Investigation of Cardioprotective Potential of Remogliflozin in preventing Streptozotocin induced Diabetic Cardiomyopathy in Male Wistar Rats

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ABBREVIATIONS

ACE	- Angiotensin-Converting Enzyme
ADA	- American Diabetes Association
AGEs	- Advanced glycation end-products
AMPK	- AMP activated Protein Kinase
ARBs	- Angiotensin II Receptor Blockers
ATP	- Adenosine triphosphate
BMI	- Body Mass Index
CCSEA	- Committee for Control and Supervision of Experiments on Animals
CK-MB	- Creatine Kinase – Myocardial Band
CRP	- C – Reactive Protein
CVD	- Cardiovascular Diseases
DC	- Diabetic control
DCM	- Diabetic Cardiomyopathy
DM	- Diabetes Mellitus
DPP-4	- Di-peptidyl peptidase 4
FDA	- Food and Drug Administration
FBS	- Fasting Blood Sugars
GIP	- Gastric Inhibitory Peptide
GLP-1	- Glucagon Like Peptide 1
H & E	- Haematoxylin & Eosin
HDL	- High Density Lipoprotein
HPE	- Histopathological Examination
IDDM	- Insulin dependent Diabetes mellitus
IL-6	- Interleukin - 6

I.P. - Intraperitoneal

LDH - Lactate Dehydrogenase

LDL - Low Density Lipoprotein

M - Metformin

MDA - Malonaldehyde

MODY - Maturity-onset diabetes of the young

mTOR - Mammalian target of Rapamycin

NA - Nicotinamide

NC - Normal Control

NF-κB - Nuclear Factor Kappa Beta

O.D - Once Daily

P.O - Per Oral

PCr - Phosphocreatine

PI3K - Phosphoinositide 3-kinase

PKC - Protein kinase C

PPAR-γ - Peroxisome proliferator-activated receptor-gamma

R - Remogliflozin

ROS - Reactive Oxygen Species

SIRT1 - Sirtuin 1

SOD - Superoxide dismutase

STZ - Streptozotocin

T1DM - Type 1 Diabetes Mellitus

T2DM - Type 2 Diabetes Mellitus

TNF-α - Tumor Necrosis Factor – alpha

WHO - World Health Organization

ABSTRACT

Introduction and objective - One of the cardiac problems that diabetes patients face is Diabetic Cardiomyopathy (DCM), which can lead to heart failure and an increase in morbidity and death. The two main pathogenic characteristics of diabetic hearts that contribute to the development of DCM are inflammation and oxidative stress. Because of its anti-oxidant and anti-inflammatory qualities, remogliflozin, an SGLT 2 inhibitor, has been demonstrated to lessen inflammation and oxidative stress. The purpose of this study was to look at how Remogliflozin protects diabetic hearts and assess via the corresponding changes in blood marker and histological characteristics.

Materials and Methods - Male Wistar rats which had developed diabetes due to streptozotocin (STZ) (55mg/ kg) were given oral gavage of Remogliflozin, Metformin alone and in co-administration with each other for eight weeks. Cardiac biomarkers viz., Cardiac Troponin I, CK – MB, LDH and histology were used to assess cardiac status. Cardiac fibrosis and morphological alterations in the heart were assessed using Masson trichrome and hematoxylin-eosin staining, respectively. Using the appropriate commercial rat ELISA kits, the levels of inflammatory markers (IL-6, IL-1β, TNF-α) and oxidative stress markers (superoxide dismutase, malonaldehyde) in blood samples were measured.

Results - Treatment with Remogliflozin alone and in co-administration with Metformin considerably reduced the aberrant morphological change in diabetic hearts and improved the cardiac fibrotic alterations. Furthermore, Remogliflozin therapy decreased the expression of TNF-α, IL-6, IL-1β, and malonaldehyde, Cardiac Troponin I, CK-MB, LDH in diabetic rats while increasing the levels of, Superoxide Dismutase.

Conclusion - Our research indicates that in male Wistar rats with STZ-induced Diabetes Mellitus, Remogliflozin alone and in co-administration with Metformin prevents the development of DCM. Remogliflozin may be a viable intervention in the treatment of DCM, according to our research.

Keywords – Type 2 Diabetes Mellitus, Streptozotocin, Diabetic Cardiomyopathy, SGLT – 2 inhibitor, Remogliflozin

TABLE OF CONTENTS

LIST OF TABLES

LIST OF GRAPHS

LIST OF IMAGES

INTRODUCTION

Introduction and Need for the Study -

It is predicted that the number of people with Diabetes Mellitus [DM] would increase sharply worldwide, anticipated to reach 380 million by 2025 and an astounding 700 million by 2045 [1]. This increase is indicative of the concerning rate at which diabetes has developed into a global epidemic in the twenty-first century, impacting roughly 1 in 11 people [2]. Lifestyle variables, particularly sedentary routines and bad eating habits, have been closely associated with the rising incidence of this metabolic illness [3]. Chronic hyperglycemia, the characteristic of diabetes, is a serious risk to many organ systems, especially the neurological, renal, and cardiovascular systems [4]. Elevated rates of morbidity and mortality are directly linked to these diabetes complications [5]. Pancreatic β cells are destroyed in Type 1 Diabetes Mellitus [T1DM], while insulin resistance varies, insulin output is decreased, and β cell apoptosis is linked to Type 2 Diabetes Mellitus [T2DM].

Diabetes ultimately results in higher rates of morbidity and mortality since it dramatically increases the risk of complications such as nephropathy, neuropathy, and cardiac myopathy. Remarkably, Diabetic Cardiomyopathy [DCM] affects about 35% of persons with diabetes, making them more susceptible to heart failure [6]. Increased intramyocardial lipid accumulation causes lipotoxicity, diastolic dysfunction, cardiac electrical abnormalities, and calcium imbalances in people with diabetes, obesity, insulin resistance, and reduced glucose tolerance [7]. Diabetes-related chronic inflammation and fibrosis are brought on by ongoing oxidative stress [8]. Regardless of other risk factors like hypertension and hypercholesterolemia, elevated levels of pro-inflammatory markers like TNF-α and NF-κB, together with increased collagen synthesis and oxidative stress, exacerbate cardiac dysfunction in people with diabetes.

Many hypoglycemic medications are used to treat diabetes and related side effects, such as insulin, biguanides, thiazolidinediones, incretin analogs, DPP-4 inhibitors, and SGLT-2 inhibitors [9]. Among these, the biguanide class of drugs includes the oral antihyperglycemic drug metformin, which is frequently used as the first line of treatment for type 2 Diabetes Mellitus (10). Its diverse modes of action, which include lowering hepatic glucose, make it unique. By decreasing intestinal glucose absorption, raising insulin sensitivity to increase peripheral glucose uptake and utilization, particularly in muscle tissues, and blocking gluconeogenesis, metformin controls blood glucose levels (11). Apart from its function in controlling blood sugar levels, metformin exhibits noteworthy

anti-inflammatory characteristics. These include blocking the NF-κB pathway, lowering pro-inflammatory cytokines like TNF-α, IL-6, and CRP, and stimulating AMPK to reduce inflammation in an all-around manner (12, 13). Metformin also protects cells from oxidative damage by increasing the activity of antioxidant enzymes like catalase and superoxide dismutase (SOD) and decreasing the formation of reactive oxygen species (ROS) (14, 15, 16). Since diabetes patients are more likely to develop cardiovascular diseases, metformin's cardioprotective qualities are especially helpful. It improves endothelial function, lowers the risk of atherosclerotic plaque formation, and helps lower blood pressure and lipid levels, including triglycerides and LDL cholesterol (17, 18). Because it effectively lowers blood glucose levels and HbA1c without significantly raising hypoglycemia, does not encourage weight gain and may even help with weight loss, and has a well-established safety profile with few side effects—mostly gastrointestinal discomfort that is usually manageable with dose adjustments—metformin is considered the gold standard for treating type 2 diabetes. Additionally, it improves circulation, lowering diabetic patients' risk of cardiovascular events (19). Its long history of clinical use and cost-effectiveness have solidified its status as a cornerstone in the management of type 2 diabetes, making it a dependable and highly recommended alternative in the treatment of diabetes (20, 21). There is insufficient evidence to support the use of metformin as an adjuvant in cardioprotective therapy for Diabetes Mellitus, despite the drug's cardioprotective properties in human patients.

For which novel therapy options include GLP-1 antagonists, GIP antagonists, SGLT-2 inhibitors, and so on. Of them, medications such as Dapagliflozin, Ertugliflozin, Canagliflozin, and Remogliflozin have become part of a new therapeutic class known as Sodium Glucose Cotransporter 2 (SGLT-2) inhibitors [22]. SGLT-2 inhibitors, which the FDA just approved, are becoming more and more well-known for their ability to successfully lower blood glucose levels in adult diabetes patients, either as monotherapy or adjunct therapy, particularly in those who have cardiovascular disease.

Remogliflozin etabonate, a prodrug of Remogliflozin (R), is a type of SGLT-2 inhibitor that specifically inhibits SGLT-2 and provides a novel insulin-independent strategy for lowering plasma glucose levels in diabetics without having a negative impact [23]. Notably, people who are insulin resistant may benefit most from this insulin-independent activity. Furthermore, it has been shown that SGLT-2 inhibitors are strong antioxidants that reduce the production of free radicals and alter antioxidant systems including glutathione peroxidases and superoxide dismutases [24]. For diabetic

patients, especially those who are at risk of cardiovascular problems, SGLT-2 inhibitors offer a viable therapeutic option by breaking the harmful cycle between insulin resistance and oxidative stress.

SGLT-2 inhibitors have been included in the treatment plans of diabetic individuals with cardiovascular illnesses because of their ability to prevent heart attacks. Remogliflozin has encouraging qualities, although its ability to prevent diabetic cardiomyopathy is still unknown. Thus, utilizing a rat model of diabetes mellitus caused by streptozotocin [STZ], the current work intends to explore the cardioprotective effects of Remogliflozin on cardiac oxidative stress, inflammation, and apoptosis [25]. This model is well-known for its ability to replicate the cardiac dysfunction and diabetes mellitus seen in diabetic individuals, including changes in the myocardium's molecular structure, local inflammation, oxidative stress, and mechanisms leading to myocardial cell death. This study may provide light on Remogliflozin's possible therapeutic effect in reducing the symptoms of diabetic cardiomyopathy, opening up important new directions for clinical treatment.

OBJECTIVES

Objectives of the Study

Primary Objective – To investigate the cardioprotective potential of Remogliflozin alone and Remogliflozin in co – administration with Metformin in preventing Streptozotocin induced Diabetic Cardiomyopathy in Male Wistar Rats by histopathology and cardiac biomarkers.

Secondary Objective – To investigate the effects of Remogliflozin alone and Remogliflozin in co-administration with Metformin by the following parameters:

- Inflammatory cytokines - IL-1β, IL-6, and Tumor Necrosis Factor (TNF) - α

- Oxidative stress markers - Superoxide dismutase (SOD), Malondialdehyde (MDA)

Review of Literature

Diabetes is defined by the American Diabetes Association (ADA) as "a group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin action, secretion, or both."[26] This definition is supported by international agencies such as the World Health Organization (WHO). All metabolic disorders associated with persistent hyperglycemia due to abnormalities in insulin action, secretion, or both are collectively referred to as diabetes mellitus. Chronic hyperglycemia associated with diabetes has been linked to long-term damage, dysfunction, and failure in many organs, including the heart, blood vessels, kidneys, eyes, and nerves.[27]

Table 1

Diabetes Mellitus Type 1 (T1DM)

Pathophysiology:

The primary cause of Type 1 Diabetes Mellitus (T1DM) is the autoimmune destruction of pancreatic beta cells in the islets of Langerhans, triggered by autoreactive T-cells. This results in a reduction or cessation of insulin production. Genetics, particularly certain HLA genotypes like HLA-DR3 and HLA-DR4, play a significant role in susceptibility [28]. Additionally, environmental triggers such as contaminants, specific food items, and viral infections (e.g., enteroviruses and Coxsackievirus) can initiate or exacerbate the autoimmune process in genetically predisposed individuals.

Type 2 Diabetes Mellitus (T2DM):

Pathophysiology

Insulin resistance, the inability of peripheral tissues (liver, muscle, and fat) to respond to insulin adequately, is a hallmark of Type 2 Diabetes Mellitus (T2DM). This leads to an increase in glucose synthesis in the liver and a decrease in glucose absorption [29]. Eventually, when insulin resistance develops, pancreatic beta cells fail to produce enough insulin to counteract it [30]. Obesity, physical inactivity, poor diet, and genetic predispositions are significantly associated with Type 2 diabetes [31].

Table 1: Classification of Diabetes Mellitus:

Diabetes is classified as follows:

Type 1 diabetes	• Also known as auto immune diabetes or Insulin dependent Diabetes mellitus (IDDM) • 5 % to 10% of all diabetic cases • Breakdown of beta cells that results in total insulin insufficiency • Type 1 diabetes also has been linked to distinct HLA types, with DR and DQ types being the most common
Type 2 diabetes	• 90 % to 95% of diabetic patients suffer from this condition • Pathophysiology spans from insulin resistance and relatively normal insulin secretion
Specific types of diabetes	Exocrine pancreas disorders such as pancreatitis and cystic fibrosis (I) can contribute to diabetes. Additionally, drug or chemical-induced diabetes, which can occur due to factors like glucocorticoid use, treatment of HIV, or following organ transplantation (II), presents another category. Furthermore, monogenic diabetes syndromes, including neonatal diabetes and maturity-onset diabetes of the young (MODY) (III), represent genetic factors that can lead to diabetes development.
Gestational diabetes mellitus	Diabetes identified in the second or third trimester of pregnancy, which was not clearly evident as overt diabetes before gestation.

Type 2 diabetes (T2DM) is a chronic metabolic disease characterized by high blood sugar levels due to impaired insulin production and insulin resistance. Its rising incidence is a major global health concern because of its noteworthy correlation with cardiovascular problems. Cardiovascular illnesses (CVDs), such as heart failure, stroke, and coronary artery disease, are the main causes of sickness and death among T2DM patients among these complications [32]. Heart failure and cardiovascular-related fatalities in diabetics are largely caused by a particular cardiac condition called diabetic cardiomyopathy [33]. Diabetic cardiomyopathy is characterized by abnormalities in the structure and function of the heart that do not correlate with traditional cardiovascular risk

factors. Its growth and progression are significantly influenced by key variables such as oxidative damage, inflammation, insulin resistance, and persistent hyperglycemia. Heart failure is the end result of these processes, which also include hypertrophy, impaired contractility, diastolic dysfunction, and myocardial fibrosis [34]. Distinguished by distinct myocardial abnormalities, this condition differs from traditional cardiovascular risk factors such as dyslipidemia, hypertension, and coronary artery disease because its primary cause is metabolic disruptions linked to diabetes, specifically chronic hyperglycemia and insulin resistance.

Mechanisms of Pathophysiology:

Long-term Hyperglycemia: Advanced glycation end-products (AGEs) are produced when blood glucose levels remain elevated over an extended period of time. Myocardial stiffness and fibrosis are caused by the disruption of normal function and structure of myocardial proteins caused by these AGEs by cross-linking. Additionally, protein kinase C (PKC) pathways are activated by hyperglycemia, leading to increased oxidative stress, endothelial dysfunction, and inflammation.

Insulin Resistance: Type 2 diabetes is characterized by insulin resistance, which reduces the ability of cardiac cells to absorb glucose and increases the heart's reliance on fatty acid oxidation as a source of energy. This change in metabolism may result in fat buildup in the heart, which could worsen cardiac dysfunction and cause lipotoxicity. The phosphoinositide 3-kinase (PI3K)/Akt pathway, which is essential for cardiomyocyte survival and function, can also be negatively impacted by reduced insulin signaling [35].

Stress by Oxidation: Increased generation of reactive oxygen species (ROS) in the heart is a result of diabetes. Increased reactive oxygen species (ROS) can be harmful to lipids, proteins, and DNA within cells, leading to cardiac cell death via apoptosis and malfunction. Diabetes-related mitochondrial dysfunction has been shown to increase ROS production, which feeds back into a harmful loop of oxidative damage [36].

Inflammatory Response: Diabetes is frequently accompanied by chronic inflammation. Increased levels of pro-inflammatory cytokines, including TNF-α, IL-6, and IL-1β, are linked to cardiac fibrosis and inflammation. Myocardial damage can be exacerbated by inflammatory signalling pathways, which can also worsen insulin signalling and increase oxidative stress [37].

Pathological Repercussions in Diabetes:

Myocardial Fibrosis: The processes stated above cause fibroblast activation and extracellular matrix protein accumulation, which in turn causes myocardial fibrosis. This fibrosis makes the heart more rigid, which exacerbates diastolic dysfunction [38].

Myocardial Hypertrophy: Cardiomyocytes experience hypertrophy in response to elevated workload and metabolic stress. Initially sustaining cardiac output, this compensatory strategy eventually leads to compromised myocardial contractility and relaxation [39].

Impaired Cardiac Contractility: Cellular dysfunction, hypertrophy, and fibrosis work together to produce impaired cardiac contractility. This dysfunction shows up as both diastolic and systolic dysfunction, which lowers the ejection fraction and causes heart failure [40].

Historically, insulin therapy, oral antidiabetic medications, and lifestyle changes have been the mainstays of T2DM management to achieve glycemic control. Recent data, however, indicates that the increased cardiovascular risk linked to diabetes may not be sufficiently addressed by traditional glucose-lowering treatments. Novel therapeutic approaches that not only target hyperglycemia but also lessen cardiovascular consequences in people with diabetes are therefore becoming more and more necessary.

In order to prevent complications from microvascular and macrovascular diseases, the therapy of diabetes, in particular type 2 diabetes mellitus (T2DM), has historically focused on establishing good glycemic control. Insulin therapy, oral antidiabetic medications, and lifestyle changes are the main therapeutic modalities.

Treatment of Diabetes Mellitus :

Non – Pharmacological treatment :

Changes in Lifestyle:

Diet: It is recommended that patients consume a diet low in refined carbs and saturated fats and high in fruits, vegetables, lean proteins, and unsaturated fats. This lowers the risk of cardiovascular disease and helps control blood sugar levels.

Exercise: Frequent resistance training and aerobic exercise increase insulin sensitivity, promote muscle uptake of glucose, and help control weight.

Weight Management: Improving insulin sensitivity and general metabolic health require achieving and maintaining a healthy body mass index (BMI).

Conventional Oral Anti Diabetic Medications

- Metformin: Decreases hepatic gluconeogenesis, raises insulin sensitivity, and boosts peripheral glucose absorption.

- Sulfonylureas: Block ATP-sensitive potassium channels to increase insulin production from pancreatic beta cells.

- Dipeptidylpeptidase-4 (DPP-4) inhibitors: Increase the activity of incretin hormones, reducing glucagon release and increasing glucose-dependent insulin production.

- SGLT2 inhibitors: Decrease renal glucose reabsorption, promoting glucosuria and lowering blood glucose levels.

- Thiazolidinediones: Increase insulin sensitivity in adipose tissue, muscle, and the liver via activating peroxisome proliferator-activated receptor-gamma (PPAR-γ).

When oral drugs and dietary modifications are insufficient to provide T2DM patients with appropriate glycemic control, insulin therapy becomes imperative. It comes in a variety of forms, including long-acting (like insulin glargine), intermediate-acting (like NPH insulin), and rapid-acting (like insulin lispro) [41]. Insulin dosages are tailored to each patient's needs in an effort to replicate the patterns of natural insulin secretion.

Management of Diabetic Cardiomyopathy

Myocardial dysfunction in diabetics, regardless of the presence of conventional cardiovascular risk factors such as hypertension, coronary artery disease, and other conditions, is the hallmark of diabetic cardiomyopathy (DCM). Chronic hyperglycemia, insulin resistance, oxidative stress, and inflammation are all part of the pathophysiology of DCM [42]. These factors combined cause myocardial fibrosis, hypertrophy, poor contractility, and diastolic dysfunction, which in turn lead to heart failure.

Glycemic Management:

Strict Glycemic Control: Required to stop DCM from getting worse. To keep HbA1c readings within goal ranges, this entails using insulin therapy, oral antidiabetic medications, and lifestyle changes. Due to their cardioprotective qualities, medications such as GLP-1 receptor agonists and SGLT2 inhibitors are especially advantageous.

SGLT2 Inhibitors: These medications, such as dapagliflozin and empagliflozin, have shown considerable cardiovascular advantages, including a decreased risk of cardiovascular death and hospitalization for heart failure, even in the absence of their glucose-lowering effects [43].

GLP-1 Receptor Agonists: These medications, such as liraglutide and semaglutide, help with weight management, blood pressure regulation, and cardiovascular outcomes in addition to enhancing glycemic control [44].

Agents that Protect the Heart:

- Angiotensin II Receptor Blockers (ARBs) and Angiotensin-Converting Enzyme (ACE) Inhibitors: By lowering blood pressure and reducing afterload, these medications assist in minimizing cardiac workload, preventing unfavorable remodeling, and enhancing heart function [45].

- Beta-Blockers: By lowering heart rate, myocardial oxygen consumption, and preventing unfavorable remodeling, these medications (such as carvedilol and metoprolol) increase survival in heart failure patients.

- Statins: Lower LDL cholesterol, lessen inflammation, and lower the risk of atherosclerotic cardiovascular disease (e.g., rosuvastatin, atorvastatin).

Innovative Medicines:

Glucagon-like peptide-1 (GLP-1) receptor agonists and sodium-glucose cotransporter 2 (SGLT2) inhibitors are two of the more recent groups of antidiabetic medications that provide benefits to the cardiovascular system in addition to glycemic control [46]. Regulatory agencies such as the European Union and the US Food and Drug Administration (FDA) have approved a number of SGLT2 inhibitors, including canagliflozin, ertugliflozin, tofogliflozin, luseogliflozin, and remogliflozin.

SGLT2 Inhibitors' Mechanisms and Benefits:

SGLT2 inhibitors primarily work by preventing glucose reabsorption in the renal tubules, raising urine glucose excretion and lowering plasma glucose levels. They also have cardioprotective benefits by modifying cardiac metabolism, enhancing myocardial energetics, and reducing arterial stiffness and afterload [47].

Clinical trials have consistently demonstrated reductions in heart failure hospitalizations, cardiovascular death, and renal outcomes with SGLT2 inhibitors. These cardiovascular advantages are combined with hemodynamic, metabolic, anti-inflammatory actions, and antioxidant effects.

Anti-Inflammatory and Antioxidant Characteristics of SGLT2 Inhibitors:

Chronic, low-grade inflammation and oxidative stress are linked to diabetes and heart failure. SGLT2 inhibitors have shown anti-inflammatory properties by downregulating proinflammatory mediators such as TGF-β, TNF-α, interleukin-6 (IL-6), NF-κB, CRP [48]. These medications also control tissue hemodynamics, redox state imbalance, and the renin-angiotensin system to modulate inflammation.

SGLT2 inhibitors demonstrate strong antioxidant effects by scavenging reactive oxygen species (ROS), increasing antioxidant enzyme activity, and restoring redox equilibrium. These antioxidant qualities guard against ischemia-reperfusion injury, reduce oxidative damage to the myocardium, and maintain heart function [49].

SGLT2 Inhibitors and their mechanisms of action in Heart Failure

A decrease in the cardiac phosphocreatine (PCr) to adenosine triphosphate (ATP) ratio indicates an energy shortage in both diabetic cardiomyopathy and heart failure. Consequently, various cellular stressors, such as hypoxia, ROS, damaged organelles, and misfolded proteins, as well as malnutrition, activate cellular low-energy sensors including sirtuin 1 (SIRT1) and AMPK [50]. SIRT1 reduces oxidative stress by increasing antioxidant activity and directly lowering the inflammatory response to oxygen free radicals. AMPK maintains mitochondrial activity, reducing ROS production and lessening proinflammatory and proapoptotic reactions. AMPK activity is elevated in failing cardiomyocytes, serving as an energy sensor to boost catabolism by promoting

glycolysis and glucose absorption while squelching anabolic processes. Targeting the AMPK pathway has shown promise in combating cardiac hypertrophy and myocardial failure [51].

The breakdown of early glycation products produces advanced glycation end-products (AGEs), which build up in vascular tissues and plasma. They cause arterial stiffness and decreased elasticity by directly interacting with the extracellular matrix. Receptors for AGEs (RAGEs) on endothelial cells, monocytes, and vascular smooth muscle cells facilitate oxidative stress, inflammation, and vascular smooth muscle cell proliferation. AGE-RAGE signaling activation is associated with heart failure and acute coronary syndrome [52].

The cardiovascular benefits of SGLT2 inhibitors have been proposed to be explained by a number of mechanisms, including decreased oxidative stress, increased hematocrit, improved myocardial energetic efficiency, decreased blood pressure, cardiac preload and afterload, and plasma volume. The potential antioxidant effects of SGLT2 inhibitors in heart failure are the main focus of this review.

It's interesting to note that SGLT2 inhibitors' ability to activate low-energy sensors is not exclusively dependent on the SGLT2 protein, as this effect is also seen in organs where SGLT2 expression is absent. SGLT2 inhibitors promote autophagy regardless of their effects on glucose or insulin because they activate SIRT1/AMPK and block AKT/mTOR signaling [53]. By activating SIRT1/AMPK and inhibiting AKT/mTOR signaling, SGLT2 inhibitors may contribute to reducing oxidative stress, improving mitochondrial structure and function, preventing inflammation, lessening coronary microvascular damage, enhancing contractility, and lowering the incidence of cardiomyopathy.

Research shows that in diabetic mice, pralgliflozin therapy improves endothelial function and decreases the generation of advanced glycation end-products (AGEs), reducing oxidative stress. In rodent diabetic models, empagliflozin has enhanced cardiac diastolic performance without correcting myocardial AGE levels [54]. Empagliflozin has been shown in cell culture models to promote cell viability and sustain ATP levels in hypoxic/reoxygenous circumstances, independent of AGE/RAGE signaling.

In people with diabetes, hyperglycemia can worsen oxidative stress and myocardial damage by increasing glucose uptake by cardiomyocytes. SGLT2 inhibitors can increase the amount of ketone used by the heart and change the body's energy metabolism from glucose and fatty acids to ketones, improving cardiac metabolic efficiency and lowering oxygen usage [55]. This theory is supported by preclinical research, but clinical evidence is still limited.

The FDA has approved SGLT-2 inhibitors such as dapagliflozin, canagliflozin, and empagliflozin for the treatment of type 2 diabetic mellitus (T2DM). Another selective SGLT2 inhibitor, remogliflozin Etabonate, is being investigated as a therapy for type 2 diabetes. By increasing the excretion of glucose through the urine, inhibition of SGLT2 lowers blood glucose levels. Remogliflozin specifically inhibits SGLT2 and shows promising results in preclinical studies.

Table 2 – Features and properties of Remogliflozin etabonate

Features and properties of remogliflozin etabonate	
Alternative names	Remo™, Remozen™, BHV 091009, GSK 189075, KGT-1681
Class	Antihyperglycaemics, glucosides hepatoprotectants, pyrans, pyrazoles
Mechanism of action	Sodium-glucose transporter 2 inhibitor
Route of administration	Oral
Pharmacodynamics	*Ki* values of 4520 and 12.4 nM for human SLGT1 and 2, respectively, (remogliflozin)
Pharmacokinetics	C_{max} and AUC_{τ} at steady state were ≈ 559 ng/mL and ≈ 1798 ng·h/mL, respectively, at 100 mg
Adverse events	
Most frequent	Urinary tract infection, pyrexia, headache, bacteriuria, constipation, diarrhoea, decreased glomerular filtration rate, ketonuria, cough, dyslipidaemia, asthenia, viral upper respiratory tract infection, hypoglycaemia, orthostatic hypotension
ATC codes	
WHO ATC code	A05B-A (liver therapy), A08 (antiobesity preparations, excl. diet products), A10X (other drugs used in diabetes)
EphMRA ATC code	A10X (other drugs used in diabetes), A5B (hepatic protectors, lipotropics), A8A (antiobesity preparations, excluding dietetics)
Chemical name	Ethyl [(2R,3S,4R,5S)-3,4,5-trihydroxy-6-[4-(4-isopropoxybenzyl)-1-isopropyl-5-methyl-1H-pyrazol-3-yloxy] tetrahydro-2H-pyran-2-yl]methyl carbonate

The study aims to explore the anti-inflammatory and antioxidant properties of Remogliflozin by investigating its cardioprotective potential in diabetic cardiomyopathy induced by Streptozotocin in male Wistar rats.

The experimental animals used to study Diabetes can be classified into three distinct types.

Table 3 - Experimental Models of Diabetes Mellitus

1. Chemically induced Models	• Adult STZ/Alloxan Models • Neonatal STZ/Alloxan Models • High Fat-diet Fed Models • Fat-Fed STZ Models • Nicotinamide STZ Models • Fructose Fed Models
2. Experimentally induced models	• Partial Pancreatectomised Models • Intrauterine Growth Retardation Models
3. Genetically derived diabetic animals	• $Lep^{ob/ob}$ mouse • db/db mice • Lepp mice • Zucker Fatty rats • Zucker Diabetic Fatty rats • OLETF rat • NZO mice • TallyHo/Jng mice • Nile grass rat • GK rat • hIAPP mice • AKITA mice
4. Miscellaneous	• Steroid hormone induced • Drug induced

Table 4 - Characteristics of T2DM animal models

Model	Features
Adult alloxan model	• Alloxan kills pancreatic beta cells, it has recognized side effects such as liver and kidney damage • Alloxan given i.p at 40-200 mg/kg BW in rats
Adult STZ model	• STZ is naturally occurring antibiotic produced by *streptomyces achromogens* bacterium. • It acts as a strong alkylating agent, disrupting glucose transport and glucokinase activity while also causing DNA strands to breakdown. • STZ given i.p. at different dose (35-65 mg/kg BW) in rats
Neonatal STZ model	• Since mid-1970's, these models have been used • STZ administered i.p. to new born rats after two days of birth. Until the 4^{th} week , FBG levels are mild hyperglycemic. When rats reach adulthood, it results in adult-onset T2DM.
High fat diet fed model	• This approach was used in 1980 • The principle of this model was that, because of obesity is a major contributor to the development of T2DM • C57BL/6 J mice used for this model • HFD (40-60 % of total calories) for atleast 10 weeks necessary to produce the primary pathology of T2DM
High Fat-Fed STZ model	• This model was originally developed in 1947 • Animals fed with High fat diet to establish insulin resistance, followed by a low dose of STZ injection to produce partial pancreatic beta cell failure. • The advantage of this model is that it replicates natural pathophysiology of T2DM.

Nicotinamide STZ model	• The rationale behind this model is that STZ causes DNA damage while nicotinamide protects pancreatic beta cells from damage caused by STZ. • The initial model used 230 mg/kg BW nicotinamide (i.p) 15 minutes before administering 65 mg/kg BW STZ (i.p) in to 3 month old wistar rats
Partial pancreatectomized model	• This model induce mild to moderate hyperglycemia after 4 days of surgery that can lasts up to 6 weeks • It mimics T2DM by having decreased pancreatic beta cell mass
Intrauterine growth retardation (IUGR) model	• IUGR has been linked to onset of disease later in life, including obesity, hypertension and T2DM • In newborns, IUGR causes significant loses in pancreatic beta cell mass, which does not recover in adulthood and results in impaired glucose tolerance and development of T2DM. • This is caused by bilateral uterine artery ligation, which results in partial reduction in blood flow to the fetus resulting in IUGR.
Zucker fatty (ZF)/ Zucker diabetic fatty rats (ZDF)	• ZF rats were created in 1961 as a result of cross between Merck M and Sherman rats • They have faulty leptin receptor which resulted in hyperglycemia and formation of obese rats at 4 weeks of age. • The ZDF strain was discovered after mutation in ZF strain which are less obese but have increased insulin resistance
Otsuka long-Evans Tokushima Fat (OLETF) rats	• This rat was created after 18 weeks of selective breeding at Tokushima Research Institute from naturally diabetic rat discovered in an outbred colony of Long Evans rats in 1984
Nile Grass rat	• The Nile Grass rat *(Arvicanthis Niloticus)* has been recommended as a model for metabolic syndrome.

	• When fed a standard chow diet in captivity, these rats develop obesity, dyslipidemia and hyperglycemia.
Goto-Kakizaki (GK) rats	• A Japanese group developed GK rats by repeatedly breeding wistar rats with the lowest glucose tolerance. • As a result, slim model of T2DM with glucose intolerance and poor glucose induced insulin production was created.
Corticosteroid induced	• When the adrenal cortex in rodent is stimulated by corticotrophin, it secretes large levels of steroids and ultimately results in steroid induced diabetes. • Dexamethasone and Prednisolone are the most prevalent glucocorticoid that cause steroid diabetes. • They enhance gluconeogenesis and inhibit insulin action which results in increased hepatic glucose production and insulin resistance.
Atypical antipsychotic induced diabetic model	• When it is given for 60 days, atypical antipsychotic such as olanzapine (10 mg/kg i.p) induce significant increase in blood glucose levels, LDL and cholesterol in rats

Mechanism of Streptozotocin and Nicotinamide

The combination of streptozotocin (STZ) and nicotinamide (NA) has been proposed as a method to induce diabetes in rats for research purposes. STZ is known to damage pancreatic B-cells, while NA helps partially protect these insulin-secreting cells from STZ's adverse effects. STZ is transported into B-cells through the glucose transporter GLUT2, leading to DNA damage and the subsequent activation of poly(ADP-ribose) polymerase (PARP-1) to repair the DNA. Excessive PARP-1 activity depletes intracellular NAD+ and ATP, causing necrosis of the insulin-secreting cells. NA inhibits PARP-1 activity, preventing the depletion of NAD+ and ATP in cells exposed to STZ. Furthermore, NA acts as a precursor to NAD+, boosting intracellular levels of NAD+. The severity of diabetes in experimental rats largely depends on the doses of STZ and NA administered. Consequently, blood glucose levels in diabetic rats can vary widely, from mild to severe hyperglycemia compared to control animals. Similarly, blood insulin levels may show slight decreases or significant

hypoinsulinemia. In vitro studies have shown that the insulin secretion response to glucose is reduced in rats with STZ-NA-induced diabetes compared to controls, due to both a decrease in beta-cell mass and metabolic defects in the insulin-secreting cells. Numerous experiments have confirmed the usefulness of this diabetes model in studying various aspects of the disease.

Streptozotocin (STZ) – It's maximum stability of STZ is at pH 4 (56). However, despite a , general belief that STZ should be dissolved immediately before use (57, 58), it is unstable at neutral pH, and low pH solution is required to maintain its stability (59); it has been reported that STZ solutions with a pH of 7.2 are as stable as those with a pH of 4.5 and STZ solution is relatively stable at a pH of 7.4 and 37º C for at least up to one hour and pH 6.7–7.8 on ice for 30 min (60). These observations have questioned the need for low pH, low temperature, and immediate use of STZ solutions (61). STZ is dissolved in saline, acidified 0.9% saline at pH 4.5, and ice-cold 0.05–0.1 M citrate buffer adjusted to pH 4.5 (62,63,64). A stable solution of STZ in citrate buffer (pH 4.5) is most suitable for injection (65). STZ could cause neoplastic growth, and precautions should be applied during its preparation (66).

Animal age, weight, sex, and the dose and time of drug injection affect sensitivity to STZ and the severity of the diabetes (67). Compared to males, female rats are less sensitive to STZ, potentially due to the anti-apoptotic activity of estradiol (68). Following STZ injection, the highest incidence of diabetes induction has been seen at 16:00 and the lowest incidence at 08:00, indicating a circadian rhythm (69). STZ dosages > 65 mg/kg BW are considered high dose, 40–55 mg/kg BW intermediate, and < 35 mg/kg BW as low dosages. A single dose of 25 mg/kg or < 35 mg/kg in rats produces no major effect; at a dose of 35 mg/kg, spontaneous recovery from the diabetic state has been reported in 25% of rats, and stable diabetes has been observed at doses of 55–65 mg/kg. The LD50 of STZ is about 130 mg/kg (70). STZ rapidly undergoes metabolic degradation in the rat liver (71) and has a half-life of 6.9 min or 15 min.

The route of STZ administration is usually intraperitoneal or intravenous (72) injections. However, other routes, including subcutaneous, intramuscular, or even intracardiac, have also been reported. It has been suggested that more stable and reproducible diabetic models could be created using intravenous injection of STZ (73), though this requires significant experience.

Nicotinamide, pyridine-3-carboxamide, is a vitamin B3 (niacin) derivative with antioxidant properties that reduce the cytotoxic actions of STZ (74). NA protects β-cells against STZ through several mechanisms; it scavenges oxygen-free radicals (75) and NO, inhibits PARP (76, 77) with an IC50 value of 210 ± 2.9 μM, inhibits cytokine-induced MHC class II expression, and provides NAD+ (78, 79, 80) NA also promotes β-cell regeneration and islet cell growth while inhibiting apoptosis. Additionally, NA may act as a methyl group acceptor, reducing DNA methylation. NA is a cytoprotective agent that inhibits apoptosis by preventing both externalization of phosphatidylserine and DNA degradation (81); however, it has been reported that the administration of NA before STZ had no effect on DNA methylation in other organs except in pancreatic β-cells, which reduced DNA methylation; the mechanism of this selective protection remains to be determined. NA has a half-life of 9 h (82) and is primarily excreted in the urine. NA is dissolved in normal saline and is typically administered intraperitoneally.

The STZ-NA rat model of type 2 diabetes relies on the protective effects of NA against the β-cytotoxic effects of STZ (83). This model was first introduced by Masiello et al. using 10-week-old male Wistar rats (84, 85). The STZ-NA model of type 2 diabetes has several features

a. Stable moderate (non-fasting) hyperglycemia that does not require exogenous insulin for survival and reduction of β-cells (–40%) and a 60% reduction in pancreatic insulin stores
b. Glucose intolerance mainly due to impaired insulin secretion (86);
c. Impaired but present glucose-stimulated insulin secretion (87);
d. Responsiveness to sulfonylureas (i.e., tolbutamide and glibenclamide) (88); and
e. Polyphagia and polydipsia (89).

   Insulin responsiveness to glucose and sulfonylureas distinguishes this model from others (90). As a model for non-obese type 2 diabetes, it is reported to be more suitable for both biochemical and pharmacological research testing potential antidiabetic effects of pharmacological and natural compounds on the course of diabetes (91). The STZ-NA model of type 2 diabetes is also noted for its suitability for studies of diabetic complications and has been used in research on diabetes complications (92), including diabetic nephropathy (93) and neuropathy, and cardiovascular complications of diabetes. However, it has been reported that STZ exerts direct renal toxicity, making it challenging to distinguish whether renal dysfunction is a complication of diabetes or due to drug-related non-specific

cytotoxicity (94); however, the kidney recovers from STZ-induced renal toxicity after three weeks. Additionally, single doses of STZ can produce renal tumors, making STZ-treated models of diabetes unsuitable for long-term studies of diabetes's effects on the kidney (95). NA can prevent renal dysfunction, further questioning the use of this model for studying diabetic nephropathy. Although single doses of STZ do not produce liver tumors, such tumors are seen with prolonged exposure. High DNA methylation levels by STZ in the liver and kidney may also influence metabolism in these organs. Moreover, NA inhibits P450 and hepatic metabolism.

Studies on Nicotinamide – Streptozotocin model of Type 2 Diabetes Mellitus

Masiello et al. examined the effects of 100–350 mg/kg NA injected intraperitoneally 15 minutes before intravenous administration of 65 mg/kg STZ and recommended a NA dosage of 230 mg/kg as the most appropriate. Following the injection of 200–230 mg/kg NA 15 minutes before STZ administration (60 mg/kg i.v.), 75–80% of rats developed diabetes with stable non-fasting hyperglycemia (150–180 mg/dL), while 20–25% of treated animals either became severely diabetic within 2–3 weeks or remained normoglycemic. Two to three weeks after diabetes induction, abnormalities in glucose tolerance and insulin responsiveness were observed. The doses of STZ and NA, the age of the animals, and the relative timing of the administration of the two compounds affect the results. Younger animals are less sensitive to STZ and are better protected by NA (96). In subsequent studies, intraperitoneal or intravenous injections of STZ at doses between 45–65 mg/kg and intraperitoneal NA injection at doses between 60–290 mg/kg have been used for diabetes induction. In most experiments, NA is given to rats 15 minutes before STZ; however, NA injection has also been done 20 minutes or 30 minutes before STZ injection. It has been reported that NA prevents the onset of diabetes when administered 15 minutes before or up to 2 hours after STZ injection; the protective effect of NA on β-cells decreases with time lapse after STZ administration (97). Additionally, it has been reported that NA offers the best protection against STZ toxicity when administered shortly after STZ. To prevent fatal hypoglycemia due to massive insulin release after STZ injection, some studies provided rats with a 10% glucose solution 6 hours after STZ injection for the next 24 hours or a 20% glucose solution for 24 hours was advised.

METHODOLOGY

Study Design:

This is an experimental animal study (chronic study, of 8 weeks duration) involving Male Wistar rats. Animals were randomly divided into 5 groups, with each group containing eight animals (n=8) with a total of 40 animals. The number of rats in the groups was higher than required for statistical significance to compensate for the expected mortality.

Ethical Committee Approval:

The study was carried out in accordance with guidelines of Committee for Control and Supervision of Experimental Animals vide resolution no 17/1, after obtaining ethical approval from the IAEC (Institutional Animal Ethics Committee) (Reg. No. 627/PO/Re/S/02/CCSEA) dated 25.06.2022.

Experimental Animals:

Adult male albino rats of the Wistar strain, weighing 200 ± 20 grams, were used in the study. The animals were sourced from the KAHER's Institutional Central Animal House, Belagavi. They were housed under standard conditions and acclimatized to a 12-hour light/dark cycle for 7 days prior to experimentation. The rats had free access to food and water ad libitum, with food provided in the form of standard chow pellets. Paddy husk was used as bedding in the cages.

Study Drug and Kits:

Drug/ Analysis Kit	**Obtained from**
Streptozotocin	Everon life sciences, New Delhi
Metformin Remogliflozin Sodium thiopentone	KLE's Hospital Pharmacy, Belagavi
Glucometer Urine Dipstick's	KLE's Hospital Pharmacy, Belagavi
Rat ELISA kits IL – 6, IL - 1β, TNF – α, SOD, MDA, Cardiac Troponin I, CK – MB, LDH	Krishgen Biosystems, Mumbai

Methodology:

- The animals obtained from Central Animal House, after the period of 7 days acclimatization, the rats weighing 200 ± 20 grams were selected and kept fasting overnight in the cages, a day prior to initiation of the study.

- On the next day, blood (2 ml) was collected from all rats from the tail veins using a 30G insulin needle and syringe. The sample was used for baseline estimation of fasting blood sugars (FBS). These were labelled as baseline values.

- Urine samples were collected using rat metabolic cages and urine glucose concentration was estimated using the dipstick test.

- 8 rats were randomly selected and assigned to the Normal control group [NC]. The rest of the 32 rats were randomly assigned into 4 groups of 8 rats each, namely, Diabetic control group [DC],

Metformin treated group [M], Remogliflozin treated group [R], Metformin + Remogliflozin treated group [MR].

- T2DM was induced in rats for the later four groups by the following procedure

Preparation of Nicotinamide

Step-by-Step Calculation:

We had determined the dose for one rat:

Weight of one rat = 200 grams = 0.2 kg

Dose per kg = 100 mg/kg

Dose for one rat = 100 mg/kg * 0.2 kg = 20 mg

In order to calculate the total dose for 32 rats:

Total dose = 20 mg/rat x 32 rats = 640 mg

We wanted to prepare a solution with a concentration of 10 mg/ml (this is a commonly used concentration for ease of calculation and administration).

To calculate the volume of 0.9% NaCl needed as solvent:

Total nicotinamide required = 640 mg

Concentration = 10 mg/ml

Volume of 0.9% NaCl needed = Total nicotinamide / Concentration

Volume of 0.9% NaCl = 640 mg / 10 mg/ml = 64 ml

Therefore, we had mixed 640 mg of nicotinamide in 64 ml of 0.9% NaCl solution to prepare the solution for 32 rats.

This solution was administered, 2 ml each 200-gram rat to achieve the desired dose of 100 mg/kg.

It was ensured that all preparations are done and injected with aseptic precautions.

20 minutes followed by the Nicotinamide injection, the rats were injected with Streptozotocin to create a model of Type 2 Diabetes Mellitus

Preparation of STZ

To calculate the amount of streptozotocin (STZ) and citrate buffer needed for administering a dose of 55 mg/kg to 32 rats, each weighing 200 grams, we followed these steps:

1. Calculate the total dose required per rat:

- Each rat weighs 200 grams (0.2 kg).
- The required dose is 55 mg of STZ per kg of body weight.
- Dose per rat = 55 mg/kg * 0.2 kg = 11 mg of STZ.

2. Calculate the total dose required for all 32 rats:

- Total dose = 11 mg/rat * 32 rats = 352 mg of STZ.

3. Prepare the STZ solution:

- Determine the volume of citrate buffer needed to dissolve the STZ.
- The concentration of the STZ solution can be decided based on practicality. For instance, a common concentration is 10 mg/ml.

4. Calculate the volume of citrate buffer:

- Desired concentration: 10 mg/ml.
- Volume of citrate buffer needed = Total dose / Concentration.
- Volume of citrate buffer = 352 mg / 10 mg/ml = 35.2 ml.

5. Procedure:

- Weigh 352 mg of STZ.
- Dissolve the 352 mg of STZ in 35.2 ml of citrate buffer.
- Ensure the solution is well mixed and administer the calculated volume to each rat.

Summary:

- STZ required: 352 mg.

-Citrate buffer required: 35.2 ml (for a 10 mg/ml concentration).

This calculation ensures that each rat receives the correct dosage of 55 mg/kg. Adjust the buffer volume accordingly if a different concentration of the STZ solution is preferred.

Step-by-Step Calculation:

We had determined the dose for one rat:

Weight of one rat = 200 grams = 0.2 kg

Dose per kg = 55 mg/kg

Dose for one rat = 55 mg/kg * 0.2 kg = 11 mg

So, in order to calculate for 32 rats:

Total dose = 11 mg/rat x 32 rats = 352 mg

Then, we made a solution of Citrate buffer i.e., of 0.05M at a pH of 4.5 was prepared immediately prior to injection. The 0.05M citrate buffer was prepared by mixing 0.05M sodium citrate & 0.05M citric acid in a ratio of 2:3 and the pH was adjusted to 4.5). with Streptozotocin with a concentration of 10 mg/ml (this is a common concentration for STZ solutions).

Then we had calculated the volume of citrate buffer needed:

Total STZ required = 352 mg

Concentration = 10 mg/ml

Volume of citrate buffer needed = Total STZ / Concentration

Volume of citrate buffer = 352 mg / 10 mg/ml = 35.2 ml

Therefore, we mixed 352 mg of streptozotocin (STZ) in 35.2 ml of citrate buffer to administer into 32 rats.

Owing to the Practical Considerations:

STZ was typically prepared fresh and used immediately as it is unstable in solution.

It was ensured that all preparations are done and injected with aseptic precautions.

Administering the Dose:

The prepared STZ solution (10 mg/ml) was administered to each rat in a volume of 1.1 ml (since 11 mg is required per rat). By mixing 352 mg of STZ in 35.2 ml of citrate buffer to get a solution of 10 mg/ml.

We administered 1.1 ml of this solution to each 200-gram rat to achieve the desired dose of 55 mg/kg.

As mentioned above, a single dose nicotinamide (100mg/kg) was injected followed by streptozotocin (55mg/kg) dissolved in 0.1 M of cold citrate buffer with pH of 4.5 intraperitoneally 20 minutes after the Nicotinamide injection, only for groups two to five. Due to streptozotocin's ability to induce fatal hypoglycemia through massive pancreatic insulin release, a 10% glucose solution was administered to the rats 24 hours after streptozotocin injection to counteract hypoglycemia in those that had received nicotinamide and streptozotocin.

After 7 days, the fasting blood sugar (FBS) levels were checked in rats from groups 2 to 5 to assess the development and exacerbation of diabetes. Only rats with fasting blood glucose concentrations exceeding 250mg/dl were selected.

Division into Groups and Treatment Protocol:

Male Wistar rats were randomly divided into five groups (n=8) and treated as follows:

Table 5 – Grouping of experimental rat groups and details of interventions

Group		**Drug**	**Dose and route**
Group - 1	Normal control group (N)	Distilled water	0.5ml, per oral, OD
Group – 2	Diabetic control group	Nicotinamide + Streptozotocin	Nicotinamide 100 mg/kg, I.P, single dose + Streptozotocini55img/kg, I.P, single dose
Group – 3	Standard group (Diabetic group treated with Metformin)	Nicotinamide + Streptozotocin + Metformin	Nicotinamide 100 mg/kg, I.P, single dose + Streptozotocin 55 mg/kg, I.P, single dose + Metformin 180mg/ kg, per oral, OD

Group 4	Remogliflozin group (Diabetic group treated with Remogliflozin)	Nicotinamide + Streptozotocin + Remogliflozin	Nicotinamide mg/kg, I.P, single dose + Streptozotocin 55img/kg, I.P, single dose + Remogliflozin 18mg/kg, per oral, OD
Group 5	Combination group (Diabetic group treated with Metformin and Remogliflozin)	Nicotinamide + Streptozotocin + Metformin + Remogliflozin	Nicotinamide 100 mg/kg, I.P, single dose + Streptozotocin 55 mg/kg, I.P, single dose + Metformin 180mg/ kg, per oral, OD + Remogliflozin 18mg/kg, per oral, OD

Metformin and Remogliflozin alone and in co – administration with each other, were given by oral gavage by dissolving in distilled water as per the treatment protocol, and the treatment lasted for 8 weeks, for the respective groups.

At the end of the treatment, the rats were anesthetized with an intraperitoneal injection of thiopentone sodium (40 mg/kg). Subsequently, 5 ml of blood was collected via cardiac puncture from each rat to measure various parameters. The rats were then euthanized. The hearts were excised, cleaned, washed with ice-cold 0.9% saline, and stored in 10% formalin for further processing and histopathological examination.

Euthanasia

Animals were sacrificed using an overdose of anesthesia as per the CCSEA guidelines. At the end of the study, thiopentone sodium at a dose of 120 mg/kg was given as an intraperitoneal injection, and the animals were sacrificed.

Outcome Measures:

a. **Body weight of the rats was measured:**

- Upon arrival
- 4th week after induction
- 8th week after induction

b. **Determination of blood and urine glucose levels:**

- **Blood glucose levels [FBS] was measured at:**
  - Onset,
  - After induction of diabetes, at 4th week, and at the end of 8th week using the standard glucometer.
- **Urine glucose levels were measured at:**
  - Upon arrival
  - After induction and at the end of the 8th week using urine dipstick test.



c. Histological Examinations:

- Cardiac tissues were analyzed for:

- Histopathological examination using Mayer's Hematoxylin & Eosin staining and Masson trichrome staining.

d. **Blood samples were directly collected from the heart by cardiac puncture technique for:**

- **Determination of Inflammatory markers:**
  - IL-1β,
  - IL-6, and
  - Tumor Necrosis Factor (TNF)-α.
- **Determination of Antioxidant markers:**
  - Superoxide Dismutase (SOD),
  - Malondialdehyde (MDA).

- **Determination of cardiac biomarkers:**
  - Cardiac troponin-I,
  - Creatine kinase-MB (CK-MB), and
  - Lactate dehydrogenase (LDH).

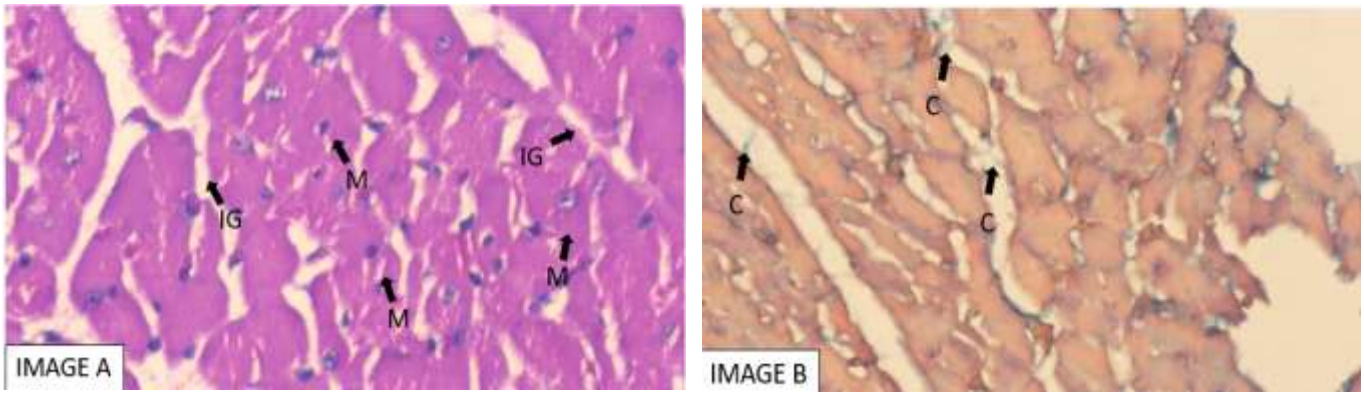
Statistical Analysis:

All the results were expressed as Mean ± Standard Deviation (SD). The data was analysed using Graphpad prism software version 8. The level of significance was set at $p < 0.05$. The study variables were analyzed using one-way ANOVA followed by post hoc Tukey's test wherever appropriate.

RESULTS

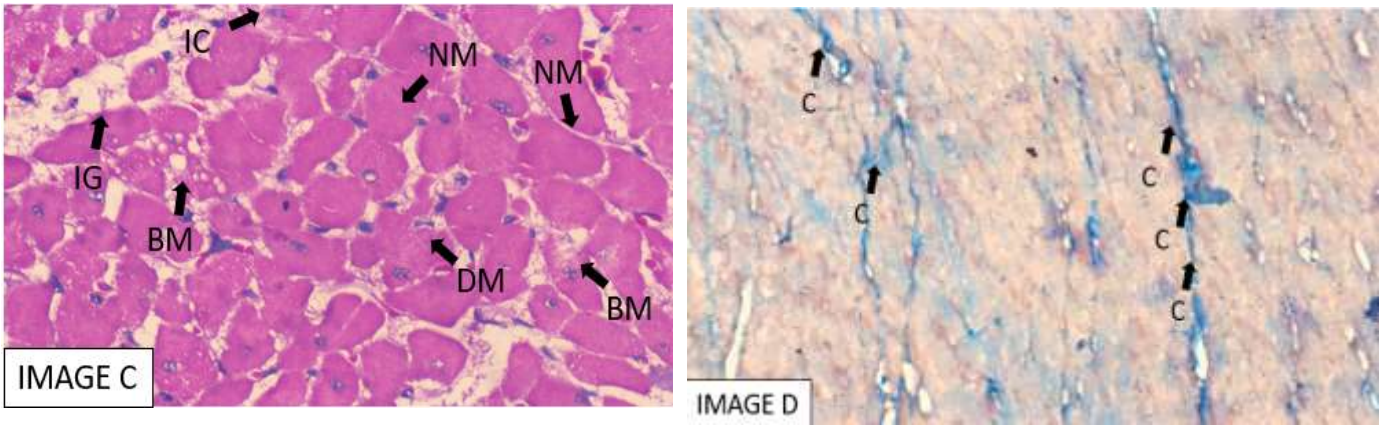
Histopathological Findings

Histopathological characteristics of Cardiac Tissue in Normal Control Group Rats (n = 8)



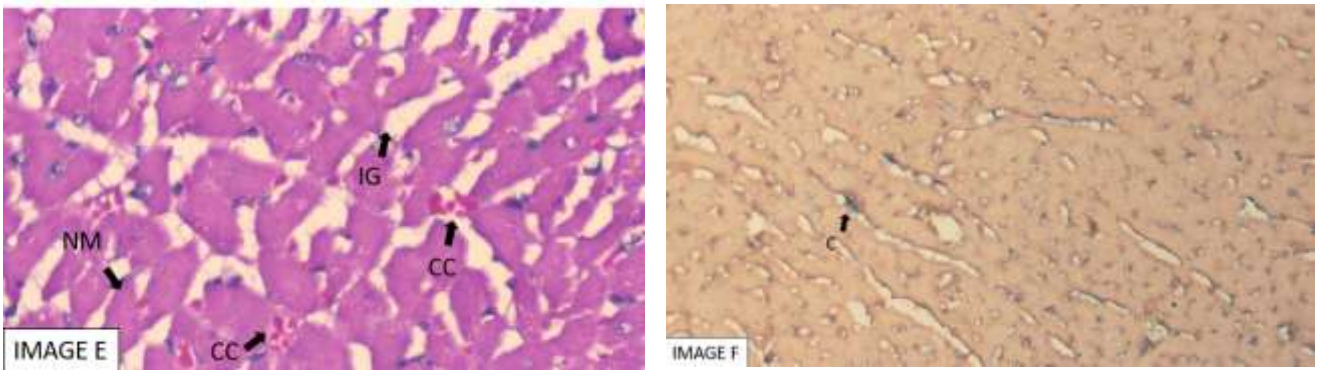
Photomicrograph's showing longitudinal section from left ventricle of heart from normal control group presenting with, Image (A) normal myocardial fibers (M) with normal intercellular gap (IG) with H & E staining under 40x magnification. Image (B) showing collagen (C) deposition (blue colour) within normal limits with MTS under 40x magnification (H & E = Hematoxylin and Eosin staining) (MTS = Masson Trichrome Staining)

Histopathological characteristics of Cardiac Tissue in Diabetic Control Group Rats (n = 8)



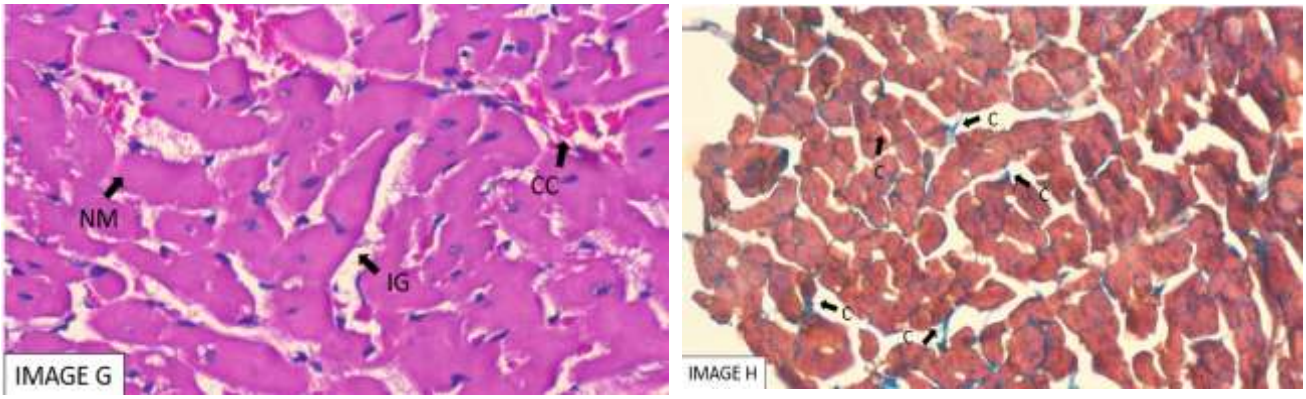
Photomicrograph's showing longitudinal section from left ventricle of heart from diabetic control group presenting with, Image (C) showing deranged myocytes (DM), necrotic myocytes (NM), broken myocardial fibers (BM) and increased intercellular gap (IG) with H & E staining under 40x magnification. Image (D) showing increased collagen (C) deposition (blue colour) with MTS under 20x magnification (H & E = Hematoxylin and Eosin staining) (MTS = Masson Trichrome

Effect of Metformin on cardiac structural changes in Diabetic rats (n = 8)



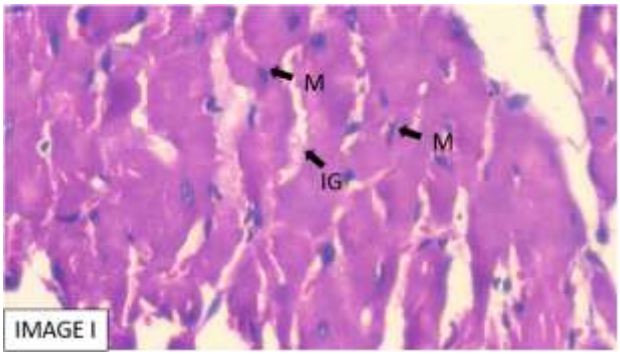
Photomicrograph's showing longitudinal section from left ventricle of heart from Metformin treated group presenting with, Image (E) showing necrotic myocytes (NM), and mild increase in intercellular gap (IG), capillary congestion (CC) with H & E staining under 40x magnification. Image (F) showing minimal collagen (C) deposition (blue colour) with MTS under 20x magnification (H & E = Hematoxylin and Eosin staining) (MTS = Masson Trichrome Staining)

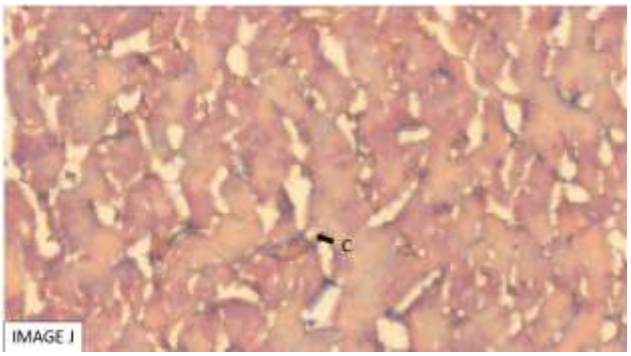
Effect of Remogliflozin on cardiac structural changes in Diabetic rats (n = 8)



Photomicrograph's showing longitudinal section from left ventricle of heart from Remogliflozin treated group presenting with, Image (G) showing necrotic myocytes (NM), and mild increase in intercellular gap (IG), capillary congestion (CC) with H & E staining under 40x magnification. Image (H) showing minimal collagen (C) deposition (blue colour) with MTS under 20x magnification (H & E = Hematoxylin and Eosin staining) (MTS = Masson Trichrome

Effect of Remogliflozin in co-administration with Metformin on cardiac structural changes in Diabetic rats (n = 8)





Photomicrograph's showing longitudinal section from left ventricle of heart from Remogliflozin in co-administration with Metformin treated group presenting with, Image (I) showing cardiac myocytes (M) within normal limits with normal intercellular gap (IG), with H & E staining under 40x magnification. Image (J) showing mere collagen (C) deposition (blue colour) with MTS under 40x magnification (H & E = Hematoxylin and Eosin staining) (MTS =

Histopathological findings in cardiac tissue of rats among various groups :

On Haematoxylin & Eosin (H & E) staining, myocardial cells in the Normal Control group appeared to be more compact and arranged in an orderly manner, with bright red cytoplasm and clear cellular nuclei. In contrast, deranged myocytic structures, broken myocardial fibres, necrotic myocytes, and inflammatory changes in cardiac myofibrils were visualized in the Diabetic control group, while treatment with Metformin, Remogliflozin, Metformin and Remogliflozin groups attenuated the above histopathological changes.

Masson's trichrome staining was used to detect interstitial fibrosis infiltration, and myocardial fibrosis.

As illustrated, no obvious myocardial interstitial collagen deposition and intact collagen fibers were observed in the Normal control group. However, in the DM group, myocardial cells showed an irregular arrangement, deranged myocytic structures, broken myocardial fibers, necrotic myocytes as visualised on H & E. The degenerative changes in the heart were significantly alleviated when diabetic rats were treated with Metformin, Remogliflozin, Metformin and Remogliflozin and also showed the signs of healing by presenting with capillary congestion in the treated groups.

Furthermore, we detected the interstitial fibrosis area of rat hearts in each group, suggested that the DM group has noticeably increased interstitial collagen fibers when compared with the Normal control group, while Metformin, Remogliflozin, Metformin and Remogliflozin

treatment groups could decrease the interstitial fibrosis area in diabetic rats and had evident differences when compared with the Diabetic control group. It is noteworthy that there was mere change between the Normal control and Metformin + Remogliflozin groups in total percentage of interstitial fibrosis area. These results suggested that the treatment groups had prevented the progression of Diabetic Cardiomyopathy as evidenced by the Histopathological studies in comparison with Diabetic control group. Amongst which, the group treated with Remogliflozin in co-administration with Metformin showed an evident prevention in progression of Diabetic Cardiomyopathy in comparison with other treatment groups as observed by the preserved heart histology with improved organization of myocytes and minor collagen deposition in diabetic rats.

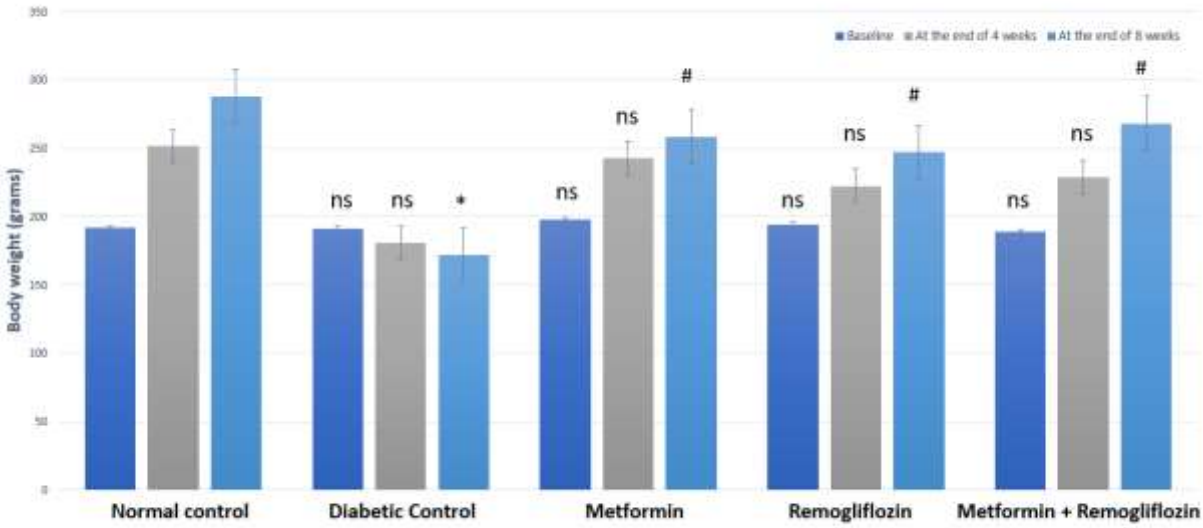
Effect of Remogliflozin alone and in co-administration with Metformin on body weight (grams) in diabetic rats

TABLE 6. Effect of Remogliflozin alone and in co-administration with Metformin on the body weight in diabetic rats (n = 8 in each group).

Day of study	Body weight in grams (Mean ± SD)				
	Normal Control (NC)	Diabetic Control (DC)	Metformin (M)	Remogliflozin (R)	Metformin + Remogliflozin (MR)
Baseline	191.5 ± 1.62	191.43 ± 2.13 ns	198.12 ± 8.21 ns	194.12 ± 2.19 ns	189.09 ± 1.19 ns
At the end of 4th week	251.67 ± 2.89	180.71 ± 4.12 ns	242.81 ± 2.09 ns	222.43 ± 2.11 ns	228.63 ± 3.78 ns
At the end of 8th week	288.01 ± 3.28	171.91 ± 3.23 *	258.29 ± 3.10 #	247.12 ± 1.28 #	268.19 ± 3.89 #

*TABLE 6: Remogliflozin, and in co-administration with Metformin alleviates the weight loss in diabetic rats. Data expressed as mean ± SD, (n = 8 in each group) statistical analysis was carried out using one – way ANOVA followed by post hoc tukey's test, * p < 0.05 versus Normal Control, # p < 0.05 versus Diabetic Control, ns – not significant*

Graph 1. Effect of Remogliflozin alone and in co-administration with Metformin on the body weight in diabetic rats (n = 8 in each group).



*Graph 1: Remogliflozin alone, and in co-administration with Metformin alleviates the weight loss in diabetic rats. Data expressed as mean ± SD, (n = 8 in each group) statistical analysis was carried out using one – way ANOVA followed by post hoc tukey's test, * p < 0.05 versus Normal Control, # p < 0.05 versus Diabetic Control*

The normal control (NC), diabetic control (DC), metformin treated (M), remogliflozin-treated (R), and metformin + remogliflozin (M + R) groups had mean body weights of (191.5 ± 1.62 grams), (191.43 ± 2.13 grams), (198.12 ± 8.21 grams), (194.12 ± 2.19 grams), and (189.09 ± 1.19 grams) on Day 0, which

was designated as the "Baseline." The one-way ANOVA followed by post hoc tukey's test showed that there was no significant difference between the groups under consideration.

Following a 4-week course of treatment, the mean body weights of the following groups were recorded: (251.67 ± 2.89 grams), (180.71 ± 4.12 grams), (242.81 ± 2.09 grams), (222.43 ± 2.11 grams), and (228.63 ± 3.78) grams for the Normal Control (NC), Diabetic Control (DC), Metformin treated (M), and Remogliflozin + Metformin + R (M + R) groups, respectively. After four weeks, the one-way ANOVA followed by post hoc tukey's test showed that there was no significant difference between the aforementioned groups.

Following an 8-week treatment period, the mean body weights (in grams) of the following groups were observed: (288.01 ± 3.28 grams), (171.91 ± 3.23 grams), (258.29 ± 3.10 grams), (250.12 ± 1.28 grams), (264.19 ± 3.89 grams), and Normal Control, Diabetic Control, Metformin treated, and Metformin + Remogliflozin treated. A one-way ANOVA followed by a post-hoc tukey's test revealed a significant decrease in body weight ($p < 0.05$) between the diabetic control group and the normal control group. Nevertheless, when compared to the diabetic control group, the considerable decrease in body weight was avoided in the Metformin, Remogliflozin, and Metformin + Remogliflozin groups ($p < 0.05$) (Graph 1).

Effect of Remogliflozin alone and in co-administration with Metformin on urine glucose (mg/dl) in diabetic rats

TABLE 7. Effect of Remogliflozin alone and in co-administration with Metformin on urine glucose in diabetic rats (n = 8 in each group).

	Normal Control (NC)	Diabetic Control (DC)	Metformin (M)	Remogliflozin (R)	Metformin + Remogliflozin (MR)
Day 0	Negative	Negative	Negative	Negative	Negative
72 hours after induction	Negative	Negative	Negative	Negative	Negative
At the end of 4th week	Negative	++	++	+++	+++
At the end of 8th week	Negative	++	+	+++	+++

Glucose levels	Negative	Trace	+	++	+++	++++
mg/dl	-	100	250	500	1000	>2000
mmol/L	-	5.5	14	28	55	> 111

The table depicts the mean values of urine glucose in mg/dl, there was no difference in the urine glucose by all rat groups on day 0 and 72 hours after induction of diabetes, whereas there was an increase in the urine glucose in Diabetic control group, Remogliflozin treated group and Metformin + Remogliflozin treated group compared to the Normal group and Metformin group by the end of 4th week. Also, there was an increase in urine glucose excretion in Remogliflozin group and Metformin + Remogliflozin group in comparison with Diabetic control group, and Metformin group by the end of 8th week (TABLE 7).

Therefore, the findings showed that, Remogliflozin by its property of SGLT – 2 inhibition had increased the urine glucose levels by inhibiting the glucose reuptake in the proximal convoluted tubules of kidney, post treatment with Remogliflozin alone and in co – administration with Metformin.

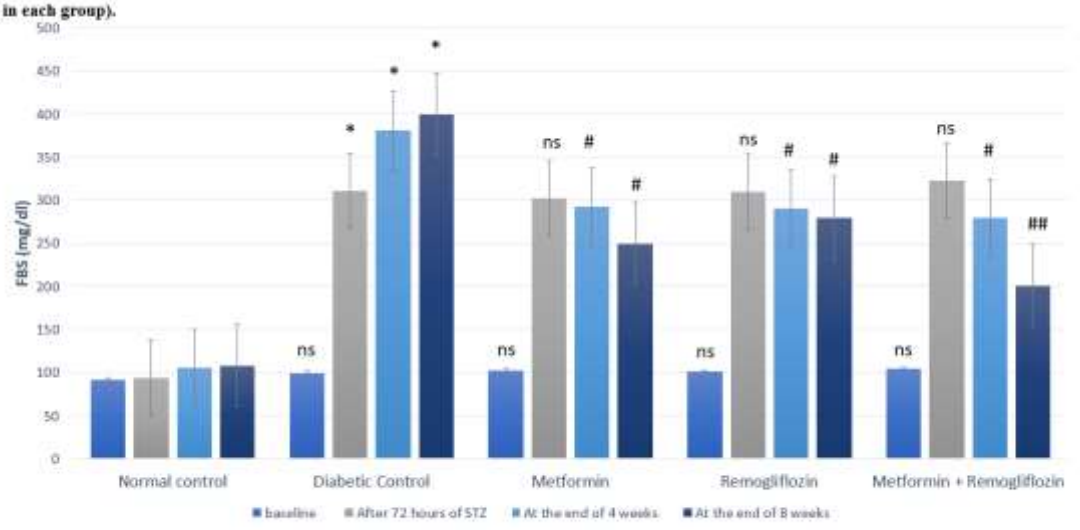
Effect of Remogliflozin alone and in co-administration with Metformin on Fasting Blood Sugar levels (mg/dl) in diabetic rats

TABLE 8. Effect of Remogliflozin alone and in co-administration with Metformin on Fasting Blood Glucose levels in diabetic rats (n = 8 in each group).

Day of study	Fasting Blood Glucose (mg/dL) (Mean ± SD)				
	Normal Control (NC)	Diabetic Control (DC)	Metformin (M)	Remogliflozin (R)	Metformin + Remogliflozin (MR)
Baseline	92.00 ± 3.86	99.58 ± 3.11	102.43 ± 1.48	101.23 ± 5.22	104.24 ± 1. 98
After 72 Hours of STZ	93.19 ± 1.22	310. 32 ± 9.21 *	302.13 ± 10.29 ns	309.27 ± 6.75 ns	323.12 ± 16.32 ns
At the end of 4th week	105.40 ± 2.08	381.21 ± 8.77 *	292.32 ± 8.78 #	290.02 ± 4.25 #	278.91 ± 12.89 #
At the end of 8th week	108.11 ± 5.34	399.72 ± 2.39 *	249.57 ± 8.43 #	279.74 ± 3.98 #	201.12 ±12.09 ##

*TABLE 8: Remogliflozin alone, and in co-administration with Metformin alleviates the Fasting Blood Glucose (mg/dl) levels in diabetic rats. Data expressed as mean ± SD, (n = 8 in each group) statistical analysis was carried out using one – way ANOVA followed by post hoc tukey's test, ns – not significant * p < 0.05 versus Normal Control, # p < 0.05 versus Diabetic Control, ## p < 0.001 versus Diabetic control*

Graph 2. Effect of Remogliflozin alone and in co-administration with Metformin on Fasting Blood Glucose levels in diabetic rats (n = 8 in each group).



*Graph 2: Remogliflozin alone, and in co-administration with Metformin alleviates the Fasting Blood Glucose (mg/dl) levels in diabetic rats. Data expressed as mean ± SD, (n = 8 in each group) statistical analysis was carried out using one – way ANOVA followed by post hoc tukey's test, ns – not significant * p < 0.05 versus Normal Control, # p < 0.05 versus Diabetic Control, ## p < 0.001 versus Diabetic control*

On Day '0', which is considered to be the baseline, the Fasting Blood Sugar (FBS) levels were compared between the groups Normal Control, Diabetic Control, Metformin treated, Remogliflozin treated, and Metformin + Remogliflozin groups and were found to be (191.5 ± 1.62 mg/dl), (191.43 ± 2.13 mg/dl), (198.12 ± 8.21 mg/dl), (194.12 ± 2.19 mg/dl), and (189.09 ± 1.19 mg/dl) respectively. For which, one-way ANOVA followed by post hoc Tukey's test showed no significant difference in the FBS levels among the groups.

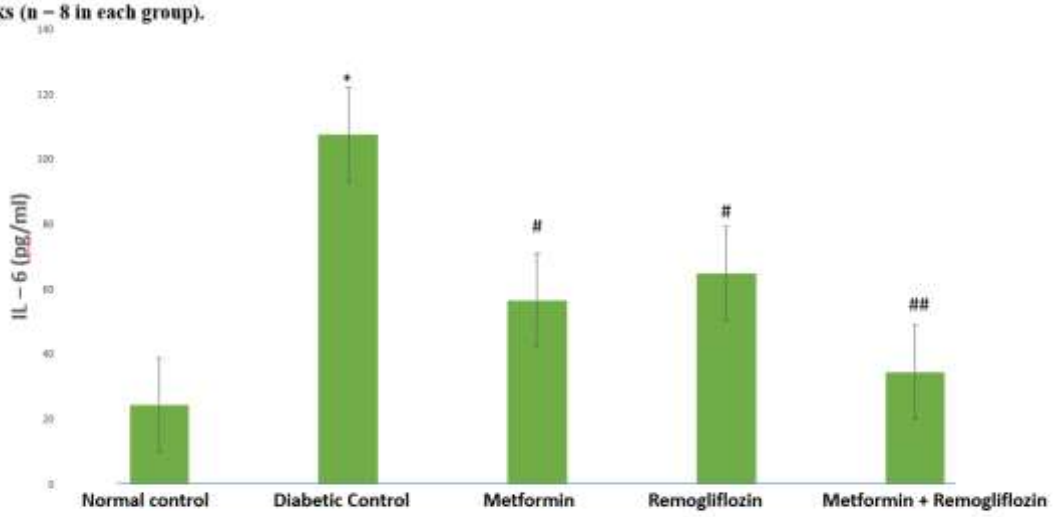
The FBS levels of the Normal Control, Diabetic Control, Metformin treated, Remogliflozin treated, and Metformin + Remogliflozin groups were, however, (93.19 ± 1.22 mg/dl), (310.32 ± 9.21 mg/dl), (302.13 ± 10.29 mg/dl), (309.27 ± 6.75 mg/dl), and (323.12 ± 16.32 mg/dl), respectively, 72 hours after the diabetes was induced. A one-way ANOVA and a post hoc Tukey's test revealed that the Diabetic control group's FBS levels were significantly higher than those of the Normal control group ($p < 0.05$). When comparing the FBS levels of the Remogliflozin and Metformin + Remogliflozin groups to those of the Diabetic control group, there was no discernible difference ($p > 0.05$). Likewise, no statistically significant variation was seen in the FBS levels between the Metformin group and the Diabetic Control group ($p > 0.05$).

By the conclusion of the fourth week, the FBS levels of the, Normal Control, Diabetic Control, Metformin treated, Remogliflozin treated and Metformin + Remogliflozin groups were, respectively, (105.40 ± 2.08 mg/dl), (381.21 ± 8.77 mg/dl), (292.32 ± 8.78 mg/dl), (290.02 ± 4.25 mg/dl), and (278.91 ± 12.89 mg/dl). A one-way ANOVA and a post hoc Tukey's test revealed that the Diabetic control group's FBS levels were significantly higher than those of the Normal control group ($p < 0.05$). Comparing the Remogliflozin and Metformin + Remogliflozin groups to the Diabetic control group, however, revealed a substantial drop in FBS levels ($p < 0.05$). Comparing the Metformin group to the Diabetic control group, there was a noteworthy drop in FBS levels ($p < 0.05$).

By the conclusion of the eighth week, the FBS levels of the Normal Control, Diabetic Control, Metformin treated, Remogliflozin treated and Metformin + Remogliflozin groups were, respectively, (108.11 ± 5.34 mg/dl), (399.72 ± 2.39 mg/dl), (249.57 ± 8.43 mg/dl), (279.74 ± 3.98 mg/dl), and (201.12 ±12.09) mg/dl. A one-way ANOVA and a post hoc Tukey's test revealed that the Diabetic control group's FBS levels were significantly higher than those of the Normal control group ($p < 0.05$). Conversely, the FBS levels in the Remogliflozin group ($p < 0.05$) and the Metformin + Remogliflozin group were significantly lower than those in the Diabetic control group ($p < 0.001$). In a similar vein, the FBS levels in the Metformin group were significantly lower ($p < 0.05$) than in the Diabetic control group (Graph 2).

Effect of Remogliflozin alone and in co-administration with Metformin on the Inflammatory parameters in diabetic rats at the end of 8 weeks

Graph 3. Effect of Remogliflozin alone and in co-administration with Metformin on the IL – 6 in diabetic rats at the end of 8 weeks (n – 8 in each group).



*Graph 3: Remogliflozin alone, and in co-administration with Metformin alleviates IL -6 (pg/ml) levels in diabetic rats. Data expressed as mean ± SD, (n = 8 in each group) statistical analysis was carried out using one – way ANOVA followed by post hoc tukey's test, * p < 0.05 versus Normal Control, # p < 0.05 versus Diabetic Control, ## p < 0.001 versus Diabetic control*

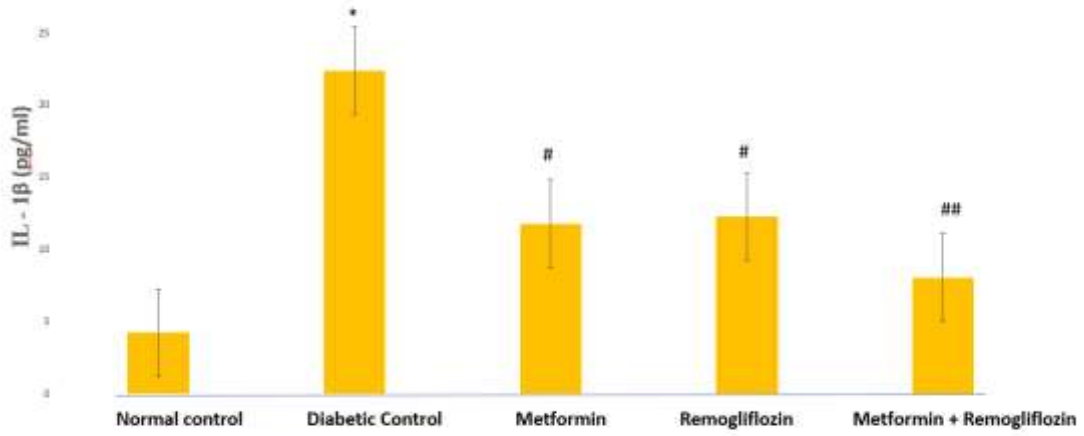
At the end of 8 weeks of treatment, the blood samples were analyzed for inflammatory parameters which included IL – 6, IL - 1β, TNF – α and the data was expressed as mean ± standard deviation.

- **Effect of Remogliflozin alone and in co-administration with Metformin on levels of IL – 6**

  After a duration of 8 weeks, the following IL-6 levels were recorded: (24.48 ± 3.87 pg/ml), (107.53 ± 1.45 pg/ml), (56.54 ± 2.15 pg/ml), (64.73 ± 5.33 pg/ml), (34.49 ± 1.78 pg/ml) for the Normal Control, Diabetic Control, Metformin treated, and Remogliflozin treated, Metformin + Remogliflozin treated groups respectively. The diabetic control group's IL-6 levels were significantly higher than those of the normal control group, as revealed by a one-way ANOVA and post hoc Tukey's test. Remogliflozin group IL-6 levels were significantly lower than those of the diabetic control group ($p < 0.05$), and Metformin + Remogliflozin group levels were significantly lower than those of the diabetic control group ($p < 0.001$). When compared to the diabetic control group, the metformin group's IL-6 levels significantly decreased ($p < 0.05$) (Graph 3).

- **Effect of Remogliflozin alone and in co-administration with Metformin on levels of IL - 1β**

Graph 4. Effect of Remogliflozin alone and in co-administration with Metformin on IL - 1β, in diabetic rats at the end of 8 weeks (n = 8 in each group).

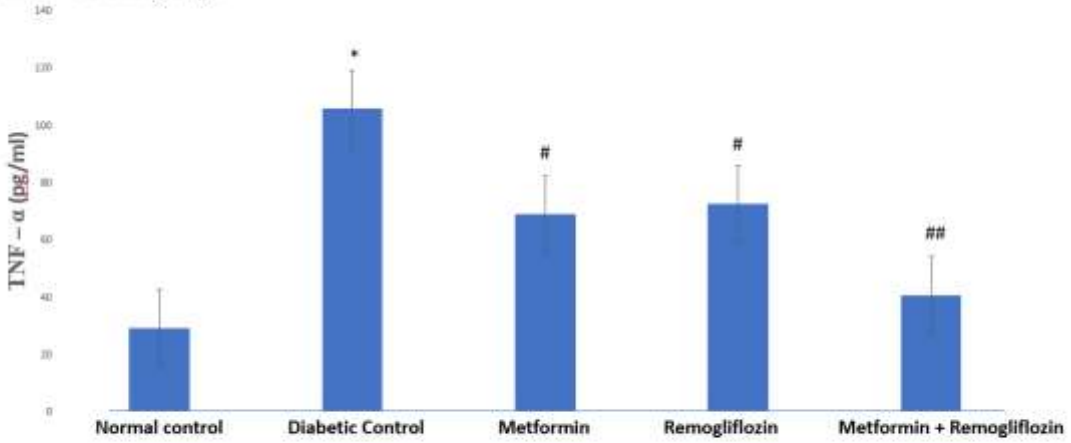


*Graph 4: Remogliflozin alone, and in co-administration with Metformin alleviates IL - 1β (pg/ml) levels in diabetic rats. Data expressed as mean ± SD, (n = 8 in each group) statistical analysis was carried out using one – way ANOVA followed by post hoc tukey's test, * p < 0.05 versus Normal Control, # p < 0.05 versus Diabetic Control, ## p < 0.001 versus Diabetic control*

The IL-6 levels were as follows at the end of the eighth week: (4.21 ± 5.21 pg/ml), (22.35 ± 2.32 pg/ml), (11.75 ± 2.15 pg/ml), (12.24 ± 7.45 pg/ml), (8.04 ± 8.31 pg/ml) for the Normal Control, Diabetic Control, Metformin treatment, Remogliflozin treatment and Metformin + Remogliflozin groups, respectively. The diabetic control group had significantly higher levels of IL-1β than the normal control group, as revealed by a one-way ANOVA and post hoc Tukey's test. Remogliflozin group IL-1β levels were significantly lower than those of the diabetic control group ($p < 0.05$), and Metformin + Remogliflozin group levels were significantly lower than those of the diabetic control group ($p < 0.001$). When compared to the diabetic control group, the metformin group's IL-1β levels significantly decreased ($p < 0.05$) (Graph 4).

- **Effect of Remogliflozin alone and in co-administration with Metformin on levels of TNF- α**

Graph 5. Effect of Remogliflozin alone and in co-administration with Metformin on TNF – α, in diabetic rats at the end of 8 weeks (n – 8 in each group).



*Graph 5: Remogliflozin alone, and in co-administration with Metformin alleviates TNF – α (pg/ml) levels in diabetic rats. Data expressed as mean ± SD, (n = 8 in each group) statistical analysis was carried out using one – way ANOVA followed by post hoc tukey's test, * p < 0.05 versus Normal Control, # p < 0.05 versus Diabetic Control, ## p < 0.001 versus Diabetic control*

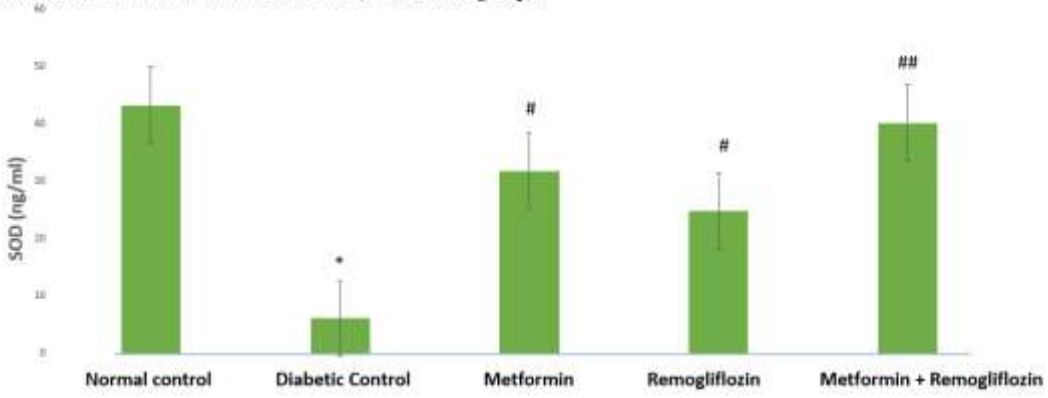
The TNF-α levels at the end of the eighth week were as follows: (29.16 ± 1.11 pg/ml), (105.71 ± 6.12 pg/ml), (68.95 ± 6.11 pg/ml), (72.46 ± 3.65 pg/ml), and (40.71 ± 5.44 pg/ml) for the Normal Control, Diabetic Control, Metformin treated, Remogliflozin treated and Metformin + Remogliflozin groups, respectively. The diabetic control group had significantly higher TNF-α levels than the normal control group, as revealed by a one-way ANOVA and post hoc Tukey's test. TNF-α levels in the Remogliflozin group were significantly lower than those in the diabetic control group ($p < 0.05$), while the Metformin + Remogliflozin group showed significantly lower TNF-α levels than the diabetic control group ($p < 0.001$). TNF-α levels were significantly lower in the metformin group compared to the diabetic control group ($p < 0.05$) (Graph 5).

- **Effect of Remogliflozin alone and in co-administration with Metformin on the Anti oxidant parameters in diabetic rats at the end of 8 weeks**

At the end of 8 weeks of treatment, the blood samples were analyzed for oxidative stress markers which included Super Oxide Dismutase (SOD), Malonaldehyde (MDA) and the data was analyzed as mean ± standard deviation.

- **Effect of Remogliflozin alone and in co-administration with Metformin on levels of Super Oxide Dismutase (SOD)**

Graph 6. Effect of Remogliflozin alone and in co-administration with Metformin on Super Oxide Dismutase (SOD) levels, in diabetic rats at the end of 8 weeks (n = 8 in each group).

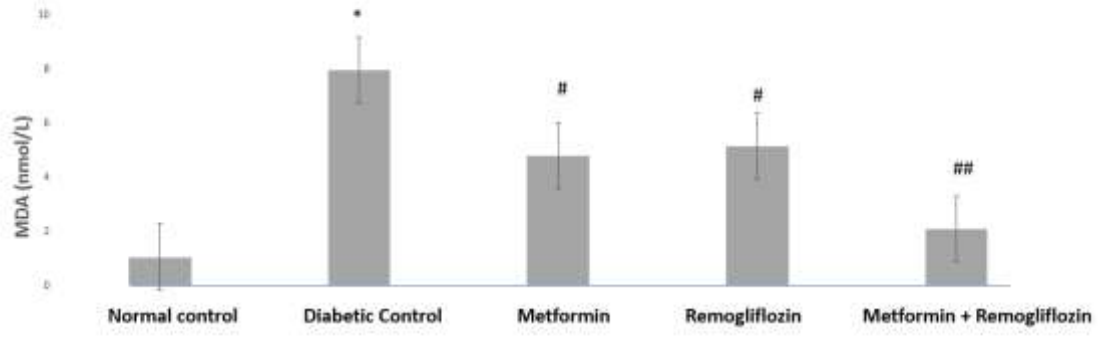


*Graph 6: Remogliflozin alone, and in co-administration with Metformin improves SOD (ng/ml) levels in diabetic rats. Data expressed as mean ± SD, (n = 8 in each group) statistical analysis was carried out using one – way ANOVA followed by post hoc tukey's test, * p < 0.05 versus Normal Control, # p < 0.05 versus Diabetic Control, ## p < 0.001 versus Diabetic control*

The SOD levels at the end of the eighth week were as follows: (43.1 ± 3.33 ng/ml), (6 ± 1.01 ng/ml), (31.67 ± 4.98 ng/ml), (24.73 ± 6.09 ng/ml), and (40.1 ± 8.34 ng/ml) for the Normal Control, Diabetic Control, Metformin treated, Remogliflozin treated, and Metformin + Remogliflozin groups, respectively. The diabetic control group's SOD levels were significantly lower than those of the normal control group, as revealed by a one-way ANOVA and post hoc Tukey's test. SOD levels were significantly higher in the Remogliflozin group compared to the Diabetic control group ($p < 0.05$), while the Metformin + Remogliflozin group exhibited a considerable increase ($p < 0.001$) when compared to the Diabetic control group. SOD levels were significantly higher in the metformin group ($p < 0.05$) than in the diabetes control group (Graph 6).

- **Effect of Remogliflozin alone and in co-administration with Metformin on levels of Malonaldehyde (MDA)**

Graph 7. Effect of Remogliflozin alone and in co-administration with Metformin on Malonaldehyde (MDA) levels, in diabetic rats at the end of 8 weeks (n = 8 in each group).



*Graph 7: Remogliflozin alone, and in co-administration with Metformin alleviates MDA (nmol/L) levels in diabetic rats. Data expressed as mean ± SD, (n = 8 in each group) statistical analysis was carried out using one – way ANOVA followed by post hoc tukey's test, * p < 0.05 versus Normal Control, # p < 0.05 versus Diabetic Control, ## p < 0.001 versus Diabetic control*

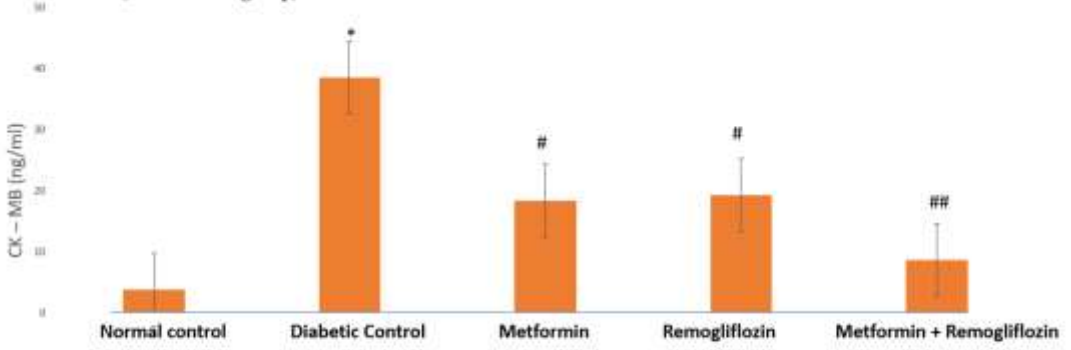
The Malonaldehyde (MDA) values at the end of the eighth week were as follows: (1.03 ± 4.32 nmol/L), (7.93 ± 6.44 nmol/L), (4.78 ± 3.41 nmol/L), (5.12 ± 2.54 nmol/L), (2.08 ± 3.33 nmol/L), for the Normal Control, Diabetic Control, Metformin treated, Remogliflozin treated and Metformin + Remogliflozin treated groups, respectively. A one-way ANOVA and a post hoc Tukey's test revealed that the diabetic control group's MDA levels were significantly higher than those of the normal control group. Remogliflozin group MDA levels were significantly lower than those of the diabetic control group ($p < 0.05$), and Metformin + Remogliflozin group MDA levels were significantly lower than those of the diabetic control group ($p < 0.001$). The MDA levels in the metformin group were significantly lower ($p < 0.05$) than in the diabetic control group (Graph 7).

- **Effect of Remogliflozin alone and in co-administration with Metformin on the Cardiac biomarkers in diabetic rats at the end of 8 weeks**

At the end of 8 weeks of treatment, the blood samples were analyzed for cardiac biomarkers which included CK – MB, Cardiac Troponin – I, Lactate Dehydrogenase (LDH) and the data was analyzed as mean ± standard deviation.

- **Effect of Remogliflozin alone and in co-administration with Metformin on levels of CK – MB**

Graph 8. Effect of Remogliflozin alone and in co-administration with Metformin on CK - MB levels, in diabetic rats at the end of 8 weeks (n – 8 in each group).

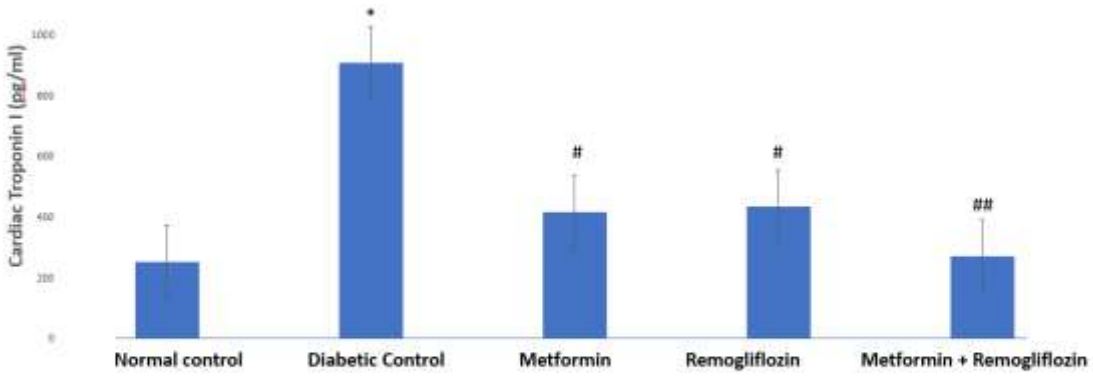


*Graph 8: Remogliflozin alone, and in co-administration with Metformin alleviates CK - MB (ng/ml) levels in diabetic rats. Data expressed as mean ± SD, (n = 8 in each group) statistical analysis was carried out using one – way ANOVA followed by post hoc tukey's test, * p < 0.05 versus Normal Control, # p < 0.05 versus Diabetic Control, ## p < 0.001 versus Diabetic control*

- By the conclusion of the eighth week, the CK-MB levels of the Metformin treated, Remogliflozin treated, Normal Control, Diabetic Control, Metformin treated, Remogliflozin treated and Metformin + Remogliflozin groups were, respectively, (3.82 ±6.01 ng/ml), (38.41 ± 4.33 ng/ml), (18.34 ± 1.23 ng/ml), (19.23 ±1.18 ng/ml), and (8.65 ± 4.72 ng/ml). A one-way ANOVA and a post hoc Tukey's test revealed that the diabetic control group's CK-MB levels were significantly higher than those of the normal control group. The Remogliflozin group's CK-MB levels were significantly lower than those of the Diabetic control group ($p < 0.05$), while the Metformin + Remogliflozin group's levels were significantly lower than those of the Diabetic control group ($p < 0.001$). When compared to the diabetic control group, the metformin group's CK-MB levels significantly decreased ($p < 0.05$) (Graph 8).

- **Effect of Remogliflozin alone and in co-administration with Metformin on levels of Cardiac Troponin I**

Graph 9. Effect of Remogliflozin alone and in co-administration with Metformin on Cardiac Troponin - I levels, in diabetic rats at the end of 8 weeks (n = 8 in each group).

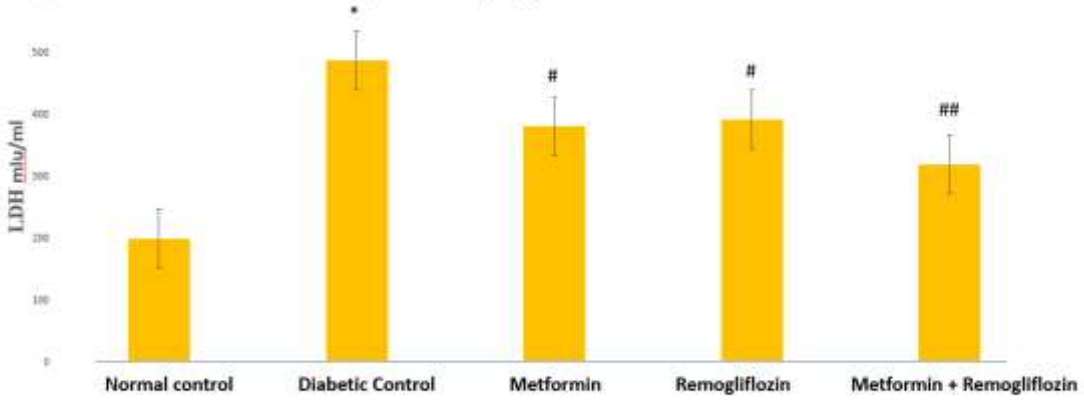


*Graph 9: Remogliflozin alone, and in co-administration with Metformin alleviates Cardiac Troponin I (pg/ml) levels in diabetic rats. Data expressed as mean ± SD, (n = 8 in each group) statistical analysis was carried out using one – way ANOVA followed by post hoc tukey's test, * p < 0.05 versus Normal Control, # p < 0.05 versus Diabetic Control, ## p < 0.001 versus Diabetic control*

Cardiac Troponin I levels at the end of the eighth week were as follows for the Normal Control, Diabetic Control, Metformin treated, Remogliflozin treated, and Metformin + Remogliflozin groups; (252.81 ± 3.32 pg/ml), (908.99 ± 3.43 pg/ml); (415.38 ± 5.43 pg/ml); (435.48 ± 1.78 pg/ml); and (269.86 ± 3.21 pg/ml), respectively. A one-way ANOVA and a post hoc Tukey's test revealed that the diabetic control group's cardiac troponin I levels were significantly higher than those of the normal control group. Remogliflozin group Cardiac Troponin I levels were significantly lower than those of the Diabetic control group ($p < 0.05$), and Metformin + Remogliflozin group levels were significantly lower than those of the Diabetic control group ($p < 0.001$). When compared to the diabetic control group, the metformin group's cardiac troponin I levels significantly decreased ($p < 0.05$) (Graph 9).

- **Effect of Remogliflozin alone and in co-administration with Metformin on levels of Lactate Dehydrogenase (LDH)**

Graph 10. Effect of Remogliflozin alone and in co-administration with Metformin on Lactate Dehydrogenase (LDH) levels, in diabetic rats at the end of 8 weeks (n = 8 in each group).



*Graph 10: Remogliflozin alone, and in co-administration with Metformin alleviates LDH (mIu/ml) levels in diabetic rats. Data expressed as mean ± SD, (n = 8 in each group) statistical analysis was carried out using one – way ANOVA followed by post hoc tukey's test, * p < 0.05 versus Normal Control, # p < 0.05 versus Diabetic Control, ## p < 0.001 versus Diabetic control*

At the conclusion of the eighth week, the LDH values for the Normal Control, Diabetic Control, Metformin treated, Remogliflozin treated, and Metformin + Remogliflozin groups were, in order, (199.01 ± 2.43 mIu/ml), (487.38 ± 4.76 mIu/ml), (380.56 ± 7.23 mIu/ml), (391.8 ± 2.88 mIu/ml), and (318.79 ± 3.90 mIu/ml), respectively. A one-way ANOVA and a post hoc Tukey's test revealed that the diabetic control group's LDH levels were significantly higher than those of the normal control group. Remogliflozin group LDH levels were significantly lower than those of the diabetic control group ($p < 0.05$), and Metformin + Remogliflozin group LDH levels were significantly lower than those of the diabetic control group ($p < 0.001$). The LDH levels in the metformin group were significantly lower ($p < 0.05$) than in the diabetes control group (Graph 10).

Discussion

A growing body of research highlights the critical roles that inflammation, cell death, and tissue oxidative damage play in causing cardiac injury in diabetes, which in turn leads to cardiac dysfunction and heart failure (HF) [98]. Innovative methods to identify new targets and treatments are desperately needed, given that diabetic patients face increased cardiac-related morbidity and mortality. The prognosis for those with diabetes is considerably poorer as they have a two to three times increased risk of acquiring cardiovascular diseases such as myocardial infarction, stroke, and heart failure [99]. By simulating cardiomyopathy in male Wistar rats with diabetes, we investigated the cardioprotective effectiveness of antidiabetic medications.

To evaluate the cardioprotective effect of the new SGLT-2 inhibitor Remogliflozin, we used the diabetic rat model generated by intra peritoneal injection of Streptozotocin [55 mg/kg] [100, 101, 102]. A popular paradigm for assessing the antidiabetic and antioxidative qualities of different medicinal drugs in vivo is Streptozotocin - induced diabetes. Streptozotocin not only produces reactive oxygen species (ROS) in pancreatic β-cells but also damages DNA and activates poly (ADP-ribose) polymerase (PARP), which reduces the amount of NAD+ in the cell and eventually causes necrosis [103]. This process could explain the lower insulin levels and hyperglycemia observed in our study's rats that received streptozotocin injections. Furthermore, sustained blood glucose elevation may accelerate the production of excess oxidative agents, leading to the breakdown of pancreatic and other tissues [104, 105]. β-cells have a limited capacity to defend against antioxidant damage, making them more susceptible to oxidative stress, which can exacerbate glycemic imbalance in animals [106].

This study's foundation lies in the absence of a cardioprotective effect associated with traditional antidiabetics. With the FDA's approval of SGLT2 inhibitors, a plethora of research has been published. Prior to this, metformin was one of the most commonly used medications to treat type 2 diabetes and was known to have cardioprotective effects in addition to its anti-hyperglycemic actions [107].

In this work, we used the SGLT2 inhibitor Remogliflozin and in co-administration with Metformin in comparison to Diabetic control rats which we used as the disease control group. According to our research, rats with type 2 diabetes treated with Remogliflozin showed improvements in abnormal blood sugar levels and weight loss. These results are consistent with clinical trials showing similar effects with other SGLT2 inhibitors, including dapagliflozin, empagliflozin, and canagliflozin [108, 109].

Our study's main finding was that, compared to the diabetes control group, all treatment groups fasting blood glucose levels were significantly lower. This implies that Remogliflozin is effective in reducing hyperglycemia, a major issue in diabetic management. Remarkably, the combination of metformin and Remogliflozin produced the greatest reduction in fasting blood glucose, suggesting synergy between the two medications.

Diabetes usually results in decreased muscle mass and inefficient glucose utilization, leading to weight loss. A study by **Wilding et al. (2009) [110]** found that Dapagliflozin in Patients with Type 2 Diabetes Receiving High Doses of Insulin Plus Insulin Sensitizers helped diabetic patients maintain or lose weight.

When compared to the normal group in our study, the diabetic control group lost a substantial amount of weight. However, there was a noticeable prevention of weight loss in the therapy groups, particularly in those receiving metformin and Remogliflozin either separately or in co-administration with each other. This suggests that Remogliflozin aids in managing body weight, likely due to enhanced glucose metabolism and overall health.

Myocardial fibrosis is a major pathophysiological process contributing to heart failure by reducing stroke volume and increasing myocardial stiffness. It is crucial to ascertain how Remogliflozin affects excessive collagen deposition in the diabetic heart and to understand its underlying mechanism of action. **Jingjing Tian et al. (2020) [111]** proved that Dapagliflozin reduces cardiac fibrosis by suppressing End-MT and fibroblast activation in T2DM. In order to identify the buildup of extracellular matrix proteins surrounding interstitial and perivascular areas—a sign of excessive collagen distribution in the hearts of type 2 diabetic rats—we performed

histopathological analyses in our study. Remogliflozin dramatically decreased the volume of collagen in these diabetic hearts, exhibiting an antifibrotic effect similar to that of metformin. Thus, in this study, we examined Remogliflozin's antifibrotic mechanism of action in detail, confirming its reported cardioprotective benefits in diabetic rats by reducing cardiac fibrosis and enhancing heart function.

Diabetic cardiomyopathy is largely caused by chronic inflammation. In this work, we investigated the effects of Remogliflozin on inflammatory parameters in diabetic rats. Important inflammatory markers like TNF-α, IL-6, and IL-1β were specifically included in the analysis. The findings demonstrated the potential anti-inflammatory qualities of Remogliflozin by showing a considerable suppression of these inflammatory cytokine levels. Furthermore, the research revealed that Remogliflozin was critical in preventing diabetic cardiomyopathy, a common complication of diabetes characterized by alterations in the structure and function of the heart muscle, leading to heart failure. **Xu et al. [112]** demonstrated that the drug Empagliflozin increased the M-2 levels in WAT and reduced the release of M-1 mediated inflammatory biomarkers in the mouse model. Similarly, Remogliflozin's capacity to reduce TNF-α, IL-6, and IL-1β levels—pro-inflammatory cytokines that lead to heart remodeling and dysfunction—is responsible for its positive effects in preventing diabetic cardiomyopathy.

The term "oxidative stress" describes a condition in which the body produces too many reactive oxygen species (ROS) than antioxidants can naturally handle. This leads to damage to proteins, lipids, and DNA, all of which are involved in the pathological processes of many diseases, including diabetes. Increases in markers such malondialdehyde (MDA) signal the generation of reactive oxygen species (ROS), which are countered by a group of enzymes including superoxide dismutase (SOD) [113]. Remarkably, recent research has demonstrated that SGLT2 inhibitors have a significant antioxidant impact on cardiomyocytes. In this work, we found that in diabetic rats, Remogliflozin markedly reduced the elevated expression of MDA. Therefore, by inhibiting oxidative stress produced by MDA and by increasing Super Oxide Dismutase levels, Remogliflozin may be able to alleviate diabetic cardiomyopathy. A study by **Hasan R. et al. (2020)** [114] demonstrated with Canagliflozin treatment that had reduced the oxidative stress pathway and restored the destroyed anti-oxidant molecules (eNOS) and inhibiting (iNOS) and NOX – 4. This is consistent with our observations in diabetic rats treated with Remogliflozin, where oxidative stress was decreased and inflammatory markers were inhibited.

Increased levels of these biomarkers are suggestive of cardiac damage and are frequently linked to diabetic cardiomyopathy, a disorder defined by anomalies in the structure and function of the heart muscle. In this work, we examined the effects of creatine kinase-MB (CK-MB), cardiac troponin I, and lactate dehydrogenase (LDH) on cardiac biomarkers in diabetic rats, both alone and in co-administration with each other, regardless of coronary artery disease and hypertension.

The findings showed that the levels of LDH, cardiac troponin I, and CK-MB were considerably lowered in diabetic rats treated with metformin and Remogliflozin, both alone and in combination. Whereas cardiac troponin I is a highly specific biomarker for myocardial infarction and cardiac stress, LDH is a marker of tissue damage, including myocardial injury [115]. This is in concordance with the study performed by **El-shafey et al** (2022), Elevated levels of the isoenzyme CK-MB, which is predominantly located in the heart muscle, are a sign of injury to the cardiac muscle. The decrease in these heart biomarkers implies that Remogliflozin has cardioprotective benefits in the setting of diabetes, whether used alone or in conjunction with metformin. Combining SGLT2 inhibitors with metformin improves glycemic management and offers further cardiovascular advantages. These findings are in support with the study conducted by **Rosa et al**. [116], where they had demonstrated the prevention of cardiac remodeling in streptozotocin-induced diabetic cardiomyopathy using the drug Dapagliflozin. Further, several clinical trials on other drugs in the same class of SGLT – 2 inhibitors where, DAPA-HF trial [117] with Dapagliflozin and EMPEROR- reduced trial with Empagliflozin had demonstrated that the drugs had reduced the risk of cardiovascular death and heart failure with reduced ejection fraction irrelevant to diabetic status of the patient.

Therefore, our study supports and was in par with the previous studies in terms of cardioprotective efficacy by other drugs such as Dapagliflozin and Empagliflozin which belongs to the same class of SGLT -2 inhibitors.

Conclusion

The study concludes with compelling evidence that remogliflozin, both by itself and in co-administration with metformin, dramatically lowers the cardiac biomarkers, oxidative stress indicators, and inflammatory markers linked to myocardial injury in diabetic rats. These results imply that these therapies can successfully stop the development and course of diabetic cardiomyopathy by shielding the heart from oxidative stress-induced damage and inflammation, as well as by enhancing overall cardiac function as seen by a decrease in cardiac markers. To confirm the therapeutic significance of these results in human individuals and to clarify the exact molecular pathways underlying these cardioprotective benefits, more investigation is necessary.

Limitations of the study :

Mechanistic Insights:

Although the study discusses potential mechanisms of action, such as anti-inflammatory and antioxidant effects, it does not delve deeply into the molecular pathways involved. Detailed mechanistic studies are required to fully elucidate how Remogliflozin confers cardioprotection.

Heart Size as a Limitation:

- **Measurement and Analysis**:

  The study does not report on the changes in heart size, such as hypertrophy or atrophy, which are important indicators of diabetic cardiomyopathy. Diabetic cardiomyopathy is often associated with changes in heart size, including left ventricular hypertrophy (LVH) and increased heart weight relative to body weight.

Immunohistochemistry Examination as a Limitation:

- **Detailed Cellular and Molecular Insights**:

  The study does not utilize immunohistochemistry (IHC), which is a powerful technique for visualizing specific cellular and molecular changes in tissue samples. IHC can provide detailed insights into the localization and expression levels of proteins related to inflammation, fibrosis, and oxidative stress.

Summary

The thesis investigates the cardioprotective effects of Remogliflozin, an SGLT2 inhibitor, in a diabetic rat model to address the growing concern of cardiovascular complications in diabetes. The study aimed to evaluate Remogliflozin's efficacy compared to traditional antidiabetic medication, metformin, in improving cardiac health in diabetic conditions.

The research established the pathological processes linking inflammation, oxidative stress, and cardiac injury in diabetic cardiomyopathy, emphasizing the urgent need for innovative treatments. Using streptozotocin + nicotinamide-induced diabetes in male Wistar rats, the study simulated cardiomyopathy to assess Remogliflozin's effectiveness.

Results indicated Remogliflozin's significant reduction in fasting blood glucose levels, demonstrating its potential in managing hyperglycemia. Furthermore, Remogliflozin attenuated weight loss, suggesting improved glucose metabolism and overall health benefits.

Histopathological analyses revealed a substantial decrease in collagen deposition in diabetic hearts treated with Remogliflozin, indicating its antifibrotic effect similar to metformin. Moreover, Remogliflozin exhibited anti-inflammatory properties by suppressing TNF-α, IL-6, and IL-1β levels, crucial in preventing diabetic cardiomyopathy.

The study also highlighted Remogliflozin's antioxidant impact by reducing markers of oxidative stress like malondialdehyde (MDA), thus potentially alleviating diabetic cardiomyopathy. Additionally, Remogliflozin treatment significantly lowered cardiac biomarkers including LDH, cardiac troponin I, and CK-MB, indicating its cardioprotective benefits.

Combining SGLT2 inhibitors with metformin showed improved glycemic control and further cardiovascular advantages. These findings align with previous studies and clinical trials, demonstrating the potential of SGLT2 inhibitors in reducing cardiovascular risk factors and diabetic complications.

Overall, the study provides valuable insights into Remogliflozin's multifaceted benefits in managing diabetic cardiomyopathy, suggesting its promising role as a cardioprotective agent in diabetes.

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KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH
(Deemed to be University)
JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI - 590010, (KARNATAKA).
INSTITUTIONAL ANIMAL ETHICS COMMITTEE.
Phone No. JNMC (0831)- 2444040

Dr.(Mrs)P.P.Patil
Chairperson, IAEC.
Prof & Head Physiology,
J.N.Medical College, Belagavi

Dr.P.A.Patil
Main Nominee - CPCSEA
Prof & Head of Pharmacology,
USM-KLE, IMP, Belagavi

Dr.(Mrs)Rekha Nayaka M.R
Member - Secretary IAEC
Asso Prof of Pharmacology
J.N.Medical College, Belagavi

CPCSEA Reg.No.: 627/PO/Re/S/02/CPCSEA

MEMBERS:

Dr.Banappa Unger
Scientist-D, RMRC,
ICMR, Belagavi.

Shri Sunil.R.Patil.
Non-scientific Social worker,
Nidasosi.

Dr. Sudha Devareddy.
Hon.Veternarian,
Belagavi.

Dr. (Mrs)S.A.Hogade,
Officer Incharge,
Central Animal House,
JNMC, Belagavi.

Dr. (Mrs)S.M.Bhimalli,
Prof of Anatomy.
JNMC,Belagavi

Dr. Vishwanatha Swamy
AHM
Link Nominee CPCSEA.
Dept of Pharmacology &
Toxicology
KLE's Coll Of Pharmacy,
Hubballi

CERTIFICATE

This is to certify that the M.D/ M.D.S/ Ph.D/ Research project Entitled "Investigation of the Cardioprotective potential of Remogliflozin in preventing Streptozotocin induced diabetic Cardiomyopathy in Male Wistar Rats.

Submitted by- [redacted]
PG Pharmacology, JNMC.

Has been approved by the Institutional Animal Ethical Committee Meeting held on 25.6.22 vide Resolution No. 17/1
For sanction of 40 Male Wistar Rats.

Main Nominee CPCSEA
IAEC-JNMC, Belagavi.
Signature and Name:
CPCSEA-Main Nominee

Member Secretary
IAEC-JNMC, Belagavi
Signature and Name:
Chairman/Mem.Secretary

No.25/1/99 – AWD (Pt.)
Government of India
Ministry of Statistics & Programme Implementation
(Committee for the Purpose of Control and Supervision of Experiments on Animals)
*****

Shastri Bhavan, New Delhi-110001.
Dated the 19th June 2002.

To -

The Principal/Director/Dean
K.L.E. Society's Jawaharlal Nehru Medical College
Nehru Nagar
Belgaum - 590 010
Karnataka

Subject: **Registration of Establishments/ Breeders under Rule 5(a) of the "Breeding of and Experiments on Animals (Control and Supervision) Rules 1998".**

Sir/Madam,

With reference to your application on the above-mentioned subject, this is to inform that your Establishment is hereby registered for "Research". Your Registration Number is **627/02/a/CPCSEA**. The nominee of CPCSEA on the Institutional Animal Ethics Committee (IAEC) of your Establishment will be intimated in due course.

1. You are requested to quote the above Registration Number in all your future correspondence with the Committee.
2. You are also requested to convene IAEC meeting at the earliest.
3. For further correspondence you are requested to contact Office of CPCSEA at Chennai, at the address given below:

Office of the CPCSEA,
Ministry of Statistics & Programme Implementation
3rd Seaward Road, Valmiki Nagar,
Thiruvanmiyur, Chennai-600 041 (Tamil Nadu)

Yours faithfully,

(R.K. JAIN)
MEMBER SECRETARY (CPCSEA) / DIRECTOR (AW)
Tel. No.3381498

Copy to: - Ms. Prema Veeraraghavan, Expert Consultant (CPCSEA), 3rd Seaward Road, Valmiki Nagar, Thiruvanmiyur, Chennai

No. 25/373/2010-AWD
Government of India
Ministry of Fisheries, Animal Husbandry and Dairying
Department of Animal Husbandry and Dairying
O/o Committee for the purpose of Control and Supervision of Experiments on Animals
(CPCSEA)
*****

Delhi Milk Scheme Complex,
Shadipur, Delhi – 110008
Date: 19.12.2022

To,

Dr Parwati Patil, Chairperson, IAEC
K.L.E.Society's Jawaharlal Nehru Medical College Nehru Nagar,
Belgaum - 590 010 Karnataka
Email: doeparwati@yahoo.co.in
Mobile: 9449019436

Subject: Renewal of Registration and Reconstitution of Institutional Animals Ethics Committee (IAEC)-regarding

Madam,

The registration of Animal House Facility of your establishment with CPCSEA has **been renewed for a period of five years from the date of issue of this letter.**

2. The registration number of Animal House Facility of your establishment is **627/PO/Re/S/02/CPCSEA for Research for Education purpose on small animals.** Henceforth, the registration number may kindly be quoted in all your future correspondence.

3. The CPCSEA has accepted the following members recommended by the establishment.

Name of the IAEC Members	Designation in IAEC
1) Dr.Parwati .P.Patil	Biological Scientist, Chairperson
2) Dr.Nerawadi A Kavi	Scientist from different biological discipline, Member Secretary
3) Dr.Veereshkumar S Shirol	Scientist from different biological discipline
4) Dr.Mohan C Singanalli	Veterinarian
5) Dr.Manjula A Yagarali	Scientist Incharge of Animal House Facility

4. CPCSEA hereby nominates the following members to the Institutional Animals Ethics Committee (IAEC) of your establishment:

Details of Nominee(s)	Nominated as
1) Dr. Manish Barvaliya Scientist-E, ICMR-National Institute of Traditional Medicine (NITM) Nehru Nagar, National Highway No. 4 Belagavi590010, Karnataka Contact No :9726901845 Email :drmanishbarvaliya@gmail.com	Main Nominee
2) Dr. Prabhakar Adake Professor of Pharmacology, KAHER's JGMM Medical College, Korgonadhurshi,Gabbur cross, Hubballi-580028 Karnataka Contact No :9886554800	Link Nominee

3) Dr. Shabbir Rafik Pendhari Department of Pharmacology Bharati Vidyapeeth (Deemed to be University) Medical College and Hospital, Sangli. 416414 Contact No :9766417420 Email :drshabbir@gmail.com	Scientist from outside the Institute
4) Mr. Atul Ramchandra Chopade Dept of Pharmacology, Rajarambapu College of Pharmacy, Kasegaon, Tal: Walwa, Dist. Sangli – 415404, Maharashtra Contact No :9226146106 Email: chopadeatul@gmail.com	Socially Aware Nominee

(Please note that any change in IAEC members can be made only with prior approval of CPCSEA.)

5. The IAEC is valid for a period of five years and is synonymous with renewed period of registration. IAEC is required to be reconstituted at the time of renewal of registration as per CPCSEA guidelines.

6. You are requested to convene the meeting of the re-constituted IAEC within a period of 30 days and upload the same on the website of the CPCSEA.

7. It is stated that only above approved IAEC members shall sign, with date, on the attendance sheet of the IAEC meetings, and decisions will be taken only in meetings where quorum is complete. The quorum for holding IAEC meeting is six (6), and Main Nominee, Scientist from outside the Institute and Socially Aware Nominee must be present in such meetings. Link Nominee can attend in case main nominee conveys his unavailability in writing to the chairman IAEC. However, the Link Nominee should be invited once a year to update him/ her about the activities of the IAEC. Any decision taken in the meetings of IAEC without quorum shall be considered invalid.

8. It is also to inform you that before commencing any research on large animals you are required to send research protocols with due recommendation of IAEC to CPCSEA for further approval (procedure for submission of Research Protocols is available on the website of CPCSEA).

Yours Sincerely,

(Dr. S. K. Dutta)
Member Secretary (CPCSEA)

Copy for necessary action to: Nominees of CPCSEA.

The Main Nominee is requested to ensure that the IAEC meetings are held regularly as stipulated in the SOP of CPCSEA and submit the Annual Inspection Reports of the Animal House Facility regularly on the Website of CPCSEA.