A COMPARATIVE STUDY OF EFFECTS OF ALPHA LIPOIC ACID, CHROMIUM PICOLINATE AND HYDROXYCHLOROQUINE ON BLOOD SUGAR IN STREPTOZOCIN INDUCED TYPE2 DIABETIC MODEL

Thesis

FOR THE DEGREE OF

Doctor of Medicine

(PHARMACOLOGY & THERAPEUTICS)

RANCHI UNIVERSITY, RANCHI

2020-2023

DR. RAJIV RANJAN DAS

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Dedicated To

MY

Parents

Patients

Teachers

Students

To My Eternal Father, My Guru, their incarnation in form of Grandparents, Parents, Teachers for their enlightenment throughout my life; No individual can achieve his/her ambition without the support of others. I would like to thank everyone who was important to the successful finalization of thesis as well as expressing my apology that I couldn't mention one by one.

For so many people who have played a role in my life as a counsellor, supporter, teacher, friend, I express my deepest appreciation from the core of my heart."

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS

RAJENDRA INSTITUTE OF MEDICAL SCIENCES

RANCHI

CERTIFICATE

This is to certify that the present work embodied in this thesis entitled **"A Comparative Study of effects of Alpha Lipoic Acid, Chromium Picolinate and Hydroxychloroquine on blood sugar in Streptozocin induced Type 2 Diabetic Model."** for the degree of Doctor of Medicine (Pharmacology and Therapeutics), Session 2020-2023, has been carried out independently by **Dr. Rajiv Ranjan Das** in this department under my direct guidance and supervision.

This thesis is hereby approved for submission to Ranchi University for the degree of Doctor of Medicine (Pharmacology and Therapeutics),

Session 2020-2023.

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DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS

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DECLARATION

I Dr. Rajiv Ranjan Das, Junior Resident, Department of Pharmacology RIMS Ranchi (session 2020-2023) do hereby declare that this thesis entitled **"A Comparative Study of effects of Alpha Lipoic Acid, Chromium Picolinate and Hydroxychloroquine on blood sugar in Streptozocin induced Type2 Diabetic Model."** is my own work and has been prepared by me under the guidance and supervision of my Guide Dr. (Prof) Uma Shanker Prasad Keshri, and Co Guide Dr. Anupa Prasad.

Proper care and handling of laboratory animals has been done in accordance with ethical norms approved by Institutional Animal Ethics Committee (IAEC) Guidelines. Permission has been sought for, in written from the head of the institution and institutional animal ethics committee, RIMS Ranchi.

I also declare that the information reported in this study is the result of my own work, except where due reference is made and no part of this study has been previously published or submitted for any degree or diploma of any university.

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Junior Resident Academic

Department of Pharmacology and Therapeutics.

Rajendra Institute of Medical Sciences,

Ranchi.

(Session 2020-2023)

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Dr. Rajiv Ranjan Das

ABBREVIATIONS

AMPK	Activated protein kinase
AGPAT	Acylglycerol phosphate acyltransferase
ADA	American Diabetic Association
ALA	Alpha lipoic acid
ANOVA	Analysis of variance
AST	Aspartate Transaminase
ATP	Adenosine triphosphate
AI	Atherogenic Index
BBB	Blood brain barrier
BLK	B Lymphocyte Kinase
CETP	Cholesteryl ester transfer protein
CI	Confidence interval
CM	Chylomicrons
CPCSEA	Committee for the Purpose of Control and Supervision on Experiments on Animals
CQ	Chloroquine
Cr	Creatinine
CrP	Chromium picolinate
CRP	C-reactive Protein
CHD	Coronary heart disease
DM	Diabetes Mellitus
DNA	-Deoxyribonucleic acid
DKA	Diabetic ketoacidosis
DR	Diabetic Retinopathy
DS	Disease
DW	Distilled water
ECM	Extracellular Matrix
FBG	Fasting Blood Glucose
ET-1	Endothelin 1
FFA	Free fatty acids
GATA	Globin transcription factor
GAD	Glutamic acid decarboxylase
GIP	Gastric inhibitory polypeptide
GDM	Gestational Diabetes Mellitus
GFR	Glomerular Filtration Rate
GHb	Glycosylated hemoglobin
GTF	Glucose tolerance factor
GLUT	Glucose transporter
GSH	Reduced Glutathione
GSH-Px	Glutathione peroxidase
GSSG	Oxidized Glutathione
HbA1c	Hemoglobin A1c
HHS	Hyperglycemic hyperosmolar syndrome
H&E	Hematoxylin and Eosin
HCQ	Hydroxychloroquine
HMG-CoA-	Hydroxy B-methyl glutaryl CoA
HSD	Honestly significant difference
HDL	High density lipoprotein
HLA	Human leucocyte antigen
HNF	Hepatocyte nuclear transcription factor
IBM	International Business Machines
IAEC	Institutional Animal Ethics Committee
ICA	Islet cell antigen
IDL	Intermediate Density Lipoprotein
IFN	Interferon
IGF	Insulin like Growth Factors
IGT	Impaired Glucose Tolerance
IL	Interleukin
IP	Intraperitoneal
IV	Intravenous

IM	Intramuscular
LDH	Lactate Dehydrogenase
LDL	Low density lipoprotein
M	Million
MAPK	Mitogen-activated protein kinase
MCP-1	Monocyte chemoattractant
m-TOR	Mammalian target of rapamycin
MODY	Maturity Onset Diabetes of Young
NMR	Nuclear Magnetic Resonance
NA/NIT	Nicotinamide
NPH	Neutral Protamine Hagedorn
NIDDM	Non-insulin-dependent diabetes mellitus
NADH	Nicotinamide Adenine Dinucleotide
NO	Nitric Oxide
OGTT	Oral glucose tolerance test
PAX	Paired-box-containing gene
PEK	Pancreatic eIF2-alpha kinase
PAI	Plasminogen Activator Inhibitor
PCOS	Polycystic ovary syndrome
PDGF	Platelet-Derived Growth Factor
PKC	Protein Kinase C
PVS	Polyvinyl sulfonic acid
PPARY	Peroxisome proliferator-activated receptor y
RBS	Random blood sugar
RAS	Renin-angiotensin system
SUR	Sulfonylurea
SES	Socio economic status
SGLT 2	Sodium glucose co-transporter 2
SOD	Superoxide Dismutase
STZ	Streptozotocin
TC	Total Cholesterol
TLR	Toll like Receptor
TNF	Tumor Necrosis Factor
TG	Triglyceride
TGF	Transforming Growth Factor
US	United States
UKPDS	The UK Prospective Diabetes Study
VEGF	Vascular Endothelial Growth factor
VLDL	Very low-density lipoprotein
WHO	World Health Organization
XO	Xanthine oxidase
ZNT	Zinc transporter

AIMS & OBJECTIVES

Alpha lipoic acid, Chromium picolinate, Hydroxychloroquine are emerging therapeutic modalities in the management of diabetes mellitus. The aims and objectives of this study were:

AIM: To explore the therapeutic effects of Alpha lipoic acid, Chromium picolinate, Hydroxychloroquine drugs on glucose and lipid metabolism and to compare them with Metformin in management of diabetes mellitus and its comorbidities (dyslipidemia, nephropathy) in experimental model of diabetic rats.

The **objectives** of this study were: -

1. To study the effect of Alpha Lipoic Acid on Weight Gain, Random Blood Glucose, Lipid Profile, Urea, Creatinine, and SGPT of streptozocin nicotinamide induced diabetic rat model.
2. To study the effect of Chromium Picolinate on Weight Gain, Random Blood Glucose, Lipid Profile, Urea, Creatinine, and SGPT of streptozocin nicotinamide induced diabetic rat model.
3. To study the effect of Hydroxychloroquine on Weight Gain, Random Blood Glucose, Lipid Profile, Urea, Creatinine, and SGPT of streptozocin nicotinamide induced diabetic rat model.
4. To compare the effects of Alpha Lipoic Acid, Chromium Picolinate and Hydroxychloroquine with Metformin on Weight Gain, Random Blood Glucose, Lipid Profile, Urea, Creatinine, and SGPT of streptozocin nicotinamide induced diabetic rat model.

1.0 INTRODUCTION

The world as we know is in an urgency for development. The technological revolution has brought both good and bad impacts on our society. On one hand it has made healthcare facilities better but on the other hand burdened us lifestyle-based disease. Diabetes Mellitus is one of the largest global health care emergencies of this country, reaching among 10 leading causes of mortality and morbidity together with CVD, respiratory disease and cancer. Over 3 in 4 adults with diabetes mellitus live in low- and middle-income countries.

"It is a syndrome of disordered metabolism and inappropriate hyperglycemia due to either a lack of insulin secretion or to a combination of insulin resistance and inadequate secretion to compensate for the resistance" [3]

Diabetes Mellitus is defined as an elevated blood glucose associated with absent or inadequate pancreatic insulin secretion, with or without concurrent impairment of insulin action. The term diabetes mellitus describes "a metabolic syndrome of multiple etiology characterized by chronic hyperglycemia with disorders of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both". It is a disorder primarily defined by the level of hyperglycemia giving rise to risk of microvascular damage (retinopathy, nephropathy and neuropathy.) [2]

It is linked to a shorter life expectancy, notable morbidity from particular microvascular diseases connected to diabetes, an elevated risk of macrovascular complications (ischemic heart disease, stroke, and peripheral vascular disease), and a lower quality of life. [4]

1.01 Epidemiology and global considerations

With an estimated 74.2 million cases currently, India accounts for 49% of the global diabetes burden by 2045, that number is projected to nearly quadruple to 124.9 million cases. However, due to the fact that many people are asymptomatic, ignorant of their condition, and fail to receive a diagnosis, "prevalence is significantly larger than this estimation." This is responsible for an additional one-third of estimated instances. [1]

The prevalence of diabetes around the world raises the risk of microvascular and macrovascular problems. Patient's morbidity has grown as a result of this. According to the International Diabetes Federation (IDF) 2021, there are currently more than 537 million people suffering from diabetes mellitus worldwide, with a prevalence of 10.5%. It is predicted that by 2030 and 2045, their numbers will increase to 643 million and 784 million, respectively, with prevalence of 11.3% and 12.2%. [1] Diabetes is thought to be responsible for 6.7 million deaths worldwide. The International Diabetes Federation (IDF) 2021 reported that, at present, approximately USD 966 billion is the total health expenditure and it is estimated that the expenditure will grow by USD 1028 billion and USD 1054 billion, respectively by 2030 and 2045. [1]

We are compelled to consider new options with fewer or no side effects and maximal therapeutic benefits due to the rising burden of diabetes and its complications due to the incapacity and death caused by this illness as well as the different adverse effects of currently available antihyperglycemic medicines. Additionally, the prevention of comorbidities necessitates the use of multiple medications, which results in polypharmacy and lower patient compliance. Additionally, in a developing nation like India, most places still lack access to inexpensive contemporary medical treatment due to financial limitations. The "Asian Indian phenotype," which includes a tendency to excess visceral adiposity, dyslipidemia with low high-density lipoprotein (HDL) cholesterol, elevated serum triglycerides (TG), and increased

small, dense low-density lipoprotein (LDL) cholesterol, as well as an increased ethnic susceptibility, makes the Asian Indians more prone to T2DM. [150] Thus, it is necessary to look for new and more efficacious drugs. Metformin is considered to be a standard drug which is usually prescribed by physicians hence, we have chosen it for my dissertation as a standard drug.

1.02 Interventional drugs

Metformin(MET) [5,62,68,151] (Standard drug) A biguanide, that lowers insulin resistance by triggering AMP-dependent protein kinase (AMPK). In STZ-induced diabetic rat, metformin lowers blood glucose levels through increasing ß-endorphin production from the adrenal glands, which stimulates opioid u-receptor linkage and increases GLUT-4 gene expression while decreasing PEPCK gene expression [151]. Lactic acidosis, bloating, nausea, metallic taste, mild diarrhea, exhaustion, and vitamin B12 deficiency are a few of the side effects of metformin.[5] With so many side effects of presently available drugs in market especially metformin, we have chosen three drugs with different mechanism of action and lesser side effects to prove as an alternative to current standard.

Hydroxychloroquine (HCQ) [6-7,67-97] Inhibiting insulin breakdown, reducing inflammation, increasing insulin sensitivity, and preservation of beta-cell are novel anti-diabetic actions. It also has antiplatelet, antithrombotic, and lipid-lowering properties. Hepatoprotection and nephroprotection are benefits. [6]. It assists in lowering cardiovascular catastrophes. Our studies on this medication and discovery of its new mechanism in diabetes were aided by its persistent usage in rheumatoid arthritis patients and my observation of its hypoglycemic effects during covid treatment. In 2014, DCGI approve this medication as a supplement to diabetic care. Its fundamental function has been observed in "Resistant Diabetes," a condition in which blood sugar cannot be controlled by using two or more conventional treatments.[7]

Alpha Lipoic acid(ALA) [8-11,98-102] Alpha-lipoic acid, commonly referred to as 1, 2-dithiolane-3-pentanoic acid or thioctic acid, is an universal antioxidant. It translocates GLUT-1 and GLUT-4 to plasmatic membrane of skeletal muscle and adipocytes [8-9] It enhances the activity of insulin signaling pathway protein. Due to these processes, it directly activates lipid, tyrosine/serine/threonine kinases in target cells, stimulating glycogenesis and glucose absorption. Endogenous antioxidants such glutathione, vitamin E, vitamin C, and coenzyme Q10 are restored by ALA. [10-11]

Chromium picolinate(CrP) [12,103-127] A study in 1989 recommended that chromium(III) picolinate may assist in weight loss and increase muscle mass which led to an increase in the usage of chromium(III) picolinate supplements.[152] It has been found to effectively improve insulin binding, receptor number and insulin receptor phosphorylation. It improves glucose tolerance by reducing insulin resistance (hence, called Glucose tolerance factor). Appropriate chromium nutrition improves lipid profile and insulin action. It increases insulin sensitivity, beta cell sensitivity and insulin internalization. [12]

Purpose of study : As far as we are aware, no research has been done to examine the effects of alpha lipoic acid, chromium picolinate, and hydroxychloroquine on both the lipid profile, SGPT and renal function test in diabetic rats. Consequently, the purpose of this study was to determine whether a single interventional therapy could prevent the comorbidities in an albino rat model of T2DM caused by streptozotocin nicotinamide. Despite the fact that insulin was discovered 100 years ago, "Rampant Diabetes" is still a pandemic and a burden on society. Together with other co-morbidities, it creates a “Lethal Nexus”. The three medications we've chosen, hydroxychloroquine, chromium picolinate, and alpha lipoic acid, may prove to be a suitable substitute for existing antihyperglycemic medications with known drawbacks for the prevention of both diabetic complications and insulin resistance and assist society in fighting against such "Man-made Pandemic."

REVIEW OF LITERATURE

2.0 Diabetes Mellitus: A Historical Review: -Past, Present, Future [13-16]

Diabetes mellitus has been recognized since antiquity. However, during that period, because of poor communication and little knowledge of human body the disease remained enormously mystifying to physicians. The word Diabetes is derivative of the *Greek* word *siphon* which suggests 'to pass through.' The *Latin* word *mellitus* means 'honey or sweet'. Aretaeus of Cappadocia, an ancient Greek Physician has described Diabetes Mellitus as: "..... *no essential part of the drink is absorbed by the body while great masses of the flesh are liquefied into Urine*" / "*liquefaction of the flesh and bones into urine*" ….. This is often for the reason that in diabetes excess sugar is found in both blood and urine. In the 17th century, it was known as "pissing evil" that is a therapeutic condition producing too much thirst, unremitting urination and unadorned weight loss. The Indian Sanskrit medicine literature, between the period of 300-600 BC. has mentioned about the sweet urine in Diabetes. The ancient physicians observed ants and insects rushing to this type of urine, and hence termed it as "sugar cane urine" (Iksumeha) or "honey urine" (Madhumeha and Hastimeha). The Chinese physicians have also described Diabetes 2000 years ago as "Xiao-ke" (wasting thirst or emaciation and thirst). Regrettably, until the initial part of twentieth century the forecast for a patient with this condition was no better than it was over 4000 years ago. Nevertheless, physicians in antiquity observed the distinctive features of diabetes and proposed several therapeutic methods. History of diabetes is still being written.

Fig: 2.1 The distinguished physician Aretaeus of Cappadocia 164AD. (Source: Wellcome Library, London). [17]

Fig: 2.2 Portrait of the French physiologist Claude Bernard 1850. (Source: Wellcome Library, London). [17]

2.0.1: Epidemiology [1]

In India diabetes is attaining the stats of a potential epidemic with 74.2M people suffering as per IDF 2021 report. The countries with the maximum number of people with undiagnosed diabetes are China, India (39.4million) and Indonesia. Still India accounts for 1 in 7 of all adults living with diabetes worldwide ,1 in 4 live births is suffering from hyperglycemia in pregnancy. India comes third with 0.6million(M) death in world after China (1.4M) and USA (0.7M). India now has the highest estimated number of prevalent type 1 diabetes mellitus cases in people under 20yrs of age (229,400) followed by the USA (157,900) and Brazil (92,300). [1] The incidence of T2DM is extremely low among pre-pubertal children but rises gradually at puberty, likely because of hormonal changes and insulin resistance associated with puberty. The countries with the largest number of adults with diabetes aged 20-79yr in 2021 are China, India (61.3M in 2011 to 74.2M in 2021) and Pakistan, they are anticipated to remain so in 2045.[1] A study conducted by the Indian Council of Medical research (ICMR) revealed that a lower proportion of the population is affected in states of Northern India (Chandigarh 0.12 million, Jharkhand 0.96 million) as compared to Maharashtra (9.2 million) and Tamil Nadu (4.8 million). [157]

South-East Asia

Country or territory	Number of adults 20–79 years with diabetes in 1,000s (95% confidence interval)	Diabetes prevalence (%) in adults 20–79 years (95% confidence interval)	Age-adjusted comparative diabetes prevalence (%) in adults 20–79 years (95% confidence interval)	Number of adults 20–79 years with undiagnosed diabetes in 1,000s (95% confidence interval)	Diabetes-related expenditure (USD) per person with diabetes (20–79 years)
South-East Asia –SEA	90,204.5 (76,848.0-99,900.5)	8.7 (7.4-9.7)	10.0 (8.6-11.1)	46,230.3 (39,636.6-51,243.3)	112.0
Bangladesh	13,136.3 (8,946.2-13,493.6)	12.5 (8.5-12.8)	14.2 (10.0-14.6)	5,712.3 (3,890.2-5,867.6)	76.5
Bhutan	44.8 (39.5-49.3)	8.8 (7.8-9.7)	10.4 (9.3-11.4)	19.5 (17.2-21.4)	194.7
India	74,194.7 (65,654.0-82,861.1)	8.3 (7.3-9.3)	9.6 (8.5-10.6)	39,397.4 (34,862.3-43,999.3)	114.4
Maldives	27.0 (26.4-63.2)	6.7 (6.5-15.6)	9.2 (9.0-22.6)	11.8 (11.5-27.5)	1,867.4
Mauritius	250.4 (98.0-257.6)	26.5 (10.4-27.3)	22.6 (9.2-23.4)	89.3 (34.9-91.9)	588.4
Nepal	1,133.5 (972.4-1,289.2)	6.3 (5.4-7.2)	8.7 (7.4-9.9)	492.9 (422.8-560.6)	102.2
Sri Lanka	1,417.6 (1,111.6-1,886.4)	9.8 (7.7-13.1)	11.3 (9.1-14.5)	507.2 (397.7-675.0)	201.6

Fig 2.3 Showing Prevalence of Diabetes Mellitus within South East Asia [1]

At a glance	2021	2030	2045
Total world population	7.9 billion	8.6 billion	9.5 billion
Adult population (20–79 years)	5.1 billion	5.7 billion	6.4 billion
Diabetes (20–79 years)			
Prevalence[i]	10.5%	11.3%	12.2%
Number of people with diabetes	536.6 million	642.7 million	783.2 million
Number of deaths due to diabetes	6.7 million	–	–
Total health expenditure due to diabetes[ii] (2021 USD)	USD 966 billion	USD 1,028 billion	USD 1,054 billion
Hyperglycaemia in pregnancy (20–49 years)			
Proportion of live births affected[iii]	16.7%	–	–
Number of live births affected	21.1 million	–	–
Impaired glucose tolerance (20–79 years)			
Prevalence[i]	10.6%	11.0%	11.4%
Number of people with impaired glucose tolerance	541.0 million	622.7 million	730.3 million
Impaired fasting glucose (20–79 years)			
Prevalence[i]	6.2%	6.5%	6.9%
Number of people with impaired glucose tolerance	319.0 million	369.7 million	440.8 million
Type 1 diabetes (0–19 years)			
Number of children and adolescents with type 1 diabetes	1.2 million	–	–
Number of newly diagnosed cases each year	184,100	–	–

i Prevalence is standardised to each national population for the respective year
ii Health expenditure for people with diabetes is assumed to be on average two-fold higher than people without diabetes
iii Prevalence is standardised to world population for the respective year

Fig-2.4 Showing present & future of Diabetes Mellitus population in world. [1]

India nearly tops the chart in many places, hence got its nickname as "विश्वस्य मधुमेहराजधानी/ Diabetic capital of the world".[1]

2.0.2 Milestones in Diabetes

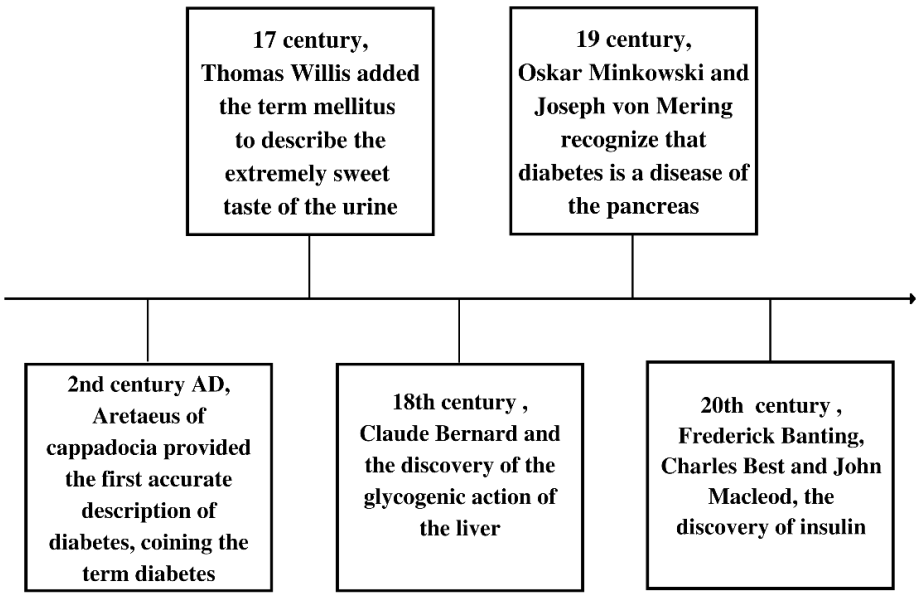


Fig: 2.5 Source: Karamanou M et al. Protagonists in diabetes mellitus [17]

For quite 4000 years physicians searched for the causes and treatment of diabetes mellitus. However, significant progress has been made over the past couple of centuries as a result of the evolution of physics, chemistry and biology. The milestones are summarized in a table at the end of my Dissertation in Annexure 1

Fig: 2.6a The Nobel laureate Frederick Banting in his laboratory with a dog, and 1st INSULIN vial (Source: Wellcome Library, London) [17]

Fig: 2.6b LEONARD THOMPSON: First patient to receive insulin (Source: Wellcome Library, London) [17]

2.1 Diabetes Mellitus: Introduction

2.1.1 Diabetes Mellitus: Definition

"Diabetes is a cluster of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action or both. The chronic hyperglycaemia of diabetes is related to long-term damage, dysfunction and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels," [18]

2.1.2 Classification of Diabetes Mellitus [19-20,28,29]

Diabetes mellitus is often classified into the subsequent general groups:

Table: -1 Etiologic Classification of Diabetes Mellitus [19-20]
1.Type 1 Diabetes Mellitus (Immune-mediated beta cell destruction usually leading to absolute insulin deficiency).
2. Type 2 Diabetes Mellitus (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly insulin secretory defect with insulin resistance).

3. Specific types of Diabetes Mellitus (monogenic or MODY)
A) **Genetic defects of beta cell development or function characterized by mutations in**:
a) Hepatocyte nuclear transcription factor (HNF) 4 alfa
b) Glucokinase
c) Hepatic nuclear factor 1 alfa
d) Insulin promoter factor- 1(IPF-1), HNF-1beta, Neuro-D1 And other pancreatic islet regulators/proteins like KLF 11, PAX 4, BLK, GATA 4, GATA 6, SLC2A2(GLUT-2), RFX 6, GLIS 3
e) Subunits of ATP sensitive $K^{+\text{ channel}}$ resulting in permanent neonatal diabetes
f) Mitochondrial DNA
g) Insulin, resulting in permanent neonatal diabetes.
B) **Genetic defects in insulin action**
a) Type A insulin resistance
b) Leprechaunism
c) Rabson Mendenhall syndrome
d) Lipodystrophy syndrome
C) **Diseases of the exocrine pancreas** – Pancreatitis, Pancreatectomy, Neoplasia, Cystic fibrosis, Hemochromatosis, Fibrocalculus pancreatopathy, mutations in carboxyl ester lipase.
D) **Endocrinopathies** – Somatostatinoma, Acromegaly, Cushing's syndrome, Glucagonoma, Pheochromocytoma, Aldosteronoma Hyperthyroidism.
E) Infections - Congenital rubella infection, Cytomegalovirus, Coxsackievirus.
F) **Drug or chemically induced**- Glucocorticoids, Vacor (rodenticide), Pentamidine, Diazoxide, Beta adrenergic agonists, Thiazides, Calcineurin and m-TOR inhibitors, Hydantoins, Asparaginase, Alfa-interferon, Protease inhibitors, Antipsychotics (atypical and others), Epinephrine, nicotinic acid.
G) **Uncommon form of immune mediated Diabetes Mellitus** - "Stiff-person" syndrome, anti-insulin receptor antibodies.
H) **Other genetic syndromes** – Wolfram's syndrome, Down's syndrome, Klinefelter's syndrome, Turner's syndrome, Friedreich's ataxia, Huntington's chorea, Laurence Moon Biedl Syndrome, myotonic muscular dystrophy, Prader –Willi Syndrome, Porphyria.
I) Transient neonatal diabetes.
4. **Type 4 Diabetes Mellitus**: Gestational Diabetes Mellitus (GDM).

2.1.3 Type 1 Diabetes Mellitus(T1DM) [153]

T1D is a diverse condition characterized by the death of pancreatic beta cells, which results in a complete lack of insulin. The onset of humoral and cellular islet autoimmunity as well as poor immunoregulation are associated with type 1 diabetes. [21]

Acute onset T1DM(children <15yrs) manifests in middle age with apparent T2DM but have evidence of autoimmunity as assessed by GAD antibody levels which ultimately become insulin-dependent. This condition is known as adult-onset latent autoimmune diabetes (LADA). [23]

2.1.3.1 Manifestation of T1 DM (By Age, Sex, Place and Time)

Children under the age of five are more likely than older children to develop T1DM. T1DM incidence data have been standardized by the World Health Organization (WHO), DIAMOND Study (Multinational Project for Childhood Diabetes) and the EURODIAB ACE Study. [23] Data on the incidence or prevalence of T1DM in India are scarce. According to a study from Chennai, it occurs 10.5 times out of every 100,000 patient years. Indian children in the UK were found to have a high incidence of T1DM (14.7/100,000 patient years), despite the fact that this rate was lower than that of non-Asians (21.5/100,000 patient years). It has been hypothesized that India has one of the highest numbers of new T1DM patients each year given that roughly 40% of the population is under 20. [1]

The prevalence of GAD antibodies (42%) are comparable to that found in white Caucasians in a study of children from North India with recently diagnosed T1DM, although IA2 frequency is significantly lower (33% vs. 70%). [1] In contrast to the 5–10% reported in European individuals, both GAD and IA2 antibodies were lacking in 45% of patients with recent onset diabetes. A low incidence of IA2 antibody (17%) is also reported in a study of Indian children with T1DM in the UK. According to research from South India, GADA was present 48% in prevalence. Additionally, patients with T1DM who were of European Caucasian descent had a decreased incidence of ZnT8 antibodies (30% vs. 65%). The frequency of autoantibody negative (Type 1B) diabetes in Indian patients is therefore significantly greater (28%) than in studies compared to that of Europe and the United States (5%), according to the measurement of all four islet autoantibodies (GAD, 1A2, IAA, and ZnT8). [23]

2.1.3.2 Pathogenesis of T1DM

T1DM is a chronic autoimmune condition marked by the autoimmune destruction of the pancreatic β-cells that secrete insulin. This leads in inadequate insulin secretion, which causes the metabolic abnormalities typical of IDDM. The pancreatic α-cells also lose their ability to operate, which causes them to secrete too much glucagon. In the absence of insulin treatment, the resulting hyperglycemia eventually causes diabetic ketoacidosis. Lack of insulin causes unchecked lipolysis, which raises the plasma level of free fatty acids and further inhibits the metabolism of glucose in the peripheral tissues. Utilization of glucose is compromised. A number of genes, including coding of glucokinase in the liver and GLUT-4 transporters in adipose tissue, whose expression is required for target tissues to respond to insulin in a normal manner are expressed less often. [154]

2.1.3.3 Human Leukocyte Antigen Association in Indian T1DM

The HLA associations in North Indian T1DM patients are often the same as those seen in white Caucasians. The strongest relationship is identified with DR3, not DR4, in contrast to findings from the West. According to Orrisa data, patients with T1DM had significantly higher associations with DRB1*03 and DQ2 (DQA1 501, DQB1*0201), but not with DR4 and DQ8. The connection of T1DM with DRB1*03, DQA1*05, and DQB1*02 was verified in association and family studies in research on north Indians who had immigrated to the United Kingdom. [23]

2.1.3.4 Mortality

Prior to the 1922 discovery of insulin, T1DM meant an almost definite early death. T1DM is linked to an approximately 2 to 10 fold increased risk of early death. [24]

2.1.3.5 Treatment of T1DM

Lifelong INSULIN medication is necessary for patients with type 1 diabetes mellitus. Most people need two or more insulin injections every day, with doses adjusted based on personal blood glucose monitoring. Treatment is covered in Table 2 later.

2.1.4. Type 2 Diabetes Mellitus (T2DM)

Insulin resistance in peripheral tissue and a beta cell malfunction in insulin secretion are two features of T2DM. 90–95% of all cases of diabetes mellitus are caused by T2DM**,** commonly known as "non-insulin dependent diabetes" or "adult-onset diabetes" in the past. In contrast to T1DM, peripheral insulin resistance is present together with a relative (but incomplete) insulin deficit [25]. Risk factors are covered in depth in Annexure 2.

Insulin resistance is a consequence of being overweight or having more visceral fat. These patient's insulin production is flawed and insufficient to compensate their insulin resistance. The typical diabetes mellitus symptoms are not severe at first because hyperglycemia develops gradually. However, the danger of macrovascular and microvascular consequences is substantial if the condition remains undetected. [23]

The liver, muscles, and pancreas are the three main organs that are altered by the metabolic abnormalities seen in T2DM. Increased hepatic output, which is a characteristic of nearly all individuals with fasting hyperglycemia, is seen at the level of the liver. The majority of the glucose is taken up by muscle during an insulin-stimulated state, and insulin resistance in target tissues is widely documented. Reduced β-cell activity and increased glucagon secretion are hallmarks in the pancreas. [23]

Individuals exposed to longer periods of hyperglycemia will undoubtedly have increased risks of developing vascular complications related to diabetes [1]

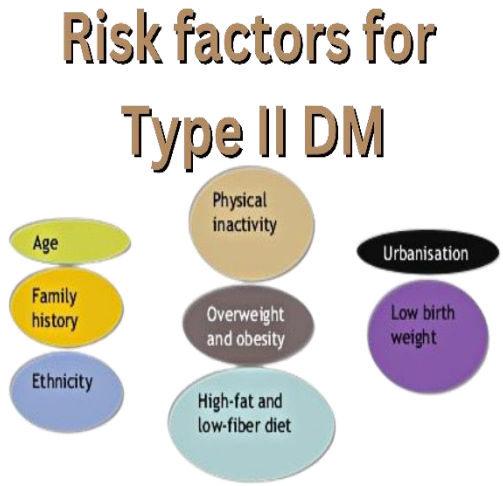


Fig: 2.7A (Risk Factors Associated with Type 2 DM)

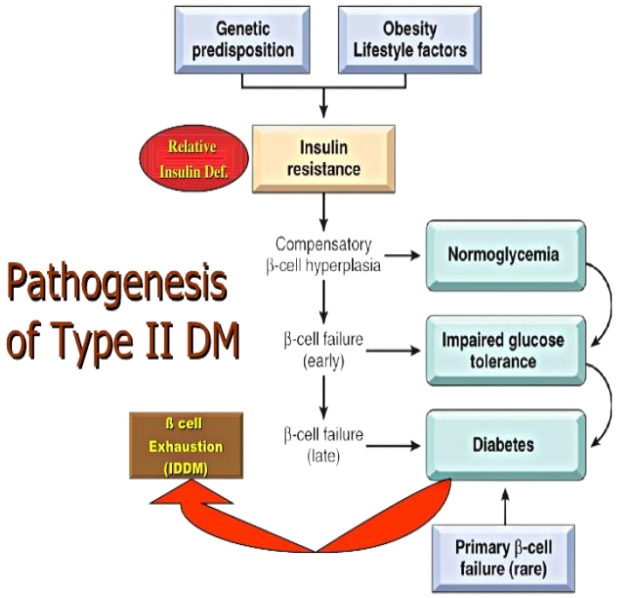


Fig: 2.7 B (Pathogenesis of Type2 DM)

2.1.4.1 Recent Emerging Risk Factors

a) Decreased Sleep

Short sleep duration is now understood to be a risk factor in addition to dietary changes and levels of physical activity. The precise mechanism by which sleep deprivation increases the risk of developing diabetes is unknown, but it may be related to sympathetic nervous system activation, a reduction in the brain's ability to use glucose, changes in the hypothalamic-pituitary-adrenal axis, or another neuroendocrine dysregulation. [26]

b) Drug - Induced Metabolic Changes

Beta-blockers can affect insulin production, and high dose thiazide diuretics have been shown to worsen insulin resistance. The usage of second-generation (atypical) antipsychotics has been connected to diabetes and hyperglycemia. Protease inhibitors and nucleoside reverse transcriptase inhibitors have been linked to insulin resistance, abnormal lipid and glucose metabolism, and a higher risk of type 2 diabetes (T2DM). [27]

c) Environmental Pollutants

Serum concentrations of chlorinated persistent organic pollutants are strongly cross-sectionally associated with both diabetes and elements of the metabolic syndrome. Another type of organic contaminants, that are linked to diabetes is brominated flame retardants. These environmental chemicals are thought to build up in adipose tissue and function as endocrine disruptors, causing disruption of lipid and glucose metabolism. [30,20]

D) Low Birth Weight and Fetal Malnutrition

An association between the intrauterine environment, fetal malnutrition, and the risk of diabetes and cardiovascular disease in later life is now more strongly supported by the available data. Rapid postnatal growth, low infant birth weight, and maternal undernutrition have all been linked to an increased risk of diabetes in the progeny. By creating a vicious cycle of "*diabetes begetting diabetes*," the rising number of women with young-onset diabetes is likely to worsen the diabetes epidemic. [30,20]

2.1.4.2 Treatment Type 2 Diabetes Mellitus:

Treatment of T2DM consist of both non-pharmacological and anti-diabetes pharmacotherapy. Former has been discussed later in a form of table in Annexure 7

Table: - 2 Summary of medications for Type 2 Diabetes (Source: Diabetes Bootcamp) [24]

Summary of Medications for Type 2 Diabetes

DIABETES MEDICATIONS			
Class of Medication	**Common Name**	**Actions**	**Potential for Hypoglycemia**
Biguanides	Metformin (Glucophage)	Reduces the amount of glucose released by the liver and decreases insulin resistance by muscle cells.	Not when used as monotherapy.
Alpha-glucosidase inhibitors	Acarbose (Precose) Miglitol (Glyset)	Slows digestion and absorption of dietary carbohydrate.	Increased risk when used in combination with insulin or sulfonylurea agents. Must treat hypoglycemia with dextrose.
Thiazolidinediones	Pioglitazone (Actos) Rosiglitazone (Avandia)	Increases insulin sensitivity, decreases liver glucose output. Takes 12 or more weeks to achieve maximal effectiveness.	Increased risk when used in combination with insulin or insulin secretagogues.
Dipeptidyl peptidase 4 (DPP-4) inhibitors	Sitagliptin (Januvia) Saxagliptin (Onglyza) Linagliptin (Tradjenta) Alogliptin (Nesina)	Slows the inactivation of glucagonlike peptide-1 (GLP-1) by the degradation enzyme DPP-4. Prolongs the action of GLP-1, thus increasing insulin secretion and decreasing glucagon secretion.	May increase risk with insulin secretagogues; not studied with insulin.
Sodium-glucose cotransporter-2 inhibitors	Canagliflozin (Invokana) Dapagliflozin (Farxiga) Empagliflozin (Jardiance)	Inhibits glucose reabsorption in the kidneys.	Low risk when used as monotherapy.
Sulfonylureas	Glimepiride (Amaryl) Glipizide (Glucotrol) Glyburide (Micronase, Glynase)	Stimulates the beta cells of the pancreas to increase insulin production.	Yes
Meglitinides	Repaglinide (Prandin) Nateglinide (Starlix)	Stimulates the beta cells of the pancreas to increase insulin production. They are shorter acting than sulfonylureas.	Yes
Incretin mimetics (injectable)	Exenatide (Byetta) Exenatide LAR (Bydureon) Liraglutide (Victoza) Dulaglutide (Trulicity)	Mimics the effect of incretin hormones to increase insulin secretion in the presence of elevated blood glucose levels, decreases glucagon production, slows gastric emptying, and improves first-phase insulin response.	Often used in combination with insulin or sulfonylureas, which may result in hypoglycemia.
Amylin analogues (injectable, taken with mealtime insulin but as a separate injection)	Pramlintide (Symlin)	Slows gastric emptying (therefore delays glucose from entering the bloodstream), suppresses glucagon.	Yes
Insulin (injectable)	Long-acting insulin: Glargine (Lantus) Detemir (Levemir) Intermediate-acting insulin: NPH (Humulin R, Novolin R) Short-acting insulin: Regular (Humulin N, Novolin N) Rapid-acting insulin: Lispro (Humalog) Aspart (NovoLog) Glulisine (Apidra)	Increases the passage of glucose from the bloodstream into the cells and decreases the production of glucose by the liver.	Yes Understanding when the insulin peaks as well as its onset and duration of action can help prevent hypoglycemic episodes.

* Not a complete list

RESOURCES

1. MENSING CE. *THE ART & SCIENCE OF DIABETES SELF-MANAGEMENT EDUCATION DESK REFERENCE*. 3RD ED. CHICAGO: IL: AMERICAN ASSOCIATION OF DIABETES EDUCATORS; 2014.
2. NAUCK MA. UPDATE ON DEVELOPMENTS WITH SGLT2 INHIBITORS IN THE MANAGEMENT OF TYPE 2 DIABETES. *DRUG DES DEVEL THER*. 2014;8:1335-1380.

2.1.5 Other Types of Diabetes

Other causes of specific diabetes are endocrinopathies, drugs and viral infections:

2.1.5.1 Maturity - Onset Diabetes of The Young

Despite its young age of onset, Maturity Onset Diabetes of Young (MODY) is an autosomal dominantly inherited form of diabetes that is not insulin dependent. It results from cell dysfunction rather than insulin resistance. They exhibit normal insulin action but impaired insulin secretion. [18] In the pre-genomic era, MODY was the most prevalent kind of monogenic diabetes. A population prevalence of 100 per million for adults and 45 per million for children has been estimated by more recent studies. This means that MODY accounts for about 5% of diabetes cases discovered before the age of 45. Since the 1990s, when the molecular genetics of MODY was clarified, mutations in at least eleven separate genes have been discovered; the details of which are listed in Annexure 3.

2.1.5.2 Drug - Induced Diabetes Tabulated in Annexure 4

2.1.5.3 Endocrine Disorders that Cause Diabetes

Chronic hormonal overproduction that affects insulin secretion or action causes hyperglycemia. Because endogenous insulin secretion continues, it is distinguished by the absence of ketosis. Endocrine factors contribute to diabetes by inhibiting insulin secretion and acting as a counter-regulatory hormone. Although insulin is crucial for metabolic regulation, other hormones such glucagon, growth hormone (GH), corticosteroids, thyroxine, and adrenaline(epinephrine) can modify or balance its effects. In cases where insulin secretion is already insufficient, an excess of these counter-regulatory hormones may result in hyperglycemia and other metabolic abnormalities. Hyperglycemia is decreased and the diseases are treated by focusing on endocrinopathy (which is typically a tumour secreting hormones). [23] Causes tabulated in Annexure 5

2.1.5.4. Pancreatic Diseases and Diabetes

When Minkowski performed a pancreatectomy on a dog in 1889, the link between the pancreas and diabetes was established. Patients with pancreatic diabetes are sensitive to the effects of insulin and experience a loss of both pancreatic glucagon and insulin. A subsequent effect of an exocrine pancreatic dysfunction may be diabetes. In 1985, the WHO classified fibro calculus pancreatopathy as a "malnutrition linked diabetes," however it has now been moved to this category. A PEK gene defect that causes Wollcot-Rallison syndrome, which is

characterized by early onset diabetes and multiple epiphyseal dysplasia, is a severe form of exocrine insufficiency.[31] Causes tabulated in Annexure 6

2.1.5.5 LADA

"Groop et al. identified latent autoimmune diabetes in people for the first time in 1986. Some persons who presented with clinical indications of T2DM in 1993 had GAD antibodies. In various publications, LADA is also referred to as slowly progressing T1DM, latent T1DM, double diabetes, and type 1.5 diabetes. Many patients with a hybrid form of diabetes who have islet autoantibodies and genetic propensity to both T1DM and T2DM are not included in the ADA classification. Latent autoimmune diabetes in adults (LADA) is the name given to this type of diabetes. Because glutamic acid decarboxylase (GAD) autoantibodies are typically identified with insulinoma-associated antigen-2 (IA-2) autoantibodies but infrequently with LADA, it is generally defined by the presence of GAD autoantibodies. The use of GAD autoantibodies in the definition of LADA, however, lacks disease specificity because they are also present in T1DM. Positive T-cell response but a negative response to autoantibodies in sera identify one more subtype of adult-onset autoimmune diabetes. According to the World Health Organization, LADA is a type 1 diabetes, as opposed to patients who are antibody negative, these patients typically have T2DM at the time of their initial diagnosis but do not need insulin for at least the first six months as they slowly progress. This necessitates a new classification for diabetes. [23]

2.1.5.6 Malnutrition modulated diabetes mellitus (MMDM):

Patients with MMDM are typically from rural areas and have low SES. Poverty contributes to nutritional deficits in both pregnant women and their unborn and young children. Malnutrition-modulated diabetic mellitus patients showed DR3 and DQ2 in 20% of cases, but a larger percentage had DQ9, clearly separating them from T1DM. None of the patients under investigation were non-Asp homozygous, according to the DQ gene study, which revealed that 70% of the MMDM were homozygous for Asp-57, DQB1. In cases of MMDM, the HLA DR7-DQ2 negative correlation with T1DM is not shown. However, there was a strong correlation between this genotype and MMDM cases with negative antibody responses. Their relationship with the MICA alleles is a more convincing discovery that set MMDM apart from T1DM. Monocytes, keratinocytes, and endothelial cells all express MHC class I polypeptide-related sequence A (MICA), which is found in the MHC class I area. Trinucleotide repeat (GCT) microsatellite polymorphism M in exon 5 was discovered by sequence analysis of the MICA gene. There are five F GCT alleles that have 4, 5, 6, and 9

repetitions, or 5 GCT repetitions plus an extra nucleotide insertion (GGCT), respectively. A4, A5, A6, and A9 are the alleles. A study was planned to determine the relationship between MICA alleles and T1DM (n = 52) and MMDM (n = 41) patients from Cuttack, Eastern India, and healthy controls (n = 73). Polymerase chain reaction amplification was used to type MICA, and an ABI prism deoxyribonucleic acid sequencer was used to calculate the sizes of the fragments. When compared to controls, allele 9 of MICA is favorably and allele 4 is adversely related with MMDM. According to the aforementioned findings, T1DM and MMDM are immunogenetically distinct diseases in eastern India, and MICA plays a significant role in the etiology of MMDM in patients from Cuttack. [23]

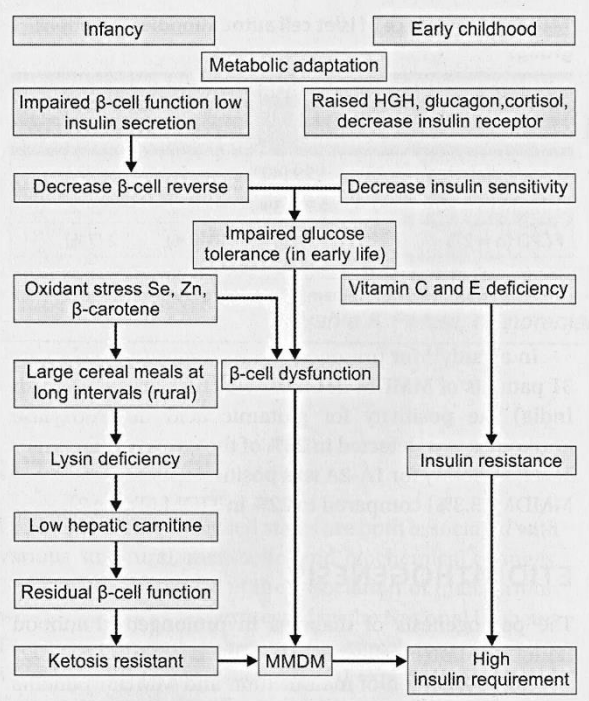


Fig: - 2.8 A Pathogenesis of diabetes in undernutrition.

- Onset by 30 years of age (mean 19.8 ± 9.7)
- Poor socioeconomic status
- Severe diabetes (FPG seldom less than 250 mg/dL)
- Absence of ketosis and ketonuria at diagnosis and on withdrawal of insulin for long period
- Persistent high insulin requirement most often over 60 U/day (2.0-3.0 U/kg).
- Absence of radiographic or sonographic evidence of pancreatic calculi, ductal dilatation or fibrosis, and laboratory evidence of pancreatic exocrine dysfunction
- Invariable rural origin (35 years of observation)
- History of malnutrition in infancy and early childhood and maternal undernutrition during pregnancy
- Leanness, BMI less than 17.0 kg/m²

Fig: - 2.8 B Clinical features of MMDM

2.1.5.7 Prediabetes: It may be a prediabetic state when the plasma glucose is higher than usual but not high enough to rule out diabetes. Impaired glucose tolerance (IGT) or impaired fasting glucose (IFG) would be symptoms of this. FPG less than 126 mg/dL (7.0 mmol/L), 2 hours post OGTT greater than or equal to 140 mg/dL (7.8 mmol/L), but less than 200 mg/dL (11.05 mmol/L) are considered to have impaired glucose tolerance. 100-125 mg/dL (6.1-6.9 mmol/L) is the threshold for impaired fasting glucose.

Glucosuria: Due to the test's lack of sensitivity, measuring urine glucose is not advised for the diagnosis of diabetes. However, because glucosuria can be caused by a failure in renal

tubular function, patients who have glucose in their urine need to have blood tests to be sure they have diabetes. [23]

2.1.5.8 New-onset Diabetes after Transplantation(NODAT)

"PTDM, or post-transplantation diabetes mellitus, is a common complication among solid organ transplant recipients regardless of when the disease first manifests itself. Individuals who acquire new-onset diabetes after transplant are known as those with NODAT (new-onset diabetes after transplant). Patients with undetected pre-transplant diabetes and post-transplant hyperglycemia that had subsided at the time of discharge are excluded. The reported incidence of NODAT among all solid organ transplants ranges from 2 to 53%. Ninety percent of kidney allograft recipients experience hyperglycemia in the first few weeks following donation. Additionally, steroid- or stress-induced hyperglycemia usually goes away by the time a patient is discharged. Immunosuppressant use is another risk factor for NODAT in addition to general diabetes risk. According to the majority of research, transplant patients with hyperglycemia had a higher risk of infection, rejection, and readmission. Therefore, there are more risks of dying and the expense to the hospital system is higher. The effectiveness of the procedure and the survival of the patient depend on early diagnosis and proper therapy. [23]

2.1.5.9 Diabetic dyslipidemia:

High fasting and postprandial triglycerides, low HDL cholesterol, elevated LDL cholesterol, and a predominance of tiny dense LDL particles are all signs of diabetic dyslipidemia.

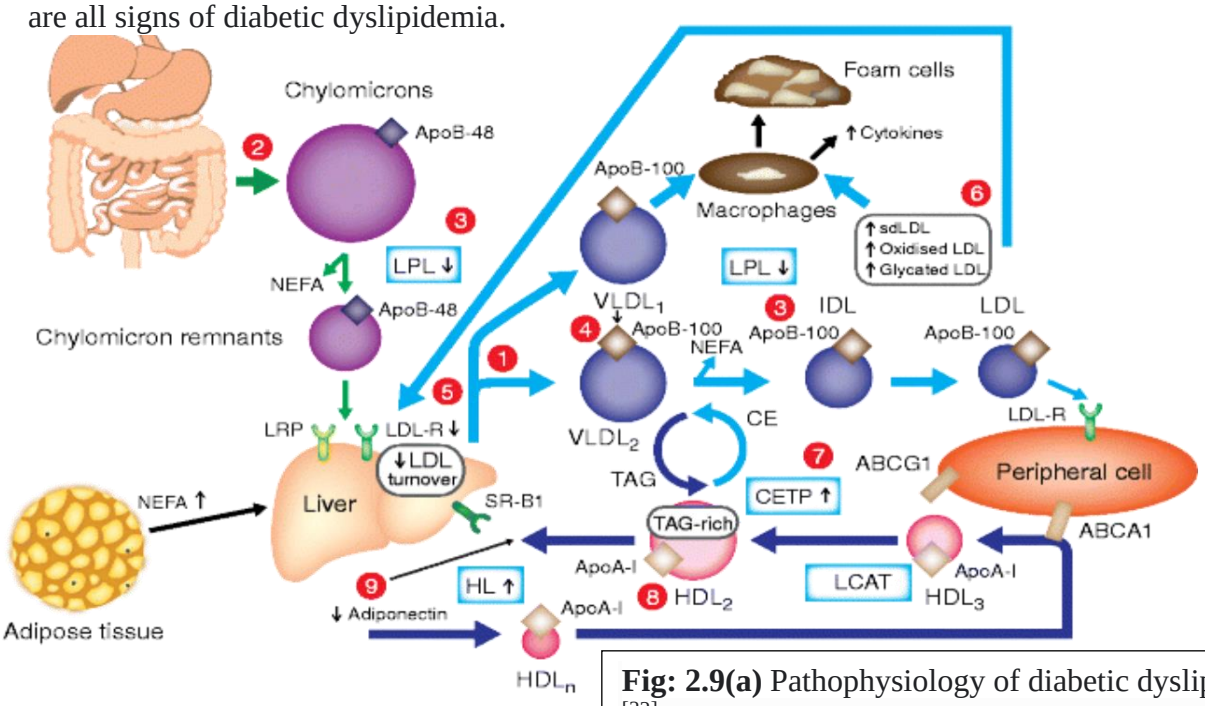


Fig: 2.9(a) Pathophysiology of diabetic dyslipidemia [32]

Main lipid abnormalities in Type 2 diabetes as shown in figure above.
Triacylglycerols (hypertriglyceridemia, qualitative and kinetic abnormalities):
(1) increased VLDL production (mostly VLDL1),
(2) increased chylomicron production,
(3) reduced catabolism of both chylomicrons and VLDL (diminished LPL activity),
(4) increased production of large VLDL (VLDL1), preferentially taken up by macrophages
LDL (qualitative and kinetic abnormalities):
(5) reduced LDL turnover (decreased LDL B/E receptor),
(6) increased number of glycated LDL small, dense LDL (TAG-rich) and oxidized LDL, which are preferentially taken up by macrophages
HDL (low HDL-cholesterol, qualitative and kinetic abnormalities):
(7) increased CETP activity (increased transfer of triacylglycerols from TAG-rich lipoproteins to LDL and HDL),
(8) increased TAG content of HDL'S, promoting HL activity and HDL catabolism,
(9) low plasma adiponectin favoring the increase in HDL catabolism.

Elevated triglycerides(TGs) are a prominent feature of the "diabetic dyslipidemia" pattern seen in type 2 diabetes mellitus (T2DM). Decreased cholesterol and high-density lipoprotein (HDL-C). Small and dense low-density lipoprotein cholesterol (LDL-C) particles that are normal, borderline, or high.

This pattern is a typical atherogenic lipid profile and is characterized by high TGs, low HDL-C, and small, dense low-density lipoprotein (LDL) particles. Compared to individuals without diabetes, adults with diabetes have a 2–4 times greater risk of developing CVD, having a stroke, and dying from heart disease. Aggressive glycemic control may not always result in a meaningful decrease in cardiovascular events or mortality, and hyperglycemia alone cannot explain the high cardiovascular risk associated with diabetes.

In recent years, approaches to addressing diabetes-related vascular problems have shifted away from a "glucocentric" focus to include additional risk factors that influence the onset and progression of atherosclerosis. Dyslipidemia is a significant factor in the onset of atherosclerosis. Treatment of dyslipidemia in patients with diabetes improves cardiovascular outcomes and is of great importance. [23]

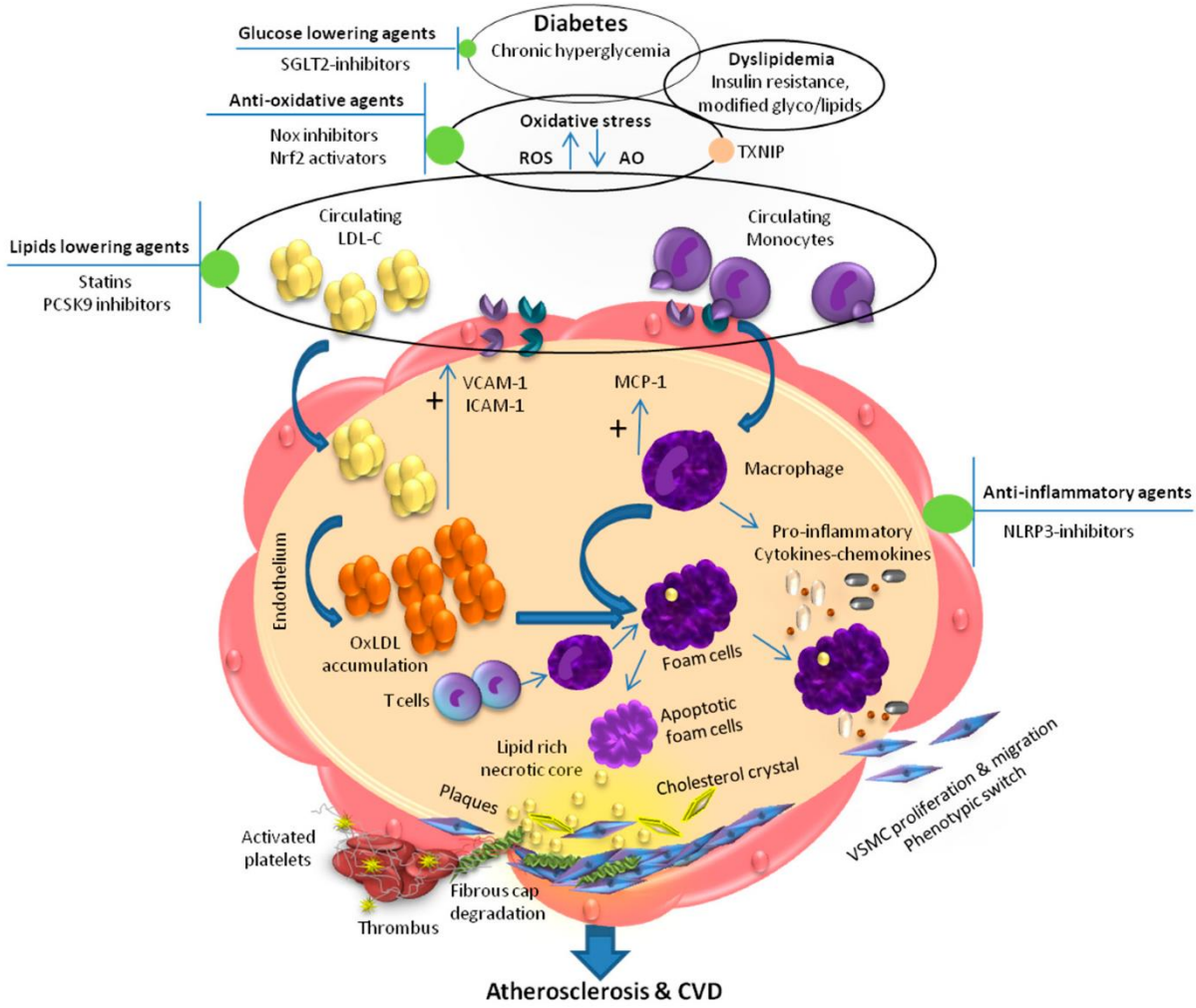


Fig: 2.9(b) Mechanism of atherosclerotic cardiovascular disease, endothelial dysfunction associated with diabetes, and anti-atherosclerotic targets/drugs: Chronic hyperglycemia and dyslipidemia increase oxidative stress, which increases the synthesis of ox-LDL, immune cell activation, and overexpression of VCAM-1, ICAM-1, and MCP-1, which encourage the creation of foam cells. Foam cells eventually experience apoptosis and develop a necrotic core. An atherosclerotic lesion develops as a result of the buildup of cholesterol crystals and foam cells that have undergone apoptosis. These pathogenic processes cause VSMC to proliferate and migrate, which aids in the creation of a necrotic core rich in lipids, which in turn encourages the development of atherosclerotic lesions and atherosclerotic cardiovascular disease. Arrows pointing upward and downward indicate an increase and a decline, respectively.[158]

2.1.5.10. Misc. [33]

- ❖ Another type of autosomal dominant diabetes results from a mutation in the sulfonylurea receptor's K-ATP channel subunit (SUR-1), which causes congenital hyperinsulinemia and loss of insulin secretory ability in young people, which causes impaired glucose tolerance and diabetes in middle age.

- ❖ Another type of hereditary beta cell malfunction results from a mitochondrial DNA abnormality. Leu-Ala at position 3243 in the mitochondrial DNA causes diabetes mellitus, which is accompanied by deafness.
- ❖ Proinsulin to insulin conversion is defective in some autosomal dominant inheritances. These individuals exhibit elevated levels of C-peptide and insulin in the blood.

Even though they are rare causes of diabetes, they should be taken into account when other insulin resistance disorders, such as acanthosis nigricans, lipodystrophy, and ovarian dysfunction, are present along with unusually high levels of circulating insulin. Sjogren's syndrome, ataxia telangiectasia, and systemic lupus erythematosus all exhibit the presence of autoantibodies against insulin receptors. Additionally, Leprechaunism, Rabson Mendenhall syndrome, and lipoatrophic diabetes share a genetic basis for insulin resistance.

2.1.6 Gestational diabetes: The term "gestational diabetes mellitus" (GDM) refers to a form of glucose intolerance that manifests itself during pregnancy. In different regions of India, the prevalence of GDM ranged from 3.8 to 21%. It has been discovered that GDM is more common in urban than rural regions. In general, gestational diabetes has two main issues: *macrosomia and hypoglycemia*. However, infants of mothers with gestational diabetes are susceptible to many chemical abnormalities, such as low blood calcium and low serum magnesium levels. Complications for babyinclude macrosomia, hypoglycemia, jaundice, low calcium and magnesium respiratory distress syndrome, increased risk for childhood and adult obesity, and risk for type 2 diabetes. Complications for mothers include hypertension, pre-eclampsia, and an increased risk for developing type 2 diabetes mellitus. [34]

Its significance in the insulin resistance of late pregnancy is confirmed by the development of severe peripheral insulin resistance in transgenic mice that overexpress human placental growth hormone, which is similar to that observed in the third trimester of pregnancy. The p85 regulatory subunit of phosphatidylinositol kinase (PI 3 - kinase) is expressed more frequently along with an increase in insulin resistance, and the activity of skeletal muscle's IRS - 1-associated PI 3 - kinase is decreased. [35]

2.1.6.1 Diagnosis of Gestational diabetes mellitus

Criteria	Fasting		1-hour		2-hour		3-hour	
	mg/dL	mmol/L	mg/dL	mmol/L	mg/dL	mmol/L	mg/dL	mmol/L
NDDG (USA)*	105	5.9	190	10.6	165	9.2	145	8.1
Carpenter Coustan (USA)*	95	5.3	180	10.0	155	8.6	140	7.8
CDA	95	5.3	191	10.6	160	9.0	–	–
WHO 1985	140	7.8	–	–	140	7.8	–	–
WHO 1999	126	7.0	–	–	140	7.8	–	–
IADPSG/ADA WHO/FIGO	92	5.1	180	10	153	8.5	–	–
(DIPSI non-fasting)	–	–	–	–	–	7.8	–	–
NICE (UK)	–	5.6	–	–	–	7.8	–	–

ADA = American Diabetes Association; NDDG = National Diabetes Data Group; CDA = Canadian Diabetes Association; DIPSI = Diabetes in Pregnancy Society of India; WHO = World Health Organization; IADPSG = International Association of the Diabetes and Pregnancy Study Groups. NICE = National Institute for Clinical Excellence; FIGO = International Federation of Gynaecology and Obstetrics

* after 50g glucose challenge test-if positive, uses 100g glucose load, at least two need to be positive

Fig 2.10: Diagnostic criteria in studies used for estimating hyperglycemia in pregnancy [1]

2.1.6.2 Metabolic changes in pregnancy in women with diabetes

By the second trimester, women with diabetes ability to tolerate carbohydrates begins to decline, concurrently with a biological loss of insulin sensitivity. Contrarily, for well-controlled women with T1DM in the first trimester, insulin sensitivity increases and insulin requirements at 14 weeks gestation are lower than at the beginning of pregnancy. Women with T1DM are more likely to develop diabetic ketoacidosis due to pregnancy-induced lipolysis (DKA). At relatively low hyperglycemia levels, this can develop quickly. If untreated, DKA is linked to a high fetal mortality rate. [23]

2.1.6.3 Treatment of GDM

The following are the ADA's suggested goals for females with type 1 or type 2 diabetes mellitus : Fasting blood sugar levels of 95 mg/dL (5.3 mmol/L), 140 mg/dL (7.8 mmol/L) one hour after eating, and 120 mg/dL (6.7 mmol/L) two hours later. The management of gestational diabetes mellitus must include a change in lifestyle. The drug of choice for treating hyperglycemia in gestational diabetes mellitus is insulin. Both metformin and glyburide may be taken, however only metformin is expected to pass the placenta to the fetus to a larger amount than glyburide. All oral medications are untested for long-term safety. [18]

2.2 Factors leading to Insulin Resistance

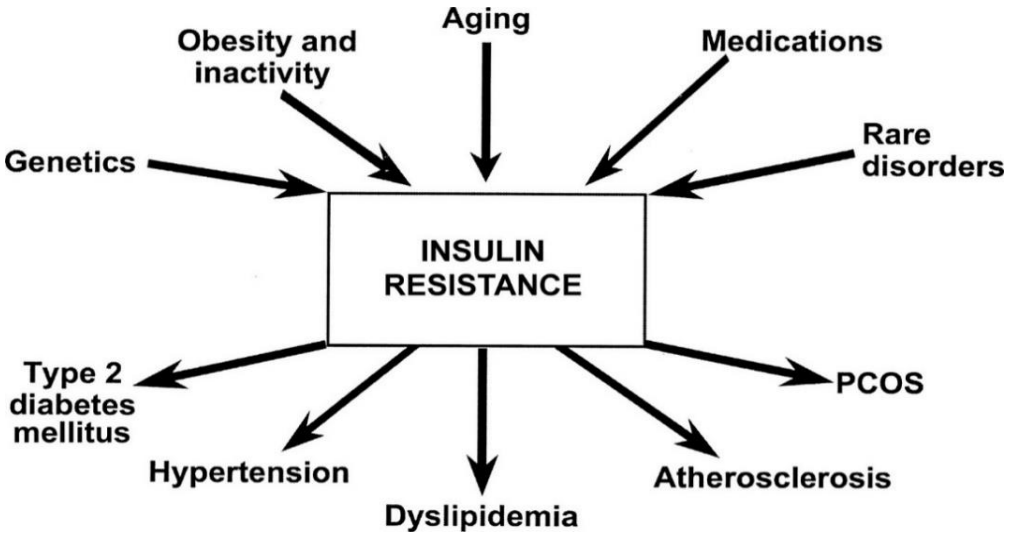


Fig: 2.11 Factors leading to Insulin Resistance

2.2.1. Genetic causes of insulin resistance

2.2.1.1 Insulin receptor mutations: More than 100 naturally occurring mutations in the insulin receptor gene as well as the total absence of insulin receptors have both been linked to severe insulin resistance. The most severe variation of these diseases is called leprechaunism, which is marked by intrauterine development retardation and distinctive dysmorphic traits such large eyes, thick lips, upturned nose, low-set posteriorly rotated ears, and thick skin devoid of subcutaneous fat. [36]

2.2.1.2 Insulin-mediated Pseudo-acromegaly: It has been reported that people with severe insulin resistance experience pathologic tissue growth that resembles acromegaly without having increased levels of growth hormone or IGF-1. The extreme hyperinsulinemia's stimulation of the intact mitogenic signaling pathways is most likely what caused the acromegalic tissue to develop. [37]

2.2.1.3 Mutations in the Peroxisome Proliferator-Activated Receptor γ: Peroxisome proliferator-activated receptor γ (PPAR γ) (a nuclear receptor) appears to be essential for insulin action and adipocyte development. Clinically significant insulin resistance and partial lipodystrophy of the limbs and buttocks, but not of the face or central abdominal adipose depots, are brought on by mutations. [38]

2.2.1.4 Insulin signaling pathway with known human monogenic disorders

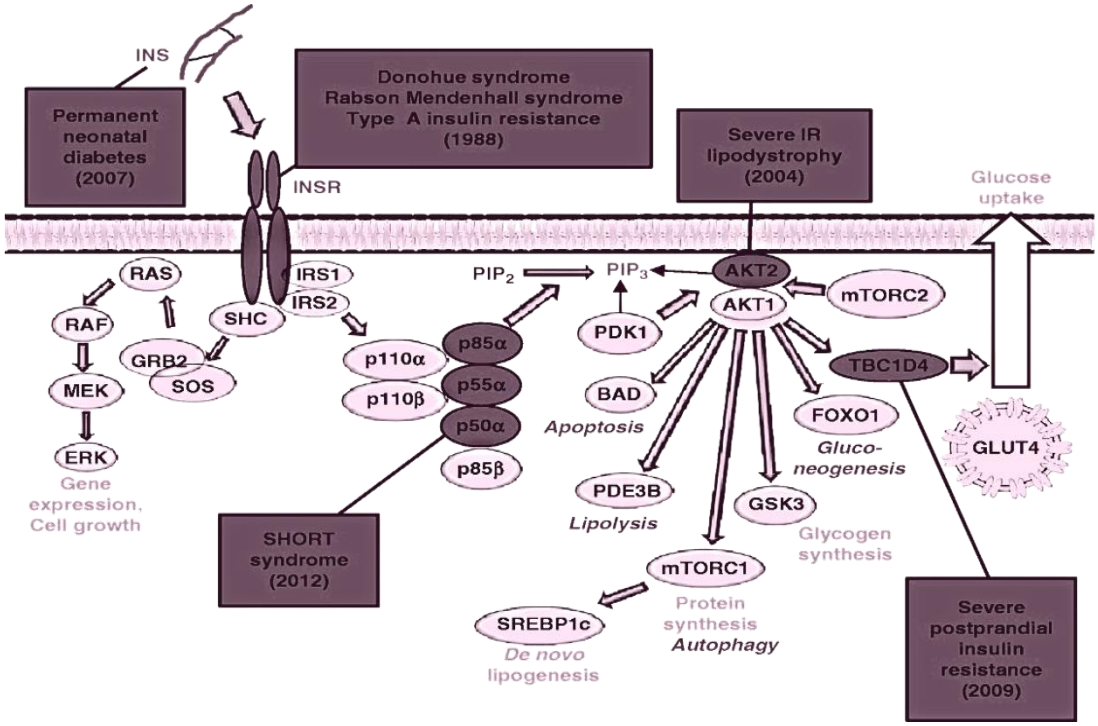


Fig: 2.12 Simplified schematic diagram of insulin signalling pathway with known human monogenic disorders. Genes that have been implicated in Mendelian disorders of insulin signalling are shown with the corresponding disease and year of discovery in linked boxes.{39}

2.2.1.5. Lipodystrophy

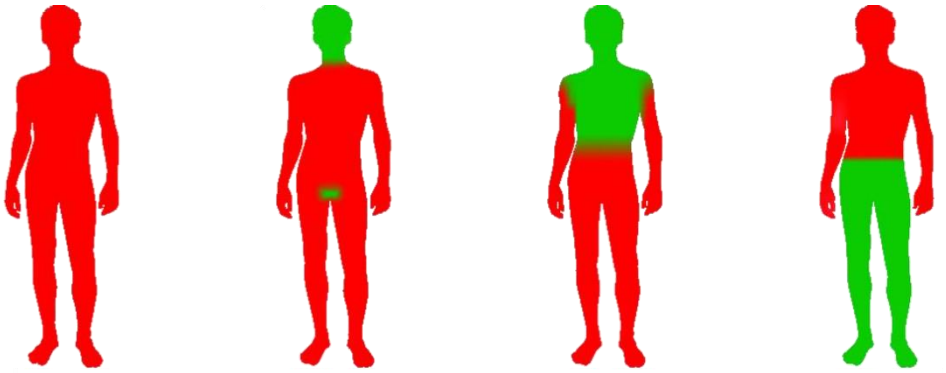
Insulin resistance from birth or early childhood and a complete lack of adipose tissue are the two main characteristics of congenital global lipodystrophy. AGPAT2 and BSCL2 gene mutations, which occur on separate chromosomes, are linked to this dramatic clinical condition. An anabolic syndrome with muscle hypertrophy, hepatomegaly, and hypertrophic cardiomyopathy, the latter of which is typically fatal by early adulthood, coexists with the clinical symptoms of insulin resistance.[40]

2.2.2. Obesity And Inactivity

Obesity, or having too much body fat, plays a significant role in the development of insulin resistance and greatly raises the risk of type 2 diabetes. White adipose tissue, which houses the majority of the body's energy reserves, affects how insulin acts throughout the body by releasing FFAs and secreting proteins generated from adipose tissue.[41]

2.2.2.1. Adiponectin

A 30-kDa adipose-specific secretory protein is called adiponectin. A much lower risk of developing type 2 diabetes is linked to high baseline plasma levels of adiponectin. In rodents, pharmacologic administration of adiponectin promotes hepatic insulin sensitivity with a reduction in glucose synthesis. In addition to stimulating fatty acid oxidation and glucose absorption in myocytes, reducing gluconeogenesis in the liver, and lowering blood glucose levels in vivo, adiponectin also increased phosphorylation and activation of the 5′AMP-activated protein kinase (AMPK) in skeletal muscle and the liver. [42]



Form of LD	CGL	FPLD2	Other FPLD	APLD
Genes	***AGPAT2, BSLC2*** *PTRF, CAV1*	***LMNA***	***PPARG***, *PLIN1* *AKT2, CIDEC*	---
Fat preserved	Sometimes mechanical WAT, BM WAT	Visceral WAT Head/neck WAT Labial WAT	Visceral WAT Some s/c Truncal WAT	Infra-umbilical WAT Visceral WAT
Metabolic profile	**Severe IR Severe DL** Severe NAFLD	**Severe IR Severe DL** Severe NAFLD	**Severe IR Severe DL** Severe NAFLD	**Moderate or no IR Little DL** Little or no NAFLD

Fig: 2.13 Distribution of adipose loss in lipodystrophies. Areas of deficient adipose tissue are illustrated in red, with preserved adipose tissue represented in green.

Source: European Journal of Endocrinology 174, 5; 10.1530/EJE-15-1131 [39]

2.2.2.2 Leptin

Leptin is a marker of obesity and the insulin resistance syndrome because it closely correlates with fasting insulin levels and the amount of body fat in individuals. Through the activation of AMPK in the liver and muscles, leptin stimulates β-oxidation. When turned on, AMPK reduces ATP-consuming anabolic pathways like glucose-regulated transcription, protein synthesis, cholesterol synthesis, and fatty acid and triglyceride synthesis while boosting ATP-producing catabolic pathways like increased glucose transport, beta-oxidation, glycolysis, and mitochondrial biogenesis.[43]

2.2.2.3. Resistin

A 10-kDa hormone, unique to fat tissue is resistin. Resistin inhibits hepatocytes quickly, but not peripheral insulin sensitivity. [44] When normal rats received doses of resistin that were nearly physiological. During a hyper insulinemic clamp research, lower rates of glucose infusion were required to maintain basal glucose levels.

2.2.2.4 Obesity and Inflammation

Obesity raises circulating levels of these "adipokines," which are proinflammatory chemicals produced by adipose tissue and include TNF-α, IL-6, transforming growth factor-β, C-reactive protein, and monocyte chemoattractant protein-1 (MCP-1). Recently, it was discovered that the chemokine MCP-1 reduces insulin sensitivity in adipocytes. [45]

In multiple rodent models, it has been shown that the proinflammatory cytokine TNF-α mediates insulin resistance brought on by fat. In adipocytes, TNF-α directly reduces insulin sensitivity and boosts lipolysis. Obesity and insulin resistance increase the expression of TNF-α in white adipose tissue. TNF-α, a transcription factor that controls the expression of several inflammatory molecules, is activated by TNF-α. Additionally increasing lipolysis, IL6 has been linked to obesity-related hypertriglyceridemia and elevated serum FFA levels. In hepatocytes, IL-6 causes cellular insulin resistance. [45]

2.2.3. Aging And Environmental Factors

2.2.3.1. Medical Conditions That Result in Insulin Resistance

Numerous medical diseases have been known to cause insulin resistance. Antibodies to the insulin receptor were one of the first medical causes of insulin resistance to be discovered (type B insulin resistance). Renal failure and uremia both exhibit insulin resistance, which gets better with dialysis. Insulin resistance and glucose intolerance are often present in hepatic cirrhosis. Early in the course of hereditary hemochromatosis (before to overt hepatic cirrhosis), iron excess appears to impair both hepatic and peripheral insulin action, and phlebotomy may improve insulin action in affected individuals.[46]

2.2.3.2. Hormonal Mediators of Insulin Action

- Cortisol, adrenaline, norepinephrine, glucagon, and growth hormone are important counter-regulatory hormones that inhibit insulin activity after hypoglycemia. It should be noted that in normal volunteers, cortisol infusions given over a 24-hour period (as

seen in Cushing syndrome) reduced the ability of insulin to suppress glucose synthesis and stimulate glucose utilization. [47]

- The insulin-stimulated PI 3-kinase activity in white adipocytes is decreased by stimulation of the α-adrenergic receptor. Isoproterenol promotes insulin-stimulated glucose uptake in white adipocytes via desensitizing α-adrenergic receptors. The W64R mutation in the beta3-adrenergic receptor has been linked to early-onset type 2 diabetes and increased belly fat. [47]
- Pheochromocytoma-related reductions in peripheral insulin action were quickly reversed after surgical removal. Growth hormone inhibits insulin effects on multiple levels, including by preventing the insulin receptor and IRS-1, one of its main signaling molecules, from being phosphorylated in response to insulin injection.[47]

2.2.3.3 Aging

Both normal ageing and progeria syndrome have been linked to higher rates of insulin resistance and type 2 diabetes. Recently, it has been suggested that insulin resistance in the elderly is caused in part by a reduction in mitochondrial function brought on by ageing. Indeed, increased intracellular and intrahepatic lipid content and decreased insulin-stimulated glucose uptake were associated with a 40% reduction in mitochondrial oxidative and phosphorylation activity as measured by in vivo 13C/31P NMR spectroscopy.[48]

2.2.3.4. Insulin Resistance Syndromes

Insulin resistance, hypertension, dyslipidemia (lower HDL and higher triglycerides), central or visceral obesity with T2DM or IGT/IFG, and accelerated cardiovascular disease are all metabolic abnormalities collectively referred to as the *metabolic syndrome*, "insulin resistance syndrome," and "syndrome X."

Type A, which affects young women and is characterized by severe hyperinsulinemia, obesity, and signs of hyperandrogenism; and type B, which affects middle-aged women and is characterized by severe hyperinsulinemia, features of hyperandrogenism, and autoimmune problems. Type A insulin resistance syndrome patients have an unidentified malfunction in the insulin signaling system, whereas type B patients have autoantibodies that are directed against the insulin receptor.

Premenopausal women are frequently affected by the condition known as polycystic ovarian syndrome (PCOS), which is characterized by chronic anovulation and

hyperandrogenism. Unaffected by the effects of obesity, PCOS significantly raises the risk for T2DM and is present in a sizable fraction of female patients.[49]

2.2.3.5. Defects in insulin secretion in diabetes

T2DM beta cell dysfunction is a result of both inherited and acquired causes. A number of genes may influence the synthesis, packaging, mobility, and release of secretory granules as well as the apoptosis, regeneration, glucose sensing, glucose metabolism, ion channels, energy transduction, microtubules/microfilaments, and other islet proteins in beta cells. Examples

1. The peroxisome proliferator-activated receptor γ (PPARγ), which is expressed in insulin target tissues and β- cells, has an amino acid polymorphism (Pro12Ala).
2. Calpain - 10, a cysteine protease that modulates insulin release as well as insulin effects on muscle and adipose tissue.
3. E23K variant of the *KIR6.2* gene (potassium inwardly rectifying channel J11 gene) [50]

2.2.3.6. Acquired factors

A) Malnutrition in utero and early childhood: - It is possible that nutritional insufficiency at this period could programme the β - cell to impair its future capacity to adjust to overnutrition. Malnutrition in utero and throughout the first few months of life may harm β - cell development. [51]

B) Glucotoxicity: - There is much of proof that both acute and persistent hyperglycemia can negatively impact β-cell function. Reactive oxygen species (ROS) formation inside β-cells, changes in gene transcription and protein expression brought on by oxidative stress, and an increase in β-cell death are all thought to be involved in these pathways. [52]

C) Lipotoxicity: - Obese people have higher plasma FFA levels, which boost UCP-2 activity in β-cells. As a result, the amount of insulin secreted in response to glucose stimulation declines due to impaired ATP production from glucose metabolism. FFA diminish the binding activity of Pdx-1 and Maf A, two transcription factors necessary for controlling the expression of numerous islet-associated genes, including the insulin gene, by increasing ceramide production. [53]

D) Inadequate stimulation by incretins: - The incretin effect is a phenomenon whereby the hormones incretins, which are generated by the gut in response to meal consumption, increase insulin secretion. The incretins GLP-1 and GIP are both important. They boost beta-cell replication, block its apoptosis, and were found to increase beta-cell bulk in a mouse model, in addition to enhancing the manufacture and secretion of insulin. Reduced insulin responses to both GLP-1 and GIP.[54]

E) Islet amyloid: - Islet amyloid deposition is a distinctive trait seen in more than 90% of T2DM patients. Islet amyloid polypeptide(IAPP), a protein, is used to make the insoluble fibrils that make up amyloid. IAPP and insulin are often released together by beta-cells in a molar ratio of 1: 10- 50. But a rare family form of T2DM has been related to the IAPP gene's S20G mutation, which makes IAPP more likely to form oligomers. [55]

2.3 Management of Diabetes

2.3.1 Clinical Presentation of Diabetes:[20]

Clinical features	Type 1 diabetes	Type 2 diabetes	Monogenic diabetes
Age of onset (years)	Most <25 but can occur at any age (but not before the age of 6 months)	Usually >25 but incidence increasing in adolescents, paralleling increasing rate of obesity in children and adolescents	Usually <25; neonatal diabetes <6 months*
Weight	Usually thin, but, with obesity epidemic, can have overweight or obesity	>90% at least overweight	Similar to general population
Islet autoantibodies	Usually present	Absent	Absent
C-peptide	Undetectable/low	Normal/high	Normal
Insulin production	Absent	Present	Usually present
First-line treatment	Insulin	Noninsulin antihyperglycemic agents, gradual dependence on insulin may occur	Depends on subtype
Family history of diabetes	Infrequent (5%–10%)	Frequent (75%–90%)	Multigenerational, autosomal pattern of inheritance
DKA	Common	Rare	Rare (except for neonatal diabetes*)

Fig: - 2.14 Clinical Features of Diabetes

2.3.1.1. Excessive Thirst, polyphagia and polyuria: These signs and symptoms are the outcome of an osmotic diuresis brought on by hyperglycemia. Although the renal threshold varies widely across individuals, often between 6 and 14 mmol/L, it is 11 mmol/L on average. Chronic hyperglycemia alters the renal sensitivity to vasopressin, making thirst less noticeable despite increasing plasma osmolarity. [56]

2.3.1.2. Sudden Weight Loss: The relative decrease of insulin's anabolic effects is what causes the majority of the weight loss. Particularly in young males, muscle loss may be noticeable. Muscle strength reduction that is related may be seen. Insulin functions primarily as an anabolic hormone to prevent protein breakdown. [56]

2.3.1.3. Blurred Vision: The patient must be informed that the visual fuzziness may get worse after the relatively quick cure of excessive hyperglycemia. [56]

2.3.1.4. Infections: When leukocytes are exposed to glucose concentrations exceeding 11 mmol/L, their phagocytic and other functions become paralyzed. Most typically, this involves male or female vaginal candidiasis or balanitis. Initial blood glucose regulation will enable infection clearance with ongoing antifungal treatment. Especially in T1DM, staphylococcal pustules, boils, and carbuncles may be present when diabetes is diagnosed. Serious infectious manifestations like necrotizing fasciitis may raise the disease's morbidity rate.[56]

2.3.1.5. Diabetic ketoacidosis (DKA): Hyperglycemia, acidosis, and ketonuria are its defining features, and it affects some T2DM patients as well as those with T1DM. Muscle cramps, malaise, and tiredness are some possible complaints from patients. Vomiting and abdominal pain could be severe enough to resemble an urgent surgical issue. Dehydration, deep, sighing respirations (also known as Kussmaul respiration), and a sweet-smelling fetor (similar to nail polish remover) on the breath are all clinical indications.[57]

2.3.1.6. Hyperosmolar hyperglycemic syndrome (HHS): HHS only happens in people with T2DM. The main clinical symptom is severe dehydration. Confusion is common, and specific neurologic symptoms such as hemi-sensory abnormalities or paralysis on one side may develop and be mistaken for stroke. The absence of severe ketoacidosis is one of the main exceptions. [58]

2.3.2 Diagnostic criteria of diabetes

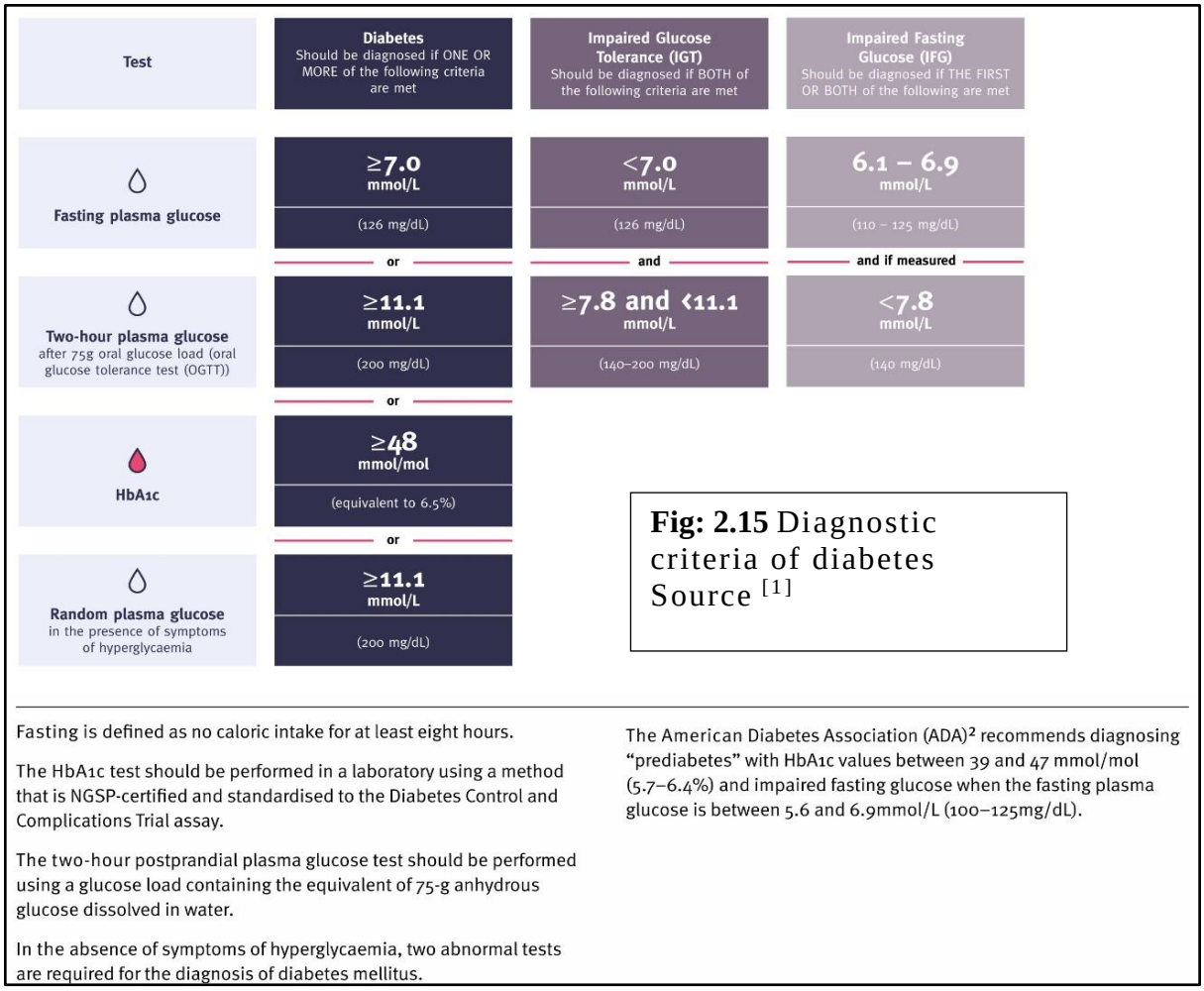


Fig: 2.15 Diagnostic criteria of diabetes Source [1]

Prediabetes is a condition that exists before the onset of overt diabetes and is marked by impaired glucose tolerance (IGT) or impaired fasting glucose (glycemia) (IFG). IFG stands for fasting glucose concentrations that are lower than those required for the diagnosis of diabetes but greater than those typically observed in persons with normal glucose tolerance. IGT can only be evaluated through the use of OGTTs. [59]

2.3.2.1. Impaired glucose tolerance: IGT, or impaired glucose tolerance, is a stage of impaired glucose regulation that is present in people whose glucose tolerance is above the typical normal range but below the level considered diagnostic of diabetes. [60]

2.3.2.2 Impaired fasting glucose: IFG is defined as people with fasting plasma glucose levels of 100 to 125 mg/dL (5.6 to 7.0 mmol/L). Some of these people will have IGT and some may develop diabetes , if an OGTT is carried out (2 hours post load plasma glucose concentration 200 mg/dL, or 11.1 mmol/L). [60]

Test	Prediabetes[1]	Diabetes[2]	Comments
A1C	A1C 5.7-6.4%	≥ 6.5%	A1C should be measured in a NGSP-certified laboratory. The purpose of the NGSP is to standardize HbA1c test results to those of the Diabetes Control and Complications Trial (DCCT) and United Kingdom Prospective Diabetes Study (UKPDS) which established the direct relationships between A1c levels and outcome risks in patients with diabetes.
Fasting plasma glucose (FPG)	100-125 mg/dL (5.6-6.9 mmol/L)	≥ 126 mg/dL (7.0 mmol/L)	No caloric intake for at least 8 hours
2-h plasma glucose during OGTT	140-199 mg/dL (7.8-11.1 mmol/ L)	≥ 200 mg/dL (11.1 mmol/L)	Using glucose load of 75 g anhydrous glucose dissolved in water
Random plasma glucose[3]		> 200 mg/dL (11.1 mmol/L)	Random glucose: Any time of the day without regard to time since last meal Symptoms: Polyuria, polydipsia, unexplained weight loss

Fig: 2.16 Diagnostic Criteria for Prediabetes and Diabetes [18,60]

2.3.3Pharmacotherapy of diabetes

Drugs are classified in a table at the end of dissertation and earlier in the form of table Annexure 7 And algorithm in Annexure 8.

2.3.3.1 Approach to diabetes patient [18,60]

A comprehensive clinical evaluation should be performed at first visit for all diabetes patients with an aim to:

- Confirm the diagnosis of diabetes
- Define the type of diabetes
- Diagnose the associated comorbidities/components of metabolic syndrome
- Define presence or absence of micro-/macrovascular complications
- Assess for the risk of developing complications
- Assess education/socioeconomic/cultural background
- Formulate a management plan
- Develop a basis for evaluation of ongoing medical care.

Additionally, one must consider past treatment, the level of past and present glucose control, and any prior comorbidities or problems and their management when evaluating a patient who has already been diagnosed with diabetes. A thorough history, thorough physical

examination, and laboratory tests are all part of a comprehensive clinical evaluation of a patient with diabetes, just like they are for any other patient. In order to make a thorough diagnosis and develop a care plan, one must also analyze one's nutritional status, diabetes self-management behavior, and psychological health. **[18,60]**

Overall, the management of Diabetes is focused on following parameters:

1. Glycemic control, diet/lifestyle, exercise, medication.

2. Treatment of related illnesses, including hypertension, obesity, coronary artery disease, and dyslipidemia.

3. Retinopathy, cardiovascular disease, nephropathy, neuropathy, and other problems should be managed. **[18,60]**

2.3.3.2 Anti-diabetes pharmacotherapy:

Modifying disease progression is the pharmacotherapy's ultimate goal. Initially, one anti-hyperglycemic medication is frequently sufficient, but as the disease progresses, another medication with a different mechanism of action is typically needed. Oral medications are frequently used as the first line of treatment for T2DM because to their ease, although oral delivery has certain disadvantages, including frequent dosage, a short half-life, and low absorption. In all stages of the disease, there is a need for adjusting the agent dose, agent type, or a combination of multiple agents. [61] Refer to Annexure 7.

2.3.3.3 Nutritional therapy: The goals of nutritional therapy are:

1. To encourage and support a range of nutrient-dense meals in sensible portion sizes in order to improve general health, and more especially to:
   a. Reach and maintain desired body weight
   b. Meet specific glycemic, blood pressure, and lipid objectives.
   c. Postpone or stop diabetes consequences.
2. To meet each person's specific nutritional needs in light of their cultural and personal preferences, health literacy, food access, willingness and ability to alter behavior, and barriers to change
3. To sustain the enjoyment of eating by conveying information about food choices that are free of judgement.
4. Instead of focusing on specific macronutrients, micronutrients, or single foods, give a diabetic person the practical tools for creating healthy eating habits.

Dietary restrictions should include: a) a low-calorie, low-fat, and low-carbohydrate diet; b) a low-trans-fat intake; and c) consumption of fruits, vegetables, and fiber-rich foods.

When a specific amount of a food is taken, the glycemic index calculates the postprandial rise in blood glucose. Low-glycemic-index food consumption appears to lessen postprandial glucose excursions and enhance glycemic control. [23]

2.3.3.4 Exercise, lifestyle modification, Yoga

The American Diabetes Association (ADA) advises individuals with diabetes to engage in 150 minutes per week (spread over at least 3 days) of moderate aerobic exercise. Even a little reduction in body weight is linked to enhanced islet ß-cell function, decreased hepatic glucose synthesis, and decreased insulin resistance. Exercise therapy plays a vital role in the prevention and treatment of diabetes mellitus (DM), both in type 1 and type 2 patients, as well as in lowering blood pressure, maintaining muscle mass, reducing body fat, and losing weight. The clinical profile of the patient, as well as his or her disease and complication status, must be taken into consideration when creating a personalized exercise prescription, but in general, it should include both resistance training and aerobic exercise. Yoga poses and pranayama have shown demonstrated benefits for those with diabetes and might be added to the recommended fitness regimen. [23,18]

2.3.3.5 Self-monitoring of blood glucose (SMBG)

Self-checking blood sugar levels complements HbAlc data, offers real-time feedback to patients and doctors, aids in the immediate detection of hypoglycemia and hyperglycemia, and enables prompt intervention. After demonstrating the advantages of glycemic management in numerous clinical studies, SMBG is now incorporated into the trial protocol. There are numerous arguments in favor of the relationship between SMBG and better clinical outcomes in diabetes. Evidence points to SMBG's therapeutic usefulness as an efficient supplemental treatment for T1DM and insulin-treated T2DM, are less obvious. Desired target blood glucose levels are outlined in both International and Indian recommendations, as are the circumstances in which SMBG should be employed. In addition to improving quality of life, preventing or slowing the progression of diabetic complications lowers the cost of diabetes treatment and financial risk. Patients and clinicians must collaborate closely and make informed decisions in order to get the most out of SMBG. Due to the perception that glucometers are expensive, a lack of knowledge about how blood glucose control can prevent or delay the onset of diabetes complications, and finally the inability of a sizable portion of

diabetes patients to engage in SMBG, obtain glucometers and test strips, or interpret longitudinal SMBG data, compliance levels for SMBG are currently low in India. [23]

2.3.3.6 Continuous glucose monitoring (CGM)

Although SMBG continues to be the gold standard for blood glucose monitoring, CGM is now viewed as an essential component of all-encompassing diabetes management. Although we have both real-time and blind gadgets, each has advantages and disadvantages of its own. Many patients will use FGM sensors for real-time or retrospective glucose monitoring in addition to CGM, MDI, insulin pumps, and AP systems. Regardless of the objective, only a skilled multidisciplinary team can interpret the readings and reports effectively. Therefore, there is still a persistent unmet demand for a team of doctors, dieticians, and other healthcare experts who are well-trained, experienced, strong, and committed and who can assist patients in modifying their therapy and behaviors. Similar to glucometers, it will take decades for CGM to become widely accepted and used. New generation sensors will improve this technology's accuracy and enable it to quickly surpass glucometers, however it may not do so anytime soon. [23]

2.3.3.7 Bionic pancreas

In the past, artificial pancreas devices were used to describe all devices that automatically checked glucose and supplied insulin based on observed glucose levels. However, insulin delivery devices have historically been referred to as "bionic pancreas" because the phrase "artificial pancreas" has been misused. The Food and Drug Administration (FDA) designated the Medtronic MiniMed 530G system (Medtronic, Northridge, CA) with the threshold suspend feature for commercial use in the United States in 2013. This system was regarded as an artificial pancreas device. Even today, the FDA's artificial pancreas device system website lists the MiniMed 670G system, the first hybrid closed-loop (HCL) insulin delivery and continuous glucose monitoring (CGM) system to receive FDA approval. Automated glucose regulation may significantly enhance glycemic control, lessen acute and long-term consequences from diabetes, and ease the burden of diabetic self-management. [23]

2.3.3.8 Stem cell therapy and transplantation

The outstanding outcomes of pancreatic and islet transplantation today are the result of numerous improvements and breakthroughs. But the reality is that a 68 kg person need between 340 and 750 million islets to effectively heal their illness, which means that at least two to three donors are required for each transplant. The complicated infrastructure required for islet purification, the scarcity of donor pancreas, and chronic immunosuppression are the main causes that have sparked the search for alternate β-cell replacement sources. The extraordinary abilities of self-renewal, proliferation, and differentiation into desired specialized cells distinguish stem cells, whether they are derived from mature adult cells or from embryonic or inducible pluripotent stem cells. Its use in clinical studies on T2DM patients has been made possible by their capacity to "homing in" to the intended site of action, promote beta-cell differentiation or regeneration, and modulate the immune-inflammatory milieu. [23]

2.4 Prevention of diabetes mellitus:

Diabetes has a severe impact on India. The best strategy for dealing with this issue is through primary prevention. Although some pharmaceutical medications, such as metformin, have also been explored and proved to be effective, numerous trials have demonstrated that lifestyle modification (LSM) is the feasible and secure intervention for primary prevention of diabetes. Identifying those who are at high risk of developing diabetes and putting primary prevention measures into action are urgent needs. Additionally, efforts are being made to strengthen the nation's ability to manage and prevent diabetes through training a significant number of medical professionals. To lessen the enormous cost of the disease, both governmental and non-governmental organizations must work together. There is plenty of data from research conducted in India and other nations to demonstrate that LSM or the use of specific pharmacological medications can prevent T2DM or at the very least delay it by many years. The community needs to be informed and inspired to adopt and adhere to preventive measures, so it is important to find a communication method that is both affordable and broadly accessible. [23]

2.5 Complications of diabetes mellitus

2.5.1 Acute Complications of diabetes mellitus

Acute consequences of diabetes include hypoglycemia, diabetic ketoacidosis (DKA), and hyperglycemic hyperosmolar state (HHS). DKA was once thought to be a characteristic of type 1 diabetes mellitus, and its initial therapy is identical. HHS is mostly observed in people with type 2 DM. Absolute or relative insulin shortage, volume loss, and acid-base imbalances are all related with both illnesses. Along a continuum of hyperglycemia, with or without ketosis, DKA and HHS exist.

Hypoglycemia: Low blood sugar, or hypoglycemia, happens when blood sugar levels fall below normal, typically to less than 70 mg/dL (3.9 mmol/L). Use Whipple's triad to appropriately identify hypoglycemic episodes. Hypoglycemia symptoms, blood sugar levels below 70 mg/dL (3.9 mmol/L), and symptom resolution with restoration to normal blood sugar levels are its defining features. Hypoglycemia can cause a variety of symptoms, including headache, tiredness, clumsiness, difficulty speaking, confusion, rapid heartbeat, perspiration, shaking, anxiety, hunger, loss of consciousness, convulsions, and even death. Symptoms frequently strike out of nowhere. Additional causes of hypoglycemia in diabetics include the following a) Fasting includes avoiding glucose for a long period of time, whether it be an overnight fast or a conscious one. b) Increased aerobic activity because it uses glucose more efficiently, especially in the muscles. c) alcohol use, especially when combined with diabetic medications since alcohol reduces glucose production. d) Kidney illness because insulin cannot be cleared from the bloodstream effectively [20]

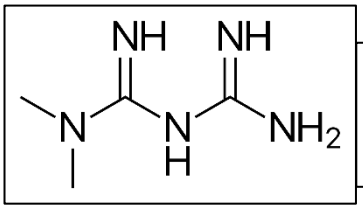
2.5.2 Chronic Complications of diabetes mellitus

Vascular and nonvascular problems are two categories of chronic complications. Microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular (coronary heart disease, peripheral artery disease, and cerebrovascular disease) complications are further separated into the vascular complications of diabetes mellitus. Among the nonvascular consequences are issues like gastroparesis, infections, and skin alterations. Diabetes that has been present for a long time may cause hearing loss. The worrisome new hot subjects of diabetes sadness and diabetes diarrhea need to be carefully examined. [20]

Table 3.: Various chronic complications of diabetes mellitus are [20] [23]:

A.	**Microvascular**	
1.	Eye	a. Retinopathy
		b. Macular edema
2.	Neuropathy	a. Sensory and motor
		b. Autonomic
3.	Nephropathy	
B.	**Macrovascular**	
1.	Coronary artery disease	Hypertension (metabolic syndrome), Heart failure
2.	Peripheral vascular disease	Diabetic foot syndrome
3.	Cerebrovascular disease	Diabetic depression, stroke
C.	**Other**	
1.	Gastrointestinal	Gastroparesis and Diabetic diarrhea, liver ds (NASH, cirrhosis, glycogen deposition), oral (periodontal ds, dental caries, oral candidiasis, halitosis)
2.	Genitourinary	Uropathy and sexual dysfunction
3.	Dermatology	Pruritus, acanthosis nigricans, NLD (necrobiosis lipodica diabeticorum), diabetic bullae
4.	Infection	Tuberculosis, chronic infections (malignant otitis externa, necrotizing fasciitis, rhino cerebral mucor mycosis, hepatitis C, HIV infection)

Metformin [62-68]



Structure of metformin

Fig: - 2.17 Galega officinalis. The plant ancestor of metformin, G. officinalis, is also known as professor weed, goat's rue, French lilac, Italian fitch, and Spanish sainfoin. Despite being classified as a noxious weed in many US states, this plant was once utilized as a traditional medicinal in mediaeval Europe. Malcolm Storey, www.bioimages.org.uk, copyright (Image taken on July 1, 2000, in Berkshire, United Kingdom) The journal Diabetes (2017) 60:1566–1576

Since the 1960s, the biguanide metformin has been used to treat T2DM. The chosen first-line oral blood glucose-lowering medication for the treatment of T2DM is metformin (dimethyl biguanide). Its origins can be traced back to Galega officinalis, commonly known as goat's rue, a traditional herbal remedy used in Europe that was discovered to be abundant in guanidine, which was first demonstrated to decrease blood sugar in 1918.[62]

In the 1920s and 1930s, metformin and other guanidine derivatives were created and used to treat diabetes; however, due to toxicity and the availability of insulin, these treatments were eventually abandoned. In the 1940s, metformin was uncovered during the quest for antimalarial drugs. Clinical trials showed that metformin may be used to treat influenza because it occasionally reduced blood glucose levels. The French physician Jean Sterne, who first described the use of metformin to treat diabetes in 1957, pursued this characteristic. Though less effective than other glucose-lowering biguanides (phenformin and buformin), which were often stopped in the late 1970s due to a high risk of lactic acidosis, metformin got less attention. [62,64]

Future prospects for metformin were uncertain because of associations with other biguanides, despite clear distinctions. After extensive testing, metformin was approved for use in the USA in 1995. Its capacity to combat insulin resistance and treat adult onset hyperglycemia without weight gain or an increased risk of hypoglycemia gradually gained acceptance in Europe. Metformin's long-term cardiovascular advantages were discovered by the UK Prospective Diabetes Study (UKPDS) in 1998, offering a fresh justification for its use as the first-line treatment for hyperglycemia in T2DM. After being used to treat T2DM for 60 years, metformin has developed into the most commonly prescribed glucose-lowering drug in the world with promise for additional therapeutic uses. The systemic (IUPAC) name of

Metformin is N, N-Dimethyl- imidodicarbonimidic diamide hydrochloride. The molecular formula is: $C_4H_{11}N_5$ .HCl and molecular weight is 165.62g/mole. [63]

Metformin only regulates insulin release indirectly through its effects on fluctuating plasma glucose levels and has no direct effects on pancreatic beta-cells. Treatment for type 2 diabetes is typically associated with a mean weight loss of 2 to 3 kg, mostly because adipose tissue decreases. [64]

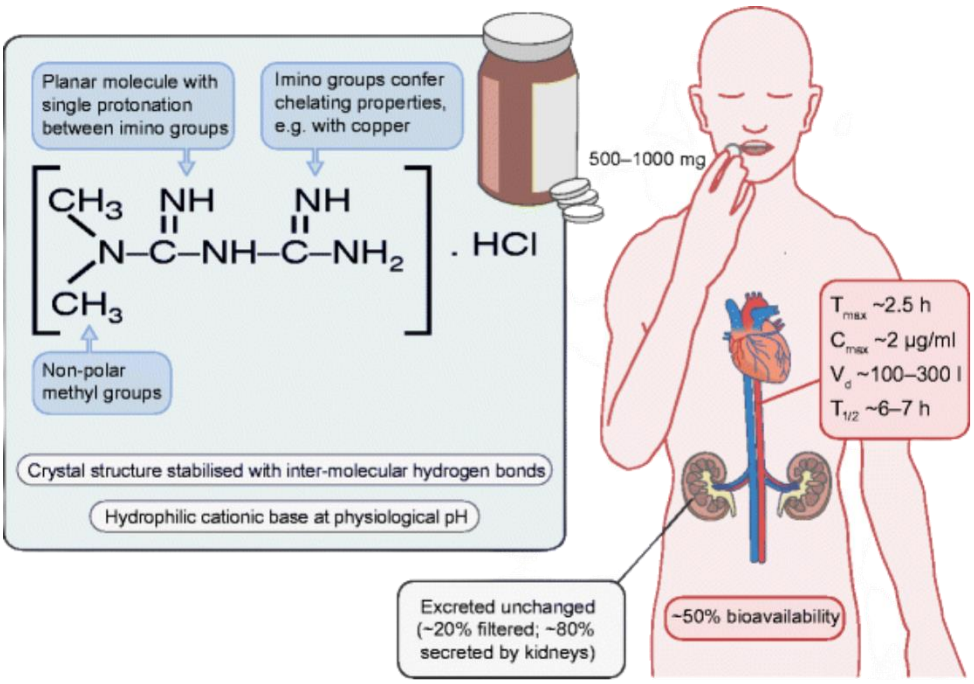


Fig: - 2.18 Metformin: Structure and Pharmacokinetics
Source: Doi: 10.1007/S00125-017-4318-Z

Mechanism of Action[20,63,65,66]

Since the vast majority of patients are obese, metformin's good effect of reducing hunger helps patients lose weight, which is advantageous. Among the currently suggested modes of action are:

1. Enhanced non-oxidative glucose disposal into skeletal muscle and improved recruitment and activity of GLUT-4 glucose transporters, which both improve insulin stimulated glucose transport in the muscle.
2. Increased secretion of glucagon-like peptide-1 (GLP-1).
3. Preserving beta-cell secretory capacity protects beta-cells against glucose toxicity and lipotoxicity.
4. Increased lipolysis inhibition and free fatty acid esterification in adipose tissue.
5. Reduction of appetite.

6. Diminished intestinal carbohydrate absorption leading to reduced post-prandial hyperglycemia.
7. Inhibition of hepatic gluconeogenesis

Anorexigenics, decreased intestinal carbohydrate absorption, suppression of hepatic gluconeogenesis, and enhanced glucose uptake by peripheral tissues are the major causes.

Clinical Use[20,64,67]

Today, metformin is the medicine of choice for all T2DM patients. Additionally, it is prescribed for polycystic ovarian disease, impaired fasting glucose, impaired glucose tolerance, and prediabetes. Advantages of metformin are:

1. Not hypoglycemic.
2. Supporting weight loss.
3. May help reduce both macrovascular and microvascular problems associated with diabetes.
4. In T2DM, there is no acceleration of cell fatigue or failure.
5. Antihyperglycemic efficacy (0.8–1.2% HbA1c reduction) comparable to other oral medications.
6. The use of metformin has positive effects on various facets of the metabolic illness syndrome (insulin resistance syndrome)
7. It also has a minor favorable effect on lowering serum triglycerides and total cholesterol
8. Reduces obesity, especially central obesity
9. Enhances fibrinolysis by lowering plasminogen activator inhibitor 1 (PAI 1).

A rise in macrovascular disease is correlated with the metabolic disease syndrome.

Side Effects[20,64-78]

The most common side effects of metformin therapy are gastrointestinal problems. The gastrointestinal symptoms that are most frequently experienced are metallic taste, anorexia, nausea, stomach pain, and diarrhea. By beginning treatment with low dosages of metformin (500 mg) and gradually increasing the dose, gastrointestinal problems can be reduced. It is beneficial to start medication therapy with the evening meal because taking the medication with meals reduces the symptoms. Another inevitable problem is lactic acidosis. Almost all individuals with compromised renal function have experienced lactic acidosis while using metformin. Thirty percent of diabetic patients receiving long-term metformin treatment have vitamin B12 malabsorption.

Hydroxychloroquine (HCQ) [69-97]

"Quinacrine was first used as an antimalarial during World War II, but it also had additional side effects such as yellow skin discoloration. Other antimalarial drugs like chloroquine and hydroxychloroquine (HCQ) were developed with considerable chemical modification as a result of quinacrine-related side effects. HCQ is an ancient medication that is still employed in clinical settings. It has been used successfully, to treat a variety of autoimmune disorders, including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and numerous skin conditions. It is also reasonably affordable and has few side effects. [69]

Blazar et al. provided the first description of chloroquine's antidiabetic effects (1984). With its post receptor suppression of insulin breakdown, HCQ possesses a novel mode of action. During the first six months of treatment with HCQ, patients with type 2 diabetes mellitus who were sulphonyl urea resistant showed improvement in their glycemic control, according to a landmark study by Gerstein et al. [69-74]

The drug hydroxychloroquine (HCQ) has demonstrated its effectiveness in the treatment of dyslipidemia and diabetes. In multiple clinical trials, HCQ demonstrated superior glycemic control in terms of lowering HbA1c, FPG, and PPG. Multiple scientific studies have suggested that HCQ has an impact on how insulin is metabolized. The rate at which insulin separates from its receptor is slowed down by direct interaction between HCQ and the insulin receptor. This has been hypothesized to lengthen the biological half-life of the receptor insulin complex and as a result, the duration of insulin activity. Insulin sensitivity is increased by HCQ's inhibition of insulin breakdown and enhancement of the hormone's metabolic effects. The metabolic activity of HCQ prolongs the action of insulin by decreasing the dissociation of insulin from its receptor (tyrosine kinase) and increasing the biologic half-life of the insulin receptor complex. [70-76]

The possible explanation for the glucose-lowering effect of HCQ maybe that HCQ stabilizes intracellular lysosomes and slows the breakdown of the internalized insulin receptor complex. When the intracellular concentration of HCQ reaches a high level, the intracellular pH is elevated, inactivating the proteolytic enzyme (insulinase), which is in charge of degrading insulin, leading to the recirculation of a significant amount of insulin in the active state.

Pharmacokinetic Properties: [20,73-75]

Hydroxychloroquine has rapid gastrointestinal absorption and elimination by the kidneys. It is metabolized to N-desethyl Hydroxychloroquine in the presence of Cytochrome P450 enzymes (CYP2D6, 2C8, 3A4 and 3A5). Orally administered hydroxychloroquine (HCQ) and chloroquine (CQ) are both well absorbed (0.7–0.8 bioavailability). Absorption is affected by severe malnutrition (such kwashiorkor), but not by diarrhea. Both hydroxychloroquine (HCQ) and chloroquine (CQ) have lengthy half-lives of 40 to 50 days and low blood clearance (e.g., hydroxychloroquine's blood clearance is 96 ml/min). [20,73]

In RA patients, blood concentrations vary greatly, with an eleven-fold variance in medication concentrations discovered after similar doses. Between 30 and 40 percent of proteins bind to albumin and alpha-glycoprotein. Between 21% and 47% of it is unchangedly excreted. With decreasing morning stiffness as HCQ concentrations rise and increasing EKG abnormalities as CQ concentrations rise, there is some evidence of a concentration response and toxicity link, but more research is necessary. While aspirin, ranitidine, and imipramine have not been identified, d-penicillamine and cimetidine have potential kinetic interactions that have been recorded. [20,75]

Pharmacodynamic Properties: [20,73-75]

A weak lipophilic base is hydroxychloroquine. The lipid cell membrane can be penetrated by HCQ, a weak diprotic base that can donate two protons or hydrogen ions, and it preferentially concentrates in acidic cytoplasmic vesicles. This causes these vesicles in macrophages or other antigen-presenting cells to have a high pH, which restricts the ability of different auto-antigenic peptides to associate with class II MHC molecules in the compartment for peptide loading and/or the processing and transport of this peptide-MHC complex to the cell membrane. Hydroxychloroquine also contributes by preventing the stimulation of the TLR9 family of receptors.

The free base form enters the lysosomes, where it builds up and undergoes protonation. As a result, the lysosome's pH rises from 4 to 6, which inhibits the activity of lysosomal acidic proteases. Reduced intracellular processing, glycosylation, and protein release also have a number of immunologic and non-immunologic effects. All of them together result in immune cell functions such neutrophil superoxide generation, phagocytosis, and chemotaxis that are reduced

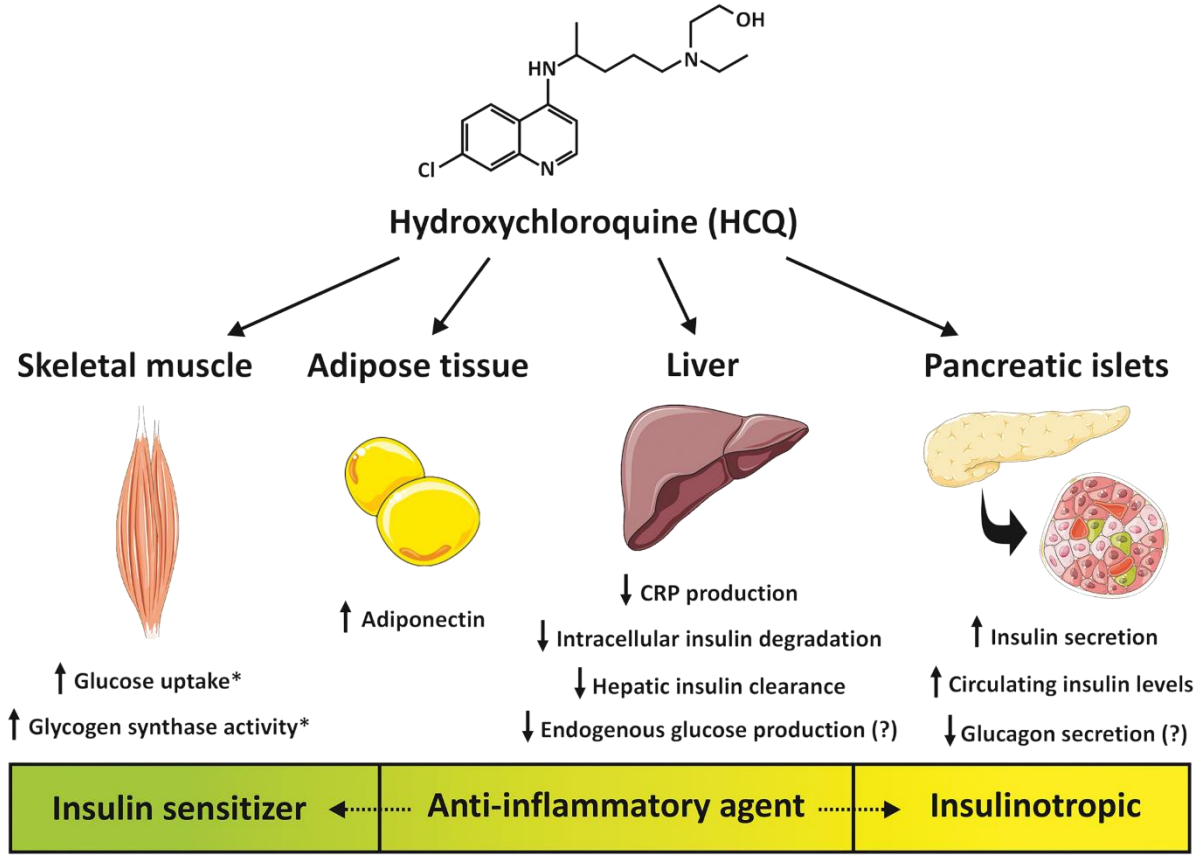


Fig: - 2.19 Mechanism of action of HCQ
SOURCE: https://doi.org/10.1111/1753-0407.13053

It has been demonstrated that cytokines including tumour necrosis factor (TNF) and interleukin-6 (IL-6) during inflammation increased obesity and insulin resistance by inducing crucial steps in the insulin signaling, hence affecting insulin and glucose metabolism. In adipocytes and skeletal muscle cells, TNF reduces the tyrosine phosphorylation of the insulin receptor and insulin receptor substrate-1 (IRS-1) kinase and increases the serine phosphorylation of IRS-1, which turns IRS-1 into an insulin receptor inhibitor. As a result, IRS-1 inhibits the insulin receptor's signaling pathway by inducing a negative feedback loop that reduces the receptor's enzymatic activity rather than just acting as a substrate for the insulin receptor. [74-82]

When compared to other nonbiologic DMARDs, TNF inhibitors and HCQ have a lower relative risk of developing diabetes, according to a retrospective cohort research on patients with RA or psoriasis who were treated with methotrexate, HCQ and other nonbiologic DMARDs. 13,669 RA patients with and without incident diabetes were included

in a retrospective cohort study by Ozen and colleagues. The patients were divided into four groups: methotrexate monotherapy, abatacept with or without synthetic DMARDs, any other DMARDs with methotrexate, and all other DMARDs without methotrexate. Separate statin, glucocorticoid, and HCQ treatments were also given. When compared to those who were not receiving HCQ medication, RA patients who were receiving HCQ exhibited a substantial risk reduction of acquiring T2DM. HCQ increases insulin sensitivity and reduces insulin resistance through its indirect effect by reducing inflammation. HCQ has been reported to improve insulin sensitivity through the activation of protein kinase β (Akt) resulting in increased glucose uptake and glycogen synthesis. [80-87]

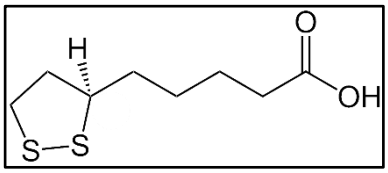
It is even endorsed by RSSDI (Research Society for the Study of Diabetes in India) clinical practice recommendations for the management of T2DM in 2017 . Hydroxychloroquine was approved by the Drug Controller General of India (DCGI) in 2014 for the management of Type 2 Diabetes (T2DM) as an adjunct to diet and exercise to improve glycemic control in patients with T2DM on combination of sulfonylurea and metformin. The recommended dose is 200mg twice daily with another diabetic drug[23]

By preventing the breakdown of insulin, hydroxychloroquine (HCQ) increases insulin sensitivity and glucose tolerance. It slows down the internalized insulin-receptor complex's decomposition and has a negligible impact on lowering glycemic indices and pro-inflammatory indicators. In individuals with type 2 diabetes, HCQ (400 mg) as compared to pioglitazone (15 mg) decreases HbA1c and LDL cholesterol levels and promotes weight loss, according to an Indian randomized controlled phase 3 trial. [88-92]

The positive effects of HCQ in a number of auxiliary illnesses, such as diabetes mellitus and dyslipidemia, are being supported by a growing body of research. The exact mechanisms of action causing these effects are still unknown, however they may involve changes in insulin metabolism and cellular receptor signaling. Further research into the underlying mechanisms of HCQ's beneficial metabolic benefits could contribute to the case for its application in the treatment of rheumatic disorders, which are linked to increased cardiovascular risk. [91-97]

Hence, we have chosen this drug for further research in the field of diabetes as very few animal studies with high dose(400mg) HCQ as antidiabetic has been done in India and abroad and no study has shown effect on organ histo-pathologically as per our knowledge.

Alpha Lipoic Acid [98-102]



Strong antioxidant alpha-lipoic acid is also referred to as 1, 2-dithiolane-3-pentanoic acid or thioctic acid. The unstable, highly reactive chemicals known as free radicals, which are byproducts of both healthy and stressed-out cell activity, cause cellular damage that alpha-lipoic acid combat. Endogenous antioxidants including glutathione, vitamin E, and vitamin C can be recovered thanks to it. It works well for a variety of medical disorders, including heart disease, type 2 diabetes, and liver illness.

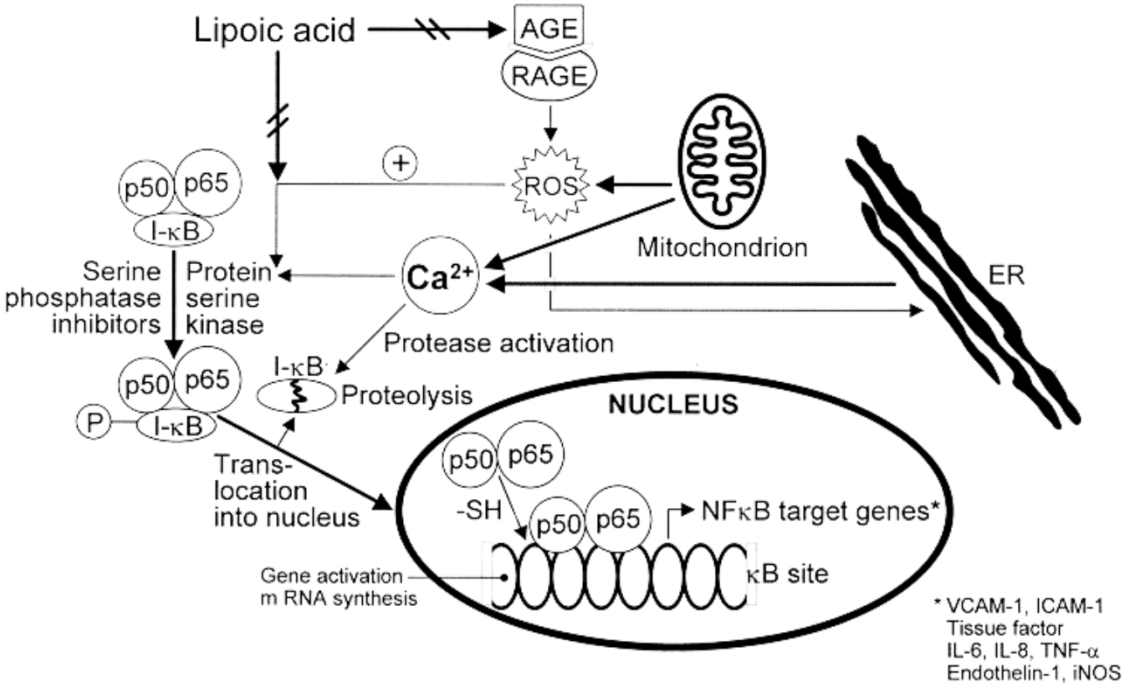


Fig 2.20: - Mechanism of action ALA at molecular level (Source; https://doi.org/10.1016/S0899-9007(01)00658-X)

In type 2 Diabetes Mellitus patients, alpha lipoic acid has been shown to advance glucose metabolism by directly activating lipid, tyrosine, and serine/threonine kinases in the target cells. These processes promote glycogenesis and glucose absorption. According to in vitro research, alpha-lipoic acid promotes the translocation of GLUT-1 and GLUT-4 to adipocytes and skeletal muscle's plasmatic membranes. It has to do with increased insulin signaling pathway protein activity. According to Budin et al., ALA consumption/intake decreased the total cholesterol and glucose levels in rats with STZ-induced diabetes. Packer et al. claim that ALA has the ability to scavenge ROS generated during lipid peroxidation and protects the cell structure.

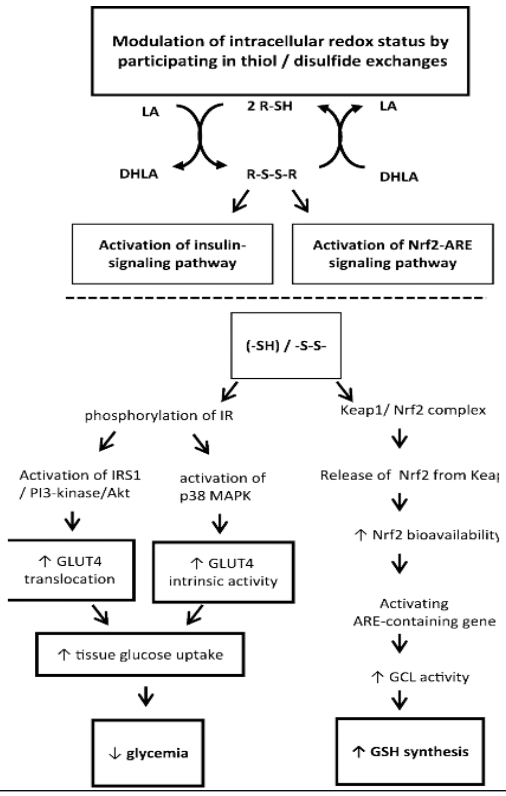


Fig: 2.21a showing hypoglycemic effect of ALA [Source: (2.21a Khalifa, Eman Aly, et al.)

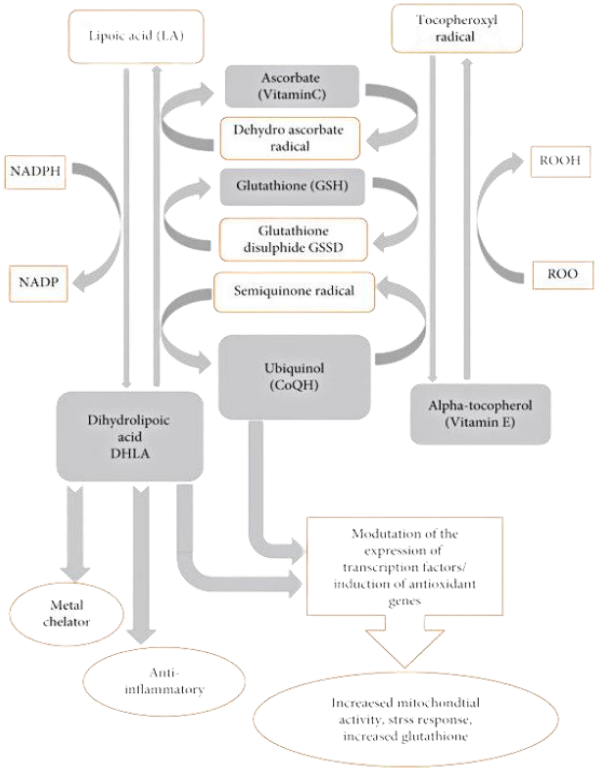


Fig: 2.21b showing ALA as a universal antioxidant (2.21b https://doi.org/10.1139/cjpp-2014-0353)]

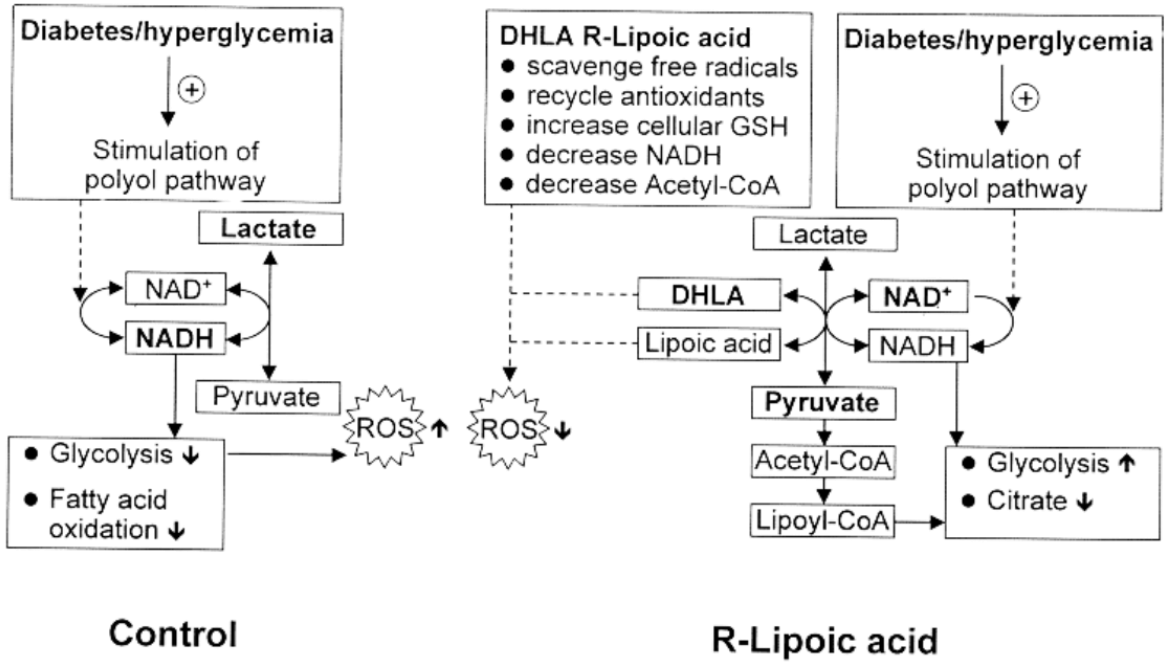


Fig 2.22: ALA As an Hypolipidemic Agent (source: https://doi.org/10.1016/S0899-9007(01)00658-X)

Pharmacokinetics

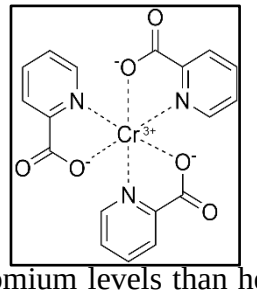
A 2007 human pharmacokinetic investigation found that sodium ALA rivals the plasma levels attained by intravenous administration of the free acid form and has a significantly higher maximum plasma concentration and bioavailability than the free acid form. High plasma concentrations were also attained, matching those observed in animal models where Nrf2 was activated. Between the various ALA formulations, there is no bioequivalence. About 27–34% of ALA taken orally is available for tissue absorption. ALA has a 30-minute plasma half-life.

Few studies have compared various enantiomers to racemic lipoic acid. It is unknown if twice as much racemic lipoic acid can be used to replace ALA. The dangerous amount of ALA in cats causes hepatocellular toxicity as opposed to humans or dogs. Through food or dietary supplements, up to 93% of an oral dose of ALA is promptly absorbed from the gastrointestinal tract (GIT). Due to its great capacity for absorption and storage, the liver is one of the essential organs for elimination. Only 3% of the body's waste is ejected in the faces, compared to around 45% in the first 24 hours. Furthermore, relatively little of the given ALA is excreted unaltered.

Pharmacodynamics

Both the oxidized and reduced forms of ALA have been found inside cells. Long incubation times of ALA allow it to scavenge reactive oxygen and nitrogen species in a biochemical experiment, but there is little proof that this happens inside of a cell or that radical scavenging is one of ALA's main modes of action. Dihydrolipoic acid, which is usually regarded as the more bioactive form of ALA and is responsible for the majority of the antioxidant effects as well as reducing the redox activities of unbound iron and copper, is formed when ALA is reduced in cells.

The majority of intracellular DHLA likely exists as mixed disulfides with different cysteine residues from cytosolic and mitochondrial proteins, even though both DHLA and ALA have been discovered inside cells following administration. According to recent research, the improvement of the cell's antioxidant status is caused by modulating gene transcription and signal transmission, which has therapeutic and anti-aging effects.



Chromium picolinate [103-127]

Diabetes patients have lower tissue chromium levels than healthy controls, and there is a link between the prevalence of type 2 diabetes and low circulating chromium levels. Since chromium (CrP) was identified in 1955 as an essential trace metal, it has been discovered to significantly increase glucose tolerance by lowering insulin resistance. Chromium supplementation may increase systemic insulin sensitivity since there is mounting evidence that it may promote insulin signaling

Nutritional chromium enhances insulin activity and blood lipid profile. Most diets fall short of the recommended 50 mg daily requirement for CrP. CrP levels that are inadequate cause symptoms and signs that resemble diabetes and cardiovascular conditions. Chromium improves the glucose/insulin levels in people who have diabetes, hyperlipidemia, hyperglycemia, and hypoglycemia, with no discernible effects in the control group. By raising insulin sensitivity, cell sensitivity, and insulin internalization, chromium also enhances insulin binding, receptor number, and insulin receptor enzymes.

Another study on the impact of chromium supplementation on blood lipid levels and glucose metabolism found that while persons without diabetes did not experience any change in their blood lipid or glucose levels, those with diabetes experienced a considerable improvement. As a result of subsequent research, dietary CrP was dubbed a "glucose tolerance factor"(GTF) since it was found to physiologically boost insulin activity. CrP contributes to increased insulin binding, increased insulin receptor quantity, and increased phosphorylation of the insulin 5 of 17 receptors. The correlation between serum CrP concentrations and T2DM was discovered by Rajendran et al. They claimed that the metabolic response to oxidative stress in T2DM patients resulted in a decrease in CrP levels.

Pharmacokinetics

When chromium (III) picolinate is consumed and reaches the stomach, the complex undergoes acidic hydrolysis when it comes into contact with the stomach mucosa. When the hydrolyzed Cr3+ reaches the alkaline pH of the small intestine, it polymerizes to create an insoluble CrP (III)-hydroxide-oxide (the process of olation), which is not soluble. Chromium (III) picolinate is the form of Cr3+ that is absorbed from the stomach on a 2% unsaturated passive transport basis. Even though it absorbs poorly, chromium picolinate (CrPic3) accumulates at higher levels in tissues than other organic and inorganic sources (such as CrCl3 and chromium nicotinate).

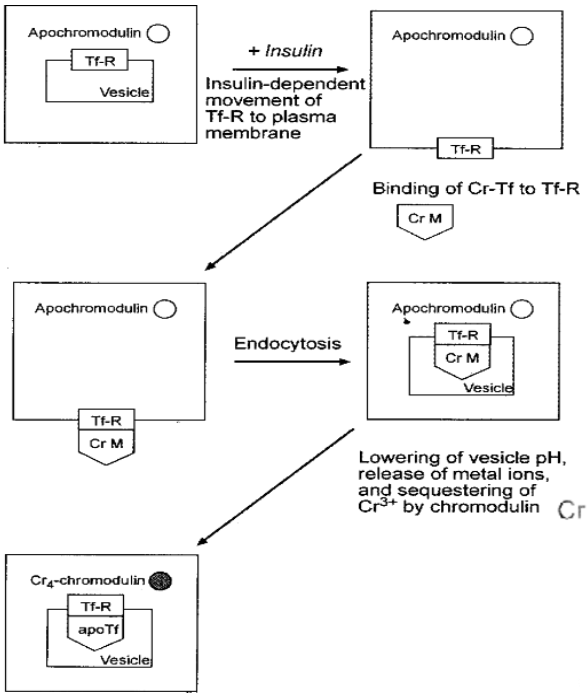


Fig: 2.23a showing chromium mechanism of action. (Source:2.23a PMID: 15505017 DOI: 10.2337/diacare.27.11.2741)

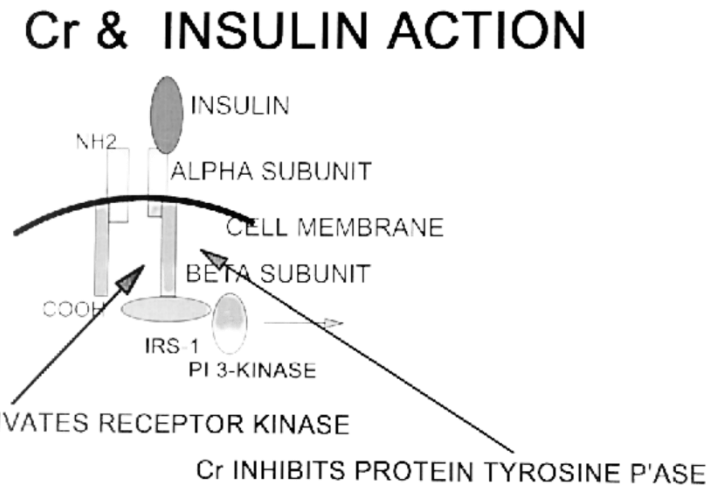


Fig: 2.23b Mode of action of chromium in potentiation of insulin (Source https://doi.org/10.1007/ BF02784583)

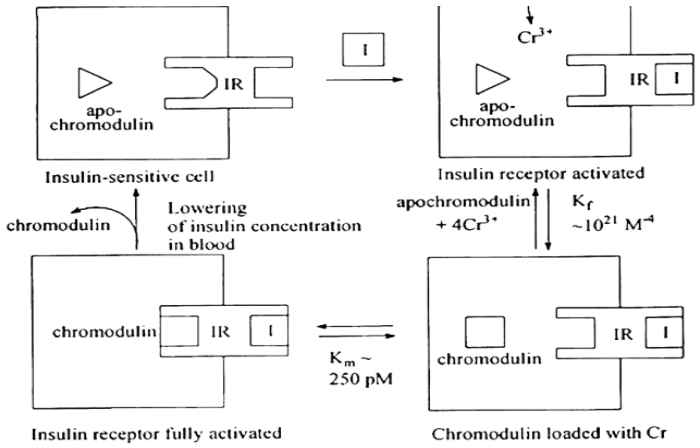


Fig 2.24: showing suggested mechanism for the activation of insulin receptor kinase activity by chromium in response to insulin (Source: PMID: 17952838 DOI: 10.1055/s-2007-985847)

Suggested mechanism for the activation of insulin receptor kinase activity by chromium in response to insulin as shown in Fig 2.24. Insulin (I) binds to an external subunit of the inactive insulin receptor (IR), bringing about a conformation change of the receptor.

The interior region of the insulin receptor's subunit undergoes autophosphorylation of tyrosine residues, which is changed into the active form by insulin binding. Chromium has been observed to migrate from the circulation to insulin-dependent cells as plasma insulin concentrations rise. The metal transport protein transferrin is probably involved in mediating this transfer (CrP-transferrin, CrP-Tf). Chromium binds to apochromodulin as a result of this entry of chromium into insulin-dependent cells (triangle). According to Figure 2.25 The insulin receptor cannot be bound by apochromodulin, and kinase activity cannot be triggered. In the end, the holochromodulin (square) attaches to the insulin receptor, stimulating the insulin-stimulated receptor kinase activity even more, assisting in maintaining its active conformation, and enhancing insulin signalling. When the insulin concentration drops, holochromodulin is released from the cell to relieve its effects (modified after).

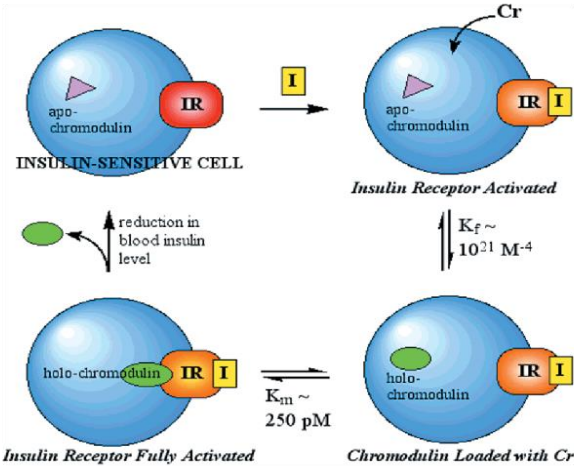


Fig 2.25: Proposed mechanism for activation of insulin receptor kinase activity by chromodulin in response to insulin (DOI: 10.1079/NRR200366)

2.7.4 Animal choice

The favored species for biomedical research animal models has long been mice and rats because of their physical, physiological, and genetic similarity to humans. Due to their small size, minimal maintenance needs, short lifespans, and abundant genetic resources, rodents are advantageous. Rats are still preferred in several scientific fields, such as toxicology, behavioral science, and cardiovascular science. Because of their tiny size, minimal maintenance requirements, and ease of access to methods for genetically changing their genomes since the 1980s, rats are typically the first choice for rodent models. [155]

2.7.5 Streptozotocin (streptozocin: STZ) [128-147]

A constant diabetes mellitus producing drug is streptozotocin. Streptozotocin is a glucosamine nitrosourea chemical with a glucose molecule and methyl group connected at one end. Its chemical name is 2-deoxy-2(methyl nitrosamine) carbonyl) amino)-D-glucose, and it was produced by the bacterial strain Streptomyces achromogenes in a fermentation broth (gram positive bacterium). Typically, it is a poisonous glucose analogue that causes experimental diabetes. The STZ-induced diabetes was originally demonstrated in an animal model by Rakieten et al. It is commonly administered as a single intravenous dose between 40 and 60 mg/kg of body weight based on the prior experimental model. Through glucose transporter 2, streptozotocin penetrates the beta cells of the pancreas (GLUT-2). DNA alkylation caused by the methyl nitrosourea moiety is the mechanism by which streptozotocin acts on the beta-cell. DNA is damaged as a result of the streptozotocin's methyl group transfer, which results in DNA fragmentation. When DNA is damaged, poly ribosylation is activated, which lowers NAD and ATP levels and eventually may cause beta-cell death. Malignant insulinomas are treated with it. STZ is transported into the cell by the low affinity glucose transporter GLUT-2 of Beta-cells, which results in DNA alkylation and the permanent necrosis of cells. The biological effects of streptozotocin, such as its antibacterial, beta-cell (beta)-cytotoxic, oncolytic, and oncogenic actions, reveal that it has four important biological qualities. Using a chromatographic approach, streptozotocin (2-deoxy-2-[3-methyl-3-nitrosourea] 1-D-glucopyranose) can be distinguished into two anomeric forms, and (HPLC).

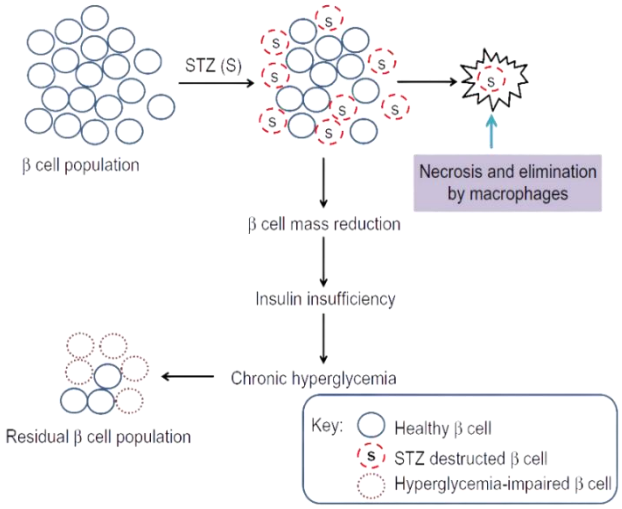


Fig: -2.26a Mechanism of action of STZ (Source: https://doi.org/10.2147/DMSO.S82272.)

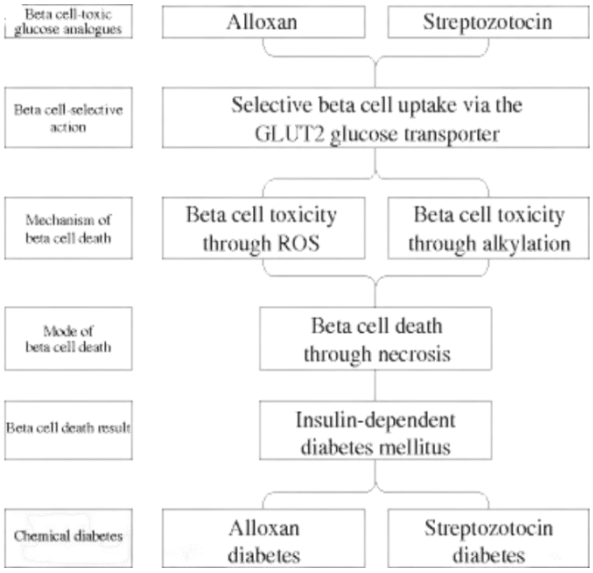


Fig: 2.26b Induction of diabetes by Alloxan & STZ (Source Busineni Jayasimha Goud et al. Ijppr. Human, 2015; Vol. 3 (1): 253-269)

In terms of solubility, STZ is largely soluble in polar organic solvents, but only to a limited level in water, ketones, and lower alcohols. This disintegrates in water at a rate of 50 mg/mL and produces a light-yellow, clear to slightly cloudy solution. Rapid mutarotation to an equilibrium blend of alpha- and beta-anomers occurs in aqueous STZ solutions. At pH 4, STZ solution is most stable, with stability rapidly dropping at higher or lower pH. Solutions that have just been made are transparent and have a faint straw tint. According to many research associations, the streptozotocin solution (in citrate or acetate buffer, pH 4.5) should be supplied "immediately" and no later than 15 to 20 minutes after dissolution.

Efficacy over Alloxan

The majority of animal research uses chemicals or medications to artificially induce diabetes. Alloxan is a well-known chemical substance that has been utilized in research on diabetes; it is poisonous and causes the pancreatic beta cells to die. In earlier studies, the drug alloxan was used to cause type 1 diabetes in rats, mice, and rabbits. Due to its toxicity and instability, Types 1 and 2 diabetes are now induced using streptozotocin rather than alloxan. STZ is an ideal agent to induce experimental type 2 diabetes, it has more advantages than alloxan over sustained hyperglycemia and the development of well characterized diabetic complications with a low incidence of ketosis and mortality.

1. The range of the STZ dose is not as narrow as in the case of alloxan.
2. In compared to alloxan, STZ's higher chemical stability and lower toxicity allow for better manipulation and more flexible dosing.
3. In addition, STZ administration is preferred to alloxan treatment due to a larger proportion of successfully inducing diabetes and lower mortality rates in experimental animals.

Route of administration

The two most usual ways to administer STZ are intraperitoneally (IP) or intravenously (IV), though rodents have also received the medication via subcutaneous, intracardiac, and intramuscular routes.

Dose of STZ

A single 100 mg/kg intraperitoneal dose causes severe islet destruction and results in insulin-dependent diabetes. The dosing range for inducing experimental type 2 diabetes is 35–65 mg IP.

Diabetogenic action of STZ

1) Alkylation of DNA by STZ causing DNA damage, PARP activation, leading to depletion of NAD+ and ATP stores that causes pancreatic beta cell death

2) Spontaneous release of NO by STZ, impairment of mitochondria by NO, inhibition of APT synthesis ultimately leading to beta cell death.

3) Generation of free radicals like superoxide (O2◦-), hydroxide (OH◦-), peroxynitrite (ONOO), causing beta cell damage.

4) Inhibition of O-GlcNAcase by STZ, formation of irreversible glycosylated proteins that damage pancreatic beta cell.

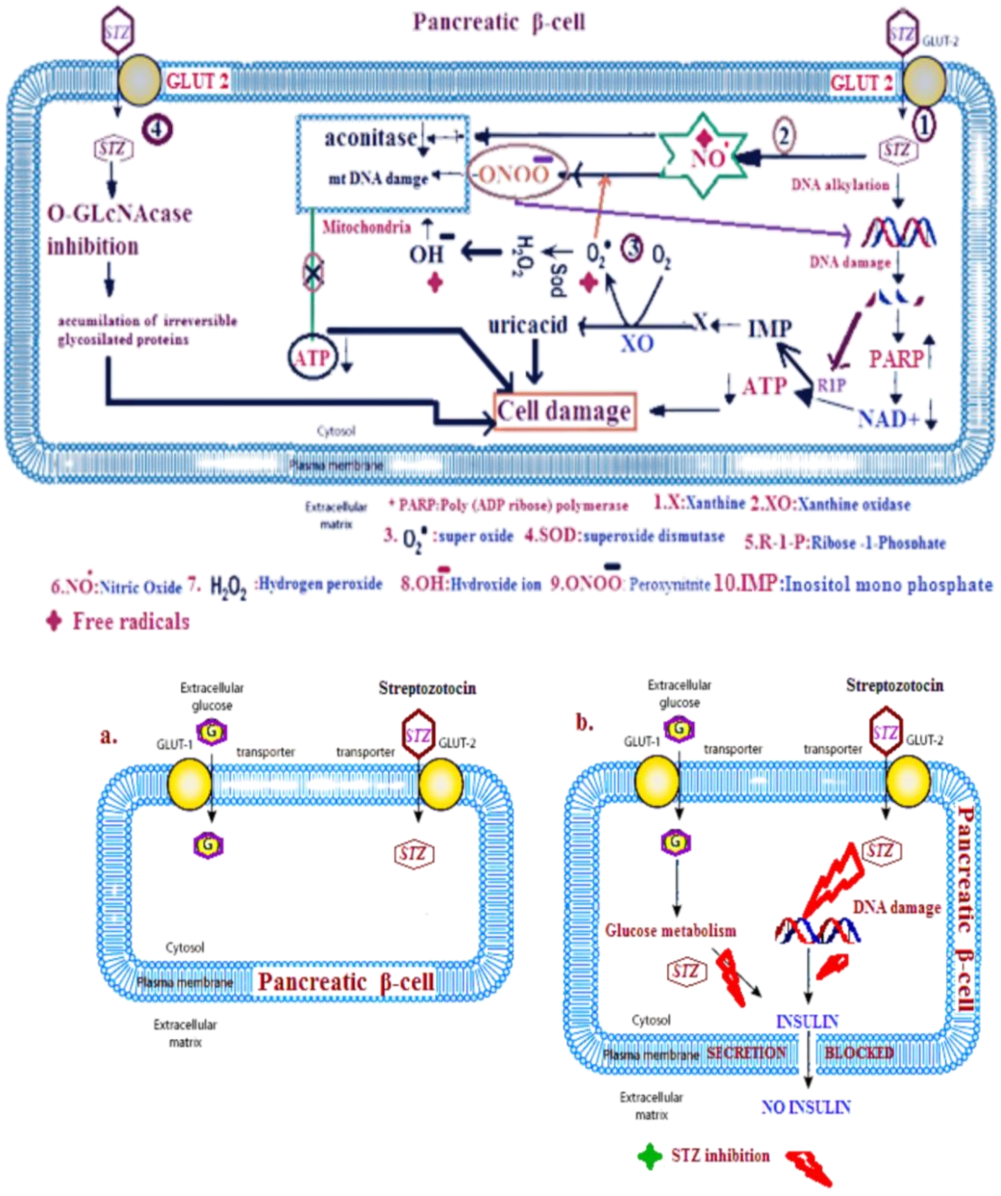


Fig: 2.27 showing: Detailed mechanism of STZ toxicity. (Source: Busineni Jayasimha Goud et al. Ijppr. Human, 2015; Vol. 3 (1): 253-269)

Comparison of the chemical properties of alloxan and streptozotocin		
	Alloxan	**Streptozotocin**
Chemical name	2,4,5,6-Tetraoxypyrimidine; 2,4,5,6-pyrimidinetetrone	2-Deoxy-2-([(methylnitrosoamino)carbonyl]amino)-D-glucopyranose
Chemical structure	Oxygenated pyrimidine derivative; barbituric acid derivative (5-ketobarbituric acid)	Cytotoxic methylnitrosourea moiety (*N*-methyl-*N*-nitrosourea) attached to the glucose (2-deoxyglucose) molecule; glucosamine derivative
Chemical properties	Very hydrophilic, beta cell-toxic glucose analogue (partition coefficient –1.8); weak acid	Hydrophilic, beta cell-toxic glucose analogue
	Chemically unstable (half-life of 1.5 min at pH 7.4 and 37° C, decomposing to alloxanic acid); Stable at acid pH[a]	Relatively stable at pH 7.4 and 37° C (at least for up to 1 h)[b]
Chemical reactivities	Thiol reagent that is reduced to dialuric acid in the presence of GSH and other thiols	DNA alkylating agent
	A protoxin; intracellular metabolism of this xenobiotic generates toxic ROS through redox cycling with dialuric acid over a long time period (>1 h)	Protein alkylating agent
	'Compound 305', a non-toxic alloxan-GSH adduct of unknown structure with a characteristic absorbance at a wavelength of 305 nm; a small amount is formed during each redox cycle	NO donor
Mode of toxicity	Generation of ROS	DNA alkylation

[a]For experimentation, concentrated stock solutions in 0.01 mol/l HCl, kept on ice, should be used and added to the test medium just prior to the start of the experiment to obtain the final concentration. For injection, the stock solution should be diluted with ice-cold saline (0.9% NaCl) immediately prior to injection.

[b]For in vitro experimentation, concentrated stock solutions in 0.01 mol/l HCl, kept on ice, should be used and added to the test medium just prior to the start of the experiment to obtain the final concentration. For injection, a stable solution in citrate buffer (pH 4.5) is most suitable.

Fig: 2.28 Comparison of the chemical properties of alloxan & streptozotocin Source: https://rdcu.be/cYm15/ https://doi.org/10.1007/s00125-007-0886-7)

Disadvantages Of Streptozotocin Model

1. STZ is a pancreatic cell chemical toxin that induces prompt and irreversible necrosis of pancreatic cells.
2. STZ-induced diabetic rats demonstrated severe hyperglycemia (FBG≥250) associated with a depletion of pancreatic insulin content/action. After a month they need insulin for survival.
3. Residual beta cell tries to overcome the insulin depletion by development of functional insulinoma.
4. STZ is a DNA alkylating substance and my lead to liver and kidney tumors in rats.

When used as an antineoplastic treatment for various cancers, STZ has been shown to be cytotoxic to pancreatic beta-cells even at therapeutic dosages (up to 15 mM), and at these concentrations, STZ causes apoptosis in pancreatic beta-cells. Recent research has demonstrated that STZ is toxic to the neuroendocrine cells of the gut as well as other organs that express GLUT 2, including the brain, liver, and kidneys. The selective uptake of STZ, its metabolic activation, and detoxification in particular cell types, as well as on the redox homeostasis and mitochondrial bioenergetics in these cells, may be associated with the differential cytotoxicity by STZ in different cellular systems because STZ competes with glucose uptake and is thought to be dependent upon the specific expression of selective GLUT transporters.

Experimental model of T2DM

For the purpose of improving our understanding of the numerous facets of its pathophysiology and ultimately discovering new treatments and a cure, experimental production of diabetes mellitus in animal models is crucial. Various techniques have been employed with varying degrees of effectiveness to cause diabetes mellitus in lab animals. The pancreas can be surgically removed, but at least 90–95 percent of the pancreas must be removed in order to cause diabetes. A less reliable method of inducing diabetes has been anterior hypophysis extract injection. [156]

For reversible diabetes induction, an alloxan-induced diabetic animal is used. On the other hand, this model has a high mortality rate. Another method which is more uniformly effective and widely used is the injection of Streptozotocin nicotinamide model.

Characteristics of the experimental model (Streptozotocin-nicotinamide)

Streptozotocin's beta cell toxicity is mostly caused by the pancreas's loss of NAD. Nicotinamide inhibits poly (ADP-ribose) synthetase activity and prevents NAD depletion in pancreatic beta-cells, which reduces the cytotoxicity caused by streptozotocin. In India, Shirwaikar A et al. (2000), Nayak Y (2014), and many others used a single intraperitoneal injection of 60 mg/kg STZ 15 minutes following a 120 mg/kg nicotinamide injection to induce type 2 NIDDM. This dosage effectively produced the moderate fasting hyperglycemia with a blood glucose range of 180–250mg. In order to obtain moderate and sustained hyperglycemia with 40% conservation of pancreatic insulin stores, Pellegrino Masiello and his team administered several dosages of nicotinamide (100-350 mg/kg body wt.) intra-peritoneally 15 min before STZ delivery (65 mg/kg i.v.). This unique NIDDM syndrome, which is identical to human NIDDM in terms of diminished pancreatic insulin storage.

It has been suggested that the administration of nicotinamide (NA) plus streptozotocin (STZ) will cause experimental diabetes in rats. Rats are given NA to partially protect insulin-secreting cells from STZ, but STZ is known to harm pancreatic B-cells. The glucose transporter GLUT2 allows STZ to enter B-cells where it damages DNA and increases the activity of the DNA repair enzyme poly (ADP-ribose) polymerase (PARP-1). However, excessive activity of this enzyme causes the cells that secrete insulin to necrotize and deplete intracellular NAD (+) and ATP. NA's protective effects result from its suppression of PARP-1 activity. Inhibiting this enzyme stops cells exposed to STZ from losing NAD (+) and ATP. Additionally, because NA is a precursor to NAD (+), it raises intracellular NAD (+) levels. The STZ and NA doses that are administered to the experimental rats have a significant impact on how severe their diabetes is.

As a result, as compared to control animals, diabetic rats' blood glucose levels might vary greatly, from mild hyperglycemia to severe hyperglycemia. The level of blood insulin may also only slightly decrease or significantly increase. The insulin-secretory response to glucose is slowed down in STZ-NA-induced diabetic rats compared to control animals, according to in vitro investigations. This results from decreased beta-cell bulk as well as metabolic issues in the cells that secrete insulin. Numerous test’s findings have shown that this diabetes model is helpful in investigations of several elements.

MATERIAL & METHODS

Study Design: Experimental

Study Period: 42 Days

Animals:

For the current diabetic study, healthy male Wistar rats weighing between 150 and 250 gms were used. The animals were kept in clean, dry cages with a 12 h:12 h light-dark cycle at ambient humidity and temperature. They were acclimated to the housing conditions and fed a typical laboratory diet of boiled eggs, soaking black gram (Kala Chana), and water, which was available at all times. Arrangements were made in place to guarantee methodological cage cleaning and excreta and urine disposal. The layout of the cages included a layer of sawdust to absorb the rat urine. This was done because rats would urinate excessively after having diabetes was induced. 'Principles of laboratory animal care' (NIH publication no. 82-23, revised 1985) guidelines were followed. The whole experiment was conducted in accordance with ethical norms approved by Institutional Animal Ethics Committee (IAEC) Guidelines *(Memo no:- 162, IAEC Rims Ranchi, dated on 26/02/2021)*

Sample Size Calculation:

Sample size is calculated on the basis of blood glucose level variation in the groups (based on the previous study) [148]

$$n = (\sigma_1^2 + \sigma_2^2) * (Z_\alpha + Z_\beta)/ d^2$$

Type I error = 5% Type II error -20% σ_1=2.36 σ_2=2.48

Power of study-80% Loss of data=10% d=4.00

n=6 each group

"The "resource equation method" is a second approach that can be applied. Based on the selected sample size, a value E (degree of freedom) is computed using this procedure. The ideal sample size for E should be between 10 and 20. More animals should be used if the value of E is less than 10, and the sample size should be reduced if it is greater than 20. However, with more than 20 DF, adding more units doesn't really add any more information." [149]

E = Total number of animals - Total number of groups

In my study, E is (36-6=30), i.e., on the higher side, to keep in mind the risk of death of rats after streptozotocin injection, and larger sample size leads to more power of study (80%).

Drugs And Chemicals:

1. Alpha lipoic acid 600mg capsule, HealthyHey Foods LLP. Mumbai
2. Chromium picolinate 600mcg capsule, Medizen Labs Pvt Ltd. Bangalore
3. Hydroxychloroquine 400mg tablet, Ipca Laboratories Ltd Mumbai
4. Metformin 500 mg tablet (Glycomet), US Vitamins Limited
5. Streptozotocin 250 mg powder, Sisco Research Laboratories (SRL) Pvt Ltd, Maharashtra
6. Nicotinamide 100 gm powder, Sisco Research Laboratories (SRL) Pvt Ltd, Maharashtra

Chemicals And Reagent Kits:

1. Distilled water	**2.** Glucometer strips
3. Dettol and sterilized cotton	**4.** 1 ml syringe
5. Normal Saline	**6.** Disposable syringe 5 ml
7. Citric acid powder	**8.** Animal feeding tube (2 Gavage tube)
9. Sodium citrate powder	**10.** Sterilized needle and surgical blades
11. Glucometer	**12.** Chloroform

Preparation Of Testing Materials:

1. **Citrate Buffer:** We brought citric acid and sodium citrate powder from the biochemistry department. There were two different solutions prepared. The first solution, designated solution "A," was created by combining 1.921 g of citric acid powder with 100 ml of deionized water. The second one, named solution B, was created by dissolving 2.941 g of sodium citrate in 100 ml of deionized water. The

following portions of solutions A and B are combined to create a volume of 50 ml, which is then increased to 100 ml by adding deionized water, in order to create 0.1 M citrate buffer with a desired pH.

ml of Citric acid	46.5	40.0	35.0	31.5	25.5	20.5	16.0	11.8	7.2
ml of Sodium citrate	3.5	10.0	15.0	18.5	24.5	29.5	34.5	38.2	42.8
pH	3.0	3.4	3.8	4.2	4.6	5.0	5.4	5.8	6.2

The ideal pH for stability of streptozotocin is 4-4.5. So, 31.5 ml of solution 'A' and 18.5 ml of solution 'B' were mixed together in a measuring cylinder and the final volume was adjusted up to 100 ml by adding deionized water. We got our desired pH of 4.2. as shown in Glossy paper Fig : 6

2. **Preparation Of Streptozotocin**: - I purchased 250 mg of streptozotocin from SRL CHEM Maharashtra in Mumbai. It was kept in dry ice for shipment, and following delivery, it was kept at -200C. Streptozotocin should be utilized within 15 to 20 minutes of being removed from the deep freeze since it is very sensitive to temperature. Streptozotocin at a dose of 60 mg/kg was used to induce moderate type 2 diabetes. It means that 12 mg of streptozotocin are needed to induce diabetes in a 200 mg rat. For that, 40 ml of 0.1 M citrate buffer and 480 mg of streptozotocin were combined (pH of 4.2). As a result, 12 mg of streptozotocin were present in 1 ml of buffer solution. Now during diabetes induction dose was adjusted according to the body weight of the rat.
3. **Gum acacia: -** By combining 1 gm of gum acacia with a little amount of distilled water in a mortar and pestle, and then creating a final suspension in 100 ml of distilled water, it was possible to create 1% gum acacia suspension. Every day, right before the administration, this was prepared.
4. **Metformin: -** The tablet was powdered, and 1% gum acacia was used to provide a consistent suspension. For mild type 2 diabetes, the recommended daily dose of metformin is 500 mg/70 kg. The daily dose for a 200 g rat will be 9 mg when 500 is multiplied by 0.018. To achieve a concentration of 10 mg/ml, 500 mg of metformin were dissolved in 50 ml of 1% gum acacia solution. The amount of metformin solution was now regulated during feeding via gavage tube in accordance with the rat's body weight.

5. **Nicotinamide: -** For this study, the nicotinamide dose was 120 mg/kg. This indicates that a 200 kg rat would receive 24 mg of nicotinamide. In order to achieve a strength of 24 mg/ml, 2400 mg of nicotinamide were dissolved in 100 ml of ordinary saline. The nicotinamide dosage was now altered during the formation of diabetes in accordance with the rat's body weight.
6. **Hydroxychloroquine: -** The tablet was powdered, and 1% gum acacia was used to provide a consistent suspension. In this study, 400 mg/70 kg of hydroxychloroquine per day was employed. The daily dose for a 200 g rat will be 7.2 mg when 400 is multiplied by 0.018. To achieve a concentration of 10 mg/ml, 400 mg of hydroxychloroquine were dissolved in 50 ml of 1% gum acacia solution. The amount of hydroxychloroquine solution was now regulated during feeding through gavage tube in accordance with the rat's body weight.
7. **Alpha lipoic acid: -** When the capsule was opened, the contents were placed into a mortar and pestle-mixed to create a homogeneous suspension using 1% gum acacia. In this study, an alpha lipoic acid dose of 600 mg/70 kg per day was employed. The daily dose for a 200 g rat will be 10.8 mg when 600 is multiplied by 0.018. To achieve a concentration of 10 mg/ml, 600 mg of alpha lipoic acid were dissolved in 50 ml of 1% gum acacia solution. The volume of the alpha lipoic acid solution was now regulated during feeding through gavage tube in accordance with the rat's body weight.
8. **Chromium picolinate: -** When the capsule was opened, the contents were placed into a mortar and pestle-mixed to create a homogeneous suspension using 1% gum acacia. In this study, an chromium picolinate dosage of 600 mcg/70 kg per day was employed. This suggests that the daily dose for a 200 kg rat will be 10.8 mcg after multiplying 600 by 0.018. To achieve a concentration of 10 mg/ml, 600 mcg of chromium picolinate were dissolved in 50 ml of 1% gum acacia solution. The amount of chromium picolinate solution was now regulated during feeding through gavage tube in accordance with the rat's body weight.

Place of study

The entire experiment was carried out in “Department of Pharmacology and Therapeutics & Biochemistry” at “Rajendra Institute of Medical Sciences, Ranchi”.

Inclusion Criteria: –

1. All the animals used for the study were healthy and active in their cage.
2. Animals were male wistar rats.
3. Weight of the animals used was 150-250grams.
4. Fasting blood sugar before the initiation of study was within the range of 200- 250 mg/dl.

Exclusion criteria: –

1. Diseased and inactive rats were excluded from the study.
2. Rats with weight less than 150 grams and above 250 grams were excluded from the study.
3. Rats with fasting blood sugar under 200 mg/dl and above 250 mg/dl were considered as improperly diabetic and excluded from the study.
4. Female rats were excluded.

Study animals were divided into six groups with six animals in each group. The rats were kept in six animal cages. Permanent markers were used to individually label and color-code each animal in its cage. For 42 days, from 9:30 am to 11 am in the morning, rats received various treatments orally.

The details of groups were as follows:

Group	Number of Rats	Drugs	Dose
A **Normal Control**	6	*Vehicle* *Gum acacia 1%*	10 ml/kg body wt
B **Diabetic control**	6	*Vehicle* *Gum acacia 1%*	10 ml/kg Body wt
C **Diabetic Control with metformin**	6	*Metformin*	500 mg/70kg
D **Diabetic control with hydroxychloroquine**	6	*Hydroxychloroquine*	400 mg/70kg
E **Diabetic control with alpha lipoic acid**	6	Alpha Lipoic Acid	600 mg/70kg
F **Diabetic control with Chromium picolinate**	6	*Chromium Picolinate*	600 mcg/70kg

Induction of Diabetes Mellitus:

Streptozotocin at a dose of 60 mg/kg was administered intraperitoneally as a single, freshly prepared inoculation to induce diabetes. Nicotinamide was administered intraperitoneally at a dose of 120 mg/kg just fifteen minutes before the streptozotocin injection. The animal was supported with its ventrum exposed and its head pointing downward in order to give streptozotocin and nicotinamide intraperitoneally. This made accidental organ puncture less likely by causing the freely moving abdominal organs to migrate in the direction of the animal's diaphragm. A 26 gauge needle with 1 ml syringe was inserted into the abdominal cavity in the lower right quadrant to avoid the caecum and urinary bladder. The needle was directed towards the animal’s head at an angle of 15-20 degrees and inserted approximately 5mm. The syringe's plunger was retracted after the needle was inserted to ensure that no liquid, including blood, was aspirated. The medication solutions were then gradually released. To combat the hypoglycemia caused by streptozotocin after intraperitoneal injections, animals were permitted to drink 5% glucose solution overnight.

After receiving a dose of streptozotocin for 72 hours, the random blood sugar level was assessed. The study only included the rats with blood glucose levels between 200 and 250 mg/dl; the other rodents were released into the wild. The diabetic animals' cages were kept at room temperature and provided with free access to tap water and a regular laboratory food. Despite the fact that diabetes was diagnosed after streptozotocin induction for 72 hours, medication wasn't started until the eighth day, which was regarded as day "0." After that, the course of treatment lasted 42 days. Before the induction of diabetes, after 72 hours, and on days 0, 7, 14, 21, 28, 35, and 42, blood samples from all groups were taken in order to measure the random glucose level using the glucometer.

Estimation of Blood Sugar:

The rats were fed overnight and were given free access to water for the calculation of random blood sugar. Since the rat's tail is the most venous portion of its body, blood samples were taken from it. The rat's tail was cleaned with spirit cotton before being severed with a sterile blade by 0.5 mm, just enough to let one drop of blood escape. The test strip was prepared in advance and affixed to the Glucometer. The relevant reaction zone of the strip could only have one drop of blood. Within a few seconds, the level of random blood sugar appeared on the display that was noted down in the master chart. After taking the blood sample, betadine ointment was applied to tail of each rat to prevent the infection.

Anesthetizing and Sacrificing rats:

The permission for sacrificing experimental animal was taken from the institutional animal ethics committee. At 42 days, last day of experiment, the rats were sacrificed to obtain appropriate amount of blood and organ specimen for histopathology as per CPCSEA guidelines. The rats were properly taken care of throughout the period of study but at the end because of very low amount of blood collected by tail method and inability of experience of withdrawal of blood by cardiac puncture, sacrifice of rats were done after proper anesthetizing the rat under aseptic condition.

Chloroform was obtained in the department from pharmacy college. Chloroform-soaked cotton was put in a container where rats were put one by one, after that rats were completely anesthetized blood from tail was taken and random blood glucose was done by glucometer, this process was also done to check the consciousness of anesthetized rat. After confirming the rat was not awake, a cut was made on the neck of rat, and blood was collected in well labeled prepared test tube. As the blood dried up and fails to come, the rat was completely decapitated. Later the viscera (liver, two kidneys, heart with blood vessel, and pancreas) were removed from the rat after proper dissection and specimen were put in formalin already put in urine collection container.

All procedures were done keeping in mind that no pain sensation was felt by the rat. Similarly, all rats were sacrificed, 12 specimen containers were conserved in formalin and 9 were sent for slide preparation (mounting and staining) and histopathological examination. Rest rat specimens were preserved in formalin and I tried to prepare a small rat museum. In my experiment, I tried every part of the rat was used in some manner so that their sacrifice to scientific study don’t go waste.

Biomedical waste management

Syringes, needles, cotton, and other organic waste were disposed of in the appropriate biomedical dustbins, which were then removed by the waste management staff. According to hospital biomedical waste management policies, all organic waste was correctly disposed of in the yellow bin and the remaining garbage, including plastic, cotton, syringes, and gloves, in the blue bin.

Estimation of Lipid Profile

Lipid profile was measured with the help of fully automated autoanalyzer (Vitros 250 Automatic Dry Chemistry Analyzer)

a) Estimation of Triglycerides: The process was done by Glycerol Phosphate Oxidase (GPO) Method

b) Estimation of Total Cholesterol: Was done using CHOD PAP reagent

c) Estimation of HDL-C

d) Estimation of LDL.

LDL-C was calculated by Friedewald formula (Friedewald et al. 1972)

LDL-C = (Total Cholesterol)- (HDL-C) -(TG/5)

e) Estimation of VLDL

VLDL-C was calculated by Friedewald formula (Friedewald et al. 1972)

VLDL = Triglycerides/5

f) Estimation of non-HDL

Non-HDL cholesterol was calculated as per the method of Kim JE et al. (2011).

Non-HDL = TC-(HDL-C)

g) Atherogenic Index

Atherogenic index (AI) was calculated as per the method of Kim JE et al (2011).

AI=TC- (HDL-C) /(HDL-C)

h) LDL / HDL Ratio

LDL / HDL Ratio was calculated as per the method of Kim JE et al (2011).

This ratio = LDL / HDL

i) Cholesterol Ratio

Cholesterol Ratio was calculated as per the method of Kim JE et al (2011).

Cholesterol Ratio = TC / HDL

Estimation of Renal Function Test & SGPT:

Lipid profile was measured with the help of fully automated autoanalyzer (Vitros 250 Automatic Dry Chemistry Analyzer)

a) Estimation of Serum Urea: was calculated as per the method of Kim JE et al. (2011)

b) Estimation of Serum Creatinine: was calculated as per the method of Kim JE et al.

c) Estimation of SGPT: was calculated as per the method of Kim JE et al. (2011)

Statistical Analysis

Study of variance was used to do a statistical analysis of the data. Sir Ronald Aylmer Fisher was the first to develop the theory of analysis of variance, and George Waddell Snedecor was the second. When more than two samples are involved, ANOVA is a helpful technique. It is a crucial step in determining whether various data groups are homogeneous. To examine how different groups responded to various medicines, a one-way ANOVA test was employed. The Tukey's HSD (honestly Significant Difference) test was used for post hoc analysis.

The single-step multiple comparison method and statistical test known as the Tukey's HSD test measures a "honestly significant difference." In conjunction with an ANOVA, it is used to identify descriptive metrics, such as means, standard deviation, and standard error, that differ significantly from one another. The Tukey test applies concurrently to the set of all pair-wise comparisons and compares the means of each treatment to the means of each other treatment.

To test theories concerning differences between two or more means, an ANOVA is utilized. Even though it contrasts mean values, analysis of variance is still used since, in ANOVA, we contrast the ratio of two variances. It shares several characteristics with the t-test. The main distinction is that while ANOVA can be used for more than two groups, t-tests are only appropriate for two groups. Although it is possible to use several t-tests to compare the mean differences of different groups with one another, doing so significantly raises the Type I error rate. In order to keep the Type I error at 5% and increase our confidence that any significant results we obtain are not just the consequence of chance, an ANOVA accounts for these errors. The t-test may therefore be thought of as a multiple group extension. The same presumptions that govern parametric tests also apply to ANOVA.

The differences between groups that are only categorised based on one independent variable are tested using a one-way ANOVA or single factor ANOVA. The factorial ANOVA method can also be used to test for interactions between many independent variables.

The test (ANOVA) contrasts differences in the mean (variance) within treatments with those between treatments. There won't be a noticeable difference between the treatment means if the between and within variations are roughly the same size. A much larger disparity between variations, on the other hand, denotes a major difference between them. ANOVA basically entails categorising and cross-categorizing data, testing if their arithmetic means differ significantly, and computing the F ratio, or the ratio of variation across groups to

variation within groups. While ANOVA was designed to compare several means it can also be used to compare two means. For intergroup comparison data were analyzed with the help of SPSS version 22 software. The significance levels for the data were 5% and 1%, respectively.

By collecting the F value and df from the common statistical table, the p value for an ANOVA test is calculated. The null hypothesis is rejected if the estimated p value is less than the predetermined cut-off value (i.e., 0.05).

The fact that ANOVA merely informs us of the differences between group averages as a whole and does not identify which particular group varies from the other is one potential downside. In order to determine where the differences are and which groups are statistically different from one another and which are not, we employ multiple group comparison tests or post hoc analyses. Most commonly used post hoc tests are Tukey's HSD test and Dunnett's t-test. But statisticians prefer Tukey's test.

The following types of ANOVA are employed in certain common designs:

1. One-way ANOVA is used to test for differences between two or more independent groups (means), for example, different levels of urea application in a crop, different levels of antibiotic action on various different bacterial species, or different levels of effect of some medication on various patient groups. If these groups are not independent, there is an order in the groups (such as mild, moderate, and severe disease), or there is a difference in the dose of a treatment (such as 5 mg/mL, 10 mg/mL, or 20 mg/mL) given to the same group of patients, a linear trend estimation should be employed.
2. When there are multiple factors, the factorial ANOVA is utilized.
3. When the same participants are utilized for each component, repeated measures ANOVA is used (e.g., in a longitudinal study).
4. When there are several response variables, multivariate analysis of variance (MANOVA) is employed.

- **Mean** - this measure implies arithmetic average or arithmetic mean, which is obtained by summing up all the observations and dividing them by number of observations.

$$\textbf{Mean} = \frac{\textbf{Sum of observed value}}{\textbf{Number of observation}}$$

- **Null hypothesis and *P*-value** : According to the null hypothesis, there should be no discernible difference between the means of the several groups; in other words, it should be zero. In hypothesis testing, the null hypothesis essentially asserts that all group means are the same. In the event that the null hypothesis is correct, it indicates the greatest chance of obtaining the observed outcome. It is the greatest likelihood that the observed result occurred by coincidence. Typically, 0.05 (or 5%) is used as the p value cutoff. P value significance: o >0.01 but 0.05 = May be Significant, o >0.05 = Not Significant. o 0.01 = Highly Significant.
- **Standard Deviation (S.D.) or σ :** Standard deviation, which is most frequently employed in statistical analysis, is an improvement over mean deviation as a measure of dispersion. It is calculated in the subsequent five steps. –

  1. Determine the mean
  2. Calculate each observation's deviation from the mean.
  3. Square the observational deviation from the mean
  4.To obtain the sum of squares, add the squared values.
  5.To get the variance, divide this sum by the number of observations less one.

Where$\Sigma(x - \bar{x})^2$= Sum of square of difference of observation from Mean

x = each observation (value)

$\bar{x}$= mean

n = number of observations.

$$S.D = \frac{\sqrt{\sum(x - \bar{x})2}}{\sqrt{n} - 1}$$

- **Standard error (S.E.) of mean:** In practice standard error of mean is calculated by following formula -

Where, S.D = Standard deviation.

n = Number of observations.

$$S.E. = \frac{SD}{\sqrt{n}}$$

- **Degree of freedom (DF)** - Degree of freedom is a term that refers to the quantity in the denominators that is one less than the independent number of observations in a sample and is frequently used instead of sample size. Where n1 and n2 are the number of observations in the two series in the paired t test, and df = n-1, the unpaired t test of difference between two means is equal to n1 + n2-2.

SPSS: SPSS stands for “Statistical Package for the Social Sciences”. It is an IBM tool. This tool first launched in 1968. This is one software package. This package is mainly used for statistical analysis of the data.

OBSERVATION & RESULTS

This study includes master charts, statistical data analysis including mean differences and significance value among various groups and graphic representations in the form of line diagram and multiple bar graphs that depicts the effect on test groups under study. The following data were obtained in all Groups of rats after induction of diabetes (except in Group 1) and administering them respective drugs for a period of 28 days. The random blood sugar value (RBS) was measured on 0,7th, 14th, 21th, 28th, 35th and 42nd day of treatment.

Fig 1: Master chart showing value of RBS (mg/dl) on 0,7th, 14th, 21th, 28th, 35th and 42nd day of all 6 Groups

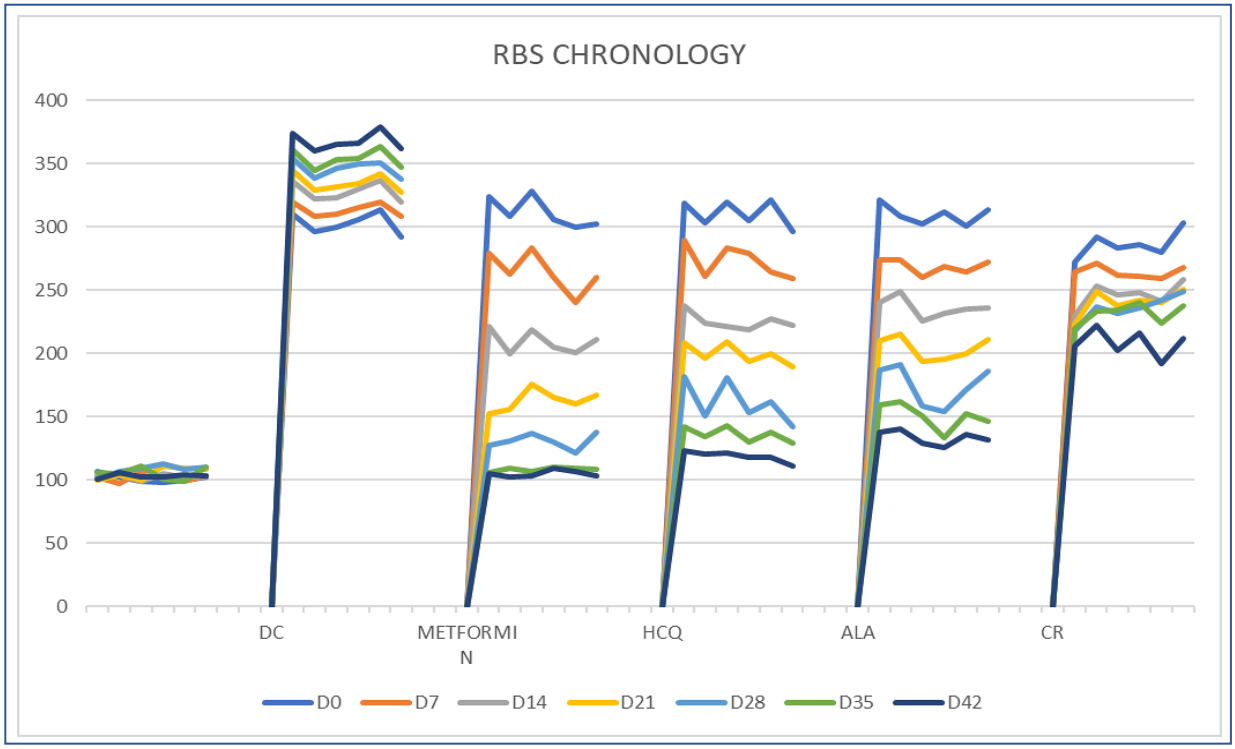


Fig 1 Represents master chart of random blood sugar of all groups throughout the study duration. The blood glucose level was increased in Group 2 progressively while, Group 3, 4, 5 showed significant($P<0.01$) reduction in blood glucose level.

Table 1(a): Master table showing value of RBS (mg/dl) on 0,7th, 14th, 21th, 28th, 35th and 42nd day in Group 1 (Normal Control Rats not induced diabetes and treated with vehicle i.e., 1% gum acacia).

RAT NO.	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28	DAY 35	DAY 42
1	107	102	103	100	101	106	101
2	102	97	104	104	107	105	106
3	99	107	99	100	109	111	102
4	98	102	105	111	113	101	102
5	100	99	102	109	108	99	104
6	102	103	103	108	110	110	103
MEAN	101.33	107.67	102.67	105.33	108.00	105.33	103.00
SD	±3.204	±3.445	±2.066	±4.719	±4.00	±4.761	±1.789

Table 1(b): Master table showing value of RBS (mg/dl) on 0,7th, 14th, 21th, 28th, 35th and 42nd day in Group 2 [Diabetic Control Rats (diabetes induced) treated with vehicle i.e., 1% gum acacia].

RAT NO.	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28	DAY 35	DAY 42
7	310	320	336	345	354	361	374
8	296	308	322	329	339	345	360
9	300	310	323	332	346	353	365
10	306	315	330	334	350	354	366
11	314	320	337	342	351	364	379
12	292	308	320	327	338	347	362
MEAN	303	313.5	328	334.83	346.33	354	367.67
SD	±8.462	±5.648	±7.403	±7.195	±6.593	±7.483	±7.339

Table 1(c): Master table showing value of RBS (mg/dl) on 0, 7th, 14th, 21th, 28th, 35th and 42nd day in Group 3 (Diabetic rats treated with Metformin)

RAT NO.	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28	DAY 35	DAY 42
13	324	279	221	152	127	106	105
14	308	263	200	156	131	109	102
15	328	283	219	176	137	107	103
16	306	260	205	165	130	110	109
17	300	240	201	160	121	109	107
18	302	260	211	167	138	108	103
MEAN	311.33	264.17	209.5	162.67	130.67	108.17	104.83
SD	±11.776	±15.459	±9.028	±8.571	±6.346	±1.472	±2.714

Table 1(d): Master table showing value of RBS (mg/dl) on 0,7th, 14th, 21th, 28th, 35th and 42nd day in Group 4 (Diabetic rats treated with Hydroxychloroquine {HCQ})

RAT NO.	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28	DAY 35	DAY 42
19	319	289	238	208	182	142	123
20	303	261	224	196	151	134	120
21	320	283	221	209	181	143	121
22	305	279	219	194	153	130	118
23	321	264	227	200	162	138	118
24	296	259	222	189	142	129	111
MEAN	310.67	272.5	225.17	199.33	161.83	136	118.5
SD	±10.671	±12.74	±6.853	±7.941	±16.510	±5.967	±4.135

Table 1(e): Master table showing value of RBS (mg/dl) on 0,7th, 14th, 21th, 28th, 35th and 42nd day in Group 5 (Diabetic rats treated with Alpha Lipoic Acid {ALA})

RAT NO.	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28	DAY 35	DAY 42
25	321	274	240	210	187	159	138
26	308	274	249	215	191	162	140
27	302	260	226	194	158	151	129
28	312	269	232	195	154	133	126
29	301	264	235	200	171	152	136
30	314	272	236	211	186	146	132
MEAN	309.67	268.83	236.33	204.17	174.5	150.5	133.5
SD	±7.607	±5.742	±7.763	±8.976	±15.909	±10.33	±5.431

Table 1(f): Master table showing value of RBS (mg/dl) on 0,7th, 14th, 21th, 28th, 35th and 42nd day in Group 6 (Diabetic rats treated with Chromium picolinate {CrP})

RAT NO.	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28	DAY 35	DAY 42
31	272	264	230	223	218	220	206
32	292	271	253	249	237	233	222
33	283	262	246	238	232	234	202
34	286	261	248	242	236	240	216
35	280	259	241	240	242	224	192
36	303	268	258	251	249	238	212
MEAN	286	264.17	246	240.5	235.67	231.5	208.33
SD	±10.640	±4.535	±9.778	±9.975	±10.443	±7.893	±10.690

Table 1 (a) to 1(f) Represents master chart with means and standard deviation of random blood sugar of all groups throughout the study duration. The blood glucose level was in increasing order for Group 2, while Group 3, 4 and 5 showed reduced blood glucose level.

Group 1	NC=NORMAL CONTROL	Group 4	HCQ=HYDROXYCHLOROQUINE
Group 2	DC=DIABETIC CONTROL	Group 5	ALA=ALPHA LIPOIC ACID
Group 3	MET=METFORMIN	Group 6	CrP=CHROMIUM PICOLINATE

Table 2: Comparison of means and standard deviations of random blood glucose level in different groups from Day '0' to Day '42'.

Table 2

Random Blood Glucose	NORMAL CONTROL (GROUP 1)	DIABETIC CONTROL (GROUP2)	DIABETIC WITH METFORMIN (GROUP 3)	DIABETIC WITH HCQ (GROUP 4)	DIABETIC WITH ALA (GROUP 5)	DIABETIC WITH CrP (GROUP 6)
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
D0	101.33±3.204	303±8.462	311.33±11.776	310.67±10.671	309.67±7.607	286±10.640
D7	107.67±3.445	313.5±5.648	264.17±15.459	272.5±12.74	268.83±5.742	264.17±4.535
D14	102.67±2.066	328±7.403	209.5±9.028	225.17±6.853	236.33±7.763	246±9.778
D21	105.33±4.719	334.83±7.195	162.67±8.571	199.33±7.941	204.17±8.976	240.5±9.975
D28	108±4	346.33±6.593	130.67±6.346	161.83±16.510	174.5±15.909	235.67±10.443
D35	105.33±4.761	354±7.483	108.17±1.472	136±5.967	150.5±10.33	231.5±7.893
D42	103±1.789	367.67±7.339	104.83±2.714	118.5±4.135	133.5±5.431	208.33±10.690

Figure 2: Comparing random blood glucose (means) of all groups from Day '0' to Day '42' among all groups

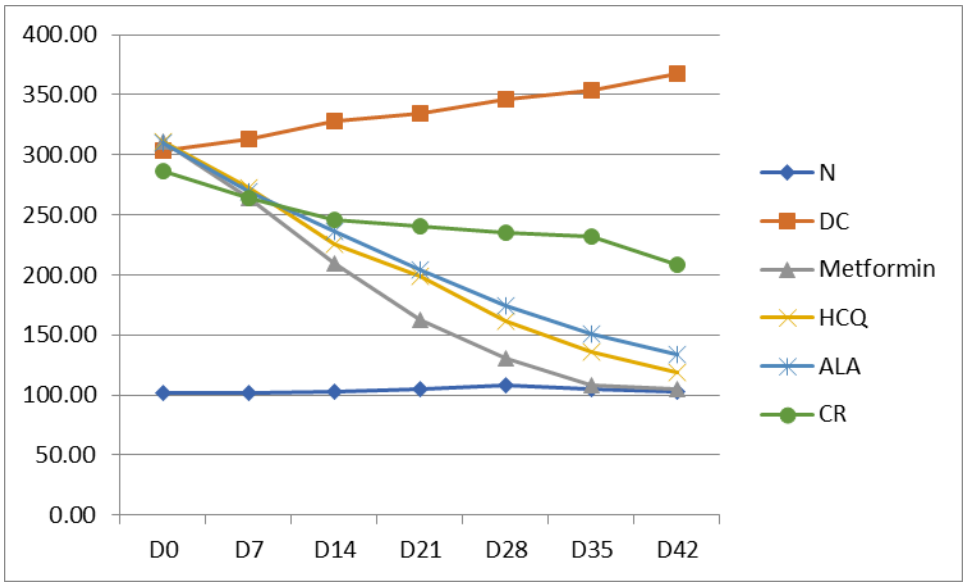


Table no 2 & Figure 2: - Represents the mean and standard deviation of random blood glucose level among all groups of experiment throughout the study period. The blood glucose level progressively increases in group 2 throughout the study period while in group 3,4 and 5 there was significant($P<0.01$) reduction of the same.

In Group 3, as compared to the Means of day ‘0’ the blood glucose level declined around 15 % by day 7, 33 % by day 14, 48 % by day 21, 58 % by day 28, 65 % by day 35, and 66 % by the day 42.

In Group 4, as compared to the Means of day ‘0’ the blood glucose level declined around 12 % by day 7, 28 % by day 14, 36 % by day 21, 48 % by day 28, 56 % by day 35, and 62 % by day 42.

In Group 5, as compared to the Means of day ‘0’ the blood glucose level declined around 13 % by day 7, 24 % by day 14, 34 % by day 21, 44 % by day 28, 51 % by day 35, and 57 % by day 42.

In Group 6, as compared to the Means of day ‘0’ the blood glucose level declined around 8% by day 7, 14% by day 14, 16% by day 21, 18 % by day 28, 19 % by day 35, and 27% by day 42.

For Group 2, the Mean of the study was progressively increased throughout the study period. It signifies that the Mean difference of Group 2 was more while comparing Group 3, 4, 5 and 6 from day 7 to day 42.

The mean values considered statistically significant ($p < 0.05$) and insignificant ($p > 0.05$)

Following tables show the mean standard deviation, difference in mean and significance level after comparing day-wise RBS with each other after analysis of the data with One-way ANOVA followed by Tukey's HSD post hoc test. P value <0.01 significant, P value >0.05 not significant, and P value > 0.01 but <0.05 may be significant

Table 3(a) & (b): Comparison of random blood glucose level (Means) of Group 1 with Group 3,4,5 and 6 from Day '0' to Day '42'

Table 3(a)

RANDOM BLOOD GLUCOSE	Group 1	Group 3	Mean Difference from Group 1	Group 4	Mean Difference from Group 1	Group 5	Mean Difference from Group 1	Group 6	Mean Difference from Group 1
D0	101.33	311.33	-210.00	310.67	-209.33	309.67	-208.33	286.00	-184.67
D7	101.67	264.17	-162.50	272.50	-170.83	268.83	-167.17	264.17	-162.50
D14	102.67	209.50	-106.83	225.17	-122.50	236.33	-133.67	246.00	-143.33
D21	105.33	162.67	-57.33	199.33	-94.00	204.17	-98.83	240.50	-135.17
D28	108.00	130.67	-22.67	161.83	-53.83	174.50	-66.50	235.67	-127.67
D35	105.33	108.17	-2.83	136.00	-30.67	150.50	-45.17	231.50	-126.17
D42	103.00	104.83	-1.83	118.50	-15.50	133.50	-30.50	208.33	-105.33

Table 3(b)

RANDOM BLOOD GLUCOSE		FROM NORMAL CONTROL(Group1) vis…			
		MET	HCQ	ALA	CrP
	Group	3	4	5	6
Day 0	Mean Difference	-210.000*	-209.333*	-208.333*	-184.667*
	Std. Error	5.299	5.299	5.299	5.299
	p Value	<0.001	<0.001	<0.001	<0.001
Day 7	Mean Difference	-162.500*	-170.833*	-167.167*	-162.500*
	Std. Error	5.263	5.263	5.263	5.263
	p Value	<0.001	<0.001	<0.001	<0.001
Day 14	Mean Difference	-106.833*	-122.500*	-133.667*	-143.333*
	Std. Error	4.368	4.368	4.368	4.368
	p Value	<0.001	<0.001	<0.001	<0.001
Day 21	Mean Difference	-57.333*	-94.000*	-98.833*	-135.167*
	Std. Error	4.659	4.659	4.659	4.659
	p Value	<0.001	<0.001	<0.001	<0.001
Day 28	Mean Difference	-22.667*	-53.833*	-66.500*	-127.667*
	Std. Error	6.388	6.388	6.388	6.388
	p Value	.015	<0.001	<0.001	<0.001
Day 35	Mean Difference	-2.833	-30.667*	-45.167*	-126.167*
	Std. Error	3.982	3.982	3.982	3.982
	p Value	.979	<0.001	<0.001	<0.001
Day 42	Mean Difference	-1.833	-15.500*	-30.500*	-105.333*
	Std. Error	3.538	3.538	3.538	3.538
	p Value	.995	.002	<0.001	<0.001

Table 3(a) & (b): Represents the statistical comparison of random blood glucose (means) level of Group 1 with Group 3, 4, 5 and 6 from Day '0' to Day '42'. Mean values of Group 3 with Group 1 were statistically significant(P<0.01) from Day0-Day21, but was statistically insignificant(P>0.05) from day28-day42. When compared with Group 3 and 4, the Mean difference declined abruptly, whereas it declined gradually for Group 5 and 6. For Group 3 and 4 the difference at Day 42 was negligible, still Group 4 was higher than Group3 showing Group 3 has better glucose control thereby significant. In Group 5 and 6 we saw statistically significant(P<0.01) changes throughout the study period but as mean difference value is quite large, hence insignificant. Thus Group 6 chromium picolinate has very less effect on control of blood sugar level when compared with rest groups.

Fig 3: Comparison of random blood glucose level (Means) of Group 1 (N=normal control) with Group 3,4,5 and 6 from Day '0' to Day '42'

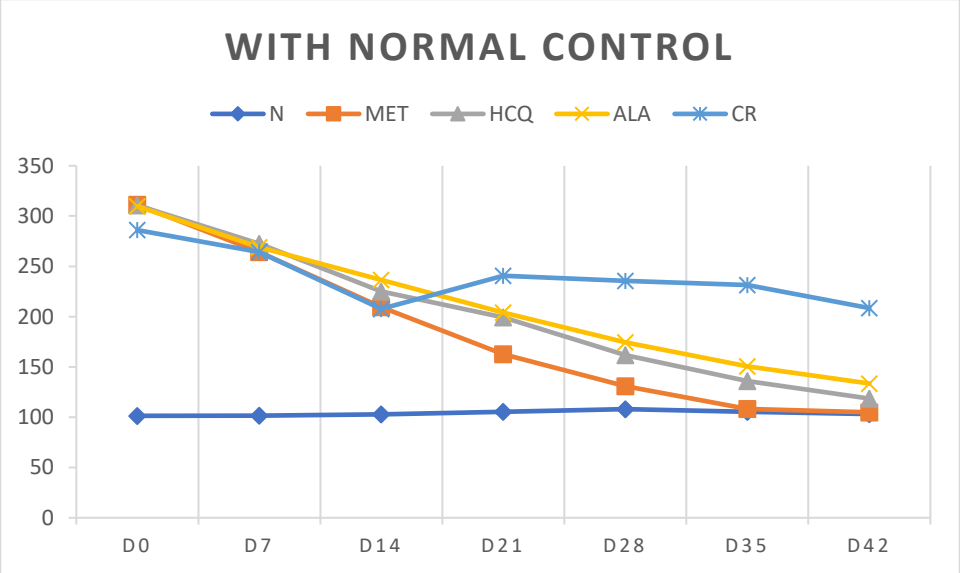


Figure 3: Represents overlapping of Group 3(MET=Metformin), Group 4(HCQ=Hydroxychloroquine), Group 5(ALA=Alpha lipoic acid) throughout study period, although within the Groups 3>4>5>6 in controlling of blood glucose level was observed. On Day42, as Group 3 graph merged with Group 1, Group 6(CR=Chromium picolinate) graph stays far behind in reaching to normal values graphically (i.e., insignificant(P>0.05))

Table 4(a) & (b): Comparison of random blood glucose level (Means) of Group 2 with Group 3, 4, 5 and 6 from Day '0' to Day '42'

Table 4(a)

RANDOM BLOOD GLUCOSE	Group 2	Group 3	Mean Difference from Group 2	Group 4	Mean Difference from Group 2	Group 5	Mean Difference from Group 2	Group 6	Mean Difference from Group 2
D0	303.00	311.33	-8.33	310.67	-7.67	309.67	-6.67	286.00	17.00
D7	313.50	264.17	49.33	272.50	41.00	268.83	44.67	264.17	49.33
D14	328.00	209.50	118.50	225.17	102.83	236.33	91.67	246.00	82.00
D21	334.83	162.67	172.17	199.33	135.50	204.17	130.67	240.50	94.33
D28	346.33	130.67	215.67	161.83	184.50	174.50	171.83	235.67	110.67
D35	354.00	108.17	245.83	136.00	218.00	150.50	203.50	231.50	122.50
D42	367.67	104.83	262.83	118.50	249.17	133.50	234.17	208.33	159.33

Table 4(b)

RANDOM BLOOD GLUCOSE		FROM DIABETIC CONTROL (Group 2) vis…			
		MET	HCQ	ALA	CrP
	Group	3	4	5	6
Day 0	Mean Difference	-8.333	-7.667	-6.667	17.000*
	Std. Error	5.299	5.299	5.299	5.299
	p Value	.622	.699	.805	.034
Day 7	Mean Difference	49.333*	41.000*	44.667*	49.333*
	Std. Error	5.263	5.263	5.263	5.263
	p Value	<0.001	<0.001	<0.001	<0.001
Day 14	Mean Difference	118.500*	102.833*	91.667*	82.000*
	Std. Error	4.368	4.368	4.368	4.368
	p Value	<0.001	<0.001	<0.001	<0.001
Day 21	Mean Difference	172.167*	135.500*	130.667*	94.333*
	Std. Error	4.659	4.659	4.659	4.659
	p Value	<0.001	<0.001	<0.001	<0.001
Day 28	Mean Difference	215.667*	184.500*	171.833*	110.667*
	Std. Error	6.388	6.388	6.388	6.388
	p Value	<0.001	<0.001	<0.001	<0.001
Day 35	Mean Difference	245.833*	218.000*	203.500*	122.500*
	Std. Error	3.982	3.982	3.982	3.982
	p Value	<0.001	<0.001	<0.001	<0.001
Day 42	Mean Difference	262.833*	249.167*	234.167*	159.333*
	Std. Error	3.538	3.538	3.538	3.538
	p Value	<0.001	<0.001	<0.001	<0.001

Table 4(a) and 4(b) Represents the statistical comparison of Group 3,4,5 and 6 with Group2 (diabetic control). All groups showed statistically significant(P<0.01) changes in Mean glucose difference from Day7 to Day42. However, Groups 3,4and 5 (3>4>5) showed decreasing order of effectiveness in controlling glucose throughout the study period. Although the Group 6 showed statistically significant(P<0.01) result when compared to Group 2.

Fig 4: Comparing random blood glucose level (Means) of Group 2 with Groups 3,4,5 and 6 from Day '0' to Day '42'

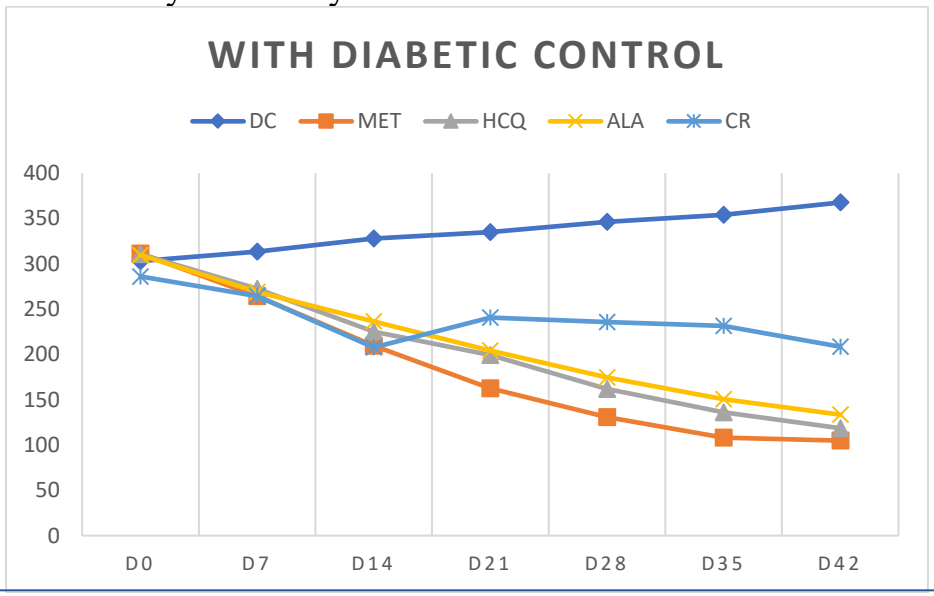


Fig 4 Represents overlapping results of Group 4, 5 with Group 3. It was better than Group 4 and Group 5. Group 6 didn't show much changes in the study from Day21-Day42.

Table 5(a) & (b): Comparison of random blood glucose level (means) of Group 3 with Group 4,5 and 6 from Day '0' to Day '42'

Table 5(a)

RANDOM BLOOD GLUCOSE	Group 3	Group 4	Mean Difference from Group 3	Group 5	Mean Difference from Group 3	Group 6	Mean Difference from Group 3
D0	311.33	310.67	0.67	309.67	1.67	286.00	25.33
D7	264.17	272.50	-8.33	268.83	-4.67	264.17	0.00
D14	209.50	225.17	-15.67	236.33	-26.83	246.00	-36.50
D21	162.67	199.33	-36.67	204.17	-41.50	240.50	-77.83
D28	130.67	161.83	-31.17	174.50	-43.83	235.67	-105.00
D35	108.17	136.00	-27.83	150.50	-42.33	231.50	-123.33
D42	104.83	118.50	-13.67	133.50	-28.67	208.33	-103.50

Table 5(b)

RANDOM BLOOD GLUCOSE		FROM METFORMIN		
		HCQ	ALA	CR
Day 0	Mean Difference	.667	1.667	25.333*
	Std. Error	5.299	5.299	5.299
	p Value	1.000	1.000	<0.001
Day 7	Mean Difference	-8.333	-4.667	0.000
	Std. Error	5.263	5.263	5.263
	p Value	.615	.947	1.000
Day 14	Mean Difference	-15.667*	-26.833*	-36.500*
	Std. Error	4.368	4.368	4.368
	p Value	.014	<0.001	<0.001
Day 21	Mean Difference	-36.667*	-41.500*	-77.833*
	Std. Error	4.659	4.659	4.659
	p Value	<0.001	<0.001	<0.001
Day 28	Mean Difference	-31.167*	-43.833*	-105.000*
	Std. Error	6.388	6.388	6.388
	p Value	<0.001	<0.001	<0.001
Day 35	Mean Difference	-27.833*	-42.333*	-123.333*
	Std. Error	3.982	3.982	3.982
	p Value	<0.001	<0.001	<0.001
Day 42	Mean Difference	-13.667*	-28.667*	-103.500*
	Std. Error	3.538	3.538	3.538
	p Value	.007	<0.001	<0.001

Table 5(a) & (b) Represents the statistical comparison of Group 4, 5, 6 with Group 3 (standard). Group 5 and 6 showed statistically significant($P<0.01$) changes from Day14-Day42. Clinically mean difference of Group 6 is very high hence insignificant($P>0.05$). Although Group 4 was statistically insignificant($P>0.05$) but showed promising results in glucose control with very less mean difference when compared to standard (Group 3) at Day42.

Fig 5: Comparing random blood glucose(means) of Group 3 with Group 4,5 and 6 from Day '0' to Day '42'

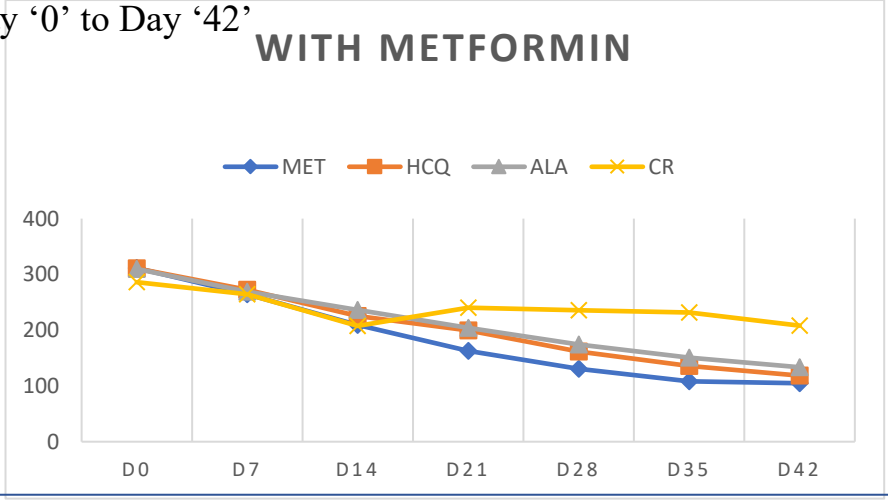


Figure 5 Represents near overlapping of graphs of Group 3, 4, 5 at day 42. Group 6 showed ineffective control in blood glucose level.

Table 6: Comparison of weight difference(mean) in percentage from Day '0' to Day '42'

	D0	D42	% CHANGE	T DIFFERENCE	DEGREE OF FREEDOM	P VALUE
	Mean±SD	Mean±SD				
NC	164.50±26.30	172.83±23.08	5.066	-4.287	5	.008
DC	176.67±24.69	213.33±25.87	20.755	-3.939	5	.011
MET	195.00±30.91	153.67±14.89	-21.197	6.159	5	.001
HCQ	170.50±21.61	164.33±20.09	-3.617	5.420	5	.003
ALA	171.00±16.35	168.33±13.19	-1.559	0.844	5	.437
CrP	177.00±18.14	184.33±15.71	4.143	-2.700	5	.043

Figure 6: Comparing weight difference(mean) in percentage from Day '0' to Day '42'

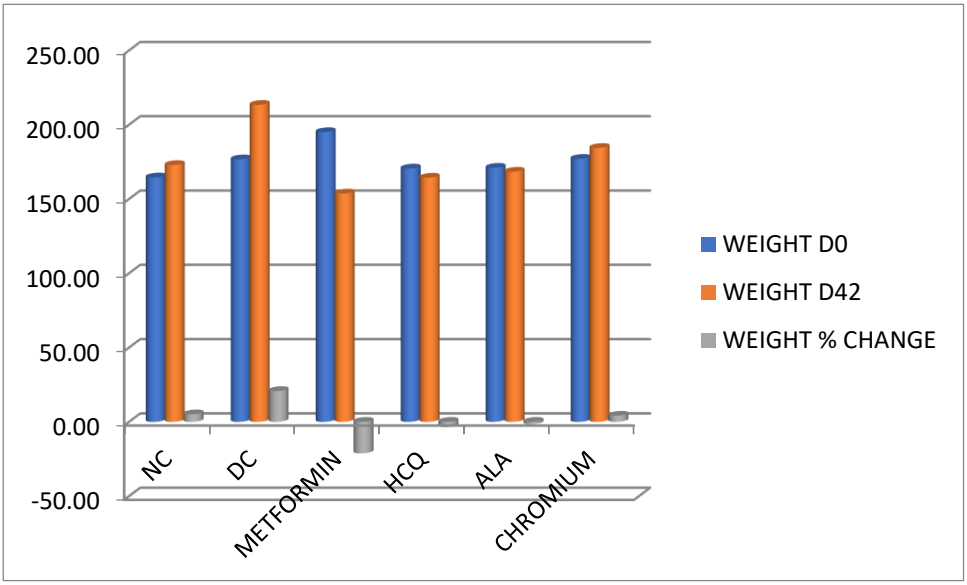


Table 6 Shows mean weight difference among all Groups across the study period, comparing Day0 and Day42. The only statistically significant(P<0.01) change was observed in Group3, whereas Group 4 showed nearby changes. Group 5 and Group 6 didn't show any statistically or clinically significant changes during study period.

Group 1	NC=NORMAL CONTROL	Group 4	HCQ=HYDROXYCHLOROQUINE
Group 2	DC=DIABETIC CONTROL	Group 5	ALA=ALPHA LIPOIC ACID
Group 3	MET=METFORMIN	Group 6	CrP=CHROMIUM PICOLINATE

Table 7: Comparing urea, creatinine and SGPT from Day '0' to Day '42' for all groups

PARAMETERS	NORMAL CONTROL (GROUP 1)	DIABETIC CONTROL (GROUP2)	DIABETIC WITH METFORMIN (GROUP 3)	DIABETIC WITH HCQ (GROUP 4)	DIABETIC WITH ALA (GROUP 5)	DIABETIC WITH CrP (GROUP 6)
LFT/KFT	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**
UREA	32.527±1.950	99.368±3.0.31	61.677±4.005	60.583±3.073	69.635±2.002	84.568±2.189
CREATININ	0.427±0.063	1.612±0.227	0.672±0.074	0.483±0.064	0.913±0.051	1.222±0.051
SGPT	104±12.028	188.33±18.282	124.5±3.202	113.833±6.866	156.167±3.078	168.833±4.18

Figure 7: Comparing urea, creatinine and SGPT from Day '0' to Day '42'for all groups

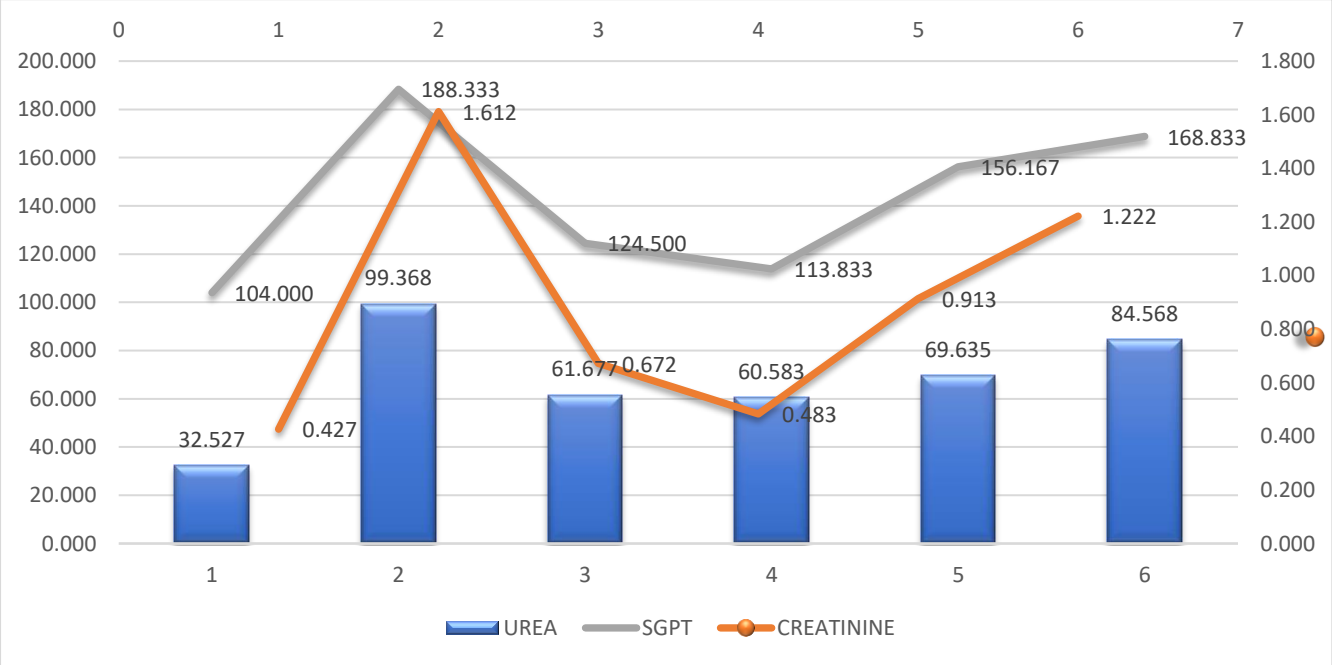


Table 7 & Figure 7 Represents the mean and standard deviation of urea, creatinine and SGPT among all interventional Groups throughout the study period. After 42 days of treatment, HCQ proves in lowering urea, creatinine and SGPT better as compared to rest interventional Groups. This proves HCQ to be renoprotective and hepatoprotective.

Table 8: Comparison of lipid profile from Day '0' to Day '42'for all groups

	NORMAL CONTROL (GROUP 1)	DIABETIC CONTROL (GROUP2)	DIABETIC WITH METFORMIN (GROUP 3)	DIABETIC WITH HCQ (GROUP 4)	DIABETIC WITH ALA (GROUP 5)	DIABETIC WITH CR (GROUP 6)
LIPID PROFILE	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**
TG	79.167±10.381	177.167±5.947	92.667±3.559	80.333±6.802	102.167±9.517	119.667±7.763
VLDL	15.833±2.076	35.433±1.189	18.533±0.712	16.067±1.360	20.433±1.903	23.933±1.553
TC	71.333±1.751	130.5±4.848	66±1.414	61.333±1.751	76.167±4.792	75.667±4.502
ATHEROGENIC INDEX	0.723±0.084	5.688±0.775	1.576±0.119	1.103±0.055	1.822±0.078	2.163±0.206
HDL	41.5±2.588	19.667±1.633	25.667±1.366	29.167±0.753	27±1.673	24±2.098
NON-HDL	29.833±1.722	110.833±6.306	40.333±1.506	32.167±1.472	49.167±3.371	51.667±3.502
LDL	14±2.574	75.4±6.709	21.8±2	16.1±0.452	28.733±3.317	27.733±2.402
LDL/HDL	0.3399±0.0755	3.877±0.650	0.8525±0.0996	0.5521±0.014	1.063±0.093	1.16±0.111

Figure 8: Comparison of lipid profile from Day '0' to Day '42'for all groups

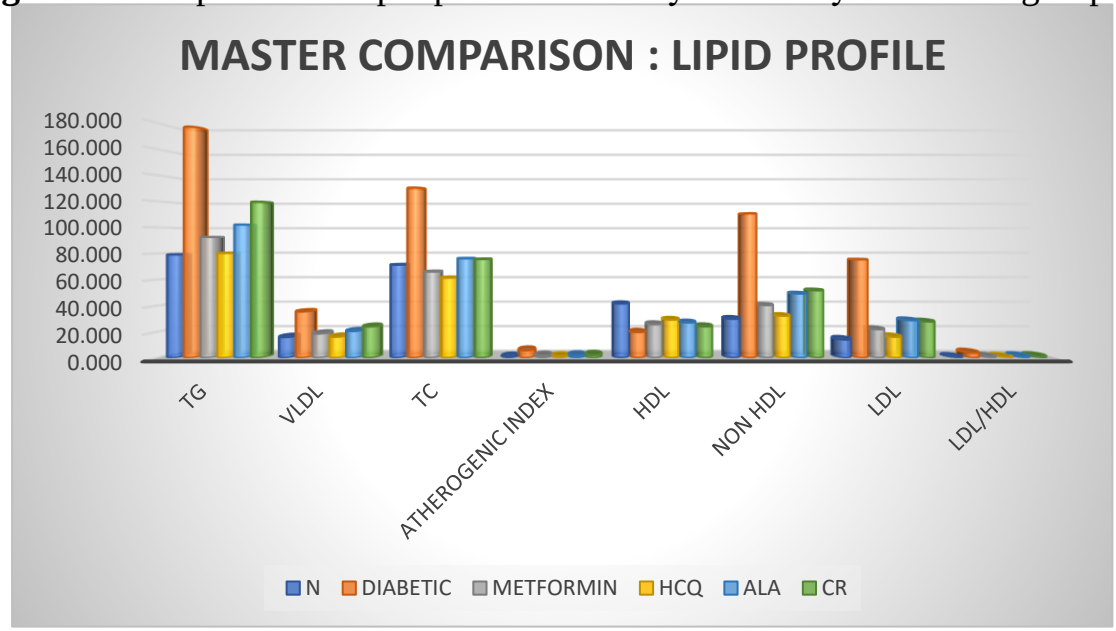


Table 8 & Figure 8: Represents the mean & standard deviation of lipid profile among all interventional Groups throughout the study period. After 42 days of treatment, HCQ causes lowering in lipid profile better than other interventional groups of comparison. ALA also showed improvement in HDL level and very less effective in lowering of other parameters of lipid profile.

		FROM NORMAL CONTROL (GROUP 1) vis…			
		METFORMIN	**HCQ**	**ALA**	**CrP**
GROUP		**3**	**4**	**5**	**6**
SGPT	Mean Difference	-20.50000*	-9.83333	-52.16667*	-64.83333*
	Std. Error	6.12765	6.12765	6.12765	6.12765
	p Value	.025	.602	.000	.000
CREATININE	Mean Difference	-29.15000*	-28.05667*	-37.10833*	-52.04167*
	Std. Error	1.77531	1.77531	1.77531	1.77531
	p Value	.000	.000	.000	.000
UREA	Mean Difference	-.24500*	-.05667	-.48667*	-.79500*
	Std. Error	.06837	.06837	.06837	.06837
	p Value	.000	.000	.000	.000
HDL	Mean Difference	15.83333*	13.00000*	14.83333*	17.50000*
	Std. Error	1.15950	1.15950	1.15950	1.15950
	p Value	.000	.000	.000	.000
TC	Mean Difference	5.33333	10.00000*	-4.83333	-4.33333
	Std. Error	2.03943	2.03943	2.03943	2.03943
	p Value	.125	.000	.199	.302
TG	Mean Difference	-13.50000*	-1.16667	-23.00000*	-40.50000*
	Std. Error	4.42782	4.42782	4.42782	4.42782
	p Value	.049	1.000	.000	.000
		FROM NORMAL CONTROL (GROUP 1) vis…			
LDL	Mean Difference	-7.80000*	-2.10000	-14.73333*	-13.73333*
	Std. Error	2.00845	2.00845	2.00845	2.00845
	p Value	.006	.898	.000	.000
VLDL	Mean Difference	-2.70000*	-.23333	-4.60000*	-8.10000*
	Std. Error	.88556	.88556	.88556	.88556
	p Value	.049	1.000	.000	.000
NON-HDL	Mean Difference	-10.50000*	-2.33333	-19.33333*	-21.83333*
	Std. Error	1.98326	1.98326	1.98326	1.98326
	p Value	.000	.844	.000	.000
NON-HDL/HDL	Mean Difference	-.85500*	-.37667	-1.09833*	-1.43833*
	Std. Error	.19413	.19413	.19413	.19413
	p Value	.002	.399	.000	.000
LDL/HDL	Mean Difference	-.51252*	-.22672	-.73895*	-.81977*
	Std. Error	.16039	.16039	.16039	.16039
	p Value	.035	.719	.001	.000
TC/HDL	Mean Difference	-.85327*	-.43770	-1.14628*	-1.44030*
	Std. Error	.20494	.20494	.20494	.20494
	p Value	.003	.297	.000	.000

Table 9: - Represents comparison of normal Group with rest of the interventional Groups on the basis of various parameter as shown above.

Table 10: - Represents comparison of diabetic Group & standard Group with rest of the interventional Groups on the basis of various parameter as shown below.

		FROM DIABETIC CONTROL vis…				FROM METFORMIN vis…			
		METFORMIN	HCQ	ALA	CR		HCQ	ALA	CR
SGPT	Mean Difference	63.83333*	74.50000*	32.16667*	19.50000*	Mean Difference	10.66667	-31.66667	-44.33333
	Std. Error	5.78657	5.78657	5.78657	5.78657	Std. Error	5.78657	5.78657	5.78657
	p Value	.000	.000	.000	.019	p Value	.372	.000	.000
CREATININE	Mean Difference	.94000*	1.12833*	.69833*	.39000*	Mean Difference	.18833	-.24167	-.55000
	Std. Error	.07277	.07277	.07277	.07277	Std. Error	.07277	.07277	.07277
	p Value	.000	.000	.000	.000	p Value	.103	.021	.000
UREA	Mean Difference	37.69167*	38.78500*	29.73333*	14.80000*	Mean Difference	1.09333	-7.95833*	-22.89167*
	Std. Error	1.86493	1.86493	1.86493	1.86493	Std. Error	1.86493	1.86493	1.86493
	p Value	.000	.000	.000	.000	p Value	.976	.002	.000
		FROM DIABETIC CONTROL vis….				FROM METFORMIN vis…			
		METFORMIN	HCQ	ALA	CR		HCQ	ALA	CR
HDL	Mean Difference	-6.00000*	-8.83333*	-7.00000*	-4.33333*	Mean Difference	-2.83333	-1.00000	1.66667
	Std. Error	1.08012	1.08012	1.08012	1.08012	Std. Error	1.08012	1.08012	1.08012
	p Value	.000	.000	.000	.004	p Value	.096	.884	.546
TC	Mean Difference	64.50000*	69.16667*	54.33333*	54.83333*	Mean Difference	4.66667	-10.16667*	-9.66667*
	Std. Error	2.18785	2.18785	2.18785	2.18785	Std. Error	2.18785	2.18785	2.18785
	p Value	.000	.000	.000	.000	p Value	.238	.001	.001
TG	Mean Difference	84.50000*	96.83333*	75.00000*	57.50000*	Mean Difference	12.33333*	-9.50000	-27.00000*
	Std. Error	4.04255	4.04255	4.04255	4.04255	Std. Error	4.04255	4.04255	4.04255
	p Value	.000	.000	.000	.000	p Value	.039	.163	.000

		FROM DIABETIC CONTROL vis…				FROM METFORMIN vis…			
		METFORMIN	HCQ	ALA	CR		HCQ	ALA	CR
LDL	Mean Difference	53.60000*	59.30000*	46.66667*	47.66667*	Mean Difference	5.70000	-6.93333*	-5.93333
	Std. Error	2.09738	2.09738	2.09738	2.09738	Std. Error	2.09738	2.09738	2.09738
	p Value	.000	.000	.000	.000	p Value	.079	.022	.063
VLDL	Mean Difference	16.90000*	19.36667*	15.00000*	11.50000*	Mean Difference	2.46667*	-1.90000	-5.40000*
	Std. Error	.80851	.80851	.80851	.80851	Std. Error	.80851	.80851	.80851
	p Value	.000	.000	.000	.000	p Value	.039	.163	.000
NON-HDL	Mean Difference	70.50000*	78.66667*	61.66667*	59.16667*	Mean Difference	8.16667*	-8.83333*	-11.33333*
	Std. Error	2.12655	2.12655	2.12655	2.12655	Std. Error	2.12655	2.12655	2.12655
	p Value	.000	.000	.000	.000	p Value	.006	.003	.000
		FROM DIABETIC CONTROL vis…				FROM METFORMIN vis….			
		METFORMIN	HCQ	ALA	CR		HCQ	ALA	CR
ATHEROGENIC INDEX (NON-HDL/HDL)	Mean Difference	4.10500*	4.58333*	3.86167*	3.52167*	Mean Difference	.47833	-.24333	-.58333
	Std. Error	.21155	.21155	.21155	.21155	Std. Error	.21155	.21155	.21155
	p Value	.000	.000	.000	.000	p Value	.191	.779	.073
LDL/HDL RATIO	Mean Difference	3.02453*	3.31034*	2.79811*	2.71729*	Mean Difference	.28581	-.22643	-.30725
	Std. Error	.16039	.16039	.16039	.16039	Std. Error	.16039	.16039	.16039
	p Value	.000	.000	.000	.000	p Value	.492	.720	.413
TC/HDL (CHOLESTEROL RATIO)	Mean Difference	4.11172*	4.52730*	3.81872*	3.52469*	Mean Difference	.41558	-.29300	-.58703
	Std. Error	.20494	.20494	.20494	.20494	Std. Error	.20494	.20494	.20494
	p Value	.000	.000	.000	.000	p Value	.351	.709	.074

Comparison of all groups in relation to SGPT

Metformin causes significant (P<0.01) reduction in SGPT level when compared to ALA group and CrP group. When we compared with HCQ group, HCQ showed much more significant(P<0.01) findings than metformin and all other groups. While comparing to normal control group, HCQ was observed to be more reliable / significant(P<0.01) and had very much close values to the normal group.

Comparison of all groups in relation to Urea and Creatinine (renal profile)

By comparing all test groups, we observe that HCQ is a drug causing significant (P<0.01) reduction in urea as well as creatinine level. When metformin, ALA and CrP groups were compared, non-significant(P>0.05) findings were found. Metformin was somehow better than ALA and CrP groups in the reduction of creatinine level. ALA and metformin groups were non-conclusive in respect to urea.

Comparison of all groups in relation to TG, TC, HDL, LDL (lipid profile)

By comparing all experimental groups , we observed that Group 4 (HCQ) causes significant (P<0.01) reduction in TG,TC,LDL level when compared to Group 1(Normal Control). The improvement in HDL level was seen in Group 4 and Group 5(ALA) significantly. Group 4(HCQ) overall shows promising result in improving lipid profile parameters. Atherogenic index (a parameter to evaluate risk of atheroma production) of HCQ is nearly equals to that of normal control (Group 1). When comparing Group 1(Normal Control) with other groups only Group 4 (HCQ) showed significant changes in triglyceride levels. Though Group 5 (ALA) & Group 6 (CrP) showed statistically significant level.

It is postulated that ALA and CrP both can lower lipid profile parameters as well. When compared with Group 2(Diabetic Control) , all other groups showed statistically significant improvement. Three indexes namely atherogenic index, cholesterol ratio and LDL/HDL ratio were calculated to evaluate different complications related to high lipid profile but showed insignificant results when compared to standard drug (Group 3). When compared with standard drug Metformin (Group 3), only CrP showed statistically significant(P<0.01) changes in TC, TG, VLDL & Non-HDL . When compared with standard drug Metformin (Group 3) ALA showed statistically significant(P<0.01) result in TC and Non-HDL. Overall, the sequence of effectiveness of our experimental drugs in controlling lipid parameters is HCQ >ALA>CrP

	DC	NC	MET	CrP1	CrP2	ALA1	ALA2	HCQ1	HCQ2
Heart With Blood Vessel									
Hyperemia	-	-	+	+	-	-	+	+	+
Hemorrhage	+	+	+	+	+	-	+	-	+
Hyaline degeneration and Zenker's necrosis	-	-	+	-	++	+	-	-	+
Interstitial cell infiltration	-	-	-	-	-	-	+	-	-
Liver (SGPT)									
Degeneration	++	-	-	+	+	+	++	+	-
Regeneration (binucleated hepatocytes)	+++	-	-	-	-	-	-	-	-
Cell infiltration in portal area	++	-	+	++	++	+	+	+	+
Bile duct hyperplasia	++	-	+	+	+	+	-	-	+
Narrowing sinusoids	+	-	-	+	+	-	+	+	-
Increase in number of large hepatocytes	+	-	-	-	-	-	+	++	++
Kidney									
Tubular degeneration	+++	-	++	++	++	+	+	-	-
Hyaline droplets	++	-	+	-	-	-	-	-	-
Hyaline cylinders	+	-	-	-	-	-	-	-	-
Pancreas									
Necrosis in Langerhans islet	-	-	-	-	-	-	-	-	-
OTHER	**NIL**	**NIL**	**NIL**	**NIL**	**NIL**	**NIL**	**PC: +++**	**NIL**	**NIL**

Table 11: Showing comparative histopathological finding among different interventional Groups, the pics of histopathological findings comparing kidney, pancreas, liver, heart with blood vessel are shown in *glossy* pages (VI - XI) as Fig-1(a - f) to 6(a - f). **[(- Absent), (+ Mild), (++ Moderate), (+++ Severe)]**

DISCUSSION

Our present study aims to evaluate the effectiveness and safe treatment of T2DM by alpha lipoic acid, hydroxychloroquine and chromium picolinate. T2DM is a disorder of the metabolism of carbohydrates, fats, and proteins that is brought on by insufficient insulin production or by its suppressive activity. It can be regarded as a major contributor to significant economic loss, which can hamper national growth. Metformin causes significant decrease in blood glucose level but also has serious side effects.

To counteract the side effects of metformin our study was to make a difference in the use of Metformin and other test drugs .The goal of the current study was to compare the roles of Chromium picolinate (group 6), Hydroxychloroquine (group 4), and Alpha lipoic acid (group 5) in the management of diabetes mellitus and the avoidance of its complications, such as diabetic nephropathy and dyslipidemia. Albino rats administered Metformin (group 3) as a routine treatment for diabetes mellitus allowed for the evaluation of its effects. Male albino Wistar rats displayed comparable baseline physical and metabolic traits. This is shown by the fact that there was no discernible change in the various groups prior to streptozotocin induction. Therefore, it may be assumed that differences in various parameters between the various groups at the time of treatment are a direct result of the various therapies that were provided to them.

In our study, we have used Streptozocin-nicotinamide induced diabetes rat model . It is the most often utilized model for screening of antidiabetic drug. Streptozocin is a common drug used to induce the diabetes in experimental animals, causing hyperglycemia whose mechanism can be understood as follows. A partial or complete lack of insulin causes an imbalance in the metabolism of carbohydrates, and a decrease in the enzyme activity of glucokinase (GK), hexokinase (HK), and phosphofructokinase (PFK) causes the liver and muscles to run out of glycogen. Glycogen synthase is activated by synthase phosphatase, which leads to glycogenesis. In STZ-diabetic rat, this activation appears to be defective. In STZ-insulin dependent diabetes, this inhibition of synthase phosphatase is nearly complete after 1-2 weeks.

Our current study utilizes the parameters like random blood glucose, weight difference, lipid profile, SGPT and renal profile of the experimental animals. As the study progresses, we have concluded that random blood glucose level was decreased significantly by hydroxychloroquine and alpha lipoic acid. When compared with metformin, HCQ has a similar effect on random blood glucose level. Chromium picolinate shows non-significant changes in random blood sugar.

As, we have discussed HCQ and ALA, reduces RBS significantly, previous few studies support our studies. According to Budin et al., ALA consumption/intake decreased the total cholesterol and glucose levels in rats with STZ-induced diabetes. Packer et al. Claim that ALA has the ability to scavenge ROS generated during lipid peroxidation and protects the cell structure. In their work, Padsalge MV et al. (2016) confirmed that HCQ inhibits the breakdown of insulin. Because insulin is quickly absorbed and broken down by insulin-degrading enzymes(IDE) in the cells, its plasma half-life is only 4-6 minutes. Due to HCQ's acidotrophic properties, which lower the pH to 6, where IDE has limited proteolytic activity, endosomal breakdown of insulin may be inhibited, leading to intracellular insulin buildup. As a result, HCQ dramatically increases blood insulin levels while lowering glucose levels. Hydroxychloroquine's peripheral activities, which result in insulin breakdown in the target cells and so facilitate insulin recycling, may be the likely explanation of its lower incidence of hypoglycemia compared to secretagogues (S.Mirza et al). According to research by Schultz O. Et al. (2012), HCQ reduced levels of TNF-, IL-1, IL-6, interferon (IFN), and other inflammatory indicators. As a result, HCQ showed a decrease in islet cell autoimmunity. Chromium supplementation can also treat corticosteroid-induced diabetes, according to research by Ravina et al. They administered 600 g/day of CrP to three individuals with steroid-induced diabetes, and they observed a reduction in fasting blood glucose levels from 250 to 150 mg/dl. In these patients, the necessity for anti-diabetic medications was also cut in half. This demonstrates a potential molecular mechanism through which CrP supplementation may enhance glycemic management by increasing insulin sensitivity and reducing oxidation.

Diabetes appears to cause an imbalance in the lipid composition of cell membranes as seen by increased non-enzymatic glycation, lipid peroxidation, and the ratio of cholesterol to phospholipids (Watala and Winocour, 1992) Increased plasma levels of triglycerides (TG), total cholesterol (TC), free fatty acids, and phospholipids were seen in STZ diabetic rats (Rodrigues et al.,1986). Numerous problems, including neuropathy, nephropathy, ketoacidosis, retinopathy, and others, can arise from diabetes mellitus (Ewing et al., 1976;

Maser et al., 1990). An important first step in preventing or correcting diabetic problems and enhancing the quality of life for those with diabetes is maintaining appropriate blood glucose levels (Diabetes control and complications trail research group, 1993; De Fronzo, 1999).

After six weeks of study, the Metformin group had higher levels of TC, TG, LDL, and VLDL and lower levels of HDL. This indicates that Metformin was ineffective in lowering the lipid level in diabetics and the need for an effective treatment to stop this comorbidity. These levels were significantly reduced after hydroxychloroquine treatment, proving the drug's ability to decrease lipids.

A potent inhibitor of cholesterol synthesis, hydroxychloroquine also increases LDL-C receptors, which improves the clearance of this lipoprotein from the plasma and lowers serum levels. Thus, based on the results of our study, we can say that hydroxychloroquine has the ability to lower cholesterol and further shield against the development of diabetic dyslipidemia. The postulated mechanism of our work is consistent with Sachet J et al. (2007), who also showed that HCQ inhibits cholesterol production, resulting in a decrease in serum lipid levels.

According to some research, hydroxychloroquine may raise LDL receptor levels, perhaps as a result of its impact on intracellular lysosomes. The consistent decline in LDL and/or total cholesterol levels observed in rheumatologic patients after hydroxychloroquine treatment may be explained by such a mechanism (Kerr G et al., 2014). To the best of our knowledge, no research has been done to determine how hydroxychloroquine affects the development of diabetes induced dyslipidemia. Non-HDL cholesterol was also observed to be decreased by HCQ. We were unable to locate any studies that discuss how HCQ affects the non-HDL cholesterol level in diabetic rats. The mechanism maybe insulin release which normalizes metabolic fat to keep lipid profile normal.

Concluding the other parameters in the study, similar effect was observed. Hydroxychloroquine also reduces urea, creatinine, LDL, TC, TG, VLDL, SGPT while it increases HDL level. Increase in HDL level was also seen with Alpha lipoic acid. In comparison to metformin, hydroxychloroquine considerably improved the LFT (liver function test), KFT (kidney function test), and lipid profile (TC-total cholesterol, TG-triglyceride, HDL-high density lipoprotein).

Previous studies seen similar findings. The findings of our investigation also showed that hydroxychloroquine plays a preventive role against renal damage in diabetic nephropathy. In comparison to the other treatment groups, the hydroxychloroquine group showed a higher reduction in the renal parameters (i.e., serum urea and creatinine). The anti-inflammatory property of HCQ, which lessens inflammatory cell infiltration, prevents congestion and fibrosis of glomerular blood vessels, and therefore preserves normal renal architecture, is one potential explanation for this.

Our study is the first to reveal lower serum urea and creatinine levels following a 6-week dose of hydroxychloroquine in Streptozotocin-induced diabetic nephropathy. To the best of our knowledge, no study has ever been done to examine its effects on animal models of diabetic nephropathy. However, when compared to the HCQ group, the metformin treatment was unable to effectively diminish the kidney damage.

As we go further, a much important parameter like weight reduction was evaluated. Metformin a stabilized and widely used drug causes significant weight reduction as expected. HCQ causes less but not significant weight reduction as compared to metformin. According to our research, there was a minor percentage decrease in body weight from the beginning of treatment to the end. Similar to our findings, a study by Pareek A. Et al. (2014) found that HCQ had an appetite-suppressing impact on T2DM patients. The anorexigenic actions of HCQ, which also increase lipolysis and triglyceride hydrolysis to diminish fat reserves and hence cause weight loss, may be the mechanism underlying this.

Predictive assay of effect of hydroxychloroquine and alpha lipoic acid over metformin had significant correlation between reading of the test and clinical potency of the drugs. As discussed earlier, hydroxychloroquine and alpha lipoic acid are individually potentiating effect in decreasing blood sugar and lipid profile level in albino rats.

The results obtained in present study indicates that hydroxychloroquine, an antimalarial drug and alpha lipoic acid a natural antioxidant shows promising antidiabetic potency in rodent models.

CONCLUSION

1. Hydroxychloroquine (400mg) and Alpha Lipoic Acid (600mg) in high dose revert random blood glucose level similar to metformin. In long term high dose Hydroxychloroquine and Alpha Lipoic Acid shows similar efficacy in reducing RBS like metformin.
2. Hydroxychloroquine in high dose improves all the parameters of lipid profile in a better way while comparing to other test drugs. Alpha Lipoic Acid also improves HDL level.
3. Hydroxychloroquine shows renoprotective and hepatoprotective property.
4. Metformin is still a better drug in weight reduction than the other test drugs.
5. Chromium picolinate is a drug which cannot cause improvement in any parameters of the study
6. Concluding all the results of this experiment, Hydroxychloroquine may be the better drug for future use in hepatorenal compromised patients of type 2 diabetes mellitus for which further clinical trials in human is required. Given its numerous benefits, hydroxychloroquine may slow the development of diabetes from the prediabetic stage as well as enhance lipid and renal profiles.
7. Finally, longer, more intensive and dose-responsive animal and human investigations(trial) are needed to correlate with the experimental data.

LIMITATIONS OF THE STUDY

There are several limitations in this study mainly due to financial constrain and lack of technical feasibilities -

1. Evaluation of HbA1C also could have given better result.
2. We have only evaluated the random blood sugar level. Evaluating the fasting sugar level could have given better predictive value.
3. Antioxidant profile could be tested.
4. Blood samples at start of study for lipid profiles and renal profile would have given a better comparison picture.
5. Brain, testes and few more organs could be screened histo-pathologically for effects of test drugs on them.

Blood sample for RBS measurement was taken from rat tail vein (tail cutting method) and Glucometer was used in this study to measure the blood glucose level. Glucometers are considered not very sensitive method for estimation of blood sugar; laboratory biochemical test of retro-orbital blood is more accurate. However retro-orbital sinus puncture may exert considerable negative impact on the animal welfare, by potentially causing subcutaneous hematomas and extensive tissue trauma as well as release of stress hormones can cause elevation of the blood glucose levels. Blood from cardiac puncture could also done be withdrawn, but lack of expertise restrained my work. Sacrificing rats became only option to obtain larger quantity of blood for testing blood parameters and histopathological examination.

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Annexure 1

The Following Table Summarizes the Milestones in Diabetes:

HISTORICAL MILESTONES
1550 BC: Written on a 3rd Dynasty Egyptian papyrus physician Hesy-Ra mentions a rare disease that causes frequent urination and lose weight rapidly as a symptom. This is the earliest known record of diabetes. Ebers Papyrus which was written around 1500 BC excavated in 1862 from an ancient grave of Thebes in Egypt and was published by an Egyptologist George Ebers in 1874. Here he mentions about the condition "too great emptying of urine" which is probably Diabetes Mellitus. The first described cases are believed to be type 1 diabetes.
1500 BC: Physicians around India (Ayurveda) at the same time noticed that urine of diabetic patients attracts ants and flies. They termed the condition 'Madhumeha'.
250 BC: Apollonius of Memphis for the first time used the term *Diabetes* which in Greek means 'to pass through'.it refers to a disease which drains patients of more fluid than they can consume
30 BC to 50 AD: The first complete clinical description of Diabetes appears to have been made by Aulus Cornelius Celsus known as 'Cicero medicorum'.
130-201 AD: Aristaeus of Cappadocia, a Greek physician wrote that Diabetes is a dreadful condition which melts flesh and limbs into urine. Life is short, unpleasant and painful, thirst unquenchable, drinking excessive and disproportionate to the large quantity of urine. If patients abstain from drinking their mouth becomes parched and their bodies dry, the viscera seem scorched up. Roman physician Galen at the same time defined the condition as weakness of kidney and termed as diarrhea of urine (diarrhea urinosa).
400-500 AD: Sushruta and Charaka, two Indian physicians were the first to differentiate between two types of diabetes mellitus. They observed that thin individuals with diabetes developed the disease in younger age in contrast to heavier individuals with diabetes who had a later onset and lived longer period of time after diagnosis.
700 AD: In China Li Hsuan noted that the patients with diabetes were prone to boils and lung infections.
980-1137 AD: Avicenna or Ibn Sina a court physician to Caliphs of Baghdad compiled an exhaustive medical text known as Cannon Avicenna which included a detailed description of diabetes. Clinical features like sweet urine, increased appetite, sexual dysfunction and gangrene were mentioned
1494-1541: Aureolus Theophrastus Bombastus von Hohenheim, a Swiss physician better known as Paracelsus allowed the urine of the diabetic patients to evaporate and observed a white residue. He incorrectly thought that this residue consisted of salt and responsible for excessive thirst and urination in these patients.
1670: Thomas Willis in Oxford noticed the sweet taste of urine of patients with diabetes.
1776: British Physiologist Matthew Dobson in his experiments and observations on the urine in diabetics was the first to show that the sweet tasting substance in the urine of patients with diabetes was sugar. He also noted the sweet taste of serum in these individuals and thus discovered hyperglycemia. Dobson put forward the theory that the diabetes was a systemic disease rather than kidney disease.
1788: Thomas Cawley was the first to suggest the link between pancreas and diabetes after he observed that people with pancreatic injury developed diabetes.
1797: John Rolo, surgeon general to the British Army added the term 'mellitus' (Greek word for honey) to diabetes in order to distinguish it from diabetes insipidus
1815: Eugene Chevreul in Paris proved that the sugar in urine of individuals with diabetes was glucose.
1848: Von Fehling developed quantitative test for diagnosing glucose in urine. After that in nineteenth century glucosuria became the diagnostic criteria for diabetes.
1850: Claude Bernard coins the term "glycogen" and developed an experimental operation during which the pancreatic ducts were ligated. This was followed by degeneration of the pancreas. In addition, Bernard also discovered that the liver stores glycogen and secretes sugar in blood in these patients. His theory got massive acceptance later on.
1850-1860: William Prout was the first to describe diabetic coma and Wilhelm Petters in 1857 demonstrated the presence of acetone in urine in patients with diabetes.
1859: Wilhelm Griesinger of Germany described renal disease in patients with diabetes.
1864: Marchal de Calvi of France discovered that neuropathy is a complication of diabetes.
1866: Willium Ogle of England described ocular nerve palsies in diabetes
1869: Henry Noyes described retinopathy in persons with advanced diabetes.
1869: Paul Langerhans of Germany discovered small cell clusters in pancreas not drained by pancreatic ducts. These cell clusters were later named as 'Islets of Langerhans'
1871: M.Troiser observed diabetes in patients with hemochromatosis and named it 'bronze diabetes'.
1874: Adolf Kussmaul proposed that acetonemia was the cause of diabetic coma.
1883: Bouchard described erectile dysfunction in poorly controlled diabetes
1886: HONK (hyperosmolar nonketotic) state was described by Dreschfield

1889: Oscar Minkowski and Joseph von Mehring of Germany observed that removal of pancreas in dogs cause immediate development of diabetes.
1890: Tropical ulcers and autonomic neuropathy were described by Auche
1907: M.A.Lane differentiated Alfa and beta cells of pancreas.
1913: Frederick Allen of USA proposed starvation diet for early onset diabetes.
1915: Karl Petren of Sweden had given the concept 'High fat low carbohydrate diet'
1921-22: Frederick Banting, Charles Best, James Collip, and John J.R. Macleod, Canada discovered insulin and awarded Noble Prize. Discovery of Insulin
1922: First clinical use of insulin
1925: Insulin made available for treatment of diabetes
1926: Insulin crystallization techniques introduced
1928: In Germany, Synthelin (a guanidine derivative) was administered for the treatment of diabetes
1936: Paul Kimmelstiel and Clifford Wilson of USA described glomerulosclerosis with heavy proteinuria in diabetic patients.
1936: Hans Christian Hagedorn of Denmark discovered protamine insulin, the first long-acting insulin
1936: Sir Harold Percival publishes research which divides into type1 and type 2 based on the degree of insulin sensitivity
1939: C.Ruiz, L.L.Silva, of Argentina observed hypoglycemic properties of sulfonamide antibiotics for the first time
1942: Auguste Loubatieres of France discovered hypoglycemic action of prototype sulfonylurea.
1946: NPH insulin introduced
1955: H.Franke and J.Fuch of Germany introduced Carbutamide, the first sulphonyl urea
1956: Lente insulin introduced
1957: G.Unger of USA introduced Phenformin, the first biguanide
1958: Metformin was introduced in United Kingdom
1958: Frederik Sanger got Noble prize for the structural formula of bovine insulin.
1959: Rosalyn Yalow and Solomon Berson of USA discovered radioimmunoassay for insulin and awarded Noble Prize.
1963: first premixed insulin introduced
1966: First transplant of the pancreas was successfully done by University of Minnesota, USA.
1967: Donald Steiner of USA discovered proinsulin
1969: Dorothy Hodgkin of Great Britain described the three-dimensional structure of porcine insulin by using X-ray crystallography.
1969: University Group Diabetes programme of USA completed first randomized trial on diabetes.
1971: Pierre Freychet of USA characterized insulin receptors.
1972: Pedro Cautrecassas of USA isolated insulin receptor protein
1975: R.Fluckiger and K.H.Winterhalter of Germany showed that HbA1c was glycated hemoglobin
1977: Alex Ullrich of USA reported sequence of rat insulin.
1978: Peter Sonksen and Robert Tattershal introduced home blood glucose monitoring (subcutaneous infusion pump) {Pickup, UK}
1978: Robert Crea and David Goeddel of USA started human insulin production by using recombinant DNA technology
1979: Ralph de Fronzo and Reuben Andres of USA discovered insulin clamp technique.
1980: Graham Bell of USA reported sequence of human insulin gene
1981: John Ireland of Scotland invented pen injection device.
1982: Recombinant human insulin approved by USFDA
1990: Thiazolidinedione were introduced
1991: WHO St Vincent declaration identified targets for diabetes care. WHO launces World Diabetic Day on 14th November the birthday of Fredrick Banting.
1992: Medtronic releases the Minimind 506 insulin pumps, which delivers meal bolus memory and daily insulin totals
1993: The landmark DCCT (diabetes control and complication trial report is published
1995: The first alpha-glucosidase inhibitor approved by USFDA
1996: First rapid acting insulin analog
1997: First thiazolidinedione introduced and Dr. Richard Bernstein published "diabetes solution".
1997: WHO authorized the classification proposed by American Diabetic Association which was based on etiology
2000: Edmonton protocol for islet cell transplant
2003: first long-acting insulin analog approved by USFDA
2005: FDA approved GLP-1 agonist Exenatide for treatment of type 2 DM, was derived from saliva of Gila

Monster(lizard)
2006: FDA approved first DPP4 inhibitor Sitagliptin for treatment of diabetes
2007: First genome wise study for diabetes.
2008: Suzanna M. de la monte proposes the term “type 3 diabetes” to describe insulin resistance in the brain.
2013: Drug control general of India approved Saroglitazar for diabetes and dyslipidemia
2013: Canagliflozin became the first SGLT2 inhibitor to be approved in the United States.
2015: Damiano describes bionic pancreas as a “bridge to a cure”, which delivers both insulin and glucagon every 5 minutes.

Annexure 2

Risk factors for type 2 diabetes

Unavoidable risk factors

- Family history
- Having a baby weighing more than 9 pounds
- Race
- Age
- Dark, thick, velvety skin appearing around the neck or armpits
- History of gestational diabetes

Risk factors that can be avoided or treated

- Obesity or being overweight, especially around the waist
- Getting little or no exercise
- High Blood Pressure
- Heart or blood vessel disease and stroke
- Low levels of "good" cholesterol (HDL)
- High levels of fats, called triglycerides
- Certain mental health conditions
- Polycystic ovary syndrome
- Smoking
- Stress
- Too much or too little sleep

Annexure 3

MODY Types with Gene Function

Type	Gene name	Gene Function	Primary defects
MODY 1	Hepatocyte nuclear factor 4 alfa (HNF4A)	Transcription nuclear factor	Pancreas
MODY 2	Glucokinase (GCK)	Hexokinase IV	Pancreas/liver
MODY 3	Hepatocyte nuclear factor 1 alfa (HNF1A)	Transcription factor(homeodomain)	Pancreas/kidney
MODY 4	Insulin promoter factor 1 beta (IPF-1)	Transcription factor(homeodomain)	Pancreas
MODY 5	Hepatocyte nuclear factor 1 beta (HNF1B)	Transcription factor(homeodomain)	Pancreas/kidney
MODY 6	Neurogenic differentiation1 (NEUROD1)	Transcription factor(bHLH)	Pancreas
MODY 7	Kruppel like factor 11	Transforming Growth Factor B-inducible early growth response 2	Pancreas
MODY 8	(KLF-11)	The endocrine cells of pancreas synthesize insulin and are involved in pathogenesis of diabetes and exocrine cell are involved in the pathogenesis of pancreatic malabsorption	Pancreas
MODY 9	Paired Domain Gene 4	Transcription factor (paired domain gene 4)	Pancreas
MODY 10	(PAX4)	B-cell of islets of Langerhans	NF-kappa-B
MODY 11	Tyrosine kinase B Lymphocytes	Tyrosine kinase (B-lymphocytes)	MIN6 beta cells

(The Journal of Medical Research, Rekha Bisht,2016)

Annexure 4 Drug Causing Diabetes

1. Drugs that cause diabetes by interfering with insulin-production and secretion –			
a.	b-receptor antagonists	b.	Tacrolimus
c.	Pyriminil (Vacor)	d.	Didansoine
e.	Pentamidine	f.	L-asparaginase
g.	Diphenylhydantoin	h.	Opiates.
2. Drugs that cause diabetes by reducing the effectiveness (sensitivity) of insulin –			
a.	Glucocorticoids	b.	b-receptor agonists
c.	Mega sterol acetate	d.	Growth hormone
e.	Oral contraceptives	f.	Protease inhibitors.
3. Drugs that act on both insulin secretion and insulin sensitivity –			
a.	Thiazide diuretics	b.	Diazoxide
c.	Cyclosporine	d.	Atypical antipsychotic
4. Treatments that induce diabetes by increasing nutrient flux –			
a.	Nicotinic acid	b.	Total parenteral nutrition.
(KK Lohani, medicine_update_2010)			

Annexure 5

Endocrine Disorder Which Causes Diabetes Mellitus
1. Acromegaly
2. Cushing's syndrome
3. Pheochromocytoma, paragangliomas, VIPoma's
4. Glucagonoma and Somatostatinoma
5. Thyrotoxicosis, hypothyroidism, hyperthyroidism
6. Other endocrinopathies such as primary aldosteronism and primary hyperparathyroidism can disturb glucose homeostasis.
7. Polycystic ovarian syndrome.
(Textbook of Diabetes, Richard I.G. Holt, Clive S. Cockram, Allan Flyvbjerg, Fourth Edition, 2010).

Annexure 6

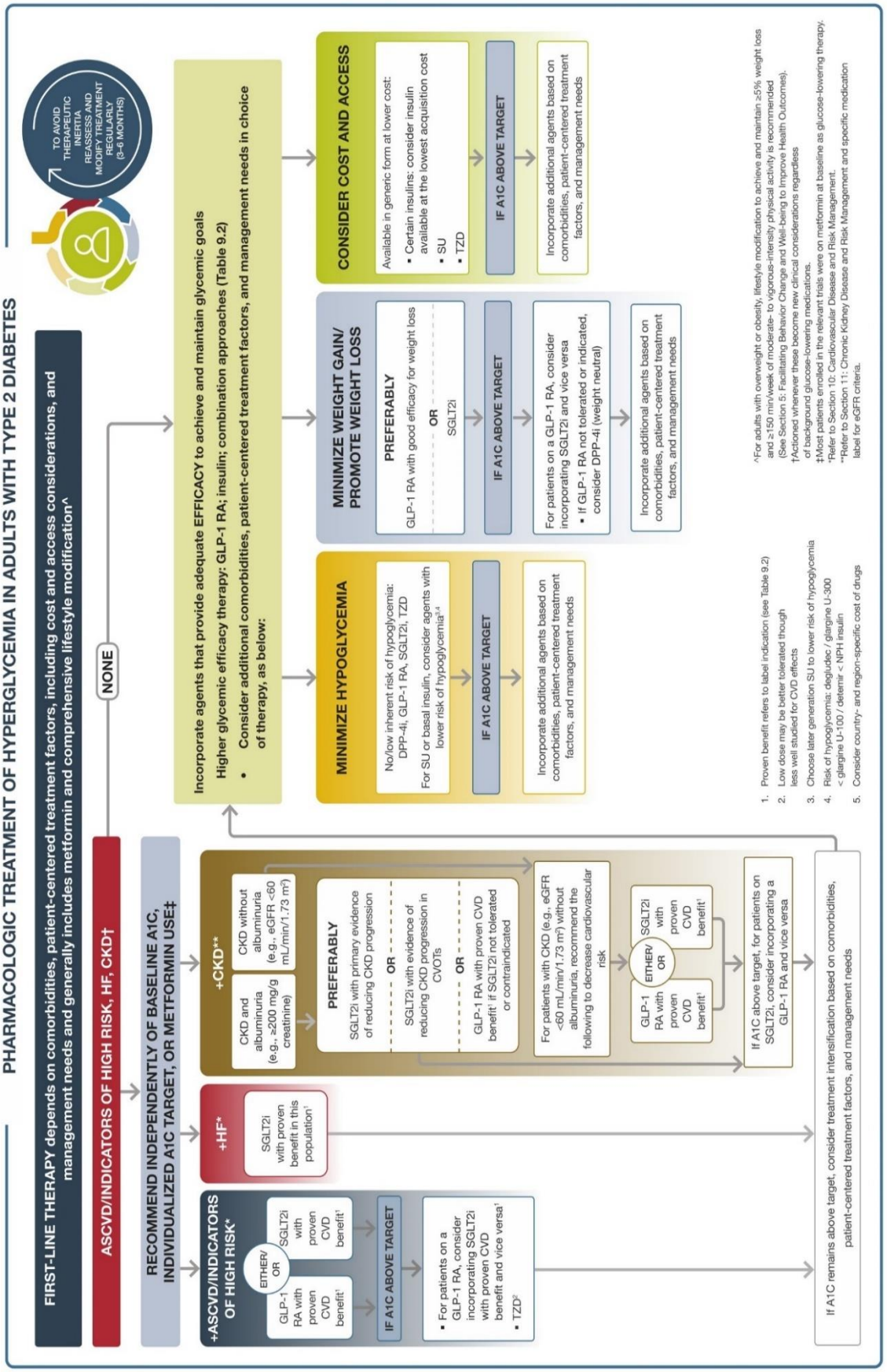
Pancreatic Diseases Associated with Glucose Intolerance and Diabetes	
1.	**Inflammatory**
a.	Acute pancreatitis
b.	Chronic pancreatitis
2.	**Infiltration**
a.	Tropical calcific pancreatitis
b.	Hereditary hemochromatosis
c.	Pancreatic carcinoma
3.	**Pancreatic surgery**
4.	**Cystic fibrosis**
(Textbook of Diabetes, Richard I.G. Holt, Clive S. Cockram, Allan Flyvbjerg, Fourth Edition, 2010).	

Annexure 7

Pharmacological Agents for Treatment of Diabetes
1. Insulin preparations
a) Ultra-short acting insulin- Insulin lispro, Insulin aspart, Insulin glulisine
b) Short acting insulin – Regular Novolin R, Regular Humulin R
c) Intermediate acting insulin- NPH Humulin N, NPH Novolin N
d) Premixed insulins- Novolin 70 NPH/30 regular, Humulin 70 NPH/30 regular, 75/25 NPL, Lispro, 70/30 NPA, Aspart
e) Long-acting insulins- Insulin detemir, Insulin glargine,
f) Ultra-long-acting insulins- Insulin degludec
2. Drugs enhancing insulin secretion
a) Sulfonylureas (KATP Channel blockers)
First generation: Tolbutamide
Second generation: Glibenclamide (Glyburide), Glipizide, Gliclazide, Glimepiride
b) Meglitinide/phenylalanine analogues: Repaglinide, Nateglinide
c) Glucagon-like peptide-1 (GLP-1) receptor agonists (Injectable drugs): Exenatide, Liraglutide
d) Dipeptidyl peptidase-4 (DPP-4) inhibitors: Sitagliptin, Vildagliptin, Saxagliptin, Alogliptin, Linagliptin
3. Overcome Insulin resistance
a) Biguanide (AMPK activator): Metformin
b) Thiazolidinediones (PPARγ activator): Pioglitazone
4. Miscellaneous antidiabetic drugs
a) α-Glucosidase inhibitors: Acarbose, Miglitol, Voglibose
b) Amylin analogue: Pramlintide
c) Dopamine-D2 receptor agonist: Bromocriptin
d) Sodium-glucose cotransport-2 (SGLT-2) inhibitor: Dapagliflozin

Annexure 8

Algorithm for management of diabetes



Annexure 9
IAEC Certificate

Memo NO:- 162, IAEC RIMS Ranchi, Dated on 26/02/2021

CERTIFICATE

This is to certify that the Institutional Animal Ethics Committee, Rajendra Institute of Medical Sciences, Ranchi approves and grants permission for academic and scientific work on the thesis : A Comparative Study of effects of Alpha Lipoic Acid, Chromium Picolinate and Hydroxychloroquine on blood sugar in Streptozocin induced Type2 Diabetic model to be done by Dr Rajiv Ranjan Das, Junior Resident (Academic), Rajendra Institute of Medical Sciences, Ranchi in the Department of Pharmacology and Therapeutics, session 2020-2023, under the guidance of Dr (Prof &HOD) Satish Chandra, Department of Pharmacology & Therapeutics, Rajendra Institute of Medical Sciences, Ranchi

26/02/21

Secretary

Secretary
Institutional Animal Ethics Committee
Rajendra Institute of Medical Sciences
Ranchi

Institutional Animal Ethics Committee

Rajendra Institute of Medical Sciences, Ranchi.

Annexure 10
GLUT types with Location

GLUTs are integral membrane proteins that contain 12 membrane-spanning helices with both the amino and carboxyl terminal exposed on the cytoplasmic side of the plasma membrane

- GLUT1 = RBC, BBB
- GLUT2 = Liver, Renal Tubular Cells, Pancreatic Beta Cells
- GLUT3 = Brain, Placenta
- GLUT4 = Striated Muscle and Adipose Tissue
- GLUT5 = Intestine (Fructose Transporter in Enterocytes)
- GLUT6 = Intestine (Transporting Glucose Out of The Endoplasmic Reticulum)

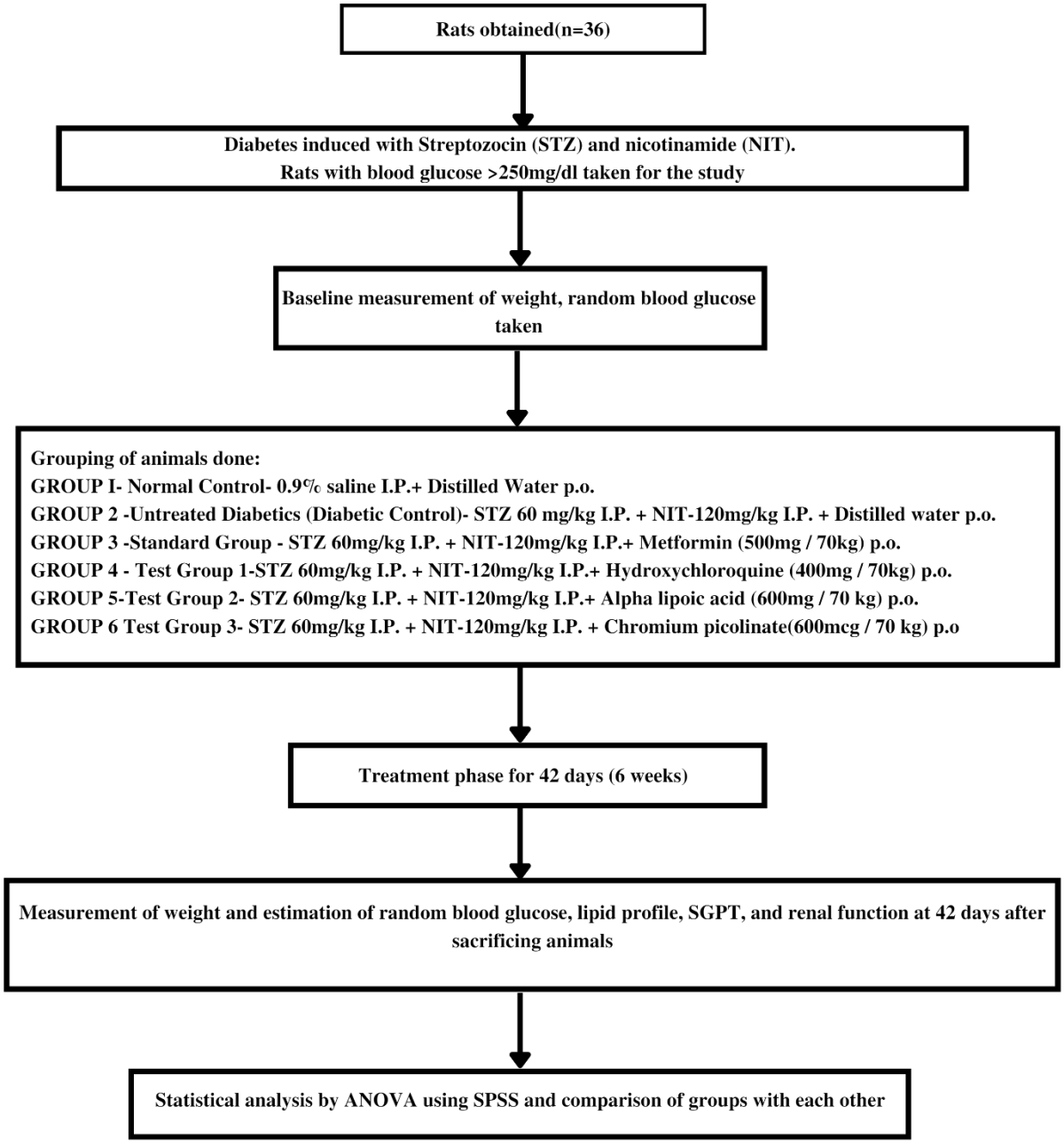
Annexure 11
List of Tables

Annexure 12
Decision Cycle for Patient-Centered Glycemic Management in Type 2 Diabetes

Annexure 13
List of Figures

Annexure 14

Diagrammatic representation of the study.



Diagrammatic representation of the study

GROUP	ID	ALT	UREA	CR	LDL	HDL	TC	TG	VLDL	NON HDL	AI			WEIGHT (D0)	WEIGHT (D42)	RDS (D0)	RBS (D7)	RBS (D14)	RBS (D21)	RBS (D28)	RBS (D35)	RBS (D42)
Normal Control	RAT1	83	34.84	0.36	16.8	37	69	76	15.2	32	0.86			154	166	107	102	103	100	101	106	101
Normal Control	RAT2	109	35.52	0.53	16.2	43	72	64	12.8	29	0.67			170	174	102	97	104	104	107	105	106
Normal Control	RAT3	121	31.4	0.44	10.6	44	71	82	16.4	27	0.61			130	146	99	107	99	100	109	111	102
Normal Control	RAT4	96	31.69	0.48	15.4	42	72	73	14.6	30	0.71			210	215	98	102	105	111	113	101	102
Normal Control	RAT5	104	31.54	0.37	13.6	43	74	87	17.4	31	0.72			166	174	100	99	102	109	108	99	104
Normal Control	RAT6	111	30.17	0.38	11.4	40	70	93	18.6	30	0.75			157	162	102	103	103	108	110	110	103
Diabetic Control	RAT7	174	95.69	1.62	81.4	18	133	168	33.6	115	6.4			156	198	310	320	336	345	354	361	374
Diabetic Control	RAT8	213	101.29	1.69	69.2	22	126	174	34.8	104	4.7			152	211	296	308	322	329	339	345	360
Diabetic Control	RAT9	192	100.6	1.63	71.4	20	128	183	36.6	108	5.4			163	180	300	310	323	332	346	353	365
Diabetic Control	RAT10	159	94.63	1.21	74.2	19	130	184	36.8	111	5.84			180	246	306	315	330	334	350	354	366
Diabetic Control	RAT11	206	102.2	1.54	85.8	18	139	176	35.2	121	6.7			193	203	314	320	337	342	351	364	379
Diabetic Control	RAT12	186	101.8	1.98	70.4	21	127	178	35.6	106	5.04			216	242	292	308	320	327	338	347	362
Standard Drug (Metformin)	RAT13	121	61.2	0.53	19.2	28	66	94	18.8	38	1.36			155	132	324	279	221	152	127	106	105
Standard Drug (Metformin)	RAT14	124	58.64	0.73	23.4	26	67	88	17.6	41	1.57			156	138	308	263	200	156	131	109	102
Standard Drug (Metformin)	RAT15	127	68.37	0.75	22.8	24	65	91	18.2	41	1.71			207	159	328	283	219	17	137	107	103
Standard Drug (Metformin)	RAT16	129	63.67	0.63	24	26	68	90	18	42	1.61			214	161	306	260	205	165	130	110	109
Standard Drug (Metformin)	RAT17	126	62.6	0.69	21.8	25	66	96	19.2	41	1.64			220	164	300	240	201	160	121	109	107
Standard Drug (Metformin)	RAT18	120	55.58	0.7	19.6	25	64	97	19.4	39	1.56			218	168	302	260	211	167	138	108	103
Test Drug (Hydroxychloroquine)	RAT19	111	63.53	0.42	16.4	29	62	83	16.6	33	1.13			157	151	319	289	238	208	182	142	123
Test Drug (Hydroxychloroquine)	RAT20	120	62.64	0.61	16.8	30	64	86	17.2	34	1.13			166	164	303	261	224	196	151	134	120
Test Drug (Hydroxychloroquine)	RAT21	112	59.48	0.42	15.6	28	61	87	17.4	33	1.17			210	201	320	283	221	209	181	143	121
Test Drug (Hydroxychloroquine)	RAT22	118	59.68	0.47	16.2	29	59	69	13.8	30	1.03			156	152	305	279	219	194	153	130	118
Test Drug (Hydroxychloroquine)	RAT23	121	63.4	0.5	15.8	30	60	76	15.2	31	1.06			154	147	321	264	227	200	162	138	118
Test Drug (Hydroxychloroquine)	RAT24	101	54.77	0.48	15.8	25	62	81	16.2	32	1.06			180	171	296	259	222	189	142	129	111
Test Drug (Alpha lipoic acid)	RAT25	159	69.57	0.9	26.8	28	72	101	20.2	47	1.88			190	180	321	274	240	210	187	159	138
Test Drug (Alpha lipoic acid)	RAT26	151	71.31	0.84	27.4	27	75	98	19.6	47	1.67			182	172	308	274	249	215	191	162	140
Test Drug (Alpha lipoic acid)	RAT27	158	69.34	0.86	32.8	28	77	86	17.2	50	1.85			185	183	302	260	226	194	158	151	129
Test Drug (Alpha lipoic acid)	RAT28	159	68.71	0.96	30.6	29	81	112	22.4	53	1.89			154	153	312	269	232	195	154	133	126
Test Drug (Alpha lipoic acid)	RAT29	157	72.62	0.98	31	25	82	110	22	53	1.82			156	152	301	264	235	200	171	152	136
Test Drug (Alpha lipoic acid)	RAT30	153	66.26	0.94	23.8	23	70	106	21.2	45	1.8			159	170	314	272	236	211	186	146	132
Test Drug (Chromium picolinate)	RAT31	162	82.81	1.22	31	23	80	130	26	57	2.47			158	164	272	264	230	223	218	220	206
Test Drug (Chromium picolinate)	RAT32	175	86.13	1.24	29	27	79	115	23	52	1.92			154	168	292	271	253	249	237	233	222
Test Drug (Chromium picolinate)	RAT33	166	83.26	1.26	28.6	26	78	117	23.4	52	2			182	196	283	262	246	238	232	234	202
Test Drug (Chromium picolinate)	RAT34	170	82.38	1.28	24	22	68	110	22	46	2.09			176	184	286	261	248	242	236	240	216
Test Drug (Chromium picolinate)	RAT35	172	84.19	1.12	26.4	24	76	128	25.6	52	2.16			194	190	280	259	241	240	242	224	192
Test Drug (Chromium picolinate)	RAT36	168	88.64	1.21	27.4	22	73	118	23.6	51	2.31			198	204	303	268	258	251	249	238	212

Abbreviations

ID	RAT IDENTIFICATION
Sgpt/ALT	ALANINE TRANSAMINASE/ALT
Urea	UREA
CR	CREATININE
LDL	LOW DENSITY LIPOPROTEIN
HDL	HIGH DENSITY LIPOPROTEIN
TC	TOTAL CHOLESTEROL
TG	TRIGLYCERIDE
VLDL	VERY LOW DENSITY LIPOPROTEIN
AI	ATHEROGENIC INDEX
NONHDL	NON-HDL
WEIGHTD0	WEIGHT AT DAY 0
WEIGHTD42	WEIGHT AT DAY 42
RBSD0	AT DAY0
RBSD7	AT DAY7
RBSD14	AT DAY14
RBSD21	AT DAY21
RBSD28	AT DAY28
RBSD35	AT DAY35
RBSD42	AT DAY42
(RANDOM BLOOD GLUCOSE-RBS)	