"PHARMACOLOGICAL SCREENING OF HERBAL FORMULATION 'BETAZEN' ON EXPERIMENTALLY INDUCED INSULIN RESISTANCE IN RATS"

DISSERTATION

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MASTER OF PHARMACY

IN

PHARMACOLOGY

By

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Affectionately Dedicated

To

MY BELOVED

FAMILY

Trust in the lord with all your heart and lean not on your own understanding; in all your ways acknowledge him, and he will make your paths straight.-Proverbs 3: 5-6.

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LIST OF ABBREVIATIONS

ATP	Adenosine Triphosphate.
AMPK	Adenosine monophosphate activated protein kinase.
ANOVA	Analysis of Variance.
BGL	Blood glucose level.
BHF	Betazen herbal formulation.
BM	*Butea monosperma*
BW	Body weight.
CAT	Catalase.
CHF	Cardiac heart failure.
ChREBP	Carbohydrate responsive element binding protein.
CVD	Cardiovascular diseases.
DEXA	Dexamethasone.
DNA	Deoxy-ribonucleic acid.
DPP-4	Dipeptidyl peptidase-4.
DM	Diabetes mellitus.
ET	Endothelin.
FFA	Free fatty acids.
FGF21	Fibroblast growth factor 21.
GC	Glucocorticoids.
GIP	Glucose dependent insulinotropic polypeptide.
GLP-1	Glucagon like peptide-1.
GLUT	Glucose transporter.
GSH	Reduced glutathione.

GWAS	Genome wise association studies.
G6Pase	Glucose-6-phosphatase.
HbA_{1c}	Glycated hemoglobin.
HDL	High density lipoprotein.
H_2O_2	Hydrogen peroxide.
IRS	Insulin receptor substrate.
IR	Insulin resistance.
IL-1b	Interleukin-1b.
IV	Intra venous.
JNK	c-Jun N terminal kinase.
Kg	Kilogram.
LDL	Low-density lipoprotein.
mTOR	Mammalian target of rapamycin.
MAPK	Mitogen activated protein kinase.
MDA	Malondialdehyde.
µg	Microgram.
mg	Miligram.
ml	Millilitre.
µM	Micromole.
mg/dl	Milligram per decilitre.
MC	*Momordica charantia*
MCP	Monocyte chemo-attractant protein.
NADPH	Nicotine amide adenine nucleotide phosphate.
NFKB	Nuclear factor activator kappa B.

NO	Nitric oxide.
PCOS	Polycystic ovary syndrome.
PDK1	Phosphoinositide-dependent protein kinase-1.
PEPCK-C	Phosphoenol pyruvate carboxykinase.
PI3-K	Phosphatidyl inositol 3-Kinase.
PIO	Pioglitazone.
PIP3	Phosphatidyl inositol 3, 4, 5-trisphosphate.
PKB	Protein kinase B.
PKC	Protein Kinase C.
PKC-z	Protein Kinase C zeta type.
PPAR γ	Peroxisome proliferator activator receptor γ.
p.o	Per oral.
RNA	Ribonucleic acid.
ROS	Reactive oxygen species.
s.c	Subcutaneous.
Ser/Thr	Serine/threonine.
SGLT-2	Sodium glucose co-transporter-2.
SIRP	Signal regulatory protein.
SOD	Superoxidismutase.
TBARS	Thiobarbituric acid reactive species.
TC	Total cholesterol.
TG	Triglycerides.
TNF-α	Tumor Necrosis Factor.
T1DM	Type 1 Diabetes mellitus.

T2DM	Type 2 Diabetes mellitus.
UCP2	Uncoupling protein 2.
VLDL	Very low density lipoprotein.
WHO	World health organisation.
4HAA	4 hydroxy amino acid.
5-HT	Serotonin.

ABSTRACT

Objective: To evaluate the anti-diabetic effect of Betazen capsules in dexamethasone and high fat-high sugar diet induced insulin resistant rats.

Methodology: Wistar rats were divided into 5 groups (n=6). Insulin resistance (IR) was induced in animals by administration of dexamethasone (2 µg/day s.c) for initial 10 days along with vanaspati ghee & fructose enriched high fat-high sugar (HFHS) diet for 42 days. Group I (normal control) received normal rat feed and water. Group II (diabetic control) received dexamethasone and HFHS diet. Group III, IV and V were IR groups treated with 2.7 mg/kg Pioglitazone, 45 mg/kg, and 90 mg/kg Betazen herbal formulation (BHF) in 1% CMC suspension respectively. Fasting blood glucose level (BGL) of rats was measured weekly. At the end of the study period, animals were been sacrificed and whole blood was utilised for HbA_{1c} estimation. Serum was collected for estimation of various biochemical parameters. Glycogen content in the liver & gastrocnemius muscle and glucose uptake in hemi-diaphragm was calculated. Levels of antioxidant biomarkers in liver was also been estimated. Pancreas was dissection out and its histopathology was been studied.

Result: Dexamethasone and HFHS diet induced IR lead to muscle proteolysis, hyperglycemia, dyslipidemia, reduced cellular glucose uptake and oxidative stress. Treatment of IR rats with BHF exhibited a significant dose dependent reduction in BGL, BW, TC, TG, LDL, VLDL, HbA_{1c} and MDA levels. In addition, there was a significant ($p<0.0001$) increase in glycogen content, glucose uptake, HDL cholesterol, and antioxidant biomarkers viz; SOD, GSH & CAT.

Conclusion: The experimental results highlighted the antioxidant, anti-glycation & anti-hyperglycemic potential of Betazen herbal formulation on IR. Furthermore, it also exhibited

an improvement in dyslipidemia, insulin sensitivity, and glycogen content. Hence, Betazen herbal formulation could be a promising future herbal treatment for insulin resistance.

Keywords: Insulin resistance, Dexamethasone, High fat-high sugar, Betazen.

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1.0 INTRODUCTION

Diabetes Mellitus (DM) is a chronic progressive metabolic disorder that takes place when the pancreas do not produce sufficient insulin or when the body cannot effectively utilise the insulin it produces.[1,2] This metabolic disorder results in abnormally high blood sugar levels (hyperglycemia) in the body. Moreover, DM is associated with micro and macro vascular diseases, leading to long-term damage, dysfunction and failure of various organs especially the eyes, nerves, heart, kidneys and blood vessels.[3] The characteristic feature of Type 1 Diabetes Mellitus (T1DM) is the loss of insulin producing pancreatic beta cells of islet of Langerhans, which lead to lack of insulin production. However, Type 2 Diabetes Mellitus (T2DM) is been associated with pancreatic beta-cell dysfunction, along with Insulin Resistance (IR).[4]

The major diabetic complications are; retinopathy (eye), cerebrovascular disease (brain and cerebral circulation), coronary heart disease (heart and coronary circulation), nephropathy (kidney), neuropathy (peripheral nervous system), peripheral vascular diseases (lower limbs), diabetic foot (ulceration and amputation), sleep apnea, gingivitis and pregnancy complications. Moreover, in-appropriately managed diabetes leads to serious complications and early death.[5]

A cardinal feature of T2DM which has been closely linked to hypertension, dyslipidemia, and obesity is IR.[6,7] IR is a pathological condition characterised by lack of physiological response from peripheral tissues, skeletal muscle, liver and adipose tissue towards insulin, leading to metabolic and hemodynamic disturbances known as the metabolic disorder. The characteristics of this metabolic disorder include coronary heart diseases, DM, hyperuricemia, abdominal obesity, defects in the fibrinolytic system, hyperandrogenism and fatty liver.[7-10]

IR involves a complex mechanism wherein there is an excess accumulation of lipids in the muscle and liver. As a result, in insulin-resistant people, normal levels of insulin do not have a similar effect on muscles, liver and adipose cells. This gives rise to glucose levels, which are higher than the normal level in the body. Hyperglycemia normally develops after a meal, when pancreatic β-cells are unable to produce sufficient insulin. However, in pre-diabetics, fasting glucose is higher than the normal level while hepatic glucose output is normal. In addition, the fasting plasma insulin raises and is inappropriately normal to the degree of insulin milieu thereby indicating the existence of hepatic IR, which is a principal component of T2DM. Furthermore, the decrease in hepatic insulin sensitivity leads to elevated hepatic glucose production, hyperinsulinemia, increased β-cells mass and hyperglycemia.[11]

Excess abdominal fat deposition in obese individuals is been found to be resistant to the anti-lipolytic effects of insulin, thereby ensuing in the release of excessive amounts of free fatty acids (FFA) and predisposing to ectopic fat accumulation, followed by IR in the liver and muscle. This ultimately contributes to an increase in gluconeogenesis in the liver and an inhibition of insulin-mediated glucose uptake in the muscles resulting in increased levels of circulating glucose.[4]

According to WHO 2015 updates, it is been established that 347 million people worldwide have diabetes. More than 80% people with diabetes live in low and middle-income countries. In 2014, 90% of adults were been diagnosed with diabetes while in 2012, diabetes was found to be the direct cause of 1.5 million deaths. In addition, WHO projects that, diabetes deaths will double between 2005 and 2030. It also predicts that diabetes will be the seventh leading cause of death by 2030.[1]

Treatment for people with DM includes advice on nutrition, physical activity, weight loss, smoking cessation and drug therapy. Dietary control is the mainstay of treatment for T1DM and T2DM. The current treatment regimens for DM include insulin as the primary treatment for almost all types of diabetic patients. T2DM can be treated with sulfonylureas, meglitinides, metformin, thiazolidinediones, incretin mimetics: GLP-1 (glucagon like peptide-1), GIP (glucose dependent insulinotropic polypeptide) and amylin analogues (Pramlintide). Although insulin therapy and oral hypoglycemics is the mainstay of treatment for diabetes and are effective in controlling hyperglycemia, they have prominent side effects like insulin allergy, resistance, lipoatropy, lipohypertrophy, insulin edema, nausea and vomiting, plasma volume expansion, myalgia, risk of fractures, fluid retention, cholestatic jaundice, agranulocytosis, aplastic and haemolytic anaemia's, generalized hypersensitivity reactions, rashes, hypoglycemia respectively.[4,12]

The prophylactic and therapeutic effect of many herbal plant extracts such as *Zingiber officinale,*[13] *Momordica charantia,*[14] *Trigonella foenum-graecum L,*[15] *Azadirachta indica*, *Gymnema sylvestre*, *Picrorrhiza kurrao*, *Pterocarpus marsupium, Tinospora cordifolia*, have been reported for the treatment of IR.[3] In addition, various chemical constituents have been reported to have anti-diabetic property viz; flavonoids, phenols, sterols, saponins, tannins, lapachol, lapachonone, de-oxylapachol, trigonelline bases, glycosides and cinnamaldehyde.[16]

As the knowledge of heterogeneity of DM increases, it necessitates the search for more efficacious agents with minimal side effects. Though development of modern medicine resulted in the advent of modern pharmacotherapeutics including insulin, biguanides, sulphonylureas, and thiazolidinediones, there is still a need to look for new drugs as no drug (except strict glycemic control with insulin) has the ability to modify the course of diabetic

complications. However, herbal formulations have shown fewer side effects but lack scientific evidence regarding their safety and efficacy.

Betazen capsules contain a combination of some of the known herbal plant extracts, which include *Curcuma longa, Melia azadirachta, Pterocarpus marsupium, Tinospora cordifolia, Phyllantus embilica, Terminalia chebula, Terminalia belerica, Zingiber officinale, Piper nigrum, Piper longum, Butea frondosa, Picrorrhiza kurroa, Gymnema sylvestre, Trigonella foenum-graecum, and Momordicia charantia* respectively.

The herbal formulation 'Betazen' has been widely prescribed by ayurvedic practitioners for insulin resistant diabetes. Despite their use, there is no scientific evidence for its potential activity.

Hence, the present study is been designed to evaluate anti-diabetic activity of Betazen herbal formulation on drug and diet induced insulin resistance in rats.

2.0 OBJECTIVES OF THE STUDY

To evaluate the anti-diabetic effect of Betazen capsules, an ayurvedic proprietary medication on drug and diet induced insulin resistant rats, by studying the following parameters:

- Body weight.
- Fasting blood glucose levels.
- Serum lipid profile (TC, TG, HDL, LDL, and VLDL).
- Estimation of HbA_{1c}.
- Estimation of glycogen content in gastrocnemius muscle and liver.
- Measurement of glucose uptake in hemi-diaphragm.
- Estimation of anti-oxidant biomarkers (SOD, MDA, GHS, CAT).
- Histopathological study of pancreas.

3.0 REVIEW OF LITERATURE

3.1 Insulin Resistance Diabetes

DM is an endocrine metabolic disorder that occurs consequently due to defective regulation of carbohydrate, protein and fat metabolism. This clinical metabolic disorder and its associated abnormalities characterized by hyperglycemia take place because of a relative or absolute deficiency of insulin or by a resistance to the action of insulin at the cellular level. [2,13,17,18]

IR is the main metabolic feature as well as a requisite precursor for the development of T2DM. It is a state characterized by hyperinsulinemia and declining insulin sensitivity exhibited by target tissues viz; skeleton muscles, liver and adipose tissue towards the biological action of insulin. This decline in insulin sensitivity occurs due to the decrease in insulin-stimulated skeletal muscle glycogen synthesis, which is been attributed to the diminished insulin-stimulated glucose transporter (Glut-4) activity. Further, tissue insensitivity towards insulin manifests either as a decrease in the number of insulin receptors or decrease in insulin's affinity towards its receptors.[6] IR refers to a pathological condition whereby insulin receptors present within the body cells are insensitive to insulin resulting in glucose not readily entering the tissues, thus ultimately leading to hyperglycemia or elevated blood glucose levels. [8,19-23]

IR can be categorised as acute or chronic wherein acute resistance develops in patients exposed to infections, surgical trauma or emotional disturbances while chronic resistance is immunological in nature and results due to formation of antibodies to insulin. IR initiates earlier than the beginning of T2DM, wherein there exists an impaired glucose tolerance because of beta cell function deterioration and comparative insulin deficiency. Due

to IR, insulin's action on peripheral tissues such as adipose tissue, skeletal muscle, and liver is been reduced. [8,24]

3.2 Glucose Homeostasis

In order to develop newer anti-diabetic agents for therapeutic interventions, it is essential to understand normal glucose metabolism. Glucose is an essential nutrient found in food that provides energy required for the appropriate functioning of various body tissues. Based on the need, glucose can either be utilised as an energetic source through glycolysis or alternatively, stored as glycogen within muscle or liver cells. In the human body, glucose utilization is either insulin-dependent or insulin-independent. The insulin-dependent actions involve glucose uptake by muscle tissue, either for glycogen storage or for glycolysis, and glucose-to-glycerol conversion by adipose tissue as well as incorporation of glycerol and free fatty acid (FFA) into stored triglyceride. Non–insulin-dependent glucose utilization occurs in other tissues, which include formed elements of blood, kidneys, and brain tissue (neurons of the central nervous system). [25] After ingestion of a meal, carbohydrates undergo break down in the small intestine and the glucose present in digested food is then absorbed by the intestinal cells into the bloodstream, wherein it is ultimately been utilized by various body tissues. [11]

Normal blood glucose levels in the body are been maintained by dietary glucose absorbed by the intestine, glucose production in the liver via glycogenolysis as well as through gluconeogenesis from various substrates, such as amino acids, lactate, glycerol and its utilisation by the tissues. Likewise, several physiological systems such as endocrine and sympathetic nervous system also contribute to maintenance of glucose homeostasis in addition to enzymes, substrates and transporters. In healthy individuals, a state of equilibrium (normo-glycemia) is been sustained through a tightly regulated interaction between tissue

sensitivity to insulin, insulin secretion and glucagon secretion. Various other hormones viz; epinephrine, cortisol and growth hormone influence glucose homeostasis by accelerating hepatic gluconeogenesis, promoting lipolysis and antagonizing cellular actions of insulin along with promotion of muscle proteolysis respectively. [25,26]

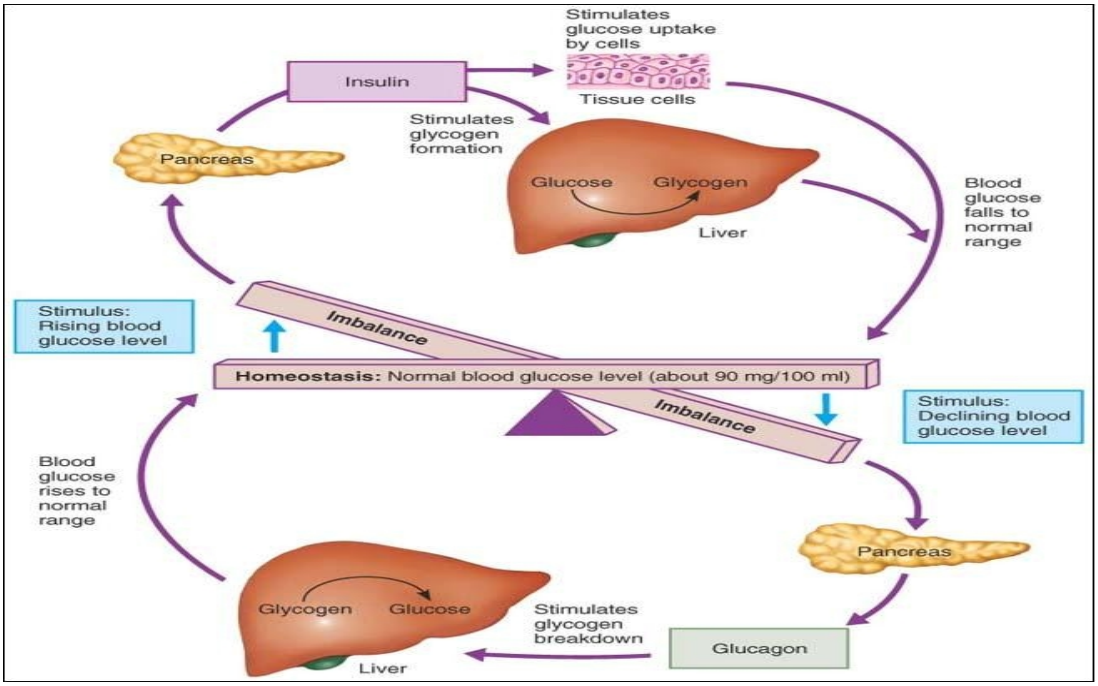


Figure No. 1: Glucose homeostasis in the body.[97]

During normal physiological conditions, glucose is usually been carried into cells via two types of carrier proteins, viz; Sodium/glucose co-transporters (S-GLUTs) and the glucose transporters (GLUTs) that permit glucose influx into various cells. The physiologically important GLUT transporters are GLUT-1 (abundantly distributed throughout the body and responsible for glucose uptake), GLUT-2 (abundant in liver and pancreatic β cells and function as a glucose sensitizer), GLUT-3 (the major non-insulin-requiring, substrate-driven glucose transporter abundant in neuronal tissue) and GLUT-4 (the major insulin-responsive glucose transporter in fat and skeletal muscles). This transport is proportional to the blood glucose concentration in the physiologic range. On the other hand, S-GLUT plays a pivotal role in glucose uptake across apical cell membranes. S-GLUT-1 and S-GLUT-2 are

responsible for sodium-dependant glucose uptake in the intestine and kidney respectively.[25,27]

Upon entry into the cell, glucose undergoes phosphorylation to glucose-6-phosphate by glucokinase. Glucose-6-phosphate further undergoes subsequent oxidation to adenosine triphosphate (ATP), which inhibit the ATP sensitive potassium channels of the cell. The cell membrane is depolarised due to closure of potassium channels thereby stimulating the opening of voltage gated calcium channels, which lead to the influx of calcium that stimulates fusion of insulin containing vesicles with the cell membrane and secretion of insulin into the extra cellular fluid by exocytosis. Moreover, glucose cannot gain entry into cells alone but requires insulin to aid its transport into the cells. In addition, inappropriate utilization of insulin leads to IR.[11,23,28-32]

3.3 Insulin Secretion and Regulation

Insulin is an anabolic pancreatic peptide hormone which is synthesized and stored as pro-insulin, comprising of two amino acids chains, A and B respectively, joined through disulphide linkages by a connecting peptide C. Insulin secretion from the pancreas is biphasic in nature and occurs when the concentration of nutrient molecules in the blood is high. In the first phase, there is a rapid release of insulin from the stored vesicles in response to rise in blood glucose, following ingestion of a meal. While in the second phase, new insulin is been synthesized and released in a slow and sustained manner. An increase in the concentration of gastrointestinal hormones like gastrin, secretin and cholecystokinin also trigger insulin release. [28-32]

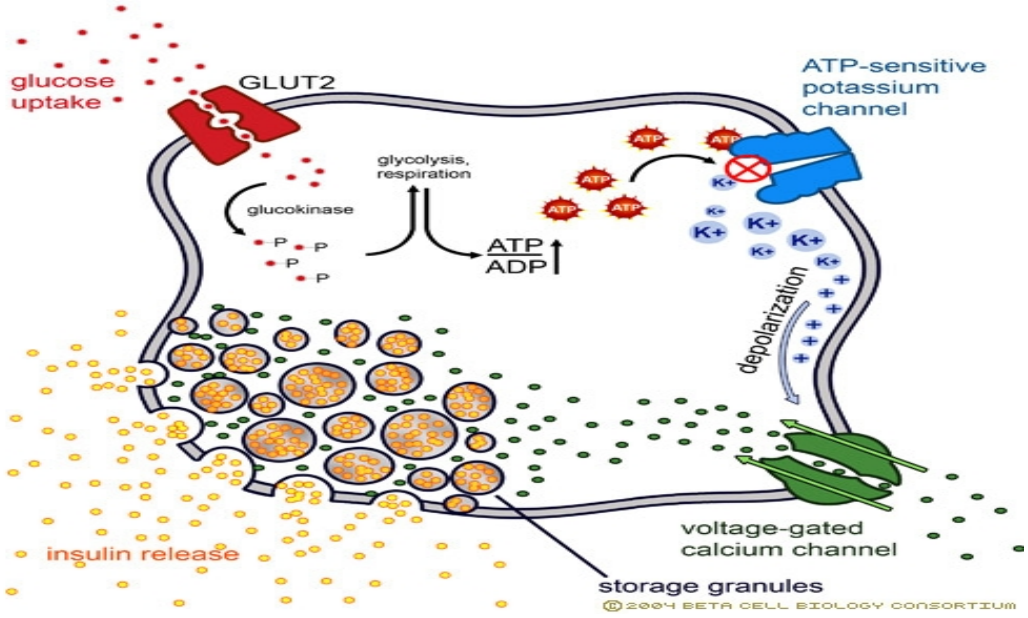


Figure No. 2: Insulin secretion by pancreatic β cells.[97]

Insulin is been secreted by the beta cells of islet of Langerhans in response to various physiological and patho-physiological conditions. A rise in blood glucose level after a meal triggers insulin release from the pancreatic β-cells. In addition, amylin hormone which is been released in comparatively smaller amounts along with insulin facilitates the regulation of meal-time glycemia, restricts postprandial glucagon secretion, retards gastric emptying, forbids absorption of nutrients from the small intestine and acts as a satiety agent.[33] Despite glucose being the main initiator for insulin secretion, intestinal incretins viz; GLP-1 and GIP, secreted by the entero-endocrine cells of the small intestine post ingestion of a meal, contribute to the release of insulin from the pancreatic β cells. Insulin potentiates glucose uptake by peripheral tissues, stimulates glucose utilisation and storage particularly by the liver (glycolysis and glycogenesis) and suppresses hepatic gluconeogenesis by inhibition of glucagon secretion from pancreatic α-cells. On the other hand, a fall in blood glucose levels subdue insulin secretion and permits glucagon secretion. In addition, somatostatin hormone released from delta cells and hypothalamus inhibits and hence, regulates the secretion of

insulin and glucagon. Furthermore, the increased blood glucagon stimulates hepatic glucose production through gluconeogenesis and glycogenolysis thereby restoring the normal blood glucose levels.[28-32]

3.4 Insulin Actions and Mechanisms

Insulin plays a pivotal role in cellular metabolism, growth and differentiation of target tissues. On binding to its respective insulin receptors, insulin stimulates its receptor tyrosine-kinase, which increases tyrosine phosphorylation of the insulin receptor substrate (IRS) proteins that further aid to recruit and activate class 1A phosphatidyl inositol 3-kinase (PI3K). Activation of PI3K catalyzes the formation of phosphatidyl inositol 3, 4, 5-trisphosphate (PIP3), which recruits both PDK1 (phosphoinositide-dependent protein kinase-1) and PKB (protein kinase B) to the phospholipid, and subsequently allows PKB to be activated through phosphorylation by PDK1 at threonine. The activated insulin receptor further initiates phosphorylation of a number of substrates belonging to the insulin receptor family and the signal regulatory protein (SIRP) family members. Activated PI3K is essential for metabolic actions, such as GLUT-4 translocation, glucose transport, glycogen synthesis and protein synthesis. Furthermore, insulin stimulates hepatocytes, myocytes, and adipocytes for glucose uptake from the circulatory system.[20,34]

The secreted insulin facilitates the glycogen synthesis pathway in muscles, wherein glucose is been taken up into the cells via glucose transporter 4 (GLUT-4) and phosphorylated by hexokinase to glucose-6-phosphate. Further, it undergoes isomerisation to glucose-1-phosphate and is activated to uridine-5'-diphosphate glucose, which is further polymerised to glycogen by the enzyme glycogen synthase. [35] Once released from the pancreas, insulin channels the human body's intracellular metabolic machinery to produce various actions on carbohydrate, protein and fat metabolism. It mainly promotes the uptake

and storage of carbohydrate, amino acids, and fat into the liver, skeletal muscle, and adipose tissue and counteracts the catabolism of these fuel reserves. As insulin increases glucose uptake and storage, blood glucose levels decline and secretion of insulin eventually stops. [16]

In addition, insulin hormone plays a vital role in inhibition of gluconeogenesis. Phosphoenolpyruvate carboxykinase (PEPCK-C) and glucose-6-phosphatase (G6Pase) significantly facilitate gluconeogenesis in liver and kidney and glyceroneogenesis in liver and adipose tissues. Insulin exerts its action by suppressing the expression of PEPCK and G6Pase genes for the gluconeogenic enzymes. [36]

3.5 Effect of Insulin on Carbohydrate Metabolism

On ingestion of a high carbohydrate meal, the glucose that is absorbed in the blood initiates rapid secretion of insulin from the pancreatic gland. The secreted insulin in turn causes rapid uptake, storage and utilization of glucose by almost all the tissues of the body, specifically the muscle, liver and adipose tissue.[29-32]

Insulin influences glucose metabolism in various tissues, specifically the liver, wherein it restricts gluconeogenesis, glycogenolysis and simultaneously accelerates glycogen synthesis. Moreover, insulin inhibits gluconeogenesis by decreasing the activity of liver enzymes required for gluconeogenesis. In addition, insulin also facilitates glucose uptake via GLUT-4 transporter in the adipose tissue and muscle, thereby enhancing glucose metabolism. It also provokes glycogen synthesis and glycolysis in the muscle tissue. Furthermore, insulin aids the esterification of glycerol (major end product of glucose metabolism) with fatty acids to form triglycerides in the adipose tissue. [29-32]

3.6 Effect of Insulin on Fat Metabolism

Excess glucose is stored as fat in the adipose tissue. Insulin increases the utilization of glucose by most body tissues and hence reduces fat storage in the adipose tissue. It does so by channelling excess nutrient molecules towards metabolic pathways thereby promoting fat synthesis.

It also activates lipoprotein lipase in the capillary wall, of the adipose tissue. Due to enzyme activation, triglycerides are been split into fatty acid so that they can be absorbed into the adipose tissue where they are further converted into triglycerides and stored. Moreover, insulin dephosphorylates lipase enzyme and hence suppresses lipolysis. It also exerts a pivotal role in the inhibition of glucagon, adrenaline and growth hormone mediated lipolysis by counteracting their action on adenylate cyclase enzyme.[29-32,37]

3.7 Effect of Insulin on Protein Metabolism

Insulin promotes protein synthesis by stimulating the transport of many amino acids into the cell. Among the amino acids most strongly transported are valine, leucine, isoleucine and phenylalanine. Insulin increases the translation of messenger RNA, thus forming new proteins. Catabolism of proteins is usually been inhibited by insulin, thus decreasing the rate of amino acid release from the cells. It also inhibits oxidation of amino acids in the liver.[29-32,37]

3.8 Etiology of Insulin Resistance

Based on relevant scientific studies the etiology of IR is been linked to;[26]

- Obesity/overweight and physical inactivity.
- Excess glucocorticoids (cushing syndrome or steroid therapy).

- Excess growth hormone (acromegaly).
- Pregnancy and Polycystic ovary syndrome (PCOS).
- Aging and Medications.
- Smoking.
- Lipodystrophy (acquired or genetic).
- Mutations of insulin receptor and insulin receptor auto antibodies.
- Mutations of the peroxisome proliferator activator receptor γ (PPAR γ).
- Mutation of glucose transporter's and signaling proteins.
- Hemochromatosis (a hereditary disease that causes tissue iron accumulation).

3.9 Pathophysiology of Insulin Resistance

Glucose homeostasis in the body exists when there is an appropriate balance between release of insulin from the pancreas, central and peripheral insulin utilisation, endogenous production and exogenous intake of glucose. Insufficient insulin release or inappropriate insulin utilisation leads to DM and its complications. Relevant scientific data suggest that IR initiates in various parts of the human body by a plethora of mechanisms.[38]

3.9.1 Defects in GLUT-4 Expression and Function.

Dysfunction in the GLUT-4 translocation machinery contributes to impaired insulin stimulated, whole-body glucose uptake. There is a reduced GLUT-4 expression in adipocytes but not skeletal muscle of obese, IR patients. However, in morbidly obese individuals, the diminished insulin-stimulated glucose transport is been attributed to inappropriate GLUT-4 function in the skeletal muscles. Therefore, the probable explanation for IR at the skeletal-muscle level is a defect either in the insulin-signaling pathways that modulate the translocation of GLUT-4 or in the molecular machinery directly involved in

the recruitment of GLUT-4 containing vesicles towards the plasma membrane, their docking and fusion with the membrane.[39]

3.9.2 Defects in insulin signaling cascade: role of the IR/IRS-1/PI3-K/PKCz/l/Akt pathway.

Prior scientific investigations suggest that impaired phosphorylation of the insulin receptor, IRS -1 and reduced PI3-K activity in response to insulin have been observed in skeletal muscles of morbidly obese individuals with or without T2DM, adipose tissue of non-obese subjects with T2DM and liver of morbidly obese patients with T2DM. Skeletal muscle biopsies of glucose-tolerant, glucose-intolerant obese subjects and T2DM patients taken during hyperinsulinemic/euglycemic clamp assays exhibited defects in insulin activation of PKC-z, but not Akt (cell signaling pathway). Defective activation of PKC-z was been found in cultured myocytes and adipocytes from obese subjects. The above observations put forth that defects in phosphorylation of the insulin receptor, IRS-1, and activation of the PI3-K & PKC-z, with or without associated defects in Akt activation, play a significant role in the development of IR.[39]

3.10 Mechanisms of Insulin Resistance.

3.10.1 Cellular Mechanism of Insulin Resistance.

Under the influence of hyperglycemia and hyperinsulinemia, the main pathway for glucose metabolism in normal and diabetic individuals is muscle glycogen synthesis. Various studies have demonstrated that impaired muscle glycogen synthesis play a pivotal role in causing IR. The study conducted by Gerald S, *et al.* postulated that defects in glucose transporters, hexokinase II and glycogen synthase enzymes contribute to the impaired muscle glycogen synthesis in T2DM individuals. Intracellular glucose-6-phosphate is the

intermediate metabolite formed during glycogen synthesis. In the course of impaired glycogen synthase activity in diabetic individuals, glucose-6-phosphate concentrations were been found to increase relative to that of normal individuals. The study further concluded that the diminished incremental changes of glucose-6-phosphate concentration in T2DM people in response to insulin stimulation could be associated with either the reduction in hexokinase II or glucose transporter activity.[40]

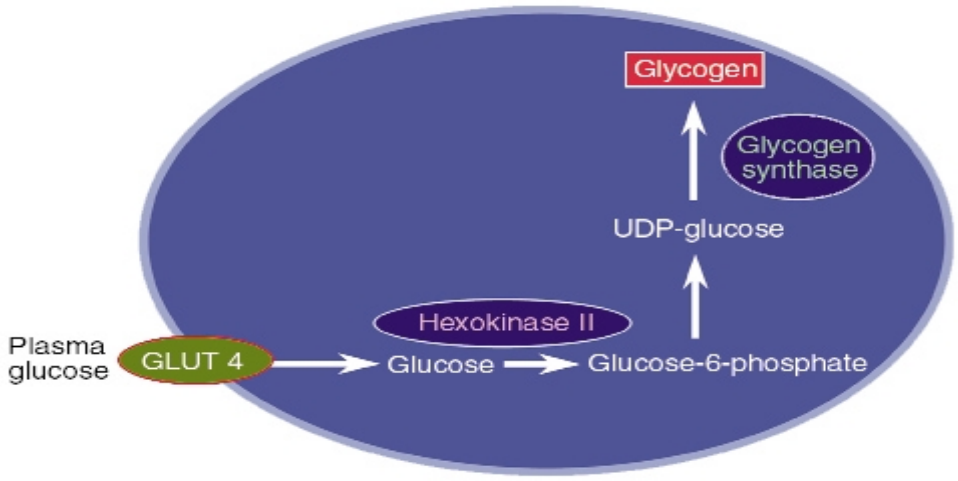


Figure No. 3: Cellular mechanism of Insulin resistance.[97]

3.10.2 Molecular Mechanism of Insulin Resistance

Experimental studies in IR animal models and humans have established defects in insulin signaling via the IRS-1/PI3K pathway, thereby contributing to the impaired glucose uptake and utilization in target tissues.

- **Serine phosphorylation of IRS-1**

Serine phosphorylation of IRS-1 protein has been determined to hinder the ability of IRS proteins to attract PI3K, thereby minimizing its activation and resulting in accelerated

degradation of IRS-1 protein. Serine phosphorylation of IRS proteins, occur in response to a number of intracellular serine kinases.

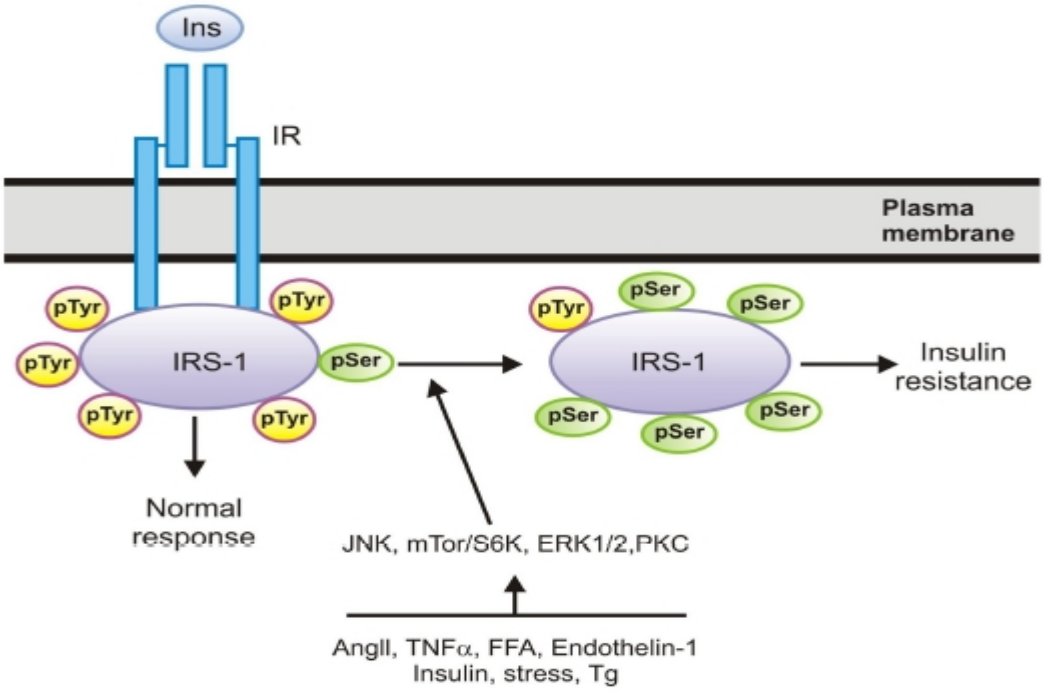


Figure No. 4: Serine phosphorylation of IRS-1.[97]

Prolonged insulin stimulation and other stimuli viz; Angiotensin-II, TNF-α, FFA, triglycerides (TG), endothelin-1 and cellular stress potentiate phosphorylation of IRS-1 proteins on Ser/Thr residues leading to impaired response towards insulin and hence IR. Furthermore, activation of JNK by FFA, stress, and inflammation exhibited an increase in serine phosphorylation of IRS-1 leading to a decline in the strength of insulin signaling along the metabolic pathway. More recently, TNF-α has been shown to hinder insulin signaling by inducing serine phosphorylation of IRS-1, with a resultant decline in IRS-1 associated PI3-K activity. In addition, mitochondrial dysfunction accompanied by reduced mitochondrial fatty acid oxidation and simultaneous accumulation of fatty acid acyl CoA and diacylglycerol can cause IR via increased serine phosphorylation of IRS-1.[41]

3.10.3 Genetics

Recent data obtained from Genome wise association studies (GWAS) on genetic architecture of T2DM exhibited that TCF7L2 gene encodes a transcription factor that has been associated with beta cell development and function. While KCNQ1 gene encodes a pore forming subunit of K^+ channel, which is usually present in several tissues including the pancreatic islet. Carriers of these risk alleles are more susceptible to T2DM. In addition, the study also brought forth that exogenous factors, which induce IR, are necessary for the manifestation of the disease.[42]

3.10.4 Raised Plasma Free Fatty Acids

Excess lipid accumulation in muscle and liver is been linked to IR. Benson M, Kitt F, *et al.* concluded from their study that enhanced plasma dietary fatty acid levels play a vital role in the pathogenesis of IR by abolishing insulin activation of IRS-1 associated PI3-K activity, altering serum lipid profile and promoting free radical generation. Various other studies conducted, including Randal's hypothesis state that this defect is due to accumulation of intracellular lipid metabolites (fatty acyl CoA, diacylglycerol etc). These metabolites activate PKC and via a serine-threonine kinase cascade, induce inhibition of IRS-1 tyrosine phosphorylation. Their inhibitory action leads to impaired glucose uptake and utilisation in insulin sensitive tissues like heart, skeleton muscles and adipose tissue. The study also stated that among the different types of fatty acids, saturated long-chain fatty acids such as palmitic and stearic acids are been found to be potent inducers of IR.[43]

In the course of normal physiological conditions, fatty acids are usually rapidly oxidized with low reactive oxygen species (ROS) production, little intracellular lipid accumulation and preservation of insulin sensitivity. Whereas under pathological conditions, chronic elevation

in circulating fatty acid levels hinder the expression of genes involved in the mitochondrial biogenesis oxidative capacity and increase production of ROS, thereby impairing mitochondrial biogenesis and function. Further Pal, *et al.* proposed that fetuin-A fatty acid complex stimulates inflammatory signaling and IR.[44] Thus, characterised by elevated free fatty acid levels in plasma, IR further leads to elevated glucose production in liver, increased breakdown of fat and in-appropriate insulin secretion by defective beta cells of pancreatic islet. It was also been suggested that augmented levels of circulating FFA disrupt pancreatic beta cells by inducing release of Interleukin-1b (IL-1b). In addition, Sajid M et al reported that like saturated fatty acids, very low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) are deleterious and behave as proapoptotic agents for the pancreatic β-cells. However, high density lipoprotein (HDL) exert a protective role over the pancreatic β-cells from the harmful effects of other lipoproteins, saturated fatty acids and glucose.[43,45]

3.10.5 Mitochondrial Dysfunction

The onset of T2DM has been associated with the loss of beta-cell mass, which occurs as an outcome of marked loss of mitochondrial mass and an increase in beta-cell apoptosis. Recent studies suggest that fructose induced uric acid release leads to mitochondrial oxidative stress that results in fat accumulation regardless of excessive calorie intake. Under the influence of normal physiological conditions, insulin-signaling molecules exists between the cytosol and internal membrane pools. These molecules facilitate the cytoskeleton reorganization and propagate the insulin signal, which further contribute to increased glucose uptake. However, under the influence of increased oxidative stress, the stress-responsive signaling cascade like mitogen activated protein kinase (MAPK) get activated and this leads to an increase in the Ser/Thr phosphorylation of the IRS molecules. As a result, modified IRS molecules are been released from the internal membrane pools and subjected to enhanced

protein degradation. In addition, intracellular lipid accumulation is been implicated in the loss of mitochondrial function. Furthermore, accumulating evidences suggest that the mitochondria play a vital role in regulating apoptotic cell death. Therefore, it is possible that decline in mitochondrial function contribute to IR in the elderly. [35,43,45-47]

Various studies have proposed that the mitochondria can be a source of TNF-α induced ROS production in the cell. TNF-α has the ability to induce vascular dysfunction via the stimulation of lipolysis, leading to an increase in the release of non-esterified fatty acids and this may further contribute to the pathogenesis of TNF-α induced IR.[48]

Metabolic stress initiated by obesity and hyperglycemia often contribute to increased rates of mitochondrial ROS production. It was been reviewed that ROS produced by β-cell mitochondria, due to metabolic stress stimulate several stress-response pathways. Further it was stated that mitochondrial superoxide trigger uncoupling protein 2 (UCP2), which leads to a proton leak across the mitochondrial inner membrane, and this has been causally linked to reduced β-cell ATP synthesis and content, which is a critical parameter in regulating glucose-stimulated insulin secretion. In addition, ROS have been involved in the oxidation of polyunsaturated fatty acids in mitochondrial cardiolipin and other phospholipids, and this impairs membrane integrity and thereby leads to cytochrome c release into cytosol and apoptosis.[11,45,46]

3.10.6 Oxidative Stress

An imbalance between generation of reactive oxygen species (ROS) and their removal by antioxidant enzymes present in the body with subsequent oxidative degradation of glycated proteins is been referred to as oxidative stress. Oxidative stress takes place in a particular cell when the generation of ROS overwhelms the cell's natural antioxidant defence. Excess

production of ROS is proficient enough to damage proteins, lipids, carbohydrates and nucleic acids thereby leading to glucose oxidation, non-enzymatic glycation of proteins, alteration in cell functions and the final pathway of cell death. Oxygen free radicals can also react with poly-unsaturated fatty acids to give rise to lipid peroxidation, which cause impairment in membrane function by decreasing membrane fluidity and altering the activity of membrane bound enzymes and receptors.[26,33,35,50]

In a diabetic patient, free radicals oxidise LDL, VLDL and HDL, resulting in various abnormalities in lipoprotein metabolism. Increasing evidence suggests that oxidative stress is a prominent feature of DM and plays a pivotal role in the pathogenesis of IR and CVD. Moreover, prior studies suggest that oxidative stress interrupts blood flow in beta cells and hence abolishes its function. ROS and lipid metabolites are been linked to IR and activation of several kinases viz: JNK, $_{P}$38 MAPK, NFKB etc. These kinases impair insulin-signalling pathways by initiating Ser/Thr phosphorylation of IRS-1 due to which there exists a decrease in glucose uptake, metabolism and hence IR.[33,43,45,46,49,50]

3.10.7 Obesity

Obesity is been implicated in the prevalence of metabolic syndrome, hypertension, IR and dyslipidemia. It is a medical condition, which occurs because of excess accumulation of body fat. Lifestyle changes like increased energy intake, decreased physical activity, and a high percentage of fat in ones diet facilitates the development of obesity. Fat accumulation in adipocytes, muscle and liver cells cause suppression of insulin receptor synthesis, organ dysfunction and hence, trigger an impaired regulation of insulin, blood sugar, cholesterol and heart functions, ultimately leading to IR in these organs. In obese individuals, calorie intake exceeds energy expenditure, contributing to excess adiposity, increased lipid peroxidation, disturbed antioxidant defence mechanism, delayed gastric empting, adipose tissue

inflammation, dysregulation of adipokines, IR mediated glucose storage, decrease in muscle mass and deterioration of glucose tolerance. Recently, a few studies have established that consumption of a fat enriched diet trigger the release of pro-oxidant and pro-inflammatory compounds that are been linked to impaired insulin sensitivity.[22,35,51]

Non-esterified fatty acids released from adipose tissue present in obese persons, predispose to ectopic fat accumulation in liver (non-alcoholic fatty liver diseases), muscle and visceral adipose tissue stores. Adipose tissue products viz; adiponectin, leptin, inflammatory cytokines, plasminogen activator inhibitor-1, resistin, and angiotensinogen are been reported to affect systemic metabolism. The accumulated ectopic fat adversely affects beta cell function through lipotoxicity.[51-54]

3.10.8 Adipocytokines

The adipocytes usually produce adipocytokines viz; Leptin and adiponectin. These adipocytokines exhibit immune-modulatory effects in T2DM. Scientific evidences have proposed that leptin induces apoptosis in pancreatic islets by initiating IL-1b secretion and suppression of IL-1Ra, which impairs insulin secretion from pancreatic b-cells. Prior evidences state that the role of adiponectin in T2DM is totally opposite to that of leptin. In obese animals, it decreases glucose levels by improving insulin sensitivity. According to some scientific studies, adiponectin deficiency is been suggested to be implicated in mitochondrial dysfunction and altered glucose homeostasis in the adipocytes. Dysregulation of adipokine function leads to subsequent deposition of ectopic fat in the abdominal viscera and liver, and thereby contribute to the induction of IR.[45]

3.10.9 Micro-vascular Dysfunction

In order to maintain the integrity of tissue and organ function it is necessary that adequate perfusion occur via the microcirculatory network (arteries, arterioles, capillaries, and venules). Accumulating evidences propose that micro-vascular dysfunction, affecting both flow resistance and tissue perfusion is been implicated not only in the development of obesity-related target-organ damage in the heart and kidney but also in the development of hypertension and IR. In obese, hypertension and IR people, insulin causes an increase in total blood flow and blood volume in skeleton muscles as its vasodilatory ability is being impaired.

Obesity induced vascular IR manifests through a complex mechanism, which involves increased free fatty acid flux, micro hypoxia in adipose tissue, secretion of adipocyte derived cytokines (IL-6 and TNF-α), increased activity of rennin-angiotensin system, overproduction of leptin, plasminogen activator inhibitor-1, MCP-1, and chronic tissue inflammation. These mechanisms hinder insulin signaling and transcription. In addition, obesity mediated micro-vascular dysfunction and IR may persist as a result of altered signaling from adipose tissue to blood vessels, which impairs the balance of NO and ET-1 production in the micro-vascular endothelium.[51]

3.10.10 Medication

Vacor, Tacrolimus, L-asparaginase, and β-adrenergic antagonists damage the β cells of pancreas. These agents produce glucose in-tolerance leading to diabetes and risk of diabetes ketoacidosis. Glucocorticoids, β-antagonists and growth hormones may induce diabetes by reducing the effectiveness of insulin to regulate metabolism. Furthermore, nicotinic acid therapy is associated with increased levels of blood glucose in both diabetic and non-diabetic

patients, and uncontrolled hyperglycemia is a frequent reason to discontinue therapy. In addition, thiazides, cyclosporine and antipsychotic drugs alter insulin secretion as well as insulin sensitivity in the body.[55,56]

3.10.11 Glucocorticoids

Glucocorticoids (GC), which are been widely used as anti-inflammatory and immunosuppressive agents have been associated with several serious adverse effects such as impaired insulin sensitivity, hyperglycemia, hyperlipidemia, Cushing syndrome, osteoporosis, obesity, and hypertension. It is been proven that dexamethasone, a frequently used member of the GC category increases triglyceride levels, causing an imbalance in lipid metabolism, leading to hyperlipidaemia, increases glucose levels leading to hyperglycemia, and reduces cellular glucose uptake thereby affecting the glucose transport system. This is been followed by complex metabolic changes resulting in decreased food consumption, bone formation, body weight, profound obesity, muscle wasting, often accompanied by diabetes and development of IR. Accumulating evidences put forth that GC affect metabolism via multiple effects on several different tissues in the body.[58,59]

- **Effect of Glucocorticoids on Muscle**

Under the influence of prolonged GC therapy, the muscle undergoes proteolysis, resulting in enhanced amino acids release. Specifically in skeletal muscle, GC cause IR by diminishing transcription of IRS-1, while increasing transcription of two proteins that counter insulin action, protein tyrosine-phosphatase type 1B and p38 MAPK.[58]

- **Effect of Glucocorticoids on Fat**

GC depresses IRS-1 and 2 levels in the fat tissue. In their scientific study Alex, *et al.* proposed that a prominent feature of excess GC administration is the enhanced accumulation

of visceral fat and loss of peripheral fat deposits in the arm and leg. They promote the expression of lipolytic enzyme sensitive lipase in peripheral fat deposits due to which TG hydrolysis occur, further leading to release of fatty acids and glycerol in the systemic circulation.[58,59]

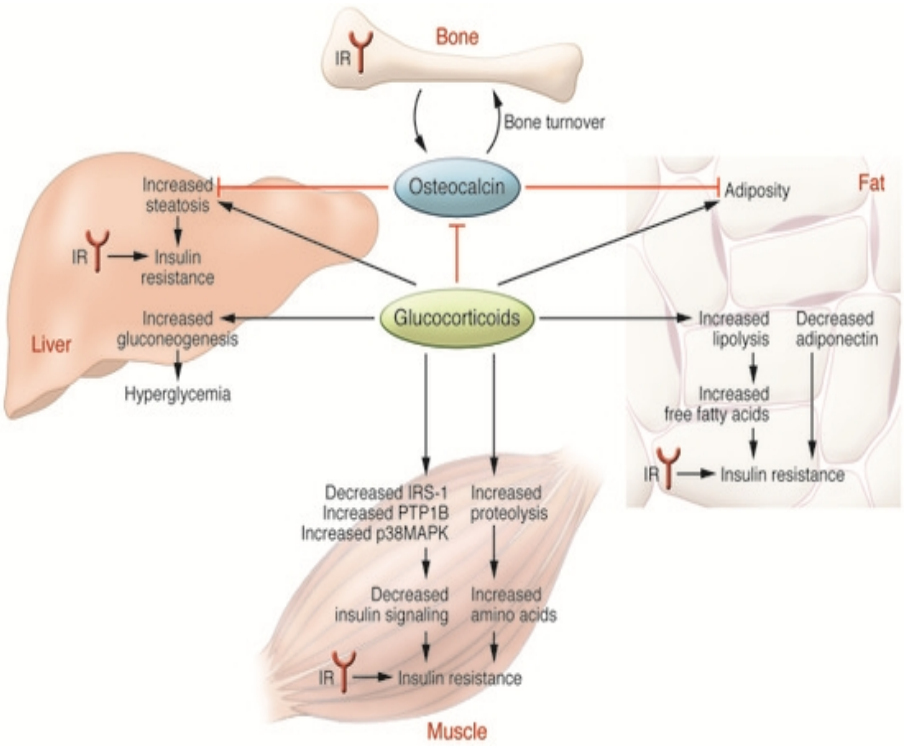


Figure No. 5: Effect of glucocorticoid therapy on various tissues.[97]

- **Effect of Glucocorticoids on Liver**

Numerous scientific investigations have proposed that GC trigger proteolysis, lipolysis, FFA production, hepatic gluconeogenesis, and steatosis in the liver. In addition, it has been proved that they down-regulate GLUT-2 expression and inhibit phosphorylation of IRS-1 in the liver, thereby decreasing glucose uptake. Moreover, GC are been implicated in the induction of liver IR by up-regulating 5-HT synthesis as well as 5-HT 2 receptors in the liver.

Furthermore, it is been proposed that 5-HT exerts its mechanism of action through the activation of the mTOR whose signaling initiates lipogenesis in the liver, and leads to serine phosphorylation of IRS-1 causing hindrance in the insulin signal transduction. All these events are been associated with hyperglycemia and hence, GC eventually impart IR.[58,60]

- **Effect of Glucocorticoids on Bone**

The bone is the main site of osteocalcin production. Osteocalcin is usually involved in bone turnover and it represses the increased adiposity and steatosis in respective tissues. However, prior research evidences suggest that GC inhibit these actions of osteocalcin and probably prolong bone resorption. The study conducted by Gordon Klein demonstrated that GC induces oxidative stress in the bone and tendon in a dose dependant manner.[58,61]

- **Effect of Glucocorticoids on Mitochondria**

Gordon Klein also proposed that GC induce oxidative stress in the mitochondria in a dose dependant manner. Under the influence of oxidative stress, various nuclear transcription factors are being stimulated which hinder the insulin transduction pathways.[58]

3.10.12 Vitamin-D Deficiency and Chemicals

Vitamin-D is been known to exhibit anti-inflammatory and immuno-modulatory effects. Based on previous studies in patients with DM, low levels of 25-hydroxy vitamin-D has been linked to higher fasting glucose and higher levels of glycated hemoglobin. Tracy M, *et al.* brought forth that vitamin-D metabolites exert their action by decreasing inflammatory cytokines, which play a pivotal role in IR and β cell apoptosis. In addition, accumulating evidences suggest that vitamin-D can improve low-grade chronic inflammation that is been implicated in IR and stimulate insulin release by pancreatic b-cells.[62]

Jeon, *et al.* reported that subjects exposed to high levels of arsenic, cadmium and mercury experienced obesity, IR, β-cell dysfunction, oxidative stress, imbalance in glucose homeostasis and hyperglycemia as a result of decreased glucose stimulated insulin secretion, suppression of insulin signaling cascade, glucose uptake, disruption of pancreatic β-cells and reduced secretion of adiponectin respectively. While in adipocytes, arsenic exposure enhanced IR by inhibiting various steps in the insulin signaling cascade and glucose uptake.[63]

3.10.13 Fructose

Numerous scientific investigations have proved that a high fructose diet can induce IR associated with hyperglycemia, hypertriglyceridemia, fatty liver and hypertension thereby, resembling the human "syndrome X". A fructose-enriched diet depresses activation of the insulin signaling cascade via IRS-1/PI3K/Akt pathway in the main target tissues viz; skeletal muscle, liver, and adipose tissue. Several evidences suggest that fructose also induces IR via effects on vasculature. Insulin accelerates the release of NO from vascular endothelium, resulting in vasodilatation, which facilitates the entry of glucose into skeletal muscles. However, fructose induced elevated uric acid levels inhibit endothelial NO generation via several mechanisms that involve blocking the uptake of the substrate, L-arginine, triggering the degradation of L-arginine by arginase, and scavenging NO by uric acid or by uric acid–generated oxidants. In addition, it has been found that fructose induced uric acid generation also induces IR via effects on adipocytes. The uptake of uric acid by adipocytes occurs via an organic anion transporter wherein it induces oxidative stress and inhibits adiponectin synthesis due to the activation of NADPH oxidase, thereby generating oxidized lipids and inflammatory mediators such as MCP-1.[47,64]

Recent scientific evidences have reported that fructose mainly exerts its action by stimulation of intracellular ATP depletion, degradation of nucleotides, and through propagation of uric acid production. Richard, *et al.* proposed that the generation of intracellular uric acid has a vital role in fat accumulation due to an increase in the expression and activity of fructokinase C. Similarly, the inhibition of uricase enzyme amplifies the ability of fructose to induce IR, raise blood pressure and increase serum triglycerides in rats. In addition, various scientific evidences have also shown that fructose induced uric acid generation leads to mitochondrial oxidative stress that stimulates fat accumulation with time and hence it is been implicated in the pathophysiology of IR.[47,64]

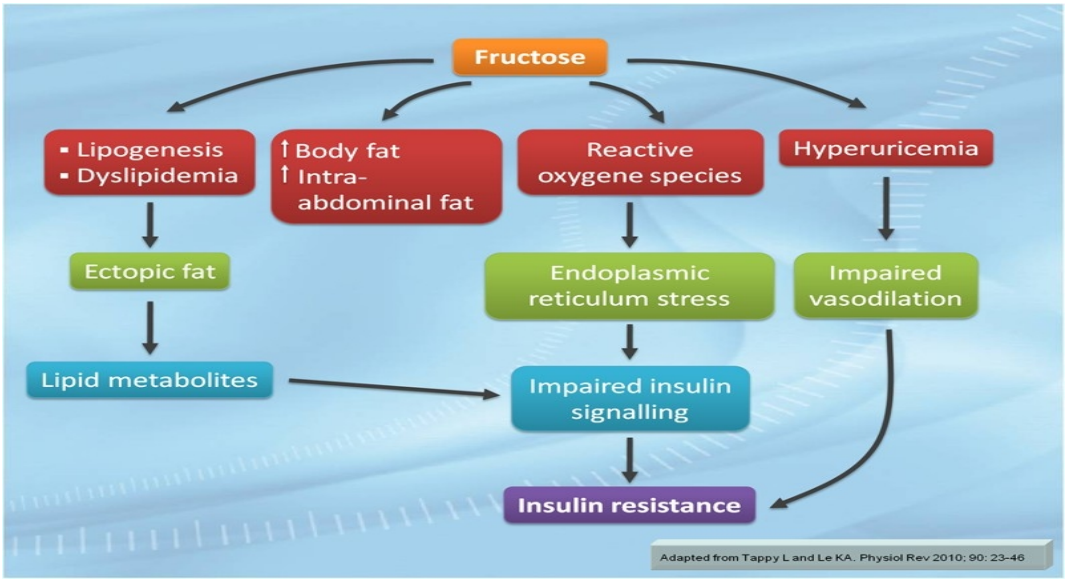


Figure No. 6: Mechanism of induction of insulin resistance by fructose.[97]

Fibroblast growth factor 21 (FGF21), which has pleiotropic action on lipid and glucose homeostasis, is a newly discovered hormone, mainly produced in key metabolic tissues viz: liver and adipose tissue. Scientific evidences prove that fructose ingestion produces an acute rapid increase in FGF21 expression via stimulation of carbohydrate responsive element binding protein (ChREBP) in the liver and adipose tissues. The study further brought forth

that ChREBP has a pivotal role in transcriptional regulation of lipogenic and glycolytic genes and thereby stimulate the expression of FGF21.[65]

3.10.14 Dysregulation of Enzyme AMPK

Based on recent studies, it is been reported that the dysregulation of enzyme, activated protein kinase (AMPK) play a pivotal role in the pathogenesis of IR and metabolic syndrome in humans as well as experimental animals. AMPK enhances cellular ATP generation via fatty acid oxidation while it diminishes ATP use for fatty acid, triglyceride, and protein synthesis. In addition, AMPK regulates glucose transport, lipid & protein synthesis, fuel metabolism, modulate cellular growth & proliferation, mitochondrial function & biogenesis, inflammation, oxidative stress, endoplasmic reticulum stress, and autophagy via phosphorylation of key enzymes viz; PEPCK and G6Pase respectively.

There exists a blunted AMPK activity in the adipose tissue of obese IR people, which is been attributed to the subsequent increase in oxidative stress and variable changes in gene expression. In addition, lack of inhibition of PEPCK and G6Pase, which play an important role in gluconeogenesis and glyceroneogenesis in liver, kidney and adipose tissue respectively, has been implicated to impart IR.[66]

3.11 Complications of IR

The major complications of IR affect both the small and large blood vessels. Hence, these complications are being categorised as micro-vascular (kidney diseases, blindness and amputation) and macro-vascular complications (heart attacks and strokes) respectively. Furthermore, acute complications comprise diabetic ketoacidosis and coma while, chronic complications include depression, dementia and sexual dysfunction.[67]

❖ Nephropathy

Diabetic nephropathy, which is clinically, represented as the evolution of proteinuria with a concomitant diminution of glomerular filtration rate results from end-stage renal failure. The diabetic kidney exhibits hypertrophy within the glomeruli and thickening of the glomerular basement membrane.[67]

❖ Retinopathy

Diabetic retinopathy, a major cause of blindness is been characterized by the presence of lesions within the retina. In diabetic individuals, the neural retina is dysfunctional thereby, disturbing retinal electrophysiology and inability to distinguish between colors.[67]

❖ Neuropathy

Neuropathy is a syndrome affecting the peripheral nervous system, mainly characterised by pain. Disease progression causes thickening of capillary basement membrane, endothelial hyperplasia and hypoxia.[67]

❖ Cardiovascular diseases

CVD are the major risk factor for mortality of diabetic patients. In diabetic people, these disorders manifest as myocardial infarction, atherosclerosis, stroke and predominantly diastolic dysfunction.[67]

❖ Hypertension

Scientific reports indicate that patients with high blood pressure are relatively glucose intolerant. This may be due to stimulation of sympathetic activity. Moreover, plasma catecholamine concentration increases with the increase in plasma insulin concentration and excessive sympathetic activity increases blood pressure.[68]

❖ Polycystic Ovary Syndrome

PCOS is an endocrine disorder of unknown etiology. IR plays a central pathogenic role in PCOS. Hyperinsulinemia and hyperandrogenemia are the principal features of PCOS.[34]

❖ Diabetic Ketoacidosis

Insulin insensitivity leads to excessive lipolysis and decreases peripheral glucose utilization. Oxidation of free fatty acids obtained via lipolysis leads to the formation of ketone bodies in the liver. As a consequence, there occurs an electrolyte imbalance, loss of vascular tone and shock.[69]

3.12 Experimental Models for induction of Insulin Resistance.

There are generally two types of animal models of IR viz; genetic and non-genetic (induced by chemical, dietary and surgical manipulations).

❖ In vivo

Generally, the genetic diabetic animals associated with IR exhibit common characteristic features such as hyperglycemia, hypertriglyceridemia, hypercholesterolemia, hyperinsulinemia, impaired glucose tolerance etc. Zucker fatty, ZDF & Wistar fatty rats and ob/ob, db/db, KK, and NZO mice are some of the genetic animal models of diabetes and IR that are been commonly used for research purpose. The development of diabetes and IR is associated with involvement of mutation either in single [dominant (KK/A^g mouse) or recessive (ZF rats)] or multiple genes (NZO mouse). Each model is significantly different from each other in its genotypic and other phenotypic characteristics.

Various non-genetic, environmentally manipulated animal models of IR are been developed. Animals are rendered insulin resistant either by feeding them high-energy diets rich in fat/fructose/sucrose, injecting into them certain chemicals/drugs, surgical manipulation in rats or mice, or a by a combination thereof (eg. combination of high fat diet + Streptozotocin (STZ) injection in rats and mice). The mechanism of development of IR is complex and varies in each model. FFA, fructose, leptin, GC, TNF-α have been reported to be involved in the development of IR in animals and human beings.[70]

❖ In vitro.

In vitro IR models utilize primary cell cultures derived from normal, diabetic/insulin resistant/transgenic animals or ex-vivo tissue, membrane preparations and tissue homogenates derived from animal models. In vitro screening via various methods can be possible by measuring the tyrosine phosphorylation of IR and IRS-1, activity of PI3K, glucose uptake, translocation of glucose transporter and glycogen content in the cell culture. Some of the cell lines used in the investigation of IR are muring C2C12 myotubes, C3H10T1-2 adipocytes, 3T3-F431A adipocytes, 3T3-L1 preadipocytes and transiently transfected Drosophilia SL-3.[70]

3.13 Treatment Modalities for Insulin Resistance

IR and beta cell dysfunction are been strongly linked to obesity and sedentary lifestyle. Presently, there is no cure for this disorder; however, treatment modalities include advice on exercise, lifestyle modification, treatment of obesity, nutrition, smoking cessation, minimal consumption of alcohol and drug therapy. Oral hypoglycemic agents and insulin sensitizers like metformin prevail as the recommended first line therapy for obese patients. Similarly, other efficacious medications include sulphonylureas, thiazolidinediones, alpha glucosidase inhibitors, and insulin. Moreover, recent approaches in research have led to the development of new medications like GLP-1 analogues: dipeptidyl peptidase-4 (DPP-4) inhibitors, inhibitors of the SGLT-2 and 11ß-hydroxysteroid dehydrogenase, insulin-releasing glucokinase activators, pancreatic G-protein-coupled fatty acid receptor agonists, glucagon-receptor antagonists, metabolic inhibitors of hepatic glucose output and quick-release bromocriptine.[12,37,71,72]

3.13.1 Oral Hypoglycemic Agents Used For Insulin Resistance.

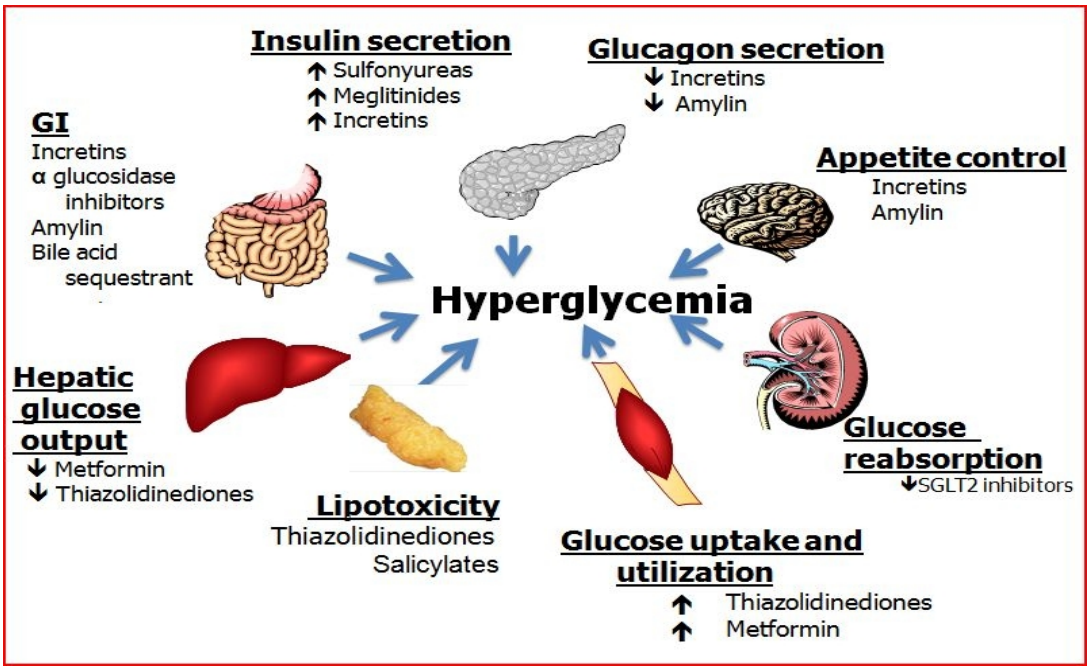


Figure No. 7: Mechanism and site of action of oral hypoglycemic agents.[97]

Various oral hypoglycemic agents are been used to treat T2DM patients. However, most of these give rise to unwanted side effects, which limit their long-term use. The oral hypoglycemic agents used as pharmacotherapy are as follows.

- Sulphonylureas: First generation: Tolbutamide, Chorpropamide.

  Second generation: Glibenclamide, Glipizide.

This class of drugs evoke a brisk release of insulin from the pancreas. They exert their mechanism by acting on the sulphonylureas receptors present on the pancreatic beta cell membrane, thereby causing depolarisation by reducing the conductance of ATP sensitive K^+ channels. This enhances Ca^{2+} influx, which induces degranulation of intracellular insulin storage vesicles. Hence, the release of insulin and subsequent reduction of raised blood

glucose levels to normal. A minor action of reducing glucagon secretion, by partially increasing insulin and somatostatin release is also been demonstrated.

Side effects: This class of drugs exerts side effects like hypoglycemia, especially in the elderly patients with impaired renal function. They cause flushing post alcohol consumption because of a disulfiram like effect and exhibit an anti-diuretic action on distal nephron, giving rise to hyponatremia. In addition, they also stimulate appetite and often cause weight gain.[31,37,55]

- BIGUANIDES: Metformin.

At present, metformin is the only drug that is been prescribed in this class. Its exact mechanism of action is unclear. However, recent studies have recognised activation of AMPK to play a crucial role in mediating its action.

Therapeutic actions: It is been reported that metformin facilitates improvement in lipid profile, suppresses hepatic gluconeogenesis, enhances insulin mediated glucose uptake in muscle and fat, reduces carbohydrate absorption as well as circulating LDL and VLDL.

Side effects: The most common unwanted effect of metformin is dose related gastro intestinal disturbances viz; anorexia, nausea and diarrhoea. Lactic acidosis is a rare but potentially fatal toxic effect. Metformin should be avoided in patients with renal and hepatic impairment, alcoholics, hypoxic pulmonary diseases or shock.[31,37,55]

- Thiazolidinediones: Troglitazone, Pioglitazone.

Currently, the only thiazolidinedione in clinical use is Pioglitazone. Ciglitazone, Troglitazone and Rosiglitazone were withdrawn since they initiated hepatotoxicity and CVD

respectively. This class of drugs are selective agonists for the nuclear PPAR-γ mainly expressed in fat, muscle cells and some other cells.

Therapeutic actions: Pioglitazone mainly binds to a nuclear receptor PPAR-γ and enhances the transcription of various insulin responsive genes. It is been reported that PIO increases glucose uptake into muscles by enhancing the effectiveness of endogenous insulin and GLUT-4 expression. In addition, it also reduces hepatic glucose output and elevated serum TG levels. Moreover, it raises abnormal HDL levels and is efficient in accelerating adipocytes turnover and differentiation. Furthermore, PIO improves glycaemic control by facilitating the decline of HbA_{1c} levels.

Side effects: The unwanted effects of PIO include weight gain, plasma volume expansion, edema, headache, myalgia and mild anaemia. Glitazones increase the risk of fractures especially in elderly women. It also promotes amiloride sensitive sodium ions reabsorption in renal collecting ducts, justifying the adverse effect of fluid retention. It is contra-indicated in pregnancy, CHF and liver diseases.[31,37,55]

- α- Glucosidase inhibitors: Acarbose, Miglitol, Voglibose.

Therapeutic actions: These agents are complex oligosaccharides that inhibit α-glucosidase, the final enzyme essential for digestion of carbohydrates in the small intestine. They act by decreasing the digestion and absorption of polysaccharides (starch) and glucose. In addition, they also promote the release of GLP-1. Furthermore, it is been reported that regular use of these agents lowers HbA_{1c} levels.

Side effects: Patient acceptability to this class of drugs is poor due to uncomfortable gastro intestinal symptoms. They also exhibit flatulence, abdominal discomfort and loose stools as adverse effects.[31,37]

- Incretine mimetics: Exenatide.

Therapeutic actions: Exenatide mimics the effect of GLP-1. It lowers blood glucose levels by increasing insulin secretion, suppressing glucagon secretion, slowing gastric emptying, reducing food intake and decreasing hepatic fat accumulation.

Side effects: It is being administered subcutaneously and can cause hypoglycemia, gastrointestinal effects and rarely pancreatitis as side effects.[31,37,55]

- Gliptins: Sitagliptin, Vildagliptine.

Therapeutic actions: This class of drugs are synthetic drugs that competitively inhibit DPP-4 thereby lowering blood glucose levels by potentiating incretins; GLP-1 and GIP. They are well tolerated and absorbed orally with an adverse effect profile similar to placebo as exhibited in clinical trials.[31,37,55]

- Dopamine D2 agonist: Bromocriptine.

  Therapeutic actions: Recently, a quick release oral formulation of Bromocriptine is been approved by US-FDA for adjunctive treatment of IR along with diet and exercise. It has been proven to efficiently improve glycaemic control and lower HbA_{1c} levels up to 0.5%.[31,55]

- Sodium-glucose co-transport-2 (SGLT-2) inhibitor: Dapaglifloxin.

Therapeutic actions: Almost all the glucose filtered at the glomerulus is been reabsorbed in the proximal tubules by SGLT-2. Inhibition of this transporter induces glucouria and lowers blood glucose levels in T2DM patients. Tolerability and safety of the drug is not yet been established.[31,55]

3.13.2 Injectables Available for IR Treatment.

- Conventional preparations of insulin:

a. Regular insulin:

It is a buffered solution of un-modified insulin stabilized by small amount of zinc. At the concentration of the injectable solution, the insulin molecules self aggregate to form hexamers around zinc ions. After injection, insulin monomers are been released gradually and absorption occurs slowly.

b. Lente insulin (insulin-zinc suspension):

Two types of insulin-zinc suspensions are been produced:

- Suspensions with large particular crystalline structure that is insoluble in water. These are long acting.
- Suspensions with small particles and which are amorphous in nature. These are short acting.

7:3 ratio of both of the above is called lente insulin and this is intermediate acting.

- Human insulin

Human insulin's (having the same amino acid sequence as human insulin) were been produced by recombinant DNA technology in bacteria (*E. coli*) and in yeast by enzymatic modification of porcine insulin.

- Insulin Analogues

An insulin analogue is an insulin molecule whose composition is been altered in order to yield some advantage over standard human insulin, while retaining its biological effect.

a. Insulin lispro (HUMALOG)

The first commercially available short-acting analog was human insulin lispro. It is been administered via IV route in conjunction with longer acting human insulin's or sulphonylureas.

b. Insulin aspart (NOVOLOG)

Insulin aspart is been formed by the replacement of proline at B-28 with aspartic acid. It is been used in combination with an intermediate or long acting insulin given at least once a day. It is not to be mixed with any other insulin due to lack of compatibility studies.[12,31]

3.13.3 Non-pharmacological Approach to Insulin Resistance

- **Exercise**

Exercise training improves insulin sensitivity. Patients with suspected IR should increase their level of physical activity. Moreover, regular sustained increases in physical activity such as daily walking can substantially decrease IR.[73]

- **Hypocaloric Diet and Weight Reduction**

Insulin sensitivity improves within a few days of calorie restriction, before any significant weight loss occurs. The amount of weight loss needed for sustained decreases in IR is still unclear. Relevant scientific investigations have reported that weight loss improves insulin sensitivity, HDL cholesterol, and blunts elevated TG levels, inflammation & the procoagulant state respectively.

- **Dietary Fibre**

The amount of dietary fibre consumed is inversely associated with the rise in insulin levels. This observation may explain the lower incidence of hypertension, hyperlipidemia and CVS among people consuming diets high in fibre. A diet high in natural sources of fibre (e.g. whole grains and vegetables) helps to reduce IR.[36-38]

- **Drawbacks of Allopathic Treatment for Insulin Resistance**

Management of diabetes without any side effects is still a challenge in the medical field, as many prominent side effects such as hypoglycemia, lactic acid intoxication, insulin allergy, lipoatropy, insulin edema, cholestatic jaundice, agranulocytosis, generalized hypersensitivity reactions, rashes, gastrointestinal upset etc. continue to exist in patients

consuming the presently available drugs and injectables for diabetes. In addition, allopathic medicines do not restore normal glucose homeostasis. Moreover, anti-diabetic medication regimen may sometimes involve prescribing more than one drug at the same time, which can augment the severity of their side effects.[2,37,74-77]

3.14 Advantages of Traditional Medicine in The Management of Insulin Resistance

Herbal medicines are been used since ages for the treatment of diabetic patients. In recent years, herbal remedies for the unsolved medical problems have been gaining importance in the research field. There is an increasing demand for herbal medications in the management and treatment of T2DM since alternative traditional medicine have numerous advantages over conventional medication. Traditional medicines are almost free from adverse drug reactions; they have flexibility, ease of accessibility, better acceptance, cost effective and positive therapeutic effects. Moreover, traditional medicinal practitioners of various countries claim to cure diabetes or at least alleviate the major symptoms and progression of this disease through efficient administration of medicinal plants. However, these herbal plant extracts lack scientific evidence regarding their safety and efficacy. In addition there exists minimal data regarding the mechanism of action of traditionally used anti-diabetic plants hence, limiting their use in standard diabetic care.[2,37,72-77]

Recent scientific evidences prove that a plethora of herbal plants possess certain chemical constituents that play a pivotal role in the management of IR and subsequent T2DM. Preliminary phytochemical investigational studies establish that saponins are utilitarian in the treatment of diabetes, phytosterols possess lipid-lowering action on hyperlipidemia and flavonoids, terpenoids, alkaloids, glycosides and polyphenols are potential anti-oxidants. The action of flavonoids is been attributed to its ability to regenerate pancreatic beta cells. In addition, it is been proposed that polyphenolics and saponins are

potent SGLT-2 inhibitors. Furthermore, the active constituents present in herbal plants have been accounted to possess pancreatic β cell regenerating ability, insulin-releasing capability, ability to improve uptake, absorption and utilization of glucose and play a significant role in fighting the problem of IR.[3,16,78-82]

Betazen capsules contain a combination of some of the known herbal plant extracts, which include *Curcuma longa, Melia azadirachta, Pterocarpus marsupium, Tinospora cordifolia, Phyllantus embilica, Terminalia chebula, Terminalia belerica, Zingiber officinale, Piper nigrum, Piper longum, Butea frondosa, Picrorrhiza kurroa, Gymnema sylvestre, Trigonella foenum-graecum, and Momordicia charantia* respectively. It is been observed that recent scientific researches aim to elucidate the mechanism of action of the active constituents which are responsible for the anti-diabetic activity of the particular plant. Relevant data obtained from various scientific investigations regarding the active constituents present in Betazen capsule contents and their possible mechanism of action are been summarised below.

3.14.1 *Butea monosperma*

Chemical Constituents:

Phytochemical investigational profile of the flower and leaves extract of *Butea monosperma* demonstrated the presence of flavonoids, chalcones, aurones, myricyl alcohol, stearic, palmitic, arachidic and lignoceric acids, glucose, fructose, histidine, aspartic acid, alanine, phenylalanine and glycosides. Among these flavonoids, phenolics and saponins were been found to be the most prominent. Moreover, phenolics and flavonoids are been reported to possess anti-oxidant action in the biological system, while acting as scavengers of singlet oxygen and free radicals.

Research and Investigation:

Pure isolated crude extracts of various parts of *Butea monosperma* (*BM*) were accounted for their antibacterial, antifungal, hypoglycemic and anti-inflammatory activities. It is been reported that *BM* flowers at the dose of 200 mg/kg p.o significantly improve glucose tolerance and cause reduction of BGL in alloxan induced diabetic rats. In addition, oral administration of the ethanolic extract of *BM* seeds at the dose of 300 mg/kg, exhibited significant anti-diabetic, hypolipaemic and antiperoxidative effects in non-insulin dependent DM rats. Scientific evidences suggest that *BM* extract may show its anti-hyperglycemic action via stimulation of insulin secretion from remnant or regenerated beta cells and improve peripheral glucose utilisation.[80,84,85]

3.14.2 Momordica charantia

Chemical Constituents:

Preliminary phytochemical analyses demonstrate that the main constituents present in *Momordica charantia* that are responsible for its biological and pharmacological actions are triterpene, protein, steroid, alkaloid, and phenolics. Due to the presence of alkaloids, insulin like peptide (lectin) and a mixture of steroidal sapogenins (charantin) it is reported to possess hypoglycemic and antioxidant potential. In addition, Khanna and Jain isolated a hypoglycemic peptide (polypeptide-P) from seeds and other tissues of *Momordica charantia (MC)* and reported that this polypeptide-P is a very effective hypoglycemic agent when administered subcutaneously to langurs and humans. Scientific studies suggested that cucurbitane triterpinoids, isolated from *MC*, reportedly stimulate GLUT-4 translocation in the cell membrane. Furthermore Singh, *et al.* have reported the hypoglycemic effect of acetone extract of whole fruit powder of *MC*.

Research and Investigation:

Accumulating scientific evidences propose that *MC* may ameliorate blood glucose levels by increasing glucose uptake and glycogen synthesis mainly in the liver, muscles and fat cells. Recent studies concerning its mechanism of action in alloxanised rats displayed that their potential anti-diabetic and anti-hyperlipidemia action may be due to inhibition of glucose-6-phosphatase and stimulation of hepatic glucose-6-phosphate dehydrogenase activity. The latest findings on high fat fed rats administered karela extract exhibited improved insulin sensitivity, glucose tolerance and insulin signaling. However, recent screening has identified triterpinoids to be the hypoglycemic components of *MC* that may be responsible for activation of AMPK.[3,11,16,18,74, 86]

3.14.3 Azadirachta indica

Chemical Constituents:

Phytochemical evaluation of *Azadirachta indica* revealed the presence of saturated & unsaturated fatty acids, bitters, nimbidin, nimbin, nimbinin, nimbidol, the main limonoid-azadirachtin, azadiradione and fraxinellone.

Research and Investigation:

Azadirachta indica seed and leaves extract have been reported to possess anti-hyperglycemic, antioxidant, antibacterial, anti-malarial, anti-fertility, anti-dyslipidemic and hepatoprotective activity. Recent investigational studies put forth that its leaf extract significantly reverses the inhibitory effect of serotonin on insulin secretion. In addition, the chloroform extract of the whole plant demonstrated significant anti-hyperglycemic effect, which is been attributed to its ability to reactivate glycogen synthase and regenerate pancreatic β-cells (attributed to presence of azadirachtin and nimbin).[3,11,16,21,36,74]

3.14.4 Picrorhizza kurrao

Chemical Constituents:

Maria, *et al.* stated in their study that iridoids (Picroside I and II), cucurbitacins and phenolic components are the major phytochemicals extracted from *Picrorrhiza kurrao*.

Research and Investigation:

According to various studies, it is been demonstrated that *Picrorrhiza kurrao* possess blood glucose reducing potential probably due to its β-cell rejuvenating ability, leading to enhanced secretion of insulin. In addition, Picroside I and II, the main constituents present in *Picrorrhiza kurrao* rhizome are been reported to exhibit significant anti-oxidant potential. This beneficial anti-oxidant potential is been attributed to its inherent ability of reducing NADPH-oxidase dependent superoxide generation and blunting the expression of MDA and advanced oxidation protein products in diabetic kidney.[3,16,87,88]

3.14.5 Pterocarpus marsupium

Chemical Constituents:

Prior phytochemical evaluation has revealed the presence of flavonoids, terpenoids, tannins, glycosides, sterols, phenols and saponins in the aqueous extract of *Pterocarpus marsupium*.

Research and Investigation:

Singh AK, *et al.* revealed that the decoction of *Pterocarpus marsupium* is effective in the management of DM. Aqueous extract of the latex of *P. marsupium* is been found to possess marked α-glucosidase inhibitory activity and exert a protective effect on HbA_{1c} levels in animal experimental models. Furthermore A Maruthupandian, *et al.* proved that ethanolic

extract of *P. marsupium* wood, exhibited anti-hyperglycemic, anti-hyperlipidemic and antioxidant properties respectively. It is possible that the flavonoids present in the plant extract regenerate the damaged beta cells while, the phenolics act as effective anti-hyperglycemic agents.[3,11,16,18,21,74,79]

3.14.6 Phyllanthus emblica

Chemical Constituents:

Phytochemical analysis of various extracts of *Phyllanthus emblica*, indicate that quercetin and sitosterol are the main chemical constituents present in this herbal plant.

Research and Investigation:

Phyllanthus emblica, belonging to the family Euphorbiaceae, is an excellent source of vitamin C and hence exhibits antioxidant property. It is also been reported that *Phyllanthus emblica* lowers cholesterol levels. The medicinal properties of *Phyllanthus emblica*, are been attributed to its quercetin (flavonoid) and sitosterol (phytosterols) content. However, further scientific evaluation is required to establish its therapeutic efficacy.[89]

3.14.7 Tinospora cordifolia

Chemical Constituents:

The presence of active constituents viz; diterpene compounds including tinosporone, tinosporic acid, cordifolisides A to E, syringen, the yellow alkaloid, berberine, Picrotene, bergenin giloin, crude giloinin and, a glucosidal bitter principle as well as polysaccharides, including arabinogalactan polysaccharide (TSP) are been reported in the plant extract of *Tinospora cordifolia.*

Research and Investigation:

Tinospora cordifolia is been extensively employed for treating DM in the traditional system of medicine. Scientific evidences have proved that it significantly reduces elevated blood glucose but not the lipid levels in alloxan-induced rabbits, through its insulin like action. Reported studies regarding *Tinospora cordifolia* state that it possesses hypoglycemic and alpha-glycosidase inhibitory activity, which is been linked to the presence of tinosporic acid in the plant.[3,11,18,74,81,90]

3.14.8 Gymnema sylvestre

Chemical Constituents:

Relevant scientific evidences propose that Gymnemic acid, the main constituent of *Gymnema sylvestre,* is composed of a mixture of at least nine closely related acidic glycosides. In addition, the extract of *Gymnema sylvestre* also shows the presence of Gymnema saponins.

Research and Investigation:

Accumulating research studies put forth that Gymnemic acid and Gymnema saponins stimulate pancreatic beta-cell function, increase the number of beta cells and trigger insulin release by increasing cell permeability towards insulin. Furthermore the ethanolic extract of Gymnema displayed hypoglycemic and anti-hyperglycemic activity in animal experimental models.[3,11,18,21,74]

3.14.9 Zingiber officinale

Chemical Constituents:

Phytochemical evaluation of various extracts of *Zingiber officinale* demonstrated the presence of active constituents viz; beta-carotene, ascorbic acid, terpenoids, alkaloids, polyphenols, flavonoids, flavones glycosides, and rutin.

Research and Investigation:

Sharma, *et al.* discovered that *Zingiber officinale* juice has glucose lowering effect in alloxan induced diabetic animals. Relevant scientific studies indicate that ginger is a potential antioxidant, α-glucosidase and amylase inhibitor, possessing properties that are relevant for use in management of T2DM. Moreover, its antioxidant potential is been attributed to its ability to reduce MDA levels in IR alloxan diabetic rats. Furthermore, it is been reported that ginger enhances serum insulin and improves insulin sensitivity in alloxan and IR diabetic rats. In addition, it is been reported to increase pancreatic insulin secretion and stimulate release of bound insulin.[11,13,18,49,74]

3.14.10 Piper nigrum and Piper longum

Chemical Constituents:

Extracts of *Piper nigrum* and longum demonstrate the presence of active constituents viz; volatile oils, resin, alkaloids and terpenoids. Among these, the main active constituent is been reported to be piperine.

Research and Investigation:

Shreya S, *et al.* put forth that piperine, exhibits fat reducing and lipid lowering effects without any change in appetite confirming its anti-obesity and anti-hyperlipidemic potential.

The possible mechanism of this action was been stated to be due to its thyrogenic activity, thus regulating apolipoprotein levels and IR in high fat diet fed rats. In addition, piperine also inhibits lipid and lipoprotein accumulation by significantly modulating the activity of enzymes viz; Lecithin-cholesterol acyltransferase (LCAT) and Lipoprotein lipase (LPL) which are involved in lipid metabolism.[54,91]

3.14.11 Trigonella foenum-graecum

Chemical Constituents:

Numerous investigational studies reveal that the defatted seed material of *Trigonella foenum-graecum* is rich in fibres, saponins, alkaloid-trigonelline, coumarin, nicotinic acid and proteins respectively.

Research and Investigation:

Jha N, *et al.* proposed that *Trigonella foenum-graecum* seed extract enhanced glucose metabolism and exhibited anti-diabetic property mainly due to the presence of its chemical constituents. Various scientific studies suggest that its mechanism of action is been mediated via stimulation of insulin synthesis, insulin secretion and its anti-oxidant potential. In addition, it is reported that, fenugreek may increase the number of glucose receptors, inhibit intestinal glucose absorption, increase insulin sensitivity and improve glucose utilisation in peripheral tissues.[3,11,16,18,74,92]

3.14.12 Curcuma longa

Chemical Constituents:

Evaluation of chemical constituents revealed the presence of alkaloids, phenolics, triterpinoids, sesquiterpines, sterol and some other moieties in *Curcuma longa*. Among these volatile oils, resins and curcuminoids were been found to be very efficient in treating DM.

Research and Investigation:

According to recent scientific studies, turmeric rhizome demonstrated concentration dependant inhibition of human pancreatic α-amylase. Curcumin, the most important chemical constituent of *Curcuma longa* has been implicated in the stimulation of insulin mediated glucose transport and inhibition of lipolysis. Recent studies also put forth that Curcumin can ameliorate IR and increase insulin sensitivity.[11,74,93,94]

3.14.13 Terminalia chebula:

Chemical Constituents:

Relevant scientific evidences have reported the presence of biologically active ingredients in *Terminalia chebula* viz; gallic acid, chebulic acid, triterpinoids, phenolics and tannins.

Research and Investigation:

Accumulating evidences demonstrated that administration of aqueous extract of *Terminalia chebula* fruits, facilitated reversal of elevated blood glucose, lowered HbA_{1c}, and lipids profile as well as decreased serum insulin levels in STZ-diabetic rats. In addition, the methanolic extract containing chebulic acid forbade endothelial cell dysfunction, and efficiently restored the levels of hepatic glycogen. Tannins present in *Terminalia chebula*

were been claimed to be responsible for antioxidant (due to presence of polyphenolics groups) and α-glucosidase inhibitory activity.[11,16,18,74,92,96]

In addition, many marketed Antidiabetic herbal formulations contain some of the contents present in Betazen capsules. The marketed herbal formulations viz; Diabecon (Himalaya), Diasulin (Tobbest Busindo), Bitter gourd powder (Garry and Sun natural remedies), Dia-care (Admark Herbals Ltd), Diabetes-Daily Care (Nature's Health Supply), Gurmar powder (Garry and Sun natural remedies), Epinsulin (Swastik Formulations), Diabecure (Nature beaute santé), Diabeta (Ayurvedic cure, Ayurvedic Herbal Health Products) and Syndrex (Plethico Laboratories) possess anti-diabetic activity.[11] However, 'Betazen' capsule contains a combination of herbal plant extracts, which have not been included in any of the marketed herbal anti-diabetic formulation. Hence, to attain scientific evidence for 'Betazen' capsule anti-diabetic efficacy and simultaneously evaluate the synergistic effect of its contents, the present study was been effectively designed.

4.0. MATERIALS AND METHODS

4.1. Source of Herbal Formulation

The crude powder contained in 'Betazen' capsules, required for the experimental study was been supplied by Progen Research Lab, Belagavi.

4.2. Selection of the dose

The formulation contains various herbal plant extracts for which LD_{50} studies are been reported safe for doses up to 3000 mg/kg. Therefore, the human dose was been converted to animal dose and each animal was been administered with 9 mg/200g rat dose for the present study.

4.3. Phytochemical investigation

The crude drug powder present in BHF capsules was been macerated for 72 hours via cold maceration technique and further subjected to different qualitative phytochemical tests to determine the chemical constituents present in the extract.[98] (Table No. 4).

4.4. Drugs and Chemicals

The various chemicals, drugs and kits used for the study are listed below.

Table No. 1: List of drugs, chemicals and kits used in present study.

Material Used	Source of the Material
1. Betazen capsules	Progen Research Lab, Belagavi.
2. Pioglitazone	Aurobindo Pharmaceuticals, Hyderabad.
3. Dexamethasone	Centaur Pharmaceuticals, Goa.
4. Ellmans reagent	Sigma-Aldrich, USA.
5.Thiobarbituric acid	Himedia Pvt. Ltd, Mumbai.
6. Anthrone	Himedia Pvt. Ltd, Mumbai.
7. Total cholesterol Kit	ERBA Diagnostics Manheim GmbH, Germany.

8. Triglyceride Kit	Yucca Diagnostics, India.
9. HDL Kit	Reckon Diagnostics Pvt. Ltd, India.
10. HbA_{1c} Kit	Asritha Diatech Pvt. Ltd, India.
11. Glucose kit	ERBA Diagnostics Manheim GmbH, Germany.
12. Glucometer	On call plus, India.
13. Glucose strips	On call plus, India.

All other reagents used were of analytical grade.

Table No. 2: List of instruments used in present study.

Sr. No.	Instruments	Description
1	Electronic balance	Adventurer, OHAUS, USA.
2	U.V. Spectrophotometer	1700 Shimadzu Corporation, Japan.
3	Auto analyzer	Star 21 plus.
4	Homogenizer	Remi Motors Pvt. Ltd, India.
5	Centrifuge	Remi Motors Pvt. Ltd, India.

4.5. Experimental animals

Wistar rats of either sex weighing 150–180g, were been procured from Shri. Venkateshwara Enterprises, Bangalore. The animals were housed in solid bottom polypropylene cages with a stainless steel grill on top and a bedding of clean paddy husk, at an ambient temperature and humidity, with 12 – 12 h light and dark cycle. The rats were been provided with normal

feed and water *ad libitum* for acclimatization to laboratory conditions for a period of 10 days and later they were been provided with a high fat-high sugar diet.

The experimental protocol was been approved by the Institutional Animal Ethics Committee (IAEC Reg. No.: 221/CPCSEA) Belagavi. All the protocols and the experiments conducted were in strict compliance with the ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

4.6. Experimental model

For the induction of experimental IR in rats, the methodology proposed by Renuka P, *et al*. was been modified slightly and utilized.[99] Wistar rats were been rendered insulin resistant by administration of dexamethasone (2 µg/day s.c) for a period of 10 days along with Vanaspati ghee and powdered normal rat feed in the ratio of 2:1 and 25% fructose (HFHS diet) for 42 days. This experimental model was been employed in order to attain drug as well as diet induced IR in rats. Dexamethasone in normal saline was been injected subcutaneously for the initial 10 days.[57] Vanaspati ghee and powdered normal rat feed (2:1) were molded into laddo's and fed to experimental rats (Group II-V) instead of normal pellets while the drinking water was been substituted with 25% fructose solution.

After 42 days of induction of IR state in rats, fasting BGL was estimated by withdrawing blood from caudal vein of rats and only experimental rats with fasting blood glucose levels of 150 mg/dl and above were included in the study. Pioglitazone API (2.7 mg/kg)[100] and Betazen formulation (9 mg/200 g rat) were triturated along with 1% CMC[101] separately and this suspension was been administered p.o to the IR rats as treatment. (Table No. 3).

Body weight, food intake and water intake was monitored daily throughout the experiment. At the end of the 30 day treatment period, blood samples were been collected from overnight fasted, ether anesthetized rats via retro orbital plexus puncture. Whole blood was being utilized for HbA_{1c} estimation, while the serum was been used for biochemical estimations. Animals were

further sacrificed by cervical dislocation and the liver homogenate supernatant was been employed for antioxidant enzymes estimation. Rat hemi-diaphragm was been used for the study of insulin and non-insulin assisted glucose uptake. The glycogen content in gastrocnemius muscle and liver was also been estimated while, the pancreas were been isolated for histological examination.

Table No. 3: Experimental Design.

Thirty Wistar rats were divided into following 5 groups for the present study. (6 animals per group)

Groups	Treatment
Group I [Normal control]	Normal rat feed and normal potable water.
Group II [Diabetic control]	Dexamethasone drug (2µg/day) for 10 days, along with High fat-high sugar diet (HFHS) for 42 days.
Group III (HFHS diet + dexamethasone + PIO) [Standard group]	Dexamethasone drug (2µg/day) for 10 days, along with HFHS for 42 days, followed by treatment with Pioglitazone standard drug (2.7 mg/kg) for further 30 days.
Group IV (HFHS diet + dexamethasone + Betazen) [Low dose]	Dexamethasone drug (2µg/day) for 10 days, along with HFHS for 42 days, followed by treatment with 45 mg/kg 'Betazen' formulation for further 30 days.
Group V (HFHS diet + dexamethasone + Betazen) [High dose]	Dexamethasone drug (2µg/day) for 10 days, along with HFHS for 42 days, followed by treatment with 90 mg/kg 'Betazen' formulation for further 30 days.

4.7. Parameters Assessed

4.7.1 Body Weight

The body weight of all the animals was been recorded initially, at the start of the study and further weekly during the entire study period. The changes in body weight were been monitored and tabulated.

4.7.2 Blood glucose level

Blood samples were been collected from rat caudal vein, and estimation of blood glucose levels was performed with the help of On Call Plus glucometer and glucose strips. The readings obtained were been recorded in mg/dl.

4.7.3 Serum Analysis

At the end of the experimental period, rats were been anaesthetized with ether. Blood samples were been collected via retro orbital puncture, stored undisturbed for 2 hours at ambient temperature and centrifuged at 2000 rpm for 5 minutes. The serum was separated and used for the estimation of biochemical parameters viz; TC, TG, HDL, LDL, VLDL. Whole blood was been utilized for HbA_{1C} estimation.

4.7.4 Procedure for Estimation of Biochemical Parameters

1) Lipid Profile:

A) Estimation of Total Cholesterol[102]

Methodology: Modified Roeschlau's Method

Clinical significance: Measurements of serum cholesterol levels are useful in evaluation of the risk of the coronary arterial occlusion, atherosclerosis, myocardial infarction and liver function.

Increase: Level increases in primary hyperlipoproteinaemias, nephritic syndrome, obstructive jaundice and DM.

Decrease: Level decreases in anemia's, hemolytic jaundice, severe malfunction, acute infections and in terminal state.

Principle: The estimation of cholesterol involves the following enzyme catalyzed reactions

1. Cholesterol ester $\xrightarrow{CE}$ Cholesterol + Fatty acid.

2. Cholesterol + O_2 $\xrightarrow{CHOD}$ Cholest-4-en-3-one + H_2O_2

3. $2H_2O_2$ + 4AAP + Phenol $\xrightarrow{POD}$ $4H_2O$ + Quinoneimine

CE: cholesterol esterase

CHOD: cholesterol oxidase

4AAP: 4-Aminoantipyrine

Absorbance of quinoneimine so formed is directly proportional to cholesterol concentration in the specimen.

Assay procedure:

Pipette	Blank	Standard	Test
Working Reagent	1000 µl	1000 µl	1000 µl
Distilled Water	20 µl	---	---
Standard	---	20 µl	---
Test	---	---	20 µl

The autoanalyser instrument was set to the specifications mentioned in the protocol supplied with the kit. The samples were prepared as per the protocol, mixed well and incubated at 37^0 C for 10 minutes. The absorbance of standard and each test was been read at 505 nm against reagent blank.

Calculation:

$$\text{Cholesterol (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of Standard}$$

B) Estimation of Triglycerides[103]

Methodology: This reaction is based on the method of Wako and the modifications by Mc G, Fossel, *et al.*

Principle: The estimation of triglycerides involves the following enzyme catalyzed reactions.

$$\text{Triglycerides} + H_2O \xrightarrow{\text{LPL}} \text{Glycerol} + \text{Free fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow[Mg^{+2}]{\text{GK}} \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-Phosphate} + O_2 \xrightarrow{\text{GPO}} \text{DAP} + H_2O_2$$

$$2H_2O_2 + \text{4AAP} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye} + 2H_2O$$

LPL - lipoprotein lipase

GK - glycerol Kinase

GPO - glycerol phosphate oxidase

DAP – dihydroxy acetone phosphate

ATP - adenosine triphosphate

4-AAP - 4-aminoantipyrine

The intensity of chromogen (quinoneimine) formed is proportional to the triglycerides concentration in the sample when measured at 505 nm.

Assay procedure:

Pipette	Blank	Standard	Test
Working Reagent	1000 µl	1000 µl	1000 µl
Distilled Water	10 µl	---	---
Standard	---	10 µl	---
Test	---	---	10 µl

The autoanalyser instrument was set to the specifications mentioned in the protocol supplied along with the kit. The samples were prepared as per the protocol, mixed well and incubated at 37^0 C for 10 minutes. The absorbance of standard and each test was been read at 505 nm against reagent blank.

Calculation:

$$\text{Triglycerides (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of Standard}$$

C) Estimation of HDL – Cholesterol[104]

Methodology: Burnstein, *et al*. Phosphotungistic Acid method, end point.

Principle: Chylomicrons, LDL and VLDL are been precipitated from the serum by phosphotungstate in the presence of divalent cations such as magnesium. The HDL cholesterol remains unaffected in the supernatant and is estimated using cholesterol reagent.

$$\text{Serum / plasma} \xrightarrow[Mg^{+2}]{\text{Phosphotungstate}} \underset{\text{(Supernatant)}}{\text{HDL}} + \underset{\text{(Precipitate)}}{\text{(LDL + VLDL + chylomicrons)}}$$

Procedure: The autoanalyser instrument was set to the specification mentioned in the protocol supplied with the kit. The samples were prepared as per the protocol, mixed well and incubated at 37^0 C for 5 minutes, followed by addition of reagent 2. The absorbance of standard and each test was been read at 505 nm against reagent blank.

Calculation: HDL- Cholesterol (mg/dl) = $\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 75$

D) Estimation of LDL and VLDL[105]

LDL and VLDL were been calculated according to Friedewald's formula.

LDL cholesterol (mg/dl) = TC – (HDL + VLDL).

VLDL cholesterol (mg/dl) = TG ÷ 5.

2) Estimation of Glycohemoglobin in Rat Blood:[106]

Methodology: Glycohemoglobin-Ion-Exchange Resin

Principle: A haemolysed preparation of the whole blood was been mixed continuously for 5 minutes with a weak binding cation-exchange resin. During this time, HbAo binds to the resin. After the mixing period, a filter was been used to separate the supernatant containing the Glycohemoglobin, from the resin.

Haemolysed Whole Blood Preparation + Cation Exchange Resin —(Mix for 5 mins)→ Fast fractions (HbA1a, HbA1b, HbA1c)

Procedure: The autoanalyser instrument was been set to the specification mentioned in the protocol supplied with the kit. The samples were prepared as per the protocol and mixed well. The absorbance of standard and each test was been read at 415 nm against reagent blank.

Calculations: % Glycohemoglobin of unknown = $\frac{\text{Abs. of Glyco hemoglobin (GHb)}}{\text{Abs.of Total Hemoglobin (THb)}} \times 4.6$

3) Estimation of Glycogen Content in Liver and Gastrocnemius Muscle of Rat:[107,108]

Reagents:

- Anthrone reagent**:** A solution containing 0.05% (w/v) anthrone, 1% (w/v) thiourea and 72% (v/v) conc. H_2SO_4. The mixture was warmed to 80-90^0 C with occasional shaking and was stored in a refrigerator after cooling.
- 30% KOH solution.
- 95% Ethanol.
- Glucose standard.

a) Preparation of stock solution:

100 mg of pure glucose was been dissolved in 100 ml of saturated benzoic acid solution.

b) Preparation of working standard:

5 ml of glucose stock solution was been placed in a 100 ml volumetric flask and the final volume was made up by addition of saturated benzoic acid. 2 ml of this solution, containing 0.1 mg of glucose was been used as standard. A reagent blank was prepared by pipetting 2 ml of distilled water.

Procedure:

Accurately weighed 1 g of liver and muscle (gastrocnemius) were been finely grounded separately with 3 ml of 30% KOH in a tissue homogenizer. The homogenate was transferred into a test tubes and 2 ml of 30% KOH was been added to it. All the tubes were been placed in a boiling water bath for 20 minutes, cooled and centrifuged at 2000 rpm for further 15 minutes. The supernatant was been transferred to a 25 ml volumetric flask and the volume was made up by addition of 30% KOH solution. To 5 ml of this solution, 10 ml of 95% ethanol was been added and mixed thoroughly. Further, all the tubes were closed with clean

rubber stoppers and allowed to stand overnight at room temperature for complete precipitation of glycogen. After precipitation was complete, the tubes were been centrifuged at 3000 rpm for 15 mins to separate the clear supernatant by gentle decantation. The residue was been dissolved in 1 ml of distilled water to which 4 ml of Anthrone reagent was added and all the test tubes were placed in a boiling water bath for 10 minutes followed by cooling in cold water. The content of each tube was further, transferred to a cuvette and its absorbance was been measured using spectrophotometer at λ 620 nm after adjusting with the reagent blank.

Calculation:

The amount of glycogen was calculated using the following formula:

$$\text{mg of glycogen/g of tissue} = \frac{\text{DU}}{\text{DS}} \times 0.1 \times \frac{\text{Volume of extract}}{\text{tissue wt}} \times 0.9$$

Where,

DU = optical density of the unknown

DS = optical density of the standard

0.1 = amount of glucose (mg) in 2 ml of standard

0.9 = factor for converting glucose in terms of glycogen.

4) Measurement of Glucose Uptake in Hemidiaphragm of Rat:[109]

Glucose uptake in rat hemidiaphragm was been estimated by the method described by Chattopadhyay, *et al*. with some modification. Diaphragms were isolated quickly, avoiding trauma and divided into two halves. The hemi-diaphragms were then rinsed in cold tyrode solution (without glucose) to remove any blood clots. In insulin-assisted glucose uptake study, one hemidiaphragm of each animal from groups I to V was been exposed to 2 ml Tyrode solution with glucose (2000 mg/l) in respective test tubes. In non-insulin assisted glucose uptake study, the remaining hemidiaphragm of each animal from groups I to V was

been exposed to 2 ml Tyrode solution with glucose (2000 mg/l) + insulin (0.25 IU/ml) in respective test tubes. All the test tubes were incubated for 30 min at 37º C in an atmosphere of 95% O_2-5% CO_2 with shaking at 140 cycles/minutes. Following incubation, the hemidiaphragm were been taken out and weighed. The glucose content of the incubated medium was measured by GOD/POD, enzymatic method. Glucose uptake was been calculated as the difference between the initial and final glucose content in the incubation medium.

Estimation of GOD/POD:[110]

Principle: Glucose oxidase oxidizes the specific substrate, D-glucose, to gluconic acid and generates hydrogen peroxide. H_2O_2 thus produced is been acted upon by peroxidase, which transfers oxygen to the chromogen system, 4-aminoantipyrine and phenolic compound. The chromogen system is been oxidized further to a red quinoneimine dye. The intensity of colour is directly proportional to the concentration of glucose and is been measured photometrically at 505 nm.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose Oxidase}} \text{Gluconate} + H_2O_2$$

$$H_2O_2 + \text{Phenolic compound} + \text{4-Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Coloured Complex} + H_2O$$

Procedure: The autoanalyser instrument was set to the specification mentioned in the protocol supplied with the kit. The samples were prepared as per the protocol, mixed well and incubated at 37^0 C for 10 minutes. The absorbance of standard and each test was been read at 505 nm against reagent blank.

4) Estimation of Hepatic Antioxidant Enzymes.

- **Preparation of Liver Homogenate:**

Animals were been sacrificed by cervical dislocation. On dissection, the livers were isolated and washed immediately with cold saline to render them free from blood clots. Liver homogenates (10% w/v) were been prepared in cold 50 mM Tris buffer (pH 7.4) using homogenizer. The unwanted cell debris was separated by centrifugation at 3000 rpm for 15 minutes (4º C), using a cold centrifuge. Furthermore, the supernatant obtained was been utilized for the estimation of superoxide dismutase (SOD), reduced glutathione (GSH), malondialdehyde (MDA) and catalase (CAT) concentration.

- **Preparation of 0.1 M Tris-HCl (ph 7.4) Buffer Solution:**

1.57 gms of Tris-HCl was dissolved in 100 ml of distilled water and pH was adjusted to 7.4 with 0.2 N NaOH.

A. Estimation of Superoxide dismutase (SOD)[111]

Principle:

SOD is a metalloprotein and the first enzyme involved in the antioxidant defense against ROS by lowering the steady state level oxygen. SOD scavenges the superoxide ions produced as cellular byproducts. SOD is a major defense for aerobic cells, combating the toxic effect of superoxide radicals. The SOD activity is been determined by the ability of the enzyme to inhibit auto oxidation of pyrogallol.

Reagent preparation:

1. **Tris-HCl buffer mixture:** 3.0275 g of tris hydrochloride and 0.186 g EDTA was been added to 300 ml of distilled water. The resultant solution's pH was been adjusted to 8.5 with 50 mM hydrogen chloride and its volume was made up to 500 ml with distilled water.

2. **Pyrogallol solution:** 25 mg of pyrogallol was been dissolved in 10 ml of distilled water.

Assay procedure:

Sr. No	Reagent	Blank (B)	Control (C)	Test (T)
1	Tris-HCl buffer (0.05M)	2.9 ml	2.9 ml	2.9 ml
2	Pyrogallol solution	-	0.1 ml	0.1 ml
3	Liver homogenate	-	-	10 µl

The reagents were been combined with the homogenate according to the assay procedure and absorbance of the resultant solution was taken at 420 nm. Absorbance was measured at two intervals, viz; 90 and 120 seconds respectively using UV spectrophotometer.

Formula for calculation:

$$\text{SOD unit/ml} = \frac{(C - T) \times 100}{(C \times 50) \times h}$$

Where, h= volume of homogenate taken.

C (Control) = (absorbance at 210 seconds - absorbance at 60 seconds) ÷ 2.

T (Test) = (absorbance at 210 seconds - absorbance at 60 seconds) ÷ 2.

Note: 1unit of SOD = Amount of enzyme required for 50% inhibition of Pyrogallol auto-oxidation.

B. Estimation of Catalase (CAT).[112]

Principle:

CAT is a heme-protein, localized in the micro-peroxisome. It reduces the hydrogen peroxide produced by dismutation reaction and prevents generation of hydroxyl radicals, thereby protecting the cellular constituents from oxidative damage in the peroxisome. The enzyme catalyses the decomposition of hydrogen peroxide to water and oxygen. Thus,

protecting the cell from oxidative damage caused by hydrogen peroxide. The assay method is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2, with the formation of perchromic acid as an unstable intermediate. The chromic acetate thus produced is been measured calorimetrically at 570-610 nm.

Requirements:

1) Dichromate/acetic acid reagent: The reagent was prepared by mixing 5% solution of $K_2Cr_2O_7$ with glacial acetic acid in the ratio 1:3.
2) Hydrogen peroxide (0.2 M).
3) Phosphate buffer (0.01 M- pH 7).

Preparation of standard graph:

Different amounts of H_2O_2 ranging from 10-160 µ moles were been taken in small test tubes (6ml). 2ml of 5% solution of $K_2Cr_2O_7$ and glacial acetic acid (1:3 ratio) was added to each of them. The resulting solution turned blue due to the unstable blue precipitate of perchromic acid. On heating all the tubes for 10mins in boiling water bath, the solution changed to a stable green color due to the formation of chromic acetate. The tubes were cooled at room temperature and then optical density was measured at 570 nm. Further, the graph of linear relationship was obtained.

Preparation of test:

4 ml of H_2O_2 solution (800 µmoles) and 5 ml phosphate buffer were been mixed together. To this 1 ml of properly diluted enzyme preparation was added and the reaction was allowed to stand at room temperature. Further,1 ml of the resulting solution was taken and blown into 2 ml of dichromate/acetic acid reagent at 60 sec intervals.

Formulae:

$$K = \frac{1}{t} \times log_{10} \frac{S_0}{S}$$

K = Pseudo monomolecular reaction velocity constant at time t

S_0 = Initial concentration of H_2O_2 (800µM).

S = Concentration of H_2O_2 at t time

K values are plotted against time in min and points were extrapolated to the Y-axis to get $K_{(0)}$ or Kat.f.

$K_{(0)}$= Pseudo monomolecular reaction velocity constant at time 0.

Kat.f = katalasefaahigkeit (catalase contents of the enzyme preparation according to von Euler and Josephson)

$$\text{Kat. f} = \frac{K_0}{\text{g of protein/ml}}$$

Calculation:

Graph between optical density and µm of H_2O_2 (standard) was plotted. At 60 sec interval different readings in the experiment were noted. Those values were been plotted in standard graph. The resultant values were put in equation (K=1/t log 10 S0/S). Different values of K were obtained. K versus time graph was plotted. K was extrapolated to 0 to obtain $K_{(0)}$ as per the formula to obtain the result.

C. Estimation of Reduced glutathione (GSH).[113]

Principle:

GSH is a major non-protein thiol, endogenous antioxidant that counters balance free radical mediated damage. It is involved in the protection of normal cell's structure and function by maintaining the redox homeostasis, quenching of free radicals and by participating in detoxification reaction.

Reagent preparation:

1. **Reaction buffer:** 0.1 M Sodium phosphate, pH 8 containing 1 mM EDTA.

Stock 1- 2.78 g Sodium phosphate monobasic was dissolved in 100 ml distilled water.

Stock 2- 2.84 g anhydrous Sodium phosphate dibasic was dissolved in 100 ml distilled water.

2.65 ml of Stock 1 and 47.35 ml of Stock 2 was been mixed together and the volume was made up to 100 ml with distilled water. Further, 37.2 mg EDTA was added to the above solution.

2. **Ellmans reagent solution:** 4 mg of Ellmans reagent was dissolved in 1 ml of reaction buffer.

Assay procedure:

Sr. No.	Test	Blank
1	50 µl of Ellaman's reagent solution.	50 µl of Ellaman's Reagent Solution.
2	2.5 ml reaction buffer.	2.5 ml reaction buffer.
3	250 µl liver homogenate.	250 µl reaction buffer.

The reagents were mixed well with the liver homogenate in the above-mentioned proportion and incubated at room temperature for 15 minutes. Absorbance of the resulting reaction was read at 412 nm using UV spectrophotometer.

Formula for calculation:

$$\text{GSH (Mole)} = \frac{\text{Absorbance}}{1.4150 \times 10^{4}} \times 11.2 \text{ mole.}$$

For the conversion of Mole to micro Mole, the whole equation is multiplied by 10^6.

$$\text{GSH } (\mu M) = \frac{\text{Absorbance}}{1.4150 \times 10^{4}} \times 11.2 \text{ mole} \times 10^{6}$$

Further, for the conversion of µM to µg, the whole equation was multiplied by Mole weight of DTNB (ie: 307.42).

$$\text{GSH } (\mu g/g \text{ of tissue}) = \frac{\text{Absorbance}}{1.4150 \times 10^{4}} \times 11.2 \text{ mole} \times 10^{6} \times 307.42$$

D. Estimation of Malondialdehyde (MDA).[114]

Principle:

Oxidative stress is associated with peroxidation of cellular lipids, which is been determined by measuring Thiobarbituric acid reacting substance (TBARS). The concentration of MDA products may reflect the degree of oxidative stress. The increased level of TBARS results in increase of oxygen free radicals, which attack the polyunsaturated fatty acids present in the cell membranes and cause lipid peroxidation. The Malondialdehyde (MDA) content, a measure of lipid peroxidation was assayed in the form of TBARS.

Requirements:

1) 1.5% KCl solution.
2) 1% phosphoric acid solution.
3) 0.6% Thio-barbituric acid solution.
4) n–butanol.

Procedure:

- Tissue malondialdehyde (MDA) levels as a marker of lipid peroxidation were been analysed using thio-barbituric acid reactive substances.

- 10% liver homogenate prepared with 1.5% cold KCl was been used for MDA analysis.
- 3 ml of 1% phosphoric acid 1 ml of 0.6% thio-barbituric acid solution was added to 0.5 ml of 10% liver homogenate contained in each of the test tubes.
- The above mixture was heated for 45 minutes and on cooling, 4 ml of n-butanol was been added to it and mixed thoroughly. Absorbance of the clear solution obtained on standing was measured at 535 and 520 nm respectively.
- The difference between the two measurements defined the level of MDA (μM/g of tissue).

4.7.5 Histopathology:

At the end of the treatment period, all animals were sacrificed, their pancreas were been dissected out and fixed overnight in 10% formalin. Sections of the tissues fixed in paraffin were prepared, stained with hematoxylin and eosin and observed for pathological changes.

4.7.6 Statistical Analysis

Results are been expressed as mean ± SEM. Differences among data were determined using one way ANOVA followed by Dunnett Multiple Comparison Test (Graph Pad Prism software, version 5.01). $p < 0.05$ was considered statistically significant.

5.0. RESULTS

5.1. Phytochemical investigation

The aqueous extract of Betazen herbal formulation was been subjected to various qualitative analysis to determine the phyto constituents present in the extract. (Table No. 4)

Table No. 4: Results of Qualitative Chemical Tests.

Sr. No.	CHEMICAL TEST		*Aqueous extract of Betazen* herbal formulation
1.	**Test for Carbohydrates** • Molisch's Test (General test)		+
	A.	**Test for Reducing Sugars**	
		• Fehling's test	-
		• Benedict's test	-
	B.	**Test for Monosaccharide's**	
		• Barfoed's test	-
	C.	**Test for Hexose sugars**	
		• Cobalt Chloride test	-
	D.	**Test for Non-reducing Polysaccharides (Starch)**	
		a. Iodine test	-
		b. Tannic acid test for starch	-
2.	**Test for proteins**		
	• Biuret test (General test)		-
	• Millon's test		-
	• Xanthoprotein test		-

3.	**Test for Steroids**	
	• Salkowski reaction	+
	• Liebermann-Burchard reaction	+
	• Liebermann reaction	+
4.	**Test for Flavonoids**	
	• Shinoda test	+
	• Ferric chloride test	+
	• Alkaline reagent test	+
	• Lead Acetate test	+
5.	**Test for Alkaloids**	
	• Dragendroff's test	+
	• Mayer's test	+
	• Hager's test	+
	• Wagner's test	+
6.	**Test for Tannins**	
	• Ferric chloride test	+
	• Lead acetate test	+
	• Bromine water test	+
	• Dilute iodine test	+
7.	**Test for Vitamin C**	+
8.	**Test for Saponins**	
	• Foam test	+

+ ⟶ Present.
- ⟶ Absent.

5.2. Pharmacological Investigations

Betazen herbal formulation (suspended in 1% CMC) was been administered at doses of 45 and 90 mg/kg to evaluate its pharmacodynamic effects on diet and drug induced insulin resistant rats.

A) Effect of Betazen herbal formulation on fasting blood glucose levels (BGL):

There was a significant ($p<0.0001$) increase in BGL in dexamethasone and high fat-high sugar diet treated insulin resistant rats (187.8±11.34 mg/dl) compared to that of the euglycaemic group (95.83±4.393 mg/dl). Treatment with BHF at 45 mg/kg and 90 mg/kg in the insulin resistant rats showed significant ($p<0.01$ & $p<0.0001$ respectively) dose dependent reduction in elevated BGL with mean value of 140.2±5.474 mg/dl and 86.5±7.14 mg/dl respectively. In addition, treatment of insulin resistant rats with standard drug PIO (2.7 mg/kg) significantly ($p<0.0001$) reduced the elevated BGL to 94±8.351 mg/dl (Table 5) (Figure 8).

B) Effect of Betazen herbal formulation on rat body weight (BW):

The change in body weight of animals in different groups was been compared at the end of study on the 72nd day. There was an initial non-significant increase in BW in the drug and diet induced IR rat's (178.1±13.24 gms), followed by a gradual decrease in weight (142.2±10.03) when compared to that of normal euglycemic group having mean BW of about 194.1±7.573 gms. Treatment of the insulin resistant rats with BHF at 45 mg/kg showed a non-significant dose dependant initial increase in BW followed by a significant ($p<0.05$) constant maintenance of BW to about 179.4±6.481. While, treatment with BHF at 90 mg/kg showed a dose dependant initial increase in BW followed by a gradual decrease in BW to about 148.1±4.875 when compared to the diabetic control group. However, treatment with

standard drug PIO (2.7 mg/kg) in insulin resistant rats showed a dose dependant improvement in BW to 153.9±10.21gms when compared to diseased group. (Table 6) (Figure 9).

C) Effect of Betazen herbal formulation on lipid profile:

1) Total Cholesterol (TC).

There was a significant ($p<0.01$) increase in TC level in dexamethasone and HFHS diet treated rats (102.6±13.93 mg/dl) when compared to that of euglycemic group (38.85±2.585 mg/dl). Treatment of IR rats with BHF at 45 and 90 mg/kg showed a dose dependent reduction in elevated TC levels with mean value 66.92±7.538 mg/dl and 78.83±11.07 mg/dl respectively. The treatment of IR rats with standard drug PIO (2.7 mg/kg) showed a significant ($p<0.05$) reduction in TC levels with mean value of 58.5±7.59 mg/dl. (Table 7) (Figure 10).

2) Triglycerides (TG).

There was a significant ($p<0.05$) increase in TG level in dexamethasone and HFHS diet induced IR group (188.5±26.76 mg/dl) when compared to that of euglycemic group (99.51±20.05 mg/dl). Treatment of insulin resistant rats with BHF at 45 mg/kg showed a dose dependent non-significant reduction in TG levels with a mean value of 132.6±12.25 mg/dl. However, treatment of IR rats at a dose of 90 mg/kg showed significant decrease ($p<0.01$) in elevated TG levels with a mean value of 94.93±7.713 mg/dl. In addition, treatment with standard drug PIO (2.7 mg/kg) exhibited a highly significantly ($p<0.0001$) reduction in TG levels with mean value of 65.88±8.889 mg/dl. (Table 7) (Figure 11).

3) High-density lipoprotein (HDL).

There was a highly significant ($p<0.0001$) decrease in HDL level in dexamethasone and HFHS diet treated rats (30.83±1.352 mg/dl), when compared to that of euglycemic group

(61.87±0.845 mg/dl). Treatment of IR rats with BHF at 45 and 90 mg/kg showed significant ($p<0.0001$) dose dependent increase in diminished HDL cholesterol levels with mean value of 52.59±3.243 mg/dl and 57.33±2.832 mg/dl respectively. Treatment with standard drug PIO (2.7 mg/kg), in IR rats significantly ($p<0.0001$) increased the HDL cholesterol levels to a mean value of around 60.12±1.603 mg/dl. (Table 7) (Figure 12).

4) Low-density lipoprotein (LDL).

There was a significant ($p<0.01$) increase in LDL level in dexamethasone and HFHS diet treated rats (45.23±4.503 mg/dl) when compared to that of euglycemic group (22.8±2.72 mg/dl). Treatment of IR rats with BHF at 45 and 90 mg/kg showed significant ($p<0.01$and $p<0.0001$) dose dependent reduction in elevated serum LDL levels with mean value of 31.98±1.107 mg/dl and 27.85±2.569 mg/dl respectively. Treatment with standard drug PIO (2.7 mg/kg) significantly ($p<0.0001$) reduced the elevated serum LDL levels with mean value of 25.58±2.037 mg/dl. (Table 7) (Figure 13).

5) Very low-density lipoprotein VLDL.

There was a significant ($p<0.0001$) increase in VLDL level in dexamethasone and HFHS diet treated rats (42.61±3.421 mg/dl) when compared to that of euglycemic group (18.97±1.725 mg/dl). Treatment of IR rats with BHF at 45 and 90 mg/kg showed significant ($p<0.0001$) dose dependent reduction in elevated VLDL levels with mean values of 25.13±2.021mg/dl and 22.9±1.389 mg/dl respectively. Treatment with standard drug PIO (2.7 mg/kg) significantly ($p<0.0001$) reduced the elevated VLDL levels with mean value 20.88±1.558 mg/dl. (Table 7) (Figure 14).

D) Effect of Betazen herbal formulation on Glycosylated Hemoglobin (HbA_{1c} %):

There was a highly significant ($p<0.0001$) increase in HbA_{1c} percentage level in dexamethasone and HFHS diet treated rats (8.367±0.319 %) when compared to that of euglycemic group (5.612±0.1195 %). Treatment of IR rats with BHF at 45 mg/kg showed a non-significant, dose dependent reduction in HbA_{1c} levels with a mean value of 7.4±0.8406 %. However, treatment of IR rats with BHF at 90 mg/kg significantly ($p<0.0001$) reduced HbA_{1c} percentage levels with a mean value of 4.05±0.3905 %. Treatment with standard drug PIO (2.7 mg/kg) significantly ($p<0.01$) reduced the elevated HbA_{1c} levels with a mean value of 5.726±0.1268 %. (Table 8) (Figure 15).

E) Effect of Betazen herbal formulation on glycogen content in Liver and Gastrocnemius muscle:

There was a significant ($p<0.0001$) decrease in glycogen content in liver and muscle of dexamethasone and HFHS diet treated rats (5.33±0.4017 and 3.46±0.2776 mg/g of tissue respectively) when compared to that of euglycemic group (33.83±1.3 and 26.59±0.7874 mg/g of tissue respectively). Treatment of IR rats with BHF at 45 and 90 mg/kg showed significant ($p<0.0001$) dose dependent increase in glycogen content with mean value of liver being 16.67±1.026 and 23.24±2.203 mg/g of tissue respectively and that of muscle 13.41±0.7231 and 16.52±1.463 mg/g of tissue respectively. Treatment with standard drug PIO (2.7 mg/kg) in IR rats, significantly ($p<0.0001$) increased the level of glycogen content with mean value of liver being 27.17±2.267 mg/g of tissue and that of muscle 21.27±1.971 mg/g of tissue respectively. (Table 9) (Figure 16).

F) Effect of Betazen herbal formulation on Glucose uptake in rat Hemi-diaphragm:

Glucose uptake in insulin assisted (8.727±0.9283 mg/dl) and non-insulin assisted (5.102±0.2459 mg/dl) isolated hemi-diaphragm's exhibited a significant ($p<0.01$ and $p<0.05$ respectively) decrease in the dexamethasone and HFHS diet treated rats when compared to

normal group (15.15±1.323 mg/dl and 8.627 ± 1.392mg/dl respectively). Insulin assisted glucose uptake (16.54±1.338 mg/dl, 14.36±0.9869 mg/dl and 15.49±0.9067 mg/dl) showed a significant ($p<0.0001$, $p<0.01$ and $p<0.0001$) increase in IR rats treated with standard drug PIO (2.7 mg/kg), BHF at 45 and 90 mg/kg respectively. In addition, non-insulin assisted glucose uptake (8.208±0.6596 mg/dl and 7.633±0.6733mg/dl) was significantly ($p<0.01$) increased in IR rats treated with standard drug PIO (2.7 mg/kg), and BHF at 90 mg/kg respectively. However, IR rats treated with BHF at 45 mg/kg showed a non-significant dose dependent increase in non-insulin assisted glucose uptake. (Table 10) (Figure 17).

G) Effect of Betazen herbal formulation on Hepatic Antioxidant Enzymes (SOD, CAT, GSH and MDA).

Liver homogenate's of experimental rats to whom dexamethasone and HFHS diet was been administered exhibited a highly significant reduction in SOD, CAT and GSH ($P<0.0001$) while, a highly significant elevation in MDA ($P<0.0001$) levels. Treatment of IR rats with BHF at 45 mg/kg showed a significant increase in SOD & CAT levels ($P<0.0001$) and a significant decrease in MDA level ($P<0.05$). However, treatment of IR rats with BHF at 90 mg/kg exhibited a significant increase in the diminished SOD, CAT ($P<0.0001$) as well as GSH levels ($P<0.01$) while a significant decrease in elevated MDA levels ($P<0.0001$). In addition, treatment with standard drug PIO (2.7 mg/kg) in IR rats, significantly increased the level of GSH ($p<0.01$), SOD & CAT ($p<0.0001$) respectively while it also exhibited a highly significant ($p<0.0001$) decrease in MDA levels. (Table 11) (Figure 18-21).

H) Effect of Betazen herbal formulation on Histopathology of Pancreas.

Histopathological studies of dexamethasone and HFHS diet treated rat pancreas demonstrated pathological changes viz; moderate decrease in size and number of islets, lymphocytic infiltration and vacuolar degeneration of islet cells, when compared to the normal group having normal cellular integrity. Mild lymphocytic infiltration, vacuolar

degeneration and increase in the number and size of islets was been observed in IR rats treated with BHF at 45 and 90 mg/kg respectively. However, treatment with standard drug PIO (2.7 mg/kg) in IR rats, showed cellular integrity comparable to normal with only mild decrease in size and number of islets. (Fig 32).

Table No. 5: Effect of various treatments on fasting Blood glucose levels.

WEEK / GROUPS	Week 1 (mg/dl)	Week 3 (mg/dl)	Week 5 (mg/dl)	Week 7 (mg/dl)	Week 9 (mg/dl)	Week 11 (mg/dl)
NORMAL GROUP	81.83±4.586	86.5±3.722	88±2.921	88.67±3.667	89.5±3.51	95.83±4.393
DIABETIC CONTROL	96.5±1.784$^{\#}$	100.3±2.512$^{\#}$	109±4.74$^{\#\#}$	140±4.115$^{\#\#\#}$	164±7.75$^{\#\#\#}$	187.8±11.34$^{\#\#\#}$
DEXA+ HFHS+ PIO	85.17±6.226	91.67±5.499	116.7±7.727	161.8±3.135***	131.2±7.153**	94±8.351***
DEXA+ HFHS+ BETAZEN (45mg/kg)	87.17±1.74	98.33±2.29	135.3±4.333**	162.5±1.803***	147.7±3.04	140.2±5.474**
DEXA+ HFHS+ BETAZEN (90mg/kg)	86.33±3.273	106±2.338	139.2±0.8724**	159.3±1.994***	128.2±2.6***	86.5±7.14***

$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.0001$ when compared with NORMAL GROUP.

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.0001$ when compared with DIABETIC CONTROL.

Results are been expressed as mean ± SEM. Differences among data were determined using one-way ANOVA followed by Dunnett Multiple Comparison Test.

Table No. 6: Effect of various treatments on rat Body weight.

WEEK / GROUPS	Week 1 (g)	Week 3 (g)	Week 5 (g)	Week 7 (g)	Week 9 (g)	Week 11 (g)
NORMAL GROUP	149±4.583	171.3±6.247	177±5.881	185.3±7.696	189.8±7.634	194.1±7.573
DIABETIC CONTROL	152.7±2.172	166±4.673	172.8±11.06	178.1±13.24	172±10.92	142.2±10.03$^{\#\#}$
DEXA+ HFHS+ PIO	119.5±13.27*	125.3±15.3**	149.8±11.63	167.6±11.38	160±12.5	153.9±10.21
DEXA+ HFHS+ BETAZEN (45mg/kg)	156.6±4.807	168.3±4.868	178.8±8.172	179.9±7.998	178.9±7.012	179.4±6.481*
DEXA+ HFHS+ BETAZEN (90mg/kg)	129.7±4.1	135.3±4.631	146±7.787	151.3±7.806	160±6.575	148.1±4.875

$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.0001$ when compared with NORMAL GROUP.

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.0001$ when compared with DIABETIC CONTROL.

Results are been expressed as mean ± SEM. Differences among data were determined using one-way ANOVA followed by Dunnett Multiple Comparison Test.

Table No. 7: Effect of various treatments on Lipid Profile.

PARAMETER / GROUPS	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
NORMAL GROUP	38.85±2.585	99.51±20.05	61.87±0.845	22.8±2.72	18.97±1.725
DIABETIC CONTROL	102.6±13.93$^{\#\#}$	188.5±26.76$^{\#}$	30.83±1.352$^{\#\#\#}$	45.23±4.503$^{\#\#}$	42.61±3.421$^{\#\#\#}$
DEXA+ HFHS+ PIO	58.5±7.59*	65.88±8.889***	60.12±1.603***	25.58±2.037***	20.88±1.558***
DEXA+ HFHS+ BETAZEN (45mg/kg)	66.92±7.538	132.6±12.25	52.59±3.243***	31.98±1.107**	25.13±2.021***
DEXA+ HFHS+ BETAZEN (90mg/kg)	78.83±11.07	94.93±7.713**	57.33±2.832***	27.85±2.569***	22.9±1.389***

$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.0001$ when compared with NORMAL GROUP.

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.0001$ when compared with DIABETIC CONTROL.

Table No. 8: Effect of various treatments on glycated haemoglobin.

PARAMETER / GROUPS	HbA_{1c} (%)
NORMAL GROUP	5.612±0.1195
DIABETIC CONTROL	8.367±0.319 ###
DEXA+ HFHS+ PIO	5.726±0.1268 **
DEXA+ HFHS+ BETAZEN (45mg/kg)	7.4±0.8406
DEXA+ HFHS+ BETAZEN (90mg/kg)	4.05±0.3905 ***

# p<0.05, ## p<0.01, ### p<0.0001 when compared with NORMAL GROUP.

*p<0.05, ** p<0.01, *** p<0.0001 when compared with DIABETIC CONTROL.

Results are been expressed as mean ± SEM. Differences among data were determined using one-way ANOVA followed by Dunnett Multiple Comparison Test.

Table No. 9: Effect of various treatments on glycogen content in Liver and Gastrocnemius muscle.

ORGANS / GROUPS	LIVER (mg/g of tissue)	MUSCLE (mg/g of tissue)
NORMAL GROUP	33.83±1.3	26.59±0.7874
DIABETIC CONTROL	5.33±0.4017 ###	3.46±0.2776 ###
DEXA+ HFHS+ PIO	27.17±2.267 ***	21.27±1.971 ***
DEXA+ HFHS+ BETAZEN (45mg/kg)	16.67±1.026 ***	13.41±0.7231 ***
DEXA+ HFHS+ BETAZEN (90mg/kg)	23.24±2.203 ***	16.52±1.463 ***

# p<0.05, ## p<0.01, ### p<0.0001 when compared with NORMAL GROUP.

*p<0.05, ** p<0.01, *** p<0.0001 when compared with DIABETIC CONTROL.

Results are been expressed as mean ± SEM. Differences among data were determined using one-way ANOVA followed by Dunnett Multiple Comparison Test.

Table No. 10: Effect of various treatments on Glucose uptake in rat Hemi-diaphragm.

PARAMETERS / GROUPS	Insulin assisted glucose uptake (mg/g/30 min)	Non-Insulin assisted glucose uptake (mg/g/30 min)
NORMAL GROUP	15.15±1.323	8.627±1.392
DIABETIC CONTROL	8.727±0.9283$^{\#\#}$	5.102±0.2459$^{\#}$
DEXA+ HFHS+ PIO	16.54±1.338***	8.208±0.6596**
DEXA+ HFHS+ BETAZEN (45mg/kg)	14.36±0.9869**	6.302±0.401
DEXA+ HFHS+ BETAZEN (90mg/kg)	15.49±0.9067***	7.633±0.6733**

$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.0001$ when compared with NORMAL GROUP.

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.0001$ when compared with DIABETIC CONTROL.

Results are been expressed as mean ± SEM. Differences among data were determined using one-way ANOVA followed by Dunnett Multiple Comparison Test.

Table No. 11: Effect of various treatments on Hepatic anti-oxidant enzymes.

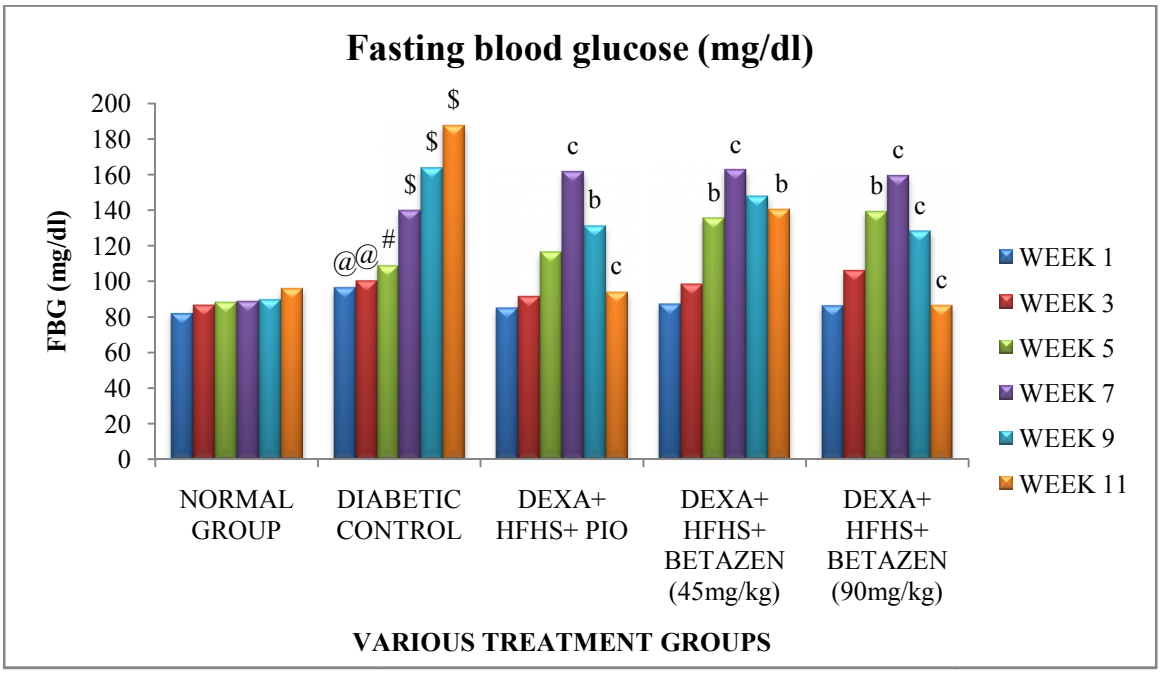
PARAMETERS / GROUPS	GSH (µg/g of tissue)	MDA (µM/g of tissue)	SOD (U/ml)	CATALASE (U/mg protein)
NORMAL GROUP	29.07±1.627	14.44±0.8316	76.55±2.318	19.45±1.88
DIABETIC CONTROL	17.46±0.8524$^{\#\#\#}$	23.32±1.25$^{\#\#\#}$	31.48±1.936$^{\#\#\#}$	6.885±0.6632$^{\#\#\#}$
DEXA+ HFHS+ PIO	24.64±1.867**	16.16±0.9175***	73.91±1.878***	17.91±1.583***
DEXA+ HFHS+ BETAZEN (45mg/kg)	21.92±1.453	19.5±0.4366*	49.95±4.123***	15.31±0.6934***
DEXA+ HFHS+ BETAZEN (90mg/kg)	24.41±1.322**	18.02±0.56***	71.51±2.123***	16.52±1.095***

$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ when compared with NORMAL GROUP.

*p<0.05, $^{**}p<0.01$, $^{***}p<0.001$ when compared with DIABETIC CONTROL.

Results are been expressed as mean ± SEM. Differences among data were determined using one-way ANOVA followed by Dunnett Multiple Comparison Test.

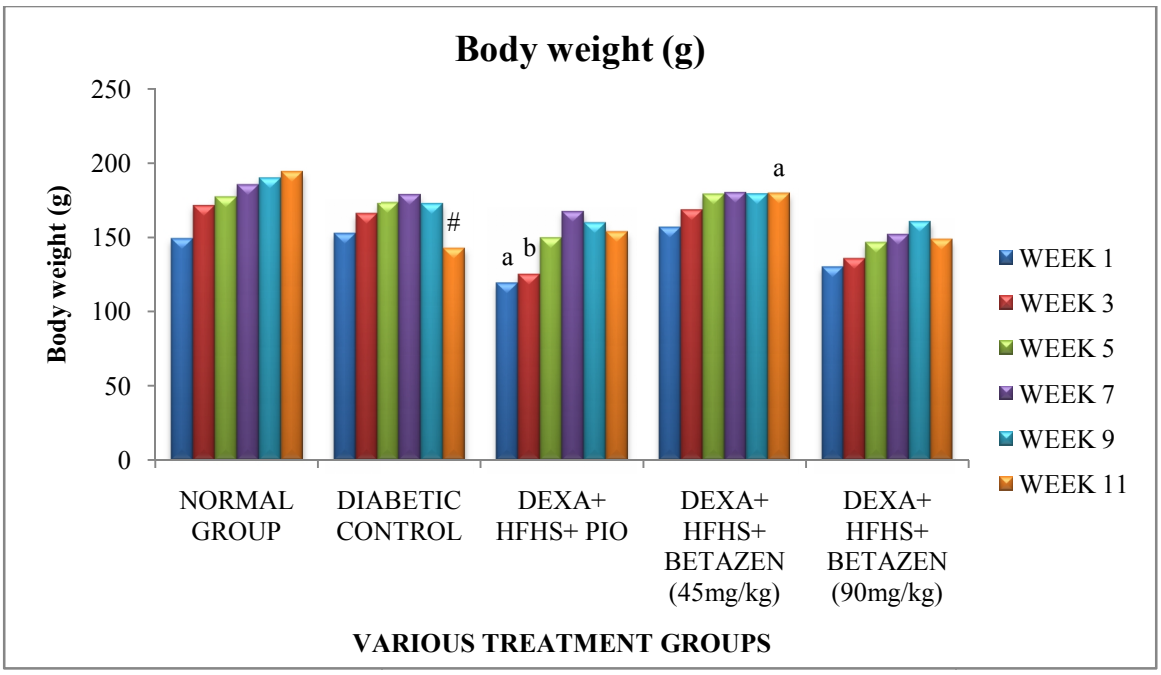
Figure No. 8: Effect of various treatments on fasting Blood glucose levels.



[@] p<0.05, [#] p<0.01, [$] p<0.0001 when compared with NORMAL GROUP.

[a] p<0.05, [b] p<0.01, [c] p<0.0001 when compared with DIABETIC CONTROL.

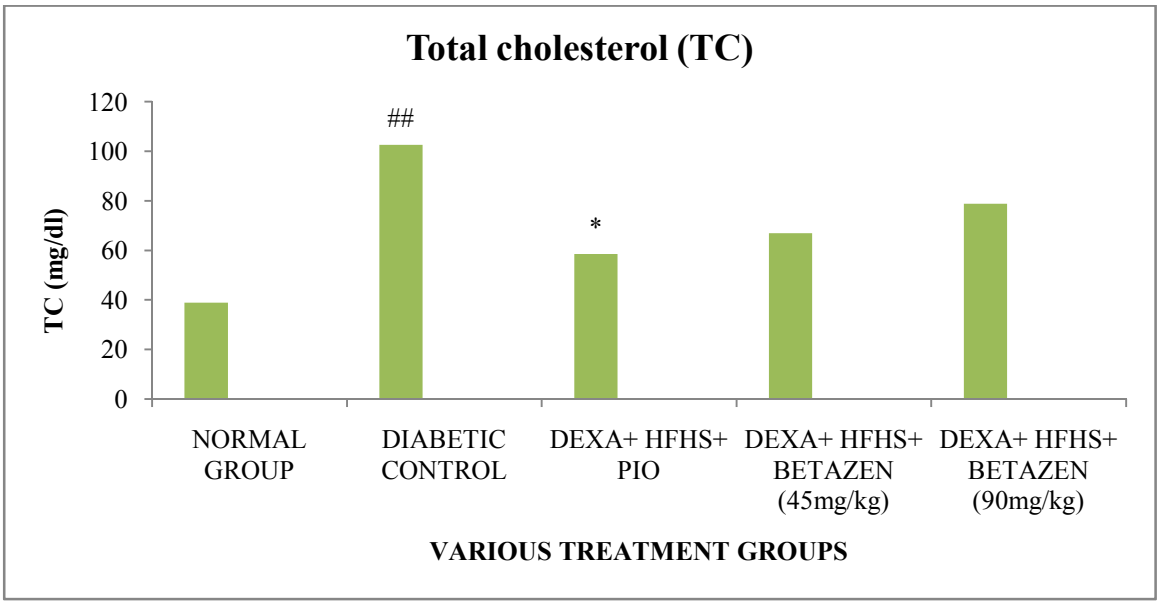
Figure No. 9: Effect of various treatments on rat Body weight.



[@] p<0.05, [#] p<0.01, [$] p<0.0001 when compared with NORMAL GROUP.

[a] p<0.05, [b] p<0.01, [c] p<0.0001 when compared with DIABETIC CONTROL.

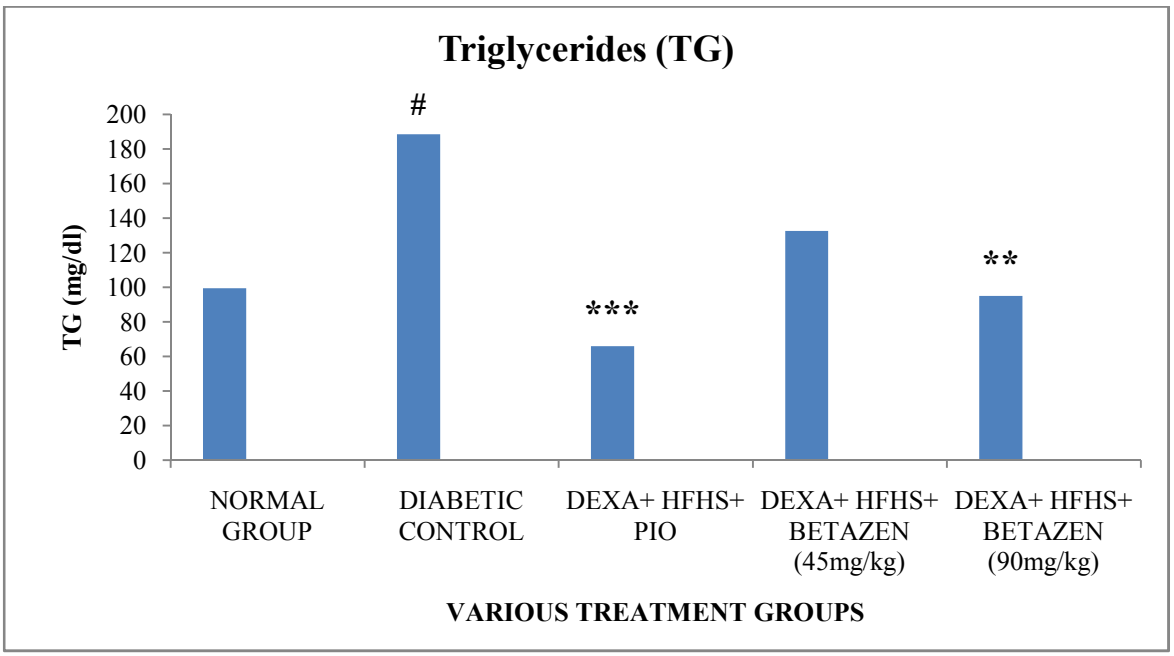
Figure No. 10: Effect of various treatments on Total Cholesterol.



# p<0.05, ## p<0.01, ### p<0.0001 when compared with NORMAL GROUP.

*p<0.05, ** p<0.01, *** p<0.0001 when compared with DIABETIC CONTROL.

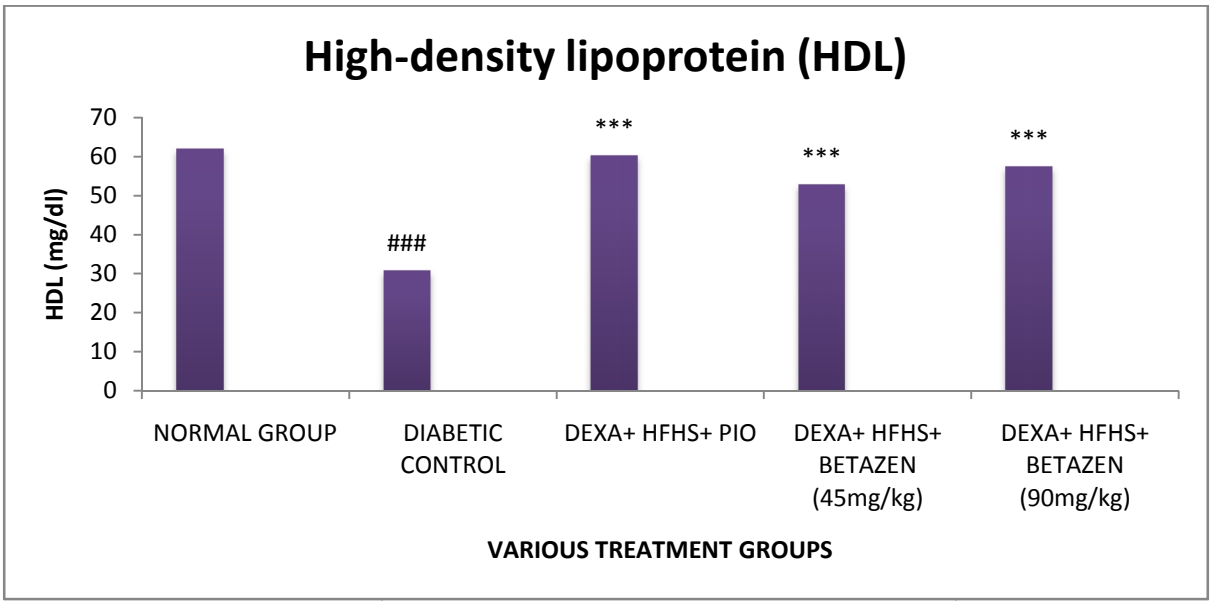
Figure No. 11: Effect of various treatments on Triglycerides.



# p<0.05, ## p<0.01, ### p<0.0001 when compared with NORMAL GROUP.

*p<0.05, ** p<0.01, *** p<0.0001 when compared with DIABETIC CONTROL.

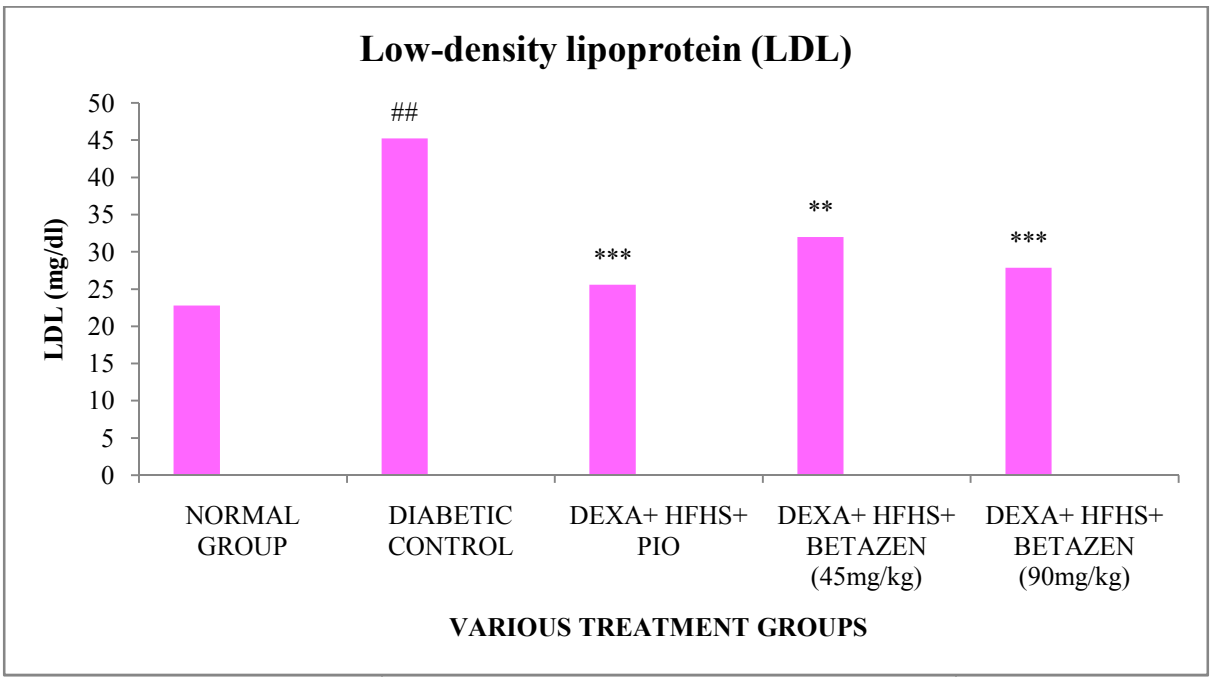
Figure No. 12: Effect of various treatments on High-density lipoprotein.



$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.0001$ when compared with NORMAL GROUP.

*$p<0.05$, $^{**}p<0.01$, $^{***}p<0.0001$ when compared with DIABETIC CONTROL.

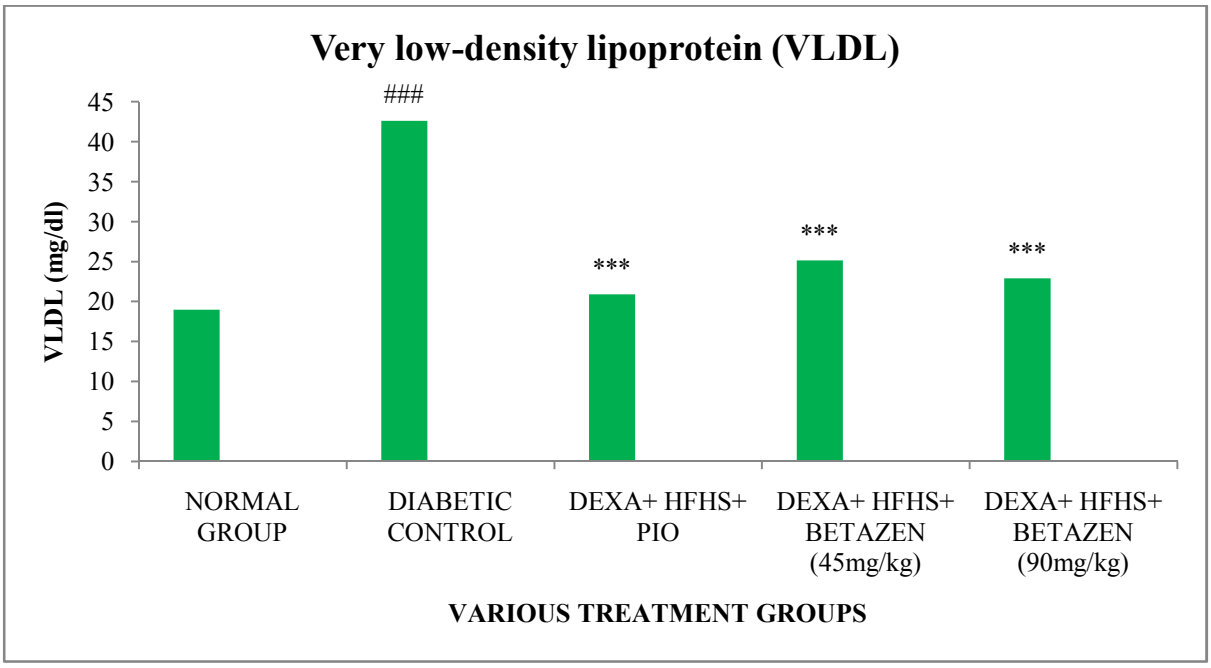
Figure No. 13: Effect of various treatments on Low-density lipoprotein.



$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.0001$ when compared with NORMAL GROUP.

*$p<0.05$, $^{**}p<0.01$, $^{***}p<0.0001$ when compared with DIABETIC CONTROL.

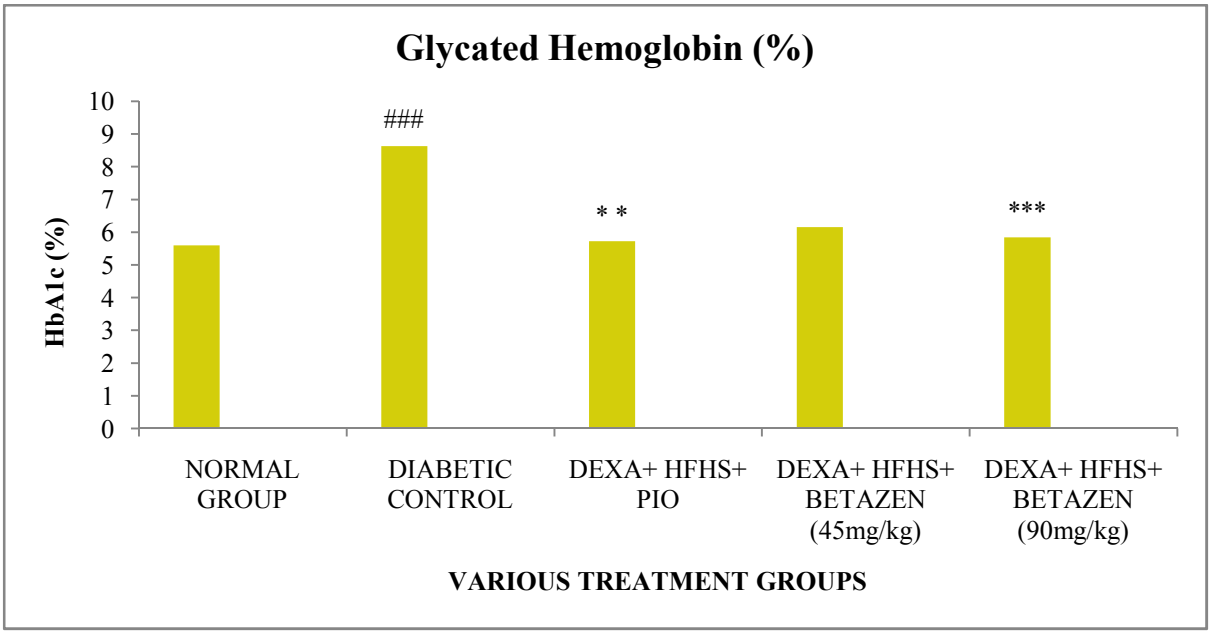
Figure No. 14: Effect of various treatments on Very low-density lipoprotein.



# p<0.05, ## p<0.01, ### p<0.0001 when compared with NORMAL GROUP.

*p<0.05, ** p<0.01, *** p<0.0001 when compared with DIABETIC CONTROL.

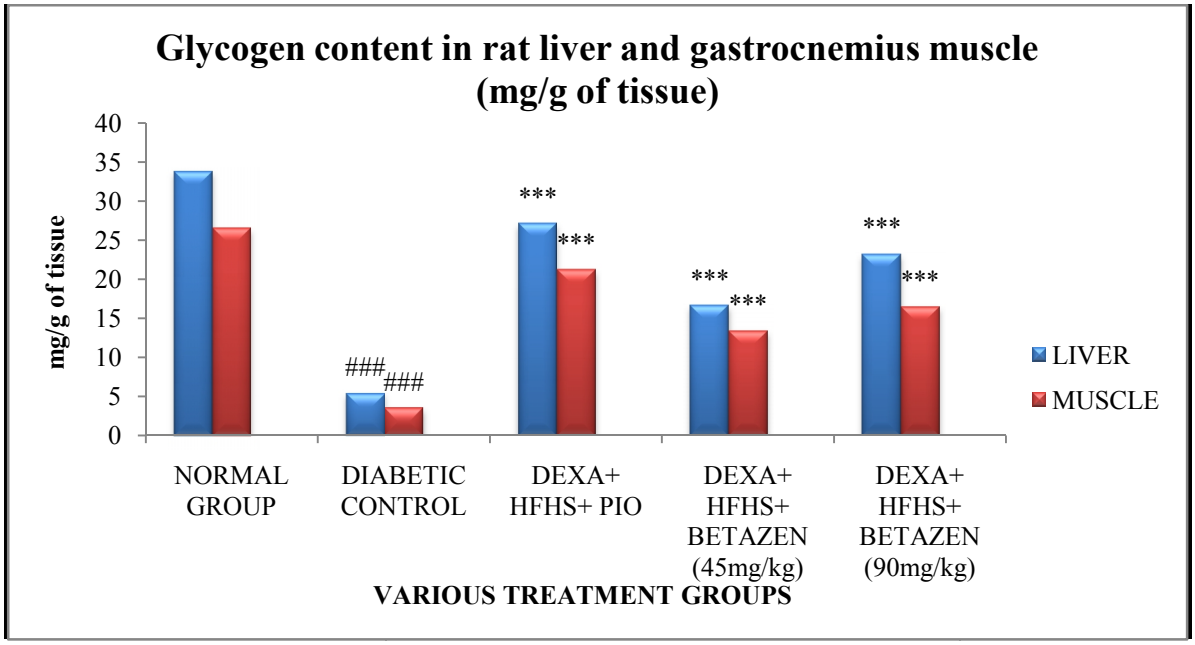
Figure No. 15: Effect of various treatments on Glycated hemoglobin.



# p<0.05, ## p<0.01, ### p<0.0001 when compared with NORMAL GROUP.

*p<0.05, ** p<0.01, *** p<0.0001 when compared with DIABETIC CONTROL.

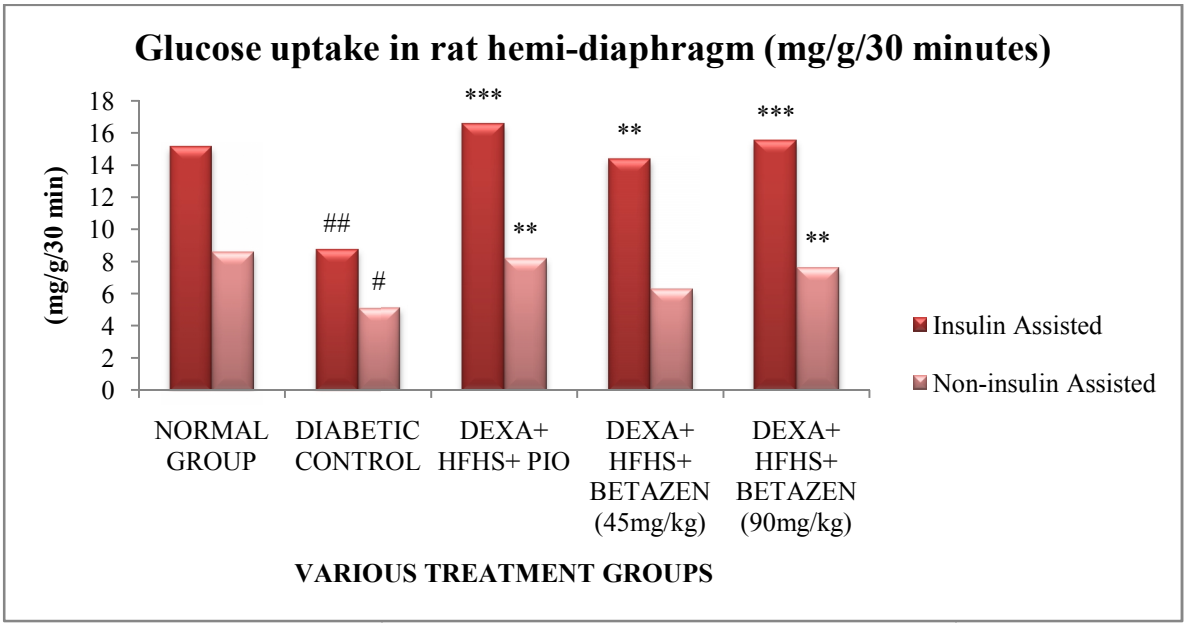
Figure No. 16: Effect of various treatments on Glycogen content in Liver and Gastrocnemius muscle.



$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.0001$ when compared with NORMAL GROUP.

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.0001$ when compared with DIABETIC CONTROL.

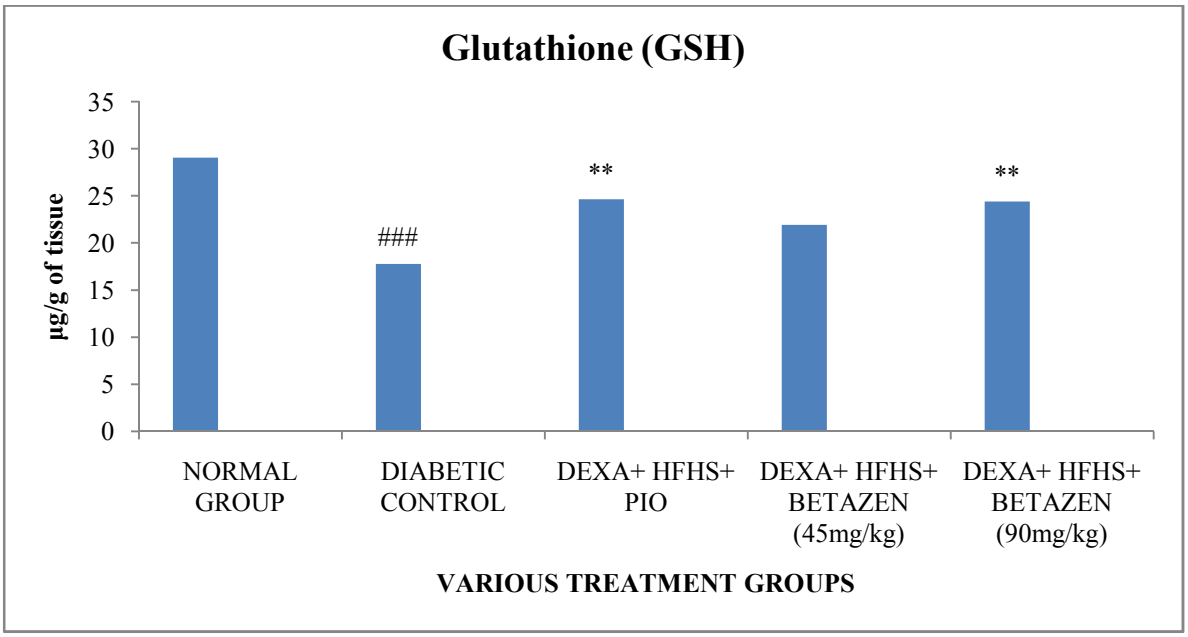
Figure No. 17: Effect of various treatments on Glucose uptake in rat Hemi-diaphragm.



$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.0001$ when compared with NORMAL GROUP.

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.0001$ when compared with DIABETIC CONTROL.

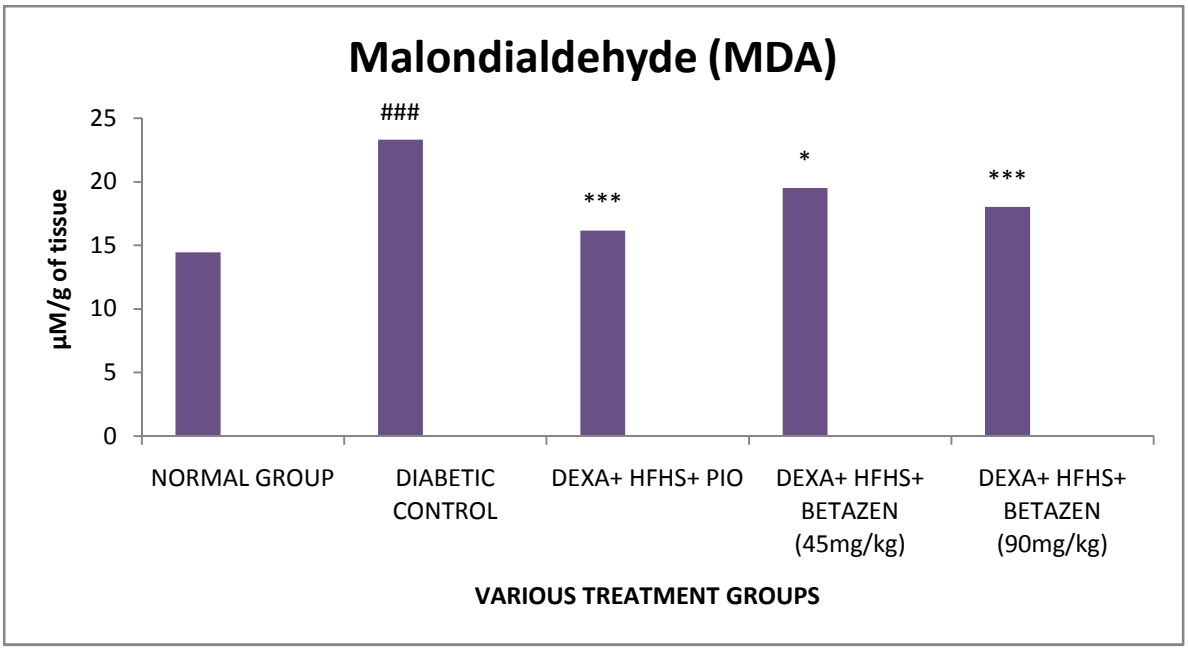
Figure No. 18: Effect of various treatments on reduced Glutathione in liver homogenate.



$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.0001$ when compared with NORMAL GROUP.

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.0001$ when compared with DIABETIC CONTROL.

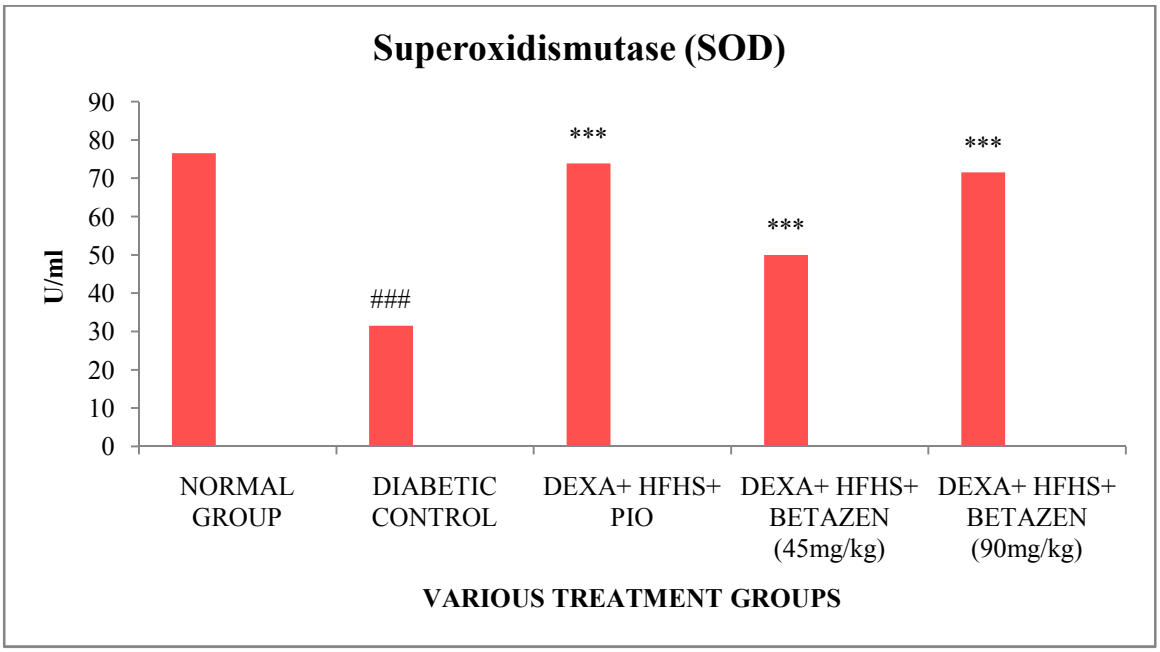
Figure No. 19: Effect of various treatments on Malondialdehyde in liver homogenate.



$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.0001$ when compared with NORMAL GROUP.

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.0001$ when compared with DIABETIC CONTROL.

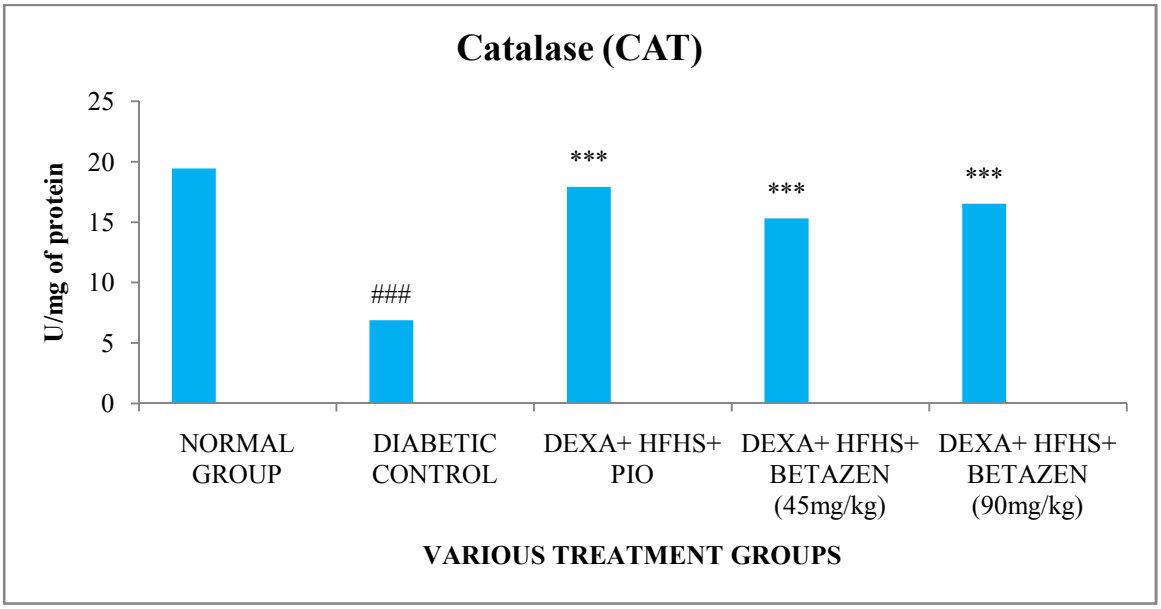
Figure No. 20: Effect of various treatments on Superoxidismutase in liver homogenate.



# p<0.05, ## p<0.01, ### p<0.0001 when compared with NORMAL GROUP.

*p<0.05, ** p<0.01, *** p<0.0001 when compared with DIABETIC CONTROL.

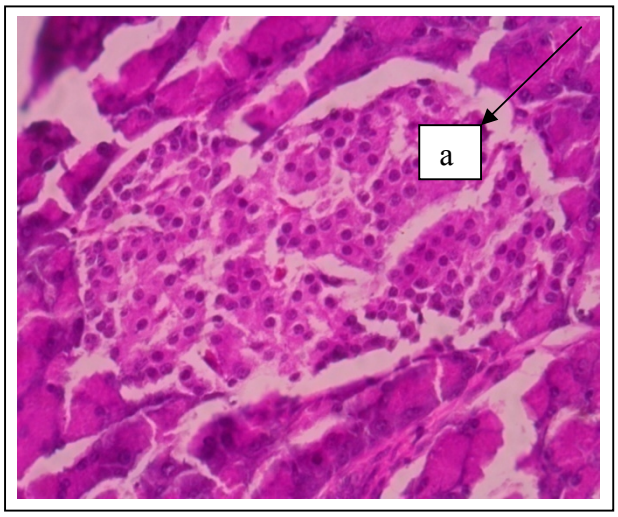
Figure No. 21: Effect of various treatments on Catalase in liver homogenate.



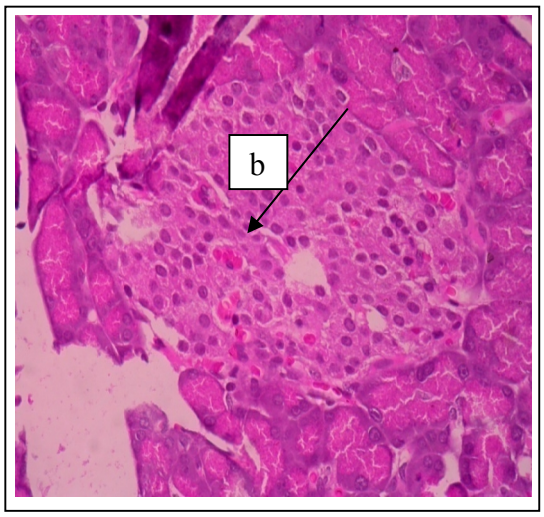
# p<0.05, ## p<0.01, ### p<0.0001 when compared with NORMAL GROUP.

*p<0.05, ** p<0.01, *** p<0.0001 when compared with DIABETIC CONTROL.

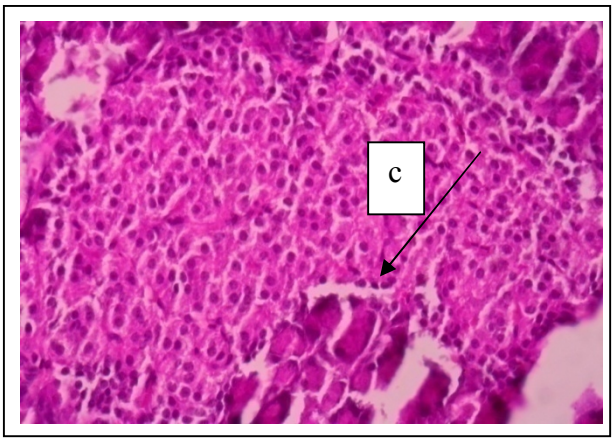
Figure No. 22: Effect of various treatments on Histopathology of Pancreas.



(A)



(B)



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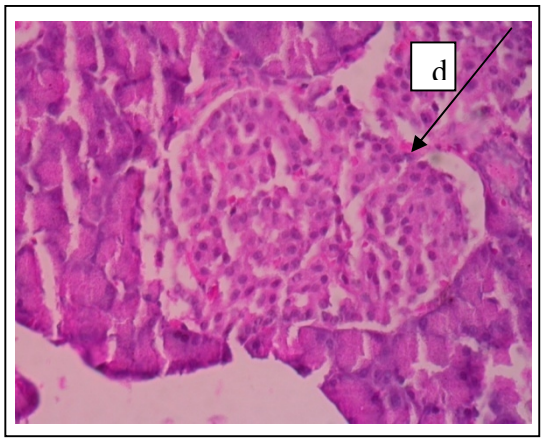

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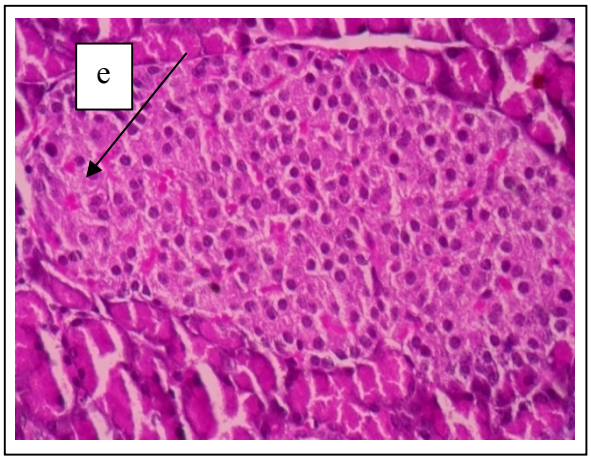

(E)

(A) Normal group: Section of pancreas showing normal architecture. (a).

(B) Diabetic control: Section of pancreas showing moderate decrease in size and number of islets, lymphocytic infiltration and vacuolar degeneration of islet cells. (b).

(C) DEXA + HFHS + PIO: Section of pancreas showing mild decrease in size and number of islets. (c).

(D) DEXA + HFHS + BETAZEN (45 mg/kg): Section of pancreas showing mild lymphocytic infiltration, vacuolar degeneration as well as an increase in the number and size of islets. (d).

(E) DEXA + HFHS + BETAZEN (90 mg/kg): Section of pancreas showing mild lymphocytic infiltration, and increase in the number and size of islets. (e).

Table No. 5: Effect of various treatments on fasting Blood glucose levels.

WEEK / GROUPS	Week 1 (mg/dl)	Week 3 (mg/dl)	Week 5 (mg/dl)	Week 7 (mg/dl)	Week 9 (mg/dl)	Week 11 (mg/dl)
NORMAL GROUP	81.83±4.586	86.5±3.722	88±2.921	88.67±3.667	89.5±3.51	95.83±4.393
DIABETIC CONTROL	96.5±1.784$^{\#}$	100.3±2.512$^{\#}$	109±4.74$^{\#\#}$	140±4.115$^{\#\#\#}$	164±7.75$^{\#\#\#}$	187.8±11.34$^{\#\#\#}$
DEXA+ HFHS+ PIO	85.17±6.226	91.67±5.499	116.7±7.727	161.8±3.135***	131.2±7.153**	94±8.351***
DEXA+ HFHS+ BETAZEN (45mg/kg)	87.17±1.74	98.33±2.29	135.3±4.333**	162.5±1.803***	147.7±3.04	140.2±5.474**
DEXA+ HFHS+ BETAZEN (90mg/kg)	86.33±3.273	106±2.338	139.2±0.8724**	159.3±1.994***	128.2±2.6***	86.5±7.14***

$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.0001$ when compared with NORMAL GROUP.

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.0001$ when compared with DIABETIC CONTROL.

Results are been expressed as mean ± SEM. Differences among data were determined using one-way ANOVA followed by Dunnett Multiple Comparison Test.

Table No. 6: Effect of various treatments on rat Body weight.

WEEK / GROUPS	Week 1 (g)	Week 3 (g)	Week 5 (g)	Week 7 (g)	Week 9 (g)	Week 11 (g)
NORMAL GROUP	149±4.583	171.3±6.247	177±5.881	185.3±7.696	189.8±7.634	194.1±7.573
DIABETIC CONTROL	152.7±2.172	166±4.673	172.8±11.06	178.1±13.24	172±10.92	142.2±10.03$^{\#\#}$
DEXA+ HFHS+ PIO	119.5±13.27*	125.3±15.3**	149.8±11.63	167.6±11.38	160±12.5	153.9±10.21
DEXA+ HFHS+ BETAZEN (45mg/kg)	156.6±4.807	168.3±4.868	178.8±8.172	179.9±7.998	178.9±7.012	179.4±6.481*
DEXA+ HFHS+ BETAZEN (90mg/kg)	129.7±4.1	135.3±4.631	146±7.787	151.3±7.806	160±6.575	148.1±4.875

$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.0001$ when compared with NORMAL GROUP.

*$p<0.05$, $^{**}p<0.01$, $^{***}p<0.0001$ when compared with DIABETIC CONTROL.

Results are been expressed as mean ± SEM. Differences among data were determined using one-way ANOVA followed by

Dunnett Multiple Comparison Test.

Table No. 7: Effect of various treatments on Lipid Profile.

PARAMETER / GROUPS	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
NORMAL GROUP	38.85±2.585	99.51±20.05	61.87±0.845	22.8±2.72	18.97±1.725
DIABETIC CONTROL	102.6±13.93$^{\#\#}$	188.5±26.76$^{\#}$	30.83±1.352$^{\#\#\#}$	45.23±4.503$^{\#\#}$	42.61±3.421$^{\#\#\#}$
DEXA+ HFHS+ PIO	58.5±7.59*	65.88±8.889***	60.12±1.603***	25.58±2.037***	20.88±1.558***
DEXA+ HFHS+ BETAZEN (45mg/kg)	66.92±7.538	132.6±12.25	52.59±3.243***	31.98±1.107**	25.13±2.021***
DEXA+ HFHS+ BETAZEN (90mg/kg)	78.83±11.07	94.93±7.713**	57.33±2.832***	27.85±2.569***	22.9±1.389***

$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.0001$ when compared with NORMAL GROUP.

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.0001$ when compared with DIABETIC CONTROL.

Results are been expressed as mean ± SEM. Differences among data were determined using one-way ANOVA followed by

Dunnett Multiple Comparison Test.

Table No. 11: Effect of various treatments on Hepatic anti-oxidant enzymes.

PARAMETERS / GROUPS	GSH (µg/g of tissue)	MDA (µM/g of tissue)	SOD (U/ml)	CATALASE (U/mg protein)
NORMAL GROUP	29.07±1.627	14.44±0.8316	76.55±2.318	19.45±1.88
DIABETIC CONTROL	17.46±0.8524$^{\#\#\#}$	23.32±1.25$^{\#\#\#}$	31.48±1.936$^{\#\#\#}$	6.885±0.6632$^{\#\#\#}$
DEXA+ HFHS+ PIO	24.64±1.867**	16.16±0.9175***	73.91±1.878***	17.91±1.583***
DEXA+ HFHS+ BETAZEN (45mg/kg)	21.92±1.453	19.5±0.4366*	49.95±4.123***	15.31±0.6934***
DEXA+ HFHS+ BETAZEN (90mg/kg)	24.41±1.322**	18.02±0.56***	71.51±2.123***	16.52±1.095***

$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.0001$ when compared with NORMAL GROUP.

*$p<0.05$, $^{**}p<0.01$, $^{***}p<0.0001$ when compared with DIABETIC CONTROL.

Results are been expressed as mean ± SEM. Differences among data were determined using one-way ANOVA followed by Dunnett Multiple Comparison Test.

6.0. DISCUSSION

The present study was been designed to evaluate the anti-diabetic effect of Betazen capsules, an ayurvedic proprietary medication in drug and diet induced insulin resistant rats. Betazen formulation contains various herbal plant extracts for which the LD_{50} value is been reported to be 3000 mg/kg. Hence, the doses selected for the present study was 500 and 1000 mg/200 g rat body weight. The results obtained from the present study, suggest that Betazen herbal formulation improves the insulin resistant state in a dose dependent manner. In addition, phytochemical investigation of the aqueous extract of Betazen capsules has revealed the presence of sterols, flavonoids, alkaloids, tannins, vitamin-C and saponins.

IR was been induced in the experimental Wistar rats via the administration of dexamethasone (2 μg/day s.c), vanaspati ghee and fructose enriched HFHS diet in place of normal rat feed and potable water respectively. Relevant scientific studies have proposed that dexamethasone, a frequently used member of the GC category increases glucose levels leading to hyperglycaemia, and reduces cellular glucose uptake thereby affecting the glucose transport system. In addition, prolonged GC therapy initiates muscle proteolysis, alters IRS-1 transcription, trigger's proteolysis, lipolysis, free fatty acid production, hepatic gluconeogenesis, and steatosis in the liver, prolongs bone resorption and induces oxidative stress in the pancreatic mitochondria, bone and tendon respectively.[58-61]

Furthermore, numerous studies have put forth that a high percentage of fat in ones diet facilitates the development of obesity. Fat accumulation in adipocytes, muscle and liver cells cause suppression of insulin receptor synthesis, organ dysfunction and hence, trigger an impaired regulation of insulin, blood sugar, cholesterol and heart functions, ultimately leading to IR in these organs. [22,35,51] Accumulating evidences have proposed that a fructose enriched diet can induce IR associated with hyperglycemia, hypertriglyceridemia, fatty liver and hypertension by depressing activation of the insulin signaling cascade via IRS-

1/PI3K/Akt pathway in the main target tissues viz; skeletal muscle, liver, and adipose tissue.[47,64]

Results obtained from the present study, confirm that administration of dexamethasone and a high fat-high sugar diet, lead to induction of IR in experimental rats. The study results exhibited a significant increase in BGL, VLDL, HbA_{1c} & MDA ($p<0.0001$), TC & LDL ($p<0.01$) and TG level ($p<0.05$) respectively in dexamethasone and HFHS treated insulin resistant rats. In addition, there was a significant ($p<0.0001$) decrease in glycogen content, HDL level, antioxidant biomarker's (SOD, GSH and CAT) as well as insulin & non-insulin assisted glucose uptake ($p<0.01$ & $p<0.05$), thereby reflecting the successful induction of IR.

According to the present study, treatment of IR rats with Betazen herbal formulation at 45 and 90 mg/kg showed significant ($p<0.01$ & $p<0.0001$ respectively) dose dependent reduction in elevated BGL. The observed anti-hyperglycemic effect in the IR rats can be attributed to the phytochemical constituents viz; flavonoids,[80,84,85] saponins, alkaloids, triterpinoids, phenolics[3,11,16,21,36,74,] and tannins,[94,96] which have been reported for their anti-diabetic potential in animal experimental studies. These phyto-constituents are been reported to exhibit their anti-hyperglycemic potential via one or more of the following mechanisms:

- By reactivating glycogen synthase enzyme.[16]
- By enhancing pancreatic β-cell function.[21]
- By inhibiting glucose absorption from the gut.[21]
- By increasing pancreatic β-cells area, size and number.[74]
- By stimulating insulin mediated glucose transport and utilisation.[74]
- By improving insulin sensitivity.[94]

In the present study, an initial gradual increase followed by a significant ($p<0.01$) decrease in body weight (BW) was been observed in the IR rats when compared to normal

euglycaemic group. This decrease in BW is been assumed to occur due to increased muscle wasting or muscle proteolysis mainly because of obesity and dexamethasone administration. Treatment of IR rats with Betazen herbal formulation at 90 mg/kg showed a dose dependant gradual decrease in BW when compared to IR group. However, treatment of IR rats with Betazen herbal formulation at 45 mg/kg showed dose dependant decrease in BW, followed by constant maintenance of BW to normal. The maintenance of constant BW can be attributed to the synergistic effects of various contents available in the formulation that due to the presence of mainly flavonoids and alkaloids exhibit thermogenic activity, thus modulating muscle wasting, protein turnover and IR in high fat diet fed rats.[54,91]

Abnormalities in lipid profile are one of the most common complications in IR. Obesity (excess accumulation of body fat) is been implicated in the prevalence of metabolic syndrome, hypertension, IR and dyslipidemia.[22,35,51] Moreover, it is been established that dexamethasone, increases triglyceride levels, causing an imbalance in lipid metabolism, thereby leading to hyperlipidaemia. In addition, glucocorticoids promote the expression of lipolytic enzyme sensitive lipase in peripheral fat deposits and trigger lipolysis, FFA production, hepatic gluconeogenesis, and steatosis in the liver. [58-60] Furthermore, numerous scientific investigations have proposed that a high fructose diet can induce IR associated with hypertriglyceridemia.[47,64]

In the present study, the levels of serum TC, TG, VLDL and LDL was significantly increased as well as marked reduction in serum HDL level was observed in IR rats when compared with euglycemic group. This increase in serum lipid levels may be due to lipolysis and resistance towards the anti-lipolytic action of insulin. Treatment of IR rats with Betazen herbal formulation at 45 mg/kg showed significant dose dependent decrease in elevated serum TC, TG, LDL ($p<0.01$), VLDL ($p<0.0001$) and a highly significant ($p<0.0001$) increase in HDL cholesterol. Likewise, treatment of IR rats with Betazen herbal formulation

at 90 mg/kg showed significant dose dependent decrease in serum TC, TG ($p<0.01$), LDL, VLDL ($p<0.0001$) as well as a highly significant ($p<0.0001$) increase in HDL cholesterol when compared to the IR group. The results clearly indicate that treatment with Betazen herbal formulation improves lipid abnormalities, and the most prominent effect was been observed at the dose of 90 mg/kg BW. The improvement in dyslipidemia may be due to the presence of phyto-constituents viz; flavonoids, phenolics and saponins that are been reported to exhibit significant anti-hyperlipidemic and antiperoxidative potential as they significantly reduce plasma cholesterol and triglycerides. [80,84,85] Curcumin, the most important constituent of curcuma longa has been implicated in the inhibition of lipolysis.[11,74,93,94] while the constituents present in *Azadirachta indica* possess anti-dyslipidemic potential.[11] In addition, Shreya S, *et al.* put forth that the alkaloid piperine, exhibits lipid lowering ability. The possible mechanism of this action was been stated to be due to its thyrogenic activity, thus regulating apolipoprotein levels and IR in high fat diet fed rats. Furthermore, piperine also inhibits lipid and lipoprotein accumulation by significantly modulating the activity of enzymes viz; Lecithin-cholesterol acyltransferase (LCAT) and Lipoprotein lipase (LPL) which are involved in lipid metabolism.[54,91]

During hyperglycemic condition, the glucose present in the blood reacts with hemoglobin to form glycosylated hemoglobin.[106] According to the present study, the IR group exhibited a significant ($p<0.0001$) increase in glycosylated hemoglobin and this increase was been found to be directly proportional to the fasting blood glucose level. However, treatment of IR rats with Betazen herbal formulation at 90 mg/kg significantly ($p<0.0001$) decreased the elevated HbA_{1c} levels. Moreover, treatment with 90 mg/kg showed better effect than the standard drug (PIO). The decline in HbA_{1c} levels may be due to the presence of flavonoids, tannins and phenolics that are been proven to have a protective effect on HbA_{1c} levels in animal experimental models.[18,21]

The content of glycogen (main intracellular storage form of glucose) in various tissues is a direct reflection of insulin activity, as insulin promotes intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase.[35] Hepatic glycogen reserves are important for whole body glucose homeostasis and are markedly low in rats with hyperglycemia. Moreover, fatigue develops when glycogen stores are been depleted in the body. In the present study, a significant ($p<0.0001$) reduction in liver and muscle glycogen was observed in dexamethasone & HFHS diet treated rats when compared to normal euglycemic rats. Treatment of IR rats with Betazen herbal formulation at 45 and 90 mg/kg showed significant ($p<0.0001$) dose dependent increase in liver and muscle glycogen content. The improvement in glycogen content may be due to the presence of alkaloids, insulin like peptide (lectin) and a mixture of steroidal sapogenins (charantin) present in *Momordica charantia,* which is been reported to increase glycogen synthesis mainly in the liver, muscles and fat cells. [86] In addition, the constituents present in *Azadirachta indica* are been reported to possess glycogen synthase activation potential.[16]

Skeletal muscle is the primary site for insulin stimulated glucose disposal. In the present study, glucose uptake in insulin and non-insulin assisted isolated rat hemi-diaphragm's exhibited a significant ($p<0.01$ and $p<0.05$ respectively) decrease in the dexamethasone and HFHS diet treated rats when compared to normal group. The decrease in diaphragm glucose uptake may be due to the insulin insensitivity exhibited by target organs. Moreover, Insulin assisted glucose uptake in IR rats treated with Betazen herbal formulation at 45 and 90 mg/kg showed a significant ($p<0.01$ and $p<0.0001$) increase. However, in non-insulin assisted glucose uptake only IR rats treated with Betazen herbal formulation at 90 mg/kg showed a significant ($p<0.01$) increase in glucose uptake. The increase in glucose uptake may be due to the flavonoids, alkaloids, phenolics and saponins present in *Momordica*

charantia, Butea monosperma and *Zingiber officinale* that have been reported to improve peripheral glucose uptake, utilisation and insulin sensitivity respectively.[13,74,80,84,85]

Gordon Klein demonstrated that GC induces oxidative stress in the bone, pancreatic mitochondria and tendon. Oxidative stress takes place in a particular cell when the generation of ROS overwhelms the cell's natural antioxidant defence. Moreover, excess production of ROS is proficient enough to damage proteins, lipids, carbohydrates and nucleic acids.[26,33,35,50] According to the present study, experimental rats to whom dexamethasone and HFHS diet was been administered exhibited a highly significant ($P<0.0001$) reduction in antioxidant biomarker's viz; SOD, CAT and GSH while, a significant ($P<0.0001$) elevation in MDA levels. The significant reduction in antioxidant biomarker's and elevation in MDA level in IR rats may be possibly due to the generation of free radicals via auto-oxidation and disturbances in the body's antioxidant defence mechanism to combat them. Treatment of IR rats with Betazen herbal formulation at 45 mg/kg showed a significant ($P<0.0001$) increase in SOD & CAT levels while a significant ($P<0.05$) decrease in MDA level. However, treatment of IR rats with Betazen herbal formulation at 90 mg/kg exhibited a significant increase in SOD, CAT ($P<0.0001$) as well as GSH level ($P<0.01$) while a significant ($P<0.0001$) decrease in MDA levels.

The improvement in antioxidant levels on treatment with Betazen herbal formulation may be because of flavonoids, phenolics, tannins and saponins present in some of its contents viz; *Zingiber officinale,*[13] *Butea monosperma,*[80,84,85] *Momordica charantia,*[86] *Trigonella foenum-graecum,*[92] *Terminalia chebula*[96] and *Pterocarpus marsupium*[79] that have been reported to possess antiperoxidative and antioxidant potential in diabetic rats. In addition, Picroside I and II, the main constituents present in *Picrorrhiza kurrao* rhizome are been reported to exhibit significant anti-oxidant potential. This beneficial anti-oxidant potential is been attributed to its inherent ability of reducing NADPH-oxidase dependent superoxide

generation and blunting the expression of MDA and advanced oxidation protein products in diabetic kidney.[3,16,87,88] Furthermore, *Phyllanthus emblica*, is an excellent source of vitamin C and hence exhibits antioxidant property.[89]

Histopathological studies of dexamethasone and HFHS diet treated rat pancreas demonstrated mild pathological changes viz; moderate decrease in size and number of islets, lymphocytic infiltration and vacuolar degeneration of islet cells, when compared to the normal group having normal cellular integrity. Thus indicating that dexamethasone and HFHS diet induced IR is not associated with beta cell damage and toxicity. Treatment of IR rats with Betazen herbal formulation significantly regulated these abnormalities. The improvement in pancreatic cell integrity is possibly because of the cucurbitane triterpinoids in *Momordica charantia* (contained in Betazen herbal formulation) that has been proposed to increase pancreatic β-cells area, size and number.[74] In addition, phyto-constituents present in *Butea monosperma* have been claimed to stimulate insulin secretion from remnant or regenerated beta cells.[80,84,85]

The results obtained from the present study indicate that Betazen herbal formulation shows significant dose dependent improvement in drug and diet induced insulin resistant in Wistar rats. Based on the present study, it is difficult to establish the exact mechanism of action for improvement in the insulin sensitivity. However, the effect of Betazen herbal formulation can be assumed to be a synergistic effect of mainly phyto-constituents like flavonoids, saponins, triterpinoids, alkaloids, sterols, tannins and vitamin-C present in its contents which act via a plethora of mechanisms to improve insulin resistance.

7.0. CONCLUSION

In conclusion, Betazen herbal formulation demonstrated a significant dose dependent increase in insulin sensitivity. Moreover, treatment of IR rats with Betazen herbal formulation exhibited an improvement in dyslipidemia, glucose uptake, and glycogen content. Furthermore, it also demonstrated antioxidant, anti-glycation & anti-hyperglycemic potential in animal experimental model. The beneficial anti-diabetic effect of Betazen herbal formulation can be attributed to the synergistic effects of phyto-constituents viz; flavonoids, saponins, triterpinoids, alkaloids, sterols, tannins and vitamin-C present in the contents of the formulation. Hence, Betazen herbal formulation could be a promising future herbal treatment for drug, and diet induced insulin resistance.

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9.0 ANNEXURES

1. Consent letter from Progen Research Lab.
2. Instituitional Animal Ethics Committee approval letter.
3. Conference/Seminars/Workshops attended:
    - Participated in Pharmacology CME 2016 on "Current Trends in the Management of Hypertension" held at J. N. Medical College, Belagavi.
    - Participated as a Delegate in the 67th Indian Pharmaceutical Congress and presented a paper titled 'Pharmacodynamic effects of hydro-alcoholic rind extract of *Garcinia indica* in Clozapine induced insulin resistant rats' in December 2015.
    - Participated as a Delegate in the 33rd Annual Conference of Indian Society for Medical Statistics in October 2015, held at J. N. Medical College, Belagavi.
    - Participated as a Delegate in UDEHP Workshop "Cell Based Assays As A Tool For Pharmacology Teaching" in September 2015, held at KLEU's College of Pharmacy, Belagavi.
    - Participated as a Delegate in APPI Workshop "A Multidisciplinary Approach To Pain And Its Management" in August 2014 And Presented A Poster Entitled "Analgesic Potential Of Garcinia Indica In Animal Experimental Models".