THE EFFECT OF RIVAROXABAN AND DABIGATRAN ON GLYCEMIC PARAMETERS AND LIPID PROFILE IN HIGH FAT DIET AND LOW DOSE STREPTOZOTOCIN INDUCED DIABETES MELLITUS IN MALE WISTAR RATS

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LIST OF ABBREVIATIONS

AA	-	Amino acids
ADA	-	American diabetic association
AGEs	-	Advanced glycation endproducts
ANOVA	-	One way analysis of Variance
BMI	-	Body mass index
CPCSEA	-	Committee for the Purpose of Control and Supervision of Experiments on Animals
CVD		Cardiovascular disease
DAG		Diacylglycerol
DC		Diabetic control group
DM		Diabetes Mellitus
DN		Diacerein group
DPP		Diabetes Prevention Program
ER		Endoplasmic Reticulum
FBG		Fasting Blood Glucose
FFA		Free Fatty Acids
FOXO1		Transcription factor forkhead box protein O1

GAD	Glutamic acid decarboxylase
GIP	Glucose-dependent insulinotropic peptide
GK	Goto-Kakizaki
GLP-1	Glucagon-like peptide-1
GWAS	Genome-wide Association Study
HDL	High Density Lipoprotein
HDL	High-density lipoproteins
HFD	High-fat diet
HOMA-IR	Homeostasis model assessment-insulin resistance
IAEC	Institutional Animal Ethics Committee
IAPP	Islet amyloid polypeptide
IAPP	Islet amyloid polypeptide
ICAs	Islet cell autoantibodies
IFN-γ	Interferon gamma
IGF-1	Insulin-like growth factor-1
IGT	Impaired glucose tolerance
IL-1β	Interleukin 1 beta
IL-6	Interleukin 6
IRPs	Insulin resistance-associated proteins
IRS	Insulin receptor substrate

JNK	c-JUN N-terminal kinase
LADA	Latent autoimmune Diabetes in adults
LDL	Low-density lipoproteins
MAPK	Mitogen-activated protein kinase
MF	Metformin group
MHO	Metabolically healthy Obesity
MODY	Maturity-onset Diabetes of the Young
NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
NC	Normal control group
NF-κB	Nuclear factor kappa-B
NLRP3	NLR family pyrin domain-containing 3
PAMPs	Pathogen-associated molecular patterns
PI3K	Phosphatidylinositol-3-kinase
PIP-3	Phosphatidylinositol 3,4,5-trisphosphate
PKB	Protein kinase B
PP	Pancreatic Polypeptide
RAGE	Receptor for Advanced glycation end products
RCT	Randomized Controlled Trial
ROS	Reactive oxygen species ROS

SEM	Standard error of mean
SFFA	Saturated free fatty acids
Shc	Src homology and collagen protein
SJS	Stevens-Johnson syndrome
STZ	Streptozotocin
SUR	Sulfonylurea receptor
SYSADOA	Symptomatic slow-acting drug in osteoarthritis
T2DM	Type 2 Diabetes Mellitus
TEN	Toxic epidermal Necrolysis
TLR	Toll-like receptor
TNF-α	Tumor Necrosis Factor alpha
TXNIP	Thioredoxin-interacting protein
WHO	World Health Organization
ZFR	Zucker fatty rats

ABSTRACT

Introduction

The current experimental study aimed to evaluate the effect of Rivaroxaban and Dabigatran on glycemic parameters in a High-fat diet and low dose Streptozotocin-induced Diabetes Mellitus in male Wistar rats. In addition, the effect on the Lipid profile and inflammatory markers were also assessed.

Methods

Animals were randomly divided into various groups. One group was Normal control while others consisted of diabetic rats without treatment or with treatment of Metformin, Diabetes was induced by feeding the rats with High-fat diet for 2 weeks followed by a single intraperitoneal injection of Streptozotocin. (30 mg/Kg). Following induction of Diabetes treatment with either Metformin, Rivaroxaban or Dabigatran and was continued for 6 weeks. Body weight, Fasting Blood Glucose and Lipid profile were measured at various time intervals. Inflammatory markers were studied at the end of the study.

Results

All three treatments significantly reduced Fasting Blood glucose compared to the untreated rats. In addition, they showed improvement in body weight as well as Lipid Profile. The inflammatory markers (IL-1β, IL-6, TNF-α) were significantly reduced in all treatment groups as compared to untreated rats.

Conclusion

The present study showed that the treatment of diabetic rats with Rivaroxaban and Dabigatran improved the High-fat diet and Streptozotocin-induced biochemical alterations. It can be concluded that Rivaroxaban and Dabigatran may be a promising option for the management of Type 2 Diabetes. Future research of Rivaroxaban and Dabigatran in combination with standard anti-diabetic drugs like Metformin in patients of diabetes, as well as clinical trials with larger sample sizes, need to be considered.

Keywords

Type 2 Diabetes Mellitus, Rivaroxaban, Dabigatran, Inflammation,Streptozotocin, High-fat diet, Wistar rats

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INTRODUCTION

Diabetes mellitus (DM) refers to a class of disease associated with metabolism which shares the phenotype of elevated blood glucose levels. It manifests in numerous types, the development of which is intricately woven through a combination of genetic susceptibilities & environmental influences. Hyperglycemia, a hallmark feature, stems from a cascade of factors including decreased secretion of insulin, compromised utilization of glucose, & heightened production of glucose. Identification of the specific cause of diabetes mellitus often hinges on understanding these underlying mechanisms. Furthermore, the metabolic dysregulation inherent in diabetes mellitus triggers a cascade of secondary pathophysiological changes across various organ systems. These systemic alterations not only profoundly impact individuals living with diabetes mellitus but also exert considerable strain on healthcare systems worldwide. [1] Over the span of 30 years, a significant surge in the global prevalence of diabetes has taken place. The anticipated growth in the worldwide adult diabetic population suggests an increase from 135 million individuals in 1995 to an estimated 300 million by 2025.[2] International Diabetes Federation paint a concerning picture, forecasting that the number of individuals affected by diabetes will skyrocket to 642 million by the year 2040. This exponential rise is closely linked to the process of industrialization observed in many countries, which correlates with elevated levels of obesity, reduced physical activity, & an aging population. Consequently, the prevalence of type 2 diabetes mellitus(T2DM) is on an alarming upward trajectory across the globe.[1] According to studies conducted by the Indian Council of Medical Research – INdia DIABetes (ICMR – INDIAB), the prevalence of diabetes in India is 7.3%, with prediabetes affecting approximately 10.3% of the population. These figures translate to a substantial burden, with an estimated 69.2

million individuals living with diabetes & 77.2 million individuals at risk of possibly developing it.[3]

Interestingly, the actions of decreasing cell insulin secretion & increasing beta insulin resistance are caused because of inflammation. Beta cell function can be affected by circulating cytokines leading to secretory dysfunction & increase in apoptosis. This is the direct effect; the indirect effect of cytokines is by increasing adipocyte formation. As a result of this glucotoxicity & lipotoxicity the inflammatory process is further enhanced resulting in a vicious cycle. [4]The overall count of white blood cells is often used as an indicator of inflammation. This is when focusing on the neutrophil count in the upper quartiles of the standard range. It shows a connection with declining insulin sensitivity, as well as the development of diabetes & cardiovascular issues. Inflammatory markers such as CRP, IL-6, & TNF-α are higher in patients with metabolic syndrome as well as those with clinically overt Type 2 Diabetes in epidemiological investigations.[5] The generation of IL-1β by the NLRP3 (NACHT, LRR, & PYD domains-containing protein 3) inflammasome has a significant role in the development of obesity & diabetes. [6] Also the primary cell implicated in inflammation & insulin resistance in type 2 diabetes mellitus (T2DM) is the adipocyte. Insulin plays a crucial role in regulating glucose uptake & triglyceride storage within adipocytes. Additionally, adipocytokines, which are secreted by adipocytes, can influence both insulin secretion & resistance. Notably, by escalating insulin resistance, adipocytokines such visfatin, omentin, resistin, leptin, & adiponectin may exacerbate beta cell dysfunction. Additionally, adipose tissue secretes dipeptidyl peptidase-4 . It stimulates the breakdown of glucagon-like peptide-1 & causes beta cells to become insulinotropic.[4] The relationship between body mass index (BMI) & the onset of diabetes is robust. In obese individuals, there

is an elevation in various substances. These include nonesterified fatty acids, glycerol & proinflammatory markers. All of these play pivotal roles in the development of insulin resistance. The pathology underlying diabetes involves impairment of the β-islet cells in the pancreas, leading to inadequate blood glucose control. Chances of developing diabetes is pronounced when this is coupled with resistance to the hormone insulin. Notably, increase in weught & body fat play central roles in emergence & increasing prevalence of type 2 diabetes.[7]

A striking observation reveals that thrombotic events contribute to mortality in a staggering 80% of individuals with diabetes. Among these, cardiovascular issues stand out as the leading cause, responsible for approximately 75% of all deaths. Within this category, cerebrovascular incidents & peripheral vascular complications account for the remaining 25% of fatalities. The main defense against thrombosis is the vascular endothelium. It is damaged in diabetes. The augmented platelet activation & clotting factors augmentation observed in diabetes- can be attributed to endothelial abnormalities, marking it as a hypercoagulable state. This assertion is reinforced by extensive studies conducted by Carr & colleagues.[8] Laboratory analyses consistently unveil heightened coagulant potential, hemostatic system remining activated sustainedly, chronic platelet activation, & diminished fibrinolytic potential in diabetic individuals. Additionally, a notable inclination toward reduced levels of natural anticoagulants is evident, while endothelial dysfunction is substantiated by histological examinations. The collective body of evidence unequivocally gives support to the observation that diabetes mellitus(DM) manifests as a profoundly hypercoagulable state, underscoring the critical need for vigilant management strategies in affected individuals.[8]

Presently, treatment options for type 2 diabetes encompass a range of medications aimed at managing the condition. This includes synthetic insulin and oral hypoglycemics. Oral hypoglycemics include drugs like metformin, sulfonylureas, thiazolidinediones, incretin - based therapies, & SGLT inhibitors. However, only some of them exhibit anti-inflammatory action. Therefore, the search for drugs with potent anti-inflammatory effects targeting the pathogenesis of Diabetes Mellitus continues.

Rivaroxaban & Dabigatran are commonly used anticoagulants with proven anti-inflammatory effect. They reduce the magnitude of inflammatory markers increased in diabetes.[9] [10] [11] Prior research has indicated that dabigatran administration significantly inhibited the activities of P65 of nuclear factor κB, tumor necrosis factor α, interleukin (IL)-1β, & IL-6. This was seen in rabbits with acute myocardial infarction. Additionally, there was a significantly higher magnitude observed in catalase & superoxide dismutase activities. Moreover, dabigatran effectively suppressed the expression of many factors. This includes Collagen I, inducible nitric oxide synthase (iNOS), α-smooth muscle actin (α-SMA), & connective tissue growth factor (CTGF) proteins.[9] Furthermore, Treatment with rivaroxaban resulted in reductions from baseline in several biomarkers. Specifically, D-dimer, interleukin-6 (hs-IL-6) and thrombin-antithrombin complex (TAT) levels decreased following rivaroxaban treatment.[12]

Given the known anti-inflammatory properties of Dabigatran & Rivaroxaban, alongside the scarcity of comprehensive studies investigating their direct effects on diabetes mellitus, this research endeavor has been meticulously designed. Our study seeks to bridge this gap by using a rodent model of Diabetes(DM) to meticulously evaluate the potential impacts of Rivaroxaban & Dabigatran. This is induced by a high-fat diet coupled with low-dose

Streptozotocin. Through a multifaceted approach encompassing rigorous experimentation & analysis, we aim to elucidate the intricate interplay between these anticoagulant agents & diabetes pathology. By shedding light on their therapeutic potential in the context of diabetes management, our findings hold promise for advancing the understanding & treatment of this complex metabolic disorder.

OBJECTIVES OF THE STUDY

✓ Primary objective: To evaluate the effect of Dabigatran & Rivaroxaban on glycemic parameters in high fat diet & low dose Streptozotocin induced Diabetes Mellitus in Male Wistar rats

✓ Secondary objective: To evaluate the effect of Dabigatran & Rivaroxaban on lipid profile in high fat diet & low dose streptozotocin induced Diabetes Mellitus in Male Wistar rats

To evaluate the effect of Dabigatran and Rivaroxaban on inflammatory markers viz. (IL-1β, TNF-α, IL-6) in high fat diet and low dose streptozotocin induced Diabetes Mellitus in Male Wistar rats.

REVIEW OF LITERATURE

A. Diabetes Mellitus

Introduction:

The American Diabetes Association(ADA) has defined Diabetes(DM) as "a group of metabolic disorders characterizedibyihyperglycemiairesultingifromidefectsiin insulin secretion, insulin action or both". This definition has been recognized & endorsed by various international organizations, including the World Health Organization(WHO).[13] Diabetes mellitus(DM) is a chronic metabolic disorder impacting multiple organ systems, leading to pathophysiological changes due to metabolic & genetic dysregulation within the body. The condition arises from a complex interaction of genetic & environmental factors, resulting in various distinct forms of diabetes mellitus.[3] [13] In US , diabetes mellitus(DM) is the primary cause of end-stage renal disease. Additionally, it increases the risk of cardiovascular diseases. With its rising incidence globally, DM is poised to remain a major contributor to morbidity & mortality. Both Type 1,2 DM significantly elevate the risk of cardiovascular diseases. Findings from the Framingham Heart Study indicate that individuals with DM have a one to five times higher risk of developing coronary artery disease.[1] India reportedly has the 2nd highest number of people with diabetes(DM) in the world [4] The Indian Council of Medical Research - India Diabetes(ICMR-INDIAB) study found that the prevalence of diabetes in India is 7.3%, while prediabetes is at 10.3%. This translates to a nationwide projection of 69.2 million people with diabetes & 77.2 million people with prediabetes. [4]

Table 1: Classification of Diabetes Mellitus:

Diabetes is classified as follows:[13],[14]

Type 1 diabetes	• Also known as autoimmune diabetes or Insulin dependent diabetes mellitus (IDDM) • 5 % to 10% of all diabetic cases • Breakdown of beta cells that results in total insulin insufficiency • Type 1 diabetes also has been linked to distinct HLA types, with DR & DQ types being the most common
Type 2 diabetes	• 90 % to 95% of diabetic patients suffer from this condition • Pathophysiology spans from insulin resistance & relatively normal insulin secretion
Specific types of diabetes	I. Exocrine pancreas disorders (eg. Pancreatitis,cystic fibrosis) II. Drug/chemical induced diabetes(DM) (eg. Glucocorticoid use, during treatment of HIV, or after organ transplantation III. Monogenic diabetes syndromes (eg. Neonatal diabetes, MODY- maturity onset diabetes of young)
Gestational diabetes mellitus	• Diabetes diagnosed in the second or third trimester of pregnancy that was not clearly over diabetes prior to gestation

Glucose metabolism and Regulation of glucose homeostasis

Carbohydrates are the primary source of energy for our bodies, with glucose being a widely utilized carbohydrate for energy by most body tissues. The main dietary sources of carbohydrates include starch from plant-based foods, glycogen from animal-based foods, sucrose from cane or beet sugar, & fructose from fruits. Most of these foods contain complex carbohydrates that must be broken down into simpler forms for absorption. This digestion process primarily occurs in the small intestine, with some digestion also taking place in the stomach. [15]

The body maintains a balance between energy intake from food, glucose production (gluconeogenesis), glucose uptake & utilization; this process is known as glucose homeostasis. This metabolic equilibrium is regulated by various factors such as neural input, metabolic signals, & hormones like glucagon, with insulin being the most crucial regulator. The various pathways of glucose depend on the individual being in a fed or fasted state. In fasting, processes are geared towards breaking down energy stores to ensure a steady supply of glucose, primarily for the brain & certain other tissues. This is achieved by decreasing insulin levels & slightly increasing glucagon levels, which promotes hepatic gluconeogenesis. Lower insulin levels also reduce uptake of glucose. This is seen in the tissues like skeletal muscle & adipose tissue, leading to the mobilization of stored proteins & fats. In the fed state, blood glucose levels rise, leading to an increase in insulin & a decrease in glucagon, reversing the process. Insulin causes glucose uptake by skeletal muscle, which consumes a significant portion of postprandial glucose. Being anabolic, insulin facilitates the storage of carbohydrates, fats, & protein biosynthesis. It promotes either glycogen formation or glycolysis, depending on the body's needs. Additionally,

substances secreted by skeletal muscle cells & adipocytes, such as leptin, adiponectin, & resistin, also play a role in glucose homeostasis [3] [15]

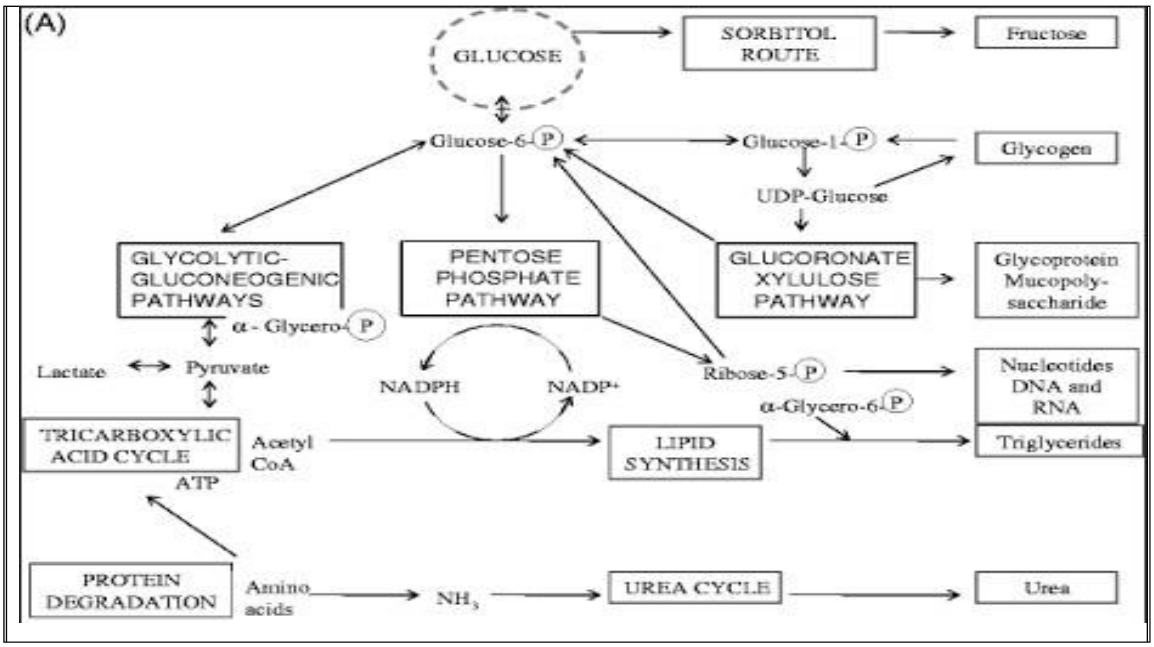

Figure 1: Central role of glucose in carbohydrate, fat and protein metabolism [15]

Insulin

The islets of the pancreas make up about 1% to 2% of the pancreas's volume. These islets have a rich blood and nerve supply and consist of five distinct types of cells: α cells, β cells, δ cells, PP (Pancreatic polypeptide) cells, and ε cells. Each cell type secretes a specific hormone: α cells produce glucagon, β cells secrete insulin, δ cells release somatostatin, PP cells produce pancreatic polypeptide, and ε cells secrete ghrelin.[16] Initial insulin production begins with the production of preproinsulin, an amino acid precursor polypeptide of 86 amino acids that are produced in a single chain. This is converted to proinsulin as a result of a proteolytic reaction. An internal 31-residue is

cleaved off proinsulin & as a result, A & B chains of insulin along with C-peptide are generated. The Golgi complex, the ER (Endoplasmic Reticulum), & β-cells of the pancreas are all involved in this complicated process. β-cell secretory granules secrete insulin & C- peptide simultaneously. C-peptide has no known physiological function or receptor. However it is useful in evaluation of β-cell function and insulin-induced hypoglycemia. Islet amyloid polypeptide (IAPP) or amylin, a peptide with 37 AA (Amino Acids), is also secreted by β-cells. Type 2 Diabetes population has amyloid fibrils in their pancreas, which are primarily composed of amylin. [3]

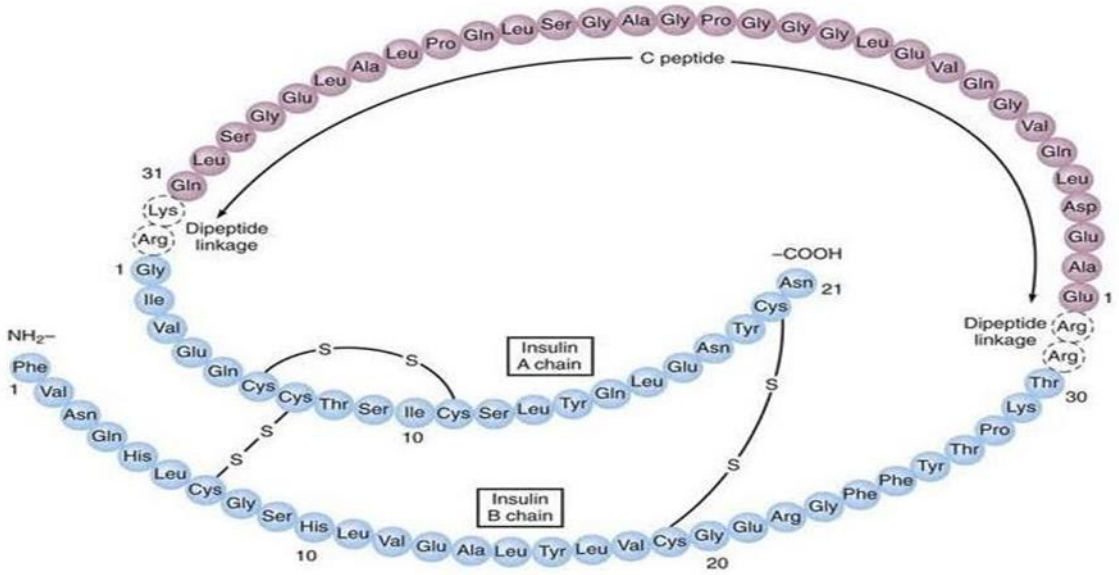

Figure 2: Structure of human proinsulin (C-peptide plus A and B chains) and insulin. Insulin is shown as the shaded (blue color) [17]

Insulin secretion is a precise process that maintains stable blood glucose levels in both fasting & fed states. This regulation involves the coordinated actions of various nutrients

& hormones. While fatty acids, amino acids, & ketone bodies aid in insulin glucose is main stimulus for its release. Its secretion is closely linked to extracellular glucose levels. Notably, orally ingested glucose triggers a higher insulin response compared to glucose administered intravenously, a phenomenon known as the incretin effect, which is due to insulinotropic gastrointestinal (GI) peptides. Pancreatic islets receive autonomic nervous system innervation. The stimulation of alpha-2 adrenergic receptors suppresses insulin secretion, whereas beta-2 adrenergic & vagal nerve stimulations increases it. Generally, conditions that activate the sympathetic nervous system like hypoglycemia and hypoxia —inhibit insulin secretion through alpha-2 adrenergic action. [16]

When blood glucose levels exceed 3.9 mmol/L (70 mg/dL), insulin synthesis is stimulated, & when glucose levels are in the range of 5–10 mM, insulin release becomes significant. The initial step in the molecular events leading to insulin secretion is the entry of glucose into pancreatic beta cells via the GLUT-1 transporter. This glucose is then phosphorylated by the enzyme glucokinase (hexokinase IV) in the beta cell, marking the rate-limiting step of this process. The resulting glucose-6-phosphate enters the glycolytic pathway, increasing ATP production. The rise in ATP levels inhibits ATP-sensitive K+ channels (KATP channels). This goes on to cause depolarization of the cell membrane. The KATP channel is heteromeric, consisting of an inward-rectifying K+ channel (Kir6.2) & a sulfonylurea receptor (SUR) protein. Mutations in KATP channel can lead to conditions such as neonatal diabetes or hyperinsulinemic hypoglycemia, depending on the type of mutation. Membrane depolarization opens voltage-dependent Ca2+ channels, allowing calcium influx. The resulting high intracellular calcium levels trigger the exocytosis of insulin from storage vesicles. These processes are modulated by transcription factors, amino acid metabolism, & changes in cAMP synthesis. Insulin secretion is pulsatile, with small bursts occurring approximately every 10 minutes,

superimposed on larger fluctuations lasting around 80-150 minutes. Besides glucose, various metabolic pathways in beta cells & external hormones enhance glucose-stimulated insulin secretion. Hormones such as glucagon-like peptide-1 (GLP-1) & glucose-dependent insulinotropic peptide (GIP), known as incretins, bind to beta-cell receptors, increase cAMP production in the fed state, & ultimately promote insulin secretion. Additionally, they suppress glucagon production & secretion. [18] [19]

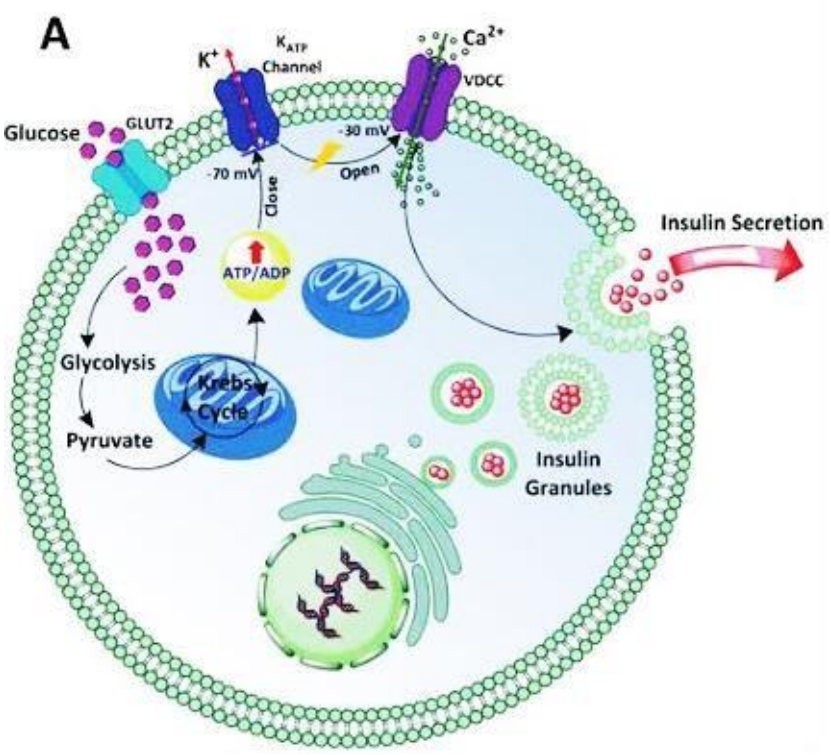

Figure 3: Mechanisms of glucose-stimulated insulin secretion[1]

Insulin acts through a tyrosine kinase receptor that is functionally similar to the insulin-like growth factor-1 (IGF-1) receptor. [20] The insulin receptor is a complex dimer made up of alpha & beta subunits, connected by disulfide bonds. This leads to the receptor being a transmembrane heterotetrameric glycoprotein with two extracellular alpha subunits & two membrane-spanning beta subunits. The beta subunits possess intrinsic tyrosine kinase activity, which is inhibited by the α subunits. When insulin binds to the α subunits, this inhibition is relieved, leading to transphosphorylation of the β subunits & subsequent autophosphorylation at specific sites. Activation of receptor initiates a signaling cascade by phosphorylating various intracellular proteins, including insulin receptor substrates (IRSs) & Src homology & collagen (Shc) proteins. These phosphorylated proteins then further go on to interact with downstream effectors, amplifying & prolonging the insulin signal. Phosphatidylinositol-3-kinase (PI3K) activation is essential for insulin's effect on glucose transport. PI3K is activated upon interaction with IRS proteins, producing phosphatidylinositol 3,4,5-trisphosphate (PIP3), which regulates the localization of several downstream kinases. These include various kinases such as protein kinase B (PKB/Akt), atypical protein kinase C isoforms (PKCς & PKCλ/τ), & the mechanistic target of rapamycin (mTOR).The isoform Akt2 plays critical roles in glucose synthesis & glucose uptake. One of the crucial actions of insulin is the production & translocation of glucose transporter type 4 (GLUT-4). This takes place to facilitate the glucose uptake in adipose tissue & skeletal muscle. [16]

Pathogenesis of Type 1 Diabetes Mellitus

Type 1 diabetes is an autoimmune disorder in which immune effector cells attack pancreatic islets in response to endogenous β-cell antigens. The development of type 1 diabetes results

from a complex interaction of genetic, environmental, & immunologic factors, ultimately leading to the destruction of pancreatic β-cells. Although this illness can be diagnosed at any age, most cases are identified before the age of 20. Since the disease is not exclusive to childhood, the term "juvenile-onset diabetes" is outdated. Similarly, the term "insulin-dependent diabetes mellitus" has been removed, as many forms of diabetes may eventually require insulin therapy. Islet-directed autoimmunity is there in the vast proportions of individuals with type 1 diabetes. However, some people without evidence of autoimmunity may develop insulin deficiency through unknown nonimmune mechanisms. [3] [21]

The destruction of the islet cells is caused by three interconnected mechanisms:

- Auto-immunity
- Environmental factors
- Genetic Susceptibility

In susceptible individuals, an infectious or environmental trigger can initiate the autoimmune response, leading to the production of autoantibodies against pancreatic beta-cell antigens. The rate at which beta-cell function declines varies among individuals, with some experiencing rapid progression while others may take several years to develop clinical diabetes. Research indicates that diabetes symptoms typically become noticeable after a significant loss of beta-cell mass, which can be as high as 70-80% in certain patients. Generally, the transition from covert glucose intolerance to overt diabetes is associated with situations that increase insulin demand, such as puberty or infections.Following the initial clinical presentation of type 1 diabetes, some patients may experience a phase known as the "honeymoon" period. During this phase, modest

insulin doses or even no insulin may be sufficient to maintain glycemic control. However, over time, the remaining beta cells cease insulin production, resulting in insulin deficiency. [1]

Genetic Susceptibility:

Several genes contribute to susceptibility to T1DM, as demonstrated by epidemiological studies. Identical twins, for instance, have a 40 to 60 percent chance of developing T1DM, suggesting the involvement of other modifying factors in the disease's development. Recently, genome-wide association studies (GWAS) have recognized numerous genetic susceptibility loci for T1DM.

Some candidate genes associated with type 1 diabetes include:

1. HLA genes
2. Superoxide dismutase G
3. Insulin gene VNTR
4. CTLA 4
5. Kidd locus

The HLA gene cluster is the most important of these, contributing as much as 50% of thegenetic vulnerability to T1D. HLA-A26 B8 DR3, HLA-A24 B8 DR3 (AH8.3), A3 B8

DR3 (AH8.4), & A31 B8 DR 3 (AH 8.5) are the common susceptibility alleles in Indian

populations. [22]

Auto-immunity:

Islet cell autoantibodies (ICAs) comprise a group of antibodies targeting various pancreatic islet components such as glutamic acid decarboxylase (GAD), insulin, ZnT-8, & IA-2/ICA-512. These antibodies are recognized as autoimmune markers of T1DM. ICAs are commonly detected in the majority of patients (>85%) with new-onset T1DM. Additionally, approximately 3–4% of first-degree relatives of these patients also exhibit these autoantibodies. The presence of ICAs often indicates an increased risk of developing T1DM, with the risk rising as the number of autoantibodies increases.[1]

Environmental factors:

Approximately half of monozygotic twins developing type 1 diabetes suggests the involvement of environmental factors. However, the specific nature of these factors is not yet fully understood. Previous viral infections have been proposed as potential triggers, but neither the type of virus nor the mechanism leading to autoimmunity has been definitively established. Evidence suggests that certain viruses may possess epitopes similar to pancreatic islet antigens, resulting in cross-reactivity that leads to the destruction of pancreatic islets—a phenomenon known as molecular mimicry. Conversely, some infections may have a protective effect against the development of type 1 diabetes. [23]

β-Cell Destruction:

The onset of the autoimmune process & the appearance of symptomatic TIDM typically have a prolonged lag period. Three distinct stages of type 1 diabetes have been identified. In the first stage, individuals develop two or more islet antibodies, but their blood glucose levels

remain normal. During the second stage, there is a progressive decline in beta-cell density, yet no overt symptoms are present. Finally, in the third stage, classic symptoms of diabetes emerge, & it is estimated that by this time, more than 90% of the beta cells have been destroyed. [23]

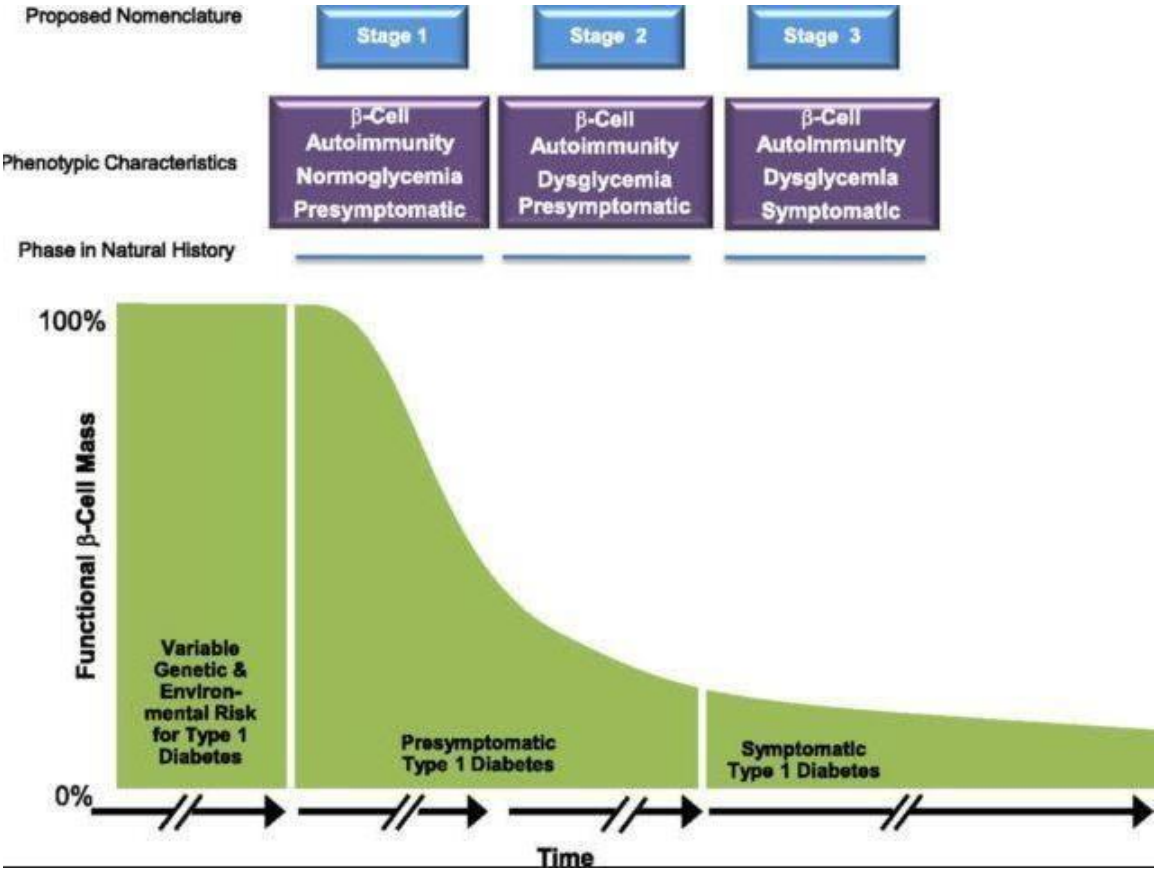

Figure 4: The three stages of type 1 Diabetes [21]

The fundamental immunological anomaly in T1DM is the inability of islet-specific T cells to achieve self-tolerance. This may result from a combination of improper clonal deletion of self-reactive T cells & abnormalities in regulatory function. Consequently, these autoreactive T cells can persist & respond to self-antigens. Initially, these cells are activated in the peripancreatic lymph nodes, likely in response to antigens released by damaged islets. Once

activated, these T lymphocytes migrate to the pancreas, where they cause beta-cell damage. Th1 cells & CD8+ cells are involved in this damage, targeting insulin, glutamic acid decarboxylase (GAD), & other antigens. It remains unclear whether autoantibodies contribute to islet damage or are a result of it.[21]

Pathogenesis of Type 2 Diabetes Mellitus

It is a multifacted disorder resulting from the interplay of genetic & environmental factors, as well as a pro-inflammatory state. The primary pathophysiological changes disrupting blood glucose control include β-cell dysfunction, insulin resistance, & chronic inflammation. While the exact defect in the pathophysiology remains debated, most research suggests that insulin resistance precedes insulin secretory deficiency, with clinical diabetes manifesting when insulin secretion becomes inadequate. T2DM encompasses a wide range of conditions that all share hyperglycemia as a defining feature. Our knowledge of the pathogenesis & genetics of T2DM is largely based on data from European populations. However, it is increasingly evident that diabetes exhibits distinct pathophysiology in different ethnic groups, which is not yet fully understood. For example, patients from East & South Asia predominantly experience beta-cell dysfunction, while Latinos tend to have more pronounced insulin resistance. Additionally, East & South Asians often develop DM at an earlier age & with a lower body mass index.[3] [21]

Genetic considerations

Type 2 diabetes (T2DM) tends to run in families & has a hereditary component. Research indicates that the relative risk of T2DM in a sibling of someone with the condition is 2-3 if

neither parent has T2DM. However, if both siblings have T2DM, the risk increases to 30%. Additionally, the risk of developing T2DM is higher when the disease is present in the mother compared to the father. Individuals with a body mass index over 30 or a fasting glucose level above 5.5 mmol/L have a significantly increased risk of developing T2DM. Identifying the specific genes responsible for the inheritance of T2DM has been challenging. However, genome-wide association studies (GWAS) have identified over 70 genes associated with a relatively small increased risk of T2DM. Among these, a variant of the transcription factor 7–like 2 (TCF7L2) gene is particularly significant. Variants in genes coding for the peroxisome proliferator-activated receptor(PPAR) and inward rectifying potassium channel, have also been linked to T2DM. [1 24]

Environmental Factors

Obesity in the central or visceral region is a significant environmental risk factor for T2DM. Over 80% of diabetic patients are affected by obesity, & the prevalence of T2DM has increased alongside the rise in obesity. Obesity contributes to the majority of metabolic abnormalities in diabetes, & even modest weight loss can improve insulin resistance & glucose tolerance. Another key risk factor is a sedentary lifestyle; regular exercise combined with weight loss can enhance insulin sensitivity.The combination of obesity, hyperglycemia, elevated serum cholesterol & triglycerides, & hypertension is referred to as metabolic syndrome. In several populations around the world, including East Asian, South Asian, & Middle Eastern populations, the incidence of T2DM is rising rapidly without a corresponding increase in obesity, indicating that the anatomical distribution of fat also plays a significant role in diabetes risk. Additional environmental risk factors for Diabetes-T2DM are sleep disorders, such as obstructive sleep apnea, & circadian disruption. Circadian disruption

occurs when there is a misalignment between the body's endogenous circadian rhythm & the rhythm created by individual behaviors. Shift workers & individuals with sleep disorders or other conditions that limit nighttime sleep & daytime wakefulness are at risk for circadian disruption. Research suggests that circadian disruption affects both insulin secretion & insulin action. Genome-wide association studies (GWAS) have found associations between circadian-controlled genes & T2DM, indicating that disruptions in these genes can influence activity levels & feeding behaviors, thereby increasing the risk for diabetes.[21] [25]

Pathophysiology

In the early stages T2DM, pancreatic beta cells secrete more insulin to counteract insulin resistance & maintain stable blood sugar levels. However, as insulin resistance & reactive hyperinsulinemia progress, beta cells in some patients are unable to sustain the increased insulin output. This leads to impaired glucose tolerance , seen by elevated postprandial glucose levels. As insulin secretion further declines & hepatic glucose production increases, overt diabetes develops, culminating in β-cell failure. Due to poor insulin regulation, glucagon secretion is upregulated, further increasing hepatic glucose production. Both resistance to insulin its diminished production cause the progression of T2DM, though the relative importance of each factor varies from patient to patient. [1] [26]

Metabolic Abnormalities

Insulin resistance is defined as the inability of target tissues—particularly muscle, liver, & fat—to respond appropriately to insulin. It is a hallmark of T2DM & results from combination of genetic susceptibility & obesity. [26]

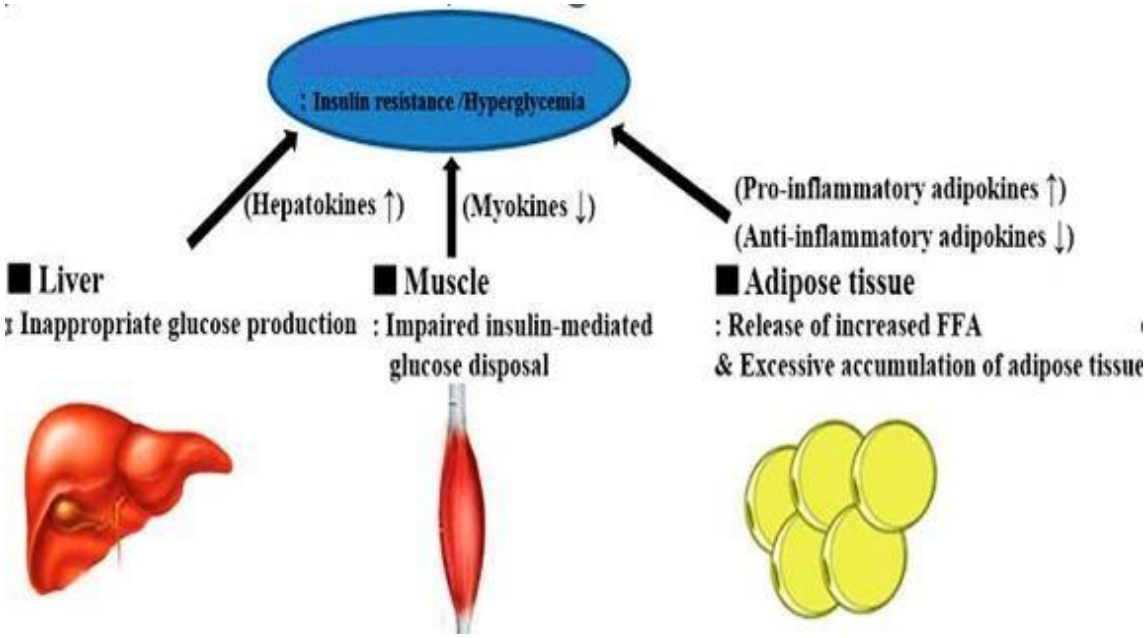

Figure 5 : Consequences of insulin resistance [27] [28]

Molecular mechanisms of insulin resistance

The insulin tyrosine kinase receptor is activated by insulin binding. This leads to the phosphorylation of multiple insulin receptor substrates , notably IRS1 & IRS2. These phosphorylated IRS then cause the activation of intracellular signaling molecules such as phosphatidylinositol 3-kinase.[29] Phosphatidylinositol 3-kinase (PI3K) promotes glucose uptake by translocating GLUT4 to the skeletal muscle plasma membrane. Additionally PI3K deactivates the transcription factor forkhead box protein O1 (FOXO1) by modifying downstream gene transcription. Additionally, insulin activates the RAS–mitogen-activated protein kinase (MAPK) pathway. Insulin resistance in obesity and T2DM is primarily attributed to disruptions in the PI3K pathway. Increased phosphorylation of IRS proteins at serine residues contributes significantly to this resistance.[30] Serine phosphorylation can also

elevate IRS degradation, leading to heightened insulin resistance, as documented in a recent journal article.[31] This form of phosphorylation can be induced by various factors, such as malfunctioning mitochondria, inflammation, abnormal lipid buildup, & stress in the endoplasmic reticulum. [32]

Mitochondrial dysfunction

Mitochondrial dysfunction has been identified in adipose tissue, liver, muscle, & even the hypothalamus in both rats & humans suffering from obesity, T2DM, & metabolic syndrome. **[33]** This is attributed to both a decrease in mitochondrial density & impaired mitochondrial function, stemming from the abnormal expression of several components of the oxidative phosphorylation system.**[34]** Mitochondrial dysfunction has been associated with a reduction in adiponectin release in adipose tissue, a potent insulin-sensitizing adipokine. In other tissues, it is suggested that mitochondrial failure results in an increase in reactive oxygen species (ROS), which subsequently activate redox-sensitive serine kinases. These kinases then trigger a phosphorylation reaction with insulin resistance-associated proteins (IRPs). Additionally, it is well-established that ectopic lipid accumulation has an important role in the causation of insulin resistance. Therefore, mitochondrial failure & diminished mitochondrial fatty acid oxidation are likely significant exacerbating factors in this process.**[35]**

Obesity

Obesity is arguably the most significant factor contributing to insulin resistance. As BMI increases, so does the risk of developing diabetes. Insulin sensitivity is influenced not only by the total amount of fat but also by its distribution: insulin resistance is more strongly associated with central obesity (abdominal fat) than with peripheral obesity (gluteal/subcutaneous fat). Observations indicate that individuals from Asia & the Middle East who develop diabetes without overt obesity tend to have more visceral adiposity.

Conversely, those with subcutaneous adiposity may have a relative protection against Type 2 diabetes. These individuals are referred to as “metabolically healthy obese,” & research on this population is an emerging field. [1]

Insulin Resistance Syndromes:

Hyperglycemia is the most easily identifiable symptom of insulin resistance, which encompasses a range of conditions. Several terms describe the cluster of metabolic abnormalities associated with insulin resistance. These terms include central/visceral obesity, T2DM, hypertension, dyslipidemia, central/visceral obesity, impaired glucose tolerance (IGT), impaired fasting glucose(IFG), & accelerated cardiovascular disease. These terms include metabolic syndrome, insulin resistance syndrome, & syndrome X. [1]

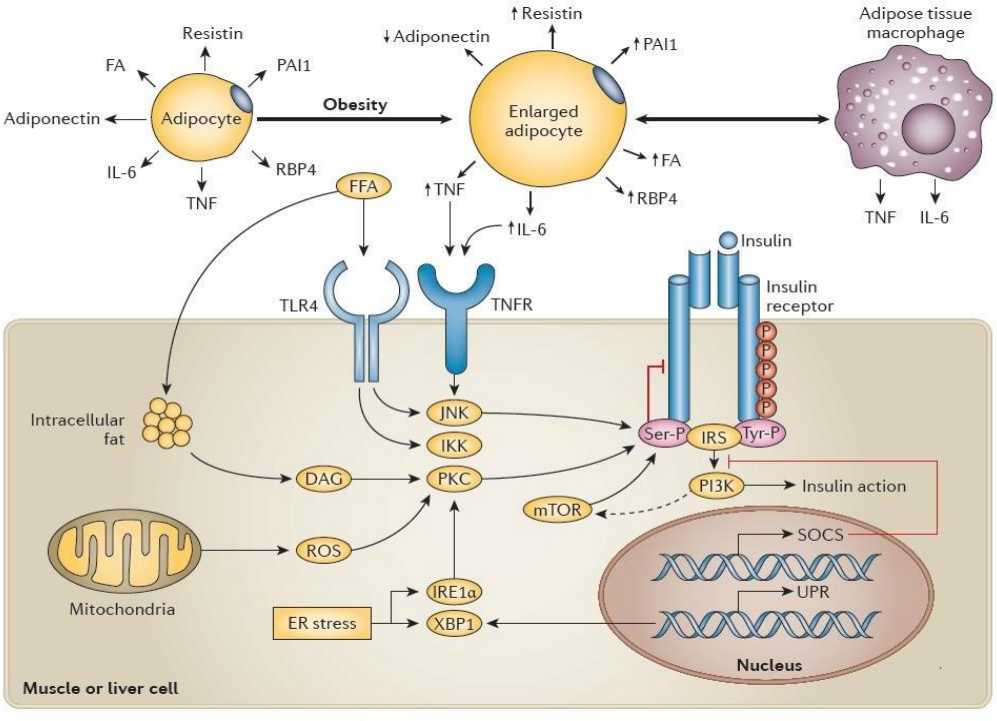

Figure 6: Mechanisms of insulin resistance[32]

Beta-Cell Dysfunction

Insulin resistance alone can lead to reduced glucose tolerance, but the onset of overt diabetes necessitates β-cell dysfunction. In patients with "sporadic" T2DM, it is not uncommon to see a significant initial improvement in β-cell activity. This is a compensatory mechanism to counteract insulin resistance & have normal blood glucose levels early in the disease. However, unlike monogenic forms of diabetes, β-cells in these patients eventually become unable to keep up with the prolonged demands of insulin resistance. This leads to a transition from a hyperinsulinemic state to one of relative insulin deficiency.

Several mechanisms contribute to β-cell dysfunction in T2D:[32 36]

i	Excess FFA (Free fatty acids) that impair β cell function and diminish insulin release (lipotoxicity)
ii	The impact of chronic hyperglycemia (glucotoxicity)
iii	An abnormal incretin effect & reduced secretions of GIP and GLP-1, hormones that enhances insulin secretions
iv	Amyloid deposition within islets: a characteristic finding in patients with long standing history of DM
v	Genetic polymorphisms

In summary, the comprehensive model that integrates the various aspects of pathophysiology is referred to as the 'ominous octet' of hyperglycemia. Recent evidence suggests the inclusion of two additional pathophysiological anomalies: the activation of inflammatory pathways & impaired insulin-mediated vasodilation, expanding the 'ominous octet'. [32]

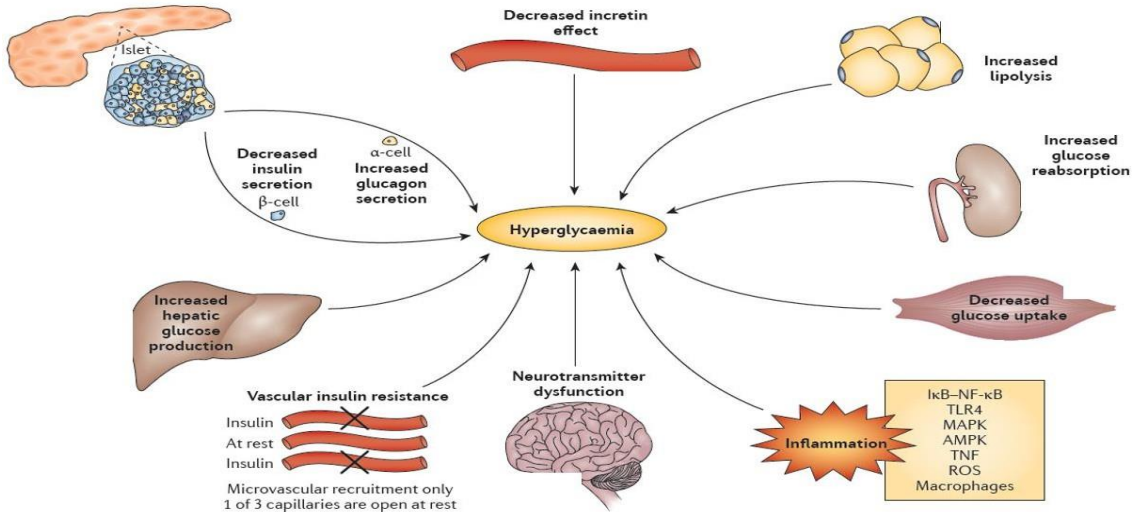

Figure 7: The 'ominous octet' of hyperglycemia in Type 2 Diabetes Mellitus[32]

Inflammation and Diabetes

Traditionally, metabolism & immunity were viewed as distinct systems with separate functions. The primary role of the immune system is to aid the body in recovering from stress. However, recent research indicates that this role extends beyond just responding to infections & injuries to also include managing the effects of overnutrition [37] Obesity & T2DM are associated with inflammation, which is a response to illness rather than its cause.

Initially, inflammation helps to contain infections & promote tissue repair. However, chronic or excessive inflammation can worsen disease by damaging tissue. This is likely true in the pathophysiology of T2DM. Inflammation exacerbates insulin resistance & pancreatic islet inflammation, leading to abnormal beta-cell secretion & diabetes. Additionally, inflammation cause high chance of cardiovascular disease observed in diabetic & obese individuals.[38] Numerous studies have demonstrated that inflammation occurs in the pancreas, muscle, liver, & adipose tissues in individuals with obesity & Type 2 diabetes mellitus(T2DM). In people with metabolic syndrome or diabetes, macrophages infiltrate these tissues. These macrophages contribute to the production of cytokines such as TNF-α, IL-6, & IL-1. [39] These pro-inflammatory cytokines promote resistance of insulin by disrupting signaling of insulin in organs by the activation of the JNK(c-JUN N-terminal kinase) & NF-kB(nuclear factor-kappa B) pathways.[40] In obesity & T2DM, the activation of these pathways leads to tissue inflammation.

Triggers of innate immunity in obesity and Type 2 DM patients

Inflammation is present in all energy-regulating tissues as well as in the arteries that transport nutrients.[41] Experimental evidence demonstrates a link between metabolic stress & inflammation. Saturated free fatty acids (FFAs) such as palmitate & stearate, along with the monounsaturated fatty acid oleate, which together make up nearly 80 percent of FFAs in human circulation, have been shown to be proinflammatory in vitro.[42] Some researchers have suggested the existence of endogenous ligands linking FFAs & Toll-like receptors (TLRs). For instance, TLR2 has been found to interact with the fatty acid transporter CD36. More recently, it has been demonstrated that the liver-derived glycoprotein fetuin-A acts as an endogenous ligand, connecting FFAs to TLR4, thereby triggering inflammation & insulin resistance in the body. [43] However, recent research has ruled out direct interaction between

FFAs & TLRs, revealing several indirect mechanisms instead. FFAs have been shown to promote the formation of lipid rafts in cell membranes, which facilitate TLR dimerization.[44] Some researchers have proposed the existence of endogenous ligands that link FFAs to Toll-like receptors (TLRs). For example, TLR2 has been found to interact with the fatty acid transporter CD36. Furthermore, lipid-induced toxicity can enhance damage signals such as HMG1. Toll-like receptors (TLRs) detect these molecules & initiate pro-inflammatory pathways. FFAs can activate inflammatory pathways independently of TLRs by releasing reactive oxygen species, which in turn activate stress kinases. ROS can also stimulate the IL-1 system by generatingNLRP3inflammasomes.[45] In human & mouse islets, FFAs induce a broad proinflammatory response primarily through IL-1 receptor activation. The production of cytokines & chemokines by FFAs was entirely prevented in human islets by inhibiting IL-1 receptor activation using either the natural antagonist IL-1Ra or a neutralizing anti-IL-1 antibody. In mouse islets deficient in TLR2 or TLR4, the absence of Myd88, a universal intracellular docking protein required for both TLR & IL-1 receptor activation, completely protected them from FFA-induced proinflammatory cytokine production. This indicates that TLR-dependent & TLR-independent pathways may coexist. [46] The systemic inflammation triggered by glucose, similar to FFAs, can be attributed to two mechanisms. Firstly, prolonged hyperglycemia leads to the nonenzymatic glycation of proteins & lipids, resulting in the formation of AGEs (Advanced Glycation Endproducts). These AGEs activate the pattern recognition receptor RAGE(Receptor for Advanced Glycation Endproducts), which subsequently activates NF-kB & stress kinases ERK1 & ERK2.[47] RAGE is present on various cell types, including smooth muscle cells, macrophages, podocytes, T cells, cardiomyocytes, & neuronal cells. The second process involves the generation of reactive oxygen species (ROS) through excessive glucose oxidative phosphorylation. ROS-induced inflammasome activation, triggered by both ROS & FFAs,

leads to the release of active IL-1 & the production of cytokines & chemokines dependent on IL-1.[46] [47]

Inflammation in adipose tissue

Hotamisligil et al. were the first to demonstrate that adipose tissue from obese individuals exhibits elevated levels of TNF-α compared to normal adipose tissue. Their findings suggested a role for TNF-α in obesity-induced insulin resistance. [48] Increasing evidence reveals an abundance of pro-inflammatory genes, cytokines, & chemokines in enlarged adipose tissue. Studies show that improvements in sensitivity of insulin is associated with loss of weight and also reductions in the expression of pro-inflammatory genes. Consequently, adipose tissue inflammation has been recognized as a precursor to metabolic syndrome, T2DM & atherosclerotic diseases. Moreover, obesity has recently been associated with an increase in immune cells within the stromovascular component of adipose tissue, which is infiltrated by macrophages.[39] While larger adipocytes indeed produce pro-inflammatory cytokines & chemokines, macrophages play a significant role in generating these inflammatory mediators as well. Increased macrophage recruitment is associated with systemic inflammation, metabolic syndrome, & insulin resistance, all of which are linked to obesity. Both surgical weight loss interventions & lifestyle modifications have been shown to reduce the number of macrophages.[49]

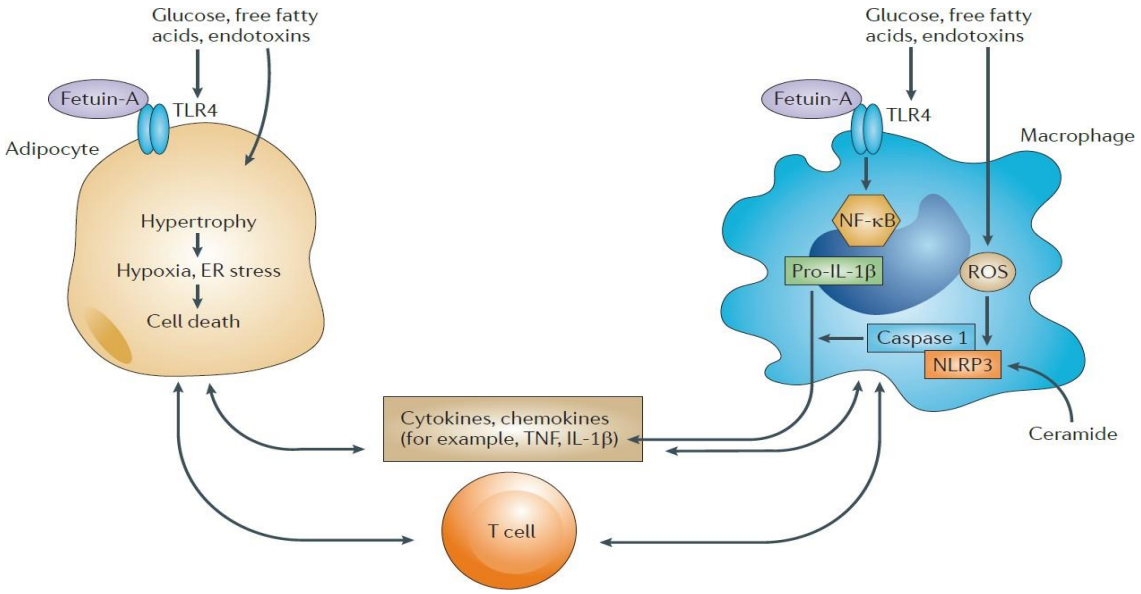

Figure 8: Storage of excessive nutrients in adipose tissues leads to an inflammatory response & insulin resistance.[50]

Macrophages can adopt two primary phenotypes: "Classically Activated" (M1) macrophages, known for releasing cytokines such as IL-1, IL-6, and TNF-α, and "Alternatively Activated" (M2) macrophages, which generate the cytokine IL-10. In the context of obesity, there is a shift from M2 to M1 macrophages, which is associated with the development of resistance of insulin. M1 macrophages can disrupt insulin signaling and inhibit adipose tissue formation in adipocytes through both direct interactions and paracrine mechanisms. Conversely, M2 macrophages are generally protective against insulin resistance associated with obesity.[39]

Obesity leads to alterations in various immune cells within adipose tissue, potentially influencing inflammation & insulin resistance. The balance between innate & adaptive immune cells appears to be critical for maintaining adipose tissue homeostasis in obesity & Type 2 diabetes mellitus. [49]

Inflammation in Islets cells

Following the discovery that hyperglycemia triggers β cell necrosis within the pancreatic islets, researchers began to suspect the involvement of an inflammatory process.[60] Through further experimentation, researchers discovered that elevated glucose levels activate the Fas receptor, leading to the production of IL-1. Recent studies have also revealed that fatty acids contribute to increased inflammation. Immune cells, cytokines, & chemokines identified in the islets of individuals with diabetes(DM) & animal models. The presence of acute inflammation in the islets is supported by reported fibrosis seen in sections of tissues from individuals suffering from T2DM, identified by deposition of amyloid, which are indicative of persistent inflammation. Notably, IL-1 is elevated and plays a significant role. This main cytokine regulates many other cytokines & chemokines, promoting the migration of immune cells that trigger widespread inflammation. Additionally, IL-1 induces its own production, creating a loop of events. Thus, insulitis, or inflammation of the islets, is factor in the causation of T2DM. β-cells exhibit up to three times the mitochondrial activity of other cells due to glucose oxidation in the mitochondria, rendering them more susceptible to reactive oxygen species (ROS) generation. This may explain why glucose-induced IL-1β affects β-cells. ROS triggers the development of inflammasome & subsequent release of IL-1 by dissociating thioredoxin-interacting protein(TXNIP) from thioredoxin. Hyperglycemia, combined with FFA, induces a proinflammatory phenotype in pancreatic islets & monocytes..

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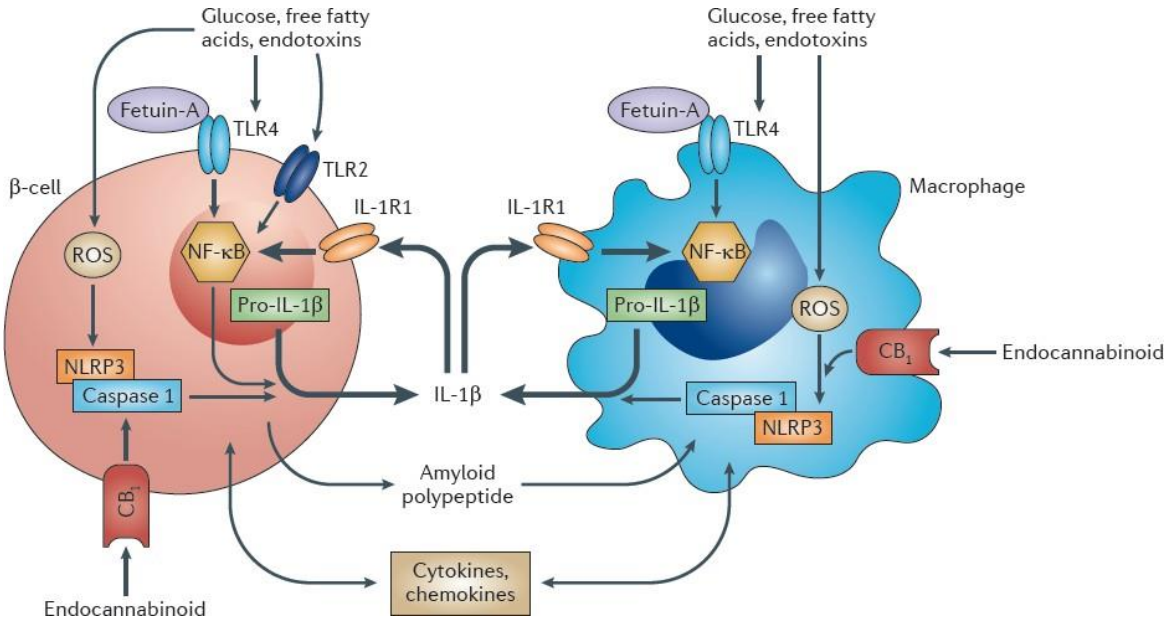


Figure 9: Islet inflammation in Type 2 Diabetes[50]

Under normal conditions, beta cells experience a high rate of protein flux through the endoplasmic reticulum (ER), which increases further with insulin resistance. This ER stress may contribute to islet cell dysfunction T2DM. It is suggested that ER stress induces thioredoxin-interacting protein(TXNIP), which leads to production of interleukin-1 (IL-1) in beta cells when NLRP3 is there.[55] In over Ninety percent of islets from individuals with T2DM IAPP (Islet amyloid polypeptide) deposition is seen. This polypeptide is converted to its mature form & secreted along with insulin. The rapid aggregation & fibril formation of human IAPP lead to amyloid deposition. An increasing array of substances, including urea crystals and asbestos, can activate theNLRP3inflammasome, with human islet amyloid polypeptide (IAPP) recently added to this list. In bone marrow-derived macrophages, oligomeric human IAPP, when combined with lipopolysaccharide (LPS) or minimally oxidized low-density lipoproteins, promotes the formation of the NLRP3inflammasome followed by release of IL-1. In these macrophages, oligomers were more effective than fibrils. Additionally, studies have shown that mice expressing human IAPP exhibit increased

IL-1 expression in their islets, indicating that IAPP can stimulate IL-1 production in vivo. [56] [57]

Sensors and mediators of inflammation in T2DM

Despite the importance of subclinical inflammation in the pathogenesis of T2DM, the processes that cause it are yet unknown. The possible mechanisms include:

i. Activation of the NF-kB and JNK pathways
ii. Generation of cytokines and chemokines
iii. Recruitment of immune cells

NLRP3 inflammasome

The notion of Type 2 diabetes mellitus as an inflammatory disease has recently gained significant support, bolstered by a growing body of evidence. Numerous studies have underscored the involvement of inflammasomes in this context.[58] The nucleotide-binding oligomerization domain-like receptor (NLR) family, pro-caspase-1, and the adaptor protein apoptosis-associated speck-like protein with a caspase recruitment domain (ASC) make up the intracellular multiprotein complex known as the inflammasome.[59] [60] The NLR family pyrin domain-containing 3 (NLRP3) inflammasome is one of the several NLRs that create inflammasomes, and it is important for the development of type 2 diabetes [59] Recent evidence also implicates theNLRP3inflammasome in the pathogenesis of many inflammatory disorders such as Alzheimer's disease and gout. [58] The NLRP3 inflammasome is a multiprotein complex that forms through the interaction of NLRP3, the adaptor protein ASC, and pro-caspase 1. Caspases-1 are activated by this assembly and go on to cleave pro-IL-1β, the inactive precursor of IL-1β, into IL-1β, the physiologically active form,

which is secreted. [59] NLRP3activation involves a 2 step process. Toll-like receptors (TLRs) or cytokine receptors are involved in the first stage, referred to as "priming," whereby they activate the NF-κB pathway and support the transcriptional production of pro-IL-1β and NLRP3. When different pathogen-associated molecular patterns(PAMPs) and danger-associated molecular patterns(DAMPs) are detected by innate pattern recognition receptors (PRRs), the second stage, known as "activation," is set off. The activation of caspase-1 is the immediate outcome of this activation signal, which also causes the NLRP3inflammasome to develop. The inactive precursor pro-IL-1β is subsequently converted by caspase-1 into the secreted active form, IL-1β. One of the main pro-inflammatory cytokines that macrophages generate, IL-1β, is essential to the pathophysiology of T2DM [59]

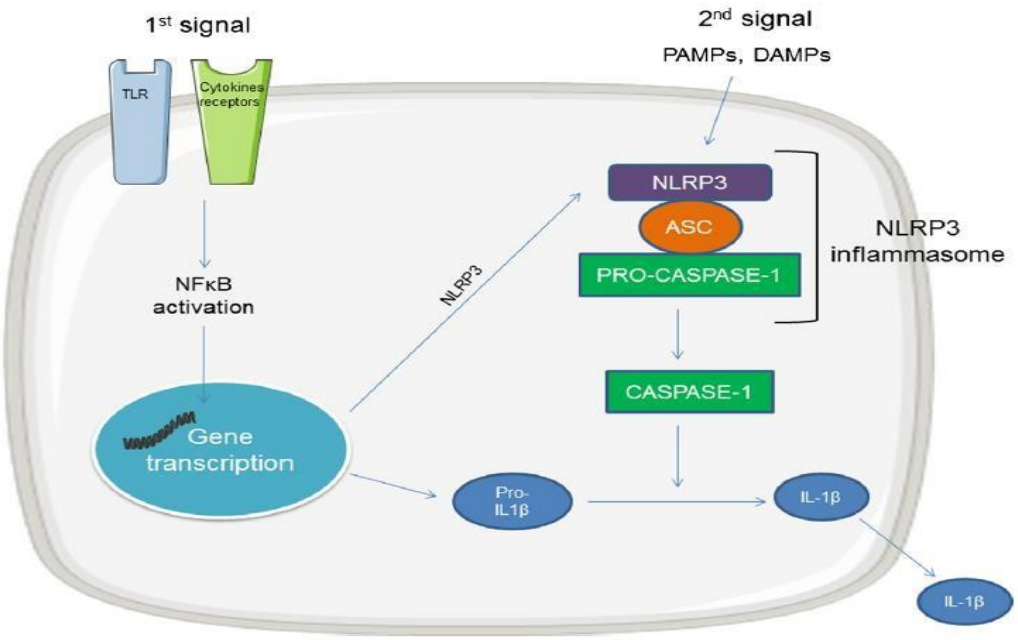


Figure 10 : TheNLRP3inflammasome [49]

The β-cells & islet-infiltrating macrophages were the first to demonstrate activation of theNLRP3inflammasome & the subsequent production of IL-1β. This process increases apoptosis & decreases insulin synthesis in β-cells. Further data indicates that islet amyloid polypeptide (IAPP) plays a role in the development of amyloid deposits in the islets, which in turn stimulates the NLRP3inflammasome to produce more IL-1β. [56] TheNLRP3inflammasome is also linked to insulin resistance induced by obesity.

Components of theNLRP3inflammasome, the action of caspase-1, & interleukin-1beta levels have all been reported to be elevated in the adipose tissue of both obese mice models & individuals with diabetes. These changes are associated with insulin resistance, metabolic syndrome, & the severity of Type 2 Diabetes(T2DM). The detrimental effects of visceral adipose tissue may stem from the higher expression & activation of theNLRP3inflammasome compared to subcutaneous adipose tissue in obese individuals. Obesity seems to trigger the activation of theNLRP3inflammasome, which serves as a sensor for metabolic danger signals such as high glucose levels, saturated free fatty acids (FFAs), & the presence of lipid intermediates like ceramides. This activation leads to the production of IL-1β & the induction of various pro-inflammatory mediators. Inhibiting theNLRP3inflammasome has been shown to improve insulin signaling in insulin-sensitive tissues & enhance pancreatic insulin production. [60]

A new study examined various aspects ofNLRP3inflammasome activation in a different subpopulation of obese individuals who do not exhibit the classical metabolic problems associated with obesity & have a reduced risk of Type 2 Diabetes & cardiovascular disease. This phenotype, referred to as "metabolically healthy obesity" (MHO), may account for up to 30 percent of the entire obese population. The research indicates that MHO is associated with less inflammation & lower levels of systemic inflammatory

markers compared to unhealthy obesity phenotypes. In MHO individuals, visceral adipose tissue shows decreasedNLRP3inflammasome activation & a more favorable inflammatory & immunological profile compared to the visceral adipose tissue of unhealthy obese individuals.[49]

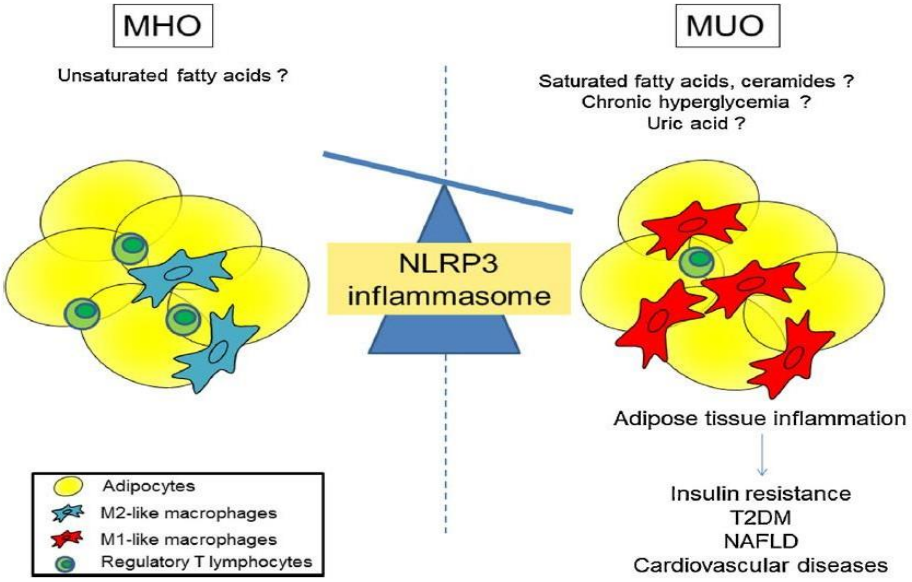


Figure 11: Adipose tissue inflammatory profile imbalance between metabolically healthy & unhealthy obese [49]

Identifying the factors that contribute to differential inflammasome activation can enhance our understanding of obesity-related metabolic diseases. Unsaturated fatty acids, which have strong anti-inflammatory properties & improve insulin sensitivity in obese & Type 2 diabetic patients, are promising candidates for studying the pathological basis of T2DM. Notably, unsaturated & omega-3 fats do not activate theNLRP3inflammasome & can inhibit its activation by other stimuli.[61] The findings of yet another recent study, which demonstrated a different fatty acid profile across MHO & unhealthy obese persons, notably for saturated

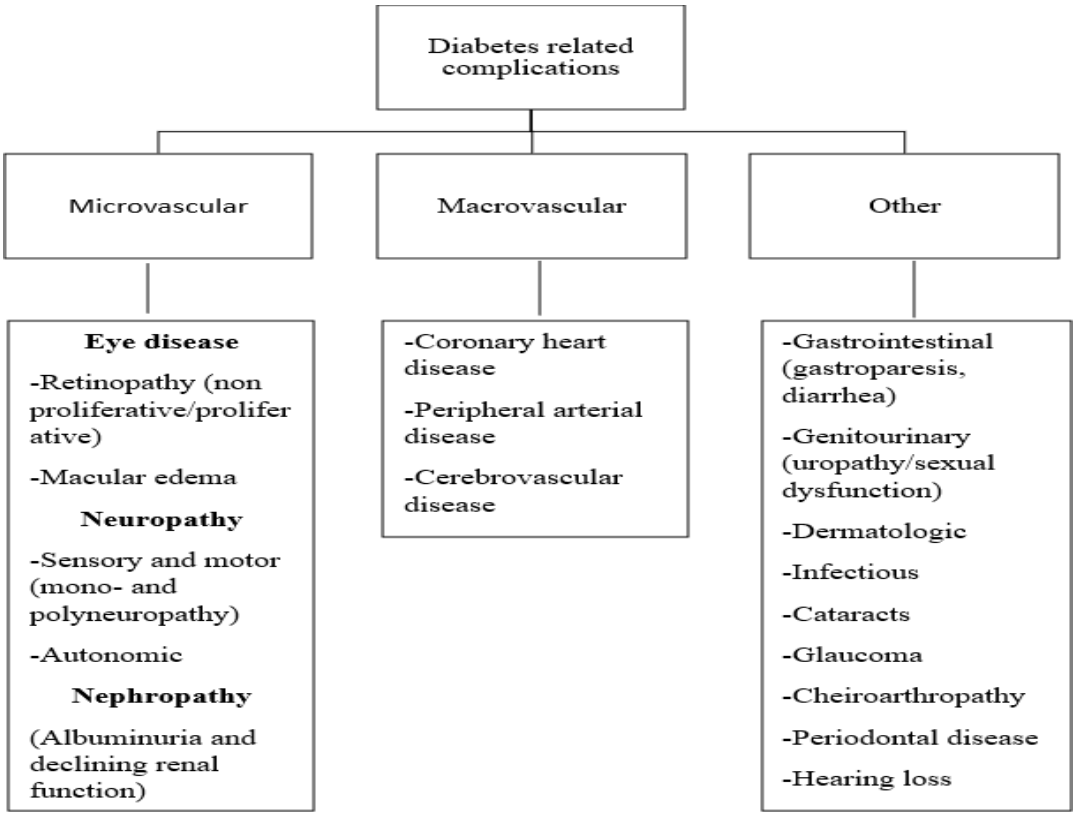
fatty acids, emphasized the importance of unsaturated fatty acids in the mediation of inflammation. [62] Therefore, more research comparing these two groups is required to develop preventive & therapeutic solutions for obesity-related insulin resistance & inflammation.

Endoplasmic reticulum stress

The endoplasmic reticulum (ER), a critical site for protein folding, maturation, & trafficking, may also be involved in macrophageNLRP3activation. Chronic nutritional overload induces ER stress, which triggers pro-inflammatory signal transduction pathways. ER stress has been observed in the adipose tissue of obese, insulin-resistant individuals, indicating that it may contribute to insulin resistance & inflammation.[63]

Diabetic Complications:

Complications of diabetes affect various organ systems & are significant contributors to morbidity & mortality. T2DM is a major cause of renal failure, adult-onset blindness, & nontraumatic lower-extremity amputation. It has also become a major risk factor for coronary heart disease (CHD). Insulin resistance & hyperglycemia are the primary drivers of diabetic complications. Typically, hyperglycemia-related complications do not appear until about a decade after the onset of diabetes. However, diabetes-associated cardiovascular disease (CVD) risk can develop even before hyperglycemia is clinically established. Due to the prolonged asymptomatic period before diagnosis, many individuals with T2DM exhibit both hyperglycemia & insulin resistance-related issues at the time of diagnosis. These complications can be categorized as vascular or nonvascular & affect individuals with both major types of diabetes. Vascular complications are further divided into microvascular & macrovascular problems. Unlike microvascular complications, which are specific to diabetes, some pathophysiological features of macrovascular problems are also found in the general population.[1]



Diagnosis of T2DM

In recent years, the diagnosticicriteriaiforidiabetesihave undergone significant changes. The American Diabetes Association (ADA) & World Health Organization (WHO) criteria for diagnosing diabetes have been widely accepted by the medical community

Table 2: Diagnostic criteria for Type 2 diabetes mellitus [64]

Diagnostic criteria for Diabetes Mellitus		
Parameter	**Values**	**Comments**
Fasting Plasma Glucose (FPG)*	≥ 126 mg/dl (7.0 mmol/l)	Fasting → no caloric intake for at least 8 h
or		
2- hour plasma glucose during OGTT*	≥ 200 mg/dL (11.1 mmol/L)	OGTT is done as per WHO description → by giving a glucose load equivalent of 75-g anhydrous glucose in water.
or		
Glycosylated Hb (HbA1C)*	≥6.5% (48 mmol/mol)	Performed in a laboratory using a that is NGSP certified method and standardized to the DCCT assay
or		
Random Plasma Glucose	≥200 mg/dL (11.1 mmol/L).	In patients with classic symptoms of hyperglycemia or hyperglycemic crisis

**In the absence of unequivocal hyperglycemia, repeat testing should be done on a different day to confirm the values*

Pharmacological therapy for T2DM

Table 3: Summary of drugs available for Type 2 diabetes mellitus(T2DM)[1 65]

Drugs	Mechanism of action & effects	Efficacy	Advantages	Disadvantages
Sulfonylureas	Bind to SURI on β cell	High	Long term	Weight gain Hypoglycemia

Glibenclamide Glimepiride Glipizide	resulting in ↑ Insulin secretion		safety data	
Biguanides Metformin	↑ Hepatic glucose production AMPK activation	High	Long term safety data Weight neutral	GI adverse events Contraindicated inrenal disease
Thiazolidinediones **Pioglitazone** **Rosiglitazone**	PPAR-γ agonists ↑ Insulin sensitivity	High	Low risk of hypoglycem ia	Edema High risk of heartfailure
GLP-1 receptor **agonists** **Exenatide** **Liraglutide** **Dulaglutide**	↑ Insulin ↓ Glucagon	High	Weight loss Minimal hypoglycem ia	Injectable GI adverse events

Albiglutide				
Insulin 1.Rapid acting(Lispro, Aspart) **2. Short acting** (Actrapid) **3. Intermediate acting** (Humulin-I, Insulatard)	↑ Glucose utilization ↓ Hepatic glucose production	High	Injectable sustained glycemic improvement	Weight gain hypoglycemia
4. Long acting (Detemir, Glargine,Degludec) **Biphasic premixed**				
Meglitinides Nateglinide Repaglinide	Inhibit SGLT-2 transportes leading to increased renal glucose excretion	Intermedi-ate to high	Weight loss Low risk of hypoglycemia	Genital infection &UTI Possible increased risk of fractures

DPP-4 inhibitors **Sitagliptin** **Vildagliptin** **Saxagliptin** **Linagliptin**	Prolong endogenous GLP-1 action ↑ Insulin ↓ Glucagon	Intermedi-ate	Weight neutral hypoglycemia	Increased risk of pancreatitis
α- glucosidase inhibitors	↓ Glucose absorpion in GI tract	Modest	Weight neutral	GI adverse effects
Dopamine-2 agonist **Bromocriptine**	Activates hypothalamic dopamine receptors leads to suprression of hepatic glucose output	Modest	Weight neutral	Dizziness Nausea Fatigue
Bile acid sequestrant **Colesevlam**	Activates liver farnesoid receptors ↑ GLP-1 secretion	Modest	Weight neutral Decrease LDL Increase HDL	Constipation ↑ Triglyceride

AMPK, 5′ AMP-activated protein kinase; DPP-4, dipeptidyl peptidase 4; GLP-1, glucagon-like peptide 1; PPAR-γ, peroxisome proliferator-activated receptor γ; SGLT2, sodium/glucose cotransporter 2; SMBG, self-monitoring of blood glucose; SUR1, sulfonylurea receptor 1; GI, gastrointestinal.

B. TARGETTING INFLAMMATION IN DIABETES

Anti-inflammatory properties of current anti-diabetic drugs

Diabetes medications & lifestyle changes offer anti-inflammatory benefits. The Diabetes Prevention Program(DPP) demonstrated that weight loss decreased C-reactive protein(CRP) levels by 31%, while Metformin lowered C-reactive protein-CRP by 13%. [66] Surgical weight loss methods yield similar results. Lifestyle adjustments can reduce insulin resistance, slow the progression from prediabetes to T2DM, & delay the development of diabetes mellitus & its complications, even without pharmacological treatment. For the same level of glucose reduction, thiazolidinediones decreased inflammation markers more effectively than other treatments.[67] This effect may be attributed to the PPAR-mediated trans repression of inflammatory response genes. The reduction in inflammation enhances the benefits of these medications, independent of their impact on glucose levels.[4] In the pancreas, prolonged exposure of β-cells to high levels of glucose & FFA increases their metabolism, leading to more reactive oxygen species(ROS) formation. This promotes the activation of theNLRP3 inflammasome & caspase-1, resulting in the production of mature IL-1β. The auto-stimulation of IL-1β further amplifies inflammation by inducing the secretion of cytokines & chemokines such as IL-6, TNF-α, & IL-8. These cytokines attract immune cells to the islets, causing insulin resistance & contributing to the progression of T2DM. [1] Given the central

role of theNLRP3inflammasome & IL-1β in the pathogenesis of T2DM, it is not surprising that blocking IL-1β has been shown to improve glucose control in pre-diabetic & T2DM populations. [59] Insulin therapy alone has been associated with a short-term reduction in inflammation. This effect is due to a decrease in the activity of nuclear factor-kappa B(NF-κB), the primary transcriptional regulator of the inflammatory response. [68] But, its temporary & often needs higher doses of insulin-administered intravenously. One benefit of initiating insulin therapy early is the potential to delay the progression of the disease & its complications. Statins, commonly used in Type 2 Diabetes management, also possess anti-inflammatory properties. These drugs lower cholesterol by inhibiting hydroxy-methyl-glutaryl-CoA reductase(HMG-CoA reductase) & reduce C-reactive protein (CRP) levels by 25%-30%. This reduction in CRP is not dose-dependent(class effect), indicating that the anti-inflammatory effect of statins is separate from their cholesterol-lowering effect. CRP levels alone can predict cardiovascular events. An interventional trial examined Rosuvastatin's impact on primary cardiovascular events in individuals with elevated CRP but without hyperlipidemia, finding a 37% reduction in C-reactive protein(CRP) & a 50% reduction in LDL. This leaves open the question of whether the benefits of statins are due to their lipid-lowering effects or their anti-inflammatory properties.[69]

Table 4: Anti-inflammatory effects of glucose-lowering agents used in the treatmentof Type 2 Diabetes[70]

Drug	Main findings	Remarks & limitation

Biguanides	• Reduced or unchanged CRP • Reduced markers of endothelial dysfunction	Good effectson chronic inflammatory disorders & cancer
Sulfonylureas	• Reduced or unchanged CRP • Reduced markers of endothelial dysfunction	Conflicting data
Thiazolidinediones	• Substantial reduction in CRP	Consistent anti-inflammatory effect
DPP-V inhibitors	• Reduced inflammatory cytokines. • Reduced CRP	Moderate effect Requires further study
GLP-1 receptor Agonists	• Reduce markers of endothelial dysfunction • Reduced inflammatory cytokines	Moderate effect Requires further study
SGLT-2 inhibitors	• Unknown	Future studies needed
Insulins	• Reduced or unchanged inflammatory cytokines & immune mediators • Reduced or unchanged CRP	Moderate effect Although data conflicting

Unmet needs in Type 2 Diabetes therapy

Challenges in the Treatment of Type 2 Diabetes

The current pharmacological landscape for T2DM treatment reveals significant unmet needs, particularly concerning the lack of disease-modifying agents that can decelerate the progressive loss of insulin secretion. Moreover, T2DM is frequently accompanied by dyslipidemia and hypertension, which markedly elevates the risk of cardiovascular complications, nephropathy, retinopathy, and neuropathy. Consequently, most patients with T2DM are prescribed complex medication regimens to both mitigate and manage these associated conditions. However, the necessity for multi-drug therapies often results in decreased patient adherence.Additionally, many anti-diabetic medications are burdened with undesirable side effects: Metformin can cause gastrointestinal disturbances; Sulphonylureas and insulin are linked to hypoglycemia and weight gain; Thiazolidinediones may result in weight gain and increased fracture risk; and SGLT-2 inhibitors are associated with urinary tract infections. Given these challenges, the ideal therapeutic strategy for diabetes should encompass comprehensive glycemic control, retard disease progression, address coexisting conditions, and provide durable efficacy with a minimal side effect profile. [50]

Immunometabolism as a therapeutic target

Recent research has established a direct connection between immune system function & metabolic changes, giving rise to a new field termed "immunometabolism." It contrasts with the conventional understanding that metabolism governs nutrition while immunity is

responsible for defending the host. However, both functions share a common goal: restoring homeostasis under stress. While immune stresses traditionally include pathogenic, mechanical, & chemical threats, overnutrition has emerged as a modern addition due to the abundance of food. This similarity between the immune system's response to pathogens & the metabolic stress response suggests a shared mechanism. Genetic predispositions may exacerbate chronic immune hyperactivation triggered by metabolic stress, potentially leading to conditions like Type 2 Diabetes. Consequently, an immunological approach to treating Type 2 Diabetes should aim to modulate or optimize the immune system's function to better manage the condition. [50]

Metabolic Effects of Anti-inflammatory Drugs

Targeted anti-inflammatory therapy has been suggested for both prevention and treatment of Diabetes.

Anti–TNF-α

TNF-α was the initial proinflammatory cytokine identified in development of insulin resistance & T2DM. However, antagonizing TNF-α has not demonstrated clear benefits in human T2DM management. [71] A comprehensive review suggests that these clinical studies were all limited by insufficient statistical power & short duration.[53] Observational studies have not demonstrated benefits from TNF-α antagonist treatment in diabetic or inflammatory disorders like rheumatoid arthritis or Crohn's disease. Most of these trials lack prospective design, & any observed benefit may not necessarily stem from improved glucose metabolism. Further research is necessary to assess the role of such treatments. [70]

Anti–IL-1β

The effects of inhibiting IL-1β on insulin resistance and T2DM have been extensively investigated since this cytokine's role in the disease was identified. Treatments with an IL-1 receptor antagonist, such as Anakinra, or IL-1β-specific antibodies, including Gevokizumab, Canakinumab, and LY21891020, have shown significant improvements in metabolic parameters. These therapies have been associated with reductions in HbA1c levels, enhanced insulin sensitivity, and improved β-cell secretory function. Furthermore, they have demonstrated a capacity to lower inflammatory markers, indicating a promising approach for addressing both the metabolic and inflammatory aspects of T2DM. [72] [80] Data from a recent study suggest that blocking IL-1β can mitigate diabetes-associated inflammation & metabolic abnormalities. Regarding safety, the drugs were generally well-tolerated. However, Anakinra required daily injections, which led to frequent injection site reactions. In contrast, humanized anti-IL-1β antibodies, which can be administered monthly, reduce these localized responses. [70]

Salicylate & salsalate

Inhibition of NF-κB activity by sodium salicylate & aspirin improves glycemic control in T2DM. Salsalate, a prodrug of salicylate, has been shown to increase insulin sensitivity and improve glucose management in both prediabetic and Type 2 Diabetes patients, with a relatively low risk of bleeding. The TINSAL-T2D trial specifically highlighted the efficacy of salsalate in improving glycemic control among T2DM patients. This was demonstrated by reductions in fasting glucose and HbA1c levels, as well as enhancements in the lipid profile, indicating its potential as a therapeutic option for metabolic improvements in these patients [73] These findings suggest that NF-κB pathways might offer a novel therapeutic approach for

preventing & treating T2DM. To ascertain if these drugs' long-term advantages can be sustained, more study is required.

Table 5: Summary of clinical trials demonstrating metabolic effects of anti-inflammatory drugs[70 71 72 73]

Drug	Mechanism of action	Main findings	Remarks & limitation
Anti TNF-α antibody	TNF α antagonism	↑ Insulin secretions ↓ CRP	Studies underpowered &of short duration
IL-1 receptor antagonist	IL-1β antagonism	↑ Insulin secretions ↑ Insulin sensitivity ↓ CRP ↓ HbA1C	Effect persisted several weeks after treatment cessation, Long term studies ongoing

Salsalate	IKK-β, NFK- β inhibition	↑ Insulin secretions ↑ Insulin sensitivity ↓ CRP ↓ HbA1C ↓ FBG	↑ LDL cholesterol & urine albumin levels
Chloroquine/ HCQ	Unknown	↑ Insulin secretions ↓ Insulin degradation ↓ HbA1C ↓ FBG	Observational study or small scale prospective RCT

FBG, fasting blood glucose; IKK-b, inhibitory kB kinase-b; RCT, randomized controlled trials.

C.DRUGS USED IN THE PRESENT STUDY:

Rivaroxaban

Rivaroxaban is a direct, specific factor Xa inhibitor that does not require a cofactor. It was a first approved by US Food & Drug administration in 2011. [74] It was obtained by the optimization of oxazolidinone factor Xa inhibitors.[75] The chemical formula for rivaroxaban is C19H18ClN3O5S, & its molecular weight is 435.88 grams per mole (g/mol).[76] Rivaroxaban

is approved for preventing strokes in patients with atrial fibrillation & for treating acute deep vein thrombosis or pulmonary embolism.[16]

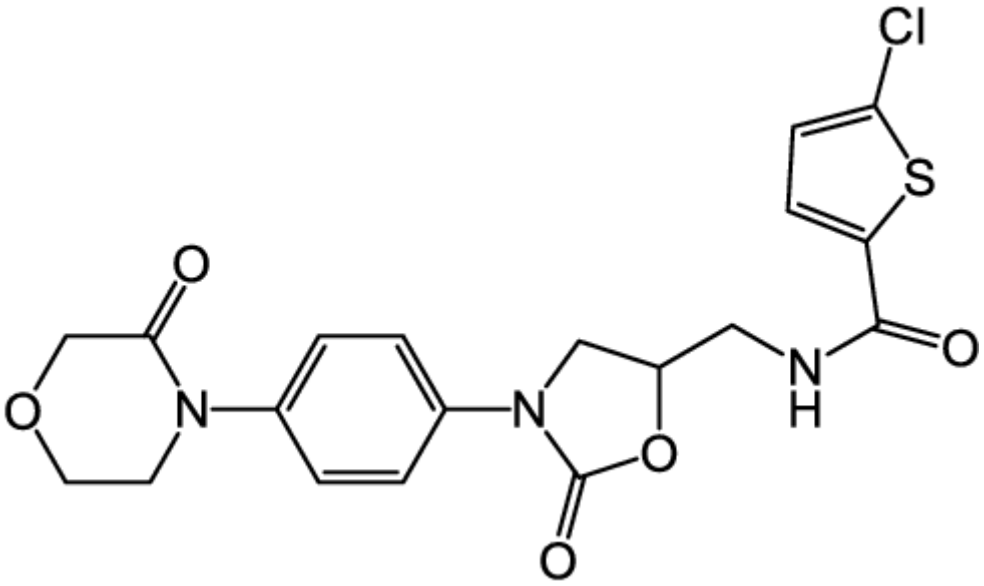


Figure 12: Structural formula of Rivaroxaban [76]

Mechanism of action:

Rivaroxaban primarily targets theNLRP3inflammasome, which is responsible for mediating the secretion of IL-1β & other inflammatory cytokines, thereby initiating & promoting diabetes mellitus. **[77]** It functions as a powerful inhibitor of theNLRP3inflammasome, which has been demonstrated to influence the activation of IL-1β.**[78]** Also, studies have demonstrated reduction in IL1b levels by rivaroxaban. [11]

Pharmacokinetics & Pharmacodynamics:

Rivaroxaban has been approved for the prevention and treatment of various thromboembolic disorders in adult patients due to its rapid onset of action, high bioavailability, & predictable

pharmacokinetics & pharmacodynamics. When administered orally, rivaroxaban attains peak plasma concentrations in about 2-4 hours, with a half-life of 5-13 hours & high bioavailability of 80-100%. The drug inhibits both free & clot-bound Factor Xa, preventing thrombus extension by blocking further thrombin generation within the clot.[79]

In terms of metabolism & elimination, rivaroxaban is metabolized via CYP3A4 & CYP2J2, with two-thirds of the drug undergoing metabolic degradation in the liver & one-third eliminated unchanged in the urine. The drug's pharmacokinetics & pharmacodynamics are predictable & dose-dependent, with inhibition of Factor Xa activity occurring approximately 3 hours after dosing. Various patient characteristics, such as age, sex, ethnicity, & body weight, do not significantly affect rivaroxaban's pharmacokinetics & pharmacodynamics, leading to fixed dosing regimens without the need for adjustment based on these factors. [79]

Safety profile of Rivaroxaban

Rivaroxaban has demonstrated a favorable safety profile in clinical trials across various indications with low rates of major bleeding across various indications. The safety outcomes were generally comparable or better than standard therapies.

In the RECORD trials for prevention of venous thromboembolism(VTE) after total hip or knee replacement surgery, the incidence of major or non-major clinically relevant bleeding, the principal safety outcome, was similar between rivaroxaban & enoxaparin groups. [80] In the EINSTEIN program for treatment of deep vein thrombosis (DVT) & pulmonary embolism (PE), the incidence of major bleeding was low in the rivaroxaban & placebo arms of the EINSTEIN Extension study. In EINSTEIN PE, there was a trend towards improved net clinical benefit with rivaroxaban compared to standard therapy. [81]

In the ROCKET AF trial for stroke prevention in patients with atrial fibrillation, the rate of major & non-major clinically relevant bleeding was similar between rivaroxaban & warfarin groups. However, fatal bleeding & intracranial bleeding occurred less frequently with rivaroxaban. [82] In the ATLAS ACS 2 TIMI 51 trial for secondary prevention in acute coronary syndrome, across both rivaroxaban doses, the rates of majoribleeding not associated with coronary artery bypass graft surgery were increased compared to placebo, as was the rate of intracranial hemorrhage. However, the rate of fatal bleeding events was significantly lower inithe rivaroxaban 2.5 mg twice-daily group compared to the 5 mg twice-daily group. [83]

Review of studies:

For the treatment of diabetes, rivaroxaban has the potential to develop into a unique therapeutic agent. Diabetes is a hypercoagulable condition. Rivaroxaban is an anticoagulant that has also been shown to reduce the inflammatory markers that are elevated in diabetics. It has been shown to inhibit caspase activity in the context of myocardial ischemia & atherosclerosis. In a rat model of myocardial ischemia, rivaroxaban markedly inhibited the pathways inducing apoptosis, including caspase 3 & caspase 9. Apoptosis is dependent on the release of cytochrome c & other pro-apoptotic factors from mitochondria in response to caspase activity.[84] Rivaroxaban reduced the expression of inflammatory molecules in aorta, including TNF-α, which is a key pro-inflammatory cytokine.[85] In vitro experiments using mouse peritoneal macrophages or murine macrophage cell line RAW264.7 demonstrated that factor Xa (FXa) promoted the pro-inflammatory activation of macrophages, increasing the production of IL-1β, which was blocked in the presence of rivaroxaban. [85] In patients with atrial fibrillation (AF) undergoing planned cardioversion, rivaroxaban

treatment resulted in a significant reduction in levels of high-sensitivity IL-6 (hsIL-6) from baseline to the end of treatment. [12]

Dabigatran

Dabigatran & its acyl glucuronides act as competitive, direct thrombin inhibitors. Thrombin, a serine protease, facilitates the conversion of fibrinogen into fibrin during the coagulation cascade. By inhibiting thrombin, dabigatran prevents thrombus formation. The active moieties inhibit both free & clot-bound thrombin, as well as thrombin-induced platelet aggregation.[86] It was a first approved by US Food & Drug administration in 2010. [86] The empirical formula is C34H41N7O5 · CH4O3S & the molecular weight is 723.86 (mesylate salt), 627.75 (free base). Dabigatran is a direct thrombin inhibitor indicated to reduce the risk of stroke & systemic embolism in patients with non-valvular atrial fibrillation. [86]

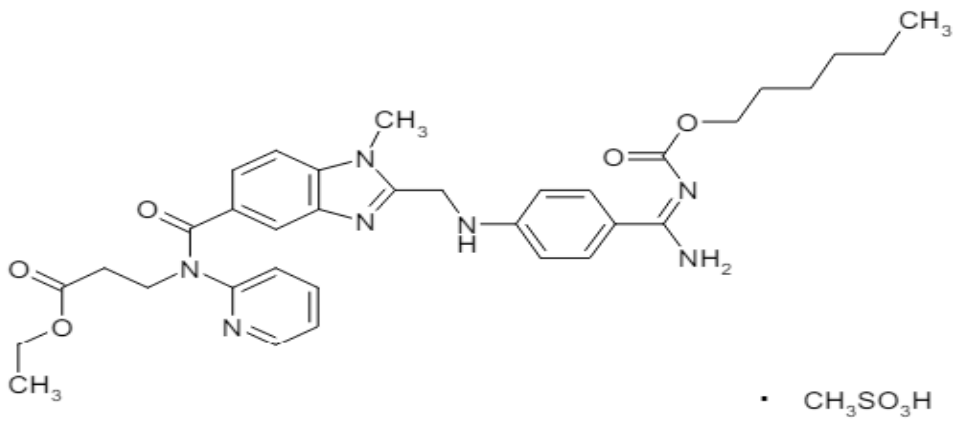


Fig 13 : Structural formula of Dabigatran [86]

Mechanism of action:

Dabigatran primarily targets theNLRP3inflammasome, which is responsible for mediating the secretion of IL-1β & other inflammatory cytokines, thereby initiating & promoting diabetes mellitus. [87] It functions as a powerful inhibitor of theNLRP3inflammasome, which has been demonstrated to influence the activation of IL-1β. [78]

Pharmacokinetics & Pharmacodynamics:

Dabigatran etexilate is absorbed as the dabigatran etexilate ester, which is then hydrolyzed to form dabigatran, the active moiety.[86] The absolute bioavailability of dabigatran following oral administration of dabigatran etexilate is approximately 3 to 7%. It is approximately 35% bound to human plasma proteins. The volume of distribution of dabigatran is 50 to 70 L[86] Dabigatran is eliminated primarily in the urine. Renal clearance of dabigatran is 80% of total clearance after intravenous administration. The half-life of dabigatran in healthy subjects is 12 to 17 hours.[86]

Dabigatran is a CYP450 enzyme substrate, inhibitor, and inducer. Dabigatran is susceptible to conjugation-forming acyl glucuronides that are pharmacologically active. There are four positional isomers of acylglucuronide: 1-O, 2-O, 3-O, and 4-O. Less than 10% of dabigatran is found in plasma overall for each of these isomers. Combining dabigatran with P-gp inducers, such as rifampin, lowers exposure to dabigatran and is typically not recommended. Increased exposure to dabigatran is primarily caused by two separate factors: P-gp inhibition and reduced renal function. The clinical significance being those pharmacokinetic interactions with P-gp inducers & inhibitors can significantly impact the efficacy & safety of dabigatran. Dose adjustments based on pharmacokinetic parameters are not necessary, but caution should be exercised in patients with severe renal impairment.[86]

Safety profile of Dabigatran

Dabigatran has a good safety profile, with low risks of major bleeding & other adverse events. Dabigatran can be identified in the bloodstream by an extended thrombin time (TT) or activated partial thromboplastin time (aPTT). However, because its anticoagulant effects are reliably predictable, regular coagulation monitoring is unnecessary. Dabigatran has a minimal likelihood of interacting with foods or other drugs, and it has a half-life of 12 to 14 hours in individuals with normal kidney function, allowing for once- or twice-daily dosing. Approximately 80% of the drug is eliminated unchanged via the kidneys, and it has a rapid offset of action[88]**.**

The RE-LY trial, which compared dabigatran (110 or 150 mg twice a day) to warfarin in 18,113 patients, showed that both doses of dabigatran were non-inferior to warfarin in preventing stroke or systemic embolism. The rates of major bleeding were comparable between the dabigatran & warfarin groups.[89] The follow-up period had a median length of two years. The annual incidence of stroke or systemic embolism was 1.53% for the dabigatran 110 mg twice day group and 1.11% for the dabigatran 150 mg twice day group. Dabigatran at any dosage did not perform worse than warfarin ($p < 0.001$). [89] The drug has a good safety profile & consistently shows a reduction in intracranial hemorrhage risk compared to warfarin. A specific study found that dabigatran in clinical practice consistently shows a reduction in intracranial hemorrhage risk compared to warfarin.[90]

REVIEW OF STUDIES

Dabigatran has the potential to become a novel therapeutic agent for the treatment of Diabetes. Diabetes is a hypercoagulable condition. Dabigatran is an anticoagulant that has also been shown to reduce the inflammatory markers that are elevated in diabetics.

Dabigatran significantly inhibits the levels of pro-inflammatory cytokines IL-1β, IL-6, & TNF-α in acute myocardial infarction (AMI) rabbits.[9] Specifically, AMI significantly increased the activities of IL-1β, IL-6, & TNF-α in rabbits compared to the normal control group. However, dabigatran treatment significantly reduced these elevated cytokine activities in AMI rabbits compared to the AMI vehicle groups.[9] These resultsisuggestithatidabigatran exerts anti-inflammatory effects in the context of AMI by reducing the levels of key pro-inflammatory mediators IL-1β, IL-6, & TNF-α. This contributes to the overall cardioprotective actions of dabigatran in regulating the no-reflow phenomenon & reducing infarct size in AMI.[9]

D. Selection of rodent model

Researchers utilize various animal models & scientific methods to induce diabetic characteristics in rodents. Non-mammalian models are favored for their short life cycles, low maintenance costs, & extensive gene-editing capabilities. However, their anatomical & physiological differences from humans limit their translational relevance. Conversely, larger animals such as dogs, pigs, & non-human primates share many physiological similarities with humans but are costly to maintain & have longer lifespans. Rats & mice offer a balance between high-throughput studies & translational relevance. Among these, laboratory mice are particularly valuable, striking an excellent balance between throughput & physiological similarity to humans. Rodents are often chosen over non-mammalian models due to their closer physiological resemblance to humans, compact size, brief life cycles, high reproductive rates, & ease of genetic modification.[91]

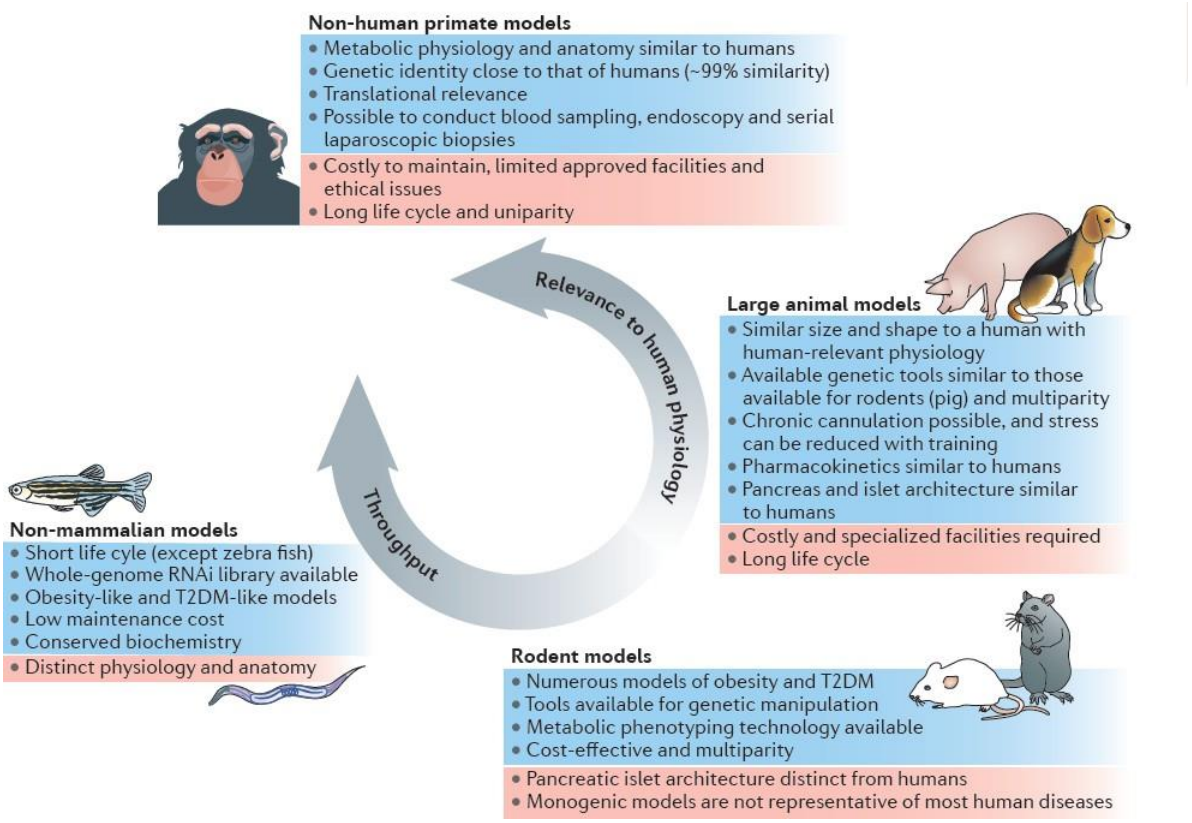


Figure 14: Key advantages & disadvantages of different classes of animal models used in Diabetes research [91]

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

Table 6: The experimental animals used to study Diabetes [92 93 94 95 96]

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

	• AKITA mice
4. Miscellaneous	• Steroid hormone induced • Drug induced

Table 7: Characteristics of T2DM animal models [92 93 94 95 96]

Model	Features
Adult alloxan model	• Alloxan kills pancreatic beta cells, it has recognized side effects such as liver & kidney damage • Alloxan given i.p at 40-200 mg/kg BW in rats

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

	• The advantage of this model is that it replicates natural pathophysiology of T2DM.
Nicotinamide STZ model	• The rationale behind this model is that STZ causes DNA damage while nicotinamide protects pancreatic beta cells from damage caused by STZ. • The initial model used 230 mg/kg BW nicotinamide (i.p) 15 minutes before administering 65 mg/kg BW STZ (i.p) in to 3 month old wistar rats
Partial pancreatectomized model	• This model inducemild to moderate hyperglycemia after 4 days of surgery that can lasts up to 6 weeks • It mimics T2DM by having decreased pancreatic beta cell mass
Intrauterine growth retardation (IUGR) model	• IUGR has been linked to onset of disease later in life, including obesity, hypertension & T2DM • In newborns, IUGR causes significant loses in pancreatic beta cell mass, which does not recover in adulthood & results in impaired glucose tolerance & development of T2DM. • This is caused by bilateral uterine artery ligation, which results in partial reduction in blood flow to the fetus

	resulting in IUGR.
Zucker fatty (ZF)/ Zucker diabetic fatty rats (ZDF)	• ZF rats were created in 1961 as a result of cross between Merck M & Sherman rats • They have faulty leptin receptor which resulted in hyperglycemia & formation of obese rats at 4 weeks of age. • The ZDF strain was discovered after mutation in ZF strain which are less obese but have increased insulin resistance
Otsuka long- Evans Tokushima Fat (OLETF) rats	• This rat was created after 18 weeks of selective breeding at Tokushima Research Institute from naturally diabetic rat discovered in an outbred colony of Long Evans rats in 1984
Nile Grass rat	• The Nile Grass rat (Arvicanthis Niloticus) has been recommended as a model for metabolic syndrome.

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

Table 8: Advantages & disadvantages of various experimental models for Diabetes [93]

Animal model	Advantages	Disadvantages
Spontaneous diabetic animals	• Involves genetic factors • Animals exhibit similar characteristics of human T2DM • Minimum variability of results and hence small sample size is enough	• Limited availability • Expensive • High mortality due to ketosis & requires insulin for survival
Diet/ Nutrition induced	• Developed diabetes with obesity which is similar to some humans • Chemical toxicity on organs can be avoided	• Long period of dietary required • Frank hyperglycemia does not develop & not suitable for screening
Chemically induced	• Selective loss of β- cells • Ketosis & mortality are low • Cheaper, easier to induce & maintain • Animals live long without exogenous insulin	• High variability of results • Hyperglycemia primarily due to insulin deficiency after than insulin resistance

Surgically induced	• Chemical toxicity on organs can be avoided • Resembles human diabetes in terms of reduced beta cell mass	• Cumbersome technical procedure & post operative care • Higher mortality • Chances of occurrence of other digestive problems
Genetically modified (transgenic/ knockout)	• Effect of single gene mutation can be studied	• Highly sophisticated & expensive

Basic properties of Streptozotocin

Streptozotocin(2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose) is a fungal antibiotic derived from Streptomycetes achromogenes. Streptozotocin represents a nitrosourea analog in which the N-methyl-N-nitrosoureamoiety is linked to the carbon-2 of a hexose. Unlike other nitrosoureas, STZ is hydrophilic due to the hexose substitution. Courtesy of glucose-like structure STZ similarly enters beta cells as glucose. [97]

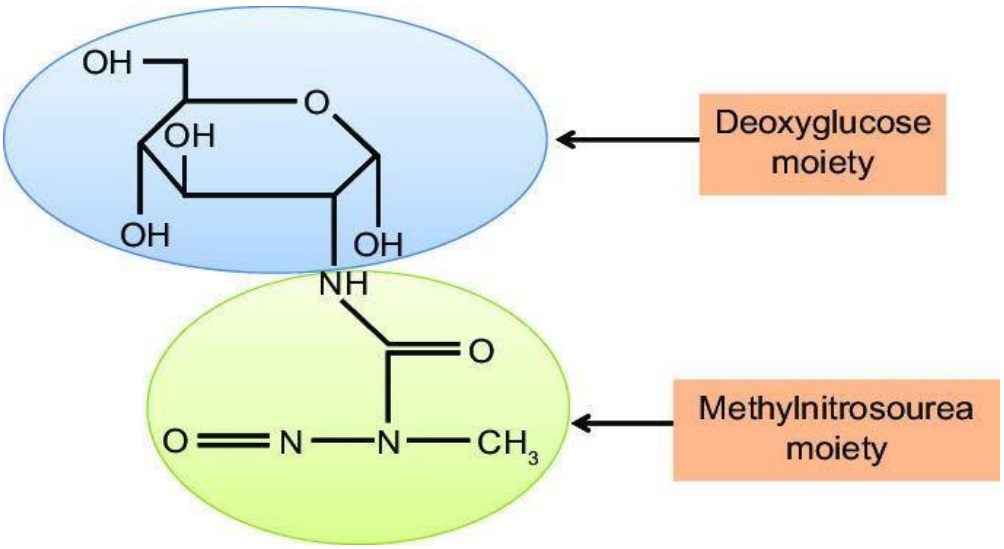


Figure 15: Chemical structure of Streptozotocin[97]

The mechanism of Streptozotocin-induced Diabetes

Streptozotocin is a hydrophilic molecule that uses GLUT2 transporters to enter the cells. It causes DNA alkylation & over activation of poly-ADPribose polymerase(PARP), resulting in NAD+ depletion, reduced cellular ATP & compromising insulin [97 98 99 100]

After the administration of STZ diabetes develops through at least three different mechanisms & the result of all these mechanisms is DNA destruction.

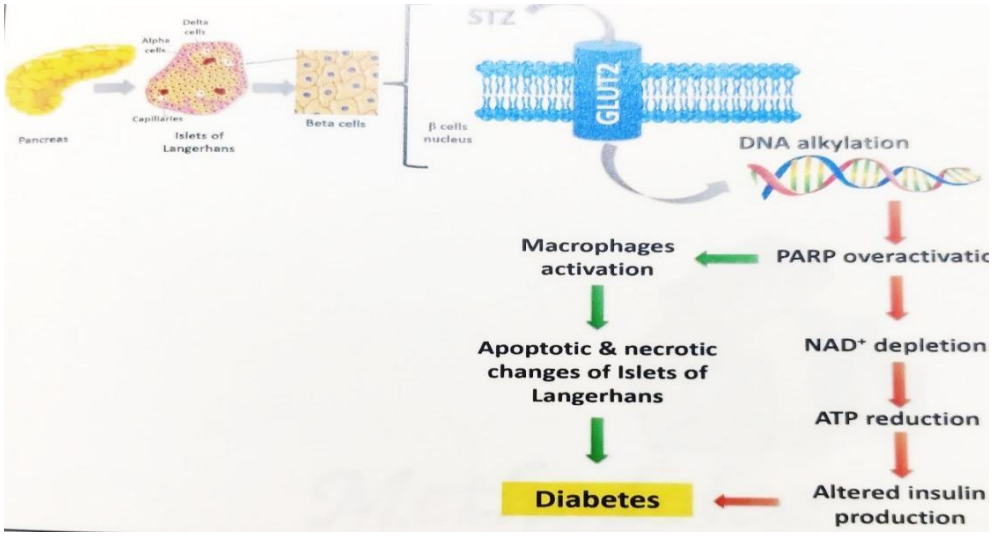


Figure 16: Mechanism of action of STZ induced Diabetes Mellitus [101]

Table 9: Summary of Mechanism of action of STZ action. [97 98 99 100]

Mechanism	Comments
DNA Alkylation	• Most important & most likely mechanism of action [84,85] • The first step is the transfer of methyl group from STZ to the beta cell DNA molecule, which is followed by cascade of events resulting in fragmentation & destruction of DNA • This further activates PARP-1 & results in diminution ofNAD+ & ATP stores [83] • The reduction in ATP of beta cells results in necrosis & NAD+

	depletion results in inhibition of insulin synthesis & secretion[83]
Nitric Oxide (NO) release	• Considered mostly as an alternative mechanism • NO is released as a result of STZ metabolism, independent of NO synthase enzyme [86]
ROS generation	• Superoxide & hydroxide radicals are the main ROS generated and are produced during the hypoxanthine metabolism

Within 48 hours of STZ injection, rats develop a state of permanent hyperglycemia, marking the onset of diabetes. During the initial 48 hours, significant changes occur in insulin & glucose levels. An hour after STZ injection, glucose levels spike significantly, followed by a drop in glycemic levels over the next 4-8 hours. This hypoglycemic phase can last several hours & may be fatal to the animals, making it the most critical period during the induction process. The final phase of this triphasic response is the establishment of persistent hyperglycemia around 72 hours post-injection. Therefore, this time point is ideal for measuring blood glucose levels to confirm the diagnosis of diabetes.

Type 2 Diabetes in rats using high fat diet & Streptozotocin

The HFD/STZ rat model, first introduced by Reed & colleagues, was designed to replicate the progression from prediabetes & insulin resistance to T2DM, mirroring the natural development of the disease. In their study, seven-weeks-aged Sprague–Dawley rats were fed a diet consisting of 40% of calories from fat for a period of two weeks. [102] Then the animals were fasted overnight & administered a single dose of STZ (50 mg/kg). Subsequently, eligible rats were given Metformin three days after receiving the STZ treatment, & the response was assessed. [94] Later, a comparable model utilizing a modest dose of STZ (35 mg/kg) was developed [95] This was altered by Zhang et al. who employed a multiple low dose regimen of STZ instead of a single dose. [96] Since the initial publication, numerous variations of HFD/STZ rat models have emerged. Feeding rats a high-fat diet induces obesity, insulin resistance, and/or glucose intolerance, closely mimicking the natural pathology of these conditions in humans. The duration & composition of the diet significantly impact weight gain & fat distribution in rats. This duration can range from a few weeks to over three months, while the nutrient sources & composition of the diets also vary. Although some studies have utilized high-carbohydrate diets, the most common approach is to use diets rich in fat & low in sugar. [103] [104]

β-cell failure is a key characteristic of T1DM & also represents the final stage in the progression of T2DM , following the onset of insulin resistance. In animal models for both T1DM & T2DM, streptozotocin (STZ) is commonly used as a β-cell toxin. Research indicates that by the time Type 1 Diabetes is diagnosed, approximately 60 to 80 percent of the fully functional β-cell mass has already been destroyed. [103] In contrast the percentage reduction of β-cell in patients with less than five years of T2DM is around 24%.[105] In rat

models, the dosage of STZ is crucial in determining the extent of β-cell destruction. While there is some debate regarding the optimal STZ dose, most researchers concur that administering either a single low dose or multiple low doses of STZ to high-fat-fed rats effectively models T2DM. [106] In this study we used High fat diet for 2 weeks followed by low dose streptozotocin (35mg/kg), single dose to induce type 2 diabetes mellitus(T2DM). [95]

METHODOLOGY

This was an experimental study involving adult healthy male Wistar rats. The total sample size was 38. Division of rats was done into 5 groups. Each group had 8 animals except for the normal control group which had 6 rats. The number of rats in the diabetic groups was higher to compensate for the expected mortality. Approval of the study was done by the IAEC (Institutional Animal Ethics Committee) (Annexure-I). The study was conducted as per the guidelines of CPCSEA (Committee for the Purpose of Control & Supervision of Experiments on Animals), New Delhi. 3–4-month-old adult healthy male Wistar rats with a mean weight of 200 ± 20g were procured from the central animal house of J.N. Medical College, KAHER, Belagavi. The animals were kept in polypropylene cages equipped with top grills made of stainless steel & supplies for food & drink. The rodents had unlimited access to food & water. Pellets were used to give the meal. In the cages, paddy husk served as the bedding.. The animals were acclimatized to a 12 -12 hour light-dark cycle for 10 days, prior to the day of experimentation. Division of rats was done into normal control, Disease Control, & treatment groups.

Study drug & kits:

- Metformin, Rivaroxaban Dabigatran & Thiopentone sodium were purchased from the hospital pharmacy.
- Streptozotocin (Cayman Chemical Company, catalog no. 13104) was procured from Everon Lifesciences, New Delhi.
- High-fat diet was purchased from VRK Nutritional Solutions, Pune, India
- Gluco One Glucometer was purchased from the hospital pharmacy.

- Rat IL-6 GENLISATM ELISA (Ref. No. KB3068), Rat TNF-alpha GENLISA (Ref. No. KB3145), & Rat Interleukin 1β, IL-1β GENLISA (Cat. No. KLR0119) were purchased from Krishgen Biosystems.

Study Methodology

The model of HFD- STZ induced T2DM was previously standardized in the department by a pilot study. The rats of the required weight range were kept fasting overnight in the cages, a day prior to initiation of the study. Of the 38 rats, 6 were a part of the normal control group. The rest of the 32 rats were given high-fat diet for 14 days (Table 1). The normal control rats were given standard chow pellet (Amrut Brand)

Table 10: Composition of High Fat Diet [HFD][107]

Ingredients	**Diet(g/kg)**
Powdered Normal Pellet diet(NPD)	365
Lard	300
Casein	280
Soya oil	50
Vitamin & Mineral Mix	50

Starch	260
Yeast Powder	01
Cholesterol	10

On day 15, blood glucose levels were measured for all rats using tail veins with a 30G insulin needle before their induction of Diabetes to check for the impact of HFD on the blood levels. Also Diabetes was induced in 32 rats as described below.

Experimental induction of Diabetes:

Type 2 diabetes mellitus was induced in rats as per the existing literature using low dose streptozotocin (35mg/kg) & High fat diet.[108] [109]

Preparation of STZ [110]

40 mg STZ was weighed in a glass beaker & the beaker was covered with aluminum foil. Fresh citrate buffer of 0.05M at a pH of 4.5 was prepared immediately prior to injection. The 0.05M citrate buffer was prepared by mixing 0.05M sodium citrate & 0.05M citric acid in a ratio of 2:3 & the pH was adjusted to 4.5). Immediately prior to injection, STZ was dissolved in the sodium citrate buffer to a final concentration of 40 mg in 8 ml.

Administration of STZ

The STZ solution was made right before injection & given out five minutes after it dissolved. Using a 1ml syringe & 23G needle, STZ was injected intraperitoneally (i.p.) into the rats belonging to various experimental groups at 35 mg/kg (6 ml/kg). An equal volume of citrate buffer was injected intraperitoneally into the normal control rats. After injection, Rats treated with STZ were given 5% glucose for 24 hours instead of water to reduce the mortality caused by hypoglycemia shock.

Confirmation of the Diabetes

After 72 hours the fasting blood glucose levels were measured. This was done using a glucometer from tail vein blood samples. Diabetic rats were defined as those with fasting blood glucose levels of more than 200 mg/dL & were included in further study.[110] [111] Diabetes was induced successfully in all rats.

Treatment Schedule

Day one of diabetes(DM) was considered the same day on which the confirmation was done. The normal control & Disease Control groups received saline as vehicle. The standard group received metformin & the treatment groups received the study drugs Rivaroxaban & Dabigatran.

Table 11: Study groups with treatment schedule

Groups	Treatment	Dose
Group I: Normal Control (NC) (n=6)	Vehicle only	1 ml
Group II: Disease Control (DC) (n=8)	Vehicle only	1 ml
Group III: Diabetic Rats + Metformin (MF) (n=8)	Metformin(standard)	180mg/kg
Group IV: Diabetic Rats + Rivaroxaban (n=8)	Rivaroxaban	1.8mg/kg
Group V: Diabetic Rats + Dabigatran mg/kg (n=8)	Dabigatran	27mg/kg

The doses of the drugs used have been calculated using the multiplication factor proposed by Paget & Barnes.[112]

Accordingly,

1) 2 gm/day maximum human dose of Metformin is equivalent to 180 mg/kg of rat.

2) 20 mg/day standard human dose of Rivaroxaban is equivalent to 1.8 mg/kg of rat.

3) 300 mg/day standard human dose of Dabigatran is equivalent to 27 mg/kg of rat.

Metformin & Rivaroxaban were dissolved in appropriate quantity of water after triturating. Dabigatran was dissolved in gum acacia after triturating. After calculating, appropriate quantities of these drug solutions were fed to respective rats by oral gavage.

All the drugs were administered orally as a single daily dose from day 1 to day 42.

Outcome measures:

The rats were observed for various parameters during study period at regular intervals. The study variables, their time & method of measurement is depicted in the following table.

Table No 12: Details of study parameters

Parameter	Timing of measurement	Method
Body weight	Baseline After 14 days of HFD	Weighing balance

	□ 21 days after DM induction, □ End of the study	
Fasting blood glucose	□ Baseline □ After 14 days of HFD □ 72-hours after STZ administration □ 21 days after DM induction □ End of the study	Rat tail vein samples were tested after overnight fasting using a glucometer
Lipid Profile 1.Total Cholesterol 2.High Density Lipoprotein 3.Low Density Lipoprotein 4.Trigkycerides	Baseline End of Study	Blood sample collected from Rat tail vein
Inflammatory markers 1.IL -1β 2.IL- 6 3.TNF- α	□ End of the study	Blood sample collected from cardiac puncture was analyzed using ELISA kits

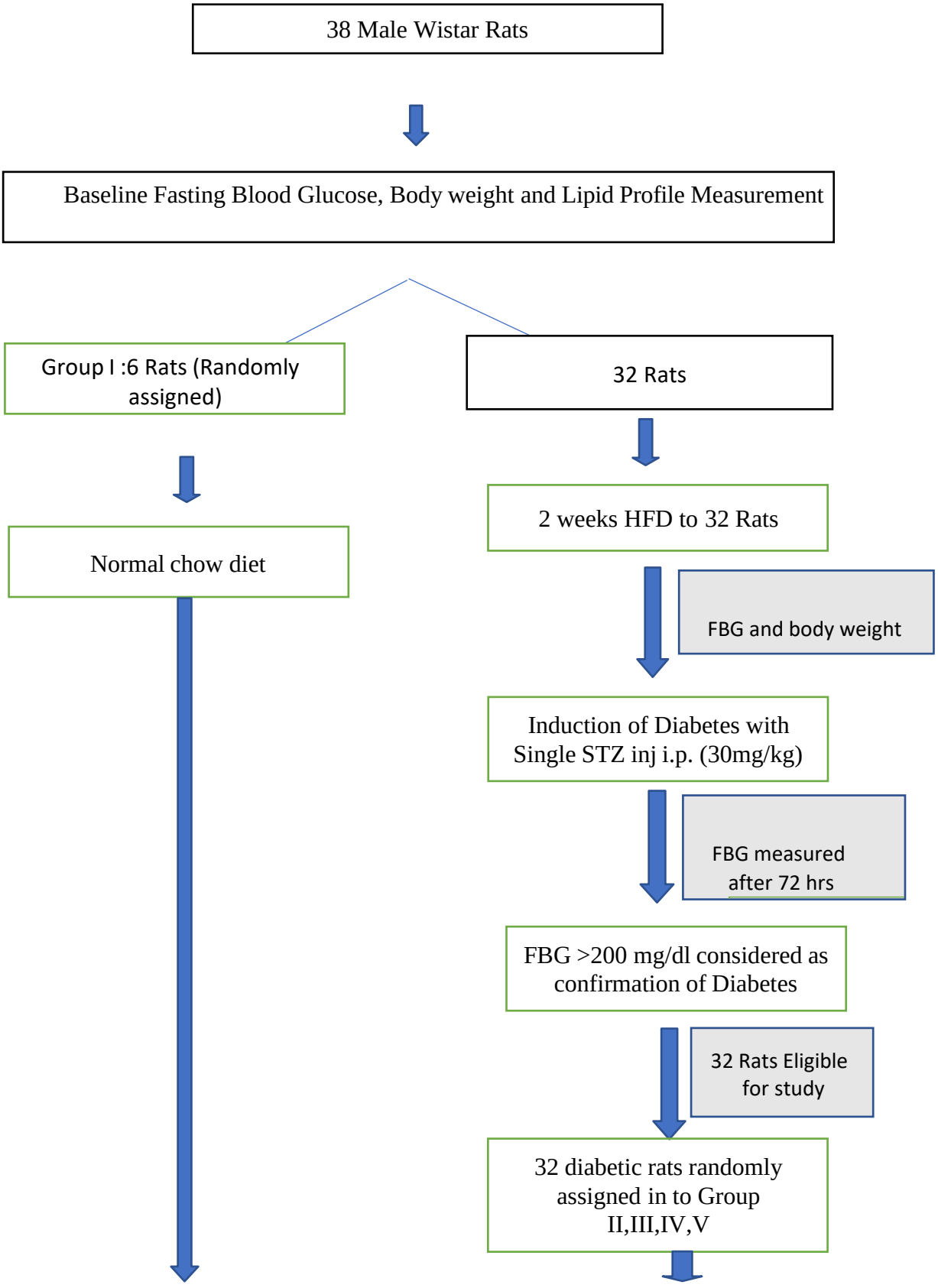
Euthanasia

On day 43 of the study, thiopentone sodium at a dose of 120 mg/kg was given as an intraperitoneal injection.[113] After this, cardiac puncture was performed for blood collection. The blood collected was then used to measure the serum lipid levels & was also centrifuged to create a homogenate to measure the levels of inflammatory makers using ELISA. Animals were then sacrificed as per the CPCSEA guidelines.

Statistical analysis

Data was expressed as Mean ± Standard Error of Mean (SEM) & was analyzed using two-way ANOVA followed by Post – hoc Tukey's test using GraphPad Prism version 9.0.

$p < 0.05$ was considered as statistically significant

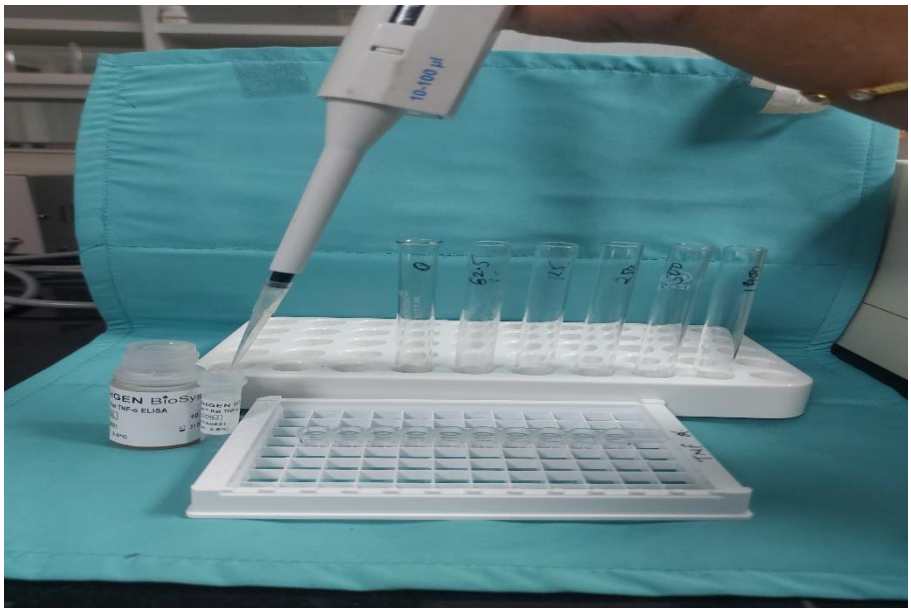

38 Male Wistar Rats
Baseline Fasting Blood Glucose, Body weight and Lipid Profile Measurement
Group I :6 Rats (Randomly assigned)
32 Rats
Normal chow diet
2 weeks HFD to 32 Rats
FBG and body weight
Induction of Diabetes with Single STZ inj i.p. (30mg/kg)
FBG measured after 72 hrs
FBG >200 mg/dl considered as confirmation of Diabetes
32 Rats Eligible for study
32 diabetic rats randomly assigned in to Group II,III,IV,V

Groups	Group I (Normal control) NC	Group II (Diabetic control) DC	Group III (Diabetic Rats + Metformin) MF	Group IV (Diabetic Rats + Rivaroxaban)	Group V (Diabetic Rats + Dabigatran)
No.of Rats	6	8	8	8	8
Treatment for 36 days	Vehicle 1 ml	Vehicle 1 ml	Metformin 180mg/kg	Rivaroxaban 1.8mg/kg	Dabigatran 27 mg/kg

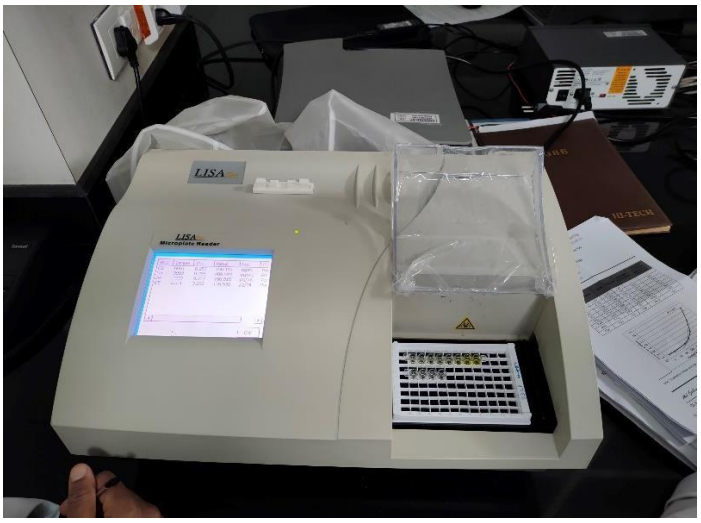
Bodyweight and FBG measured on day 21 and at the end of study

Day 43	Cardiac puncture to obtain blood from all groups to estimate FBG, Lipid profile, IL -1β, IL -6 and TNF- α, followed by euthanasia with overdose of anesthesia

Figure 17: Schematic representation of the study design

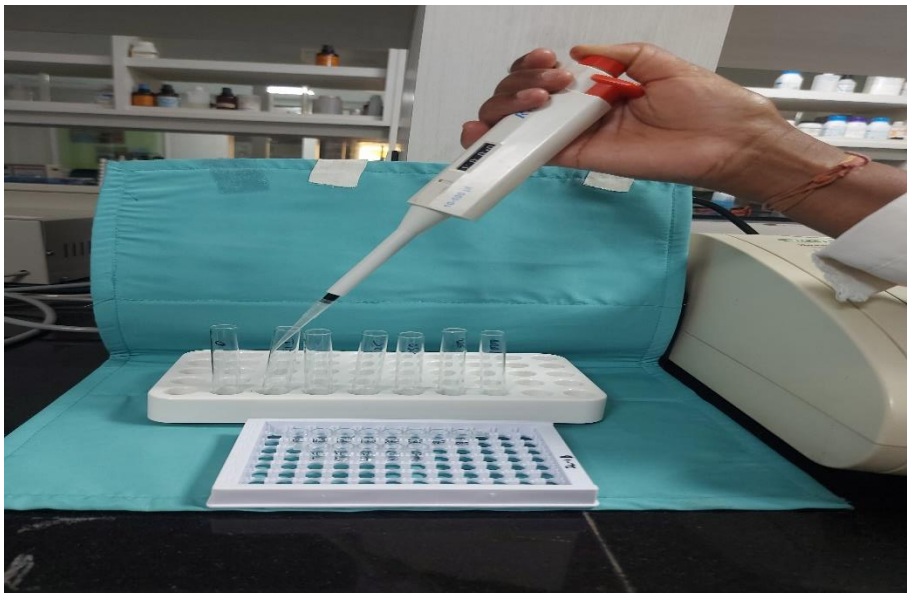


a)Rat TNF-alpha GENLISA (Ref. No. KB3145,Krishgen Biosystems)



b)Elisa microplate reader machine

Figure 18: Sample images of test kits, instruments and selected procedures



c)Creating serial dilutions of the stock solution

d)Transferring diluted stock to microtiter coated plate wells

Figure19 : Sample images of test kits, instruments and selected procedures

e) Incubating microtiter plates after adding conjugation antibody

Figure 20: Sample images of test kits, instruments and selected procedures

RESULTS

This study was done to determine the impact of Dabigatran and Rivaroxaban on glycemic parameters, lipid profile and inflammatory markers in high fat diet and low dose Streptozotocin induced Diabetes Mellitus in Male Wistar rats.

Data collected in the study was compiled into an excel sheet and was analyzed using GraphPad Prism Version 10.0. Parameters like Body Weight, Blood Glucose, Lipid levels, and Inflammatory markers were analyzed. This was done using appropriate statistical tests. On doing the tests if the p value was 0.05 was considered statistically significant. Data is expressed as Mean ± SEM.

1. **Body weight**

   Body weight was measured at baseline, following 14 days of the high-fat diet, 21 days after treatment, and at the end of the study.

   The mean body weights of all the groups were comparable at baseline. After 14 days of the high-fat diet, the mean body weights (in grams) of Normal Control, Disease Control, Metformin, Rivaroxaban and Dabigatran groups were 251±5.865, 260±2.853, 244±10.71, 258±4.7, 248.3±4.78 respectively. Two-way ANOVA showed no statistically significant difference between the groups (Table no 13 Graph no 1)

   On Day 21 of treatment, the mean body weights (in grams) of Normal Control, Disease Control, Metformin, Rivaroxaban and Dabigatran groups were 290±3.990, 232.3±2.684, 275.3±9.531, 273±4.54, 268.8±5.219 respectively. Two-way ANOVA revealed a statistically significant weight reduction between groups with $p<0.0001$.

Post hoc Tukey's test showed there was statistically significant difference between the Disease Control group in comparison with treatment groups ($p < 0.0001$). (Table no 13 Graph no 1)

At the end of the study mean body weights (in grams) of Normal Control, Disease Control, Metformin, Rivaroxaban and Dabigatran groups were 337.5±3.819, 180.8±4.729, 304.6±10.25, 286.7±3.727, 292.4±5.34 respectively. Two-way ANOVA revealed a statistically significant weight reduction between groups with $p<0.0001$. Post hoc Tukey's test showed there was statistically significant difference between the Disease Control group in comparison with treatment groups($p < 0.0001$) and also between the Disease Control and Normal control ($p<0.001$). (Table no 13 Graph no 1)

Table no 13 : The Effect of various drugs on Body Weight in High Fat Diet and Streptozotocin induced diabetes in Wistar rats.

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Day of study	Body weight in grams (Mean ±SEM)				
	Normal Control (NC)	Disease Control (DC)	Metformin (MF)	Rivaroxaban (RIVA)	Dabigatran (DABI)
	207.2 ±5.108	214.1±1.903	208.1±3.889	205±4.2	196.9±3.884

Baseline					
After 14Days ofHFD	251±5.865	260±2.853	244±10.71	258±4.7	248.3±4.78
After 21 Days of induction	290±3.990	232.3±2.684	275.3±9.531 *	273±4.54 *	268.8±5.219 *
End of Study	337.5±3.819 #	180.8±4.729	304.6±10.25 *	286.7±3.727 *	292.4±5.34 *

Values are expressed as Mean ± SEM, Analysis was done by ANOVA and post hoc Tukey's test. *****p < 0.0001, indicates the significant difference between Disease Control and treatment groups. #p < 0.001, indicates the significant difference between Disease Control and normal control group.

Graph no 1 : The Effect of various drugs on Body Weight in High Fat Diet and Streptozotocin induced diabetes in Wistar rats.

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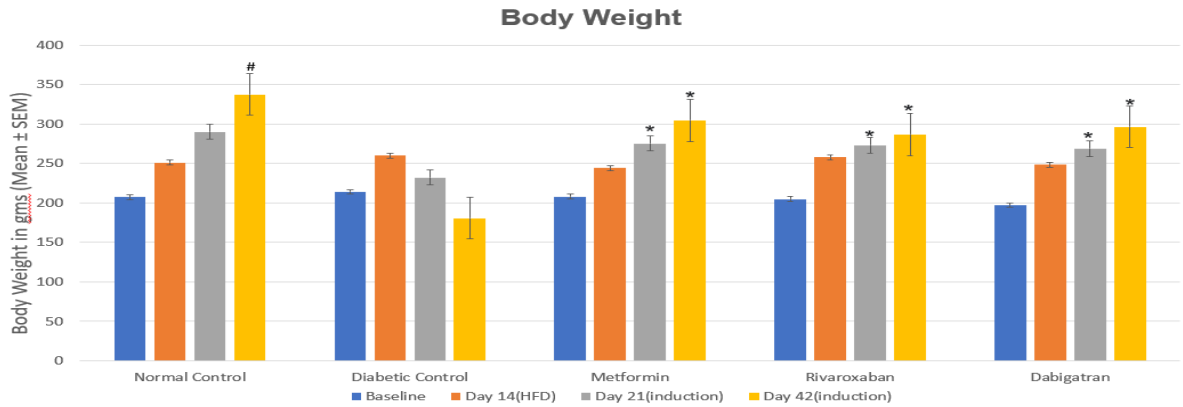


Values are expressed as Mean ± SEM, Analysis was done by ANOVA and post hoc Tukey's test.*$p < 0.0001$, indicates the significant difference between Disease Control and treatment groups. #$p < 0.001$, indicates the significant difference between Disease Control and normal control group

2. Fasting Blood Glucose (FBS) at various intervals

Fasting blood glucose was measured at baseline, following 14 days of the high-fat diet, 72 hours after Streptozotocin injection, 21 days after treatment, and at the end of the study.

The mean FBS of all the groups were comparable at baseline. Two-way ANOVA revealed that there was no significant difference between various groups at baseline and after 14 days of High fat diet administration. Following 72 hours of STZ administration the mean FBS values (mg/dL) of Disease Control, Metformin, Rivaroxaban and Dabigatran groups were - 103.7±1.909, 341.5±12.51, 316.5 ± 10.99, 316.5 ± 13.93, 322.6 ± 13.27 respectively with no statistical difference detected between them using two way ANOVA. (Table no 14 Graph no 2)

After 21 days of treatment the mean FBS values (mg/dL) of Diabetic Control, Metformin, Rivaroxaban and Dabigatran groups were 368.5±12.35, 288.8±10.21, 310.4±10.43 and 313.8±13.02 respectively Two-way ANOVA revealed a statistically significant reduction in blood glucose levels between groups with $p<0.0001$. Post hoc Tukey's test showed there was statistically significant difference between the Diabetic Control group in comparison with treatment groups ($p < 0.0001$) (Table no 14 Graph no 2).

At the end of the study, the mean FBS values (mg/dL) of Normal Control, Diabetic Control, Metformin, Rivaroxaban and Dabigatran groups were 102.3±1.838, 397.5±10.06, 275.9±9.33, 299.3±13.54 and 296.4±12.12 respectively. Two-way ANOVA revealed a statistically significant reduction in blood glucose levels between groups with $p<0.0001$. Post hoc Tukey's test showed there was statistically significant difference between the Diabetic Control group in comparison with treatment groups ($p < 0.0001$). (Table no 14 Graph no 2).

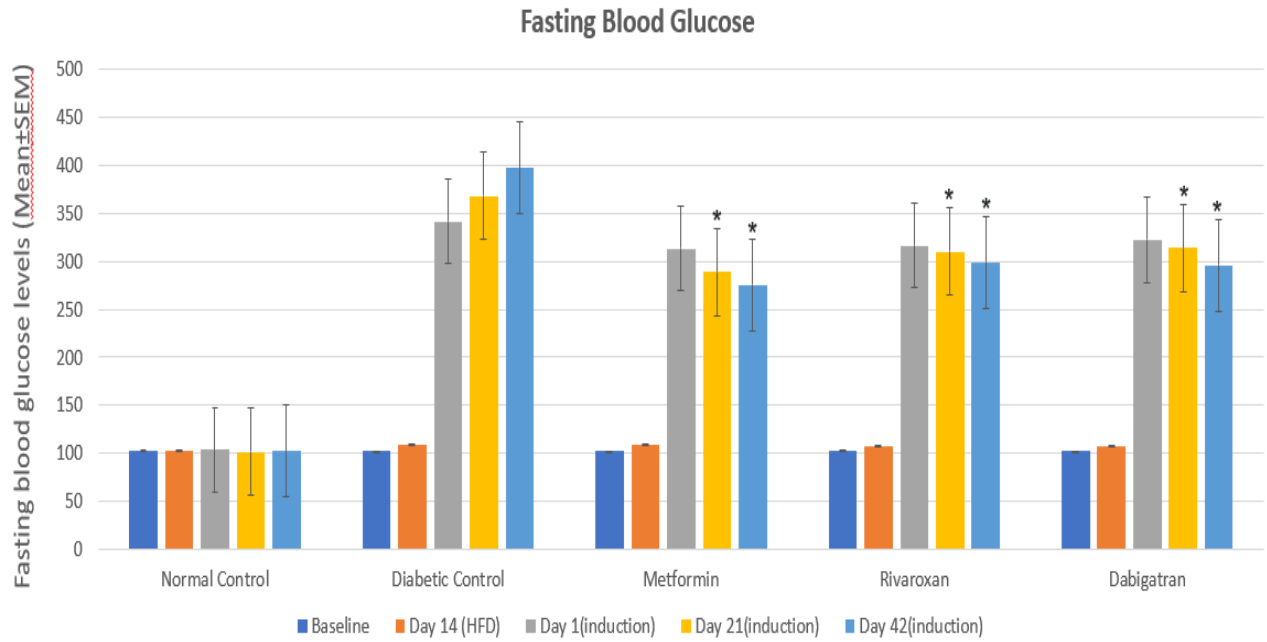
Table no. 14 : The Effect of various drugs on Fasting Blood Glucose in High Fat Diet and Streptozotocin induced diabetes in Wistar rats.

Day of study	Fasting Blood Glucose (mg/dL) (Mean ±SEM)				
	Normal Control (NC)	Disease Control (DC)	Metformin (MF)	Rivaroxaban (RIVA)	Dabigatran (DABI)
Baseline	103.2 ± 1.138	102.1± 1.394	102.1 ±1.109	103±1.102	102.4±1.068
After 14Days ofHFD	103.2 ± 1.352	108.8±1.130	108.5±1.225	107.4±1.133	107.3±1.411
Day 1 - Following 72	103.7±1.909	341.5±12.51	316.5 ± 10.99	316.5 ± 13.93	322.6 ± 13.27

hours of STZ administration					
After 21 Days of induction	101.5±1.052	368.5±12.35	288.8±10.21 *	310.4±10.43 *	313.8±13.02 *
End of Study	102.3±1.838	397.5±10.06	275.9±9.33 *	299.3±13.54 *	296.4±12.12 *

Values are expressed as Mean ± SEM, Analysis was done by ANOVA and post hoc Tukey's test.*****$p < 0.0001$, indicates the significant difference between Disease Control and treatment groups.

Graph no 2 :The Effect of various drugs on Fasting Blood Glucose in High Fat Diet and Streptozotocin induced diabetes in Wistar rats.



Values are expressed as Mean ± SEM, Analysis was done by ANOVA and post hoc Tukey's test.*****$p < 0.0001$, indicates the significant difference between Disease Control and treatment groups.

4. Lipid profile

Lipid profile was assessed at baseline and at the end of the study.

i) Total Cholesterol

The mean total cholesterol (mg/dL) values of all the groups were comparable at baseline. A two-way ANOVA revealed that there was no significant difference between various groups at baseline. At the end of the study the total cholesterol values of Normal Control, Diabetic Control, Metformin, Rivaroxaban and Dabigatran groups were 79±8.536, 88.5±5.714, 131.6±1.569, 151.7±3.63, 142.2±2.773 respectively. Two-way ANOVA revealed a statistically significant reduction in Total Cholesterol levels between groups with $p<0.05$. Post hoc Tukey's

test showed there was statistically significant difference between the Diabetic Control group in comparison with treatment groups ($p < 0.05$). (Table no 15)

ii) Triglycerides

The mean Triglycerides (mg/dL) values of all the groups were comparable at baseline. A two-way ANOVA revealed that there was no significant difference between various groups at baseline. At the end of the study the Triglycerides values of Normal Control, Diabetic Control, Metformin, Rivaroxaban and Dabigatran groups were 97.3±1.116, 177.8±1.493, 147±1.493, 168.3±1.445, 168.3±1.445 respectively. Two-way ANOVA revealed a statistically significant reduction in Triglycerides levels between groups with $p<0.05$. Post hoc Tukey's test showed there was statistically significant difference between the Diabetic Control group in comparison with Metformin and Dabigatran groups ($p < 0.05$). (Table no 15)

iii) Low-density lipoproteins

The mean Low-density lipoproteins (mg/dL) values of all the groups were comparable at baseline. A two-way ANOVA revealed that there was no significant difference between various groups at baseline. At the end of the study the Low-density lipoproteins values of Normal Control, Diabetic Control, Metformin, Rivaroxaban and Dabigatran were 39±1.528, 74.7±3.667, 59.8±2.128, 71.1±2.219, and 64.4±1.631 respectively. Two-way ANOVA revealed a statistically significant reduction in Low-density lipoproteins levels between groups with $p<0.05$. Post hoc Tukey's test showed there was statistically significant difference between the Diabetic Control group in comparison with Metformin group ($p < 0.05$). (Table no 15)

iv) High-density lipoproteins

The mean High-density lipoproteins (mg/dL) values of all the groups were comparable at baseline. At the end of the study the High -density lipoproteins values of Noraml Control, Disease Control, Metformin, Rivaroxaban and Dabigatran were 33.8±1.447, 23.3±1.25, 30.9±1.060, 25.7±1.614, 26.3±0.8921 respectively. A two-way ANOVA revealed that there was no significant difference between various groups at baseline. Two-way ANOVA revealed no statistically significant reduction in High-density lipoproteins levels between groups. (Table no 15)

Table no. 15 : The Effect of various drugs on Lipid Profile in High Fat Diet and Streptozotocin induced diabetes in Wistar rats.

	Total Cholesterol (Mean ± SEM)	HDL (Mean ± SEM)	LDL (Mean ± SEM)	Triglycerides (Mean ± SEM)
Normal control	79±8.536	33.8±1.447	39±1.528	97.3±1.116
Disease Control	88.5±5.714	23.3±1.25	74.7±3.667	177.8±1.493
Metformin	131.6±1.569 *	30.9±1.060	59.8±2.128 *	147±1.493 *
Rivaroxaban	151.7±3.63	25.7±1.614	71.1±2.219	168.3±1.445

	*			
Dabigatran	142.2±2.773 *	26.3±0.8921	64.4±1.631	164.3±1.643 *

Values are expressed as Mean ± SEM, Analysis was done by ANOVA and post hoc Tukey's test.*****$p < 0.05$, indicates the significant difference between Disease Control and treatment groups.

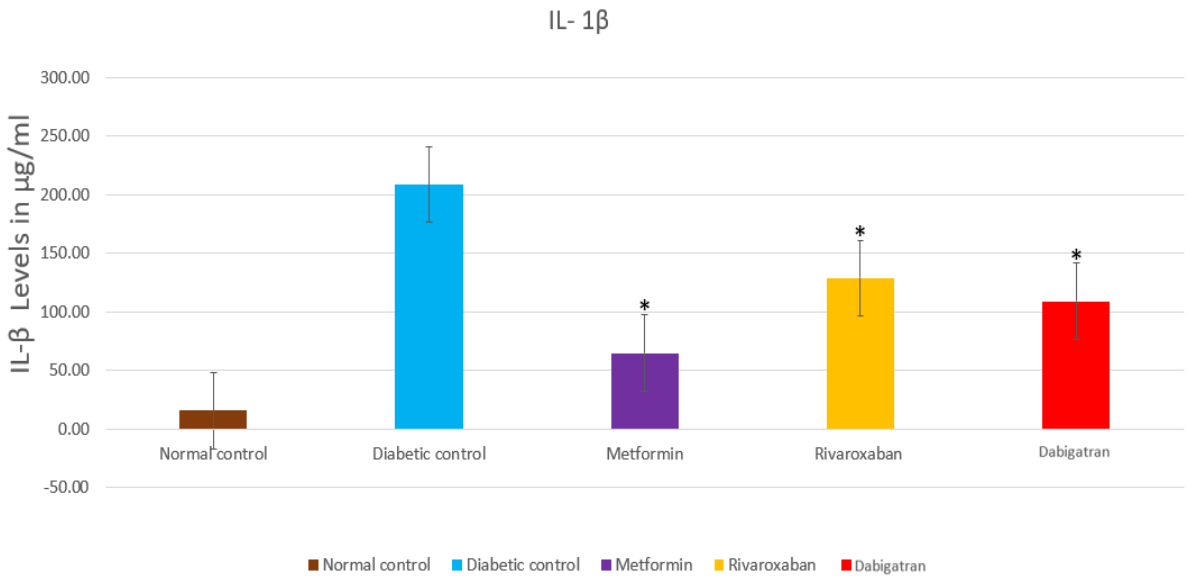
5. Inflammatory markers

Serum Il-1β, TNF- α and IL-6 levels were measured at the end of the study.

i. IL -1β

At the end of the study the mean IL-1β values of Normal Control, Disease Control, Metformin, Rivaroxaban and Dabigatran groups were 15.65 ± 0.622, 208.53 ± 2.743 , 64.94 ± 2.872, 128.86 ± 2.434, 109.11 ± 2.32 respectively. Two-way ANOVA revealed a statistically significant reduction in **IL -1β** levels between groups with $p<0.001$. Post hoc Tukey's test showed there was statistically significant difference between the Disease Control group in comparison with Treatment groups ($p < 0.001$). (Graph no 3)

Graph no 3 : The Effect of various drugs on IL-1β levels in High Fat Diet and Streptozotocin induced diabetic rat model



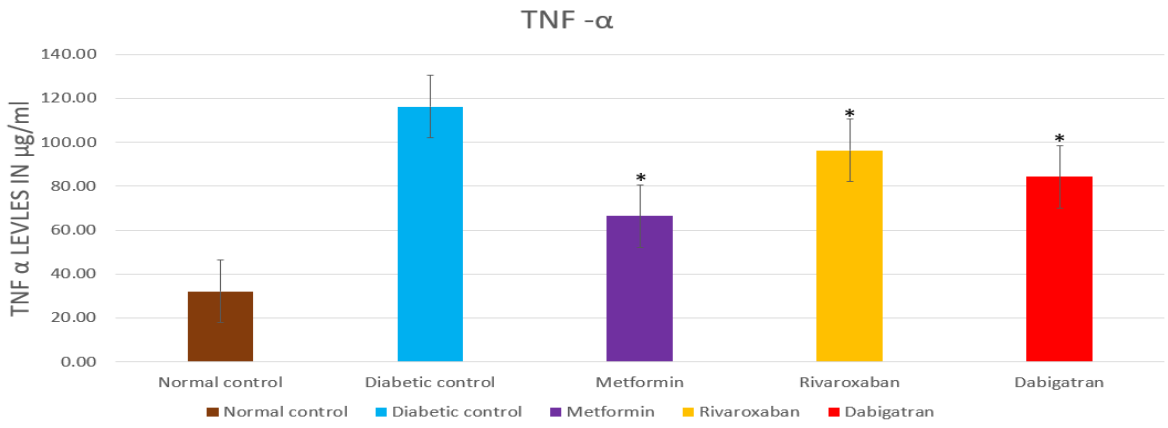
Values are expressed as Mean ± SEM, Analysis was done by ANOVA and post hoc Tukey's test.*****$p < 0.001$, indicates the significant difference between Disease Control and treatment groups.

ii. TNF-α

At the end of the study the mean TNF- α values of Normal Control , Disease Control, Metformin, Rivaroxaban And Dabigatran groups were 32.13 ± 2.411, 116.33 ± 1.797, 66.41 ±1.404, 96.41 ±1.272, 84.29 ± 1.945 respectively. Two-way ANOVA revealed a statistically significant reduction in TNF- α levels between groups with $p<0.001$. Post hoc Tukey's test showed there

was statistically significant difference between the Disease Control group in comparison with Treatment groups ($p < 0.001$). (Graph no 4)

Graph no 4 : The Effect of various drugs on TNF-α levels in High Fat Diet and Streptozotocin induced diabetic rat model



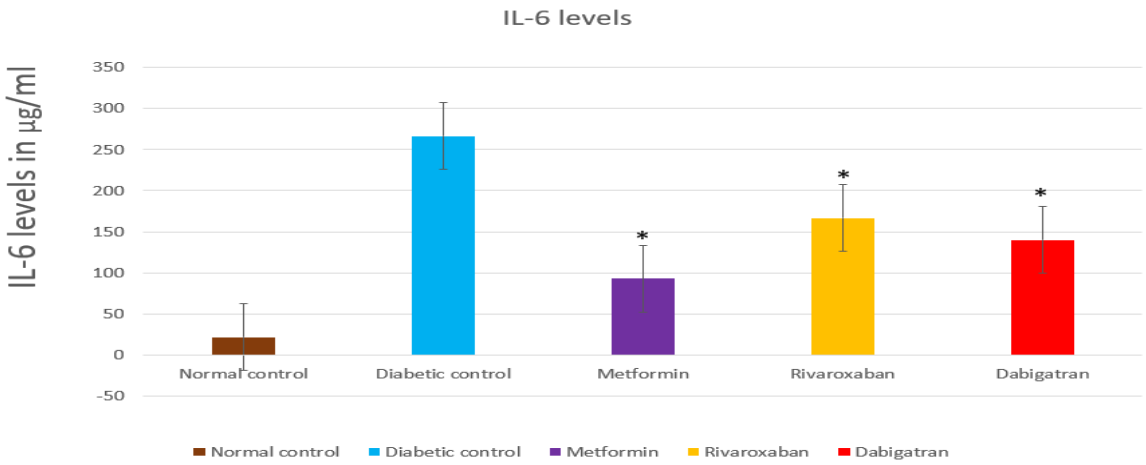
Values are expressed as Mean ± SEM, Analysis was done by ANOVA and post hoc Tukey's test.*****$p < 0.001$, indicates the significant difference between Disease Control and treatment groups.

iii. IL-6

At the end of the study the mean IL-6 values of Normal Control, Diabetic Control, Metformin, Rivaroxaban and Dabigatran groupswere 21.3 ± 2.038, 266.5 ± 3.646, 92.88 ± 2.003, 166.37 ± 3.298, 140.14 ± 2.361 respectively. Two-way ANOVA revealed a statistically significant reduction in IL-6 levels between groups with $p<0.001$. Post hoc

Tukey's test showed there was statistically significant difference between the Diabetic Control group in comparison with Treatment groups ($p < 0.001$). (Graph no 5)

Graph no 5 : The Effect of various drugs on IL-6 levels in High Fat Diet and Streptozotocin induced diabetic rat model



Values are expressed as Mean ± SEM, Analysis was done by ANOVA and post hoc Tukey's test.*****$p < 0.001$, indicates the significant difference between Disease Control and treatment groups.

DISCUSSION

The present study was conducted to evaluate the effect of anticoagulant drugs, Rivaroxaban and Dabigatran on glycemic parameters in high-fat diet and Streptozotocin-induced diabetes model in adult male Wistar rats. The anti-inflammatory effects of Rivaroxaban and Dabigatran are well established. However, the evidence on the effects of Rivaroxaban and Dabigatran on blood glucose level is inconclusive which has been the objective of the present study.

A high-fat diet induces insulin resistance and/or glucose intolerance in rats similarly to humans. Streptozotocin (STZ) induces β-cell failure through mechanisms such as DNA alkylation, nitric oxide release, and reactive oxygen species (ROS) generation. STZ is preferred over alloxan for diabetes induction because it selectively targets β-cells via GLUT-2 receptors, has lower toxicity, and results in a lower mortality rate.[111] [100] As the HFD-STZ model mimics the natural pathology of human diabetes, it was used for induction of diabetes in the current study. A state of permanent hyperglycemia develops in the rats 48 hours after STZ injection, and this is considered the start of the disease. Also, 72 hours post-injection is considered as the best time point for measuring the blood glucose level to confirm the diagnosis of diabetes.

Effect on body weight

In our study, after 14 days on a high-fat diet, all groups exhibited higher body weight compared to the normal control group. However, the weight gain was not statistically significant. A study by Marques, Cláudia et al suggested that weight gain with high fat diet in Wistar rats were statistically significant only after 4 weeks of high fat diet, although weight gain was reported after 2 weeks of High fat diet but weight gain was not statistically

significant which supports our study.[114] Wistar rats fed with HF diet had higher amounts of calorie intake compared to the normal control rats fed with chow diet. As a result, weight growth was greater in these animals, owing primarily to an increase in adipose tissue mass. [115] [116] [117] [118]

With the progression of diabetes, there was significant weight reduction in the untreated diabetic rats compared to the non-diabetic animals. Such a drastic weight loss was also observed in studies done by Cheng, Daye et al. [119] and Mestry, Snehal Nitin et al.[120] This can be explained on the basis that STZ-induced diabetes is accompanied by a substantial decrease in body weight as a result of hyperglycemia, hypoinsulinemia, muscle wasting, and protein loss [119] [121]. Treatment of diabetic rats with Metformin, Rivaroxaban and Dabigatran significantly improved body weight, indicating that muscle tissue damage caused by hyperglycemia was prevented. It is known that Metformin's weight-neutral or weight-sparing effects provide a therapeutic benefit in diabetes care, while other first-line oral antidiabetic medications frequently cause clinically substantial weight gain. [122]

The current investigation found that Rivaroxaban and Dabigatran were effective at preventing diabetes-induced dramatic weight loss and was comparable to Metformin therapy.

Effect on Fasting Blood Glucose

In our experiment, rats fed a high-fat diet exhibited a significant increase in fasting blood glucose levels after 14 days compared to those fed a regular chow diet. These findings align with previous research demonstrating that high-fat diets result in elevated blood glucose levels in rats. Fat metabolites and free fatty acids (FFAs) entering the liver impair insulin sensitivity in liver cells and disrupt glucose metabolism, leading to increased glucose levels. [107] [123]

A state of hyperglycemia was successfully generated following a single dose of Streptozotocin in this study. The fasting blood glucose levels of untreated diabetic rats were consistently raised throughout the trial. The FBS of the rats treated with Metformin was substantially lower than that of the untreated rats halfway through the study. Rivaroxaban and Dabigatran were also successful in bringing down the glucose levels, though the difference was not statistically significant. At the end of 6 weeks, Rivaroxaban and Dabigatran significantly reduced the FBS in comparison with the Disease Control rats. Moreover, the results of Rivaroxaban and Dabigatran were comparable with Metformin therapy. These findings are in alignment with studies by Lie et al and Cheung et al who have demonstrated the reduction in blood glucose by Rivaroxaban and Dabigatran respectively. [124] [125]

Effects on Lipid profile

Akiyama et al. for the first time established unequivocally that a high-fat diet causes an increase in serum cholesterol and triglycerides in Wistar rats because of the obesity that is resulting from the high-fat intake. They reasoned that the increased serum lipid concentrations impeded glycolysis and decreased insulin action, resulting in insulin resistance.[123] These findings were replicated in subsequent animal experiments using a high-fat diet, which indicated significant increases in blood cholesterol, triglycerides, and LDL, along with a decrease in HDL.[116] Likewise in our study the lipid profile was deranged significantly in HFD rats compared to the rats on a normal diet.

The Diabetes induction worsened this derangement in lipid profile in our study. The untreated rats had significantly high total cholesterol, triglycerides, LDL values compared to the non-diabetic rats, along with a significantly low HDL value. Although dyslipidemia is common in Diabetes, the pathophysiology underlying it is only partially understood. Recent research

indicates that altered insulin sensitivity pathways, elevated FFA concentrations, and low-grade inflammation all contribute to the overproduction and impaired metabolism of TG-rich lipoproteins of intestinal and hepatic origin. The observed alterations in HDL and LDL cholesterol levels are mostly a result of this. These lipid alterations are the main link between Diabetes and increased cardiovascular risk. There is significant evidence that decreasing cholesterol improves cardiovascular outcomes, even in persons with normal lipid profiles. [117] [118] Hence it is desirable to have anti-diabetic drugs with additional hypolipidemic potential.

In our study, Metformin monotherapy significantly reduced the total cholesterol, triglycerides, and LDL levels compared to the untreated diabetic rats. Metformin is known to have a beneficial effect on lipid profiles, and this finding is consistent with the existing research.[116] [115] The impact of Rivaroxaban and Dabigatran on the lipid profile has been not been studied in detail. Our study demonstrated that Metformin, Rivaroxaban and Dabigatran reduced the total cholesterol significantly in comparison to Disease Control group by the end of the study. These findings are similar to studies done by Luo et al and Rocha et al. [126] [127] Metformin and Dabigatran reduced triglycerides significantly in comparison to Disease Control, however Rivaroxaban didn't affect it. These findings are similar to studies done by Gillani et al and Rocha et al. [128] [126] There was no impact on the HDL levels in any of the treatment groups.

Effects on Inflammatory markers

In our study, all three treatment groups were able to significantly lower serum IL-1, IL-6, and TNF- levels in diabetic rats when compared to untreated diabetic rats. Metformin has previously been reported to lower these inflammatory markers in animal models of diabetes.

In a rodent study by Mohamed-I Kotb El-Sayed et al., it was observed that Metformin treatment significantly reduced the serum TNF and IL-6 compared to the Disease Control rats. [129] In a 2018 study by Ling Kou et al., Metformin significantly reduced serum IL-6, TNF-, and IFN- when compared to untreated diabetic rats.[123] In a similar study which used STZ induced diabetic rats Metformin reduced the IL-1 levels significantly compared to the untreated rats.. [130] Our study also confirmed the beneficial effect of Metformin on these inflammatory markers.

We found that Rivaroxaban and Dabigatran were effective in reducing these inflammatory parameters. The anti-inflammatory characteristics of Rivaroxaban and Dabigatran are well documented; they are powerful inhibitors of IL-6, IL-1, and TNF-alpha. Dabigatran is an anticoagulant that has also been shown to reduce the inflammatory markers that are elevated in diabetics. Dabigatran significantly inhibits the levels of pro-inflammatory cytokines IL-1β, IL-6, and TNF-α in acute myocardial infarction (AMI) rabbits.[9] Specifically, AMI significantly increased the activities of IL-1β, IL-6, and TNF-α in rabbits compared to the normal control group. However, dabigatran treatment significantly reduced these elevated cytokine activities in AMI rabbits compared to the AMI vehicle groups.[9]These results suggest that dabigatran exerts anti-inflammatory effects in the context of AMI by reducing the levels of key pro-inflammatory mediators IL-1β, IL-6, and TNF-α. This contributes to the overall cardioprotective actions of dabigatran in regulating the no-reflow phenomenon and reducing infarct size in AMI.[9]

Rivaroxaban has been shown to inhibit caspase activity in the context of myocardial ischemia and atherosclerosis. In a rat model of myocardial ischemia, rivaroxaban markedly inhibited the pathways inducing apoptosis, including caspase 3 and caspase 9. Apoptosis is dependent on the release of cytochrome c and other pro-apoptotic factors from mitochondria in response to caspase activity.[84] Rivaroxaban reduced the expression of inflammatory molecules in

aorta, including TNF-α, which is a key pro-inflammatory cytokine.[85] In vitro experiments using mouse peritoneal macrophages or murine macrophage cell line RAW264.7 demonstrated that factor Xa (FXa) promoted the pro-inflammatory activation of macrophages, increasing the production of IL-1β, which was blocked in the presence of rivaroxaban. [85] In patients with atrial fibrillation (AF) undergoing planned cardioversion, rivaroxaban treatment resulted in a significant reduction in levels of high-sensitivity IL-6 (hsIL-6) from baseline to the end of treatment. [12]

The findings of present study thus support that Rivaroxaban and Dabigatran exert beneficial effects on glycemic parameters of diabetes that could be due to its effect on inflammatory markers. However, further studies are required to establish the effect of Rivaroxaban and Dabigatran in combination with standard anti-diabetic drugs like Metformin to supports their addition in the treatment of diabetes.

LIMITATIONS AND FUTURE RECOMMENDATIONS:

Rivaroxaban and Dabigatran have been employed in the present study. Our study has reported the effects of Rivaroxaban and Dabigatran onglycemic parameters of diabetes. Further studies can be conducted utilizing Rivaroxaban and Dabigatran in combination with Metformin (Standard drug for diabetes) for maximum benefit in treatment of diabetes.

Our study did not determine the histopathologic effect of Rivaroxaban and Dabigatran also it did not estimate the effect of the drugs on HOMA-IR (Homeostasis model assessment-insulin resistance), which is considered as a valid estimate of insulin sensitivity. Further studies can be planned to study the same.

CONCLUSION

The present study showed that the treatment of diabetic rats with Rivaroxaban and Dabigatran improved the HFD-STZ induced biochemical alterations in glycemic parameters and inflammatory markers. Furthermore, Metformin and Rivaroxaban and Dabigatran was found to be equally efficacious across all trial variables. Based on the findings of this study, it can be concluded that Rivaroxaban and Dabigatran may be a promising option for the management of Type 2 Diabetes. Future research of Rivaroxaban and Dabigatran in combination with standard antidiabetic drugs like Metformin in patients of diabetes, as well as clinical trials with larger sample sizes, are recommended

SUMMARY

The present study was conducted to evaluate the effects of Rivaroxaban and Dabigatran in a High-fat diet low dose Streptozotocin-induced rodent model of diabetes. In this study, the effect of Rivaroxaban and Dabigatran on glycemic parameters and inflammatory markers was evaluated. We found that treatment of diabetic rats with Rivaroxaban and Dabigatran improved the HFD-STZ induced biochemical alterations in all these parameters.

Key findings of the study:

- Rivaroxaban and Dabigatran was effective at preventing Diabetes-induced weight loss.
- Rivaroxaban and Dabigatran was effective in reducing the Fasting Blood Glucose.
- Rivaroxaban and Dabigatran significantly lowered serum IL-1β, IL-6, and TNF-α levels in comparison with the untreated rats.

According to the findings of this study, Rivaroxaban and Dabigatran may be a viable choice in the management of Type 2 Diabetes. Clinical trials with bigger sample sizes, as well as further research of Rivaroxaban and Dabigatran in combination with standard anti-diabetic drugs like Metformin in patients of diabetes need to be considered.

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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
A.H.M
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 : Effect of Dabigatran and Rivaroxaban on Glycemic Parameters in High Fat Diet and Low dose Streptozotocin Induced Diabetes mellitus in Male Wistar Rats.

Submitted by- BO0121004 PG Pharmacology, JNMC.

Has been approved by the Institutional Animal Ethical Committee Meeting held on 25-6-22 vide Resolution No. 17/4.

For sanction of 38 Male Wistar Rats.

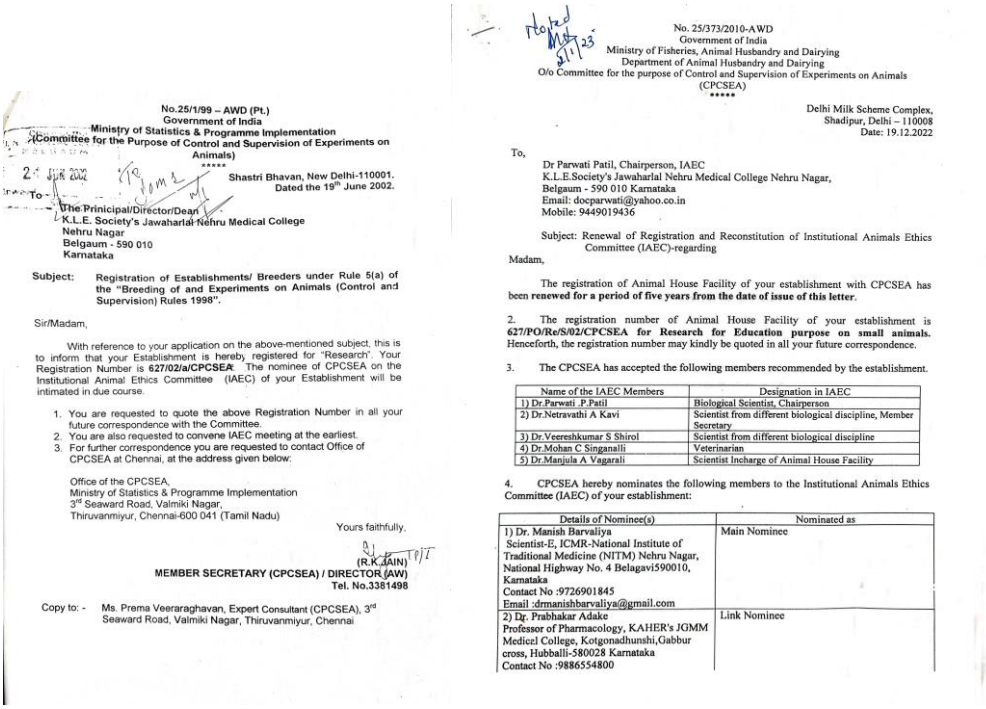
Main Nominee CPCSEA
IAEC-JNMC, Belagavi.

Signature and Name:
CPCSEA-Main Nominee

Member Secretary
IAEC-JNMC, Belagavi.

Signature and Name:
Chairman/Mem.Secretary

ANNEXURE - II - CPCSEA REGISTRATION & RENEWAL



No.25/1/99 – AWD (Pt.)
Government of India
Ministry of Statistics & Programme Implementation
(Committee for the Purpose of Control and Supervision of Experiments on Animals)
*****
Shastri Bhavan, New Delhi-110001.
Dated the 19th June 2002.

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

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

Sir/Madam,

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

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

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

Yours faithfully,

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

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

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

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

To,

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

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

Madam,

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

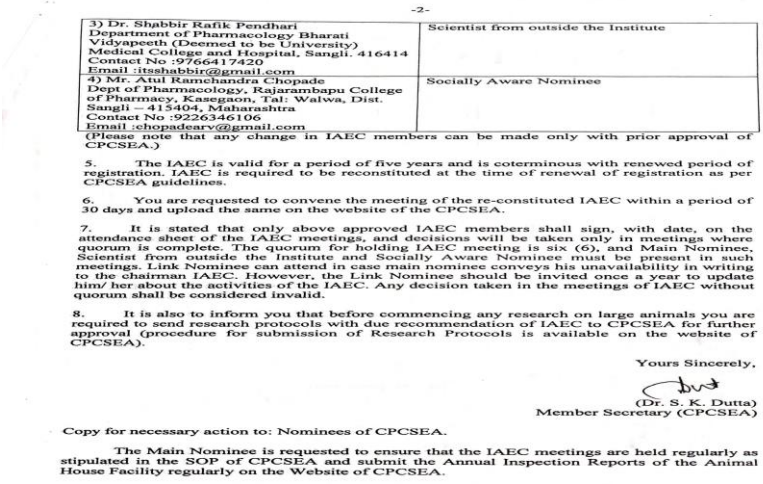
2. The registration number of Animal House Facility of your establishment is **627/PO/Re/S/02/CPCSEA for Research for Education purpose on small animals.** Henceforth, the registration number may kindly be quoted in all your future correspondence.

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

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

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

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



-2-

3) Dr. Shabbir Rafik Pendhari Department of Pharmacology Bharati Vidyapeeth (Deemed to be University) Medical College and Hospital, Sangli. 416414 Contact No :9766417420 Email :itsshabbir@gmail.com	Scientist from outside the Institute
4) Mr. Atul Ramchandra Chopade Dept of Pharmacology, Rajarambapu College of Pharmacy, Kasegaon, Tal: Walwa, Dist. Sangli – 415404, Maharashtra Contact No :9226346106 Email :chopadearv@gmail.com	Socially Aware Nominee

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

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

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

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

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

Yours Sincerely,

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

Copy for necessary action to: Nominees of CPCSEA.

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