# Comparative Effect Of Roflumilast, Dapagliflozin And Etanercept On Cardiovascular Outcomes In Diabetic Rats

## Thesis

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## By

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# ***Summary***

The current work was conducted to clarify the cardioprotective effect of Roflumilast (a phosphodiesterase-4 (PDE4) inhibitor), Dapagliflozin (an inhibitor of renal sodium-glucose cotransporter -2 (SGLT-2)) and Etanercept (a competitive inhibitor of TNF-α) on cardiovascular outcomes in diabetic rats.

In the present study, a total of 48 male albino rats (weighted 150-200 g) were used. The animals were divided into 2 main groups according to the following:

## 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.

**Subgroup 3- Dapagliflozin Control:**

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

**Subgroup 4 - Etanercept Control:**

Rats received single dose of distilled water(1ml) intraperitoneally and Etanercept

1 mg\kg subcutaneous twice\week for 21 days.

## Group II: Diabetic Groups (24 rats):

The diabetic rats were divided into four subgroups of six rats each:

**Subgroup 1- Diabetic group:**

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

**Subgroup 2- Streptozotocin + Roflumilast:**

Rats received Streptozotocin by single intraperitoneal injection of 65mg \kg and Roflumilast 0.5 mg \ kg orally daily for 21 days.

**Subgroup 3- Streptozotocin + Dapagliflozin:**

Rats received Streptozotocin by single intraperitoneal injection of 65 mg\kg and Dapagliflozin 1mg\kg orally daily for 21 days.

**Subgroup 4- Streptozotocin + Etanercept:**

Rats received Streptozotocin by single intraperitoneal injection of 65mg \kg and Etanercept 1 mg \kg subcutaneous twice \week for 21 days.

At the end of 21 days, ECG was traced to asses heart rate, QT, QTc intervals, R-wave amplitude and ST deviation. Blood of fasting rats was collected through retro orbital puncture then serum was separated for detection of **blood glucose, glycosylated hemoglobin** and **serum insulin** levels.

Rats were sacrificed by cervical dislocation and hearts were excised, then cannulated and **cardiac contractility** was estimated by Langendorff technique. After that, hearts were washed with ice-cold saline and preserved for estimation of **TNF-α**, **cardiotrophin, MMP-13** expression by enzyme-linked immunosorbent assay (ELISA) and for **histopathological** examination.

The current study revealed that diabetic rats showed a significant increase in blood glucose and HbA1c by 213% and 140% respectively. Diabetic rats also showed a significant decrease in serum insulin by 66.4%.

Roflumilast significantly decreased blood glucose and HbA1c by 37% and 42% respectively and significantly increased serum insulin by 85%. Dapagliflozin significantly decreased blood glucose and HbA1c by 35% and 49% respectively and significantly increased serum insulin by 65%. Etanercept significantly decreased blood glucose and HbA1c by 42.5% and 48% respectively and significantly increased serum insulin by 97%.

Cardiac level of TNF-α, cardiotrophin and MMP-13 were significantly increased in diabetic rats by 688.5%, 340% and 428.5% respectively. Roflumilast significantly decreased TNF-α, cardiotrophin and MMP-13 in cardiac tissue by 47.1%, 47.5% and 63.5% respectively. Dapagliflozin significantly decreased TNF-α, cardiotrophin and MMP-13 in cardiac tissue by 47%, 58% and 60 % respectively. Etanercept significantly decreased TNF-α, cardiotrophin and MMP-13 in cardiac tissue by 54.5%, 54% and 65 % respectively.

The present work revealed that diabetic rats presented a significant decrease in heart rate (HR) and a significant increase in QT, QTc intervals and ST deviation. Roflumilast significantly increased HR and significantly decreased QT, QTc intervals. Roflumilast did not cause significant change in ST deviation comparing to diabetic group. Dapagliflozin significantly decreased QTc interval and ST deviation. Dapagliflozin did not cause significant change in HR and QT interval comparing to diabetic group. Etanercept significantly increased

HR and significantly decreased QT, QTc intervals. Etanercept significantly increased ST deviation. There was significant increase in R wave amplitude in diabetic group compared to Roflumilast, Dapagliflozin control groups. On the other hand, there was no significant change in R wave amplitude between Etanercept and normal control groups compared to diabetic group. There was significant decrease in R wave amplitude in (STZ+Roflumilast) group compared to (STZ+Dapagliflozin) group.

The current work revealed that there was significant decrease in response of cardiac contractility percentage to isoproterenol in diabetic group comparing to all control groups. Roflumilast, Dapagliflozin and Etanercept did not cause any significant improvement in cardiac contractility comparing to diabetic 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 moderate and marked collagen deposition around cardiomyocytes and blood vessels. These changes were ameliorated with Roflumilast, Dapagliflozin and Etanercept treatment.