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Captopril pretreatment augments diabetogenic response to streptozotocin administration: experimental in vivo rat model



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Abstract

Background Streptozotocin (STZ) is a glucose analogue commonly used for inducing diabetes in experimental animals. This study is intended to investigate the ability of captopril (Cap) pretreatment to augment STZ-induced diabetogenic effect in an experimental rat model. If this hypothesis were proven, Cap administration to rats could reduce the dosage of STZ by augmenting its effect and resulting in a subsequent reduction in STZ cost. Forty-two adult male Wistar rats were randomly divided into seven groups: a control group that fed a normal diet, whereas the other six experimental groups were fed a high-fat diet (HFD). The six groups were then divided into STZ-30, STZ-30-Cap, STZ-40, STZ-40-Cap, STZ-50, and STZ-50-Cap. All Cap-received groups were supplemented with 50 mg/kg Cap orally one hour just before intraperitoneal (I.P.) injection of STZ. 30-STZ, 40-STZ, and 50-STZ-treated groups were injected once with STZ I.P. at doses of 30, 40, and 50 mg/kg, respectively. An intraperitoneal glucose tolerance test (IPGTT) was done. Pancreatic tissue was obtained to measure Tumor necrosis factor alpha (TNF- α), interleukin one beta (IL-1 β), and nitric oxide (NO) by enzyme-linked immunosorbent assay (ELISA) and glucose transporter 2 (GLUT2) gene expression by reverse transcription polymerase chain reaction (RT-PCR). Pancreatic sections were examined by hematoxylin and eosin (H&E) stain, and immunohistochemical staining by anti-insulin and anti-TNF- α antibodies.

Results Results indicated that administration of Cap before STZ in different doses significantly augmented the hyperglycemic state that was evident by intraperitoneal glucose tolerance test, and markedly increased pancreatic proinflammatory markers. Histological analysis of islets of Langerhans indicated degeneration with extensive vacuolations associated with a significant decrease in mean area % of insulin immunoreactivity and an increase in optical density of TNF-α immunoreactivity.

Conclusion These findings pointed to the ability of captopril pretreatment to augment the hyperglycemic state and the diabetogenic response that was induced secondary to STZ injection in an experimental rat model.

Keywords Captopril, Streptozotocin, Diabetes, Experimental rats, Vascular mechanism, Biochemical markers, Immunohistochemistry

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Introduction

The animal models played a crucial role in studying how disease pathogenesis develops and evaluating novel therapeutic agents. Diabetes mellitus (DM) was one of the disorders that necessitated ongoing creative therapy approaches [1]. DM refers to a set of metabolic illnesses that result in high blood glucose levels and a state of chronic hyperglycemia. In this context, the two most prevalent types of diabetes mellitus are type-1 diabetes and type-2 [2].

Type-1 diabetes is classified as an autoimmune disease due to the attack and destruction of insulin-producing pancreatic β -cells [3], while type-2 diabetes is accompanied by insulin resistance and insufficient insulin secretion due to a lack of appropriate compensation by the beta-cells. Consequently, the selection of appropriate animal models for diabetes research should be contingent upon the specific aspects of the disease under investigation [4].

To induce DM. in animal models, the traditional toxic chemicals that selectively target pancreatic β -cells have been used by many researchers. For instance, alloxan has been utilized to induce β -cell destruction through the production of free radicals. However, alloxan demonstrated several prominent drawbacks, including high animal mortality, the generation of animal ketosis due to the formation of free fatty acids, reversible diabetic states, species-specific resistance to its diabetogenic effects as in guinea pigs, and renal toxicity within a narrow effective dose range [5]. As a result, streptozotocin (STZ) has emerged as a substitute for alloxan due to its more favorable characteristics for diabetes induction [6]. STZ can be used to induce animal models of both type-1 and type-2 diabetes by applying either high or low doses [7]. High doses of STZ can severely impair insulin release resembling what happened in type-1 diabetes and can also lead to the formation of ketone bodies, a feature of uncontrolled type-1 diabetes. In contrast, low doses of STZ can cause mild impairment in insulin secretion that mimics type-2 diabetes. However, the low-dose STZinduced animal model does not address insulin resistance which is in general a feature of type-2 diabetes [8].

A model, that combines a high-fat diet (HFD) followed by a low STZ dose to induce peripheral insulin resistance, would closely resemble human type-2 diabetes [9]. Although there are several experimental models of DM reported to date, the HFD-STZ-type-2 diabetes model is still considered the preferable one [10, 11]. The STZ dose-dependent direct and indirect toxicity is still the main significant drawback. Studies reported that some albino rats developed stomach ulcers, decreased bone volume and muscle mass, reproductive problems, hepatotoxicity, and nephrotoxicity at a dose of 65 mg/kg and 100% mortality in Wistar rats due to the lethal end-point at a dose \geq 70 mg/kg [11].

Even while it appears to be easy to induce diabetes in many different animal species with STZ, it is difficult because of factors like unpredictability, high cost, and high mortality rates. Based on their particular experiences, the investigators employ different techniques when implementing this paradigm. The STZ dosage, the way it is administered, the animal species, the gender, and the body weight are all subject to variation. The investigators have made numerous attempts aiming to create more stable and consistent hyperglycemia [12].

The local renin–angiotensin system (RAS) has been discovered to play a crucial role in pancreatic physiology and is involved in pancreatic function. Angiotensin II receptors include two types 1 and 2 (AT1R and AT2R), they have been demonstrated to be present in pancreatic islets, whereas the function of AT2R is still controversial and not fully understood [13, 14].

Recent evidence revealed that RAS could regulate the endocrine and exocrine function of the pancreas and short-term Angiotensin II (Ag II) infusion changes the intra-islet blood flow while the chronic exposure effects are still unknown [15]. Another study found that Ang II has a significant vasoconstrictive effect on islet vasculature and reduces islet blood flow. In contrast, the angiotensin receptor blockers vasodilate islet arterioles and stimulates insulin secretion in response to high glucose levels [16, 17].

Captopril (Cap), an angiotensin-converting enzyme (ACE) inhibitor medication, is used in controlling hypertension, management of left ventricular dysfunction after myocardial infarction, and diabetic nephropathy. Many researchers discovered its role in increased glucose uptake by pancreatic tissues, the exact mechanism is unclear, but a vasodilation effect may be incorporated [18].

This study aimed to investigate the possibility of developing a modified experimental model of diabetes mellitus by estimating the adding effect of captopril on different low doses of STZ in HFD adult Wistar rats. The authors compared the changes in serum glucose levels in response to the intraperitoneal glucose tolerance test (IPGTT), and local pro-inflammatory markers in pancreatic tissues secondary to injecting different doses of STZ that were given either alone or in combination with captopril for inducing animal model diabetes.

Material and methods

Experimental animal & grouping

Forty-two adult male Wistar rats (8–10 weeks in age and 180–200 g weight) were purchased from and housed in the Animal House of the Faculty of Medicine, Cairo

University. Animals were left for seven days to acclimate to ordinary environmental living conditions regarding humidity, temperature, and dark/light cycles. Rats had free access to food and water to ensure normal growth and behavior; they were kept in wire mesh cages (three rats in each) before starting the experimental procedures. All animal procedures were done in accordance with the highest International Criteria for Animal Experimentation of Helsinki. The Faculty of Medicine, Cairo University Ethical Approval No is; CU/III/F/38/22.

Drugs and chemicals

STZ was purchased from Sigma-Aldrich, MO, USA, and was given at doses of (30, 40 &50 mg/kg). STZ was dissolved in freshly prepared 0.1 M citrate buffer (pH 4.5; Talpate, Bhosale, Zambare, &Somani). Captopril purchased from Sigma-Aldrich, MO, USA, as a powder, was dissolved in sterile distilled water and was given at 50 mg/kg orally. All other chemicals and reagents were of high analytical grade.

Investigation protocols

Rats were randomly divided into seven groups (six rats each), Group I; control normal rats (fed a normal rat chow (12% fat, 60% carbohydrate, and 28% protein), and the other six main experimental groups were maintained on a HFD; 41% fat, 41% carbohydrate, and 18% protein) for 2 weeks. Then, the overnight-fasted rats in the experimental groups were injected with STZ. The rats were allocated to: Group II (STZ-30); the rats in this group received STZ at a dose of 30 mg/kg (I.P.) [19], Group III (STZ-30-Cap); the rats were subjected to the intake of captopril (50 mg/kg orally) [20, 21] one hour just before injecting STZ at a dose of 30 mg/kg, group IV (STZ-40); the rats in this group were subjected to STZ injection at a dose of 40 mg/kg I.P.), group V (STZ-40-Cap): the rats were subjected to oral administration of captopril at the same of group III one hour just before injecting 40 mg/ kg of STZ, group VI (STZ-50); the rats were subjected to STZ injection at a dose of 50 mg/kg (I.P.), and group VII (STZ-50-Cap); the rats were subjected to oral administration of captopril (at the same of group III, and V one hour just before injecting 50 mg/kg of STZ.

At the end of the study, after 28 days (one week allowed for acclimatization of the experimental rats, followed by a two-week span during which the rats were maintained on a HFD). The final week was dedicated to the implementation of the treatment protocol and served as the end-point of the study, the blood pressure of each rat was measured using the tail-cuff method. The animals were fasted overnight for about 12 h, and then the blood samples were obtained from the femoral vein under ketamine/xylazine (60/7.5 mg/kg, i.p) [22] anesthesia using non-heparinized capillary tubes for separation of sera. Samples were kept immediately in ice-chilled, siliconized disposable glass tubes. The serum samples were obtained by centrifuging blood samples at 4,000 g for 15 min at 4 °C and kept at - 80 °C until further analysis. After that, the rats were sacrificed by cervical dislocation under anesthesia, and the pancreatic tissues were excised and fixed in a 10% buffered formalin solution for histopathologic analysis. More treatment protocol clarification is shown in Fig. 1.

Noninvasive blood pressure measurement

The noninvasive rat tail systolic arterial blood pressure was recorded at the end of the study using a noninvasive blood pressure meter (LE 5002, Harvard Apparatus). The rat is placed in a restrainer and warmed. A pneumatic pulse sensor is attached to its tail. A cuff is placed around the tail, and slowly inflated above the systolic pressure until it causes pulsations to cease, measured by the piezoelectric pulse sensor. The cuff pressure at which pulsations cease is taken to be the SBP in the tail.

Verification of diabetes

Intraperitoneal glucose tolerance test (IPGTT)

All rats were subjected to IPGTT [23, 24]. Male rats were fasted overnight and injected with glucose at a dose of 2 g/kg (I.P.). Blood glucose levels were measured at times of fasting (time zero), 30, 60, and 120 min with a glucometer (GlucoDrTM, All Medicus Co. Ltd, Gyeonggi, Korea) [25].

Biochemical measurement

TNF-α in pancreatic tissue by ELISA

TNF- α in pancreatic tissue protein was assessed by ELISA technique according to kit instructions; Rat TNF- α ELISA Kit, Catalog No: MBS2507393. MyBio-Source, Inc. San Diego, CA 92195–3308, USA.

IL-1 β in pancreatic tissue by ELISA

IL-1 β in pancreatic tissue protein was assessed by ELISA technique according to kit instructions; Rat IL-1 β ELISA Kit. Catalog No: MBS824956.MyBioSource, Inc. San Diego, CA 92195–3308.

NO level in pancreatic tissue

NO level in pancreatic tissue was assessed according to kit instruction; Nitric Oxide Assay Kit (Colorimetric). Catalog No: ab65328, Abcam, Waltham, USA.

GLTU2 gene expression in pancreatic tissue by RT-PCR

The pancreatic tissues from all studied groups were homogenized and total RNA was isolated with Gene JET Kit (Thermo Fisher Scientific Inc., Germany,



Fig. 1 Schematic illustration of the timeline of scheduled interventions for all examined groups. STZ Streptozotocin, Cap Captopril

#K0732). A one-step qRT-PCR reaction was done; for reverse transcription; about 5 μ l from the total RNA from each sample (once for the GLUT2 gene and once for ß actin gene) was used with subsequent amplification with Bioline, A median Life Science company, UK (SensiFASTTM SYBR R Hi-ROX) One-step Kit (catalog number PI-50217 V) in a 48-well plate using the Stepone instrument (Applied Biosystems, USA). Thermal profile was as follows: 45 °C for 15 min in one cycle (for cDNA synthesis), 10 min at 95 °C for reverse transcriptase enzyme inactivation, followed by 40 cycles of PCR amplification. 10 s at 95 °C, 30 s at 60 °C, and 30 s at 72 °C were adjusted for each cycle.

RNA quantitation assessment was carried out using a spectrophotometer the Nano $\text{Drop}^{(0)}$ (ND)-1000 (Nano Drop Technologies, Inc.) by measuring the absorbance at 260 nm.

RNA purity was assessed by comparing the absorbance ratio using a spectrophotometer the Nano Drop[®] (ND)-1000 (Nano Drop Technologies, Inc.) as we measured the absorbance at 280 to exclude contamination with protein and reagent that absorbed near 280, the 260/280 ratio was 2, also the absorbance at 230 was measured to exclude contamination with another compound as carbohydrate and phenol that absorb near 230, the 260/230 ratio was 2.1

From the expression of the studied genes, genes were normalized relative to the mean critical threshold (CT) values of β actin as the housekeeping gene by the Ct method. Primers' sequences for studied genes are listed in Table 1.

Pancreatic tissues histological study

The Pancreas was dissected, fixed in 10% formaldehyde solution, and processed into paraffin blocks. Five-micron sections were cut and mounted on glass slides for hematoxylin and eosin (H&E) staining to examine structural changes in islets of Langerhans in the pancreas.

Immunohistochemical staining of the pancreatic tissues

Pancreatic sections were de-paraffinized, hydrated with descending grades of alcohol, and treated with citrate buffer pH 6 in the microwave for antigen retrieval, then incubated overnight with: 1-anti-insulin antibody (ab63820; rabbit polyclonal antibody, dilution (1:100), Abcam, Cambridge, UK). 2-anti-TNF- α receptor (ab220210; mouse monoclonal antibody, dilution (1:100), Abcam, Cambridge, UK). Two drops of biotinylated secondary antibody were applied for 20 min and then two drops of streptavidin peroxidase for 10 min. The reaction was visualized with diaminobenzidine (DAB) as a

Гal	ble	e '	1	Primer	sequence	for a	a studied	l gene
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Gene	Primer sequence from 5'-3'		
GLTU2	Forward: GCACACTCTTGGCCCGG Reverse: GCCTGGGAAGAAGAAGACT		
ßactin	Forward: GCA CCA CAC CTT CTA CAA TG Reverse: TGC TTG CTG ATC CAC ATC TG		

chromogen and Mayer's hematoxylin as a counter-stain. The primary antibodies were replaced in negative control serial sections with PBS.

Morphometric assessment

The "Leica Qwin 500 C" image analyzer computer system Ltd. (Cambridge, UK) was used to collect the data. The image analyzer was made up of an Olympus color video camera, a colored monitor, and an IBM hard drive that was attached to a light microscope and ran "Leica Qwin 500 C" software. To automatically translate the measurement units (pixels) generated by the image analyzer program into actual micrometer units, the image analyzer was first calibrated.

The mean diameter of the islet of Langerhans, area % of anti-insulin immunoreactivity, and optical density of TNF- α immunoreactivity were assessed in 10 non-overlapping randomly selected high-power fields (×400).

Statistical analysis

The results were obtained as mean ± standard deviation (SD). Statistical analysis for parametric measures was done via one-way analysis of variance (ANOVA) version 25, followed by the Tukey–Kramer multiple comparisons test as a post hoc test. A comparison of glucose levels in each group with time was done using repeated measure ANOVA. Statistical significance was considered when P < 0.05. Statistical analysis will be carried out using IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, N.Y., USA).

Results

The intraperitoneal glucose tolerance test (IPGTT) and the systolic and diastolic blood pressure result in all studied groups

The blood pressure was recorded both before and after the administration of STZ and Cap to confirm any changes in blood pressure. The findings showed that there were no significant differences in the reading among all studied groups.

As shown in Table 2, all treated groups showed a significant increase in fasting, and estimated blood glucose was measured after 30, 60, and 120 min following I.P. injection of glucose compared to the control group. Moreover, receiving captopril before injecting STZ at 30 mg/kg and 40 mg/kg was able to significantly increase the levels of blood glucose measured at different times compared to STZ-40 and STZ-50, respectively. Additionally, the maximum results of estimated blood glucose were obtained STZ-50-Cap group at different times compared to other groups.

Captopril administration was able to enhance the expression of GLUT2 and increased nitric oxide levels in pancreatic tissues in the studied groups

The expression levels of GLUT2 were estimated in all studied groups and the pre-intake of captopril before the administration of STZ was able to enhance its expression levels that were documented by PCR. Our data documented enhanced expression levels in the STZ-30-Cap compared to STZ-30, and a significant increase in the same parameter in the STZ-40-Cap compared to its corresponding values in the STZ-40 group, additionally significant increase in GLUT2 expression was recorded in the STZ-50-Cap compared to its corresponding values in the STZ-50 group, without recording any significant difference between STZ-30-Cap, STZ-40-Cap, STZ-50-Cap groups (Table 3). Additionally, the levels of nitric oxide were significantly increased in all STZ-treated groups compared to the control group, and captopril administration before STZ injection was able to significantly increase the levels of NO in these groups compared to the corresponding values of the STZ-treated groups (Table 3).

Table 2 Timeline changes in fasting blood sugar, estimated blood glucose 30 min, 60 min, and 120 min (mg/dl) after IPGTT in all studied groups

	Control	STZ-30	STZ-30-Cap	STZ-40	STZ-40-Cap	STZ-50	STZ-50-Cap
Fasting	88.17±1.17	198.17±7.28*	355.5±6.35*#	222.5±10.39*\$	380.5±3.94*#€	345±48.2*#€¥	490±10.49*#\$€¥@
30 min	144.5 ± 10.56	239.83±9.26*	436.33±22.43*#	259.17±9.35*\$	498.83±15.99*#\$€	414±28.48*#€¥	487.67±4.97*#\$€¥@
60 min	172±3.79	231.5±6.5*	466.67±4.46*#	246.5±3.21*\$	523.83±8.84*#\$€	421.33±25.15*#\$€¥	481.17±9*#€¥@
120 min	116.83±2.64	214.5±4.14*	387.5±82.13*#	227.17±8.18*\$	490±5.37*#\$€	385.5±20.27*#€¥	506.5±1.38*#\$€@

Data presented by mean \pm SD No = 6

Statistical analysis was done using one-way ANOVA test followed by Tukey-Kramer post hoc test

*denotes significant difference compared to control, # denotes significant difference compared to STZ-30, \$ denotes significant difference compared to STZ-30-Cap, € denotes significant difference compared to STZ-40, ¥ denotes significant difference compared to STZ-40-Cap, and @ denotes significant difference compared to STZ-50

IPGTT Intraperitoneal glucose tolerance test, STZ Streptozotocin, Cap Captopril

	Control	STZ-30	STZ-30-Cap	STZ-40	STZ-40-Cap	STZ-50	STZ-50-Cap
NO nmol/ml	10.4±0.21	29.88±6.28*	59.38±5.73*#	30.45±7.6*\$	58.03±4.34*#€	33.33±2.98*\$¥	61.62±2.4*#€@
GLUT2 relative expression	1.02 ± 0.18	1.07 ± 0.08	1.73±0.12*#	1.02±0.03\$	1.77±0.08*#€	1.01±0.1\$¥	1.73±0.12*#€@

Table 3 The levels of NO (nmol/ml) and the expression levels of GLUT2 in all studied groups

Data presented by mean \pm SD No = 6

Statistical analysis was done using one-way ANOVA test followed by Tukey-Kramer post hoc test

*denotes significant difference compared to control, # denotes significant difference compared to STZ-30, \$ denotes significant difference compared to STZ-30-Cap, € denotes significant difference compared to STZ-40, ¥ denotes significant difference compared to STZ-40-Cap, and @ denotes significant difference compared to STZ-50

NO Nitric oxide, GLUT2 Glucose transporter 2, STZ Streptozotocin, Cap Captopril

Augmenting pro-inflammatory state secondary to captopril pre-intake with an increased dose of STZ

As shown in Fig. 2, the levels of pro-inflammatory cytokines (TNF- α and IL-1 β) were significantly increased in all STZ and STZ-Cap-treated groups compared to the corresponding results of the control group that denotes the effectiveness of STZ to produce the pro-inflammatory condition. Moreover, the captopril intake before STZ administration was able to augment the production of both TNF- α and IL-1 β in STZ-30-Cap compared to STZ-30, and STZ-40-Cap compared to STZ-40 and STZ-50-Cap compared to SZT-50.

Effect of STZ and captopril pre-intake on pancreatic tissue degeneration

As shown in Fig. 3, the histological examination using H&E staining of the pancreatic tissues demonstrated that the control group showed normal architecture of the pancreas with the islet of Langerhans surrounded by pancreatic acini. The experimental groups, STZ-30;

STZ-30-Cap; and STZ-40, exhibited a degenerated islet of Langerhans. However, STZ-40-Cap; STZ-50; and STZ-50-Cap groups revealed massive degeneration of islets of Langerhans with large areas of vacuolations and numerous depleted β -cells.

Immunohistochemical assessment of the pancreas stained with anti-insulin

As shown in Fig. 4; the control group revealed strong widespread insulin immunostaining in the islet of Langerhans. All STZ-treated groups exhibited a significant decrease in the mean area % of insulin immunoreactivity in comparison with the control group. β -cells in experimental groups, STZ-30; STZ-30-Cap; and STZ-40, demonstrated moderate heterogeneous insulin immunostaining. STZ-40-Cap group showed weak insulin immunostaining. Moreover, STZ-50 and STZ-50-Cap groups exhibited faint heterogeneous insulin immunostaining in β -cells, revealing a significant decrease in mean area % of insulin immunoreactivity







Fig. 3 Representative histological photomicrographs of H&E-stained rat pancreatic Sects. (400x). **A** The control group shows a pale-stained islet of Langerhans surrounded by a darkly stained closely packed pancreatic acini. The acini are formed of pyramidal cells displaying apical acidophilia and basal basophilia with vesicular nuclei and prominent nucleoli. **B** & **C** Groups STZ-30 and STZ-30-Cap illustrate degenerated islets of Langerhans with vacuolations (arrows) and a few depleted β -cells that display darkly stained nuclei (arrow heads). **D** & **E** Group STZ-40 illustrates a degenerated islet of Langerhans with few vacuolations (arrows) and few depleted β -cells that display darkly stained nuclei (arrow heads). **D** & **E** Group STZ-40 illustrates a degenerated islet of Langerhans with few vacuolations (arrows) and few depleted β -cells that display darkly stained nuclei (arrow heads). Group STZ-40-Cap exhibits more vacuolations (arrows) in islets of Langerhans and more depleted B-cells with darkly stained nuclei (arrow heads). **F** & **G**. Group STZ-50 displays marked degeneration of islets of Langerhans with vacuolations (arrows) and many depleted β -cells. Group STZ-50-Cap shows massive degeneration with vacuolations (arrows) and most of the cells are depleted (arrow heads). **H**. Histogram representing mean area % Islet of Langerhans size; *denotes significant difference compared to control, # denotes significant difference compared to STZ-30, \$ denotes significant difference compared to STZ-40-Cap, and @ denotes significant difference compared to STZ-40-Cap.



Fig. 4 Representative photomicrographs of anti-insulin immunohistochemistry-stained rat pancreatic Sects. (400x). **A** Control group shows a strong homogeneous distribution of insulin immunostaining in β cells. **B** & **C** Groups STZ-30 and STZ-30-Cap illustrate moderate heterogeneous insulin immunostaining in β cells. **D** STZ-40 group exhibits moderate insulin immunostaining in β cells. **E** Group STZ-40-Cap demonstrates weak insulin immunostaining of β cells. **F** & **G** STZ-50 and STZ-50-Cap exhibit faint heterogeneous insulin immunostaining in β cells. **H** Histogram representing mean area % anti-insulin immunostained sections; * denotes significant difference compared to control, # denotes significant difference compared to STZ-40, ¥ denotes significant difference compared to STZ-40, ¥ denotes significant difference compared to STZ-50. *STZ* Streptozotocin, *Cap* Captopril

when compared to groups STZ-30; STZ-30-Cap; and STZ-40. Furthermore, the STZ-50-Cap group demonstrated a significant decrease in mean area % in comparison with all experimental groups. A significant difference was recorded between STZ-30 and STZ-30-Cap groups as well as between STZ-40 and STZ-40-Cap groups in addition between STZ-50 and STZ-50-Cap groups.

Immunohistochemical assessment of the pancreas stained with anti-TNF- $\!\alpha$

As shown in Fig. 5, β -cells of the control group exhibited negative TNF- α immunostaining. The optical density of TNF- α immunostaining in all experimental groups revealed a significant increase when compared to the control group. β cells of STZ-30; STZ-30-Cap; and STZ-40 groups revealed moderate TNF- α immunostaining. In addition, groups STZ-40-Cap; STZ-50; and STZ-50-Cap illustrated strong TNF-α immunostaining of β cells. STZ-40 and STZ-40-Cap groups revealed a significant increase in the optical density of TNF- α immunostaining when compared to STZ-30 and STZ-30-Cap groups. Moreover, STZ-50 and STZ-50-Cap groups demonstrated a significant increase of optical density of TNF- α immunostaining in comparison with all experimental groups. A significant difference was recorded between STZ-30 and STZ-30-Cap groups as well as between STZ-40 and STZ-40-Cap groups in addition between STZ-50, and STZ-50-Cap groups.

Mean area % of insulin antibody immunoreactivity result

In addition, STZ-30-Cap, STZ-40-Cap, and STZ-50-Cap revealed a significant decrease when compared to group STZ-30, STZ-40, and STZ-50, respectively (Fig. 4).

Optical density of TNF-a antibody immunoreactivity result

The optical density of TNF- α immunoreactivity in all experimental groups revealed a significant increase when compared to the control group. Groups STZ-30-Cap, STZ-40-Cap, and STZ-50-Cap exhibited a significant increase in comparison with groups STZ-30, STZ-40, and STZ-50, respectively (Fig. 5).

Discussion

To our knowledge, this study is the first to investigate the possibility of developing a modified experimental model of DM by estimating the adding effect of captopril on different low doses of STZ in HFD adult Wistar rats. Our goal was to reduce the high doses of STZ and to reduce the cost of the experimental protocols.

The components of RAS have been proven to be expressed locally in rodent and human pancreatic islets a long time ago. Twenty years ago, researchers [26] discovered the intrinsic angiotensin system in dog exocrine pancreas, and numerous data support the idea of an intrinsic RAS in the rat pancreas indicating its role in pancreatic function regulation and insulin secretion [27].

In this study, we tried to explore a new modified experimental model for the induction of DM in which we investigated the effect of the addition of ACE inhibitor, captopril, to different doses (30, 40, & 50 mg/kg) of STZ aiming to augment the diabetic response caused by low doses of STZ by combining it with captopril. This



Fig. 5 Representative photomicrographs of anti-TNF- α immunohistochemistry-stained rat pancreatic Sects. (400x). **A** The control group shows negative immunostaining of β cells with TNF- α . **B** & **C** The groups STZ-30 and STZ-30-Cap exhibit moderate TNF- α immunostaining in β cells. **D** Group STZ-40 exhibits moderate TNF- α immunostaining of β cells. **F** STZ-50 illustrates strong TNF- α immunostaining of β cells. **G** STZ-50-Cap exhibits intensive immunostaining of β cells. **F** STZ-50 illustrates strong TNF- α immunostaining of β cells. **G** STZ-50-Cap exhibits intensive immunostaining of β cells with TNF- α . **H** Histogram illustrating optical density of anti-TNF- α immunostained sections. * denotes significant difference compared to control, # denotes significant difference compared to STZ-30, \$ denotes significant difference compared to STZ-30, \$ denotes significant difference compared to STZ-50. *TNF*- α Tumor necrosis alpha, *STZ* Streptozotocin, *Cap* Captopril

approach assists in achieving a more efficient model of diabetes while reducing the hazards associated with giving high doses of STZ; which can sometimes be lethal to the animals.

Our results were consistent with our working hypothesis as the administration of 50 mg/kg of captopril just an hour before the I.P. injection of 30 mg/kg STZ to the HFD rats resulted in a significant increase in both fasting and postprandial glucose levels more than in groups that were injected with STZ alone and all animals were still alive till the end of the experiment. However, there is some mortality at the other two higher doses (40 & 50 mg/kg) in STZ combined with captopril. Captopril is a medication used as an antihypertensive drug that prevents the conversion of angiotensin I to the potent vasoconstrictor angiotensin II [28]. However, its precise mechanism in producing hyperglycemia is not evident but a long time ago, several studies have suggested the relationship between RAS and both pancreatic and islet blood flow.

Studies revealed that islet RAS exhibits a significant role in regulating islet glucose-stimulated insulin release and the locally produced Ang II markedly impair islet blood flow and blocks glucose-induced insulin secretion. RAS antagonists, such as enalaprilat and irbesartan (an AT1 receptor antagonist), can selectively augment pancreatic islet blood flow and improve glucose-stimulated insulin release [29].

A study was done by Olsson et al. [30] to investigate the effect of Ang II on revascularization and local blood flow regulation in transplanted rat pancreatic islets and they concluded that Ang II decreased the vascular conductance of the islet grafts in both nondiabetic and diabetic animals. Carlsson et al. [29] suggested that RAS has marked impacts on the growth, differentiation, secretion, and insulin sensitivity of pancreatic islets, which in turn affect glucose uptake, metabolism, and pathogenesis of DM. Campbell and his colleague [31] 1986 have reported that mRNA encoding angiotensinogen, renin, and Ang II have been discovered in the pancreas of various species. This Ang II can adversely influence pancreatic and islet blood flow through the vasoconstrictive mechanism. A further study was conducted by Lau et al. [32], which used a double immunostaining technique to detect high-affinity binding sites for Ang. Results showed that Ang II was specifically located in islet betacells, and it was found to block glucose-induced insulin release either directly by inhibiting β -cell insulin exocytosis or indirectly through suppressing islet blood perfusion. This effect was fully reversed by losartan indicating a potential role of locally produced pancreatic Ang II in glucose homeostasis. Based on these earlier studies, RAS inhibitors may have a promising role in increasing local pancreatic and islet blood flow which may increase glucose and glucose analog (STZ) uptake through the GLUT2 mechanism. According to these prior studies, the authors of the current study found that the biochemical and both histological and immunohistochemical findings support this theory.

In the present study, the histological examination of pancreatic sections revealed degeneration of islets of Langerhans with β-cell necrosis after STZ administration which is augmented with captopril intake. These results agree with Raza and John [33], stating that administration of STZ at low doses results in β-cell dysfunction and apoptosis while at higher doses leads to β -cell necrosis. Various data explained that STZ induces diabetes in experimental animals through different pathways, The first mechanism involves DNA fragmentation due to the STZ nitrosourea moiety, the second is releasing nitric oxide (NO), the third is creating reactive oxygen species (ROS) and inducing oxidative stress, the fourth mechanism is inhibiting the activity of the glycoside hydrolase O-GlcNAcase enzyme in the β -cell, which is responsible for removing O-GlcNA from proteins. This produces irreversible O-glycosylation of intracellular proteins causing β -cell apoptosis [34].

In addition, STZ contains deoxyglucose molecules that are very reactive and can produce direct cytotoxic damage on pancreatic β -cells. It targets the GLUT2 receptor, which is copious on β -cells plasma membranes making them its primary target [35]. Hence the STZ mechanism begins with GLUT2, a transmembrane carrier protein found in β -cells of the pancreas, the absorptive epithelial cells of the intestinal mucosa, liver, and renal cells [36]. The current results showed that the administration of captopril before STZ exposure resulted in more damage of β -cells, this may be due to enhanced expression of GLUT2 in pancreatic tissues of all groups regardless of the dosage compared to the groups that only received STZ.

Furthermore, this could be attributed to the fact that pancreatic β -cells selectively accumulate STZ via GLUT2 and STZ damage to the β -cells is dependent on GLUT2 expression [37].

The contribution of NO and oxidative stress is a crucial aspect of STZ toxicity [38]. Since β -cells have lower levels of enzymes that scavenge NO and free radicals, they are particularly vulnerable to damage caused by NO and free radicals [39]. In our study, we found that NO levels in pancreatic tissues were increased significantly in all groups that administered STZ. This increase was particularly significant in the STZ-Cap groups, and this may be related to the marked uptake of STZ by captopril. Results were supported by the work done by Eleazu et al. [40] who

explained the mechanism of increased NO as the nitrosamine group found in STZ serves as an intracellular donor of NO in pancreatic β -cells, and the amount of NO released locally in pancreatic tissues increased with a high level of STZ. When released in excessive amounts NO hinders the action DNA enzyme, aconitase, which ultimately leads to alkylation and damage of DNA. Aconitase is an enzyme that safeguards mitochondrial DNA from degradation. This enzyme can be inhibited by various ROS as well as reactive nitrogen species [41].

Selective and progressive destruction of insulinsecreting β -cells secondary to the diabetogenic action of STZ can enhance local pancreatic infiltration with inflammatory cells and cytokines, such as TNF- α , IL-1 β , and interferon- γ (IFN- γ). Furthermore, IFN- γ and TNF- α , either alone or in combination, produce high levels of NO in pancreatic cells [42, 43].

The current results reported a significant increase in pancreatic TNF- α and IL-1 β levels in STZ and STZ-Cap-treated groups which reflect the inflammatory state of the pancreatic tissues. Moreover, the captopril intake before STZ administration was able to augment the production of both TNF- α and IL-1 β in STZ-Cap which might be due to increased STZ uptake. Also, histopathological findings of the pancreas showed a significant degeneration of islets of Langerhans and an increase in the optical density of TNF- α immunoreactivity at the doses 30 mg/kg in both STZ and STZ-Cap and the degree of degeneration increased by increase STZ-Cap doses. Also, the mean area % of antiinsulin immunoreactivity had a significant decrease in all groups STZ and STZ-Cap. This decrease ranges from moderate heterogeneous insulin immunostaining in β -cells to faint immunostaining according to STZ doses. Our results are consistent with Akash et al. [44] who denoted that the first pro-inflammatory cytokine that was recognized due to its involvement in insulin resistance pathogenesis and T2DM was TNF-a and the release of other cytokines like IL-6 and IL-1 β is enhanced by the pro-inflammatory TNF- α . During acute inflammation, the elevation in both TNF- α and IL-1 β impairs β -cell activity and disrupts the insulin signaling pathway.

In conclusion, our study suggests that captopril may be a crucial regulator of glucose analog uptake (STZ) by β -cell. We found that the prior administration of captopril significantly enhanced the effect of low-dose STZ, helped the induction of diabetes, and decreased the mortality rate that occurred at the other higher doses this could make it a potential novel modified experimental model for the induction of diabetes in experimental animals.

Limitation and future recommendations

Future work is highly recommended to elucidate the long-term effect of coadministration of captopril and STZ regarding the state of hyperglycemia. We combined captopril with different doses of STZ, and we found that the intake of STZ at a dose of 40 mg/kg produced the same results of 50 mg/kg. By introducing the appropriate dose and route of administration for STZ and Alloxan chemical compounds, it could be possible to produce an optimal experimental model for type II DM with a low dose of STZ in rats. However, the long-term effect on plasma glucose and insulin levels, and islet morphology was not investigated.

In addition, it is highly recommended to investigate the effect of the combined treatment protocol versus STZ on the liver and kidney.

Abbreviations

Streptozotocin
Captopril
Intraperitoneal glucose tolerance test
Tumor necrosis factor alpha
Interleukin one beta
Enzyme-linked immunosorbent assay
Glucose transporter 2
Reverse transcription polymerase chain reaction
Hematoxylin and eosin
Diabetes mellitus
Renin–angiotensin system
Angiotensin II receptor types 1
Angiotensin II receptor types 2
Angiotensin converting enzyme
Analysis of variance
Nitric oxide
Reactive oxygen species

Author contributions

The authors declare that all data were generated in-house and that no paper mill was used. All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Ateyya Hayam; Harb Inas; ShamsEldeen Asmaa; Adel Sara; Samir Samaa; Rashed Laila; Mostafa Abeer. HL, AH., and S A. conceived and designed research. AH, HI, and HS conducted experiments. RL, SA, and KS contributed new reagents or analytical tools. AH, HI, AM, and AH analyzed data. AH and SA wrote the manuscript. All authors read and approved the manuscript.

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Availability of data and materials

The data that support the findings of this study are available from the authors upon reasonable request.

Declarations

Ethics approval and consent to participate

The study was done in accordance with the highest International Criteria of Animal Experimentation of Helsinki and was approved by the animal house of the faculty of medicine, Cairo University, approval No; CU/III/F/38/22. The authors confirm that neither the manuscript nor any parts of its content are currently under consideration or published in another journal.

Consent for publication

Not applicable.

Competing interests

We would like to declare that there were no conflicts of interest in conducting this research.

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