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Comparative Effect of Roflumilast, Dapagliflozin and Etanercept on Cardiovascular Outcomes in Diabetic Rats

Ghada S. Ewies, Sawsan Sadik, Sara Wahib Fayek, Aymen Helal

Faculty of Medicine, Fayoum University, Egypt.
Email: gsa03@fayoum.edu.eg

Abstract: The aim of the present work is to clarify the potential protective effect of Roflumilast, Dapagliflozin and Etanercept on cardiovascular outcomes in diabetic rats. Diabetes mellitus was induced in male albino rats by intraperitoneal injection of a single dose of Streptozotocin (STZ) (65 mg/kg). Rats were divided into two main groups; control groups (Normal, Roflumilast, Dapagliflozin and Etanercept Control groups) and diabetic groups (Diabetic, STZ+Roflumilast, STZ+Dapagliflozin and STZ+Etanercept groups). Roflumilast (0.5 mg\kg orally daily), Dapagliflozin (1mg\kg orally daily) and Etanercept (1 mg\kg subcutaneous twice \week) were administered for 21 days to study their effects on (blood glucose, glycosylated hemoglobin (HbA1c) and serum insulin) and (tumor necrosis factor-α (TNF-α), cardiotrophin and metalloproteinase-13 (MMP-13) in cardiac tissue). Electrocardiogram (ECG) data (heart rate HR, QT interval, QTc interval, R wave amplitude and ST deviation) were assessed. Cardiac contractility and histopathological examination were also performed. The present study revealed that diabetic rats showed a significant increase in blood glucose and HbA1c and a significant decrease in serum insulin. Roflumilast, Dapagliflozin and Etanercept significantly decreased blood glucose and HbA1c and significantly increased serum insulin. There was significant decrease in serum glucose level in (STZ+etanercept) group compared to (STZ+dapagliflozin) group. Moreover, there was significant decrease in glycosylated hemoglobin level in (STZ+dapagliflozin) compared to (STZ+roflumilast). Cardiac level of TNF-α, cardiotrophin and MMP-13 were significantly increased in diabetic rats. Roflumilast, Dapagliflozin and Etanercept significantly decreased TNF-α, cardiotrophin and MMP-13 in cardiac tissue. There was significant decrease in TNF-α in (STZ+etanercept) group compared to (STZ+roflumilast) and (STZ+dapagliflozin) groups. There was also significant decrease in cardiotrophin level in (STZ+dapagliflozin) and (STZ+etanercept) groups compared to (STZ+roflumilast) group. The present work revealed that diabetic rats presented a significant decrease in HR and a significant increase in QT, QTc intervals and ST deviation. Roflumilast and Etanercept significantly increased HR and significantly decreased QT, QTc intervals. Dapagliflozin significantly decreased QTc interval and ST deviation. Etanercept significantly increased ST deviation. There was significant decrease in QTc interval in (STZ+roflumilast) group compared to (STZ+dapagliflozin) and (STZ+etanercept) groups. There was also significant increase in ST deviation in (STZ+roflumilast), and (STZ+etanercept) groups compared to (STZ+dapagliflozin) group. Histopathological examination by H & E staining revealed that STZ-induced diabetes caused prominent widening between cardiomyocytes, degenerative changes of cardiomyocytes, vacuolation and inflammatory cell infiltration. Sections stained by Masson's Trichrome showed marked collagen deposition around cardiomyocytes and blood vessels. These changes were ameliorated with Roflumilast, Dapagliflozin and Etanercept treatment.
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Keywords: Diabetes mellitus; diabetic cardiomyopathy; cardiac dysfunction, Roflumilast; Dapagliflozin and Etanercept.

1. Introduction:

Diabetes mellitus increases the risk of cardiovascular diseases which are the leading causes of morbidity and mortality in diabetic patients. Heart failure (HF) is a particularly common complication of diabetes. Poorer glycemic control is an important predictor of HF development **(1).**

Diabetic cardiomyopathy (DCM), one of the cardiovascular complications in diabetic patients which leads to HF. DCM is defined as the existence of abnormal cardiac structure and performance in the absence of coronary artery disease, hypertension, and significant valvular disease. Hyperglycemia, hyperinsulinemia and insulin resistance mediate the

pathological remodeling of the heart which are characterized by left ventricle concentric hypertrophy and perivascular and interstitial fibrosis leading to diastolic dysfunction. A change in the metabolic status, impaired calcium homeostasis and energy production, increased inflammation and oxidative stress, as well as an accumulation of advanced glycation end products are among the mechanisms implicated in the pathogenesis of diabetic cardiomyopathy **(2).**

Roflumilast is a potent inhibitor of the phosphodiesterase-4 (PDE-4) pathway and is reported to have potent anti-inflammatory properties such as inhibiting hydrolysis of cyclic adenosine monophosphate (c-AMP) in inflammatory cells and decreasing neutrophilic release of inflammatory mediators and cytokines while decreasing apoptosis and expression of cell surface markers **(3).** The increased cAMP levels directly block secretion of various cytokines especially tumor necrosis factor-α (TNF-α), interleukin-1b (IL-1b) and transforming growth factor-β (TGF-β) in various cells. The decreased (TGF-β) levels and the direct effects of Roflumilast on fibroblast functions ameliorate fibrosis. Furthermore, Roflumilast diminished reactive oxygen species (ROS) production which can lead to fibrotic changes **(4).**

Dapagliflozin is an oral anti-hyperglycemic drug, classified as a selective sodium-glucose cotransporter 2 inhibitor (SGLT2- inhibitor). Its mode of action is inhibition of glucose reabsorption in the renal proximal tubule leading to intensified glucose and sodium excretion, loss of caloric value and lowering of blood pressure **(5).** Sodium-glucose cotransporter 2 inhibitors (SGLT-2) enhance glucosuria independently of insulin so they promote weight loss and do not induce hypoglycemia. Furthermore, by reducing glucotoxicity, they indirectly improve both β-cell function and insulin. They affect positively several recognized cardiovascular risk factors: weight reduction, drop in arterial blood pressure and reduction in serum uric acid levels which is attributed to enhanced urinary excretion. **(6).** Recently, despite the lack of myocardial SGLT-2-expression, direct effects of the drug on heart muscle cells have even been suggested. There is evidence that the sodium hydrogen exchanger (NHE) may play an important role in the interplay of heart failure and diabetes since renal and cardiac isoforms of the NHE are upregulated in both conditions. Inhibition of NHE by SGLT-2 inhibitors and modulation of intramyocardial Ca+2 and Na+ fluxes seems to have a beneficial impact on diastolic myocardial function **(7).**

Etanercept, a human recombinant soluble tumor necrosis factor-α (TNF-α) receptor protein, is considered to be an effective TNF-α inhibitor. It can competitively combine with TNF-α and then block the combination of TNF-α and its receptor, resulting in inhibition of TNF-α activity. Tumor necrosis factor-α (TNF-α) level is increased in patients with heart failure. A previous study has further revealed that overexpressed TNF-α in rats exerts a detrimental role and may promote the occurrence and development of cardiomyocyte hypertrophy and dilated cardiomyopathy. Thus, inhibition of TNF-α expression may be beneficial for cardiomyocyte hypertrophy **(8).**

2. Materials and methods

2.1. Animals:

In the present study, 48 adults, age matched male albino rats of Wistar strain weighing about 150-200 g were used. The rats were obtained from the animal house of the National Research institute-Egypt. All rats were kept under observation for at least one week prior to the study with free access to food and water. Rats were exposed to daily light/ dark cycle. All rats were put in pairs in wire cages under good sanitary conditions and normal humidity in accordance with the guidelines of the Institutional Animal Care and Use Committee.

2.2. Drugs and experimental design:

2.2.1 Drugs:

Streptozotocin (powder): supplied by (Sigma Aldrich Co). Roflumilast (Daliresp powder): supplied by (Sigma Aldrich Co). Dapagliflozin: (Farxiga tablet): supplied by (Bristol-Myers Squibb) Each tablet contains 10mg. Etanercept (Enbrel vial): supplied by (Pfizer), Each vial contains (25 mg).

All drugs were dissolved in distilled water before use. The doses were chosen according to the previous studies.

2.2.2. Induction of diabetes mellitus in rats:

Twenty-four rats received Streptozotocin (STZ) by a single intraperitoneal (i.p) injection of 65 mg/kg (freshly dissolved in 0.1 mol/l sodium citrate buffer (pH 4.5) after an overnight fast. 72 hours after the STZ injection, rats fasted for 6 hours. Blood glucose was analyzed from a tail-vein blood sample using a One Touch Basic blood glucose monitoring system to ensure hyperglycemia in the STZ-treated rats. Rats with blood glucose level higher than 250 mg/dL were considered diabetic (**9**).

2.2.3 Experimental groups: Animals were divided into two main groups:

Group I: Control Groups (24 rats):

Consist of four subgroups of six rats each:

Subgroup 1- Normal Control:

Rats received single dose of distilled water (1ml) intraperitoneally and distilled water orally daily for 21 days.

Subgroup 2- Roflumilast Control:

Rats received single dose of distilled water (1ml) intraperitoneally and Roflumilast 0.5 mg/kg orally daily for 21 days **(10).**

Subgroup 3- Dapagliflozin Control:

Rats received single dose of distilled water (1ml) intraperitoneally and Dapagliflozin 1mg/kg orally daily for 21 days **(11).**

Subgroup 4 - Etanercept Control:

Rats received single dose of distilled water (1ml) intraperitoneally and Etanercept 1mg/kg subcutaneous twice/week for 21 days **(12).**

Group II: Diabetic Groups (24 rats).

The diabetic rats were divided into four subgroups:

Subgroup 1- Diabetic group:

Rats received Streptozotocin (STZ) by a single intraperitoneal (i.p.) injection of 65 mg/kg **(9).**

Subgroup 2- Streptozotocin + Roflumilast:

Rats received Streptozotocin by single intraperitoneal injection of 65mg/kg **(9)** and Roflumilast 0.5 mg/kg orally daily for 21 days **(10).**

Subgroup 3- Streptozotocin + Dapagliflozin:

Rats received Streptozotocin by single intraperitoneal injection of 65 mg/kg (9) and Dapagliflozin 1mg/kg orally daily for 21 days (11).

Subgroup 4- Streptozotocin + Etanercept:

Rats received Streptozotocin by single intraperitoneal injection of 65mg /kg **(9)** and Etanercept 1 mg /kg subcutaneous twice/week for 21 days **(12).**

2.2.4 Measurements:

At the end of the experimental period (21 days):

- **ECG** was traced to asses heart rate (HR), QT, QTc intervals, R-wave amplitude and ST deviation.
- Blood of fasting rats was collected through retro orbital puncture using 10µl x20mm x 0.8 mm glass capillary under light anesthesia for estimation of blood glucose and glycosylated hemoglobin levels, then serum was separated for detection of insulin level.
- After that, hearts were washed with ice-cold saline and preserved for estimation of TNF-α, cardiotrophin, metalloproteinase 13 expression by enzyme-linked immunosorbent assay (ELISA). and for histopathological examination.
- The hearts were excised and preserved in 10% formalin solution, after fixation, Serial sections of 4 microns thickness were obtained and stained with: Hematoxylin and Eosin (H & E) and Masson's trichrome stains for light microscopic examination. H & E staining for detecting degeneration, hypertrophy, widening, inflammatory cell infiltration and vacuolation. Masson's trichrome staining for detecting fibrosis.

3. Statistical analysis:

The collected data was organized, tabulated and statistically analyzed using SPSS software (Version 16) on Windows 7. Mean and S.D. were calculated for quantitative variables in the form of simple descriptive analysis. Categorical data was analyzed by computing percentages, and differences were tested statistically by applying chi square tests for comparisons between groups; p-value of <0.05 was considered statistically significant. Comparison of quantitative values between groups with done using ANOVA test, post hoc test (LSD) was further done after ANOVA test for intergroup comparisons. Correlation of quantitative variables was done using Pearson correlation and expressed using correlation coefficient (r) with its value ranged from (-1 to 1).

4. Results:

4.1. Biochemistry data:

4.1.1. Blood glucose:

There was no significant difference in blood glucose level in Roflumilast, Dapagliflozin and Etanercept control groups compared to normal control group ($p>0.05$). On the other hand, there was significant increase in blood glucose level in diabetic group compared to normal, Roflumilast, Dapagliflozin and Etanercept control groups ($p<0.05$).

There was significant decrease in blood glucose level in (STZ+Roflumilast), (STZ+Dapagliflozin) and (STZ+Etanercept) groups compared to diabetic group There was significant decrease in serum glucose level in (STZ+Etanercept) group compared to (STZ+Dapagliflozin) group ($p<0.05$) (table 1).

4.1.2. Glycosylated hemoglobin (HbA1c):

There was no significant difference in glycosylated hemoglobin level in Roflumilast, Dapagliflozin and Etanercept control groups compared to normal control group ($p>0.05$). On the other hand, there was significant increase in glycosylated hemoglobin level in diabetic group compared to normal, Roflumilast, Dapagliflozin and Etanercept control groups ($p<0.05$).

There was significant decrease in glycosylated hemoglobin level in (STZ+Roflumilast), (STZ+Dapagliflozin) and (STZ+Etanercept) groups compared to diabetic group ($p<0.05$). There was significant decrease in HbA1c level in (STZ+Dapagliflozin) compared to (STZ+Roflumilast) ($p<0.05$) (table 1).

4.1.3. Serum insulin:

There was no significant difference in serum insulin level in Roflumilast and Dapagliflozin and Etanercept control groups compared to normal control group ($p>0.05$). On the other hand, there was significant decrease in serum insulin level in diabetic

group compared to normal, Roflumilast, Dapagliflozin and Etanercept control groups ($p<0.05$).

There was significant increase in serum insulin level in (STZ+Roflumilast), (STZ+Dapagliflozin) and (STZ+Etanercept) groups compared to diabetic group ($p<0.05$). On the other hand, there was no significant change in serum insulin level between (STZ+Roflumilast), (STZ+Dapagliflozin) and (STZ+Etanercept) groups ($p>0.05$) (table 1).

Table (1): Effect of Roflumilast, Dapagliflozin and Etanercept on blood glucose, serum insulin and glycosylated hemoglobin in diabetic and diabetic treated rats:

	Diabetic group	Diabetic-treated groups		
		STZ+Roflumilast	STZ+Dapagliflozin	STZ+Etanercept
Glucose (mg/dl)	270.1±25.27	169.73±14.66 ♠	175.85±20.5 **µ**	155.1±14.87 © **Θ**
Insulin (ng/ml)	0.94±0.07	1.74±0.23 ♠	1.55±0.27 **µ**	1.85±0.34 **Θ**
HbA1c (%)	9.83±0.72	5.73±0.53 ♠	5±0.32 **µ** #	5.1±0.67 **Θ**

Each value represents (mean ± standard deviation) (n=6).
♠ Significant at ($p<0.05$) compared diabetic group to (STZ+Roflumilast) group.
µ Significant at ($p<0.05$) compared diabetic group to (STZ+Dapagliflozin) group.
Θ Significant at ($p<0.05$) compared diabetic group to (STZ+Etanercept) group.
Significant at ($p<0.05$) compared (STZ+Roflumilast) group to (STZ+Dapagliflozin) group.
© Significant at ($p<0.05$) compared (STZ+Dapagliflozin) group to (STZ+Etanercept) group.

Table (2): Effect of Roflumilast, Dapagliflozin and Etanercept on cardiac TNF-α, cardiotrophin and MMP-13 in diabetic and diabetic treated rats

	Diabetic group	Diabetic-treated groups		
		STZ+Roflumilast	STZ+Dapagliflozin	STZ+Etanercept
TNF-α (pg/ml)	108.83±8.9	57.50±6.42 ♠	57.6±4.16 **µ**	49.46±6.5 ©∞ **Θ**
Cardiotrophin (pg/ml)	150.03±9.16	78.40±7.97♠	63.10±11.39 **µ** #	69.13±7.1 ∞ **Θ**
MMP-13 (ng/ml)	2.96±0.659	1.08±0.102 ♠	1.19±0.115 **µ**	1.03±0.079 **Θ**

Each value represents (mean ± standard deviation) (n=6).
♠ Significant at ($p<0.05$) compared diabetic group to (STZ+Roflumilast) group.
µ Significant at ($p<0.05$) compared diabetic group to (STZ+Dapagliflozin) group.
Θ Significant at ($p<0.05$) compared diabetic group to (STZ+Etanercept) group.
Significant at ($p<0.05$) compared (STZ+Roflumilast) group to (STZ+Dapagliflozin) group.
∞ Significant at ($p<0.05$) compared (STZ+Roflumilast) group to (STZ+Etanercept) group.

4.1.4. Tumor necrosis factor-α (TNF-α):

There was significant increase in TNF-α level in Roflumilast control group compared to normal control group ($p<0.05$). There was no significant difference in TNF-α level in Dapagliflozin and Etanercept control groups compared to normal control group ($p>0.05$). On the other hand, there was significant increase in TNF-α level in diabetic group compared to normal, Roflumilast, Dapagliflozin and Etanercept control groups ($p<0.05$).

There was significant decrease in TNF-α in (STZ+Roflumilast), (STZ+Dapagliflozin) and (STZ+Etanercept) groups compared to diabetic group ($p<0.05$). There was also significant decrease in TNF-α in (STZ+Etanercept) group compared to (STZ+Roflumilast) and (STZ+Dapagliflozin) groups ($p<0.05$) (table 2).

4.1.5. Cardiotrophin:

There was no significant difference in cardiotrophin level in Roflumilast, Dapagliflozin and Etanercept control groups compared to normal control group ($p>0.05$). On the other hand, there was significant increase in cardiotrophin level in diabetic group compared to normal, Roflumilast and Dapagliflozin and Etanercept control groups ($p<0.05$).

There was significant decrease in cardiotrophin level in (STZ+Roflumilast), (STZ+Dapagliflozin) and (STZ+Etanercept) groups compared to diabetic group

(p<0.05). There was significant decrease in cardiotrophin level in (STZ+Dapagliflozin) and (STZ+Etanercept) groups compared to (STZ+Roflumilast) group (p<0.05) (table 2).

4.1.6. Metalloproteinase -13 (MMP-13):

There was significant increase in MMP-13 level in Roflumilast control group compared to normal control group (p<0.05). On the other hand, there was no significant difference in MMP-13 level in Dapagliflozin and Etanercept control groups compared to normal control group (p>0.05). There was significant increase in MMP-13 level in diabetic group compared to normal, Roflumilast, Dapagliflozin and Etanercept control groups (p<0.05).

There was significant decrease in MMP-13 level in (STZ+Roflumilast), (STZ+Dapagliflozin) and (STZ+Etanercept) groups compared to diabetic group (p<0.05). On the other hand, there was no significant difference between (STZ+Roflumilast), (STZ+Dapagliflozin) and (STZ+Etanercept) groups (p>0.05) (table 2).

4.2. ECG data:

4.2.1. Heart rate (HR):

There was no significant change in heart rate between Roflumilast, Dapagliflozin and Etanercept control groups compared to normal control group (p>0.05). There was significant decrease in heart rate in diabetic group compared to normal, Dapagliflozin and Etanercept control groups (p<0.05). On the other hand, there was no significant change in heart rate between Roflumilast control and diabetic groups (p>0.05).

There was significant increase in heart rate in (STZ+Roflumilast) and (STZ+Etanercept) groups compared to diabetic group (p<0.05). On the other hand, there was no significant change in heart rate between (STZ+Dapagliflozin) and diabetic groups (p>0.05). There was also no significant change in heart rate between (STZ+Roflumilast), (STZ+Dapagliflozin) and (STZ+Etanercept) groups (p>0.05) (figure 1).

4.2.2. QT interval:

There was no significant change in QT interval between Roflumilast, Dapagliflozin and Etanercept control groups compared to normal control group (p>0.05). There was significant increase in QT interval in diabetic group compared to normal, Dapagliflozin and Etanercept control groups (p<0.05). On the other hand, there was no significant change in QT interval between Roflumilast control and diabetic groups (p>0.05).

There was significant decrease in QT interval in (STZ+Roflumilast) and (STZ+Etanercept) groups compared to diabetic group (p<0.05). On the other hand, there was no significant change in QT interval between (STZ+Dapagliflozin) and diabetic groups (p>0.05). There was also no significant change in QT interval between (STZ+Roflumilast), (STZ+Dapagliflozin) and (STZ+Etanercept) groups (p>0.05) (figure2).

4.2.3. QTc interval:

There was no significant change in QTc interval between Roflumilast, Dapagliflozin and Etanercept control groups compared to normal control group (p>0.05). There was significant increase in QTc interval in diabetic group compared to normal, Dapagliflozin and Etanercept control groups (p<0.05). On the other hand, there was no significant change in QTc interval between Roflumilast control and diabetic groups (p>0.05).

There was significant decrease in QTc interval in (STZ+Roflumilast), (STZ+Dapagliflozin) and (STZ+Etanercept) groups compared to diabetic group (p<0.05). There was also significant decrease in QTc interval in (STZ+Roflumilast) group compared to (STZ+Dapagliflozin) and (STZ+Etanercept) groups (p<0.05) (figure 3).

4.2.4. R wave amplitude:

There was significant decrease in R wave amplitude in Roflumilast and Dapagliflozin groups control compared to normal control group (p<0.05). There was no significant change in R wave amplitude between Etanercept and normal control groups (p>0.05). There was significant increase in R wave amplitude in diabetic group compared to Roflumilast, Dapagliflozin control groups (p<0.05). On the other hand, there was no significant change in R wave amplitude between Etanercept and normal control groups compared to diabetic group (p>0.05).

There was no significant change between (STZ+Roflumilast), (STZ+Dapagliflozin) and (STZ+Etanercept) groups compared to diabetic group (p>0.05). There was significant decrease in R wave amplitude in (STZ+Roflumilast) group compared to (STZ+Dapagliflozin) group (p<0.05) (figure 4).

4.2.5. ST deviation:

There was no significant change in ST deviation between all control groups (p>0.05). There was significant increase in ST deviation in diabetic group compared to normal, Roflumilast, Dapagliflozin and Etanercept control groups (p<0.05).

There was significant decrease in ST deviation in (STZ+Dapagliflozin) and compared to diabetic group (p<0.05). There was significant increase in ST deviation in (STZ+Etanercept) compared to diabetic group (p<0.05). There was significant increase in ST deviation in (STZ+Roflumilast), and (STZ+Etanercept) groups compared to (STZ+Dapagliflozin) group (p<0.05). On the other hand, there was no significant change in ST deviation in (STZ+Roflumilast) group compared to diabetic group (p>0.05) (Figure 5).

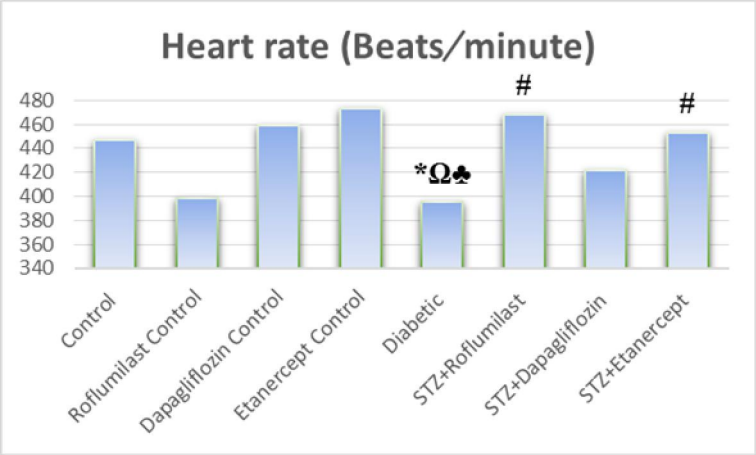

Figure (1): Heart rate (beats/minute) in all studied groups.
* (p<0.05) compared to control group. Ω (p<0.05) compared to Dapagliflozin group.
# (p<0.05) compared to diabetic group. ♣ (p<0.05) compared to Etanercept group.

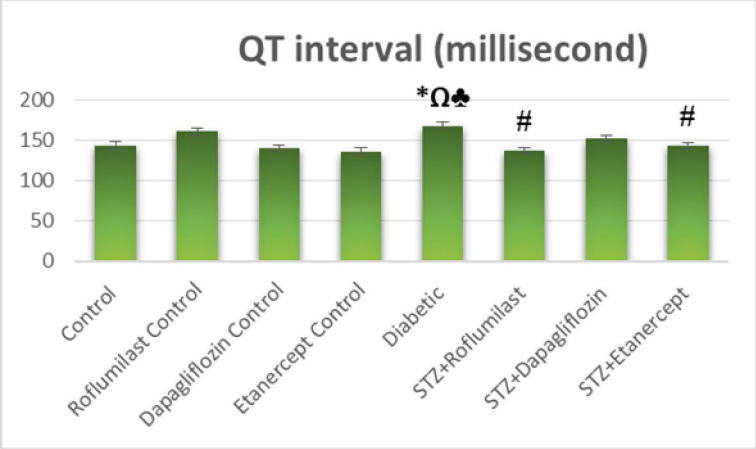

Figure (2): QT interval (millisecond) in all studied groups.
* (p<0.05) compared to control group . ♣ (p<0.05) compared to Etanercept group.
Ω (p<0.05) compared to Dapagliflozin group. # (p<0.05) compared to diabetic group.

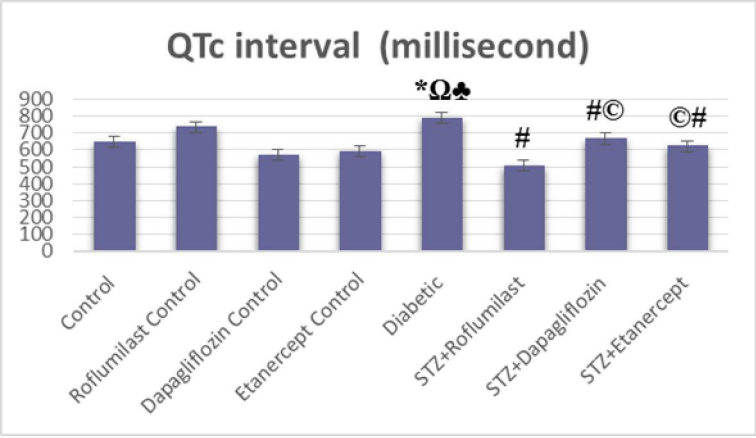

Figure (3): QTc (millisecond) interval in all studied groups.
* (p<0.05) compared to control group. Ω (p<0.05) compared to Dapagliflozin group.
♣ (p<0.05) compared to Etanercept group. # (p<0.05) compared to diabetic group.
© (p<0.05) compared to (STZ+Roflumilast) group.

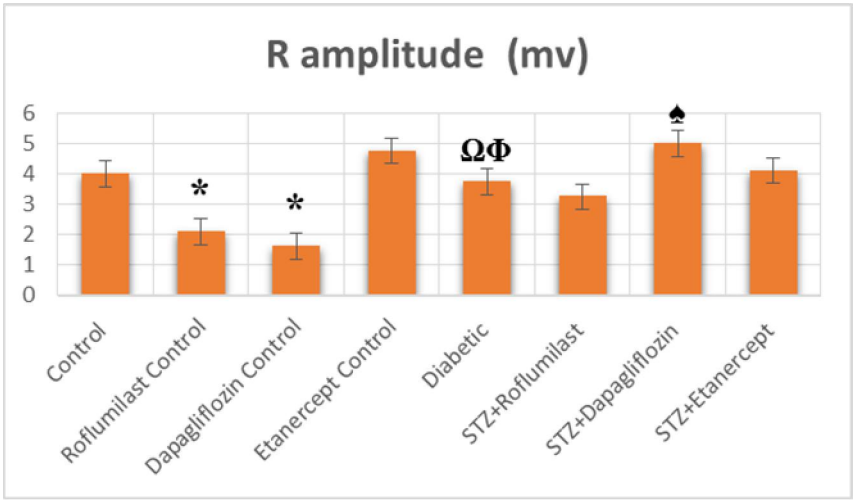

Figure (4): R wave amplitude (mv) in all studied groups.
* ($p<0.05$) compared to control group. Ω ($p<0.05$) compared to Dapagliflozin group.
♠ ($p<0.05$) compared to (STZ+Roflumilast) group. ϕ ($p<0.05$) compared to Roflumilast group.

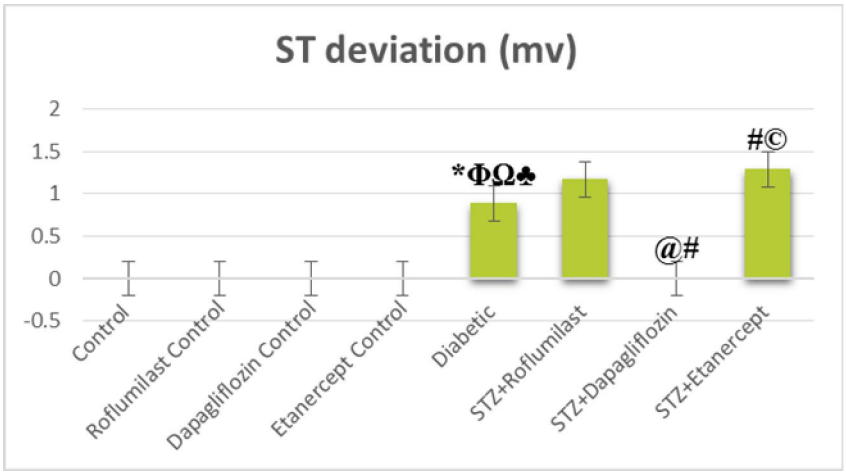

Figure (5): ST deviation (mv) in all studied groups.
* ($p<0.05$) compared to control group. ♣ ($p<0.05$) compared to Etanercept group.
# ($p<0.05$) compared to diabetic group. Φ ($p<0.05$) compared to Roflumilast group.
@ ($p<0.05$) compared to (STZ+Roflumilast) group. © ($p<0.05$) compared to (STZ+Dapagliflozin) group.
Ω ($p<0.05$) compared to Dapagliflozin group.

4.3. Histopathological findings:

In the present study the light microscopic examination using hematoxylin, and eosin revealed all four control groups showed the normal appearance of the cardiomyocytes. They were short, branched and arranged in interlacing bundles with alternating light and dark bands. They had spindle shaped nuclei and abundant eosinophilic cytoplasm (photo 1). By Masson trichrome stain there was minimal collagen deposition around cardiomyocytes and around blood vessels (photo 2).

In diabetic group, there was prominent widening between cardiomyocytes, degenerative changes of cardiomyocytes, vacuolation and inflammatory cell infiltration (photo 3). Masson trichrome stain revealed moderate and marked collagen deposition around cardiomyocytes and blood vessels (photo4).

Treated groups showed decrease of STZ induced histopathological changes but with widening between cardiomyocytes and minimal residual degenerative changes of cardiomyocytes (photo 5, 7and9). Masson trichrome stain revealed minimal collagen deposition between cardiomyocytes and around blood vessels (photo 6, 8, and 10).

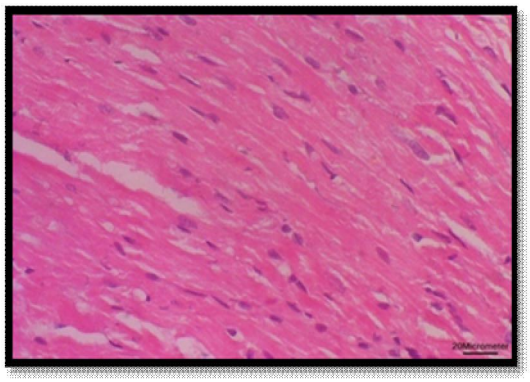

Photo (1): Sections in rat's heart muscle of **"Control Group"** showing normal myocardial architecture. **(Hx & E, x 400)**.

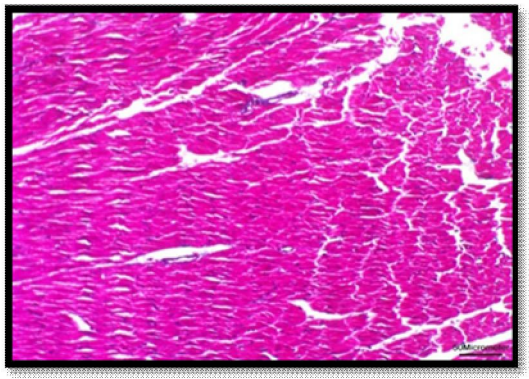

Photo (2): Sections in rat's heart muscle of **"Control Group"** showing normal myocardial architecture and minimal collagen deposition around cardiomyocytes and around blood vessels **(Masson's trichrome, x 200).**

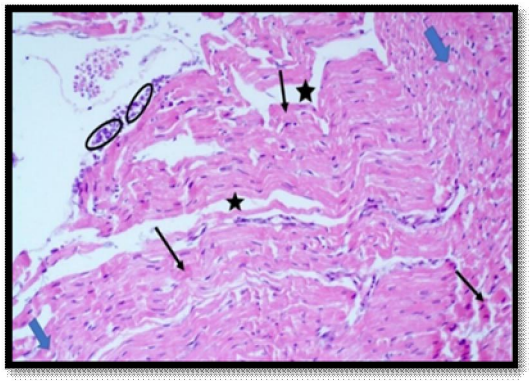

Photo (3): Sections in rat's heart muscle of **" Diabetic Group"** showing loss of normal myocardial architecture with widening between cardiomyocytes (star), degeneration (black arrow), vacuolation (blue arrow) and inflammatory cell infiltration (circle). **(Hx & E, x 200)**

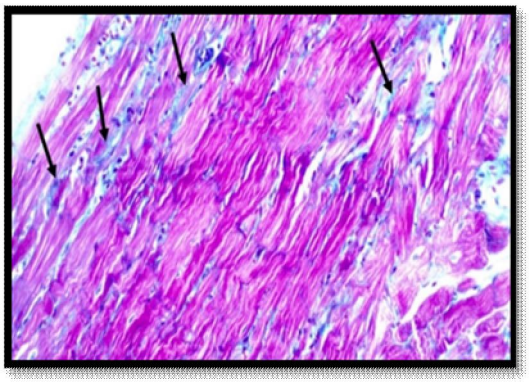

Photo (4): Sections in rat's heart muscle of **" Diabetic Group"** showing marked collagen deposition between cardiomyocytes and around blood vessels (arrow). **(Masson's trichrome, x 200)**

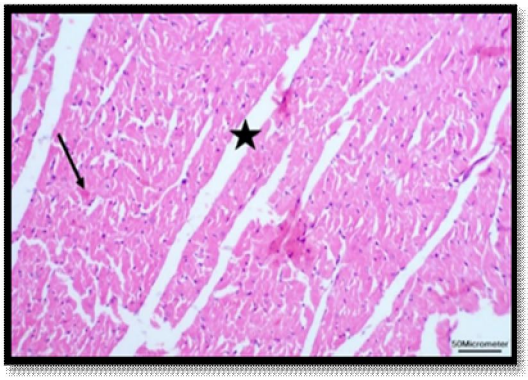

Photo (5): Sections in rat's heart muscle of **" STZ+Roflumilast Group"** showing widening between cardiomyocytes (star) and minimal residual degenerative changes of cardiomyocytes (arrow). **(Hx & E, x 200).**

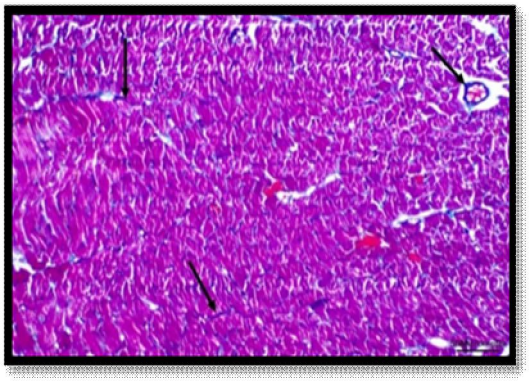

Photo (6): Sections in rat's heart muscle of **" STZ+Roflumilast Group"** showing moderate collagen deposition between cardiomyocytes and around blood vessels (arrow). **(Masson's trichrome, x 200)**

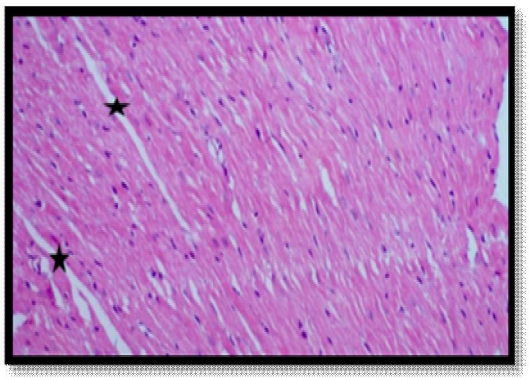

Photo (7): Sections in rat's heart muscle of **"STZ+Dapagliflozin Group"** showing slight widening between cardiomyocytes (star). **(Hx & E, x 200)**

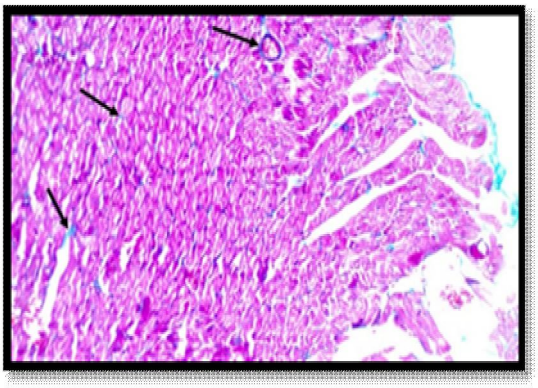

Photo (8): Sections in rat's heart muscle of **" STZ+Dapagliflozin Group"** showing mild collagen deposition between cardiomyocytes and around blood vessels (arrow). **(Masson's trichrome, x 200)**

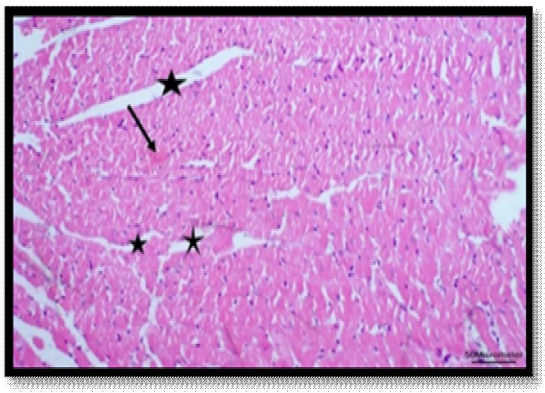

Photo (9): Sections in rat's heart muscle of **" STZ+Etanercept Group"** showing slight widening between cardiomyocytes (star) and minimal residual degenerative changes of cardiomyocytes (arrow). **(Hx & E, x 200)**

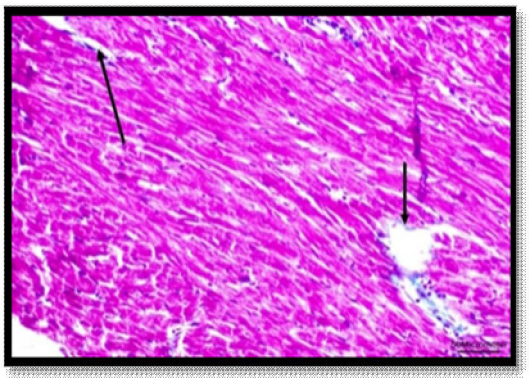

Photo (10): Sections in rat's heart muscle of **"STZ+Etanercept Group"** showing minimal collagen deposition between cardiomyocytes and around blood vessels (arrow). **(Masson's trichrome, x 200)**

5. Discussion

The present study demonstrates the effect of Roflumilast (a phosphodiesterase-4 (PDE-4) inhibitor), Dapagliflozin (an inhibitor of renal sodium-glucose cotransporter -2 (SGLT-2) and Etanercept (a competitive inhibitor of TNF-α) on cardiovascular outcomes in streptozotocin-induced diabetes in rats.

Diabetes mellitus was induced by a single intraperitoneal injection of Streptozotocin (STZ) at a dose of 65 mg/kg **(9)**. Streptozotocin (STZ, causes selective destruction of insulin-producing β-cells of the pancreas. This is mediated by induction of high levels of DNA strand breaks in these cells, causing activation of poly ADP-ribose polymerase (PARP), resulting in reduction of cellular NAD+. Poly ADP ribose polymerase (PARP) redirects glucose metabolism from its usual glycolytic pathway to an alternative biochemical pathway that results in the development of various mediators and causes hyperglycemia-induced cellular injury. These injuries include increased hexosamine and polyol flux, protein kinase C activation, and advanced glycation end product (AGE) levels **(13).**

Excessive accumulation of fatty acids in cardiac tissue and the associated lipotoxicity impairs insulin signaling and reduces normal physiological autophagy, which leads to morphological and structural alterations, as well as impaired myocardial performance. These abnormalities increase myocardial oxygen use and can reduce the efficiency of the function of muscle fibers in response to electrical stimuli (electrical–mechanical coupling) **(14).**

In the current study, single intraperitoneal injection of STZ (in a dose of 65 mg/kg) produced significant hyperglycemia after three days of injection which is in agreement with Salahuddin and Katary,

2017. Moreover, clinical symptoms of diabetes such as polyphagia, polydipsia and polyuria were appeared on rats which is consistent with Niyomchan et al., 2019 **(15).**

In the present study, diabetic group presented a significant increase in serum glucose, HbA1C, cardiac level of (TNF-α), cardiotrophin & MMP-13, and a significant decrease in (serum insulin) comparing to all four control groups. These results coincide with previous studies **(16/17/18/19).**

As regards ECG data in the present work, diabetic group presented a significant decrease in (heart rate) and (R wave amplitude), significant prolongation in (QT, QTc intervals) and increase in (ST deviation) comparing to normal control group, these results are in agreement with previous studies **(20/21).**

Regarding treated groups, there was a significant decrease in serum glucose, HbA1C, cardiac level of TNF-α, cardiotrophin, MMP-13 and a significant increase in serum insulin comparing to diabetic group. As regards ECG data, treated groups presented a significant increase in heart rate and a significant shortening in QT and QTc intervals.

Hypoglycemic activity of Roflumilast may be due to changes in the cytoplasmic cAMP concentration which play an important role in the adjustment of cellular processes initiated by hormones, neurotransmitters and nutrients. In insulin-secreting pancreatic B-cells, cAMP has been found to potentiate Ca2+-dependent exocytosis. This is likely to be the mechanism by which the paracrine and systemic hormones glucagon, glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1 enhance glucose-stimulated insulin secretion. The action of these hormones involves activation of protein kinase a (PKA) which occurs by increase in cytoplasmic cAMP concentration by inhibition of phosphodiesterases or stimulation of adenyl cyclase. In accordance with this concept, the ability of cAMP to stimulate secretion can be prevented by inhibition of PKA **(22).**

Sodium glucose cotransporter-2 (SGLT2) inhibitors directly attenuate cardiac inflammation, oxidative stress which lead to improving both cardiac structure and function, and finally result in decreased mortality rate from cardiovascular causes. Natriuretic effect of SGLT-2 inhibitors results in lowering plasma volume and blood pressure, which are subsequently decreasing cardiac preload and after load **(23**). Sodium glucose cotransporter-2 (SGLT-2) inhibitors also directly improve cardiac calcium handling via inhibiting myocardial Na+/ H+ exchange (NHE) which subsequently decrease intracellular Na+ and Ca2+ loading mostly found in heart failure. Therefore, cardiac contractility and cardiac output could be improved in heart failure patients as observed in clinical trials. It may be concluded that the direct effects of SGLT-2 inhibitors are potentially mediated through their ability to reduce cardiac inflammation, oxidative stress, apoptosis, mitochondrial dysfunction and ionic dyshomeostasis **(24).**

Etanercept inhibit cardiomyocyte hypertrophy by inhibiting inflammatory cytokines (IL-1b, IL-6) and cardiotrophin and cell apoptosis. Etanercept inhibited the expression of Bax but increased the expression of Bcl-2 genes. The down-regulated Bax and upregulated Bcl-2 were consistent with the anti-apoptotic role of etanercept in the cell model of cardiomyocyte hypertrophy. The production and release of MMPs play an important role during the ventricular remodeling. MMP-9 and MMP-13 were overexpressed during the progression of remodeling. Etanercept remarkably reduced the mRNA levels of myocardial hypertrophy marker genes (atrial natriuretic factor, MMP-9 and MMP-13**(8).**

Histopathological examination in our study by hematoxylin and eosin (H & E) stain showed that STZ administration (diabetic group) caused loss of normal myocardial pattern with prominent widening between cardiomyocytes, degenerative changes of cardiomyocytes, vacuolation and inflammatory cell infiltration. Masson's trichrome stain revealed moderate and marked collagen deposition around cardiomyocytes and blood vessels, these results are in accordance with previous studies **(25**). Treated groups presented with a decrease of STZ induced histopathological changes but with residual widening between cardiomyocytes and minimal residual degenerative changes of cardiomyocytes by H & E staining. Masson trichrome stain revealed moderate collagen deposition around cardiomyocytes and blood vessels. These results become in compatible with previous studies **(26/27).**

6. Conclusions

In conclusion, Roflumilast, Dapagliflozin and Etanercept were able to alleviate STZ-induced cardiac dysfunction in rats. Our results in the present study give an idea about cardioprotective role of Roflumilast, Dapagliflozin and Etanercept as indicated by ameliorating the hyperglycemic state and reductions in cardiac inflammation (TNF-α), hypertrophy (cardiotrophin) and fibrosis (MMP-13). The electrocardiographic and histopathological changes were also corrected.

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