Original Article

Effect of Enoxaparin and Swertiamarin on Streptozotocin-Induced Diabetes Mellitus in Rats: Biochemical, Histological and Immunohistochemical Study

Samah Kandeel and Mona Tayssir Sadek

Department of Histology and Cell Biology, Faculty of Medicine, Tanta University, Egypt

ABSTRACT

Introduction: Diabetes mellitus (DM) is a chronic metabolic disorder characterized by elevated blood glucose levels. Enoxaparin is low molecular weight heparin with anticoagulant, anti-inflammatory and antifibrotic actions. Swertiamarin is secoiridoid glycoside with hypoglycemic, hypolipidemic, anti-inflammatory and antioxidant properties.

Aim of the Work: To evaluate the effect of enoxaparin and swertiamarin on streptozotocin (STZ)-induced DM using histological, immunohistochemical and biochemical techniques.

Materials and Methods: Seventy Wistar rats (10-12 weeks) weighing 120-150 gram divided into 2 main groups. Group 1 (control: 55 rats) and group 2 (STZ-induced DM: 55 rats): Diabetes mellitus induced by 50 mg/kg STZ single dose intraperitoneal; only 40 rats became diabetic and subdivided into 4 equal subgroups. Subgroup 2a (STZ): left without treatment, subgroup 2b (STZ+Enoxaparin): treated with enoxaparin 2 mg/kg/day subcutaneously for 3 weeks, subgroup 2c (STZ+Swertiamrin): treated with swertiamarin 50 mg/kg/day orally for 3 weeks and subgroup 2d (STZ+Enoxaparin+Swertiamarin): treated with enoxaparin and swertiamarin as previous doses and period.

Results: Subgroup 2a (STZ) showed decreased body & pancreas weight and ratio, increased blood glucose level, plasma HbA1c%, total cholesterol, TGs, LDL and decreased HDL, reduced tissue SOD & GSH, elevated tissue MDA, TNF-α & IL-6. Congested dilated blood capillaries, β& α cell cytoplasmic vacuolations & nuclear pyknosis by H&E, increased collagen area percentage, HSP60 color intensity & decreased PCNA percentage were detected. Treated subgroups (2b,2c,2d) showed marked amelioration of previously reported results especially in subgroup 2d.

Conclusion: Combined use of enoxaparin and swertiamarin showed the best results in improvement of biochemical and structural changes on islet cells of the pancreas in type 2 diabetes mellitus in rats than their separate use.

Received: 14 June 2022, **Accepted:** 07 July 2022

Key Words: Diabetes mellitus, enoxaparin, swertiamarin, streptozotocin.

Corresponding Author: Samah Kandeel, MD, Department of Histology and Cell Biology, Faculty of Medicine, Tanta University, Egypt, **Tel.**: +20 15 5722 9520, **E-mail:** samah.kandeel@med.tanta.edu.eg

ISSN: 1110-0559, Vol. 46, No. 3

INTRODUCTION

Diabetes mellitus (DM) is a serious worldwide medical condition especially in developing countries with great concern because of its increased rate of mortality with high management costs. It is a chronic metabolic disorder characterized by elevated blood glucose level (hyperglycemia). The World Health Organization (WHO) expected that more than 19% of the worldwide adult general population will suffer from DM by 2030. Moreover, the International Diabetes Federation expected increased total number of individuals suffering from DM to reach about 693 million by the year 2045[1]. On the other hand, the prevalence of type 2 diabetes in Egypt is about 15.6% of all adult people, aged from 20 to 79 years old. According to the International Diabetes Federation (IDF); Egypt is the 9th foremost country in the world as regards the number of type 2 diabetic patients[2].

There are two main types of DM; Type 1 and Type 2. Type 1 diabetes mellitus (T1DM) predominates in children and is caused by decreased insulin production. On the other hand, type 2 diabetes mellitus (T2DM) predominates in middle-aged and adults. It is caused by defective insulin action. mostly due to an unhealthy lifestyle[1,3].

In T2DM, the insulin plasma level becomes higher than normal as a result of hypersensitivity of the β-cells of the islets of Langerhans to the glucose plasma levels that consequently causes a further deterioration of β-cell functions[4]. Moreover, DM induces disturbance of the body metabolism with subsequent dyslipidemia, atherosclerosis and other microvascular complications that account for most cases of disease morbidity and mortality[5,6]. In addition, DM causes platelet function disorders with activation of platelet aggregation and coagulation and subsequent different organ affection including; pancreatic islets of Langerhans, eyes, kidneys, heart and brain[7,8].

The DM-associated insulin resistance (IR) could lead to elevation of the free fatty acids as a result of their decreased intake by the skeletal muscles as well as their increased production by the adipose tissue in addition to adipokine dysregulation with subsequent increase in both adipose

 DOI: 10.21608/ejh.2022.144798.1703

tissue mass and adipose cell size[9,10]. This in turn could be associated with tissue hypoxia, fibrosis and inflammation with increased release of different pro-inflammatory cytokines such as IL-6 & TNF-α[11]. Besides, oxidative stress with the release of reactive oxygen species (ROS) from the mitochondria participates in the pathophysiology of both DM and IR[12].

Yet, no treatment is currently effective in the management of either DM or its complications especially vascular affection as well as the associated inflammation and oxidative stress. So, the need for a new strategy for DM management is required.

Enoxaparin is a low molecular weight heparin with in *vivo* long half-life[13,14]. It is used in prevention as well as in treatment of many different thrombotic disorders such as; deep vein thrombosis, myocardial infarction and unstable angina[15]. Currently, it is considered a promising anticoagulant drug because of its safety, bioavailability, efficiency in addition to its antifibrotic effects[16]. It was proved that enoxaparin also has the ability to decrease microvascular permeability, influx of neutrophils, dysregulated coagulation and fibrinolysis besides its anti-hypoxic effects[15].

Herbal medicine succeeded to provide various therapeutic agents that could be used in treatment of many human diseases due to their different chemical structures with their ability to modify biological tissue functions. Swertiamarin is a secoiridoid glycoside, the main component of Enicostemma littorale plants[17]. It is a famous medicinal herb found throughout India, being used in the treatment of DM because of its hypoglycemic and hypolipidemic efficiency. Moreover, it was documented to exert many beneficial effects in different pathological conditions due to its analgesic, gastroprotective, anti-microbial as well as anti-inflammatory and antioxidant properties[18].

So, the present research was conducted to evaluate the effect of enoxaparin in combination with swertiamarin over their separate use on streptozotocin-induced diabetes mellitus in rats using biochemical parameters in addition to histological, and immunohistochemical methods.

MATERIALS AND METHODS

This study was conducted using 70 adult Wistar rats; aged 10-12 weeks, obtained from the animal house, Faculty of Medicine, Tanta University, Egypt. Their weight ranged from 120 to 150 grams. They were housed in clean ventilated cages at room temperature with 12 hours' light/dark cycle and humidity about 60%. The rats were fed a standard commercial diet and were given water ad libitum.

The experiment was carried out in accordance with the guidelines for care and use of the experimental animals in Research, Ethical Committee of Faculty of Medicine, Tanta University, Egypt. Approval code No. 35314/3/22.

Chemicals

Both streptozotocin (pale yellow, crystalline solid) and swertiamarin (white powder) used in the current study were purchased from Sigma-Aldrich, Egypt. Enoxaparin (colourless to pale yellow solution of enoxaparin sodium injection, USP, 150mg/ml) was purchased from Sandoz Inc., Egypt. Cold citrate buffer, DMSO (dimethyl sulfoxide), Tris-HCl buffer, diaminobenzidine, hematoxylin, and eosin were purchased from El Gomhuria Co., Tanta, El-Gharbeya, Egypt.

Induction of DM

A single dose (50 mg/kg b.w) of freshly prepared STZ solution (5 mg dissolved in 0.1 M cold citrate buffer) was given intraperitoneally (i.p.) in order to induce type-2 DM in the overnight fasted animals[19].

Animals were allowed to drink 5% glucose solution overnight in order to reduce the STZ-induced hypoglycemia. Then, 72 hours after STZ injection, blood was collected through the rat tail vein and blood glucose level was evaluated using an automated glucometer (Accu-Check Active, Roche Diagnostics, Germany). Finally, rats with a fasting blood glucose level that more than 250 mg/dl, were considered diabetic and selected to complete the experiment[19].

Experimental Groups

Rats were randomly divided into two main groups as follows:

Group 1 (Control): included 15 rats that were further randomly subdivided into 3 equal subgroups (5 rats each):

- Subgroup 1a: were left without any treatments.
- Subgroup 1b: were given single intraperitoneal injection of 0.1 M cold citrate buffer in a dose corresponding to STZ.
- Subgroup 1c: were given 0.5% DMSO orally through an intragastric tube in a dose corresponding to swertiamarin.

Group 2 (STZ-induced DM): included 55 rats in which DM was induced by STZ injection and confirmed by measuring the blood glucose level. In this work, only 40 rats became diabetic and were further randomly subdivided into 4 equal subgroups, each included 10 rats:

- Subgroup 2a (STZ): were left without further treatment.
- Subgroup 2b (STZ+Enoxaparin): in which the diabetic rats were treated with enoxaparin in a dose of 2 mg/kg/day subcutaneously for 3 weeks[20].
- Subgroup 2c (STZ+Swertiamrin): in which the diabetic rats were treated with swertiamarin in a dose of 50 mg/kg/day orally through an intragastric tube for 3 weeks[21].

- Subgroup 2d (STZ+Enoxaparin+Swertiamarin): in which the diabetic rats were treated with both enoxaparin and swertiamarin in the same doses and routes and for the same duration as subgroups 2b and 2c respectively.

Samples collection and tissue preparation

One day after the last dose treatment, while left fasting overnight for 18 hours, rats were weighed then anesthetized with i.p. injection of 50 mg/kg pentobarbital sodium[22]. Blood was collected through the tail vein, centrifuged at 5000 rpm for 10 minutes for separation of the blood serum that was then stored at -80 oC for further biochemical analysis. For measurement of the glycosylated hemoglobin A1c (HbA1c), 0.5 ml of blood was collected in an EDTA coated tube[23,24].

Moreover, the pancreas was rapidly dissected out, first weighed, then part of the samples was homogenized in 2 ml of 0.1 mM Tris-HCl buffer (pH = 7) and about 1 ml of the homogenate was centrifuged at 5000 rpm for 15 minutes, with collection of the supernatant. Then, both the homogenate as well as the supernatant were stored at -80 oC for further biochemical investigations[23]. On the other hand, the other parts of the pancreatic samples were processed for histopathological and immunohistochemical examination.

Biochemical parameters

Blood glucose level and plasma HbA1c (hemoglobin A1c) percentage

For evaluation of the blood glucose level (mg/dl), an automated glucometer (Accu-Check Active, Roche Diagnostics, Germany) was used. Meanwhile, the plasma HbA1c % was assessed using (sELISA) kits (Biol. Tech. Co., China). Then, an automated plate reader (Stat Fax 2100, France) was used to analyze its optical density at 450 nm, with a minimal detection limit about 0.938 ng/ml[23].

Serum lipid profile

Serum lipid profile parameters including [Total cholesterol, triglycerides (TGs), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels] were estimated using the standard assay kits (Bio-Diagnostic Co., Egypt). The results were expressed as mg/dl[25,26].

Tissue oxidative stress markers

Levels of the tissue antioxidant superoxide dismutase (SOD) (U/mg protein) and reduced glutathione (GSH) (µM/mg protein) were estimated besides, the lipid peroxidation end-product malondialdehyde (MDA) (nM/mg protein) by using the colorimetric assay kits (Bio-Diagnostic Co., Giza, Egypt)[27,28].

Tissue cytokine levels

The levels of tissue TNF-α & IL-6 (pg/mg protein) were measured by ELISA kits (Biotechnology Co., China) according to the manufacturer's instructions[29].

Histological examination

Pancreatic specimens were fixed in 10% formalin buffered saline, then, dehydrated, cleared and finally embedded in paraffin. After that, 5 µm thick sections were obtained through using the rotatory microtome from Leica, US[30].

Haematoxylin & Eosin (H&E) staining

Pancreatic sections were deparaffinized, hydrated, then stained with hematoxylin for 10 minutes, followed by counterstaining in 1% eosin. After that, dehydration, clearance in xylol and mounting in Canada balsam were done. The results were expressed as blue-stained nuclei with hematoxylin and pink-stained cytoplasm and connective tissue fibers with eosin.

Mallory's trichrome staining

Specimens were deparaffinized, rehydrated, then, were re-fixed in Bouin's solution and stained with Wiegert's iron hematoxylin in addition to Biebrich scarlet-acid fuchsine. After that, sections were differentiated in phosphotungstic acid solution followed by aniline blue staining with differentiation in 1% acetic acid. At last, sections were dehydrated, and cleared in xylol. The collagen fibers were appeared blue-stained while the nuclei were stained red.

Immunohistochemistry of anti-PCNA (proliferating cell nuclear antigen) and anti- HSP60 (heat shock protein-60)

Sections were deparaffinized, rehydrated and placed in a buffer solution and boiled in a microwave oven for 5 minutes (Kenmore, USA). The sections were allowed to cool down at room temperature then placed in 0.3% hydrogen peroxide/methanol for 15 minutes. The rabbit polyclonal primary antibodies; anti-PCNA at 1:300 dilutions (Santa Cruz Biotechnology) and anti- HSP60 at 1:200 dilutions (Dako, UK) were added overnight at 4 °C. Then, 1:50 secondary antibody (Termo, USA) was added for 30 minutes at room temperature. Finally, 2-3 drops of diaminobenzidine were added then, counterstained with Mayer's hematoxylin. At last, sections were dehydrated, cleared and mounted, then examined by an Olympus, Japan light microscope. Sections stained with HSP60 expressed brown cytoplasmic reaction while, positive nuclear staining for PCNA stained sections.

Negative control sections were obtained through the omission of the primary antibody. On the other hand, the positive control for HSP60 was mammary cancer while that for PCNA was skin[31-33].

Morphometry

Morphometric analysis was carried out using "Image J" software program (version 1.48v. National Institute of Health, Bethesda, USA). Ten different images from each experimental group (x 400) were used to evaluate the followings:

1. Mean area percentage (%) of collagen fibers in Mallory's trichrome stained sections.
2. Mean area percentage (%) of islet cells positive for PCNA in anti-PCNA immunostained sections.
3. Mean color intensity of HSP60 positive cytoplasmic reaction in islet cells in anti-HSP60 immunostained sections.

Statistical analysis

Data of the present research were collected. Statistical analysis program (IBM SPSS Statistics for Windows, IBM Corp, Version 22.0., USA) was used to evaluate the statistical difference between the experimental groups. Two-sample Student's t-test was used to compare between subgroup 2a (STZ) and group 1 (control). Additionally, One-way analysis of variance (ANOVA) test was used to compare subgroups; 2b (STZ+Enoxaparin), 2c (STZ+Swertiamrin) and 2d (STZ+Enoxaparin+Swertiamarin) to subgroup 2a (STZ). The results were expressed as mean ± standard deviation (SD). Values were significant when *P value* was ≤ 0.05.

RESULTS

Mean body weight (gm), mean pancreas weight (gm), and pancreas/body weight ratio percentage (%)

As regards the mean body weight, the pancreas weight and the pancreas/body weight ratio (%), there was a significant decrease in subgroup 2a (STZ) as compared to group 1 (control). While, a significant increase in subgroups 2b (STZ+Enoxaparin), 2c (STZ+Swertiamrin) and 2d (STZ+Enoxaparin+Swertiamarin) when compared to subgroup 2a with the highest significance in subgroup 2d (Figure 1).

Blood glucose level and plasma HbA1c %

Subgroup 2a (STZ) showed significantly increased blood glucose level as well as plasma HbA1c % when compared to group 1 (control). On the other hand, subgroup 2b (STZ+Enoxaparin) showed a non-significant difference as regards the two parameters when compared to subgroup 2a. For subgroups 2c (STZ+Swertiamrin) and 2d (STZ+Enoxaparin+Swertiamarin), a significant reduction of the blood glucose level as well as plasma HbA1c % was noticed when compared to subgroup 2a and was more prominent in subgroup 2d (Figure 2).

Serum lipid profile results

As regards the serum lipids profile parameters of subgroup 2a, there was a significant increase in total cholesterol, TGs and LDL levels, whereas HDL level was significantly lower as compared to group 1 (control group). Considering subgroup 2b, a non-significant difference of total cholesterol, TGs, LDL and HDL levels was seen compared to those of subgroup 2a. While, subgroups 2c and 2d showed significantly decreased levels of total cholesterol, TGs and LDL levels and increased HDL levels in respect to subgroup 2a with the upper hand to subgroup 2d (Figure 3).

Pancreatic tissue oxidative stress markers

There was a significant reduction of tissue SOD as well as GSH levels besides a significant elevation of tissue MDA level at subgroup 2a (STZ) compared to group 1 (control). A non-significant difference was documented for subgroup 2b when compared to subgroup 2a. Conversely, significantly increased SOD and GSH levels while decreased MDA level were reported in both subgroups 2c and 2d compared to subgroup 2a with the best results reported in subgroup 2d (Figure 4).

Pancreatic tissue cytokine levels

As regards TNF-α and IL-6, a significant increase in their levels was recorded in subgroup 2a when compared with group 1 (control). Meanwhile, their levels were significantly decreased in subgroups 2b, 2c and 2d when compared to subgroup 2a with the most significance decrease in subgroup 2d (Figure 5).

H & E results

Group 1 (control group) revealed pancreatic sections with a normal histological structure of the islets of Langerhans. They appeared as pale stained areas with a delicate connective tissue capsule. Cells of the islets were arranged in anastomosing cords separated by blood capillaries. Two types of cells were recognized; central beta (β) cells with large vesicular rounded nuclei and peripheral alpha (α) cells with small dark nuclei (Figure 6A).

Subgroup 2a (STZ) showed the islets with congested apparently dilated blood capillaries. Regarding the islets' cells, β &α-cells appeared vacuolated while others revealed increased cytoplasmic eosinophilia besides pyknotic or karyolitic nuclei of both cell respectively. Also, there were mild mononuclear cell infiltrations situated peripherally (Figure 6B).

Subgroup 2b (STZ+Eoxaparin) showed nearly normal blood capillaries. Although, some β & α cells showed vacuolated cytoplasm, and few β cells with increased cytoplasmic eosinophilia and pyknotic nuclei (Figure 6C). Subgroup 2c (STZ+Swertiamrin) showed moderately dilated and congested blood capillaries. In addition, few β cells showed vacuolated cytoplasm and pyknotic nuclei (Figure 6D). Subgroup 2d (STZ+Enoxaparin+Swertiamarin) showed marked improvement of the islets of Langerhans with nearly normal histological structure of β & α cells, and nearly normal blood capillaries (Figure 6E).

Mallory's trichrome results

Group 1 revealed delicate blue-stained collagen fibers surrounding the pancreatic islets, around the blood capillaries and in-between the islets' cells (Figure 7A). Subgroup 2a showed apparently increased collagen fibers deposition around the islets and the blood capillaries as

well as in-between the islets' cells (Figure 7B). Subgroup 2b showed little deposition of collagen fibers (Figure 7C) while subgroup 2c showed apparently increased collagen deposition (Figure 7D). In subgroup 2d, nearly normal appearance was observed with deposition of delicate collagen fibers surrounding the islets, around the blood capillaries and in-between the islets' cells (Figure 7E).

As regards the mean area percentage (%) of collagen fibers, there was a significant increase in subgroup 2a as compared to group 1. However, a significant decrease was reported in subgroups 2b, 2c and 2d in respect to subgroup 2a (Figure 8).

PCNA results

Regarding the negative control section, it showed no PCNA immunohistochemical reaction (Figure 9A). For group 1, many cells of the islets with a positive nuclear immune reaction for PCNA were detected (Figure 9B). Subgroup 2a showed markedly decreased PCNA reaction and only few cells showed a positive nuclear immune reaction (Figure 9C). On the other hand, subgroup 2b showed some cells with moderate positive PCNA reaction (Figure 9D). As regards subgroup 2c, many cells expressed moderate positive reactions for PCNA (Figure 9E), while in subgroup 2d, most of the cells showed strong positive PCNA immune reaction as in group 1 (Figure 9F).

Regarding the mean area percentage (%) of islet cells positive for PCNA, subgroup 2a (STZ) showed a significant decrease in comparison to group 1. On the other hand, a significant increase was observed in subgroups 2b, 2c and 2d when compared to subgroup 2a (Figure 10).

HSP60 results

The negative control specimen showed no islet cells' immunohistochemical reaction for HSP60 (Figure 11A). Regarding group 1, there was a weak brownish cytoplasmic reaction for HSP60 of the islet cells (Figure 11B). In subgroup 2a, a strong positive HSP60 immunohistochemical reaction of islet cells was observed (Figure 11C). While, in subgroups 2b & 2c, a moderate positive reaction of islet cells was noticed (Figures 11 D, E respectively). As regards subgroup 2d, a weak positive cytoplasmic reaction of the islet cells for HSP60 was observed (Figure 11F).

As regards the mean color intensity of islet cells' HSP60 positive cytoplasmic immunoreaction, there was a significant increase in subgroup 2a as compared to group 1. While, a significant decrease was recorded in subgroups 2b, 2c and 2d compared to subgroup 2a (Figure 12).

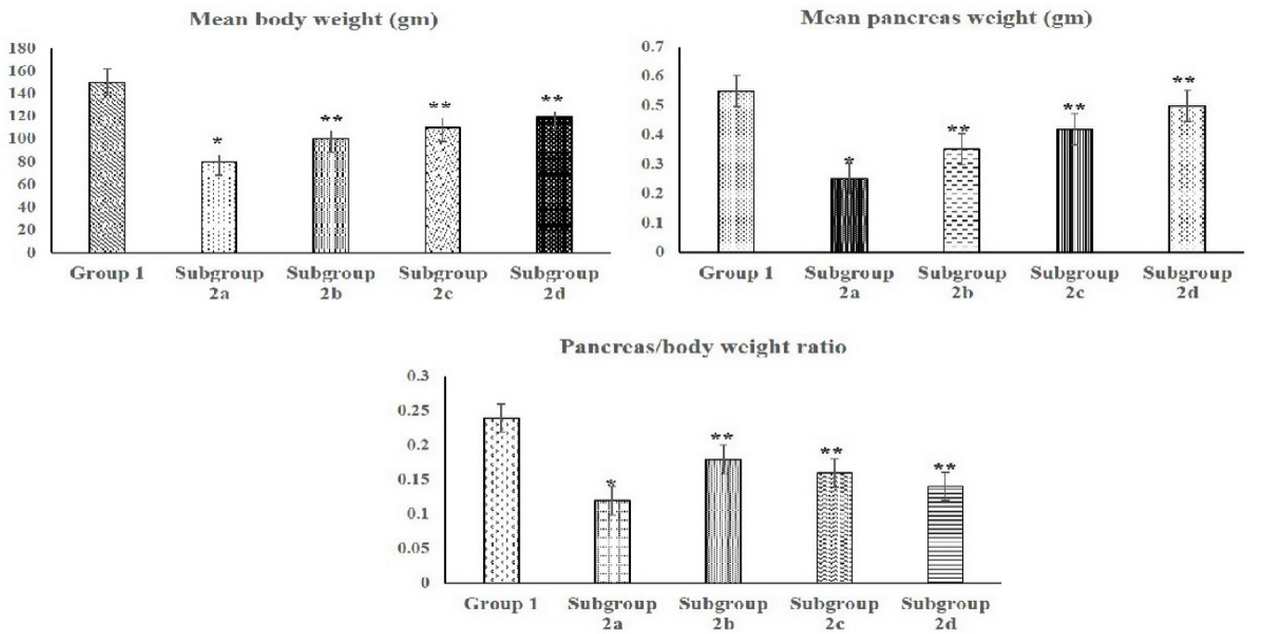

Fig. 1: Mean body weight (gm), mean pancreas weight (gm) and pancreas/body weight ratio percentage (%). Data expressed as mean ± SD (Standard deviation). * $P<0.05$; subgroup 2a (STZ-induced DM) compared to group 1 (Control). ** $P<0.05$; subgroup 2b (STZ+Enoxaparin), subgroup 2c (STZ+Swertiamrin) and subgroup 2d (STZ+Enoxaparin+Swertiamarin) compared to subgroup 2a (STZ-induced DM).

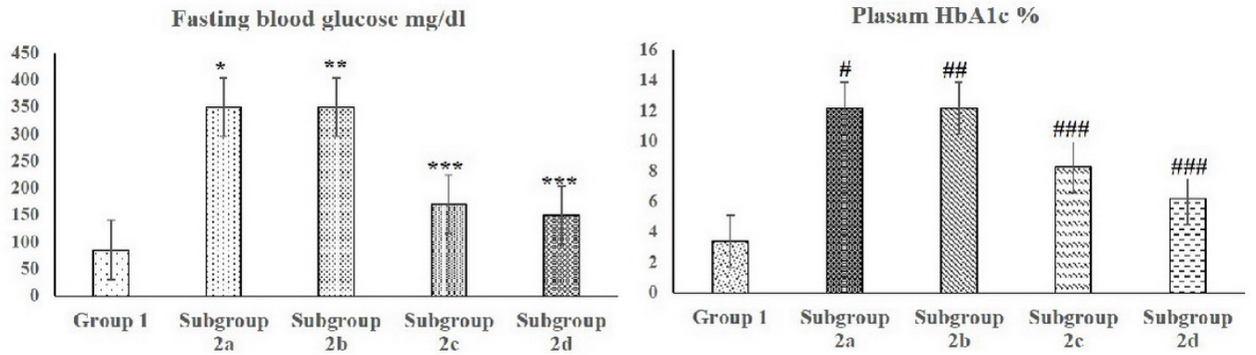

Fig. 2: Fasting blood glucose level (mg/dl) and Plasma HbA1c %. Data expressed as mean ± SD.
* and # $P<0.05$; subgroup 2a (STZ-induced DM) compared to group 1 (Control). ** and ## $P>0.05$; subgroup 2b (STZ+Enoxaparin) compared to subgroup 2a (STZ-induced DM). *** and ### $P<0.05$; subgroup 2c (STZ+Swertiamrin) and subgroup 2d (STZ+Enoxaparin+Swertiamarin) compared to subgroup 2a (STZ-induced DM).

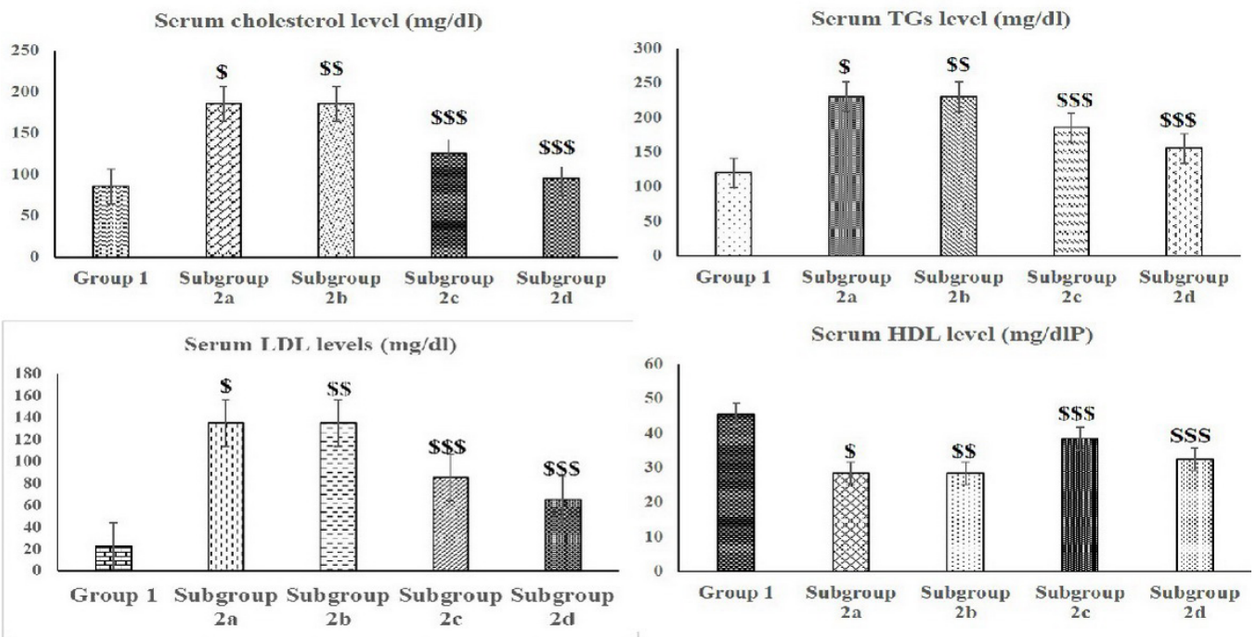

Fig. 3: Serum lipid profile (mg/dl). Data expressed as mean ± SD.
$ $P<0.05$; subgroup 2a (STZ-induced DM) compared to group 1 (control). $$ $P>0.05$; subgroup 2b (STZ+Enoxaparin) compared to subgroup 2a (STZ-induced DM). $$$ $P<0.05$; subgroup 2c (STZ+Swertiamrin) and subgroup 2d (STZ+Enoxaparin+Swertiamarin) compared to subgroup 2a (STZ-induced DM).

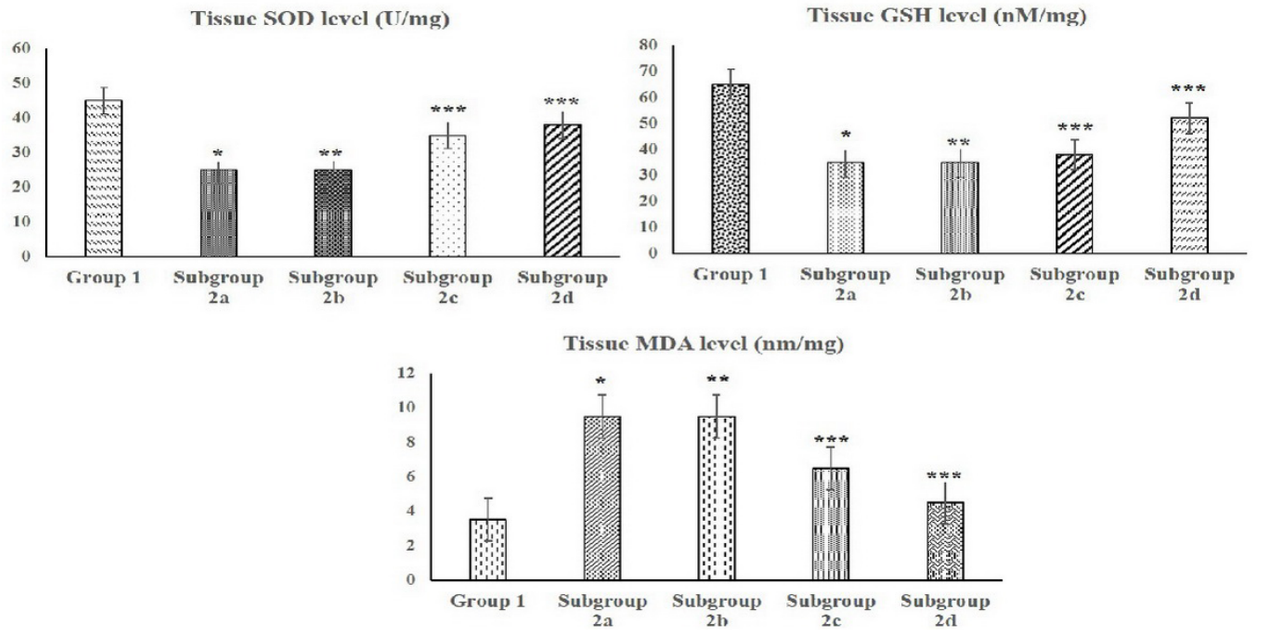

Fig. 4: Tissue levels of oxidative stress markers. Data expressed as mean ± SD.
* *P*<0.05; subgroup 2a (STZ-induced DM) compared to group 1 (control). ** *P*>0.05; subgroup 2b (STZ+Enoxaparin) compared to subgroup 2a (STZ-induced DM). *** *P*<0.05; subgroup 2c (STZ+Swertiamrin) and subgroup 2d (STZ+Enoxaparin+Swertiamarin) compared to subgroup 2a (STZ-induced DM).

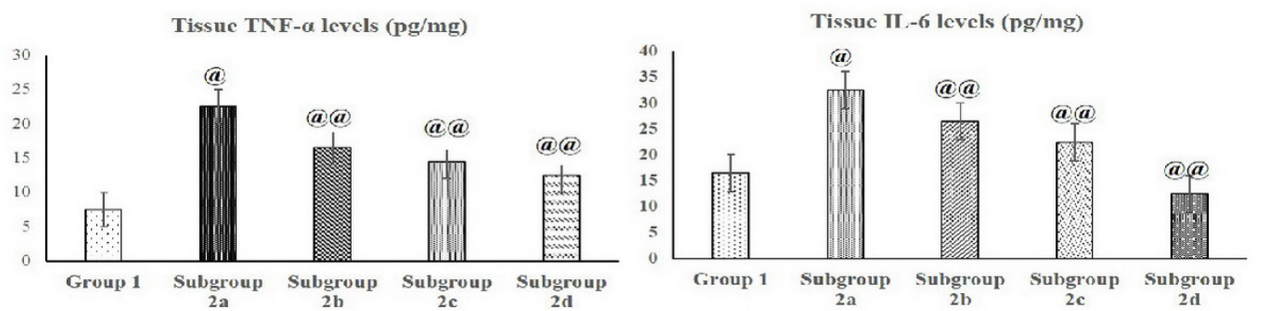

Fig. 5: Tissue cytokine levels. Data expressed as mean ± SD.
@ *P*<0.05; subgroup 2a (STZ-induced DM) compared to group 1 (control). @@ *P*<0.05; subgroup 2b (STZ+Enoxaparin), subgroup 2c (STZ+Swertiamrin) and subgroup 2d (STZ+Enoxaparin+Swertiamarin) compared to subgroup 2a (STZ-induced DM).

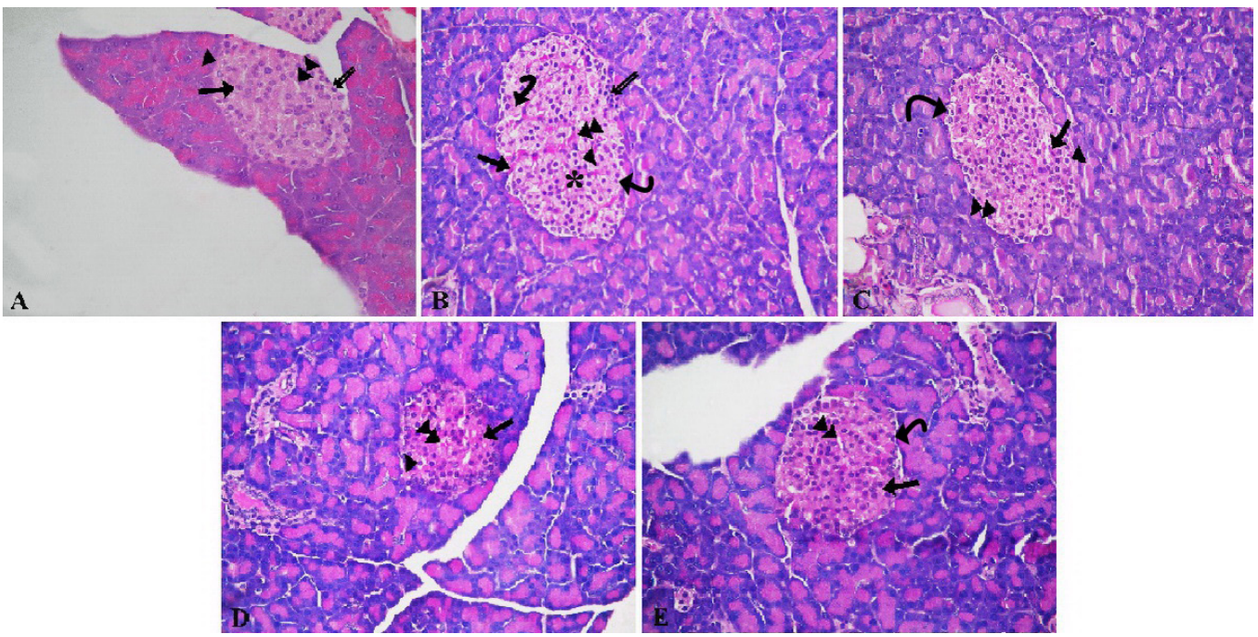

Fig. 6: H & E-stained pancreatic sections (H&E x400).
A) Group 1 (control): revealing pale stained areas of islets of Langerhans with a delicate connective tissue capsule (►), blood capillaries (►►), central beta (β) cells with large vesicular rounded nuclei (→) and peripheral alpha (α) cells with small dark nuclei (double arrow). B) Subgroup 2a (STZ-induced DM): showing congested apparently dilated blood capillaries (►►), vacuolated β-cells (►), β (*) &α cells (→) with increased cytoplasmic eosinophilia with nuclear pyknosis, karyolsis (curved arrow), and mild mononuclear cell infiltrations situated peripherally (double arrow). C) Subgroup 2b (STZ+Eoxaparin): showing nearly normal blood capillaries (►►), some β (►) & α cells (curved arrow) with vacuolated cytoplasm, few β cells with increased cytoplasmic eosinophilia and pyknotic nuclei (→). D) Subgroup 2c (STZ+Swertiamrin): showing moderately dilated congested blood capillaries (►►), few β cells with vacuolated cytoplasm (►) and pyknotic nuclei (→). E) Subgroup 2d (STZ+ Enoxaparin + Swertiamarin): showing nearly normal architecture with nearly normal β (→) & α (curved arrow) cells, and blood capillaries (►►).

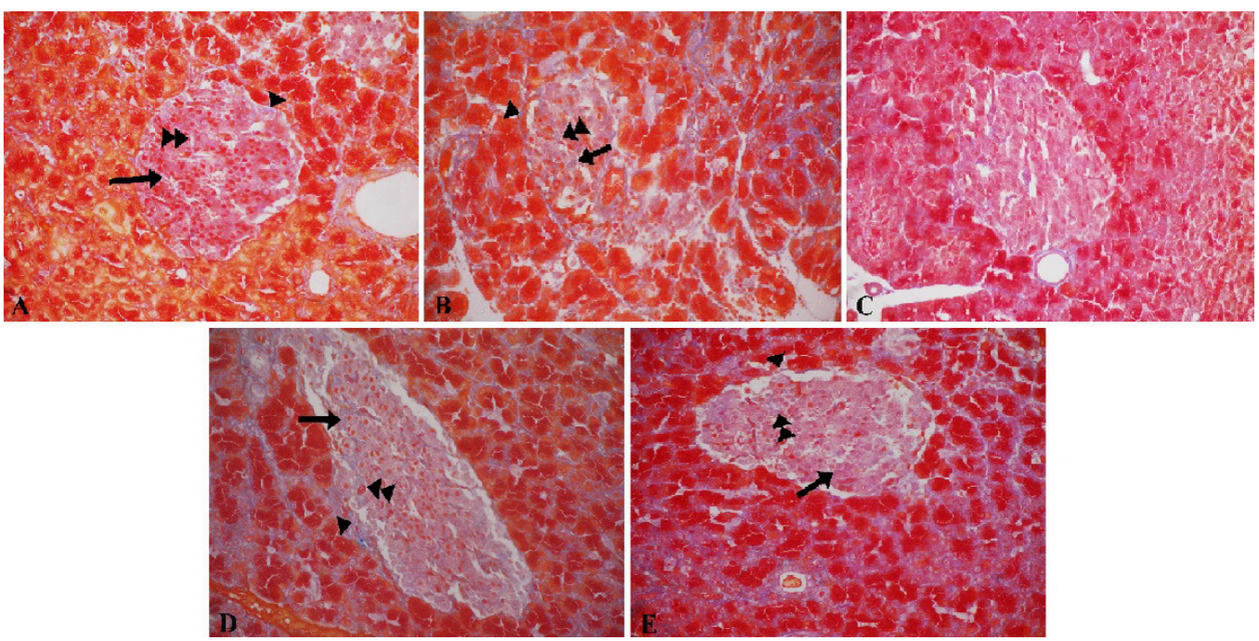

Fig. 7: Mallory's trichrome stained pancreatic sections (Mallory's Trichrome x400).
A) Group 1: revealing delicate collagen fibers surrounding the islets (►), around the blood capillaries (►►) and in-between the islet cells (→). B) Subgroup 2a: showing apparently increased collagen fibers surrounding the islets (►), around the blood capillaries (►►) and in-between the islet cells (→). C) Subgroup 2b: showing apparently decreased collagen fibers. D) Subgroup 2c: showing moderately increased collagen fibers surrounding the islets (►), around the blood capillaries (►►) and in-between the islet cells (→). E) Subgroup 2d: showing delicate collagen fibers surrounding the islets (►) and the blood capillaries (►►) and in-between the islet cells (→).

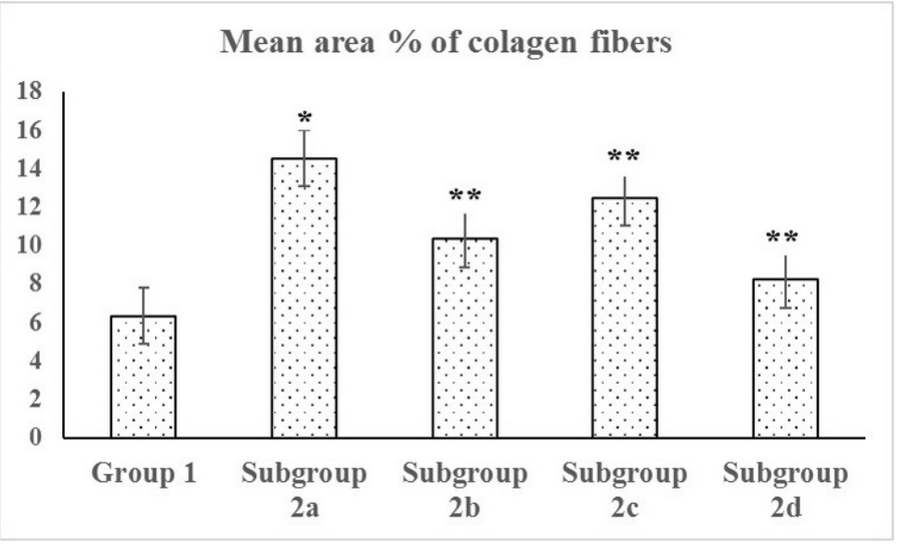

Fig. 8: Mean area % of collagen fibers. Data expressed as mean ± SD.
* *P*<0.05; subgroup 2a (STZ-induced DM) compared to group 1 (control). ** *P*<0.05; subgroup 2b (STZ+Enoxaparin), subgroup 2c (STZ+Swertiamrin) and subgroup 2d (STZ+Enoxaparin+Swertiamarin) compared to subgroup 2a (STZ-induced DM).

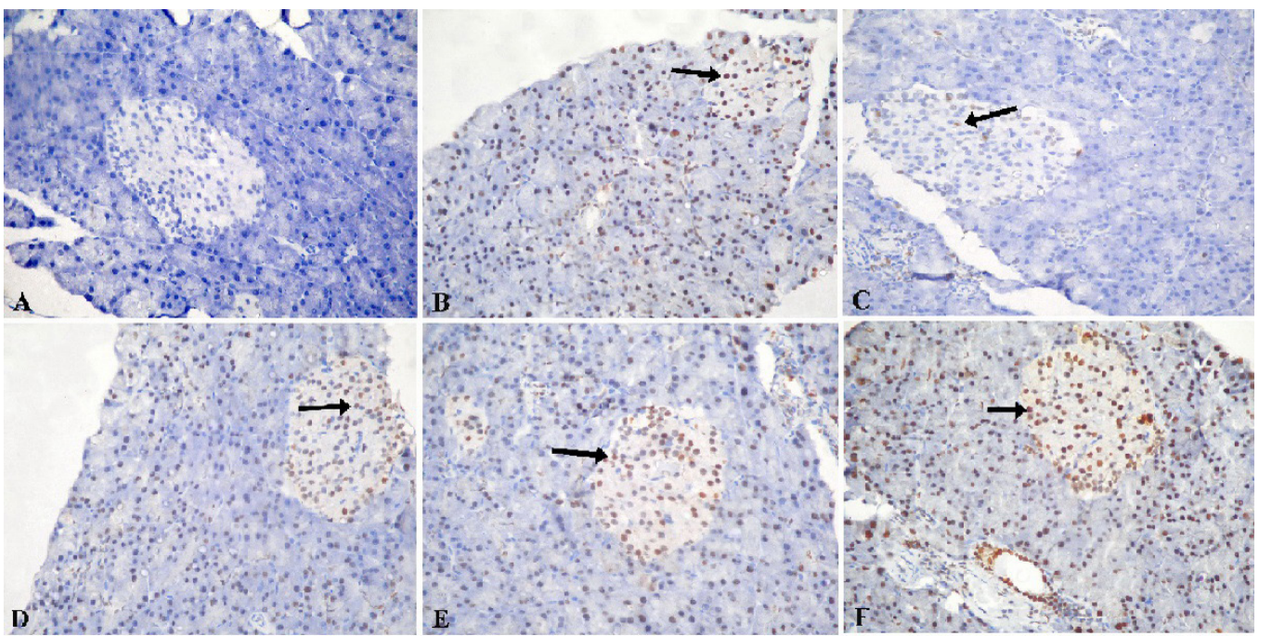

Fig. 9: PCNA immunostained pancreatic sections (PCNA x 400).
A) Negative control: showing no PCNA immunohistochemical reaction. B) Group 1 (Control): showing many cells of the islet with positive brownish nuclear immune reaction for PCNA (→). C) Subgroup 2a: showing markedly decreased PCNA immunoreaction and only few islet cells showing positive nuclear immunoreaction (→). D) Subgroup 2b: showing some islet cells with positive PCNA reaction (→). E) Subgroup 2c: showing many islet cells expressing positive reaction for PCNA (→). F) Subgroup 2d: showing most of the islet cells revealing positive PCNA immune reaction (→).

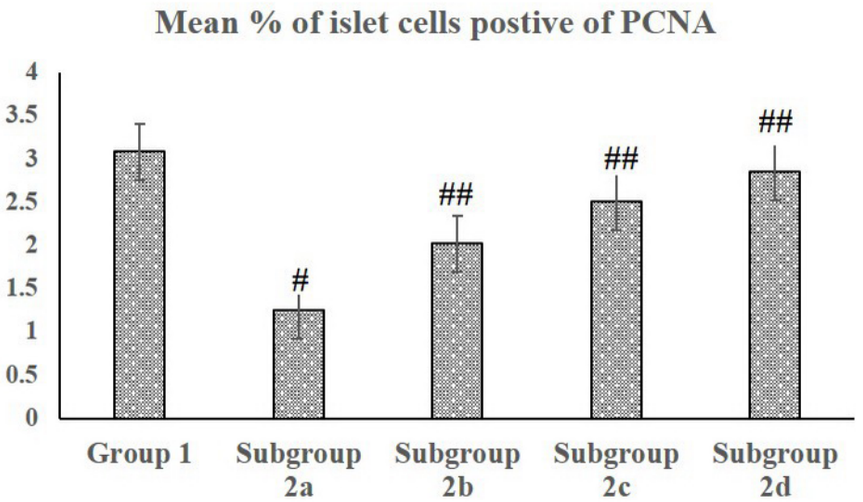

Fig. 10: Mean percentage (%) of islet cells positive for PCNA. Data expressed as mean ± SD.
# $P<0.05$; subgroup 2a (STZ-induced DM) compared to group 1 (control). ## $P<0.05$; subgroup 2b (STZ+Enoxaparin), subgroup 2c (STZ+Swertiamrin) and subgroup 2d (STZ+Enoxaparin+Swertiamarin) compared to subgroup 2a (STZ-induced DM).

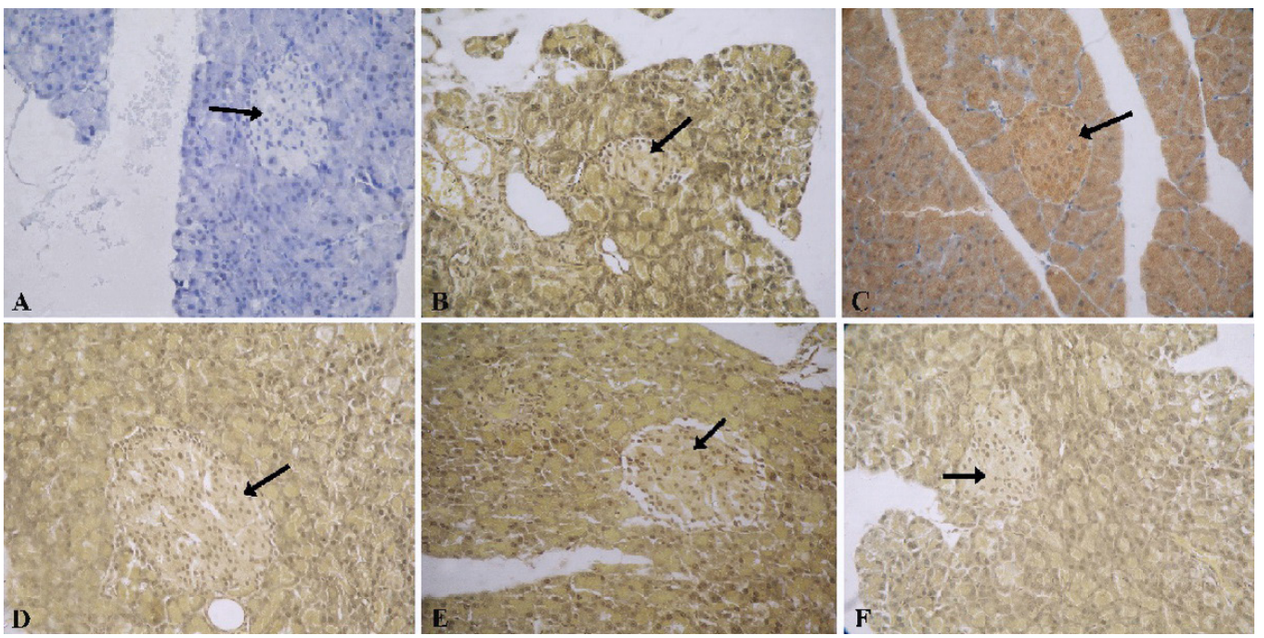

Fig. 11: HSP60 immunostained pancreatic sections (HSP60 x 400).
A) Negative control: revealing no HSP60 immunohistochemical reaction of islet cells (→). B) Group 1: showing islet cells' weak cytoplasmic brownish reaction for HSP60 (→). C) Subgroup 2a: showing islet cells' strong HSP60 immunohistochemical reaction (→). D) Subgroups 2b: showing islet cells' moderate HSP60 immunoreaction (→). E) Subgroup 2c: showing islet cells' moderate HSP60 immunoreaction (→). F) Subgroup 2d: showing islet cells' weak cytoplasmic reaction for HSP60 (→).

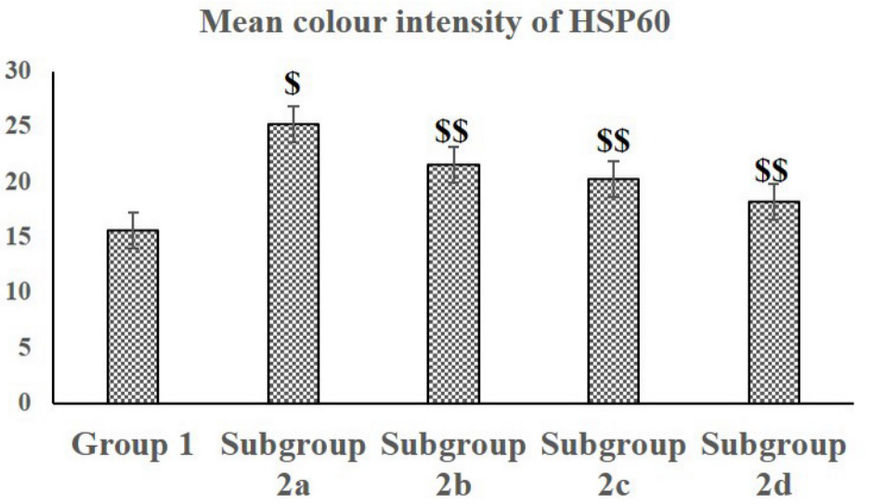

Fig. 12: Mean color intensity of islet cells' HSP60 positive cytoplasmic reaction. Data expressed as mean ± SD.
$ P<0.05; subgroup 2a (STZ-induced DM) compared to group 1 (control). $$ P<0.05; subgroup 2b (STZ+Enoxaparin), subgroup 2c (STZ+Swertiamrin) and subgroup 2d (STZ+Enoxaparin+Swertiamarin) compared to subgroup 2a (STZ-induced DM).

DISCUSSION

The unhealthy sedentary lifestyle leads to DM, makes is a serious public health problem. It is associated with different microvascular and cellular complications which requires novel strategies for their management[34].

In the present study, STZ was used to induce insulin-dependent DM in rats through causing toxic damage and cellular injury of the beta cells of islets of Langerhans with their subsequent inability to produce insulin[35]. This study also investigated the possible therapeutic effect of separate and combined use of enoxaparin and swertiamarin in treating the diabetic rats.

The results of the present work proved that the use of enoxaparin and swertiamarin greatly ameliorated the STZ-induced biochemical, histological and immunohistochemical changes with the best results in their combined use.

In the present research, subgroup 2a (STZ) showed significant decrease in mean body weight and pancreatic weight as well as pancreas/body weight ratio percentage. Previous studies also confirmed the ill-appearance of the experimental animals with decreased body and pancreas weights in STZ-induced diabetic rats[35,36]. This could be attributed to the injurious effect of STZ on the islet cells, especially β- cells resulting in DNA alkylation[37], tissue necrosis and decreased organ weight[38]. Moreover, the associated diabetic hyperglycemia increases protein catabolism with subsequent skeletal muscle protein depletion that eventually causes wasting and weight loss[38].

However, a significant increase in body weight, pancreas weight as well as pancreas/body weight ratio percentage were recorded in the diabetic rats treated with either enoxaparin (subgroup 2b) or swertiamarin (subgroup 2c) with the highest increase in their combined use in subgroup 2d. This could be owed to the beneficial effect of enoxaparin on the pancreatic vascular micro-environment with the preservation of a good blood supply to the islet cells and the subsequent improved tissue perfusion, circulation, vitality and activity resulting in restoration of the normal metabolism[39]. Meanwhile, swertiamarin through its hypoglycemic, antioxidant and anti-inflammatory effects that were proved in the present research could enhance the survival of the islet β-cells with subsequent normal release of insulin and balanced metabolism that both help regaining body and pancreatic weights[19].

The present work showed significantly increased blood glucose level and plasma HbA1c% in STZ subgroup 2a when compared to group 1 (control) while showing a non-significant difference in both parameters in subgroup 2b (STZ+Enoxaparin) and a significant decrease in subgroup 2c (STZ+Swertiamrin) and subgroup 2d (STZ+Enoxaparin+Swertiamarin) when compared to subgroup 2a. Both blood glucose level and HbA1c% are long term biomarkers used for diagnosis and follow up of diabetic glycaemia. Also, HbA1c% is considered a post-synthetic modification of HbA throughout the lifespan of the red blood cells. Its amount is affected by the condition of carbohydrate metabolism, that if it is uncontrolled, increased glycosylation of proteins such as hemoglobin will occur[40].

The destructive effect of DM on β-cells of the islet of Langerhans causes liberation of nitric oxide (NO) with inhibition of aconitase enzyme activity, both causing more destruction of the pancreatic β-cells with subsequent inhibition of insulin secretion, hyperglycemia and decreased uptake of glucose by skeletal muscles, liver and adipose tissue. Moreover, the disturbed carbohydrate metabolism could lead to excess glucose in the blood,

which in-turn will react with hemoglobin irreversibly, and so increases the levels of HbA1c%[41].

Swertiamarin was proved to has a hypoglycemic activity[19]. This could be attributed to increased survival of the pancreatic β-cells trough the reversal of the condition of cellular oxidative stress with subsequent increased insulin secretion and enhanced glucose uptake by the cells so, maintaining glucose homeostasis. Swertiamarin was also reported to improve insulin sensitivity as well as glucose tolerance and uptake[23].

The current research proved the hyperlipidemia effect of STZ through the elevated serum levels of total cholesterol, TGs and LDL together with the decreased levels of HDL. This could be explained by the disturbed lipid metabolism secondary to several factors such as increased glucose levels, insulin resistance besides the adipokine and adipocytokines abnormalities[11]. Moreover, and as insulin inhibits lipolysis, the adipocytes' insulin resistance leads to impaired suppression of lipolysis and impaired glucose uptake with the enhancement of the free fatty acid release into the bloodstream that in-turn will be accumulated in other tissues[9,10].

However, a non-significant difference was noticed as regards total cholesterol, TGs, LDL and HDL levels in subgroup 2b compared to subgroup 2a. While, both subgroups 2c and 2d showed significantly decreased levels of the total cholesterol, TGs and LDL with increased HDL levels in respect to subgroup 2a that were more prominent in subgroup 2d. These findings came in line with previous studies that proved the beneficial effects of swertiamarin on the hyperlipidemia state in STZ-induced DM. Furthermore, swertiamarin was also reported to has the ability to increase the mRNA expression of adiponectin, a protein secreted by the adipocytes that plays a role in regulation of lipid and carbohydrate metabolism as well as insulin sensitivity[19].

Moreover, swertiamarin is known to stimulate the genes of peroxisome proliferator- activated receptor (PPAR). These are nuclear genes of two isoforms; PPAR-γ1 that are present in all tissues except muscle and PPAR-γ2 that are present in intestine and adipose tissue[42]. These genes reduce insulin tissue resistance and regulate fatty acids storage as well as glucose metabolism through stimulation of lipid uptake and adipogenesis by the fat cells[43]. Additionally, other studies proved the anti-atherogenic activity of swertiamarin through the inhibition of β-hydroxy β-methylglutaryl-CoA (HMG-CoA) reductase enzyme activity in high cholesterol fed rats[44].

Oxidative stress is considered a chief factor in development and progression of DM. There is also great evidence of DM associated mitochondrial dysfunction which is an important organelle for reactive oxygen species (ROS) clearance[45]. Therefore, the present work assessed the pancreatic tissue oxidative stress markers. A significant reduction of tissue SOD and GSH levels besides a significantly elevated tissue MDA (lipid peroxidation product) level was recorded in subgroup 2a (STZ) compared to control group 1. The hyperglycemia associated with DM increases levels of ROS secondary to mitochondrial dysfunction in addition to the increased levels of glycation end products besides inhibited glycosylation by the effect of free radicals[12].

On the other hand, a non-significant difference in SOD, GSH and MDA levels was observed in subgroup 2b in comparison to subgroup 2a. However, significantly increased SOD and GSH levels with decreased MDA level were reported in subgroups 2c & 2d as compared to subgroup 2a with better results in subgroup 2c. Similar findings were documented in previous studies that also added that swertiamarin could protect the pancreatic β-cells from oxidative stress damage through free radicals scavenging action and increased levels of tissue antioxidants besides lipid peroxidation inhibition[46].

Low grade inflammation was observed with STZ-DM with the release of different pro-inflammatory cytokines from the macrophages[34]. The present research revealed a significant increase of the levels of pancreatic tissue inflammatory cytokines (TNF-α and the IL-6) in subgroup 2a when compared with control group 1. This could be owed to the strong relation between hyperglycemia, oxidative stress and inflammation[47]. The increased protein glycation and the subsequent production of advanced glucose end products (AGEs) activate the nuclear factor kappa B protein complex signaling pathway (NF-κB) which controls the inflammatory cytokines production and in turn increases the release of TNF-α and IL-6. In addition, hyperglycemia interferes with the phagocytic activity of the macrophages and increases the incidence of infection, which in turn could stimulate release of inflammatory cytokines and so triggers the process of inflammation[47].

However, significantly decreased levels of both TNF-α and IL-6 were recorded in the subgroups treated with either enoxaparin or swertiamarin (2b and 2c respectively) with the most decreased levels in the combined treated subgroup 2d, when all compared to the STZ subgroup 2a. Enoxaparin was proved to have anti-inflammatory actions through inhibition of macrophages activity with the inhibition of cytokines release, and so suppressing the inflammatory progress[48]. Moreover, enoxaparin was documented to decrease the microvascular permeability and neutrophil recruitment; so counteracting inflammation[15]. Another study documented that enoxaparin can stabilize the endothelial cells and inhibit Intercellular adhesion molecule-1 (ICAM-1) that prevents neutrophils adhesions[49].

Meanwhile, the anti-inflammatory effect of swertiamarin was reported in previous studies who illustrated that it has the ability to suppress the activated NF-κB pathway with the consequent suppressed release of TNF-α and IL-6[50]. Moreover, the antioxidant effect of swertiamarin that was evidenced in the present study causes suppression of the release of ROS which ameliorates the inflammatory process[18].

The histopathological assessment of H&E-stained sections from the STZ-diabetic subgroup 2a showed nuclear pyknosis and karyolsis together with vacuolations and increased eosinophilia of the cytoplasm of the pancreatic β& α cells besides presence of apparently dilated congested blood capillaries as well as mild mononuclear cellular infiltration. These findings could be due to the direct injurious effect of STZ-induced DM on the islets of Langerhans especially on the β-cells. As well, the nuclear changes could be due to the DNA damaging effects of the released ROS[51].

Moreover, the associated hyperglycemia state due to IR leads to disturbed metabolism, especially for lipids and proteins with abnormality of the adipose tissue structure and functions. Consequently, tissue hypoxia, oxidative stress and inflammation[52]. Also, the endothelial dysfunction associated with DM leads to defective production of NO which is very important for vascular viability and consequently causes increased release of pro-inflammatory cytokines and increased expression of leukocyte adhesion molecules besides platelet hyper-reactivity[8]. As well, the nuclear changes could be due to the released ROS by STZ with DNA damaging effects[51].

The previously listed histopathological changes were improved in the treated subgroups 2b, 2c and 2d. However, the synergistic and combined effect of both enoxaparin and swertiamarin in subgroup 2d in the present study was found to greatly ameliorate the H&E structural changes more than with their separate use in subgroups 2b and 2c. Enoxaparin was reported to exert beneficial effects in improving functions of the vascular endothelial cells, that together with the good tissue circulation and oxygenation improve the pancreatic β-cell structure and function[13,39]. On the other hand, swertiamarin was proved to have anti-hyperglycemic, anti-inflammatory and anti-oxidative effects that could protect the pancreatic β-cells and ameliorate the injurious effects of DM[19]. So, protects the pancreatic β-cells as well as ameliorates the injurious effects of STZ[19].

As regards Mallory's trichrome stained sections, increased collagen fibers deposition with significant increase in their mean area percentage (%) were reported in subgroup 2a (STZ) as compared to the control group (group 1). This could be attributed to hyperglycemic-induced disturbed protein metabolism with the resulting disturbed deposition of extracellular matrix proteins ending with fibrosis. Moreover, activation of the macrophages with the consequent release of the inflammatory cytokines stimulates fibroblasts proliferation with increased deposition of collagen fibers. Also, hyperglycemia is associated with coagulation disorders with activation of thrombin that ends with tissue hypoxia and fibrosis[11].

Moreover, stimulation of macrophages to release several inflammatory cytokines, including IL-6 which in turn attracts more inflammatory cells. So, stimulates fibroblast proliferation with fibrosis. Also, hyperglycemia is associated with coagulation disorders with activation of thrombin that follow a coagulation cascade ends with hypoxia and tissue fibrosis[11].

Oppositely, decreased deposition of collagen fibers as well as their significantly decreased mean area percentage were observed in subgroups 2b, 2c and 2d as compared to subgroup 2a with the best results with the combination of enoxaparin and swertiamarin in subgroup 2d. This could be attributed to the antifibrotic effect of enoxaparin that was previously proved in other studies owed to its anticoagulant effect as thrombin was found to induce tissue fibrosis during cell injury[20]. Additionally, it was stated that swertiamarin could modulate the extracellular matrix protein deposition and so reduce collagen fibers deposition through reducing the hyperglycemia and improving the tissue metabolism[20,53].

Regarding the number and mean percentage of PCNA-positive islet cells, subgroup 2a showed a significant decrease in their values in comparison to control group 1. The finding could be explained by the diabetic-associated oxidative stress that causes β-cell dysfunction and death secondary to defective synthesis of important macromolecules including proteins, lipids and DNA. Also, the oxidative stress increases the activity of the DNA repair enzyme, poly ADP-Ribose polymerase-1 (PARP-1) with the resulting reduction of the intracellular NAD that ends with ATP depletion and consequently, pancreatic β-cell death. Moreover, inflammation induces the apoptotic signaling cascade that ends with cell death[54].

However, a significant increase in the number and mean percentage of the islet cells positive for PCNA was observed in the STZ treated subgroups 2b, 2c and 2d especially in the subgroup 2d with enoxaparin, and swertiamarin compared to subgroup 2b. This could be attributed to the improvement of the vascular environment by enoxaparin as well as the antioxidant and anti-inflammatory actions of swertiamarin that together improve both cell survival and tissue renewal[15,55].

Heat shock proteins (HSPs) are important proteins that are synthesized inside the cells with important physiological functions. They are important for the assembly, translocation, and maintenance of the integrity of polypeptides as molecular chaperones. Their expression increases when the cells are exposed to stressful conditions like oxidative stress, inflammation and apoptosis[31]. In the present research, a positive cytoplasmic reaction of HSP60 in the islet cells was recorded with a significant increase in its mean color intensity in subgroup 2a in respect to group 1. Earlier studies recorded that DM-induced hyperglycemia causes modification of the cellular proteins with altered expression of the molecular chaperones[56]. Other studies also illustrated that the DM- associated oxidative stress as well as the release of pro-inflammatory cytokines like TNF-α and IL-6 induces release of cell stress proteins such as HSP60 in order to protect the cells from the DM injurious effects[57].

The present research also showed a decreased HSP60 cytoplasmic expression in the islet cells with a significantly decreased mean color intensity in subgroups 2b, 2c and 2d in respect to subgroup 2a with the best results in the combined treatment with both enoxaparin and swertiamarin in subgroup 2d. Previous studies proved that enoxaparin as well as swertiamarin have hypoglycemic, hypolipidemic as well as anti-inflammatory and antioxidant effects. Taken together, these effects could restore the normal pancreatic β- cell functions with restoration of the normal metabolism so, decreasing cell stress and HSP60 expression[48,58].

CONCLUSION

Results of the current study revealed that STZ- induced type 2 DM caused structural and biochemical changes in the islet cells of the pancreas especially β-cells with insulin resistance and altered cell metabolism. Additionally, the combined use of enoxaparin and swertiamarin ameliorated these previously mentioned changes more than their separate use, which was proved through the antifibrotic and anti-inflammatory effects of enoxaparin, and the hypoglycemic, hypolipidemic, antioxidant and anti-inflammatory actions of swertiamarin.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Ohiagu FO, Chikezie PC, Chikezie CM: Pathophysiology of diabetes mellitus complications: Metabolic events and control. Biomed. Res. Ther. (2021) 8(3) 4243-4257.
2. Hegazi R, El-Gamal M, Abdel-Hady N, Hamdy O: Epidemiology of and risk factors for type 2 diabetes in Egypt. Annals of global health. (2015) 81(6) 814-820.
3. American Diabetes Association: 2. Classification and diagnosis of diabetes: Standards of Medical Care in Diabetes—2021. Diabetes care. (2021) 44(Supplement 1) S15-S33.
4. Cho N, Shaw J, Karuranga S, Huang Y, da Rocha Fernandes J, Ohlrogge A, Malanda B: IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res. Clin. Pract. (2018) 138 271-281.
5. Keenan HA, Sun JK, Levine J, Doria A, Aiello LP, Eisenbarth G, Bonner-Weir S, King GL: Residual insulin production and pancreatic β-cell turnover after 50 years of diabetes: Joslin Medalist Study. Diabetes. (2010) 59(11- 22) 2846-2853.
6. Cerf ME: Beta cell dysfunction and insulin resistance. Front. Endocrinol. (2013) 4 37- 48.
7. Kitada M, Zhang Z, Mima A, King GL: Molecular mechanisms of diabetic vascular complications. J. Diabetes Investig. (2010) 1(3) 77-89.
8. Paneni F, Beckman JA, Creager MA, Cosentino F: Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: part I. Eur. Heart J. (2013) 34(31) 2436-2443.
9. Scherer PE: The many secret lives of adipocytes: implications for diabetes. Diabeto. (2019) 62(2) 223-232.
10. Czech MP: Mechanisms of insulin resistance related to white, beige, and brown adipocytes. Mol. Metab. (2020) 34 27-42.
11. Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, Ostolaza H, Martín C: Pathophysiology of type 2 diabetes mellitus. Int. J. Mol. Sci. (2020) 21(17) 6275.
12. Schofield JH, Schafer ZT: Mitochondrial reactive oxygen species and mitophagy: a complex and nuanced relationship. Antioxid. Redox Signal. (2021) 34(7) 517-530.
13. Kalaska B, Miklosz J, Kamiński K, Swieton J, Jakimczuk A, Yusa SI, Pawlak D, Nowakowska M, Szczubiałka K, Mogielnicki A: Heparin-binding copolymer as a complete antidote for low molecular weight heparins in rats. J. Pharmacol. Exp. Ther. (2020) 373(1) 51-61.
14. Tang B, Qian Y, Fang G: Development of lipid polymer hybrid nanoparticles for improving oral absorption of enoxaparin. Pharmaceutics. (2020) 12(7) 607- 618.
15. Li LF, Liu YY, Lin SW, Chang CH, Chen NH, Hung CY, Lee CS: Low molecular weight heparin reduces ventilation-induced lung injury through hypoxia inducible factor-1α in a murine endotoxemia model. Int. J. Mol. Sci. (2020) 21(9) 3097- 3105.
16. Guzman-Valdivia Gomez G, Linares-Rivera E, Tena-Betancourt E, Arroyo-Del Castillo G, Reipen L: Prevention of postoperative abdominal adhesions using systemic enoxaparin and local diclofenac. An experimental study. Surg. Pract. (2020) 24(1) 4-10.
17. Ahamad J, Uthirapathy ST, Anwer ES, Mohammed Ameen M, Samad A: A critical review on potential pharmacological activity and pharmacokinetic perspective of swertiamarin. Res. J. Phytochem. (2021) 15(1) 1-13.
18. Fadzil NSM, Sekar M, Gan SH, Bonam SR, Wu YS, Vaijanathappa J, Ravi S, Lum PT, Dhadde SB: Chemistry, pharmacology and therapeutic potential of swertiamarin. A promising natural lead for new drug discovery and development. Drug Des. Devel. Ther. (2021) 15 2721.
19. Jaishree V, Narsimha S: Swertiamarin and quercetin combination ameliorates hyperglycemia, hyperlipidemia and oxidative stress in streptozotocin-induced type 2 diabetes mellitus in wistar rats. Biomed. Pharmacother. (2020) 130 110561- 110572.

20. Li CJ, Yang ZH, Shi XL, Liu DL: Effects of aspirin and enoxaparin in a rat model of liver fibrosis. World J. Gastroenterol. (2017) 23(35) 6412- 6423.

21. Sonawane RD, Vishwakarma SL, Lakshmi S, Rajani M, Padh H, Goyal RK: Amelioration of STZ-induced type 1 diabetic nephropathy by aqueous extract of Enicostemma littorale Blume and swertiamarin in rats. Mol. Cell. Biochem. (2010) 340(1) 1-6.

22. Laferriere CA, Pang DS: Review of intraperitoneal injection of sodium pentobarbital as a method of euthanasia in laboratory rodents. J. Am. Assoc. Lab. Anim. Sci. (2020) 59(3) 254-263.

23. Selvam R, Muruganantham K, Subramanian S: Biochemical evaluation of antidiabetic properties of swertiamarin, a secoiridoid glycoside of enicostemma littorale leaves, studied in high-fat diet-fed low-dose streptozotocin-induced type 2 diabetic rats. Asian J. Pharm. Clin. Res. (2018) 11(10) 486-492.

24. Sacks DB: Measurement of hemoglobin A1c: a new twist on the path to harmony. Diabetes care. (2012) 35(12) 2674-2680.

25. Abdel-Moneim AMH, Lutfi MF, Alsharidah AS, Shaker G, Faisal W, Abdellatif AA, Rugaie OA, Mohany KM, Eid SYEl-Readi MZ: Short-term treatment of metformin and glipizide on oxidative stress, lipid profile and renal function in a rat model with diabetes mellitus. Appl. Sci. (2022) 12(4) 2019.

26. Mohamed RAA, Noor BYM, Ahmed SM, Dafalla AM, Abdelhameed Mohammed Y, Modawe GO: Assessment of lipid profile among sudanese patients with type 2 Diabetes Mellitus. AL-Kindy Col. Med J. (2022) 18(1) 30-35.

27. Okaichi Y, Ishikura Y, Akimoto K, Kawashima H, Toyoda-Ono Y, Kiso Y, Okaichi H: Arachidonic acid improves aged rats' spatial cognition. Physiol. Behav. (2005) 84(4) 617-623.

28. Selvam R, Muruganantham K, Subramanian S: Antioxidant properties of swertiamarin, from blume. leaves Enicostemma littorale studied in high fat diet fed and low dose streptozotocin induced diabetic rats. Asian J. Pharm. Pharmacol. (2019) 5(2) 344-352.

29. Ali EN, Eltyeaf EM, Kadhem AA, Jouda J: Effect of Artemisia fruit extract on TNF-α and IL-6 levels in streptozotocin-induced diabetic mice. J. contemp. Med. Sci. (2019) 5 51-58.

30. Suvarna KS, Layton C, Bancroft JD: Bancroft's theory and practice of histological techniques E-Book. 8th edition., Elsevier Health Sciences. UK. (2018)pp: 50-120.

31. Mallard K, Jones DB, Richmond J, McGill M, Foulis AK: Expression of the human heat shock protein 60 in thyroid, pancreatic, hepatic and adrenal autoimmunity. J. Autoimmun. (1996) 9(1) 89-96.

32. Zanin-Zhorov A, Cahalon L, Tal G, Margalit R, Lider O, Cohen IR: Heat shock protein 60 enhances CD4+ CD25+ regulatory T cell function via innate TLR2 signaling. J. Clin Investig. (2006) 116(7) 2022-2032.

33. Koivisto C, Flake GP, Kolenda-Roberts H, Masinde T, Kissling GE, Sills RC, Hoenerhoff MJ: Immunohistochemical investigation of F344/N rat islet cell tumors from national toxicology program studies. Toxicol. Pathol. (2012) 40(5) 751-763.

34. Zhu L, Sha L, Li K, Wang Z, Wang T, Li Y, Liu P,Dong X, Dong Y, Zhang X: Dietary flaxseed oil rich in omega-3 suppresses severity of type 2 diabetes mellitus via anti-inflammation and modulating gut microbiota in rats. Lipids Health Dis. (2020) 19(1) 1-16.

35. Zafar M, Naqvi SNul-H: Effects of STZ-Induced diabetes on the relative weights of kidney, liver and pancreas in albino rats: a comparative study. Int. J. Morphol. (2010) 28(1) 135- 142.

36. Karganov MY, Alchinova IB, Tinkov AA, Medvedeva YS, Lebedeva MA, Ajsuvakova OP, Polyakova MV, Skalnaya MG, Burtseva Tinotova SV: Streptozotocin (STZ)-induced diabetes affects tissue trace element content in rats in a dose-dependent manner. Biol. Trace Elem Res. (2020) 198(2) 567-574.

37. Magalhaes DA, Kume WT, Correia FS, Queiroz TS, Allebrandt EW, Santos MP, Kawashita NH, Franca SA: High-fat diet and streptozotocin in the induction of type 2 diabetes mellitus: a new proposal. An. Acad. Bras. Cienc. (2019) 91(1) e20180314- e20180328.

38. Vique-Sanchez JL, Lopez-Palacios TP, Miranda-Ozuna JF, Benitez-Cardoza CG: Effects of W100E-Leptin in streptozotocin-induced diabetic mice. Nutr. Clin. Diet Hosp. (2020) 40(3) 153-161.

39. Georgescu A, Popov D, Capraru M, Simionescu M: Enoxaparin-a low molecular weight heparin, restores the altered vascular reactivity of resistance arteries in aged and aged–diabetic hamsters. Vascu. Pharmacol. (2003) 40(3) 167-174.

40. McLaughlin CM, Sharkey SJ, Harnedy-Rothwell P, Parthsarathy V, Allsopp PJ, McSorley EM, FitzGerald RJ, O 'Harte FP: Twice daily oral administration of Palmaria palmata protein hydrolysate reduces food intake in streptozotocin induced diabetic mice, improving glycaemic control and lipid profiles. J. Funct. Foods. (2020) 73 104101- 104112.

41. Durg S, Bavage S, Shivaram SB: Withania somnifera (Indian ginseng) in diabetes mellitus: a systematic review and meta-analysis of scientific evidence from experimental research to clinical application. Phytother. Res. (2020) 34(5) 1041-1059.

42. Vaidya H, Goyal RK, Cheema SK: Anti-diabetic activity of swertiamarin is due to an active metabolite, gentianine, that upregulates PPAR-γ gene expression in 3T3-L1 cells. Phytother. Res. (2013) 27(4) 624-627.

43. Chigurupati S, Dhanaraj SA, Balakumar P: A step ahead of PPARγ full agonists to PPARγ partial agonists: therapeutic perspectives in the management of diabetic insulin resistance. Eur. J. Pharmacol. (2015) 755 50-57.

44. Patel TP, Soni S, Parikh P, Gosai J, Chruvattil R, Gupta S: Swertiamarin: An active lead from Enicostemma littorale regulates hepatic and adipose tissue gene expression by targeting PPAR-γ and improves insulin sensitivity in experimental NIDDM rat model. Evid. Based complement. Alternat. Med. (2013) 2013.

45. Blake R, Trounce IA: Mitochondrial dysfunction and complications associated with diabetes. Biophys. Acta Gen. Subj. (2014) 1840(4) 1404-1412.

46. Vasu VT, Modi H, Thaikoottathil JV, Gupta S: Hypolipidaemic and antioxidant effect of Enicostemma littorale Blume aqueous extract in cholesterol fed rats. J. Ethnopharmacol. (2005) 101(1-3) 277-282.

47. Joshi MB, Lad A, Prasad ASB, Balakrishnan A, Ramachandra L, Satyamoorthy K: High glucose modulates IL-6 mediated immune homeostasis through impeding neutrophil extracellular trap formation. FEBS lett. (2013) 587(14) 2241-2246.

48. Lean QY, Eri RD, Randall-Demllo S, Sohal SS, Stewart N, Peterson GM, Gueven N, Patel RP: Orally administered enoxaparin ameliorates acute colitis by reducing macrophage-associated inflammatory responses. PLoS One. (2015) 10(7) e0134259- e0134270.

49. Koksoy FN, Yankol Y, Sen Oran E, Ozkan Gurdal S, Yuksel M, Akyildiz Igdem A, Yildirim Yazgan N, Soybir GR: Preventive effects of enoxaparin and hesperidin in cerulein-induced acute pancreatitis in rats. Turk. J. Gastroenterol. (2013) 24(6) 495-501.

50. Lad H, Bhatnagar D: Amelioration of oxidative and inflammatory changes by Swertia chirayita leaves in experimental arthritis. Inflammopharmacol. (2016) 24(6) 363-375.

51. Abunasef SK, Amin HA, Abdel-Hamid GA: A histological and immunohistochemical study of beta cells in streptozotocin diabetic rats treated with caffeine. Folia Histochem. Cytobiol. (2014) 52(1) 42-50.

52. Morey M, O'Gaora P, Pandit A, Hélary C: Hyperglycemia acts in synergy with hypoxia to maintain the pro-inflammatory phenotype of macrophages. PloS one. (2019) 14(8) e0220577- e0220588.

53. Chen J, Liu J, Lei Y, Liu M: The anti-inflammation, anti-oxidative and anti-fibrosis properties of swertiamarin in cigarette smoke exposure-induced prostate dysfunction in rats. Aging (Albany NY). (2019) 11(22) 10409- 10420.

54. Sadi G, Sahin G, Bostanci A: Modulation of renal insulin signaling pathway and antioxidant enzymes with streptozotocin-induced diabetes: effects of resveratrol. Medicina. (2019) 55(1) 3- 11.

55. Dhanavathy G: Immunohistochemistry, histopathology, and biomarker studies of swertiamarin, a secoiridoid glycoside, prevents and protects streptozotocin-induced β-cell damage in Wistar rat pancreas. . J. Endocrinol. Invest. (2015) 38(6) 669- 684.

56. Yamagishi N, Nakayama K, Wakatsuki T, Hatayama T: Characteristic changes of stress protein expression in streptozotocin-induced diabetic rats. Life sci. (2001) 69(22) 2603-2609.

57. Bellini S, Barutta F, Mastrocola R, Imperatore L, Bruno G, Gruden G: Heat shock proteins in vascular diabetic complications: Review and future perspective. Int. J. Mol. Sci. (2017) 18(12) 2709- 2720.

58. Konda PY, Dasari S, Konanki S, Nagarajan P: In *vivo* antihyperglycemic, antihyperlipidemic, antioxidative stress and antioxidant potential activities of Syzygium paniculatum Gaertn. in Streptozotocin-induced diabetic rats. Heliyon. (2019) 5(3) e01373- e01383.

الملخص العربى

تأثير الإنوكسابارين وسوارتيامارين علي داء السكري المستحث بالستربتوزوتوسين في الجرذان: دراسة كيميائية حيوية ونسيجية وكيميائية مناعية

سماح قنديل، منى تيسير صادق

قسم الهستولوجى وبيولوجيا الخلية - كلية الطب - جامعة طنطا

المقدمة: داء السكري هو إضطراب أيضي مزمن يتميز بإرتفاع مستويات السكر في الدم. إنوكسابارين هوهيبارين منخفض الوزن الجزيئي له نشاط مضاد للتخثرومضاد للإلتهابات ومضاد للتليف. سوارتيامارين هوجليكوزيد سيكوريدويد له نشاط خافض لسكر ودهون الدم ومضاد للإلتهابات ومضاد للأكسدة.

الهدف: تقييم تأثيرالإنوكسابارين وسوارتيامارين علي داء السكري المستحث بالستربتوزوتوسين في الجرذان بإستخدام دراسات نسيجية وكيميائية مناعية وكيميائية حيوية .

المواد والطرق: تم إستخدام سبعون جرذا ًمن جرذان ويستار(١٠-١٢ أسبوعًا) ,وزن ١٢٠-١٥٠ جرام مقسمة إلى مجموعتين رئيسيتين ؛المجموعة ١ (المجموعة الضابطة: ٢٠ جرذًا) والمجموعة ٢ (داء السكرى الناجم عن ستربتوزوتوسين : ٥٠ جرذًا): داء السكرى الناجم عن إعطاء ٥٠ مجم/كجم جرعة واحدة من ستربتوزوتوسين داخل الصفاق ؛ أصيب ٤٠ جرذًا فقط بالسكري وتم تقسيمهم إلى ٤ مجموعات فرعية متساوية ؛المجموعة الفرعية ٢ أ :(داء السكري المستحث بستربتوزوتوسين) تركت دون علاج، المجموعة الفرعية ٢ ب (ستربتوزوتوسين+ إنوكسابارين): عولجت بـإنوكسابارين ٢ مجم/كجم/يوم تحت الجلد لمدة ٣ أسابيع ، المجموعة الفرعية ٢ س (ستربتوزوتوسين + سوارتيامارين): عولجت بـسوارتيامارين ٥٠ مجم/كجم/يوم عن طريق الفم لمدة ٣ أسابيع والمجموعة الفرعية ٢ د (ستربتوزوتوسين+ إنوكسابارين + سوارتيامارين) :عولجت بإنوكسابارين وسوارتيامارين بنفس الجرعات والفترة السابقة.

النتائج: أظهرت المجموعة الفرعية ٢ أ ((STZ) إنخفاضًا في وزن الجسم والبنكرياس والنسبة مع إرتفاع مستوى السكر في الدم ،والبلازما HbA١c ، الكوليسترول الكلي ، LDL ، TGs ، إنخفاض HDL ، إنخفاض SOD و GSH في الأنسجة مع إرتفاع MDA و TNF-α و IL-٦ فى الأنسجة. وقد تبين وجود إحتقان وإتساع الشعيرات الدموية ، وجود فجوات سيتوبلازمية وإنكماش أنوية خلايا ألف وبيتا فى نتائج الهيماتوكسلين والإيوسن مع زيادة نسبة منطقة الكولاجين، وكثافة لون HSP٦٠ وإنخفاض نسبةPCNA . أظهرت المجموعات الفرعية المعالجة (٢ ب ، ٢ ج ، ٢ د) تحسنًا ملحوظًا في النتائج التى ذكرت مسبقًا خاصة في المجموعة الفرعية ٢ د.

الخلاصة: أظهر الجمع بين إستخدام الإنوكسابارين وسوارتيامارين أفضل النتائج في تحسين التغيرات الكيميائية الحيوية والتركيبية التي يسببها النوع الثانى من داء السكرى على خلايا جزرالبنكرياس فى الجرذان مقارنة بإستخدامهما منفصلين.