. Louis, MO, USA) and RPMI-1640 medium (Sigma-Aldrich, St. Louis, MO, USA), respectively. The media were supplemented with 10% FBS and 1% penicillin-streptomycin [26].

Cell viability test: A cell viability assay was conducted according to the protocol of Jayash *et al.* [27]. Briefly, the subcultured cells of RIN-14B and WRL 68 were trypsinized, seeded in triplicate onto a 96-well plate with a density of 5×10^3 cells/well, and then incubated for 24 h (at 37°C in 5% CO_2) to allow cell attachment to the plate surface. Then a volume of 20 μl from each serial dilution of the ethanolic extract of khat (1,000, 500, 250, 125, 62.5, 31.25, 15.625, and 7.8125 $\mu\text{g/ml}$) was introduced in triplicate into the cell-containing wells, and the plates were incubated again for 24, 48, and 72 h at 37°C in 5% CO_2 . In the dark, 20 μl of MTT solution (5 mg/ml) was pipetted into the treated cells, and the plate was then covered with aluminum foil and incubated again for 4 h. Finally, all media were removed, and a total of 100 μl of dimethyl sulfoxide (DMSO) was added to each well until the purple formazan crystals were dissolved. The negative control (untreated cells) had to undergo the same procedure. Plates were read with a microplate reader at 570 nm. The test was repeated three times to calculate the IC_{50} . The inhibitory effect of each serial dilution of khat was calculated as a percentage of the difference between the average absorbance of the treated cells and the untreated cells (negative control), which in turn was divided by the average absorbance of the untreated cells (negative control). Then the viability of cells at each serial dilution of khat was calculated by subtracting the percentage inhibition from the constant 100. The cytotoxic effect of each serial dilution of khat was rated as severe, moderate, mild, and noncytotoxic when the percentage cell viability was <30 , 30–59, 60–90, and $>90\%$, respectively. The percent inhibition of the serial dilutions after 24, 48, or 72 h was plotted graphically against their corresponding serial dilutions. The IC_{50} from each experiment after 24, 48, or 72 h of exposure to khat was calculated from the trend using a Microsoft excel 2016 spreadsheet. The IC_{50} was considered $>1,000 \mu\text{g/ml}$ in the event that no IC_{50} could be obtained with the highest concentration of the treatment.

Statistical analysis

Data were analyzed statistically using the IBM SPSS

software package (version 20). For normally distributed data, one-way ANOVA was used followed by a post hoc Tukey test. Otherwise, the Games-Howell post hoc test was applied if homogeneity was violated. In addition, the independent samples *t*-test was used to compare the mean difference of pairs of variables [23]. $P \leq 0.05$ represented a significant difference, $P \leq 0.01$ represented a highly significant difference, and $P \leq 0.001$ represented a very highly significant difference. Differences in means were expressed as the mean \pm SD or mean \pm SE.

Results

In vivo effect of the ethanolic khat extract in T1DM-induced rats

Baseline FBS after induction of T1DM: The baseline mean FBS levels of STZ-induced diabetic rats were 21.40 ± 2.25 mmol/l in the DC group, 22.90 ± 1.45 mmol/l in the DI group, 21.40 ± 3.73 mmol/l in the DK200 group, and 22.46 ± 4.04 mmol/l in the DK400, all of which were significantly ($P \leq 0.001$) higher than the levels of the NC group (5.02 ± 0.70 mmol/l).

Weekly body weight (BW): At the end of the 1st, 2nd, 3rd, and 4th weeks, the body weight of rats in the NC group increased significantly ($P \leq 0.001$) as compared with those of diabetic rats in the DC, DI, DK200, and DK400 groups. At the end of the 4th week, the body weight of diabetic rats in the DI group was significantly ($P \leq 0.01$) more than those of diabetic rats in the DC, DK200, and DK400 groups; however, there was no significant difference in the body weights of rats between the DC, DK200 and DK400 groups. As compared with the baseline BWs (Week 0), only diabetic rats in DK200 showed a significant reduction in body weight at the end of the 4th week ($P \leq 0.05$) (Fig. 1).

Weekly caloric intake: As compared with week 1, caloric intake at week 2 was decreased in almost all groups, and the caloric intakes of the nondiabetic control rats (NC) and insulin-treated diabetic rats (DI) showed significant decreases ($P \leq 0.001$ and $P \leq 0.01$, respectively). On the other hand, there was no significant difference in caloric intake between any groups at the end of the 1st, 2nd, 3rd, and 4th weeks of treatment (Fig. 2).

Weekly fasting blood sugar (FBS): FBS of non-diabetic control remained significantly ($P \leq 0.01$) lower than that of all diabetic rats in DC, DK200 and DK400 at the end of the 1st, 2nd, 3rd and 4th week. Similarly, insulin treated diabetic rats (DI) showed significantly ($P \leq 0.001$)

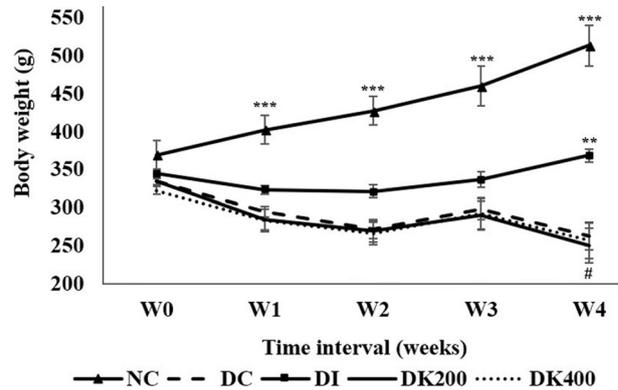


Fig. 1. Body weight of treated and untreated rats. Data are graphically presented as the mean \pm SE (n=5). **body weight of DI group at the end of 4th week was significantly higher than those of the DC, DK200, and DK400 groups ($P \leq 0.01$). ***body weights of the NC group at the end of the 1st, 2nd, 3rd, and 4th weeks were significantly higher than that of the DC, DI, DK200 and DK400 groups ($P \leq 0.001$). #body weight of the DK200 group decreased significantly at the end of the 4th week as compared with week 0 ($P \leq 0.05$). NC, nondiabetic control group; DC, diabetic control group; DI, insulin-treated diabetic group; DK200, low dose khat-treated diabetic group; DK400, high dose khat-treated diabetic group.

lower FBS at the end of 1st, 2nd, 3rd and 4th week of treatment as compared to DC, DK200 and DK400 groups. On the other hand, there was no significant difference in FBS between DC, DK200 and DK400. As compared to baseline (week 0), FBS of DC significantly ($P \leq 0.05$) increased only at the end of the 2nd and 3rd week. Conversely, FBS of DI significantly ($P \leq 0.001$) declined reaching a minimum at the end of the 4th week as compared to week 0 (Fig. 3).

Biochemistry: Induction of T1DM in rats using STZ resulted in a considerable reduction of insulin in diabetic-induced rats as compared to non-diabetic control rats, however, insulin level of insulin treated rats (DI) was significantly ($P \leq 0.01$) higher than that of DC as a result of insulin treatment. Amylase in NC, DK400 and DI was significantly higher than that of DC ($P \leq 0.001$, $P \leq 0.05$ and $P \leq 0.001$, respectively). Triglyceride, total cholesterol, HDL, and lipase were non-significantly different between treated rats and diabetic control. Total protein of DI was significantly ($P \leq 0.01$) higher than that of DC group. On the other hand, LDL of DI, DK200 and DK400 was significantly lower than that of DC (Table 1).

Histopathology: Quantitative morphometric evaluation: Number and volume density of islets of diabetic rats in DC, DI, DK200 and DK400 groups were sig-

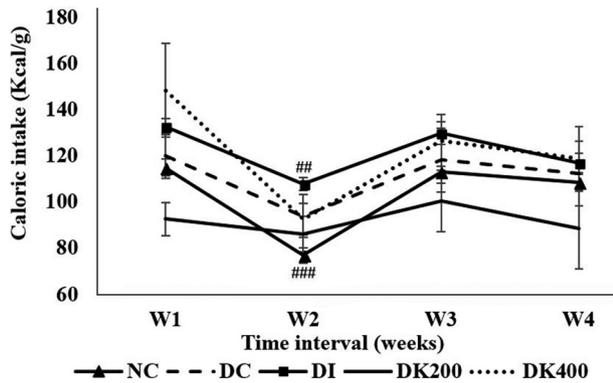


Fig. 2. Caloric intake of treated and untreated rats. Data are graphically presented as the mean \pm SE (n=5). ##caloric intake of the DI group decreased significantly at the end of the 2nd week as compared with week 0 ($P \leq 0.01$). ###caloric intake of the NC group decreased significantly at the end of the 2nd week as compared with week 0 ($P \leq 0.001$). NC, nondiabetic control group; DC, diabetic control group; DI, insulin-treated diabetic group; DK200, low dose khat-treated diabetic group; DK400, high dose khat-treated diabetic group.

nificantly ($P \leq 0.001$) lower than those of non-diabetic control rats (NC). Interestingly, it was obviously that DK400 treated diabetic rats showed a significant ($P \leq 0.05$) higher number and volume density of islets as compared to DK200 treated diabetic rats. Similarly, size of islets of diabetic rats in DC, DI, DK200 and DK400 groups was significantly ($P \leq 0.001$) lower than that of NC group. In addition, DK200 treated diabetic rats showed a significant ($P \leq 0.05$) lower size of islets than those in DK400 and DC groups. On the other hand, number of α -cells of non-diabetic and diabetic rats was non-significantly different, while the number of β -cells of all diabetic rats in DC, DI, DK200 and DK400 groups was significantly ($P \leq 0.01$) lower than that of NC group. However, DK400 treated diabetic rats demonstrated a higher significant ($P \leq 0.01$) number of β -cells than DK200 treated diabetic rats, while rats treated with insulin (DI) showed a significant ($P \leq 0.05$) higher number of β -cells than diabetic control rats (DC). Nonetheless, the volume density of β -cells of all diabetic rats in DC, DI, DK200 and DK400 groups was significantly ($P \leq 0.001$) lower than that of NC group. Otherwise, the volume density of β -cells of diabetic rats in DK200 was non-significantly lower than that of DK400 (Table 2).

Histology examination Histology examination of pancreas of NC group shows a typical architecture and the islets of Langerhans were well-defined in shape, compact

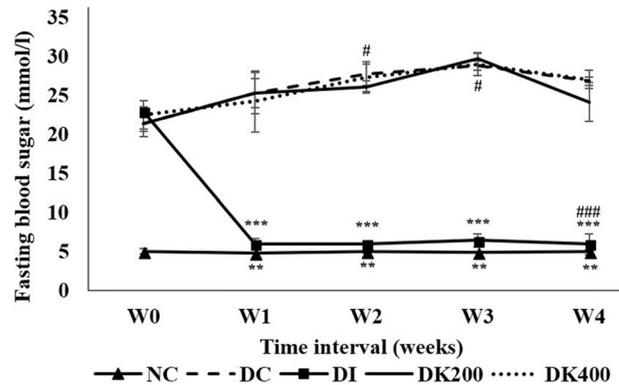


Fig. 3. Fasting blood sugar of treated and untreated rats. Data are graphically presented as the mean \pm SE (n=5). **fasting blood sugar levels of the NC group at the end of the 1st, 2nd, 3rd, and 4th weeks were significantly lower than those of the DC, DK200, and DK400 groups ($P \leq 0.01$). ***fasting blood sugar levels of the DI group at the end of the 1st, 2nd, 3rd, and 4th weeks were significantly lower than those of the DC, DK200, and DK400 groups ($P \leq 0.001$). #fasting blood sugar levels of the DC group at the end of the 2nd and 3rd weeks increased significantly as compared with week 0 ($P \leq 0.05$). ###The fasting blood sugar level of the DI group at the end of the 4th week was significantly lower as compared with week 0 ($P \leq 0.001$). NC, nondiabetic control group; DC, diabetic control group; DI, insulin-treated diabetic group; DK200, low dose khat-treated diabetic group; DK400, high dose khat-treated diabetic group.

in size, well-demarcated outlines and free of vacuoles. Conversely, architecture of DC was disorganized and the islets of Langerhans were vacuolated, lost their regular shape, their β -cells and α -cells appeared to be diffused with the adjacent acinar cells because of the rupture of the demarcated outlines. In addition, the nuclei of β -cells appeared eccentric-located and degranulated. Unlike DC, the architecture of pancreatic sections of DI were slightly more organized than that of DC, the islets of Langerhans were more demarcated in outlines, and the nuclei of β -cells appeared dark granulated centrally-located, however, the islets of Langerhans were vacuolated and had irregular shape. Like DC, sections of DK200 showed similar changes in the architecture of islets of Langerhans, while the changes in the architecture of islets of Langerhans of DK400 was more organized and less damaged than that of DK200 and DC (Fig. 4).

In vitro cytotoxic effect of the ethanolic extract of khat

RIN-14B cells (rat pancreatic β -cells): The IC_{50} values of khat after 24, 48, and 72 h of treatment were significantly different ($P \leq 0.001$). Accordingly, the IC_{50} value

Table 1. Effect of khat treatment on biochemistry parameters in rats

	NC	DC	DI	DK200	DK400
INS	5.34 ± 2.73	0.63 ± 0.51	139.77 ± 45.91*	1.01 ± 1.04	2.71 ± 1.65
Amy	2,006.56 ± 56.17	746.20 ± 124.50 ^{##}	2,297.36 ± 81.21***	916.80 ± 218.01	1,044.72 ± 74.66*
Lip	5.47 ± 0.92	10.18 ± 3.42 [#]	13.68 ± 3.59	7.61 ± 1.34	7.27 ± 1.58
TP	72.94 ± 4.20	57.44 ± 2.75 [#]	75.74 ± 12.94**	56.20 ± 5.45	55.32 ± 5.06
TG	0.41 ± 0.05	0.98 ± 0.81	0.99 ± 0.16	0.48 ± 0.34	1.13 ± 0.52
TC	1.86 ± 0.32	1.86 ± 0.62	2.34 ± 0.59	1.34 ± 0.53	1.18 ± 0.28
LDL	0.25 ± 0.06	0.55 ± 0.13 ^{##}	0.35 ± 0.05*	0.23 ± 0.05***	0.21 ± 0.08***
HDL	1.44 ± 0.25	1.00 ± 0.27	1.78 ± 0.40**	1.08 ± 0.26	0.68 ± 0.08

Data are expressed as the mean ± SD, (n=5). * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ vs. DC group, [#] $P \leq 0.05$, ^{##} $P \leq 0.01$ vs. NC group. NC, nondiabetic control group; DC, diabetic control group; DI, insulin-treated group; DK200, low dose khat-treated group; DK400, high dose khat-treated group. INS, insulin ($\mu\text{U/l}$); Amy, amylase (U/l); Lip, lipase (U/l); TP, total protein (g/l); TG, triglyceride (mmol/l); TC, total cholesterol (mmol/l); LDL, low density lipoprotein (mmol/l); HDL, high density lipoprotein (mmol/l).

Table 2. Morphometric quantitative histological evaluation of pancreatic sections

	NC	DC	DI	DK200	DK400
No. of islets/50 fields	39.25 ± 5.74	8.25 ± 2.06 ^{###}	14.50 ± 3.87 ^{###}	4.50 ± 1.29 ^{###, 0}	12.25 ± 1.71 ^{###}
Volume density of islets (%)	78.50 ± 11.47	16.50 ± 4.12 ^{###}	29.00 ± 7.75 ^{###}	9.00 ± 2.58 ^{###, 0}	24.50 ± 3.42 ^{###}
No. of β -cells/10 islets	318.00 ± 46.57	27.50 ± 2.65 ^{##}	39.50 ± 4.20 ^{##, *}	24.50 ± 1.29 ^{##, 0 0}	34.25 ± 2.63 ^{##}
No. of α -cells/10 islets	81.00 ± 11.43	65.25 ± 7.72	75.75 ± 4.43	66.50 ± 1.73	76.50 ± 5.51
volume density of β -cells (%)	79.62 ± 2.15	29.74 ± 2.72 ^{###}	34.25 ± 2.78 ^{###}	26.92 ± 0.93 ^{###}	30.98 ± 2.93 ^{###}
size of islets (μm^2)	27,688.82 ± 3,240.23	15,351.41 ± 2,559.37 ^{###}	19,754.31 ± 2,093.52 ^{##}	9,129.35 ± 587.17 ^{###, *, 0}	15,448.80 ± 3,926.47 ^{###}

Data are expressed as the mean ± SD (n=4). * $P \leq 0.05$ vs. DC group, ^{##} $P \leq 0.01$, ^{###} $P \leq 0.001$ vs. NC group, ⁰ $P \leq 0.05$ and ⁰⁰ $P \leq 0.01$ vs. DK400. NC, nondiabetic control group; DC, diabetic control group; DI, insulin-treated group; DK200, low dose khat-treated group; DK400, high dose khat-treated group.

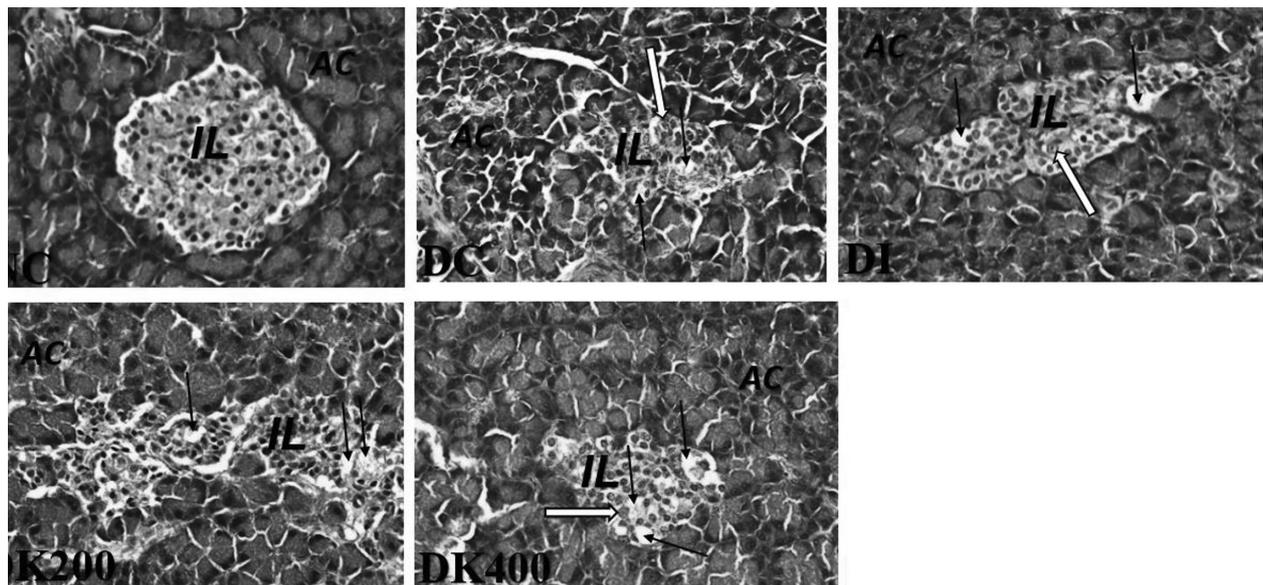


Fig. 4. Pancreatic histological sections of treated and untreated rats. Black arrows point to vacuolated areas. White arrows point to dark, granulated, centrally-located nuclei of β -cells, AC, Acinar cells; IL, islet of Langerhans; NC, nondiabetic control; DC, diabetic control; DI, insulin-treated group; DK200, low dose khat-treated group; DK400, high dose khat-treated group. H&E stain; 40 \times .

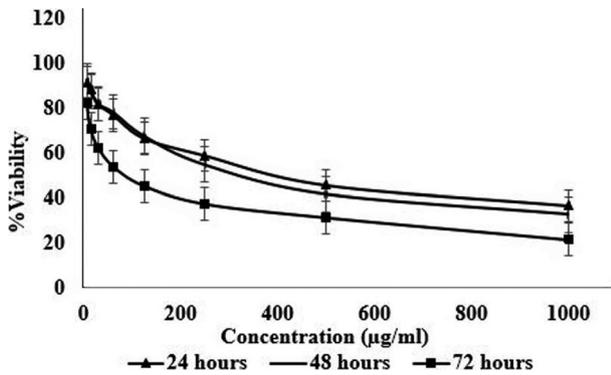


Fig. 5. Percentage of cell viability of RIN-14β cells.

after 72 h ($88.12 \pm 27.59 \mu\text{g/ml}$) was significantly ($P \leq 0.001$) lower than those after 24 h ($417.43 \pm 44.56 \mu\text{g/ml}$) and 48 h ($345.49 \pm 13.44 \mu\text{g/ml}$), while the IC_{50} values after 24 and 48 h were not significantly different. Khat exhibited moderate cytotoxicity at 1,000, 500, and 250 $\mu\text{g/ml}$ and mild toxicity at 125, 62.5, and 15.652 $\mu\text{g/ml}$ after 24 and 48 h of treatment. Conversely, khat exhibited severe cytotoxicity at 1,000 $\mu\text{g/ml}$, moderate cytotoxicity at 500, 125, and 62.5 $\mu\text{g/ml}$, and mild toxicity at 31.25, 15.625, and 7.8125 $\mu\text{g/ml}$ after 72 h of treatment (Fig. 5).

WRL 68 cells (hepatocytes): Results showed that the IC_{50} values of khat after 24 and 48 h of treatment were more than 1,000 $\mu\text{g/ml}$, whereas the IC_{50} value of khat after 72 h of treatment was $642.40 \pm 40.32 \mu\text{g/ml}$. Based upon the percentage of cell viability, khat exhibited a mild cytotoxicity at 1,000, 500, 250, and 125 $\mu\text{g/ml}$ after 24 h of exposure. After 48 h, khat exhibited a moderate cytotoxicity at 1,000 $\mu\text{g/ml}$ and mild cytotoxicity at 500, 125, and 62.5 $\mu\text{g/ml}$. After 72 h, khat exhibited moderate cytotoxicity at 1,000 and 500 $\mu\text{g/ml}$ and a mild cytotoxicity at 250, 125, and 62.5 $\mu\text{g/ml}$ (Fig. 6).

Discussion

The glycemic activity of khat in a type 1 diabetes-induced animal model has not yet been elucidated. Sprague-Dawley rats are widely used to investigate the *in vivo* glycemic activity of medicinal plants [24] and are more susceptible to development of T1DM with a single dose of STZ (75 mg/kg) [18]. STZ selectivity destroys pancreatic β -cells, which leads to induction of T1DM, as demonstrated in the insulin-deficient animal model [18, 24]. In our experiment, the STZ-injected rats developed FBS levels of $21.40 \pm 2.25 \text{ mmol/l}$ or more,

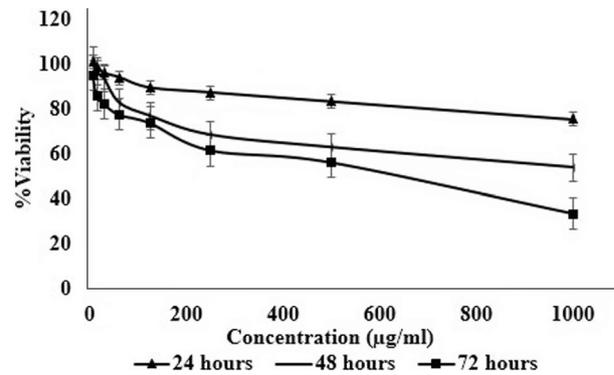


Fig. 6. Percentage of cell viability of WRL 68 cells.

and this is more than the suggested lower limit of FBS (13.9–33.3 mmol/l) for rats to be enrolled in T1DM studies [24, 25].

Obviously, high and low doses of khat were associated with a nonsignificant difference in BW gain, caloric intake, FBS, and total proteins. However, the low dose of khat was associated with a decline in BW from one week to another, which might be due to the catabolic effects of the consistent uncontrolled hyperglycemia of T1DM [32]. Accordingly, neither high nor low doses of khat could produce a significant reduction in the FBS levels of T1DM-induced rats [11]. Insulin-treated rats showed an increase in body weight, reduced hyperglycemia, and significantly higher total protein because insulin has anabolic [32] and euglycemic actions [10]. On the other hand, the very low level of serum insulin in the diabetic control rats and rats treated with the high and low doses of khat emphasized that the rats were insulin deficient due to the destruction of pancreatic β -cells by the administration of STZ [18, 24], while insulin-treated rats showed a significantly higher insulin level, which was due to daily injection of those rats with exogenous insulin (NovoMix 30 FlexPen). On the other hand, the significant increase in amylase with the slight increase in insulin in the high dose khat-treated rats could be due to the interrelation between the secretion of insulin and amylase [35]; such findings could also indicate that high dose khat-treated rats were able to preserve some β -cells, which might have been because the high dose of khat provides some antioxidant activity because of its higher content of phenolic compounds [7, 12, 20, 41]. On the other hand, the low-density lipoprotein levels of the rats treated with the high and low doses of khat were significantly lower than that of the diabetic control, which could indicate that the considerable antioxidant

activity of khat [41] could have ameliorated lipid peroxidation [37].

Morphometric evaluation indicated that nondiabetic control rats showed a significantly higher number of islets, volume density of islets, size of islets, number of β -cell, and volume density of β -cells than diabetic rats in the DC, DI, DK200, and DK400 groups, which could be due to the destructive effect of STZ [18, 24], taking into consideration that the numbers of α -cells in nondiabetic and diabetic rats were not significantly different, which could be due to the selective toxicity of STZ in β -cells rather than α -cells [36]. However, the obviously higher number, volume density, and size of islets, as well as number of β -cells, in DK400-treated diabetic rats compared with DK200-treated diabetic rats could indicate a sign of protection [28]; particularly, our findings indicated that high dose khat-treated rats showed a relatively higher insulin level than low dose khat-treated rats. On the other hand, it was noticed that the antioxidant activity of khat is dose dependent so the higher the concentration of khat is, the higher the scavenging activity is against the free radicals of DPPH [7]. Perhaps the persistent uncontrolled hyperglycemia after induction of diabetes mellitus resulted in glucotoxicity that resulted in further destruction of β -cells in the DK200 group [42]; the high dose of khat might provide some antioxidant activity that protected the remaining β -cells against glucotoxicity, as it contains higher amounts of phenolic compounds than at a low dose [7, 12, 20, 41].

The histomorphological abnormalities in the pancreatic sections showed a destruction of the pancreatic architecture of the islets of Langerhans and even acinar cells, which could be relevant to the destructive effect of STZ [40], excluding the sections from of insulin-treated rats which, showed less destructive changes. However, the abnormalities in the structures of the islets of Langerhans of rats treated with the high and low doses of khat were greater than in the diabetic control and insulin-treated rats, which could support the earlier suggestion of the destructive effect of khat on pancreatic β -cells [21] as well as our results *in vitro* showing dose- and time-dependent cytotoxic effects of khat on pancreatic β -cells. However, the histopathological change in the pancreas of the DK400 group were less severe than those in the DK200 and DC groups and might be due to the higher contents of phenolic compounds than at the low dose [7, 8, 20, 41].

Previous studies have attributed the destructive effect

of khat on pancreatic β -cells to the pesticides that are sprayed on the khat trees to improve the harvest [21], which could be misunderstood to mean that khat itself is safe for the pancreas. Conversely, our findings concerning *in vitro* exposure of pancreatic β -cells to khat indicated dose- and time-dependent cytotoxicity. On the other hand, the toxicity of khat has been established in several body organs, particularly the liver as evidenced by the increase in liver enzymes or abnormalities in histological sections of the liver [2, 4, 6, 8, 17]. Accordingly, to further support the *in vitro* findings indicating the cytotoxicity of khat in pancreatic β -cells, human hepatocytes cells were treated with similar concentrations of khat. However, the results revealed only time-dependent evidence indicating that pancreatic β -cells are more susceptible to the toxic effect of khat as compared with hepatocytes cells. These findings emphasized the earlier suggestion regarding the destructive effect of khat on pancreatic β -cells of chronic chewers [21].

Finally, the *in vivo* finding of the absence of hypoglycemic activity of khat in T1DM-induced rats refutes the claim that khat could reduce elevated blood sugar levels in T1DM patients. On the other hand, the *in vitro* cytotoxicity of khat in pancreatic β -cells could support our earlier systematic review finding that chronic khat chewing might be a risk factor in the development of DM [9]. Nonetheless, further investigations are required to elucidate the expression level of glucagon and insulin to confirm the morphometric quantitative evaluation of α - and β -cell using immunohistochemistry. In addition, investigation of the *in vitro* cytotoxicity of khat in human pancreatic cell lines should be carried out to prove if the findings are similar to those in rat pancreatic cell lines.

Conclusion

Khat can exert a direct cytotoxic effect on pancreatic β -cells. Neither a high nor low dose of khat could produce a hypoglycemic effect in T1DM-induced rats. However, the low dose of khat contributed to exacerbation of hyperglycemia by provoking further destruction of pancreatic β -cells, which emphasized the direct *in vitro* cytotoxicity of khat in pancreatic β -cells. Accordingly, the suggested association between chewing khat and the possibility of development DM is evident, and further investigation is needed to elucidate how khat acts to cause destruction of pancreatic β -cells.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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