"To Evaluate The Effect Of Ranolazine Alone And Coadministration With Metformin On Streptozotocin Induced Diabetic Nephropathy In Male Wister Rats"

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Dr. Rahul Vaish

LIST OF ABBREVIATIONS

ADA	-	American diabetic association
AGE	-	Advanced glycation end products
ANOVA	-	One way analysis of Variance
CNS	-	Central Nervous System
CVD	-	Cardiovascular Disease
FPG	-	Fasting Blood Glucose
CPCSEA	-	Committee for the Purpose of Control and Supervision of Experiments on Animals
CRP	-	C-reactive protein
ELISA	-	Enzyme Linked Immuno Sorbent Assay
DM	-	Diabetes Mellitus
DN	-	Diabetic nephropathy
GLUT	-	Glucose transporter
H & E	-	Haematoxylin and eosin
HDL-C	-	High Density Lipoprotein Cholesterol
MBW	-	Mean body weight
RBS	-	Random blood sugar
OGTT	-	Oral Glucose Tolerance Test
STZ	-	Streptozotocin
IAEC	-	Institutional Animal Ethics Committee
ICAM-1	-	Interstitial Cellular Adhesion Molecules-1
TFN – α	-	Tumour necrosis factor
PAS	-	Periodic Acid Schiff

IL	-	Interleukin
IRS-1	-	Insulin receptor substrate 1
Sr. Cr.	-	Serum Creatinine
ACE	-	Angiotensinogen converting Enzyme
MCP	-	Monocyte Chemoattractant Protein
MIP	-	Macrophage Inflammatory Protein
BP	-	Blood Pressure
RPC	-	Renal Parenchymal Cell
VEGF	-	Vascular Endothelial Growth Factor
TGF	-	Transforming Growth Factor
IP	-	Intraperitoneal
ESRD	-	End Stage Renal Disease
GFR	-	Glomerular filtration Rate
GBM	-	Glomerular Basement Membrane
PCT	-	Proximal Convoluted Tubule
PG	-	Prostaglandins
NIDDM	-	Non-Insulin Dependent Diabetes Mellitus
PDGF	-	Platelet Derived Growth Factor
PGF-2α	-	Prostaglandin F – 2 alpha
DPP	-	Dipeptidyl Peptidase
ARB	-	Angiotensin Receptor Blocker
ROS	-	Reactive oxygen species
SGLT	-	Sodium glucose cotransporter
SEM	-	Standard Error of Mean

T1DM	-	Type 1 Diabetes Mellitus
T2DM	-	Type 2 Diabetes Mellitus
TG	-	Triglyceride
TGF	-	Transforming growth factor
ZFR	-	Zucker Fatty Rats
TZD	-	Thiazolidinedione

ABSTRACT

Introduction: Diabetic nephropathy (DN) is a consequence of diabetes mellitus (DM) that affects the kidneys. Inflammation and excessive blood sugar are two factors in the pathophysiology of Diabetic nephropathy. So the primary objective of this study was to evaluate the efficacy of ranolazine monotherapy and combination therapy of ranolazine and metformin on streptozotocin induced diabetic nephropathy in male Wistar rats. The secondary objective of this study to evaluate the effect of these drugs on inflammatory markers like TNF – α, CRP, IL-6.

Materials and Methods: For induction of diabetic nephropathy, rats were injected with a single dose of intraperitoneal streptozotocin (45 mg/kg). After 72.hr random blood sugar were measured and a value of >200mg/dl was considered as diagnostic of diabetes mellitus. After induction of diabetes, oral metformin (180 mg/kg; once daily), ranolazine (90 mg/kg; once daily) and ranolazine with metformin i.e. combination therapy (90 /180 mg/kg; once daily) were administered for eight weeks. Body weight was measured weekly and RBS was measured every 15 days. HbA1C, Serum Creatinine, Urine proteinuria were assed at the onset and at the end of study. At the end of the experiment, rats were scarified and serum was analysed for TNF-α, IL-6 and CRP levels. Histopathological examination of kidneys was done using haematoxylin and eosin (H & E) and PAS.

Results: Ranolazine monotherapy significantly reduced HbA1C level, RBS, Serum creatinine and inflammatory markers compared to the diabetic control group. But, did not show significant reduction in proteinuria. Ranolazine also failed to show protective effect against the histopathological changes of diabetic nephropathy. The

improvement in RBS, HbA1C and inflammatory markers by Ranolazine was less compared to metformin monotherapy or the combination group.

The combination therapy demonstrated a significant reduction in HbA1C level, RBS, Serum creatinine, proteinuria and inflammatory markers compared to the diabetic control rats. Additionally combination therapy was successful in preventing the changes of diabetic nephropathy in rat kidneys such as basement membrane thickening, mesangial matrix expansion, interstitial nephritis and focal tubular necrosis compared to the diabetic rats. When compared with the metformin monotherapy and ranolazine monotherapy, the combination treatment showed more reduction in RBS, HbA1C and inflammatory markers.

Conclusion: The present study demonstrated that the combination treatment of Ranolazine and Metformin exert significant nephroprotective effect in chronic model of diabetic nephropathy in male Wistar rats. The anti-diabetic and anti-inflammatory effect seen in this study could be attributed to their reduced blood sugar effect and direct anti-inflammatory effect. However, ranolazine alone requires additional investigation in order to provide further insight and understanding into the mechanism of action and efficacy of this compound in diabetic nephropathy.

Key words: Ranolazine, Metformin, Diabetic nephropathy, Inflammation, Wistar rats.

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INTRODUCTION

Diabetes is caused by a combination of impaired insulin-mediated glucose elimination (insulin resistance) and a deficiency in pancreatic beta cell insulin secretion.[1] Complex interplay of genetic and environmental variables produce DM. It is divided into two categories based on the underlying pathogenic process: type 1 and type 2. Type 1diabetes is caused by a complete or near-complete lack of insulin, whereas type 2 diabetes is marked by varying degrees of insulin resistance, decreased insulin secretion, and rise glucose production.[2]

Diabetes was anticipated to affect 171 million people worldwide in 2000, rising to 463 million in 2019 and expected to reach 700 million by 2045. This equates to around 8%– 10% of the global population, necessitating at least 760 billion US dollars in diabetes-related health expenditures.[3] DM has progressively rise in India and around the world over the last quarter-century, with India accounting for a significant portion of the worldwide hardship. Diabetes has gone up significantly in India from 26 million in 1990 to 65 million in 2016, with the highest prevalence in Tamil Nadu, Kerala, and New Delhi.[4] India now has roughly 69.1 million diabetics and is predicted to have the second highest number of diabetes cases behind China, with the number of cases expected to reach 79.4 million by 2030. [5, 6]

Diabetes is also a potential risk for diabetes - related complications such as retinopathy, nephropathy, neuropathy (microvascular), cardiovascular disease, and stroke (macro vascular) and it usually coincide with dyslipidaemia and hypertension, making it a major cause of diabetic complications mortality.[7] Diabetic nephropathy (DN) is a serious microvascular consequence of both type 1 and type 2 diabetes that is a significant cause of kidney failure (ESRD). More than 40% of persons with diabetes

develop chronic kidney disease, and a large number of them require renal replacement therapy as a result of ESRD. [8, 9]

In India, one population-based previous research from 2002 to 2005 found that diabetic nephropathy was responsible for 44% of ESRD patients. [10] The 'CURE' According to the Study 2.2% of people with kidney disease had overt nephropathy and microalbuminuria. 26.9 percent of people with diabetes live in urban areas. Among the elder people also, it's much more common, with diabetic nephropathy accounting for about 46% of all chronic illness of the kidneys. [11, 12]

Genetic predisposition, acceleration of the polyol pathway, stimulation of the renin–angiotensin system, reactive oxygen species (ROS), activation of the protein kinase C pathway, a rise in advanced glycation end products (AGE), & glomerular hyperfiltration are all potential causes for diabetic nephropathy.[13, 14] There seems to be no symptoms or indicators of diabetic nephropathy in its early stages. It is important to note that as the disease progresses, changes in blood pressure, fluid balance, increased excretion of urinary albumin and protein, as well as a decrease in the kidney's ability to filter blood occur as a result of the chronically elevated blood glucose levels and inflammatory markers. [15]

For diabetic nephropathy proteinuria and glomerulosclerosis are the two most common medical conditions treated with drugs now on the market.[16] There is a significant risk of diabetic nephropathy advancement while taking these drugs. Preventing or slowing the progression of diabetic nephropathy is impossible with the help of a single drug. New therapeutic drugs targeting kidney-specific disease processes and new strong biomarkers for assessing their efficacy are therefore

urgently required.[9] To address the clinical demand, further research is required in this therapeutic field.

Renal parenchyma secretes MCP-1, a monocyte chemo-attractant protein, which attracts other immune cells to the kidneys in response to chronic hyperglycemia and AGE (Advanced Glycation End Products) accumulation. Monocytes are activated and differentiated by MCP-1. Monocytes that have been stimulated then start releasing inflammatory cytokines and ROS. RPC death and necrosis are induced by as IL-1, IL-6, IL-18, CRP and ROS. (TGF) and (VEGF) are examples of Profibrotic growth factors that stimulate the formation of myofibroblasts, leading to fibroblast proliferation and the deposition of extracellular matrix. As a result, inflammation-induced fibrosis has emerged as a new area of research for new therapeutic agents in both laboratory investigations and preclinical studies.[16, 17, 18]

Metformin, sulfonylureas, and insulin are the mainstays of diabetes pharmacotherapy today. Some of the most recent additions include the dipeptidyl peptidase IV (DDP IV) and SGLT II inhibitors (Gliflozins) as well as thiazolidinedione (TZD) and alpha glucosidase inhibitors. [19] These medications help manage blood sugar levels, but they can't stop diabetes and its complications from progressing or eliminate the need for insulin altogether.

For the treatment of T2DM, metformin is recommended as a first-line medicine because of its beneficial anti-inflammatory and hypoglycaemic properties. An AMPK (AMP protein kinase) activator, it works by decreasing hepatic glucose synthesis, lowering intestinal absorption, and enhancing peripheral insulin sensitivity. It showed in murine macrophages that AGEs - driven inflammatory response was inhibited. Because of its glycaemic effectiveness, weight reduction, minimal risk of

hypoglycemia, good tolerability, and low cost, metformin is suggested as an initial pharmacological therapy for patients with type 2DM.[20] And it also reduce the initial progression of diabetic nephropathy in many studies. Despite this, metformin frequently loses its effectiveness with time, necessitating the use of a second-line therapy. [21]

The lacks of present treatments will have to be talked with a variety of new therapeutic substances.

In CAD patients with cardio protective qualities and no effect on heart rate or blood pressure, ranolazine is the first-in-class antianginal medication. Its anti-ischemic actions are thought to be caused by an inhibition of late sodium current and caused by blocking of the cardiac sodium channel isoform Nav1.5. [22, 23] In chronic stable angina, a sneaky symptom of CAD, ranolazine is a unique anti-ischemic and antianginal medication that reduces angina frequency while also successful workout performance.[24] In two clinical studies, ranolazine reduced HbA1c in patients of diabetes with CAD in addition to its antianginal benefits. Like in the CARISA research found that ranolazine reduced HbA1c in patients with chronic angina and type 2 diabetes in a dose-dependent manner. [25] And also Researchers found that ranolazine reduced HbA1c levels in diabetics and prevented those with previously normal HbA1c levels from developing newly increased levels in the MERLIN-TIMI-36 research.[26 ,27] Ranolazine, an antianginal medication, has also been found to have an anti-inflammatory effect in animal experiments using acute and subacute models. There is some evidence that ranolazine can help treat diabetes and inflammation that may also help with post-diabetic complication consequences.[28] However, to date there have been no animal studies which have tested the effects of ranolazine alone

and in combination with metformin on inflammation and diabetic complication like nephropathy.

Therefore, considering the burden of the disease, its Complications, role of inflammation in the pathogenesis of diabetes and its complications, combined use of antidiabetic agents that lower blood glucose level and possess anti-inflammatory actions can be explored, since it would be beneficial to the patients with comorbid conditions like diabetes with nephropathy from an efficacy, safety and economical point of aspect.

In this study we used streptozotocin (STZ) induced diabetic nephropathy rats model and observed whether ranolazine alone and in combination with metformin exerted renal protective effect by regulating glucose level and mediated by inflammatory markers (CRP, IL- 6, TNF – alpha) in DN rats.

OBJECTIVES

- **Primary Objective**

- To assess the efficacy of ranolazine alone and coadministration with metformin to prevent diabetic nephropathy in male Wister rats.

- **Secondary Objective**

- To assess the effect of ranolazine alone and coadministration with metformin on inflammatory markers TNF-Alpha, CRP, IL-6, level in male Wister rats.

REVIEW OF LITERATURE

A. Diabetes mellitus:

Definition:

WHO has defined diabetes as a metabolic disorder of multiple aetiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both.[29]

History of Diabetes mellitus:

Aristotle's Roman physician Aretaeus first used the term "diabetes" in the second century AD, which literally translates as "passing through." For their part, accounts of a disease condition that is comparable to diabetes have been bare in texts that date back thousands of years and come from a variety of different countries around the world. Early records of a condition that is comparable to diabetes may be found in the Egyptian Ebers Papyrus, which goes back to 1552 BC. It is the earliest documented mention of the name for this condition. A group of ancient Indian medical writings published between 400 and 500 BCE, known as Charaka and Sushruta, are widely regarded as the first medical texts ever written in the world to contain comprehensive information on diabetes, including its categorization and treatment. In the latter half of the nineteenth century, notably in the United States, the understanding of human physiology and fundamental biochemistry experienced a rapid growth. Several researchers, including Minkowski and von Mering, Paulescu and Moses Barron, and others, made significant contributions to the discovery that DM results from a malfunctioning pancreas. However, it was not until the ground breaking work of Banting and colleagues in 1921 that the precise chemical secreted

by the pancreas that prevented the development of diabetes was discovered and named. Originally known as "isletin" insulin is the term given to a substance that was later renamed once it was discovered that it contained the substance insulin.[30]

It was far from perfect when Banting and Best first created their "thick brown sludge," as they discovered while they were experimenting with it. The product was discovered to contain high quantities of impurities, and the clinical effect of each batch differed significantly from the previous one. The development of improved insulin formulations in the twentieth century led in the development of high purity insulin and mono component insulin formulations, as well as improved pharmacokinetic properties (mono component insulin) (NPH and Lente insulin). The introduction of human insulin in the 1980s and insulin analogues in the last decade of the twentieth century marked the culmination of this process.

As with insulin therapies, non-insulin therapy for diabetes gained a significant boost in the later part of the twentieth century with the development of Biguanides and sulfonylureas. The development of novel compounds such as thiazolidinedione, alpha glucosidase inhibitors, incretin-based treatments, and most recently, SGLT2 inhibitors, has occurred in the latter part of the twentieth century and the early years of the twenty first century, respectively.

The advancements in medicine over the last 150 years have made a significant difference in the lives of people suffering from diabetes. Type 1 diabetes is no longer considered to be deadly in all cases. Patients with type 2 diabetes can now expect to live longer and more comfortably than they could in the past, thanks to advances in medical science. We do, however, have a long way to go before we are finished. A cure for diabetes, whether type 1 or type 2, is still decades away from being

discovered. The currently available medications do not exactly restore normoglycaemic in a physiologic manner and they are not completely free of adverse effects, either and diabetic complications kill millions of lives every year, despite our best efforts. It is hoped that the research being conducted now all over the world will bring solutions to these difficulties during our lifetime. The number of people suffering from diabetes is increasing at an alarming rate. In the past, we have overcome significantly more tough challenges than this one!

Diagnostic criteria for Diabetes mellitus:

In order to diagnose diabetes mellitus, blood glucose levels are measured (fasting plasma glucose [FPG] and oral glucose tolerance test [OGTT]) or HbA1c is measured. Testing the connection of FPG and HbA1c with retinopathy helps to determine the optimal cut-off estimation of plasma glucose and HbA1c values for diabetic patients.[31]

TABLE 1: Diagonstic criteria for diabeties mellitus

HbA1C> 6.5%. The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay. *
OR
FPG >126 mg/dl (7.0 mmol/l). Fasting is defined as no caloric intake for at least 8 h.*
OR
2-h plasma glucose > 200 mg/dl (11.1 mmol/l) during an OGTT. The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water. *
OR
In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose > 200 mg/dl (11.1 mmol/l).

Classification of Diabetes mellitus: American diabetic association (ADA) classified diabetes as type 1, type 2 that is most widely accepted worldwide.

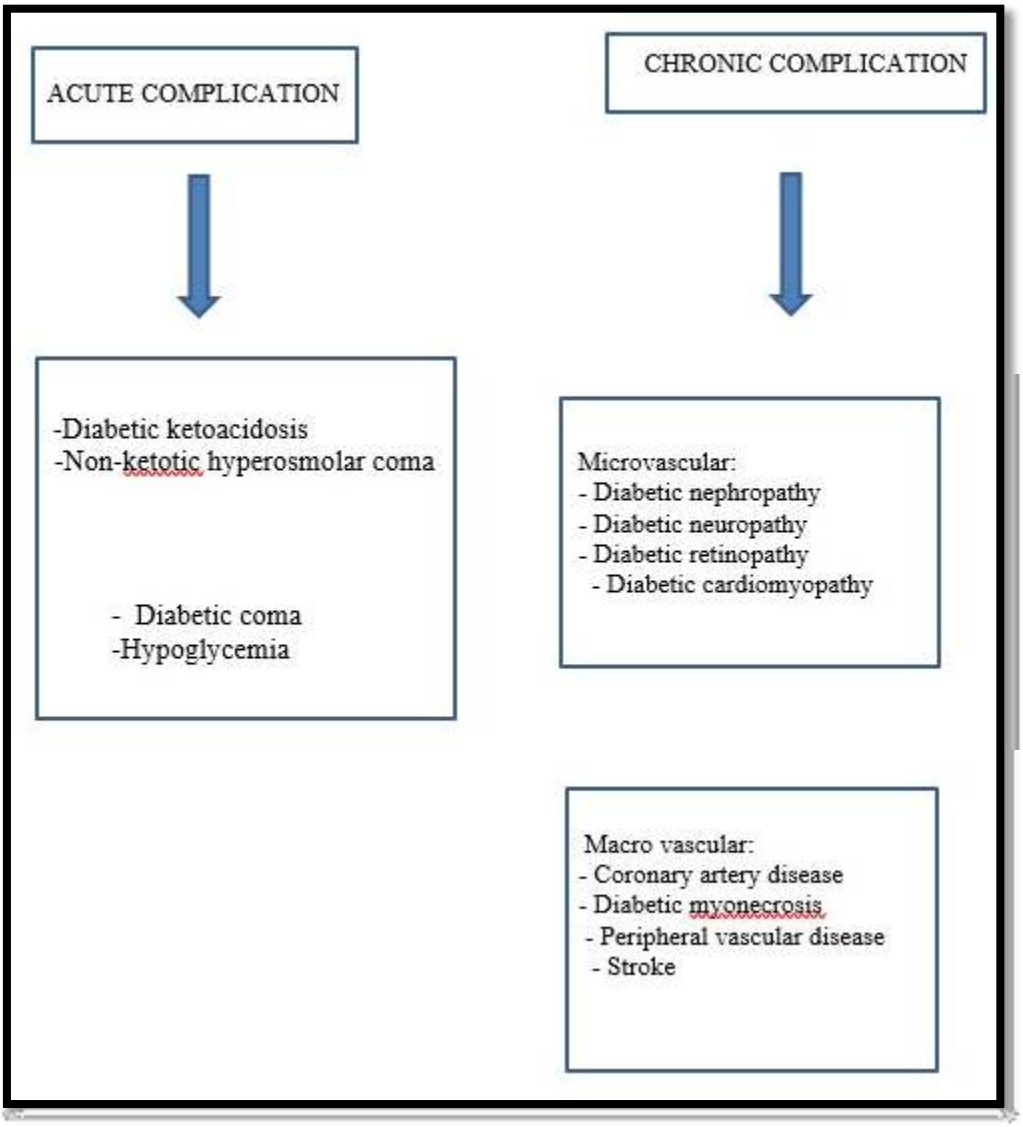
Type 1 diabetes mellitus: Approximately 5-10 percent of diabetic cases are caused by type 1 diabetes mellitus, commonly known as autoimmune diabetes or non-insulin dependent diabetes mellitus (NIDDM). It is believed that the primary cause of diabetes in these patients is autoimmune T-cell driven inflammation and beta cell death. It is responsible for around 80-90 percent of all diabetes cases in children and adolescents. Type 1 diabetes has also been linked to a number of different HLA types, with the majority of these being linked to the DR or DQ kinds.[32]

Type 2 diabetes mellitus: More than 90-95 percent of diabetes patients suffer from this type of condition, which accounts for the vast majority of diabetic individuals. Insulin resistance is the most common cause of type 2 diabetes mellitus, and it occurs when the body's organs have an increased demand for insulin. There is no insulin dependency in these people, which is the primary difference between them and those who have type 1 diabetes, as previously stated. Obesity, essential hypertension, nephropathy, dyslipidaemia, low HDL, and high LDL cholesterol are all connected with insulin resistance, as are other risk factors.[33]

TABLE 2: Current pharmacotherapy for diabetes mellitus:[2]

Drug class	Mechanism of action	Examples
Oral preparation		
Biguanides	Decrease hepatic glucose production	Metformin
α- glucosidase inhibitors	Decrease glucose absorption	Acarbose, Miglitol, Voglibose
Dipeptidyl peptidase IV (DPP-IV)	inhibitors Prolong endogenous GLP-1 action	Linagliptin, Sitagliptin, Vildaglipti Saxagliptin, Teneligliptin,
Insulin secretagogues Sulfonylureas	Increase insulin secretion	Gliclazide, Glipizide, Glyburide, Gly Glimepiride, Gliquidone, clopyramide
Insulin secretagogues Nonsulfonylureas	Increase insulin secretion	Nateglinide, Repaglinide, Mitiglinide
Sodium glucose cotransporter 2 (SGLT-2) inhibitors	Increase urinary glucose excretion	Canagliflozin, Dapagliflozin, Empagliflozin
Thiazolidinedione's	Decrease insulin resistance, increase glucose utilization	Rosiglitazone, Pioglitazone
Parenteral preparation		
Amylin agonists	Slow gastric emptying, decreases glucagon	Pramlintide
GLP-1 receptor agonists	Increase insulin, decrease glucagon, slow gastric emptying, satiety	Exenatide, Liraglutide, Dulaglutide
Insulin	Increase glucose utilization, decrease hepatic glucose production and other anabolic actions	
Medical nutrition therapy and physical activity	Decrease insulin resistance, Increase insulin secretion	Low calorie, Low fat diet, Exercise

TABLE 3: Complications associated with Diabetes mellitus: [33]

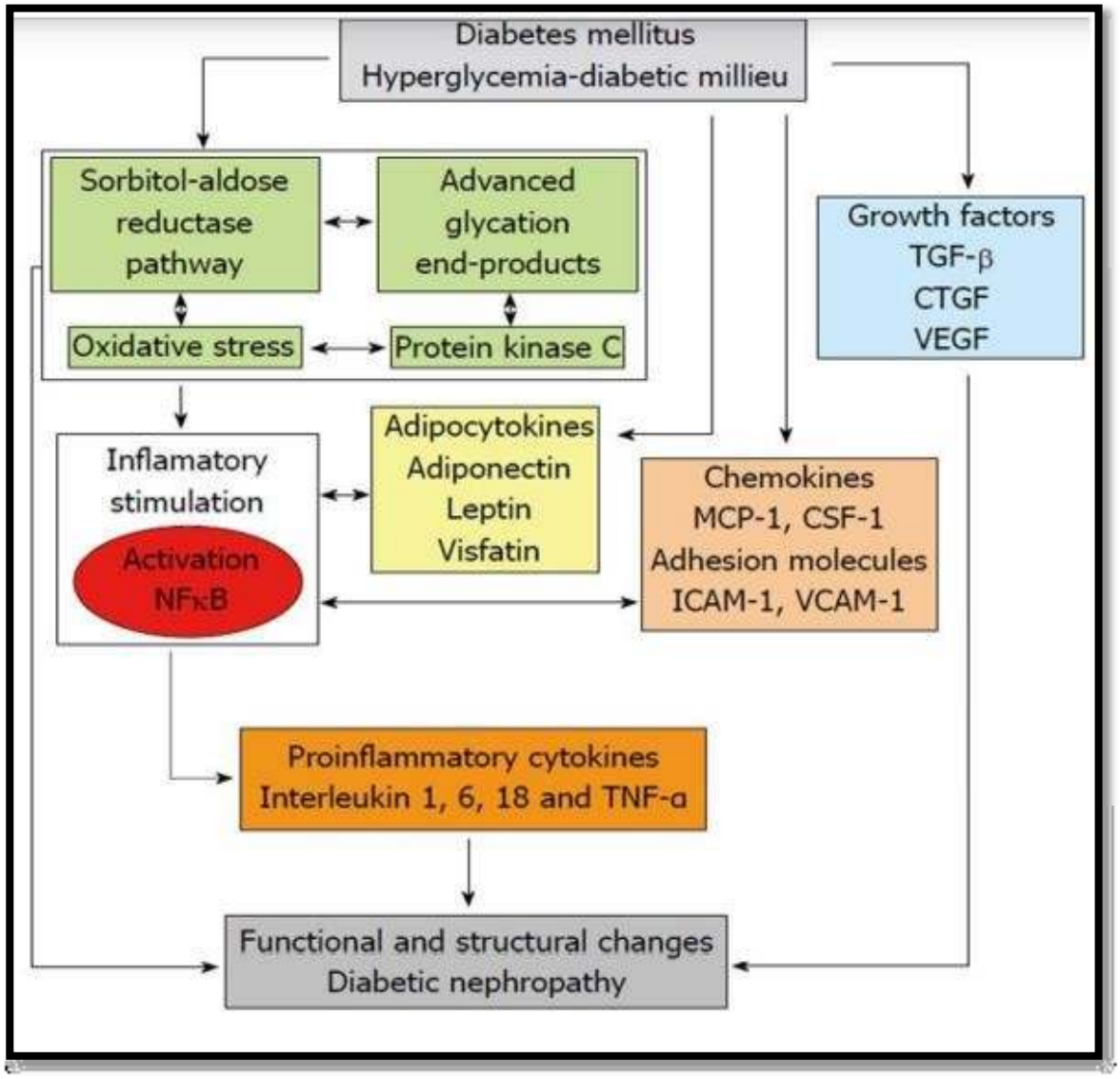


Diabetic nephropathy: [34]

Microalbuminuria precedes proteinuria >500 mg in 24 hours, which is what is known as Diabetic Nephropathy or Diabetic Nephropathy. Albumin excretion of 30-299 mg/24 hours is considered microalbuminuria. About 7% of people with type 2 diabetes may already have microalbuminuria when they are diagnosed with the disease, according to current estimates.

Diabetic nephropathy is related with an increase in glomerular basement membrane thickness, the production of mesangial nodules (Kimmelstiel-Wilson bodies), and the development of micro aneurysms.

FIGURE 1: Pathophysiology of diabetic nephropathy and other microvascular complications in diabetes: [16, 17, 18]



It has been discovered that the pathophysiology of microvascular problems is connected with a variety of processes. The names of them are as follows:

- High glucose levels produce an increase in the influx of sugar through the polyol pathway, which results in an increase in the production of sorbitol.
- High sorbitol concentrations produce osmotic stress, which ultimately results in microvascular difficulties.
- High glucose concentrations cause an increase in the non-enzymatic creation of advanced glycosylated end products, which finally results in microvascular complications (AGEs). AGEs have been demonstrated to be related with the production of microaneurysms and the loss of pericytes.
- Glucose concentrations above a certain threshold result in the creation of free radicals and reactive oxygen species (ROS), which ultimately induce oxidative stress in the tissues.
- As a result of persistent hyperglycemia, the body secretes more (MCP-1), which draws monocytes towards the kidneys, where they undergo proliferation and differentiation. These inflammatory cells emit IL-1, IL-6, and TNF-alpha, which lead to structural and functional abnormalities in the kidneys.
- Increased production of TGF-β as a result of elevated blood sugar levels causes myofibroblasts to become activated, which results in increased fibroblast proliferation and matrix deposition.

Multiple growth factors, including (VEGF), (TGF beta), and growth hormone, have been linked to microvascular problems in diabetic patients. It has been demonstrated that increased production of VEGF is connected with the development of DN.

In diabetic individuals with cerebrovascular disease, it appears that both major and small blood arteries are damaged in some way. Small subcortical infarcts or lacunar strokes are caused by fibrinoid necrosis, which is a type of microvascular illness.

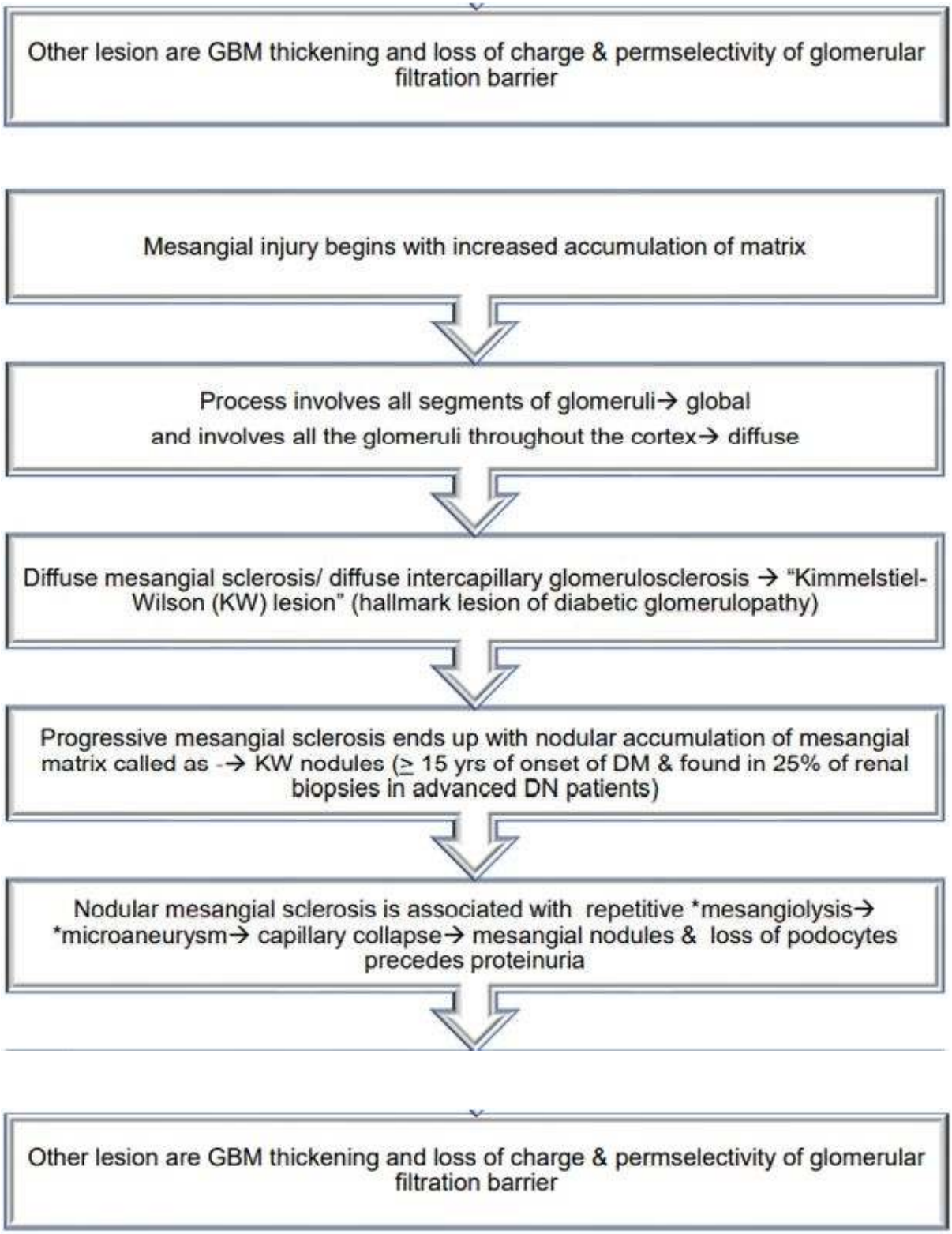
TABLE 4: Stages of Kidney disease in diabetes mellitus [2, 9]

Stage	Designation	Characteristics	Structural changes	GFR ml/min	Urinary albumin excretion	Blood pressure
I	Hyperfunction/ Hypertrophy	Glomerular hyperfiltration	Glomerular hypertrophy	~150	May be increased	Normal
II	Normoalbuminuric	Normal urinary albumin excretion	Increase Basement membrane thickness	Hyperfiltration	Normal (high during stress)	Normal
Transition (II→ III)	Transition phase	High urinary albumin excretion	Not known	Hyperfiltration	Increasing	Increase
III	Incipient DN, microalbuminuria	Raised urinary albumin secretion	Urinary albumin excretion correlated to structural damage	Increased more	20→200 mcg/min	Raised compared to stage II
IV	Overt DN	Clinical proteinuria or urinary albumin excretion > 200 mcg/ min	Advanced structural damage	Normal to advanced reduction	>200 (~10000mcg /min)	Often frank hypertension (5% yearly)
V	End stage renal Disease	Uraemia	General glomerular closure	Very low	Decreasing albuminuria	Often high related to volume expansion

Poor glycaemic management is a significant determinant in the development of microalbuminuria and the course of the disease. Patients with microalbuminuria have a significantly higher prevalence of hypertension, and the vast majority of patients with overt proteinuria have typical hypertension as well. BP may rise by around 4 mm of mercury per year shortly after the onset of microalbuminuria. Because of this, patients with T1DM who have microalbuminuria or overt renal

impairment comprise the majority of those who receive antihypertensive treatment. On the other hand, certain people with insulin-dependent diabetes may have elevated blood pressure prior to the development of microalbuminuria, a condition known as coincidental essential hypertension. Additionally, some people with normoalbuminuria will acquire microalbuminuria. This is a normal component of the disease's progression because, throughout the first few years of diabetes, the vast widely held of individuals will have normal albuminuria after their metabolic status has been stabilized. Patients with diabetic nephropathy have persistent abnormalities in glomerular structure that can be detected in biopsy material, according to the researchers. Renal damage manifests itself in the form of hemodynamic alterations such as hyperfiltration and glomerular hypertrophy in the early stages.[35]

FIGURE 2: Characteristics of diabetic nephropathy in humans [35]



Current pharmacotherapy for DN: [2]

The current standard of care for patients with diabetic nephropathy who have microalbuminuria and concomitant hypertension is a class of medicines known as (ACEIs) or (ARBs).

ACE inhibitors are medications that work by inhibiting the angiotensin converting enzyme (ACE), which is originate in lungs, responsible for conversion of angiotensin I, a precursor hormone, into the vasoconstrictor hormone angiotensin II. As effect of ACE inhibitors on nephrons of the kidney, there is no vasoconstrictive and salt-retaining effect of angiotensin II and aldosterone on these cells.

Selection of rodent model of streptozotocin induced diabetic nephropathy:

Mouse models of diabetic nephropathy:

Numerous publications have lately examined the numerous spontaneous and acquired rat models of diabetes that have been developed. The widely held of these experiments work on the NOD mouse model or the STZ induced type 1 diabetes paradigm, with the db/db strain being the most often utilized to create type II diabetes. All of these diabetic mice develop hyperglycaemia, which is followed by both functional and morphologic renal problems. The functional anomaly consists of hyperfiltration and proteinuria, whereas the morphological abnormality consists of adaptable degrees of mesangial matrix expansion and GBM thickening in the kidney. Furthermore, renal alterations caused by prolonged hyperglycemia are a common early symptom of diabetic nephropathy. [36]

Limitations of mouse models:

Only a few rodent models may develop hallmarks of advanced human DN, such as nodular glomerulosclerosis, and these models are quite rare. Even the time span necessary for the development of such extensive DN lesions is excessively protracted and unreliable.[37]

Perfect Criteria for an animal model: [37]

Ideal animal model of DN should have all of the following features:

Should produce most or all lesions resembling to human DN,

- Avoid the use of non-human primates like dogs and swine
- Specifically damage the kidney within the limited duration
- Should produce renal damage in short term with alternation in doses of inducing agent
- Nephropathy lesions should get differentiated between Type 1 & type 2 diabetes mellitus
- Animals used can breed in large numbers so to prevent scarcity of animals as mortality rate varies with different models

(AMDCC) after lot of efforts and developed, three criteria for perfect model. [37]

1) Progressive renal failure in the setting of hyperglycemia
2) Proteinuria (Albuminuria)
3) Characteristic pathologic changes that includes basement membrane thickening by electron microscopy, mesangial matrix expansion ± nodular mesangial sclerosis, interstitial fibrosis and arteriolar hyalinosis.

These criteria were updated with addition of few more features:

- Greater than 50% decline in GFR over the lifetime of the animal.
- More than >10-fold increase in albuminuria associated with controls for that strain at the same age and gender.

Additional histologic phenotyping for DN animals with advanced disease should include the following: [38]

1. Quantification of mesangial matrix expression, ideally with morphometric analyses.
2. Immunohistochemistry on frozen sections for IgG, IgM, and IgA antibodies to rule out immunological complex illness IgA.
3. Electron microscopy was used to demonstrate the thickening of the glomerular basement membrane.
4. Establishing an acceptable morphometric technique to demonstrate podocyte loss in a reasonable time frame.

Recently, it has been revealed that podocyte loss is one of the most important events recognized in the progression of human diabetic nephropathy. The identity and density of podocytes cannot be determined by routine histology and necessitates the use of immunohistochemical staining techniques, which yields a large amount of information about growing renal injury. A diabetic nephropathy model that fits the majority of these requirements is helpful for the investigation of novel medications.

There are plentiful causes of nephropathy in humans, each of which results in a distinct kidney lesion that is currently being lengthily researched in various species of animals that can mimic specific lesions. In instance, diabetic nephropathy, which is

associated with streptozotocin-induced pancreatic islet cell destruction, is one of the most commonly investigated nephropathy prototypes.[39]

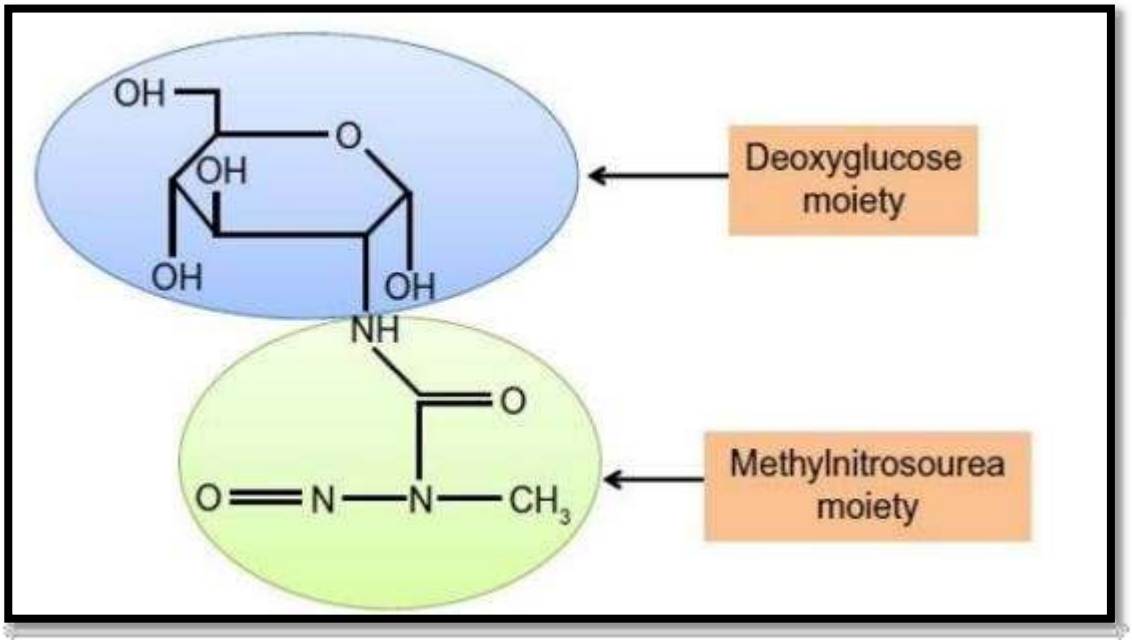
Other experimental models of nephropathy consist of: [40]

1. Diabetes-induced nephropathy
2. Ciclosporin A-induced nephropathy
3. Anthracycline-induced nephropathy
4. Electrolyte nephropathy
5. Cadmium-induced nephropathy
6. Aminoglycoside-induced nephropathy
7. Carbon tetrachloride-induced nephropathy
8. Germanium dioxide-induced nephropathy
9. Mercury chloride-induced nephropathy
10. Vomitoxin-induced nephropathy
11. Maleic acid-induced nephropathy

Streptozotocin (STZ) induced diabetic nephropathy: [39, 40]

STZ is one of the most extensively used agents for the induction of type 1 and type 2 diabetes, and it is one of the most widely used agents for the induction of nephropathy among the agents described above. Nephropathy can be generated in rats with STZ over a period of 4–8 weeks with a single intraperitoneal dose of 40–60 mg/kg administered intraperitoneally. This can be determined by a considerable rise in proteinuria, serum creatinine, blood urea nitrogen (BUN), extracellular matrix deposition, and thickening of the glomerular basement membrane, among other parameters.

FIGURE 3: Streptozotocin molecule [41]



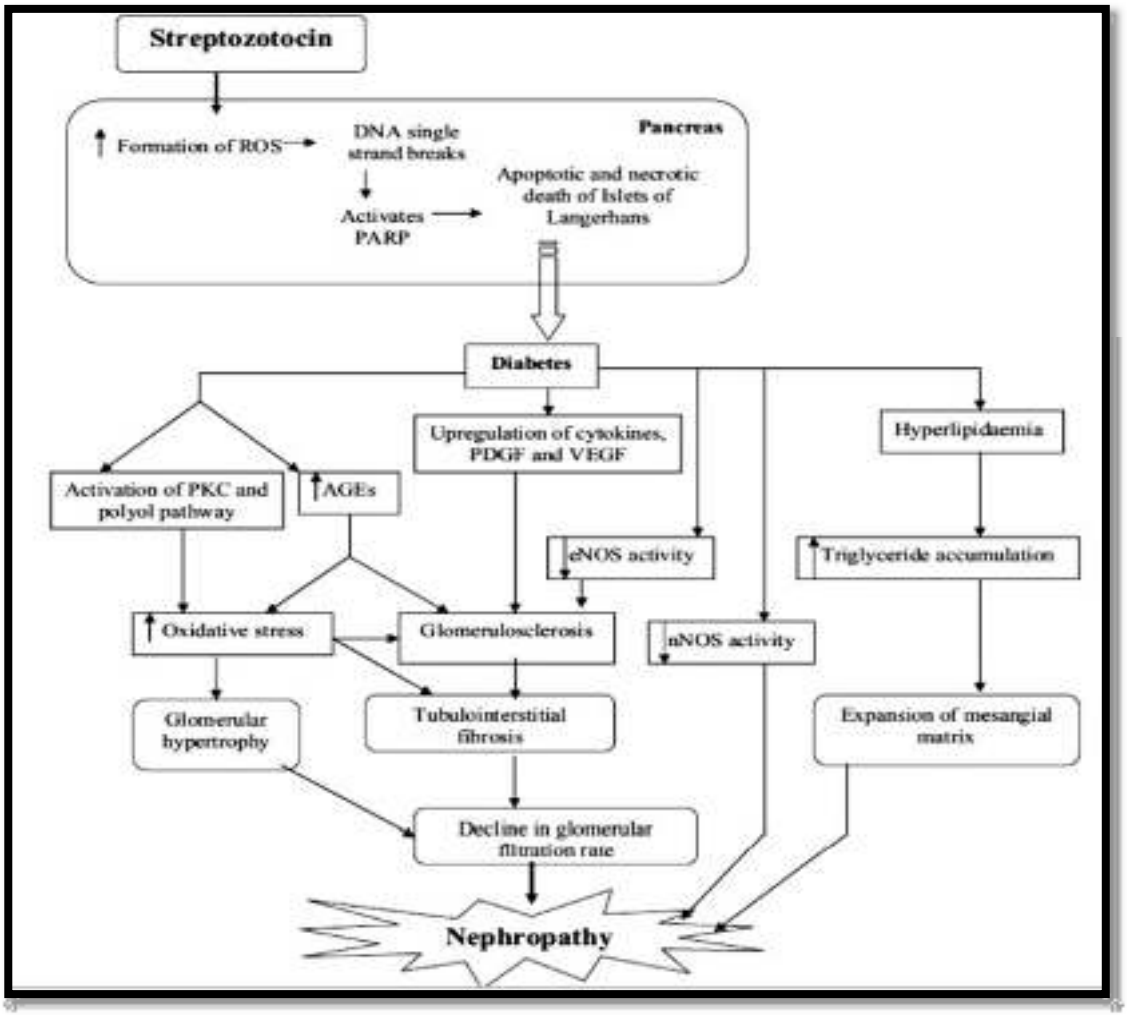
MOA: [41]

The glucose transporter GLUT2 is responsible for the uptake of STZ [2-deoxy-2-(3methyl-3-nitrosoureido)-D-glucopyranose] by the pancreatic cells. The alkylation of DNA and fragmentation of DNA occur as a result of the STZ's intracellular action. The nitrosourea moiety of STZ, particularly at the O6 position of guanine, is thought to be responsible for its alkylating activity. (Produced by the bacteria Streptomycetes Achromogens).

The impact of streptozotocin on beta cells is defined by distinctive variations in insulin and glucose concentrations in the bloodstream. Two hours after the injection, hyperglycemia is detected, which is accompanied by a decrease in insulin levels in the blood. Hypoglycemia occurs approximately six hours later as a result of the release of high levels of stored insulin from the body into the circulation. Finally, hyperglycemia occurs, and insulin levels in the circulation fall.

It is believed that these alterations in BG and insulin concentrations are caused by anomalies in beta cell activity. Nephropathy can be induced in rats with STZ over a period of 4–8 weeks with a single intraperitoneal dose of 40–60 mg/kg given every 4–8 weeks (i.p.). This can be determined by a considerable rise in proteinuria, serum creatinine, blood urea nitrogen (BUN), extracellular matrix deposition, and thickening of the glomerular basement membrane, among other things.

FIGURE 4: Pathophysiology of STZ induced diabetic nephropathy [41]



Single and multiple doses STZ models in mice [39]

Streptozotocin (STZ) is a cytotoxic drug that is preferentially toxic to pancreatic islet -cells. As a result, it is traditionally administered as a single large dose that causes total islet necrosis and diabetes within 48 hours.

Low-dose STZ injection in mice is administered multiple times, resulting in partial destruction to the pancreatic islets cells each time. As a result, it initiates an inflammatory response that results in additional loss of -cell function, which leads to insulin insufficiency and hyperglycemia. In terms of aetiology and morphologic alterations, this is similar to (T1DM) in appearance.

Diabetes mouse model is created by administering several modest doses of STZ (40 mg/kg) IP to mice over a 5-day period, resulting in the development of diabetes in the mice. Using this diabetic mouse model, researchers can evaluate the efficacy of possible antidiabetic drugs.

Depending on the experimental objectives, the administration of the test substances can begin prior to and/or post the production of diabetes. The outcomes of intravenous and intraperitoneal injections are almost identical. In 1976, the authors, Like and Rossini, published a paper titled.

A single large IP dose of STZ (60 mg/kg) produces total -cell necrosis, which results in fast expansion of diabetes, with BG levels reaching >500 mg/dl within 48 hours (Like 1976). Although STZ at small doses at several intervals has been shown to be safe in STZ-induced diabetic mice and has mostly replaced a single high dose of STZ as the primary regimen in these animals, a single high-dose STZ approach is still the most often used rat model for T1DM.

Single dose STZ model in rats: [42]

STZ-induced diabetes in rats is one of the earliest and most widely used rat models of diabetes, having been utilised since the 1940s. It has also been shown that a STZ-induced diabetes state in rats is dose-dependent (Arison et al., 1967; Junod et al. 1969; Ganda et al., 1976; Arison and colleagues, 1977). The most frequently provided STZ single intraperitoneal dose in rats (aged 8 to 10 weeks) varies between 40 and 70 mg/kg, with the most frequently administered dose being 40 mg/kg (Brondum et al., 2005). Several researchers have used a single STZ dose of 60-65 mg/kg to develop diabetes in their studies. In order to induce a T1DM condition in rats, we employed a single intraperitoneal dosage of STZ (60 mg/kg) administered intraperitoneally. It is possible to utilise diabetic rats to explore the pathophysiology of type 1 diabetes in addition to test potential antidiabetic drugs. (Bond et al.,1983).

STZ dose range and features: [43]

Type 1 DM: The dosing range for STZ is not as limited as it is for alloxan, which is a good thing.

When inducing type 1 diabetes in adult rats, the most frequently utilised single intravenous dose is between 40 and 60 mg/kg body weight, but greater doses are also employed. After intraperitoneal injection of a similar or greater dose (60 to 75 mg/kg), STZ is likewise beneficial; however, a single dose of less than 40 mg/kg may be unsuccessful. In a similar vein, Junoid et al. (1979) discovered that the lesser amount is ineffectual in causing frank hyperglycemia, but that when the dose (25 mg/kg) was repeated, a severe form of diabetes occurred, albeit with less beta cell damage than when the dose (65 mg/kg) was given once.

STZ may also be administered in a series of modest dosages. This sort of treatment is most commonly employed in mice, and the production of T1DM is mediated through the stimulation of the immune system.

T2DM can be easily generated in rats by administering STZ dose of 100 Mg/kg intravenously, intraperitoneally on same day as birth. Earlier this year, Portha et al. published the first description of this approach of NIDDM induction (1974). At 8-10 weeks of age and thereafter, rats neonatally treated with STZ develop modest baseline hyperglycemia, a decreased response to the glucose tolerance test (Portha et al. 1979), and a decrease in the sensitivity of B cells to glucose (Portha et al. 1979). Insulin levels in these animals remain normal or may even modestly fall in some circumstances (Bailey. et al, 2003). Other researchers have then experimented with different doses and timings of injection, and at least three separate models have emerged as a result. The first of these models, as previously mentioned, is referred to as the n-0 STZ model. In addition, the n-2 STZ model and the n-5 STZ model, which are administered on days 2 and 5, respectively, contain doses of 80-100 mg/kg on those days. A higher level of hyperglycemia and depletion of insulin reserves are observed in both models with the exception of the n-2 STZ model, which is similar to the n-0 model.

Neonatal growth of pancreas in rats and β- cell regeneration:

Growing the beta cell mass in new born rats is still possible, however at a slower rate than in late foetal rodents. Evidence has been shown indicating the occurrence of both beta-cell replication and neogenesis, the latter of which is derived from duct-like cells that surround neonatal islets and have a significantly higher replicative activity than mature islet cells, according to the findings. These islet cell

precursor cells are no longer visible during the first week of life, and no additional morphological evidence of neogenesis from such precursors can be identified after that time period. After the first week of life, the beta cell mass grows mostly through self-replication, rather than through neogenesis (Bouwens et al, 2005) It is possible to achieve various stages of insulin resistance in neonatal STZ models by varying both the dose and the day of STZ injection. These stages include impaired glucose tolerance, mild, moderate, and severe hyperglycemia, as well as differences in insulin levels and the extent of insulin damage. Because of these characteristics, it is considered to be one of the most appropriate experimental models for type 2 diabetes.

Genetic rodent models of diabetes:

Diabetic nephropathy can affect up to one-third of diabetic people with type 1 or type 2 diabetes.

A great deal of research has been done on diabetic nephropathy in rodent models of diabetes, such as rats and mice, to better understand the pathophysiology of the disease. Although mice are smaller in stature than rats, it was more difficult to undertake surgical procedures and micro puncture experiments on them when comparing the two species. With the introduction of knockout mice strains, it is now able to investigate the influence of single gene deletion on a wide range of illnesses, including diabetes and its complications, among others.

1) **Rat models of diabetes:** It has been decades since the Zucker Diabetic Fatty (ZDF) rat and the Zucker Fatty rat (ZFR) were introduced into the world of metabolic illness research. A mild hyperglycemic condition is initially observed in the ZDF strain, but this is quickly followed by a substantial hyperinsulinemic state, which indicates a state of significant insulin

resistance. Because of this exhaustion, insulin production falls (just as it does in diabetes humans), and plasma glucose levels rise steadily over time. The ZFR strain presents as an obese mouse with an elevated serum lipid profile, progressive hepatic steatosis, and resembles a mild profile of decreased insulin sensitivity, all of which are characteristic of obesity.

2) **Mouse Models of Diabetes:** [44] It is also possible to create an experimental diabetic model by using genetically engineered mice with metabolic illness. There are just a few genetic mice models that can accurately replicate the human disease of type 2 diabetes and can create images that are similar to the natural history of diabetic nephropathy in humans. This information is also useful in the investigation of novel pharmacological compounds that block the primary pathways involved in the advancement of diabetic nephropathy. These three mice models of diabetes are frequently used because they reproduce a well-defined and robust whole-body phenotype of diabetes in a consistent and reproducible manner.

- The ob/ob and db/db mice have a particular obese phenotype in addition to having a diabetic profile, respectively.
- The ob/ob and db/db mice strains have defects in the leptin signalling system, respectively (leptin deficiency and lacking leptin receptor, respectively). In the ob/ob mouse, this leads in hyperphagia as well as a decreased glucose tolerance and insulin sensitivity profile, as well as hyperglycemia (which is temporary in this model) and hyperinsulinemia.
- This mouse is a more aggressive model of type 2 diabetes that will develop to pancreatic failure and frank diabetes in the absence of treatment.

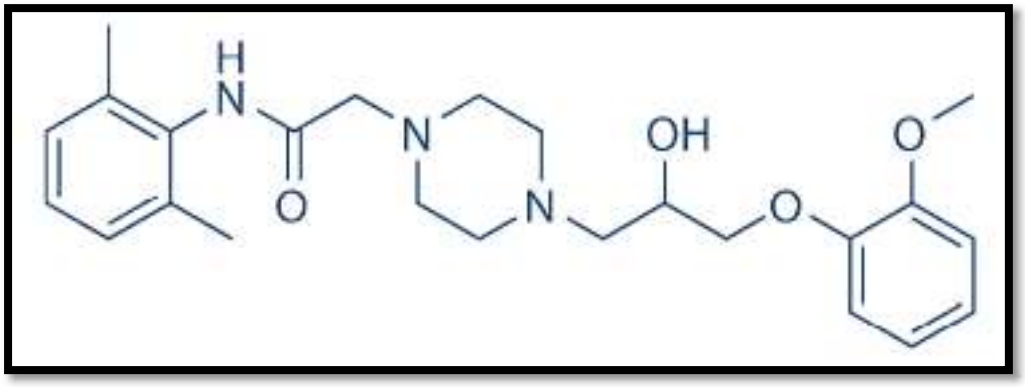
- The NOD mouse is an animal model that is analogous to the aetiology of type 1 diabetes in terms of its genetic makeup.
- When the severe combined immunodeficiency (SCID) mutation is present, insulitis can develop in the pancreatic beta cell as a result of leukocyte infiltration, culminating in the development of a type 1 diabetic-specific phenotype of glycosuria and hyperglycemia.
- The streptozotocin induced type 1 diabetic rat model, is better for studying certain diseases such as nephropathy, particularly when physiologic and metabolic aspects are being investigated.
- However, the advantage of using murine model is being readily able to induce specific genetic manipulations, both transgenic and knockout.

TEST DRUG USED IN PRESENT STUDY:

Ranolazine are second-line antianginal agent for the treatment of chronic angina.

Ranolazine - Ranolazine is being be used with a number of different drugs, including blockers, Ca2+ channel blockers, ACEIs, ARBs, and lipid-lowering and platelet-aggregation-reducing medicines. [45]

Fig 5: Ranolazine Structure



Mechanism of action: Ranolazine works by inhibiting a late sodium current that allows calcium to enter through the sodium-calcium exchanger. Ranolazine lowers diastolic tension, cardiac contractility, and workload by decreasing intracellular calcium concentration. [45]

Pleotropic effects

1. **Anti-inflammatory effects:** Studies conducted show that Ranolazine inhibits the inflammatory markers such as IL1β and TNF-α, and increases anti-inflammatory PPAR-γ.

2. **Anti-Oxidant effects:** Furthermore, antioxidant proteins Cu/Zn SOD and Mn-SOD significantly increased after Ranolazine addition in cultured astrocytes.[46]

Pharmacokinetics: The medicine has a 75 percent oral bioavailability; Pgp inhibitors can enhance ranolazine absorption and exposure to both ranolazine and the competing drug. The terminal t 1/2 of ranolazine is approximately 7 hours; with repeated doses, a steady-state Cp is established in 3 days. Ranolazine is metabolised primarily by CYP3A4 and to a lesser amount by CYP2D6; both the unmodified drug and its metabolites are eliminated in the urine (5 percent). Strong CYP3A4 inhibitors (e.g., macrolide and imidazole antibiotics, HIV protease inhibitors) should not be used with ranolazine, and dosages should be reduced when moderate CYP3A4 inhibitors (e.g., verapamil, diltiazem, and erythromycin) are taken simultaneously. CYP3A4 inducers (e.g., rifampin, carbamazepine, and hypericum) can lower ranolazine plasma levels, necessitating dosage adjustments.[47]

Adverse effects and drug interactions: Additional CYP3A4 substrates, such as simvastatin and its active metabolite, can be affected by ranolazine, necessitating

dosage adjustments; dose reduction may be required for other CYP3A4 substrates (e.g., lovastatin), especially for those having a limited therapeutic range (e.g., cyclosporine, tacrolimus, sirolimus). Ranolazine may enhance exposure to other CYP2D6 substrates, such as tricyclic antidepressants and antipsychotics, when used together.[47]

Dizziness, headache, nausea, and constipation are the most common side effects. Some CNS effects resemble those of class I antiarrhythmics (e.g., dizziness, fuzzy vision, and disorientation). Although QT prolongations must be noted, there have been no reports of torsades de pointes arrhythmias or associated occurrences.[47]

Therapeutic use: Ranolazine is a drug that is used to treat chronic angina. It comes in extended release tablets and is taken twice a day without regard for meals at 500 to 1000mg twice daily.

Selection of Ranolazine as the study drug: Ranolazine is prescribed for the treatment of chronic angina in conjunction with other antianginal medications such as beta blockers, calcium channel blockers, and long acting nitrates, among other things. Ranolazine has been shown to be safe in patients with cardiovascular disease and has also been shown to result in a drop in HbA1C without an increase in blood glucose levels, according to a prior study that evaluated "Ranolazine and Its Effects on Haemoglobin A1C. [48]

According to the findings of a study titled "Blockade of Na+ Channels in Pancreatic a-Cells Has Antidiabetic Effects," Na Ch. blockers inhibit glucagon secretion by blocking the Nav1.3 isoform of pancreatic alpha-cells, which results in glucagon- and glucose-lowering effects in animal models of diabetes. The function of Na+-dependent action potentials of pancreatic α-cells in the release of glucagon

suggests that Na v1.3 specific Na Ch.blockers may provide a novel mechanism of action for ranolazine in the treatment of diabetes. [49]

Ranolazine has also been proven in clinical trials to have positive metabolic benefits in diabetic people, as evidenced by considerable reductions in HbA1c levels. [50]

Ranolazine has also been proven to have anti-inflammatory qualities, as demonstrated by the studies listed below. This is according to the findings of a study entitled 'Vagal stimulation Facilitates Improving Effects of Ranolazine on Cardiac Function in Rats with Chronic Ischemic Heart Failure,' which suggested that a combination of vagal stimulation and ranolazine improved cardiac function by attenuating the exaggerated levels of NE/BNP-45 and cytokines in the rats with chronic ischemic heart failure (IL-1beta, IL-6, and TNF-alpha).[51]

According to the findings of another study titled 'Ranolazine improves endothelial function in patients with stable coronary artery disease,' ranolazine improved endothelial function and C- reactive protein levels in patients with stable CAD, suggesting that ranolazine may have a novel mechanism of action in this setting.[52]

Because of this, ranolazine advantages may extend far beyond its antianginal capabilities. Along with these things ranolazine also increase the efficacy of metformin in body. As a result, ranolazine may be a viable treatment

MATERIALS AND METHODS

The present study was an experimental study and it involves the use of adult healthy male Wistar rats weighing 200 ± 20 gm body weight which was obtained from central animal house. Animals was housed under standard conditions and acclimatized to 12-h light/dark cycle for 10 days prior to the day of experimentation. They were have free access to food (standard rat chow pellet, Amrut brand) and water ad libitum.

All Test drugs (Ranolazine and Metformin) required for this experiment were procured hospital pharmacy of KLE University's Dr. Prabhakar kore hospital and medical research Centre, Belagavi and all required laboratory equipment and reagents was obtained from standard laboratory equipment and reagent suppliers. All guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) for use, care and sacrificing of experimental animals was followed.

Study Design this is a chronic animal experimental study involving male Wistar rats. Animals was randomly divided into 5 study groups, with each group containing eight animals (n=8) to total 40 animals.

Study duration 8 wk. (56 days)

Study Period Present study was carried out in one year time period from April 2020 to March 2021.

Study Population: Adult healthy male Wistar rats weighing 200±20 gm. Healthy male Wister rats weighing 200+20 gm was injected with single dose STZ 45 mg /kg I/p. Random blood sugar level was monitored after 72 hr. after STZ treatment. Diabetic rats showing random blood sugar level above 200 mg /dl was included in the

study. Rats with random blood sugar below 200mg/dl was excluded from the study. Male Wister rats was divided into five study groups, with each group contain eight animals (n=6) in to total 38 animals as described below:

TABLE 5: ANIMALS EXPERIMENTAL GROUPS:

S.NO	Groups	Drugs Administered	Dose
1	Group I: Normal group	Vehicle only	Distilled water– 0.5ml I/p (Daily)
2	Group II: Control Group	Streptozotocin induced diabetic group treated with vehicle	(STZ)45mg/kg I /p (Once)
3	Group III:	Streptozotocin induced diabetic group treated with ranolazine	Ranolazine 90 mg/kg (Daily) orally
4	Group IV:	Streptozotocin induced diabetic group treated with metformin	Metformin 180mg/kg (Daily) orally
5	Group V:	Streptozotocin induced diabetic group treated with ranolazine and metformin	Ranolazine 90mg/ kg and Metformin 180 mg/kg (Daily) orally

Procedure

Animals was assigned to each group randomly. Blinding was introduced to eliminate observer bias. It was achieved by coding and masking of all the drugs used in the experiment by the guide before starting the experiments. Procedure for the experiments is as follows.

INDUCTION OF DIABETES:

Diabetes was induced in overnight fasted Wister rats by using single intraperitoneal Injection of 45 mg/kg streptozotocin. STZ received rats was given 5% of glucose instead of water for 24 h after diabetic induction in demand to diminish hypoglycemic shock related mortality.[16] Blood samples was collected from the tail vein after 72 hr. of STZ treatment to quota the random glucose levels. Animals with random blood glucose levels above 200 mg/dl was considered diabetic rats and used for study. Following confirmation of diabetic state, the animals in each group was treated with one of the treatment protocols as described for a period of 8 weeks. Assessment for diabetic nephropathy: Body weight of the rats was estimated 15 days by weighing machine. Blood glucose was estimated every 15 Days using glucometer. HbA1c levels was measured using a chemical analyser at baseline and 8th week. Urine was collected at the end of study to measure urine proteinuria concentration which was done by urine dipsticks kit. Before the animal are scarified blood sample was collected by cardiac puncture in order to estimate the level of CRP, TNF -alpha, IL-6. At the end of 8th week, animals was sacrificed using an overdose of thiopentone and kidneys were obtained and preserved for histopathological studies using Haematoxylin and Eosin (H&E) staining. Periodic Acid Schiff (PAS) stain use to identify for interstitial fibrosis, glomerular hypertrophy, thickening of the glomerular basement membrane and mesangial matrix expansion.

Histopathological Examination of Kidneys

The kidney sections in the formalin glass bulbs were sent to the laboratory for preparation of the slides and the slides were stained using haematoxylin and eosin staining. The slides were examined by a trained and experienced pathologist. As there

was no validated scoring system for reporting of histopathological changes in STZ induced diabetic nephropathy, based on the current knowledge regarding histopathological changes that occur in STZ induced diabetic nephropathy in rats, we devised a scoring system in consultation with the pathologist. This scoring is indirectly based on a study by Ozdemir O et al as shown in the Table 6. [53]

Table 6: Scoring System for Histopathological Examination of Kidneys

Score	Description
0	No light microscopy changes
1	Minimal changes, >5 and <10 tubules
2	Mild changes, >10 and <15 tubules in 5 LPF with vacuolar degeneration and cystic dilatation of tubules
3	Moderate changes, >15 and <20 tubules in 5 LPF with vacuolar degeneration and cystic dilatation of tubules
4	Severe changes, >20 tubules in 5 LPF with vacuolar degeneration and cystic dilatation of tubules OR mesangial expansion.

Statistical analysis

The data was expressed as Mean ± SEM for all the groups. Data was analysed by one way ANOVA (Analysis of variance). Post hoc Dunnett's test was used to compare treatment (ranolazine and ranolazine +metformin) groups with diabetic control group. Evaluation between metformin and treatment groups (ranolazine and ranolazine with metformin) was done by one way ANOVA followed by Bonferroni's test. Analysis was done by using Graph pad prism software and $p \leq 0.05$ was considered as statistically significant.

RESULT

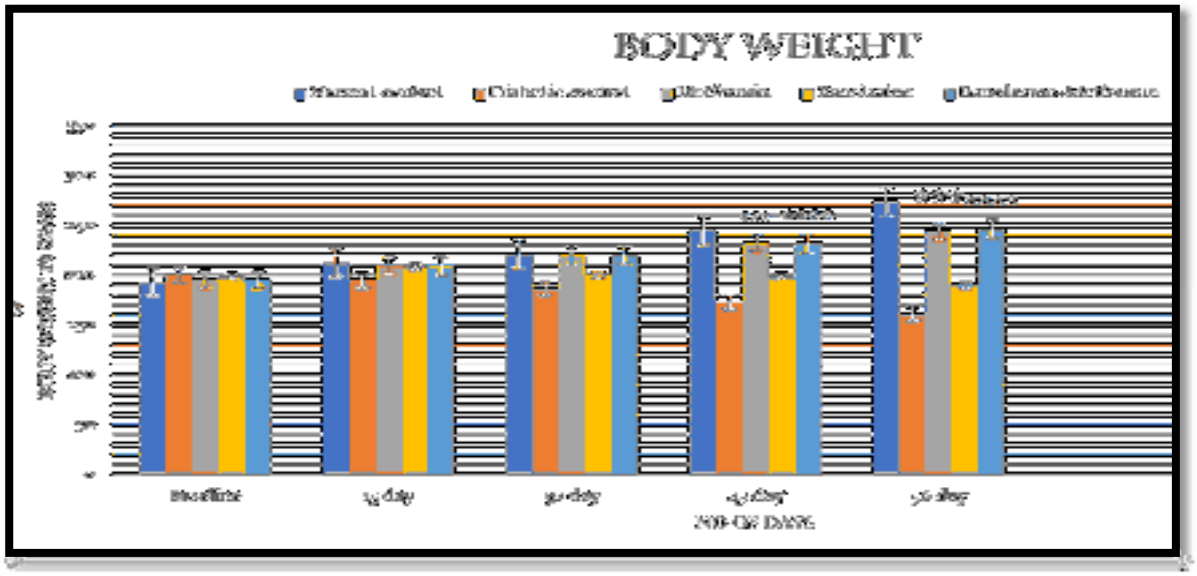
Data generated in the study were compiled into an excel sheet and were analysed using appropriate statistical tests. The results of the study are described in this chapter using graphs and tables for easy understanding. For analysis Dunnett's test were used for comparisons between treated group with diabetic control group and Bonferroni's s test used for comparisons between standard drug and test drugs.

Estimation of Body weight:

Table 7: Comparison of Rat Body Weight (grams) at Day 0 and Day 56

Days	Body Weight In Gm(Mean ± SEM)					ANOVA Result
	Normal control	Diabetic Control	Metformin	Ranolazine	Ranolazine+ Metformin	F(4,25)
Baseline	192.6 ±1.69	199.6 ±1.5	195.3 ±2.65	198.1 ±1.74	195.1 ± 1.96	0.8024
15 days	211.5 ±3.23	194.5 ±1.96	208.6 ±2.23	208.3 ±1.6	209.5 ± 1.5	2.343
30 days	220.5 ±1.9	185.8 ±2.3	220.3 ±1.12	200.6 ±3.04	220.16 ± 0.95	0.9546
45 days	244.3 ±1.5	172.6 ± 1.8	231.8 ±1.6	198.1 ±2.2	231.1 ± 1.5	0.1490
56 days	272.6 ±2.5	160.6 ± 2.0	242.6 ±1.47 *** p <.001	190.5 ±2.37	245.6 ± 1.5 **** p <.0001	0.7013

Values are expressed as mean +/- SEM, n=6, *p < 0.05,*p< 0.001, ****p< 0.0001 indicates the significant difference between diabetic control and treatment groups by using ANOVA followed by Dunnett's multiple comparison test.**



Graph 1: Mean Body Weight (grams) at Day 0 and Day 56

Values are expressed as mean +/- SEM, n=6, ***p<0.001,**p< 0.0001 indicates the significant difference between diabetic control and treatment groups by using ANOVA followed by Dunnett's multiple comparison test.**

The mean body weight in grams (gm), as measured by weighing machine, for diabetic control group at baseline, 15day, 30 day, 45day, 56 day was 199.6 ± 1.5, 194.5 ± 1.96, 185.8 ± 2.3, 172.6 ± 1.8, 160.6 ± 2.0 (Table-6) respectively, while the corresponding mean body weight in metformin (180 mg/kg) treated group were 195.3 ± 2.65, 208.6 ± 2.23, 220.3 ± 1.12, 231.8±1.6, 242.6±1.47, 0. Respectively (Table-7, Graph-1).

The body weight (gm) in ranolazine treated group (90 mg/kg) at baseline, 15day, 30 day, 45day, 56 day was respectively 198.1 ± 1.74 , 208.3 ± 1.6 , 200.6 ± 3.04 , 198.1 ± 2.2 , 190.5 ± 2.37 (Table-6, Graph-5).The ranolazine with metformin treated group (90/180 mg/kg) at baseline, 15day, 30 day, 45day, 56 day was respectively 195.1 ± 1.96, 209.5 ± 1.5, 220.16 ± 0.95, 231.1 ± 1.5, 245.6 ± 1.5 (Table-7, Graph-1).

According to post-hoc analysis by Dunnett's test, there was statically significant ($p < 0.05$) increase in body weight value at 56 days in the combination (ranolazine with metformin) treated group and metformin treated group but with ranolazine treated groups increase in body weight value was not significant compared to the diabetic control group (Table-7, Graph-1). According to the post-hoc Bonferroni's analysis there was no significant difference in body weight observed in combination (ranolazine with metformin) treated group compared to the metformin treated group.

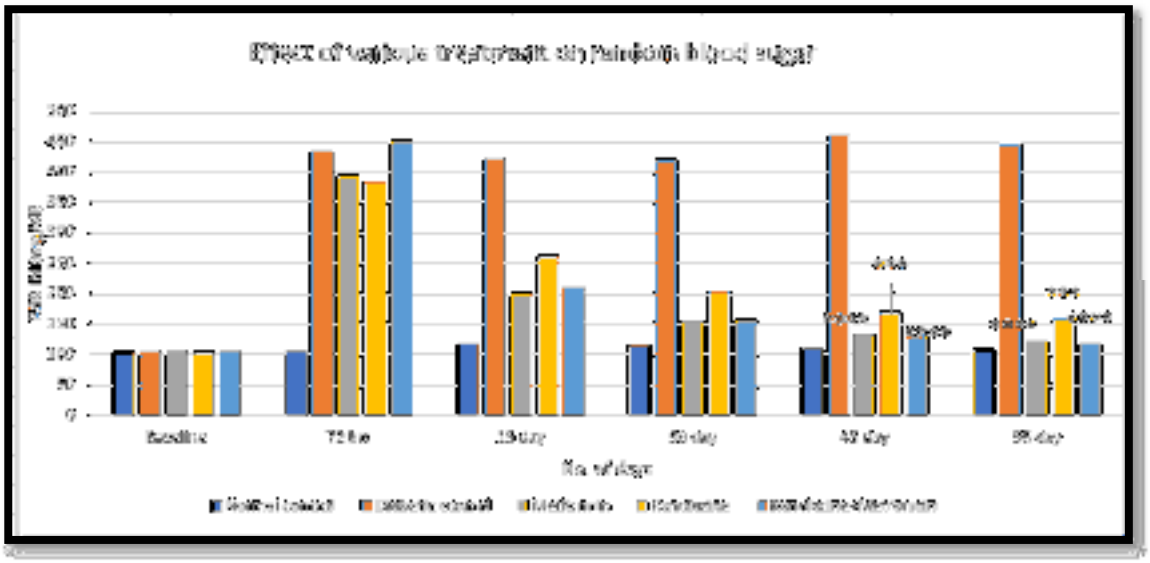
Above results indicate that ranolazine with metformin have shown statistically significant gain in body weight in diabetic nephropathy model during the study as compared with diabetic control group.

Estimation of Random blood sugar:

Table 8: Comparison of Rat GRBS (Mg/dl) at Day 0 and Day 56

Days	GRBS In Mg/dl (Mean ± SEM)					ANOVA Result	
	Normal control	Diabetic Control	Metformin	Ranolazine	Ranolazine+ Metformin	F (4,30)	P value
Baseline	104.6 ± 3.07	105.1 ± 4.9	106.3 ± 3.9	103.6 ± 5.1	104.8 ± 4.7	0.2755	0.8915
72 hrs.	105.3 ± 3.42	433.67 ± 36.9	393 ± 24.7	384.0 ± 8.5	452.8 ± 28.4	2.879	0.0395
15 days	117.8 ± 2.9	423.1 ± 33.8	200.5 ±13.6	263.0 ± 19.7	212.5 ± 5.49	2.857	0.0406
30 days	116.5 ± 3.46	419.5 ± 28.03	154.0 ± 11.35	204.5 ± 10.8	157.3 ± 2.6	3.689	0.0147
45 days	111.8 ± 4.5	462.5 ± 28.23	133.6 ± 4.2	169.5 ± 9.0	131.8 ± 3.0	3.855	0.0121
56 days	109.1 ± 2.9	447.0 ± 17.29	114.3 ± 1.47 **** p<.0001	159.3 ± 5.7 *** p<0.001	108.5 ± 3.03 **** p<.0001	10.12	≤0.001

Values are expressed as mean +/- SEM, n=6, *p < 0.05, **p< 0.001, ****p< 0.0001 indicates the significant difference between diabetic control and treatment groups by using ANOVA followed by Dunnett's multiple comparison test.



Graph 2: GRBS (mg/dl) at Day 0 and Day 56

Values are expressed as mean +/- SEM, n=6, *p < 0.05, ***p< 0.001, ****p< 0.0001 indicates the significant difference between diabetic control and treatment groups by using ANOVA followed by Dunnett's multiple comparison test.

The mean RBS in mg/dl, as measured by glucometer, for diabetic control group at baseline, after STZ injection 72 hrs, 15day, 30 day,45day, 56 day was 105.1 ± 4.9, 433.67 ± 36.9, 423.1 ± 33.8, 419.5 ± 28.03, 462.5 ± 28.23, 447.0 ± 17.29 (Table-8, graph -2) respectively, while the corresponding mean RBS in metformin (180 mg/kg) treated group were 106.3 ± 3.9, 393 ± 24.7, 200.5 ± 13.6, 154.0 ± 11.35, 133.6 ± 4.2, 114.3 ± 1.47 respectively (Table-8, graph -2)

The RBS (mg/dl) in ranolazine treated group (90 mg/kg) at baseline,72hrs, 15day, 30 day, 45day, 56 day was respectively 103.6 ± 5.1 , 384.0 ±8.5, 263.0 ± 19.7 , 204.5 ± 10.8 , 169.5 ± 9.0 , 159.3 ± 5.7 (Table-8, graph -2). The ranolazine with metformin treated group (90/180 mg/kg) at baseline, after STZ injection 72hrs, 15day, 30 day, 45day, 56 day was respectively 104.8 ± 4.7, 452.8 ±28.4 , 212.5 ± 5.49, 157.3 ± 2.6, 131.8 ± 3.0, 108.5 ± 3.03 (Table-8, graph -2).

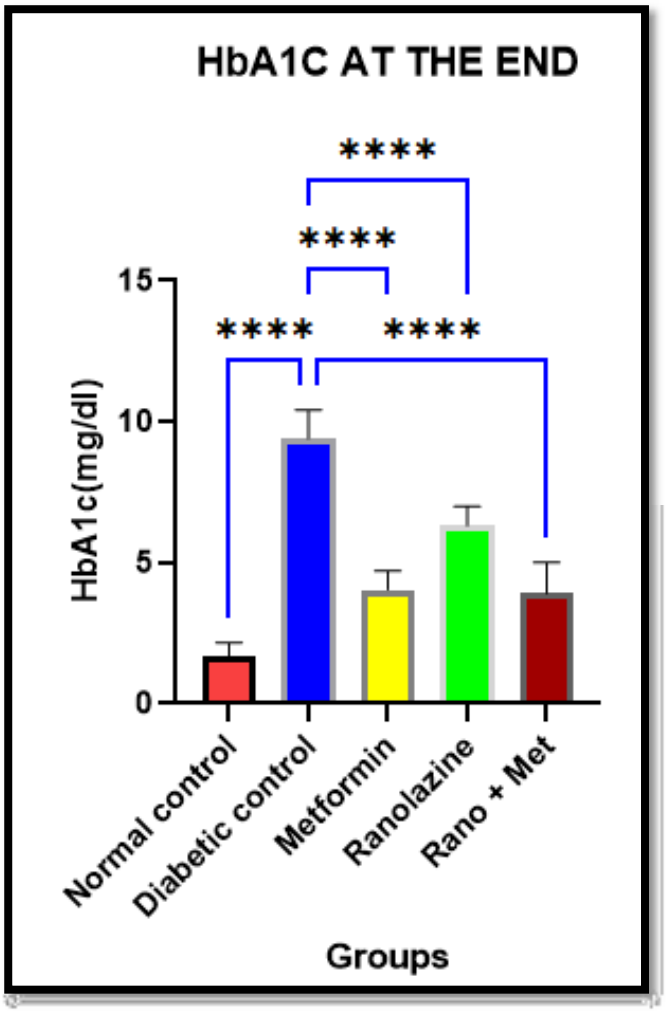
According to post-hoc analysis by Dunnett's test, there was statically significant ($p < 0.05$) reduction in RBS at 15, 30 , 45 and 56 days in the all treated groups when compared to the diabetic control group (Table-7, Graph-6). According to the post-hoc Bonferroni's analysis there was no significant difference in the RBS observed in combination (ranolazine with metformin) treated group when compared to the metformin treated group but combination (ranolazine with metformin) treated group when compared with ranolazine alone treated group there is more reduction in RBS level. Above results indicate that ranolazine with metformin have shown statistically significant reduction in GRBS in diabetic nephropathy model during the study as compared with diabetic control group.

Estimation of HbA1C:

Table 9: Effect of various treatments on HbA1C

Day of study	HbA1C (%) (Mean ±SEM)					ANOVA Result	
	Normal Control	**Diabetic Control**	**Metformin**	**Ranolazine**	**Ranolazine +metformin**	**F(4,25)**	**P value**
Baseline	1.60 ±0.34	1.61 ±0.34	1.84 ±0.4	1.62 ±0.32	1.83 ±0.43	0.7119 (4, 25)	0.8443
56 days	1.66 ± 0.39	9.41 ± 0.38,	4.05 ± 0.3, **** p<.0001	6.32 ± 0.27, **** # p<.05	3.11 ± 0.23 **** ###p<.0001	0.9275 (4, 25)	<0.0001

Values are expressed as mean +/- SEM, n=6, *p < 0.05, ****p< 0.0001indicates the significant difference between diabetic control and treatment groups by using ANOVA followed by Dunnett's multiple comparison test. #p < 0.5, ###p< 0.001 indicates the significant difference between the other treatment group vs the metformin monotherapy group by using Bonferroni's test.



GRAPH 3: Effect of various treatment on HbA1c

Values are expressed as mean +/- SEM, n=6, *p < 0.05, ***p< 0.001, ****p< 0.0001 indicates the significant difference between diabetic control and treatment groups by using ANOVA followed by Dunnett's multiple comparison test.

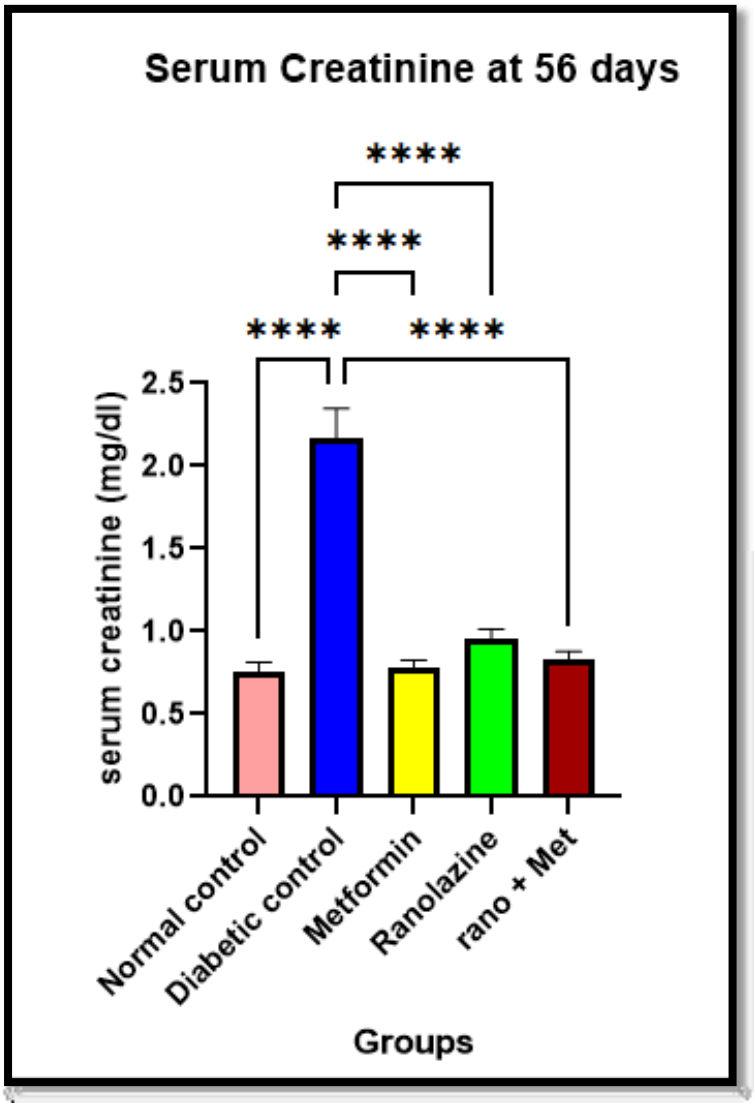
HbA1C was measured at baseline and at the end of the study (56 days). The mean HbA1C (%) of all the groups was comparable at baseline. A one-way ANOVA revealed that there was no significant difference between various groups at baseline. Baseline mean HbA1C values of normal control, diabetic control, metformin, ranolazine alone and combination groups were 1.60±0.34, 1.61±0.34, 1.84±0.4, 1.62±0.32, 1.83±0.43 respectively. The one-way ANOVA test showed no significant difference among the groups.

At the end of the study the mean HbA1C values (%) of Normal control, diabetic control, metformin, ranolazine and combination (ranolazine + metformin) groups were 1.66 ± 0.39, 9.41 ± 0.38, 4.05 ± 0.3, 6.32 ± 0.27, and 3.11 ± 0.23 respectively. One-way ANOVA followed by Dunnett's test revealed that the HbA1C value in the diabetic control group was significantly high compared to normal control ($p< 0.0001$), Metformin ($p< 0.0001$), ranolazine ($p <0.0001$), and combination groups ($p< 0.0001$).

According to the post-hoc Bonferroni's analysis, HbA1c level in the metformin treated group showed statically significantly ($p < 0.0006$) reduction when compare to ranolazine treated group. Also, there was statically significantly ($p < 0.0003$) reduction in HbA1c level in the combination (ranolazine + metformin) treated group when compared to the ranolazine treated group. But there was no statically significant reduction in HbA1c level in metformin treated group when compare with combination treated group.

A student's paired t-test was performed to compare HbA1C levels before and after therapy/intervention in each group. At the end of the study, the diabetic control group showed a statistically significant rise in HbA1C compared to the value at baseline ($p < 0.0001$) and all treated groups (metformin , ranolazine , combination) showed statically significant ($p<0.0021$),($p<0.004$),($p<0.0013$) increased in HbA1c level when compare to baseline values.

Estimation of Serum creatinine:



GRAPH 4: Effect of various treatment on Serum Creatinine

Values are expressed as mean +/- SEM, n=6, *p < 0.05, ***p< 0.001, ****p< 0.0001 indicates the significant difference between diabetic control and treatment groups by using ANOVA followed by Dennett's multiple comparison test.

Serum creatinine was measured at baseline and at the end of the study (56 days). The mean Serum creatinine of all the groups was comparable at baseline. A one-way ANOVA revealed that there was no significant difference between various groups at baseline. Baseline mean Serum creatinine Values of normal control, diabetic control, metformin, ranolazine alone and combination groups were 0.74±0.34,

0.73±0.34, 0.77±0.4, 0.81±0.37 and 0.80±0.43 respectively. The one-way ANOVA test showed no significant difference among the groups.

At the end of the study the mean serum creatinine values (%) of Normal control, diabetic control, metformin, ranolazine and combination (ranolazine + metformin) groups were 0.74 ± 0.34, 3.50 ± 0.34, 0.78 ± 0.4, 1.21 ± 0.37, and 0.73 ± 0.43 respectively. One-way ANOVA followed by Dunnett's test revealed that the serum creatinine in the diabetic control group showed significantly high value compared to normal control ($p< 0.0001$), metformin ($p< 0.0001$), ranolazine ($p < 0.0001$), and combination groups ($p< 0.0001$). According to the post-hoc Bonferroni's analysis, Serum creatinine in the metformin group showed statically significantly ($p < 0.0001$) reduction in compare to diabetic control group. Also, the combination (ranolazine with metformin) treated group showed reduction in serum creatinine value when compared to the metformin and ranolazine treated group.

A student's paired t-test was performed to compare Serum creatinine levels before and after therapy/intervention in each group. At the end of the study, the diabetic control group and ranolazine treated group showed a statistically significant rise in Serum creatinine compared to the value at baseline ($p < 0.0007$), ($p<.0132$) and metformin treated group and combination treated group showed non-significant ($p<0.7030$), ($p<0.9490$) increased in Serum creatinine level when compare to baseline values.

Estimation of Inflammatory markers:

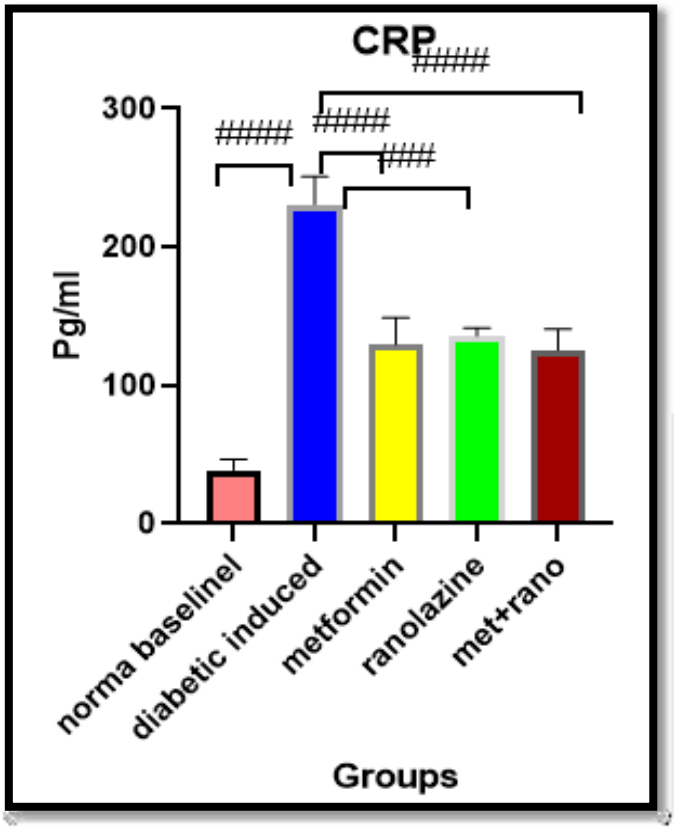
Before the animal are scarified blood sample was collected by cardiac puncture in order to estimate the level of CRP, TNF -alpha, IL-6. At the end of 8th week, animals was sacrificed using an overdose of thiopentone.

Table 10: Effect of various treatments on inflammatory markers at 56 days

	Normal control	**Diabetic control**	**Metformin**	**Ranolazine**	**Ranolazine + Metformin**
CRP (pg/ml)	38.85±3.19	230.8±8.25	129.6±7.93 **** p<0.0001	135.9±2.37 **** p<0.0001	125.6±6.1 **** p <0.0001
TNF– α (pg/ml)	37.3±1.8	142.8±12.25	56.2±6.8 **** p<0.0001	78.45±2.37 *** p<0.001	51.5±5.43 **** p<0.0001
IL-6 (pg/ml)	89.31 ± 7.7	578.9 ± 24.6	247.28 ± 26.1 **** p<0.0001	295.03 ± 41.9 *** p<0.001	213.7 ± 17.9 **** p<0.0001

Values are expressed as mean +/- SEM, n=6, *p < 0.05, ***p< 0.001, ****p< 0.0001 indicates the significant difference between diabetic control and treatment groups by using ANOVA followed by Dennett's multiple comparison test.

Estimation of CRP value at 56 day



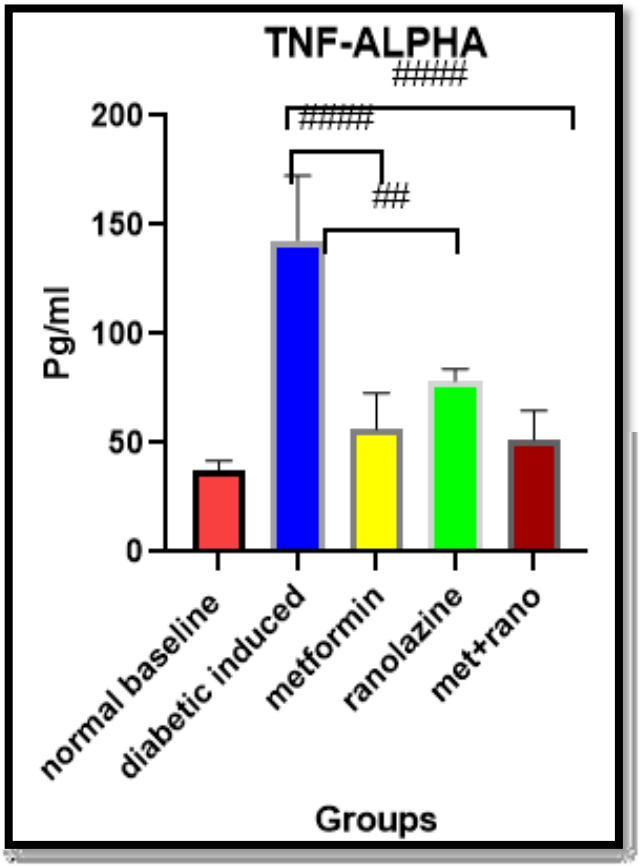
GRAPH 5: Effect of various treatment on CRP

Values are expressed as mean +/- SEM, n=6, #p < 0.05, ## p<0.01, ###p< 0.001, ####p< 0.0001 indicates the significant difference between diabetic control and treatment groups by using ANOVA followed by Bonferroni's multiple comparison test.

CRP

At the end of the study the mean CRP values of normal control, diabetic control, metformin, ranolazine and the combination groups were 38.85±3.19, 230.8±8.25, 129.6±7.93, 135.9±2.37 and 125.6±6.1 respectively. The post-hoc Dunnett's test showed that the CRP value of the diabetic control group was significantly high compared to normal control ($p < 0.0001$), metformin ($p = 0.0001$), ranolazine ($p < 0.001$), and the combination groups. ($p < 0.0001$). According to Bonferroni's analysis, the combination (ranolazine with metformin) treated group showed more reduction in CRP level when compared to metformin and ranolazine alone treated groups but it's not statically significant difference.

Estimation of TNF –α at 56 day



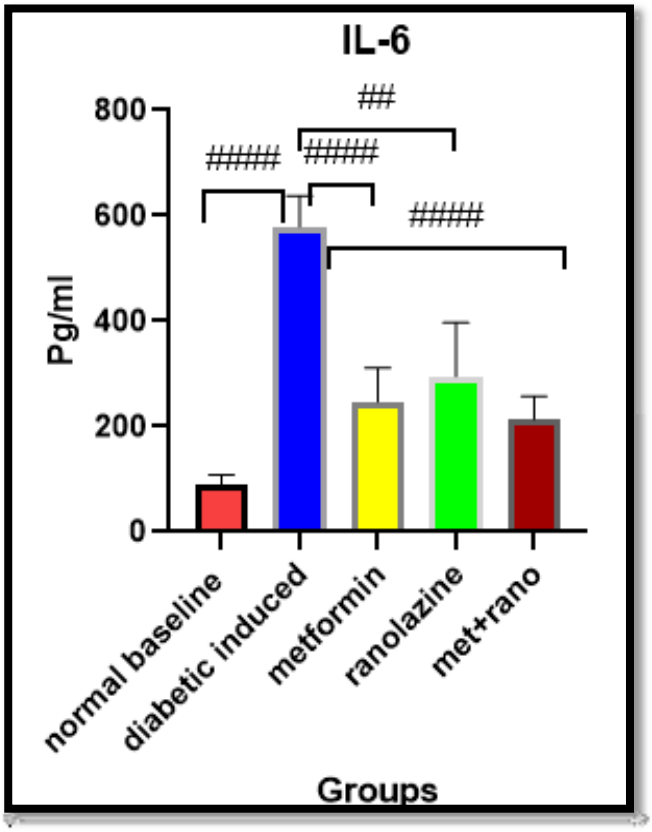
GRAPH 6: Effect of various treatment on TNF-α

Values are expressed as mean +/- SEM, n=6, #p < 0.05, ## p<0.01, ###p< 0.001, ####p< 0.0001 indicates the significant difference between diabetic control and treatment groups by using ANOVA followed by Bonferroni's multiple comparison test.

TNF-α

At the end of the study the mean TNF- alpha values of normal control, diabetic control, metformin, ranolazine and the combination groups were 37.3±1.8, 142.8±12.25, 56.2±6.8, 78.45±2.37 and 51.5±5.43 respectively. The post-hoc Dunnett's test showed that the TNF-α value of the diabetic control group was significantly high compared to normal control ($p < 0.0001$) and all treated groups. According to Bonferroni's analysis, the combination (ranolazine with metformin) treated group showed more reduction in TNF-α level when compared to metformin and ranolazine treated group but value were non-significant.

Estimation of IL-6 Value at 56 day



GRAPH 7: Effect of various treatment on IL-6

Values are expressed as mean +/- SEM, n=6, #p < 0.05, ## p<0.01, ###p< 0.001, ####p< 0.0001 indicates the significant difference between diabetic control and treatment groups by using ANOVA followed by Bonferroni's multiple comparison test.

IL-6

At the end of the study the mean IL- 6 values of normal control, diabetic control, metformin, ranolazine and the combination groups were 89.31 ± 7.7, 578.9 ± 24.6,247.28 ± 26.1, 295.03 ± 41.9and 213.7 ± 17.9 respectively. The post-hoc Dunnett's test showed that the IL-6 value of the diabetic control group was significantly high compared to normal control ($p < 0.0001$), metformin ($p = 0.0001$), ranolazine ($p < 0.001$), and the combination groups ($p < 0.0001$). According to Bonferroni's analysis, the combination (ranolazine with metformin) treated group showed more reduction in IL-6 level when compared to metformin and ranolazine treated group.

Table 11: **Comparison of Rat urine proteinuria through dipstick test at Day 56**

	Urine protein after 56 days				
Rats	**Normal control**	**Diabetic Control**	**Metformin**	**Ranolazine**	**Ranolazine + Metformin**
R1	**-**	++	+	+	**Trace**
R2	**Trace**	+++	**Trace**	+	**Trace**
R3	**Trace**	++	**-**	+	**-**
R4	**-**	+++	**Trace**	**Trace**	**Trace**
R5	**Trace**	+++	**Trace**	++	**Trace**
R6	**Trace**	+++	+	+	+

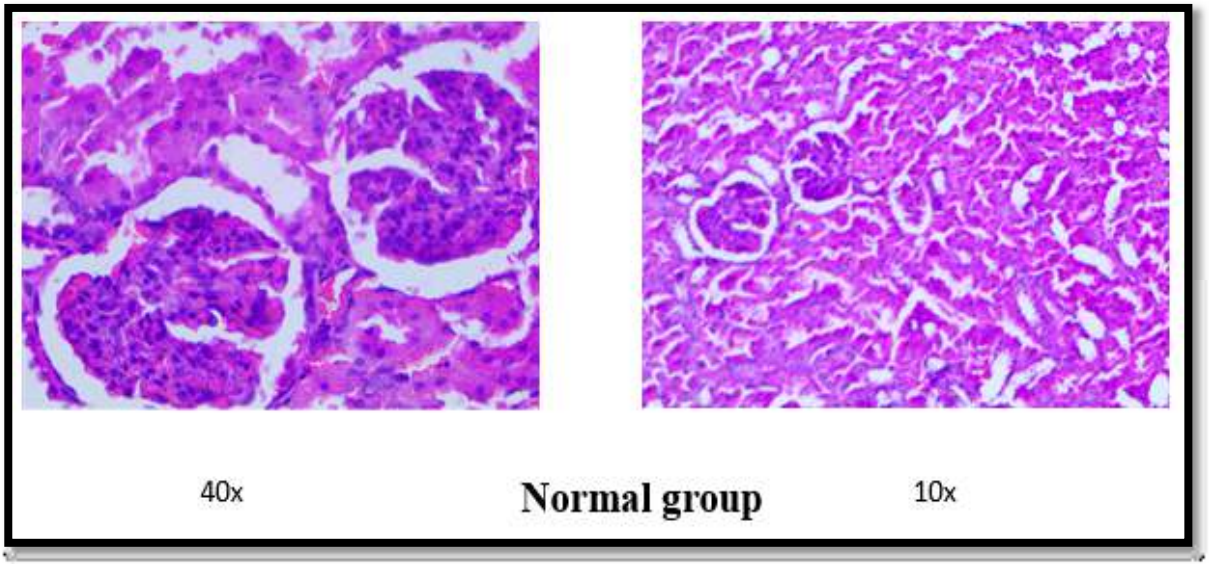
Urine Proteinuria:

Proteinuria was assessed at the end of the study by using urine dipstick kit. The level of excretion urine protein >300mg/dl is considered as significant proteinuria. Normal control rats did not exhibit proteinuria, but in diabetic control group 4 rats showed >300 mg /dl and two rat showed >100 mg/dl of proteinuria. In the metformin group only 2 rats showed >30mg/dl proteinuria rest 4 rats showed Trace amount of proteinuria. In ranolazine group one rats showed >300mg/dl, one showed >100mg/dl and 4 rats showed >30mg/dl proteinuria. In ranolazine + metformin group only one showed >30mg/dl rest showed trace amount of proteinuria.

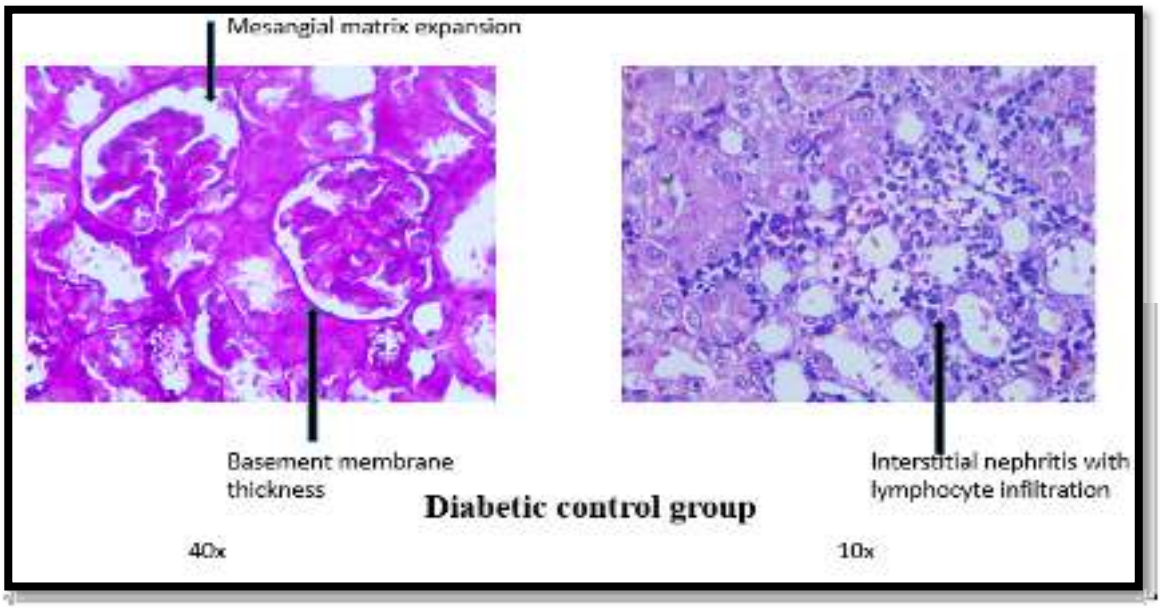
NOTE: Diabetic group showed significant proteinuria, where treating group i.e ranolazine showed mild proteinuria and metformin, combination (ranolazine with metformin) treated group showed non-significant proteinuria.

Histopathological examination of rat Kidneys:

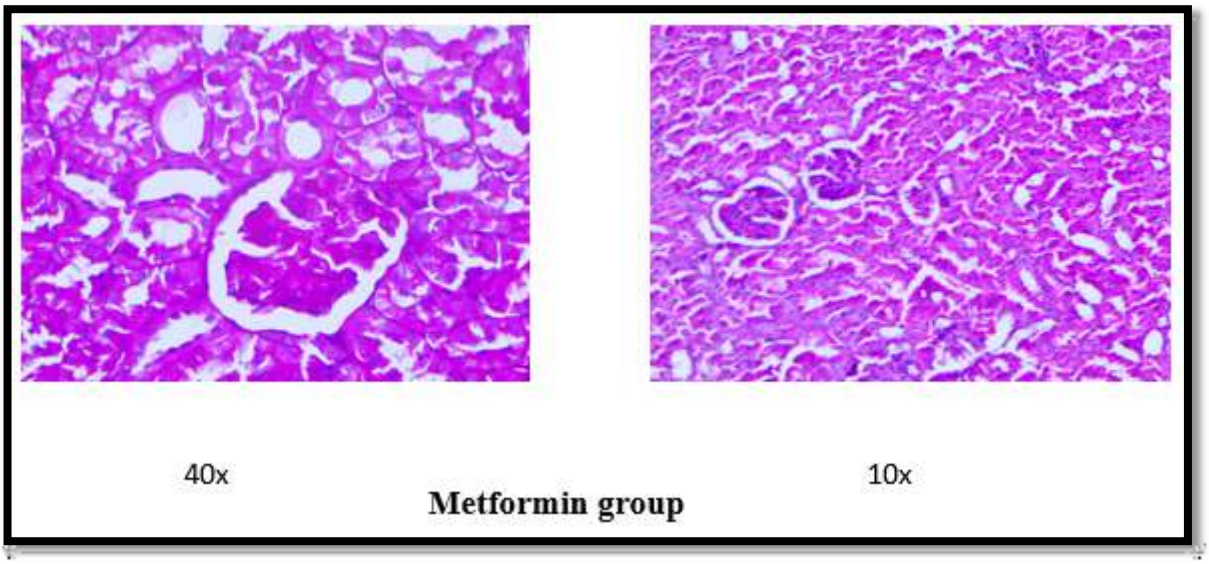
PHOTO-MICROGRAPH: Histopathological Sections of Rat Kidney



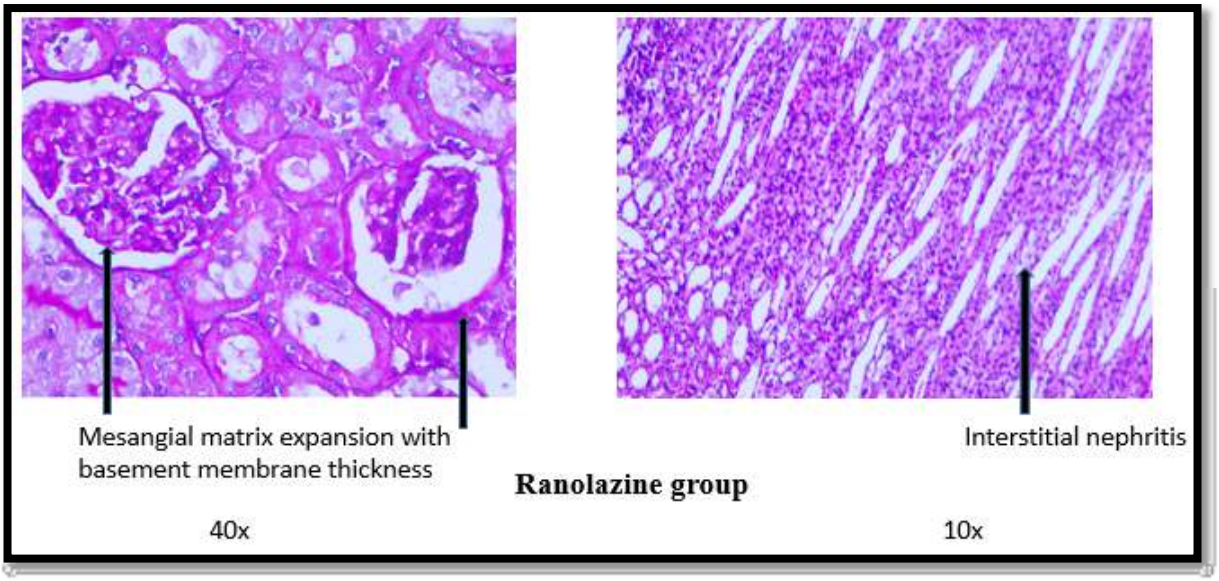
1: (NORMAL CONTROL)



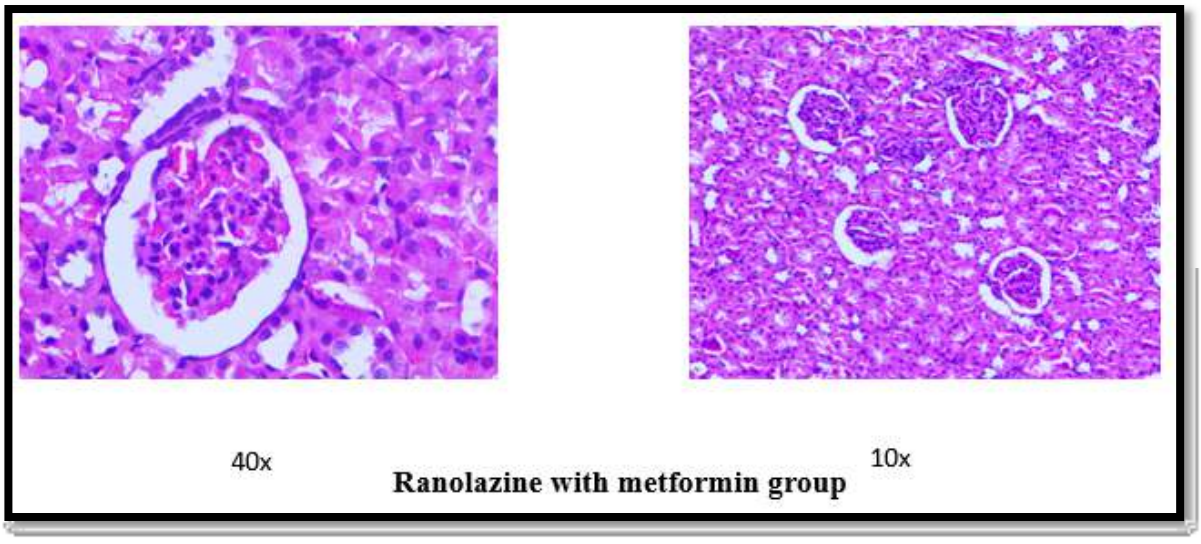
2: (DIABETIC CONTROL)



3: (METFORMIN)



4: (RANOLAZINE)



5: (RANOLAZINE +METFORMIN)

MICROSCOPY AND IMPRESSION: Normal group kidney specimen showed normal interstitum with normal appearing glomeruli. Diabetic group kidney specimen showed moderate interstitial nephritis with areas of focal tubular necrosis and with basement membrane thickness.in metformin group kidney specimen showed mild nephritis in 2 rats out of 6 rats. In ranolazine group kidney specimen showed interstitial nephritis with mild basement membrane thickness in 3 rats out of 6 rats. In ranolazine with metformin group kidney specimen showed mild interstitial nephritis only in one rat out of 6 rats.

Summary: In conclusion we have noted that ranolazine with metformin group showed better outcome and effect as compared to ranolazine treated and metformin treated group. The kidney sections were stained using haematoxylin and eosin staining (Images: H&E stain under PAS 40x magnification). The slides were examined by a trained and experienced pathologist.

Histopathology grades given to kidneys expressed as median and range. Based on the current knowledge regarding histopathological changes that occur in STZ induced diabetic nephropathy in rats, we devised a scoring system indirectly based on a study by Ozdemir O et. al.[50]

Median score that was observed in diabetic control (range 1-2) and ranolazine (range 0-2) group was 2 indicating vacuolar degeneration and cystic dilatation of tubules which was seen in both the groups.

While, metformin and ranolazine with metformin group showed median score of 0 (range 0-1) indicating near normal kidney histology with minimal changes.

KITS AND PROCEDURE OF IL-6, TNF-α

Interleukin 6 Standard

Biotinylated Mouse Interleukin 6 Antibody

Elisa microplate reader machine

Tumor necrosis factor α Standard

Biotinylated RAT Tumor necrosis factor α Antibody, Wash Buffer

DISCUSSION

In India, the number of instances with diabetic nephropathy (DN) has been increasing steadily over the past few decades. A structural and functional alteration in the kidney characterizes diabetic neuropathy.[5] Diabetic nephropathy is related with the presence of pathological levels of albumin excretion in the urine, diabetic glomerular lesions, and a decrease in the glomerular filtration rate. Diabetic nephropathy progression and tissue damage are exacerbated by poor glucose control, advanced glycation end products (AGEs), genetic predisposition, activation of the renin–angiotensin system, reactive oxygen species (ROS), and oxidative stress, all of which contribute to tissue damage and advancement. [54]

Antihypertensive medications, particularly those that target the renin-angiotensin system, such as ACE inhibitors and ARB-1, proven valuable in the treatment of diabetic nephropathy. These medications, on the other hand, are ineffective in the prevention of diabetic nephropathy.[55] There seem to be a number of medications that have demonstrated therapeutic benefits in animal research but have failed in clinical trials, either because of a lack of efficacy or because of safety concerns. There is a significant unmet demand in this therapeutic area. As a result, it was of interest to investigate novel agents for the therapy of diabetic nephropathy. [56]

In accordance with current understanding of the pathophysiology of diabetes, persistently elevated blood glucose levels and the generation of advanced glycation products result in increased production of Profibrotic growth factors and chemokines such as (MCP-1). Those chemokines attract inflammatory cells within the kidneys, which then cause them to become activated and differentiated. The activation of inflammatory cells results in the creation of reactive oxygen species (ROS) and pro-

inflammatory cytokines. Proinflammatory cytokines generated within the kidneys are responsible for parenchymal cellular degeneration. Furthermore, the release of Profibrotic growth factors results in the proliferation of fibroblasts and the deposition of matrix. [16, 17, 18]

Diabetic nephropathy advances with mesangial expansion caused by matrix deposition, thickening of the basement membrane, and the creation of Kimmelstiel–Wilson nodules in the glomeruli as the disease progresses. As a result of the foregoing facts, it is reasonable to conclude that inflammation and oxidative stress are the primary mechanisms behind the development of diabetic nephropathy. [57, 58]

A permission letter from the Institutional Animal Ethics Committee was obtained before the study could begin to be conducted. Wistar rats of either sex (n=38) were given a single IP injection of STZ (45 Mg/kg), which resulted development of diabetes. The presence of diabetes was confirmed by measuring random blood glucose (RBG) on the third day following the injection. The animals were placed into five groups: a normal control group that received normal saline, a diabetic control group, metformin group, ranolazine group and ranolazine with metformin group. In this investigation, the study medications were delivered once daily via oral gavage with a rat feeding needle from day third day to day 56 of the study period. Body weight, random blood glucose (RBG), serum creatinine, and urine proteinuria levels were measured at the start of the trial and again at the conclusion. A number of parameters like CRP, TNF- ALPHA, IL-6 and histological inspection of the kidney were measured when all of the animals were sacrificed at the conclusion of the study.

Weight loss in rodents has been observed following the administration of STZ and the induction of diabetes. [59] In our investigation, the mean body weights of the rats at the start of the experiment were comparable among the groups. During the 8-week study period, the diabetic control group experienced the minimum weight increase, showing that the weight loss caused by the introduction of diabetes was the leading. With ranolazine, weight gain was initially observed, but as the study progressed, weight loss was observed many study show with ranolazine there is weight loss occur. Additionally, previously conducted research studies examining nephroprotective medications have revealed a significant reduction in MBW in diabetic rats treated with STZ. [60, 61] It is possible that the significant loss of muscle mass in diabetic rats is caused by tissue protein catabolism in a condition of hyperglycemia, which could explain the weight loss.

Despite the fact that all other groups gained weight over the course of eight weeks, metformin and ranolazine with metformin groups demonstrated statistical significance at the end of the study in comparison to diabetic control. In addition, the MBW in the ranolazine with metformin treatment group was considerably higher than the MBW in the metformin group.

There have been a number of preclinical studies utilizing the STZ model that have examined the nephroprotective activity of experimental medicines using the conventional biochemical assays such as RBG, serum creatinine, and urine proteinuria. Streptozotocin is an antibiotic with a broad spectrum of activity that is toxic to the insulin-producing beta cells found in pancreatic islets. STZ is taken up by the GLUT2 glucose transporter in the cell membrane, where it induces DNA

alkylation and, eventually, beta cell death. Hyperglycemia is caused by the death of beta cells in the body. [59]

As a result, random blood glucose (RBG) is a regularly utilized variable in this model to assess the induction of diabetes. In our study, we measured the RBG levels of the rats at their baseline, and we discovered that they were comparable across all of the animals and within the normal range. After third day of STZ administration, there was a significant increase in RBG, indicating that diabetes had been successfully induced. Founded on the results of prior preclinical investigations, the cut off level of RBG was > 200 mg/dL. When comparing the metformin, ranolazine group and the ranolazine with metformin group to the diabetic control group, there was a statistically significant difference in blood glucose levels, demonstrating that these medications have a protective effect against diabetes. Furthermore, the RBG levels in the metformin group were comparable to those in the ranolazine and ranolazine with metformin group. Ranolazine treated rats showed decreased RBG level, however the reduction in RBG was not significant when compared to metformin treated group and combination of ranolazine with metformin treated group.

Diabetic nephropathy is characterised by the development of proteinuria (microalbuminuria), which is a significant clinical characteristic and the first symptom of the disease. [62] In the STZ-induced diabetes paradigm, proteinuria is generated by thickening of the glomerular basement membrane, death of podocytes, and an increase in intracellular gaps. In previous preclinical research testing nephroprotective drugs in STZ induced diabetic nephropathy, urine proteinuria was employed as a variable for identifying nephropathy. [59]

The results of a preclinical investigation conducted by Bahaa Al-Trad et al. revealed that a reduction in podocytes, as well as a decreased expression of mRNA and proteins associated to Nephrin and Podocin, resulted in albuminuria following a single dosage of STZ therapy. [63]

A study conducted by Dubey V et al. revealed that albuminuria was significantly increased after 4 weeks of STZ therapy. [64]

In the present study, the diabetic control group showed proteinuria which is specific investigation parameter for diabetic nephropathy. In terms of protection against Proteinuria, metformin treated, ranolazine treated and ranolazine with metformin treated groups performed well. Additionally, the increase in urine proteinuria levels from baseline to 8 weeks was statistically significant in all groups except the metformin treated and ranolazine with metformin treated group, demonstrating that the drug has a nephroprotective outcome in the early stages of diabetes.

Renal biopsy is the gold standard for the diagnosis of diabetic nephropathy. As the disease progresses, the pathological alterations in the kidney grow more severe, beginning with glomerular basement membrane thickening and advancing to worldwide glomerulosclerosis. According to Zhao Y. et al., diabetic rats with increased matrix in the mesangium and vacuolar degeneration of glomerular epithelial cells after 28 days of STZ injection revealed increased matrix in the mesangium. [65] In other preclinical trials, researchers such as Zhou X. et al, Han H. et al, Elbe H. et al, and Yuan H. et al found that glomerular basement membrane thickening, vacuolar degeneration, and renal tubular injury were seen. The formation of reactive radicals

and lipid peroxidation in the STZ induced model of diabetic nephropathy is likely to be the origin of the glomerular and tubular damage seen.[66, 67, 68]

In the present study, histopathological examination of the kidney was analysed by a scoring system based on study by ozdemir O et al. a median score of 2 indicating vacuolar degeneration of the kidney and cystic dilatation of the tubule was observed in the diabetic control group (range 1 to 2) and ranolazine treated group (range 0-2).

The metformin treated and ranolazine with metformin treated groups, was scored with median score of 0 (range 0-1), suggesting that their kidney histology was close to normal with little differences. As a result, metformin and ranolazine with metformin were able to maintain renal architecture despite to interstitial nephritis. The discovery is intriguing, and ranolazine may have potential as a preventive drug for the prevention of diabetic nephropathy if it is investigated thoroughly for this feature in future trials.

Our results showed that STZ-induced diabetic nephropathy was associated with increased levels of pro-inflammatory mediators (TNF-α, IL-6 and CRP). These current findings are consistent with previous evidences. In chronic models of diabetic nephropathy, cytokines and chemokines released from immune cells in kidney were correlated with renal changes. Hyperglycemia activates inflammatory pathways, and prolonged inflammation exacerbates renal damage through sustained overexpression of pro-inflammatory factors that induce more inflammation and enhances other pathogenic mechanisms, like oxidative stress. High levels of CRP, TNF-α and IL-6 partly mediated alterations in histopathological changes detected in diabetic nephropathy. According to Shereen E. Elkholya et al.[69] The anti-inflammatory effects of ranolazine are well established in various animal studies, in their study on diabetic

neuropathy concluded that ranolazine improved evoked-pain behaviours, by reducing sciatic TNF-α and 1L-1B levels.[70] In a randomise control trail by Andreja Rehberger Likozar et al. ranolazine significantly lowered CRP in patients with coronary artery disease. Similarly in another animal study ranolazine significantly reduced cytokines such as IL-6 and TNF-α.[71]

The findings of our study are in line with these existing evidence. We found that ranolazine significantly reduced CRP, TNF – ALPHA, IL- 6 compared to diabetic controlled rats. However this anti-inflammatory action of Ranolazine was not reflected in its efficacy against the diabetic nephropathy. We also found that the combination was more effective in suppressing these inflammatory markers in comparison to ranolazine or Metformin monotherapy. The anti-inflammatory effects of Metformin are well known. Hence, the efficacy of the combination against the diabetic nephropathy could be possibly because of the additive anti-inflammatory effects of these individual drugs.

The outcomes of this trial point to metformin's potential role as a nephroprotective agent in the treatment of diabetic nephropathy, and the combination of ranolazine with metformin produced results comparable to metformin alone, which is now the standard of care for the treatment of diabetic nephropathy. However, significant limitations should be acknowledged when interpreting the results and drawing conclusions from the current study.

Future studies could use diabetic nephropathy models with stronger predictive and face value to validate the effect and move this medicine further in clinical development to meet the unmet demand for a treatment to prevent microvascular complications of diabetes. In addition, it may be worthwhile to investigate whether

the effect of ranolazine when combined with an antidiabetic medication or insulin in diabetic nephropathy is enhanced. To summarise, ranolazine with metformin had a potential nephroprotective effect, as evidenced by improved renal biochemical and histopathological variables in the STZ induced diabetic nephropathy model used in this study, and the protective effect was shown to be mediated by its anti-inflammatory effect.

SUMMARY AND CONCLUSION

Long term diabetes, have risk for developing a condition known as Diabetic Nephropathy (DN), which includes kidney damage, low GFR, and the excretion of albumin in the urine. End-stage renal disease is most commonly brought on by diabetic nephropathy, which is a progressive disease with no known cure. Oxidative stress and a variety of inflammatory mediators have a significant influence in the course of this disease. Pharmacological treatment for diabetic nephropathy now relies on medications that affect the renin-angiotensin system. There is no single licenced treatment that can slow down the progression of the disease, despite the existence of many drugs. Pre-clinical investigations have demonstrated that ranolazine, an antianginal medication, has antihyperglycemic and anti-inflammatory characteristics. In pathophysiology of diabetic nephropathy hyperglycemia and increases in inflammatory marker play major role. Therefore, ranolazine was tested in a diabetic nephropathy model generated by STZ. The current chronic study was conducted for 8 weeks, employing a model that has been used in many earlier studies. A single IP dosage of STZ (45 Mg/kg) produced diabetes in 40 Wistar rats of male sex. Rats were divided into five groups and randomly distributed to normal group, diabetic group, metformin, ranolazine and ranolazine with metformin treated groups (n=6). Except normal group all 4 group were tested for diabetic induction on day 3 of the experiment using random blood glucose level. The animals in control group received normal saline, the positive control group received metformin (90 mg/kg), ranolazine plus metformin (90 mg/kg and 180 mg/kg) group and the received ranolazine alone. Diabetic Rats were treated with drugs from day 4 to day 56. Body weight, RBG, serum creatinine, and HbA1c levels were measured at the start and end of the study, and urine proteinuria, TNF alpha, CRP, IL-6, and histopathological scores were

measured at the conclusion of the study. Ranolazine (90 mg/kg) demonstrated a statistically significant reduction in RBG, HbA1c, CRP, TNF- alpha, IL-6, serum creatinine but variable viz. urine proteinuria and histopathological levels when compared to the diabetic controls the difference was not statistically significant. Nevertheless, ranolazine group had much less proteinuria than the diabetic control group. After matched to the diabetic control group, ranolazine with metformin not just to lowered RBG, serum creatinine, HbA1c, urine proteinuria, CRP, TNF-α, and IL-6 levels, but also exhibited statistical significance. Furthermore, the combination of ranolazine with metformin improved histopathological scores and all the factors in comparison to metformin treated group.

To summarise, the present study demonstrated that the combination treatment of Ranolazine and Metformin exert significant nephroprotective effect in chronic model of diabetic nephropathy in male Wistar rats. The anti-diabetic and anti-inflammatory effect seen in this study could be attributed to their hypoglycaemic effect and direct anti-inflammatory effect. Furthermore, the combination of ranolazine with metformin improved histopathological scores and all the factors in comparison to metformin treated group. However, ranolazine alone requires additional investigation in order to provide further insight and understanding into the mechanism of action and efficacy of this compound in diabetic nephropathy.

BIBLIOGRAPHY

1. Global Report on Diabetes, World Health Organization (WHO), 2016.

2. Longo, Fauci, Kasper, Houser, Jamson, Loscalzo, Harrison's principle of internal medicine. 2015; 19(2):2422 6.

3. International Diabetes Federation. IDF Diabetes Atlas, 9th edition. International Diabetes Federation, 2019. http://diabetesatlas.org/ resources/2019-atlas.html (accessed December 24, 2019).

4. Tandon N, Anjana RM, Mohan V, Kaur T, Afshin A, Ong K, Mukhopadhyay S, Thomas N, Bhatia E, Krishnan A, Mathur P. The increasing burden of diabetes and variations among the states of India: The Global Burden of Disease Study 1990–2016. The Lancet Global health. 2018 Dec 1; 6(12): e1352-62.

5. Tripathy JP, Thakur JS, Jeet G, Chawla S, Jain S, Pal A, et al. Prevalence and risk factors of diabetes in a large community-based study in North India: results from a STEPS survey in Punjab, 2017;18.

6. Kaveeshwar SA, Cornwall J. The current state of diabetes mellitus in India. Australasian Medical Journal.2014; 7:45-8.

7. Timmis AD, Chaitman BR, and Crager M Effects of ranolazine on exercise tolerance and HbA1c in patients with chronic angina and diabetes. Eur Heart J 2006; 27:42–48.

8. American Diabetes Association. Standards of medical care in diabetes-2014. Diabetes Care. 2014; 37 Suppl1:S14-80.

9. Alicic RZ, Rooney MT, Tuttle KR. Diabetic kidney disease: challenges, progress, and possibilities. Clinical Journal of the American Society of Nephrology. 2017 Dec 7; 12(12):2032-45. 90.

10. Modi GK, Jha V. The incidence of end stage renal disease in India: A population based study.2006; 70(12):2131 3.

11. Unnikrishnan RI, Rema M, Pradeepa R, Deepa M, Shanthirani CS, Deepa R, et al. Prevalence and risk factors of diabetic nephropathy in an urban South Indian population: The Chennai Urban Rural Epidemiology Study (CURES 45) Diabetes Care.2007;30:2019–24.

12. Prakash J, Hota JK, Singh S, Sharma OP. Clinical spectrum of chronic renal failure in the elderly: A hospital-based study from eastern India. Int Urol Nephrol. 2006; 38:821–7.

13. Klemens R, Angela G, Sabine H, et al. Diabetic nephropathy in 27,805 children, adolescents, and adults with type 1 diabetes: effect of diabetes duration, A1C, hypertension, dyslipidaemia, diabetes onset and sex. Diabetes Care. 2007; 30:2523– 8.

14. Eberhard R. Diabetic nephropathy. Saudi J Kidney Dis Transplant. 2006; 17:481– 90.

15. Nazar CM. Diabetic nephropathy; principles of diagnosis and treatment of diabetic kidney disease. Journal of nephropharmacology. 2014; 3(1):15.

16. Ritz E. Limitations and future treatment options in type 2 diabetes with renal impairment. Diabetes Care. 2011 May 1; 34(Supplement 2): S330-4.

17. Banba N, Nakamura T, Matsumura M, Kuroda H, Hattori Y, Kasai K. Possible relationship of monocyte chemoattractant protein-1 with diabetic nephropathy. Kidney international. 2000 Aug 1; 58(2):684-90.

18. Tesch GH. MCP-1/CCL2: a new diagnostic marker and therapeutic target for progressive renal injury in diabetic nephropathy. American Journal of PhysiologyRenal Physiology. 2008 Apr; 294 (4): F697-701

19. Goring S, Hawkins N, Wygant G, Roudaut M, Townsend R, Wood I, & Barnett A. H. Dapagliflozin compared with other oral anti-diabetes treatments when added to metformin monotherapy: a systematic review and network meta-analysis. Diabetes, Obesity and Metabolism. 2013; 16(5), 433–442.

20. Matthaei S, Bowering K, Rohwedder K, Grohl A. Dapagliflozin improves glycaemic control and reduces body weight as add-on therapy to metformin plus sulfonylurea: a 24-week randomized, double-blind clinical trial. 2015 Mar;38(3):365-72

21. Gupta P, Bala M, Gupta S, Dua A, Dabur R, et al. Efficacy and risk profile of anti-diabetic therapies: Conventional vs traditional drugs—A mechanistic revisit to understand their mode of action. Pharmacological Research. 2016; 113, 636–674.

22. Chaitman BR, Pepine CJ, Parker JO, et al.; Combination Assessment of Ranolazine In Stable Angina (CARISA) Investigators. Effects of ranolazine with atenolol, amlodipine, or diltiazem on exercise tolerance and angina frequency in patients with severe chronic angina: a randomized controlled trial. JAMA 2004;291:309–316

23. Belardinelli L, Shryock JC, Fraser H. The mechanism of ranolazine action to reduce ischemia-induced diastolic dysfunction. Eur Heart J 2006;8:A10–A13

24. Chaitman BR, Skettino SL, Parker JO, Hanley P, Meluzin J, Kuch J, et al. Anti-ischemic effects and long-term survival during ranolazine monotherapy in patients with chronic severe angina. J Am Coll Cardiol 2004 43:1375–1382.

25. Timmis AD, Chaitman BR, and Crager M Effects of ranolazine on exercise tolerance and HbA1c in patients with chronic angina and diabetes. Eur Heart J 2006; 27:42–48.

26. Morrow DA, Scirica BM, Chaitman BR, et al.; MERLIN-TIMI 36 Investigators. Evaluation of the glycometabolic effects of ranolazine in patients with and without diabetes mellitus in the MERLIN-TIMI 36 randomized controlled trial. Circulation 2009; 119:2032–2039

27. Chisholm JW, Goldfine AB, Dhalla AK, et al. Effect of ranolazine on A1C and glucose levels in hyperglycemic patients with non-ST elevation acute coronary syndrome. Diabetes Care 2010; 33:1163–1168

28. Naveena R, Hashilkar N.K, Davangeri R, & Majagi S. I. Effect of anti-inflammatory activity of ranolazine in rat model of Inflammation. Indian journal of medical research. December 2018; 743-747.

29. Diabetes, WHO definition. http://www.who.int/diabetes/en/ last accessed on 14 December 2019.

30. Unnikrishnan R. Landmarks in the history of diabetes.in: chandalia HB et al, RSSDI textbook of diabetes mellitus (3rd edn.)2014 .pp 3-16

31. American Diabetes Association. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes—2019. Diabetes Care. 2019 Jan 1; 42(Supplement 1):S13-28.

32. Craig ME, Hattersley A, Donaghue KC. Definition, epidemiology and classification of diabetes in children and adolescents. Pediatric diabetes. 2009 Sep; 10:3-12.

33. American Diabetes Association. 11. Microvascular complications and foot care: Standards of Medical Care in Diabetes—2019. Diabetes Care. 2019 Jan 1;42(Supplement 1):S124-38

34. Robles NR, Villa J, Gallego RH. Non-proteinuric diabetic nephropathy. Journal of clinical medicine. 2015 Sep; 4(9):1761-73.

35. Tervaert TW, Mooyaart AL, Amann K, Cohen AH, Cook HT, Drachenberg CB, Ferrario F, Fogo AB, Haas M, de Heer E, Joh K. Pathologic classification of diabetic nephropathy. Journal of the American Society of Nephrology. 2010 Apr 1; 21(4):556- 63.

36. Alpers CE, Hudkins KL. Mouse models of diabetic nephropathy. Current opinion in nephrology and hypertension. 2011 May; 20(3):278.

37. Brosius FC, Alpers CE, Bottinger EP, Breyer MD, Coffman TM, Gurley SB, Harris RC, Kakoki M, Kretzler M, Leiter EH, Levi M. Mouse models of diabetic nephropathy. Journal of the American Society of Nephrology. 2009 Dec 1; 20(12):2503-12.

38. Ponchiardi C, Mauer M, Najafian B. Temporal profile of diabetic nephropathy pathologic changes. Current diabetes reports. 2013 Aug 1; 13(4):592-9.

39. Furman BL. Streptozotocin-induced diabetic models in mice and rats. Current protocols in pharmacology. 2015 Sep; 70(1):5-47.

40. Balakumar P, Chakkarwar VA, Kumar V, Jain A, Reddy J, Singh M. Experimental models for nephropathy. Journal of the Renin-Angiotensin-Aldosterone System. 2008 Dec; 9(4):189-95.

41. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiological research. 2001 Jan 1; 50(6):537-46

42. Like AA, Rossini AA. Streptozotocin-induced pancreatic insulitis: new model of diabetes mellitus. Science. 1976 Jul 30; 193(4251):415-7.

43. Arulmozhi DK, Veeranjaneyulu A, Bodhankar SL. Neonatal streptozotocin-induced rat model of Type 2 diabetes mellitus: A glance. Indian Journal of Pharmacology. 2004 Jul 1; 36(4):217.

44. Velasquez MT, Kimmel PL, Michaelis 4th OE. Animal models of spontaneous diabetic kidney disease. The FASEB Journal. 1990 Aug; 4(11):2850-9.

45. BruntonL, KnollmannB, Hilal-Dandan R. goodman and gilman's. New York,N.Y :Mcgraw-Hill Education LLC.2018

46. Aldasoro M, Guerra-Ojeda S, Aguirre-Rueda D, Mauricio M, Vila J, Marchio P et al. Effects of Ranolazine on Astrocytes and Neurons in Primary Culture. PLOS ONE. 2016; 11(3):0150619.

47. Goodman L, Gilman A, Brunton L, Hilal-Dandan R, Knollmann B. Goodman & Gilman's the pharmacological basis of therapeutics. New York [etc.]: McGraw Hill Education; 2018.

48. Greiner L, Hurren K, & Brenner M. Ranolazine and Its Effects on Hemoglobin A1C. Annals of Pharmacotherapy. 2016; 50(5), 410–415.

49. Dhalla A.K, Yang M, Ning Y. Blockade of Na+ Channels in Pancreatic a-Cells Has Antidiabetic Effects 2014;63:3545–3556

50. Gupta P, Bala M, Gupta S, Dua A, Dabur R, et al. Efficacy and risk profile of anti-diabetic therapies: Conventional vs traditional drugs—A mechanistic revisit to understand their mode of action. Pharmacological Research. 2016; 113, 636–674.

51. S. Teng, Z. Ren and K. Zhao. Vagal Stimulation Facilitates Improving Effects of Ranolazine on Cardiac Function in Rats with Chronic Ischemic Heart Failure. 2018; Vol 5 (8), 36-43,

52. Deshmukh S.H, Patil S R, Elsa Pinassi, et al. Ranolazine improves endothelial function in patients with stable coronary artery disease. 2009; 20:343–347

53. Ozdemir O, Akalin PP, Baspinar N, Hatipoglu F. Pathological changes in the acute phase of streptozotocin-induced diabetic rats. Bull Vet Inst Pulawy. 2009 Jan 1; 53(4):783-90.

54. Pourghasem M, Shafi H, Babazadeh Z. Histological changes of kidney in diabetic nephropathy. Caspian journal of internal medicine. 2015; 6(3):120. 97

55. Forbes JM, Coughlan MT, Cooper ME. Oxidative stress as a major culprit in kidney disease in diabetes. Diabetes. 2008 Jun 1;57(6):1446-54

56. Perez-Gomez MV, Sanchez-Niño MD, Sanz AB, Martín-Cleary C, Ruiz-Ortega M, Egido J, Navarro-González JF, Ortiz A, Fernandez-Fernandez B. Horizon

2020 in diabetic kidney disease: the clinical trial pipeline for add-on therapies on top of renin angiotensin system blockade. Journal of clinical medicine. 2015 Jun; 4(6):1325-47.

57. Weil EJ, Lemley KV, Mason CC, et al. Podocyte detachment and reduced glomerular capillary endothelial fenestration promote kidney disease in type 2 diabetic nephropathy. Kidney Int. 2012; 82(9):1010–1017.

58. Toyoda M, Najafian B, Kim Y, Caramori ML, Mauer M. Podocyte detachment and reduced glomerular capillary endothelial fenestration in human type 1 diabetic nephropathy. Diabetes. 2007; 56(8):2155–2160.

59. Deeds MC, Anderson JM, Armstrong AS, Gastineau DA, Hiddinga HJ, Jahangir A, Eberhardt NL, Kudva YC. Single dose streptozotocin-induced diabetes: considerations for study design in islet transplantation models. Laboratory animals. 2011 Jul; 45(3):131-40.

60. Elbe H, Vardi N, Esrefoglu M, Ates B, Yologlu S, Taskapan C. Amelioration of streptozotocin-induced diabetic nephropathy by melatonin, quercetin, and resveratrol in rats. Human & experimental toxicology. 2015 Jan; 34(1):100-13.

61. Hasanvand A, Amini-Khoei H, Jahanabadi S, Mehr SE, Dehpour AR. Metformin attenuates streptozotocin-induced diabetic nephropathy in rats through activation of AMPK signaling pathway. Journal of Nephropathology. 2018 Jan 1; 7(1).

62. Roshan B, Stanton RC. A story of microalbuminuria and diabetic nephropathy. Journal of nephropathology. 2013 Oct;2(4):234

63. Al-Trad B, Ashankyty IM, Alaraj M. Progesterone ameliorates diabetic nephropathy in streptozotocin-induced diabetic Rats. Diabetology & metabolic syndrome. 2015 Dec; 7(1):97.

64. Dubey VK, Patil CR, Kamble SM, Tidke PS, Patil KR, Maniya PJ, Jadhav RB, Patil SP. Oleanolic acid prevents progression of streptozotocin induced diabetic nephropathy and protects renal microstructures in Sprague Dawley rats. Journal of pharmacology & pharmacotherapeutics. 2013 Jan; 4(1):47.

65. Zhao Y, Huang W, Wang J, Chen Y, Huang W, Zhu Y. Taxifolin attenuates diabetic nephropathy in streptozotocin-induced diabetic rats. American journal of translational research. 2018; 10(4):1205.

66. Han H, Cao A, Wang L, Guo H, Zang Y, Li Z, Zhang X, Peng W. Huangqi decoction ameliorates streptozotocin-induced rat diabetic nephropathy through antioxidant and regulation of the TGF-β/MAPK/PPAR-γ signaling. Cellular Physiology and Biochemistry. 2017; 42(5):1934-44.

67. Zhou X, Feng Y, Zhan Z, Chen J. Hydrogen sulfide alleviates diabetic nephropathy in a streptozotocin-induced diabetic rat model. Journal of Biological Chemistry. 2014 Oct 17; 289(42):28827-34. 100

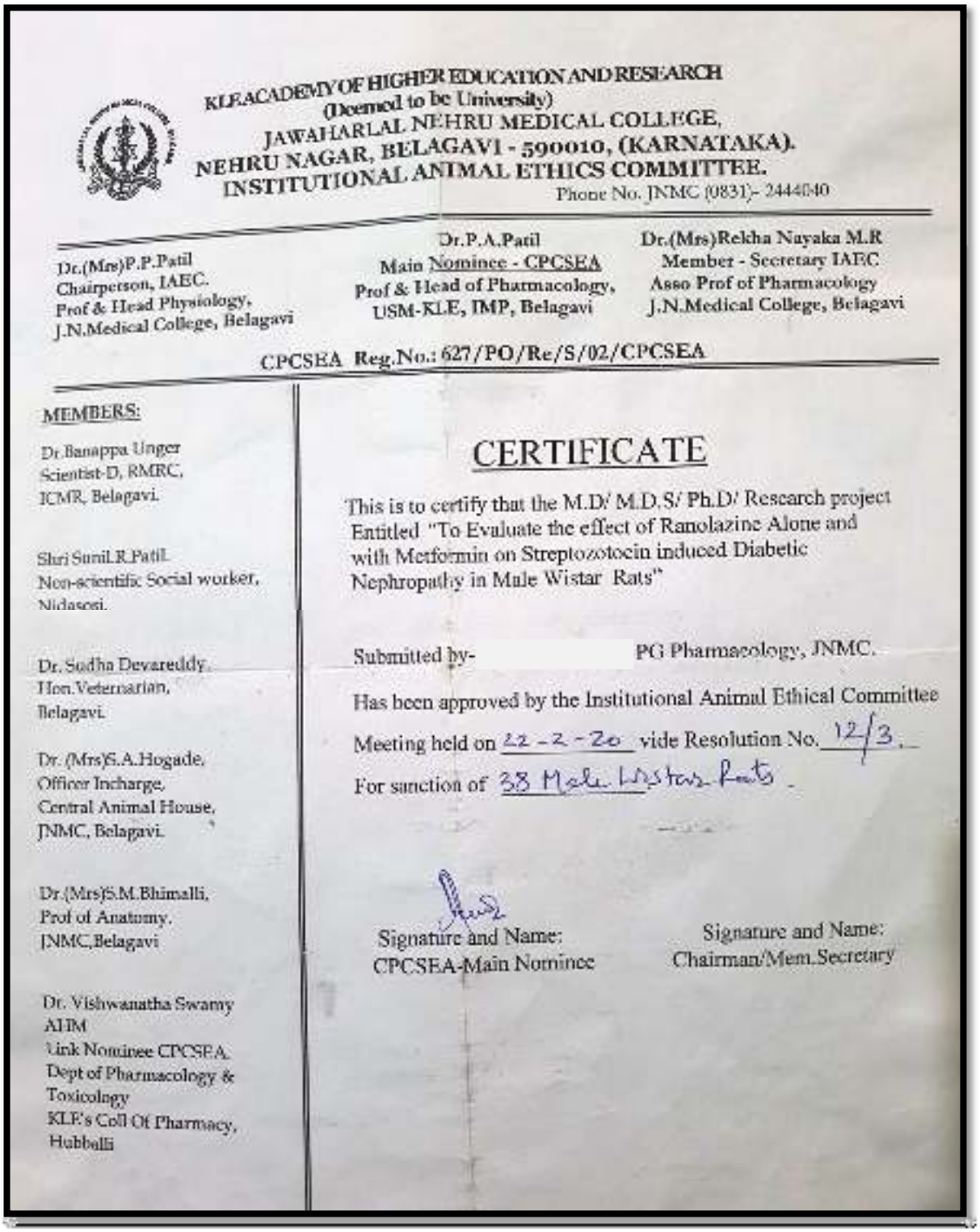
68. Yuan H, Zhang X, Zheng W, Zhou H, Zhang BY, Zhao. D. Minocycline attenuates kidney injury in a rat model of streptozotocin-induced diabetic nephropathy. Biological and Pharmaceutical Bulletin. 2016 Aug 1; 39(8): 1231-7.

69. Shereen E. Elkholya, Samah M. Elaidy; Neuroprotective effects of ranolazine versus pioglitazone in experimental diabetic neuropathy: Targeting Nav1.7 channels and PPAR-γ: Life Sciences 250 (2020).

70. S. Teng, Z. Ren and K. Zhao; Vagal Stimulation Facilitates Improving Effects of Ranolazine on Cardiac Function in Rats with Chronic Ischemic Heart Failure; Current Molecular Medicine 2018, 18, 36-43.

71. Andreja Rehberger Likozar, Miran Šebeštjen. Ranolazine, But not Trimetazidine, Influences Markers of Inflammation in Patients with Stable Coronary Artery Disease. Biomed J Sci & Tech Res 34(4)-2021. BJSTR. MS.ID.005598.

ANNEXURE – I - IAEC APPROVAL CERTIFICATE



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 "To Evaluate the effect of Ranolazine Alone and with Metformin on Streptozotocin induced Diabetic Nephropathy in Male Wistar Rats"

Submitted by- PG Pharmacology, JNMC.

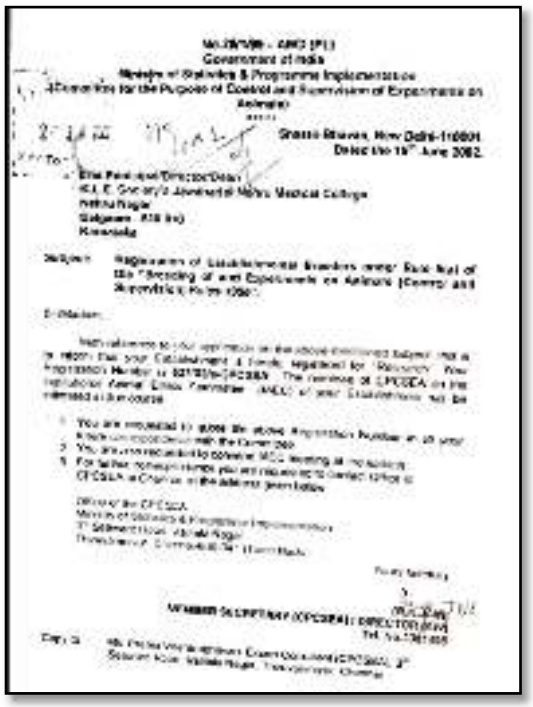
Has been approved by the Institutional Animal Ethical Committee Meeting held on 22-2-20 vide Resolution No. 12/3.

For sanction of 38 Male Wistar Rats.

Signature and Name:
CPCSEA-Main Nominee

Signature and Name:
Chairman/Mem.Secretary

ANNEXURE - II - CPCSEA REGISTRATION & RENEWAL



Government of India

Ministry of Environment & Programme Implementation

Committee for the Purpose of Control and Supervision of Experiments on Animals

Shastri Bhawan, 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/ Stock Unit of the Breeding of and Experiments on Animals (Control and Supervision) Rules 1998.

Sir/Madam,

You are requested to quote the above Registration Number in all your

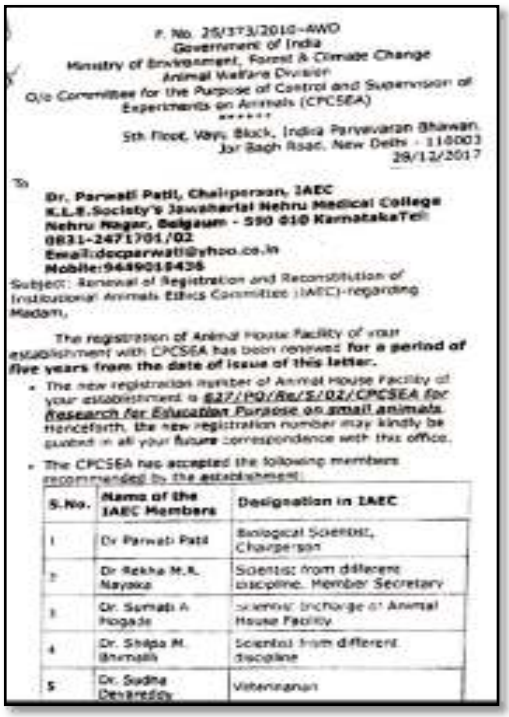
For further correspondence you are requested to contact Officer in

Officer in the CPCSEA

Ministry of Environment & Forest

Secretary

Tel. No.



F. No. 25/373/2010-AWO

Government of India

Ministry of Environment, Forest & Climate Change

Animal Welfare Division

O/o Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)

5th Floor, Vayu Block, Indira Paryavaran Bhawan,

Jor Bagh Road, New Delhi - 110003

28/12/2017

To

Dr. Parwati Patil, Chairperson, IAEC
K.L.E.Society's Jawaharlal Nehru Medical College
Nehru Nagar, Belgaum - 590 010 KarnatakaTel:
0831-2471701/02
Email:docparwati@yhoo.co.in
Mobile:9480018436

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.**

- The new registration number of Animal House Facility of your establishment is ***827/HO/Re/S/02/CPCSEA for Research for Education Purpose on small animals.*** Henceforth, the new registration number may kindly be quoted in all your future correspondence with this office.
- The CPCSEA has accepted the following members recommended by the establishment:

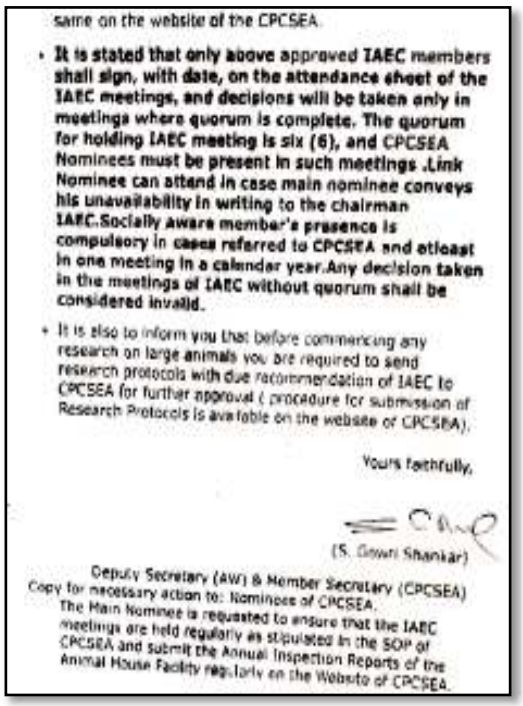
S.No.	Name of the IAEC Members	Designation in IAEC
1	Dr Parwati Patil	Biological Scientist, Chairperson
2	Dr Rekha M.R. Nayaka	Scientist from different discipline, Member Secretary
3	Dr. Sumati A Hogade	Scientist incharge of Animal House Facility
4	Dr. Shilpa M. Bhemalli	Scientist from different discipline
5	Dr. Sudha Devareddy	Veterinarian

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

S.No.	Name	Nominated as
1	**Dr. P.A. Patil** 'Vinlaq' 23-A, II Main, II Cross, Basava Road, Belagavi - 590016, Karnataka Contact No :9448889519 Email :drpapatil@yahoo.co.in	Main Nominee
2	**Dr. Viswanatha Swamy A.H.M.** Associate Professor, Deptt. Of Pharmacology & Toxicology, Karnataka Lingayat Education Society's College of Pharmacy, Vidyanagar, Hubli - 580 031, Karnataka Contact No :9449667355 Email :(Withheld)	Link Nominee
3	**Dr. Basappa S Unger** Scientist –D (Pharmacology), Regional Medical Research Centre, Indian Council of Medical Research, Nehru Nagar, Belgaum-590010, Karnataka Contact No :9916373018 Email :(basappa@gmail.com	Scientist from outside the Institute
4	**Shri. Sunil B Patil** At/po: Nidasoshi, Tq: Hukkeri, Dist: Belgaum, Karnataka - 591236 Contact No :9902263037 Email :(unilab@rediffmail.com	Socially Aware Nominee

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

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



same on the website of the CPCSEA.

- **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 CPCSEA Nominees must be present in such meetings .Link Nominee can attend in case main nominee conveys his unavailability in writing to the chairman IAEC.Socially aware member's presence is compulsory in cases referred to CPCSEA and atleast in one meeting in a calendar year.Any decision taken in the meetings of IAEC without quorum shall be considered invalid.**
- 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 faithfully,

(S. Gowri Shankar)

Deputy Secretary (AW) & 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.