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Dedication

My most sincere gratitude goes to my family for their unconditional love and constant encouragement.

In loving memory of my dearest sisters ***Ida Everline Taaka*** *and* ***Sumaya Ali****.*

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Abstract

Diabetes mellitus, characterized by hyperglycemia and metabolic dysregulation, represents a global health crisis affecting 537 million adults, with projections identifying it as the 7th leading cause of mortality by 2030. Glucocorticoids like dexamethasone are widely used for immunosuppression. They induce severe metabolic complications, including steroid-induced diabetes. This study investigated dexamethasone induced diabetes and hepatic toxicity in a male *Albin wistar* rat model. Rats received intraperitoneal injection of dexamethasone (10 mg/kg/day) for 7 days to induce metabolic disturbance and toxicity.

Obtained results showed that dexamethasone induced an increase in rat's body weight, lipid profile parameters mainly; triglycerides and cholesterol and hyperglycaemia confirming the metabolic disturbance. Also, a rise in plasma transaminases (AST and ALT) were registered, indicating hepatic injury. Furthermore, obtained results showed an increase in hepatic malondialdehyde (MDA) and reduced glutathione (GSH), confirming redox imbalance and oxidative stress state flowing dexamethasone treatment.

This work establishes dexamethasone as a model for metabolic associated hepatic injury and underscores oxidative stress as a central mediator in glucocorticoid induced diabetes.

Keywords: *Dexamethasone, Glucocorticoid, Hepatotoxicity, Oxidative stress, Diabetes.*

Résumé

Le diabète mellites caractérisé par une hyperglycémie et un dérèglement métabolique, représente une crise sanitaire mondiale touchant 537 millions d'adultes. Les projections le désignent comme la ^{7éme} cause de mortalité d'ici 2030. Les glucocorticoïdes comme la dexaméthasone sont largement utilisés pour l'immunosuppression. Ils induisent de graves complications métaboliques, notamment un diabète induit par les stéroïdes. Cette étude a examiné l'éffet de la dexaméthasone d'induire le diabète et la toxicité hépatique chez de rat *Albino Wistar.* Les rats ont reçu une injection intrapéritonéale de dexaméthasone (10 mg/kg/jour) pendant 7 jours afin d'induire des troubles métaboliques et une toxicité.

Les résultats obtenus ont montré que la dexaméthasone a induit une augmentation du poids corporel des rats, des paramètres lipidiques principalement (triglycérides et cholestérol) et une hyperglycémie confirmant les troubles métaboliques. Egalement, une augmentation des transaminases plasmatiques (ASAT et ALAT) a été enregistrée, indiquant une atteinte hépatique. De plus, les résultats obtenus ont montré une augmentation du malondialdéhyde hépatique (MDA) et une diminution du glutathion (GSH), confirmant ainsi le déséquilibre redox et l'état de stress oxydatif induit par le traitement à la dexaméthasone.

Ces travaux établissent la dexaméthasone comme modèle de lésion hépatique associée au métabolisme et soulignent le rôle du stress oxydatif comme un médiateur central du diabète induit par les glucocorticoïdes.

Mots clés: *Dexamethasone, Glucocorticoide, Hepatotoxicité, Stress oxydative, Diabètes.*

ملخص

يُمثل داء السكري، الذي يتميز بارتفاع سكر الدم واضطراب التمثيل الغذائي، أزمة صحية عالمية تؤثر على 537 مليون بالغ. وتشير التوقعات إلى أنه سيصبح سابع سبب رئيسي للوفاة بحلول عام 2030. تُستخدم الجلوكوكورتيكويدات مثل ديكساميثازون على نطاق واسع لكبت المناعة. إنها تُسبب مضاعفات أيضية خطيرة، بما في ذلك داء السكري الناجم عن الستيرويدات. تناولت هذه الدراسة داء السكري الناجم عن ديكساميثازون وسمية الكبد في نموذج فأر ألبين ويستار الذكر. تلقت الفئران حقنة داخل الصفاق من ديكساميثازون (10 ملغ/كغ/يوم) لمدة 7 أيام لإحداث اضطرابات أيضية وسمية.

أظهرت النتائج أن ديكساميثازون تسبب في زيادة وزن جسم الفئران، وخاصةً معايير الدهون (الدهون الثلاثية والكوليسترول)، وفرط سكر الدم، مما يؤكد الاضطرابات الأيضية. كما لاحظنا زيادة في ناقلات الأمين في البلازما (AST وALT)، مما يشير إلى تلف الكبد. بالإضافة إلى ذلك، أظهرت النتائج زيادة في مالونديالدهيد الكبدي (MDA) وانخفاض في الجلوتاثيون (GSH)، مما يؤكد اختلال التوازن الأكسدة والاختزال والإجهاد التأكسدي الناجم عن علاج ديكساميثازون

يثبت هذا العمل أن ديكساميثازون هو نموذج لإصابة الكبد المرتبطة بالتمثيل الغذائي ويسلط الضوء على دور الإجهاد التأكسدي كوسيط مركزي لمرض السكري الناجم عن الجلوكوكورتيكويد.

الكلمات المفتاحية:

ديكساميثازون، الجلوكوكورتيكويد، السمية الكبدية، الإجهاد التأكسدي، مرض السكري

List of Abbriviations

8-OHdG : 8-hydroxy-2'-deoxyguanosine
AGEs: Advanced glycosylation end products
ALT: Alanine aminotranferase
AST: Aspartate aminotransferase
CCK: Cholecystokinin
CNS: Central Nervous System
DAG: Diacylglycerol
G3P: Glycerol-3-phosphate
GLP-1:gluco-dependent insulinotropic polypeptide
GSH: Rreduced glutathione
H_2O_2: hydrogen peroxide
HbA: Adult haemoglobin hemoglobin
HDL: High-density lipoprotein
HLA: human leukocyte antigen
LDH : Lactate dehydrogenase
LDL: Low density lipoprotein
MDA: Malondialdehyde
MODY : Monogenic diabetes
NAFLD : Non-alcoholic fatty liver disease
PKC : Protein kinase C
ROS: Reactive oxygen species
T1DM : Type 1 diabetes mellitus
T2DM: Type 2 diabetes mellitus
WHO : World Health Organaisation

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Introduction

High blood glucose levels (hyperglycemia), polydipsia, and polyphagia primarily characterize diabetes mellitus (DM). DM is one of the most common metabolic disorders that are increasing at an alarming rate all over the world. The number of patients with DM has quadrupled (from 108 million in 1980 to 422 million in 2014) within 34 years only, while the worldwide incidence of diabetes among adults over 18 years of age has risen to 8.5% (2014) from 4.7% (1980). The WHO estimates that diabetes will be the 7th primary cause of fatality by 2030 (**Alam *et al.*, 2021**), as the medical condition has increased in prevalence over the past few decades to constitute a major public health challenge of the twenty-first century (**Tomic *et al*., 2022**). Diabetes is a complex, chronic condition requiring continuous medical care with multifactorial risk-reduction strategies beyond glycaemic management (**ADA, 2023**).

Diabetes increases the risk of debilitating complications such as amputation, vision loss, and renal failure, and is associated with cardiovascular disease, dementia, some cancers, and infections such as tuberculosis and severe COVID-19 (**Zhou *et al*., 2024**). The physiology of glucose homeostasis is complicated with involvement of several organs including brain, liver, pancreas, skeletal muscle and adipose tissues. Among these, liver is a pivotal site of glucose synthesis and storage (**Sakharkar and Deb, 2021**). The liver also plays a significant role in lipid metabolism by manufacturing, storing, and transporting lipid metabolites. Thus, abnormality in the lipid level can lead to a change in liver metabolism and can damage the hepatic tissue. The most common chronic liver disease is represented by excess accumulation of lipids in the liver is known as non-alcoholic fatty liver disease (**Kathak *et al*., 2022**).

Glucocorticoids are a class of steroid hormone that regulate glucose and lipid metabolism. They are used in various clinical situations to suppress immunological responses in autoimmune diseases, inflammatory diseases, and after organ transplantation; however, the adverse effects sometimes cause negative clinical outcomes (**Uto *et al.*, 2021**). Pharmacologic doses of glucocorticoid hormones are used in a variety of disease conditions. Although they are usually well tolerated, especially if used for short durations, their usage is associated with a number of adverse effects. A common adverse effect is the development of hyperglycemia even in the absence of known type 2 diabetes mellitus (**Abdelmannan *et al*., 2010**).

Glucocorticoid-induced hyperglycemia is one of the most frequent adverse effects that are often difficult to control with ordinary lifestyle modification and caloric restrictions (**Uto *et al*., 2021**). On the other hand, Dexamethasone, in excess inhibits insulin secretion from pancreatic β- cells, decrease glucose utilization, and stimulate glucagon secretion, lipolysis, proteolysis, and hepatic glucose production. Glucocorticoids also cause insulin resistance by decreasing hepatic glucose utilization, decreasing glycogen synthesis. Free fatty acids may be elevated in insulin resistance because of impaired insulin-dependent down-regulation of lipolysis, hence leading to increase in triglyceride levels, which are then deposited in these organs (**Ghaisas *et al*., 2009**).

This study aims to investigate the metabolic and hepatic toxicity of dexamethasone (10 mg/kg/day *via* intraperetoneal injection) for seven days to *Albino wistar*, with a focus on elucidating the mechanisms underlying dexamethasone-induced metabolic disturbance and, toxicty.

Our thesis will be articulated in the following way:

- ***A first part which is devoted to the bibliographic synthesis is divided into two chapters:***
  - Chapter 1 establishes the anatomical and physiological foundation of the pancreas, detailing embryological development, histophysiology, and endocrine/exocrine functions.
  - Chapter 2 extensively examines diabetes mellitus; covering its definition, classification, genetic/environmental risk factors, pathophysiological mechanisms (insulin resistance, oxidative stress, inflammation..etc), and complications.
- ***The second part describes the experimental work and is divided into two chapters:***
  - The first chapter describes the materials and methods used in this study
  - The second chapter is devoted to the obtained results, their interpretations..

Finally, a general conclusion summarizes the whole study in highlighting interesting results and recommending future prospect.

1. Embryological Aspects

The pancreas is a complex organ made up of both exocrine glands, which secrete digestive enzymes into the intestinal lumen, and endocrine glands, called Langerhans islets, which secrete hormones directly into the blood stream and help regulate carbohydrate metabolism. The pancreas develops like other glands, starting with the formation of the duct, followed by cells growing around it to create gland lobules. The endocrine and exocrine tissues of the pancreas come from the endodermal epithelium of the duodenum. During the second and third weeks of pregnancy, when the embryo is about 3.4 mm long, the pancreatic tissue begins to form from the duodenum as two buds, known as the ventral and dorsal buds (**Ahmed and Reda, 2024**). The right and left ventral buds are distinct anatomical entities. The left ventral bud does not develop further and eventually regresses, whereas the right ventral bud, located between the duodenum and the common bile duct bud, continues to grow **(Pan and Brissova, 2014).** Compared to the ventral buds, the dorsal bud is larger and positioned more superiorly. It grows toward the spine and is situated between the stomach and the dorsal mesentery of the duodenum. Ultimately, this development results in the formation of the superior part of the head, the entire body, and the pancreatic tail **(Polak *et al.* (2000).**

2. Anatomical Aspects

Healthy human adult pancreas weighs approximately 100 g, has a length of 14 to 25 cm, a volume of approximately 72.4 ± 25.8 cm^3. The pancreas is a single and retroperitoneal organ. It is the second largest gland of our body in terms of size. It lies between the 12th dorsal vertebra and the 2nd lumbar vertebra, so its topographical projection on the abdomen is in the epigastric area **(Ellis, 2007).** The pancreas is a mixed gland, able to orchestrate endocrine and exocrine physiological responses due to the anatomical and functional interrelationship of its three constituents (pancreatic islets, acini, and duct) **(Atkinson *et al.*, 2020; Yuan *et al.*, 2021**). The pancreas has an elongated, hooked appearance and is divided macroscopically into the head, neck, body, and tail **(Figure 1).**

It is located on the posterior wall of the abdominal cavity as a retroperitoneal organ **(Ellis, 2007)**. The duodenum surrounds the head of the pancreas, followed by the neck, which is situated near the superior mesenteric arteries. The body lies behind the posterior wall of the stomach, while the tail extends toward the hilum of the spleen (**Ahmed and Reda., 2024**).

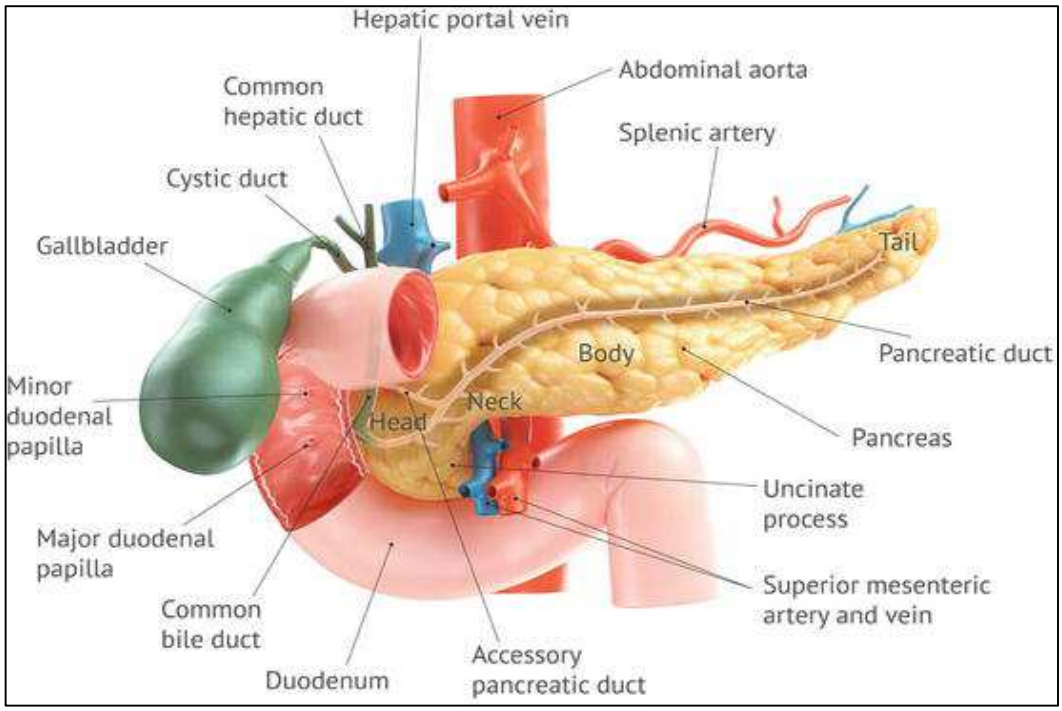


Figure1. Anterior vision of the pancreas with duodenum section and vision of pancreatic ducts **(Di Dato *et al.,* 2025).**

➢ The head of the pancreas is the enlarged part of the gland surrounded by the C-shaped curve of the duodenum (**Talathi *et al*., 2023).** Posteriorly, the head lies against the inferior vena cava, both renal veins, and the right renal artery. The common bile duct passes caudally and to the right, behind the upper portion of the head, and enters the gland's substance. Due to the close relationship of the head of the pancreas with the common bile duct and duoden um, these organs are often affected by tumors or pancreatitis (**Di Dato *et al.* ,2025).**

➢ The tail is loosely developed in between the double peritoneal layer of the spleno-renal ligament. The space behind the pancreatic tail is an extension of the peritoneal space and it is referred to as the retropancreatic recess which forms a potential pathway for intraperitoneal spread of pancreatic disease **(Van Hoe and Claikens, 1999).**

➢ The neck of the pancreas lies immediately in front of the commencement of the portal vein which is formed by the union of the splenic and superior mesenteric veins Mahadevan (2019). The neck of the pancreas is a very important surgical landmark, especially its upper margin; there can be isolated the origin of the gastroduodenal artery from the common hepatic artery **(Dato and Bellino, 2025).**

3. Pancreas blood supply, lymphatic system and innervation

➢ The main arterial blood supply to the human pancreas is from the coeliac trunk, a branch of the descending aorta located below the diaphragm **(Figure 2).** Blood flow to the pancreas is 1% of the total, which equates to 50 ml/min **(Muratore *et al*., 2021).** The head of the pancreas receives most of its blood supply via the gastroduodenal artery with some contribution via the superior mesenteric artery. The body of the pancreas is mainly perfused by the splenic artery and dorsal pancreatic artery **(Rorsman and Ashcroft, 2018).** The greater pancreatic artery branches off from the splenic artery and the side branches anastomose with the transverse pancreatic artery (inferior) to supply blood to both head (close to duodenum) and tail (close to spleen) of the pancreas. The pancreas is drained via the splenic (body and tail) and pancreatico-duodeal veins (head) into the portal vein. Thus, the liver is the first organ to be exposed to the hormones released by the pancreas **(Coyle and Kulendran, 2024).**

➢ Pancreatic lymphatic drainage starts in little vases that originate in the pancreatic stroma, and they confluence into bigger lymph vessels that reach the pancreas surface. These vessels connect to the intricate complex of lymph stations that surround the pancreatic space; of great importance are the superior mesenteric lymphatic group and the coeliac group (**Di Dato *et al.,* 2025**).

➢ The pancreas exerts endocrine and exocrine functions in energy balance. The neural innervation and immune milieu are both crucial in supporting

pancreatic homeostasis. The neuronal network connects the pancreas with the central nervous system (CNS) and the enteric nervous system (ENS) and sustains metabolic activities. The nerves in the pancreas are categorized as spinal sensory afferent fibers, vagal sensory afferent nerves, autonomic fibers of both sympathetic and parasympathetic divisions, and fibers from the ENS and intrapancreatic ganglia **(Figure 3).** They innervate different regions and various cell types, which collectively determine physiological functions (Ding *et al.*, 2024).

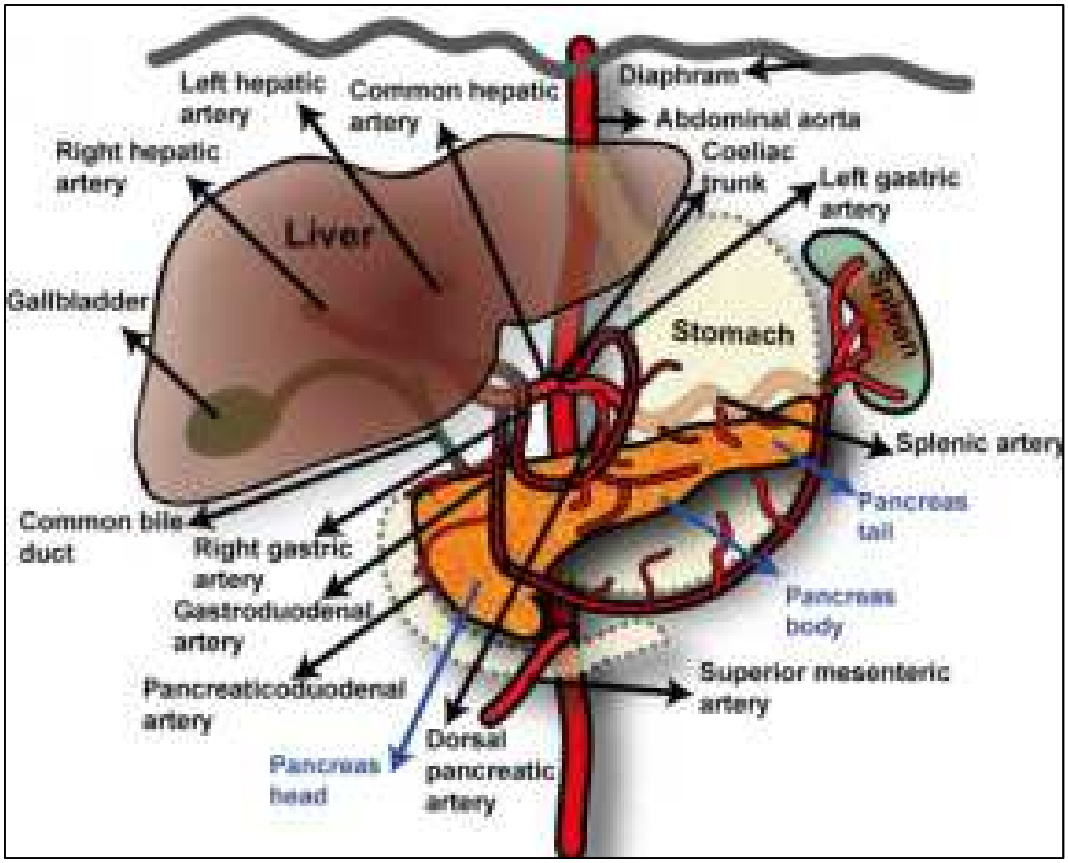


Figure 2. Pancreas main blood supply **(Muratore *et al.*, 2021)**

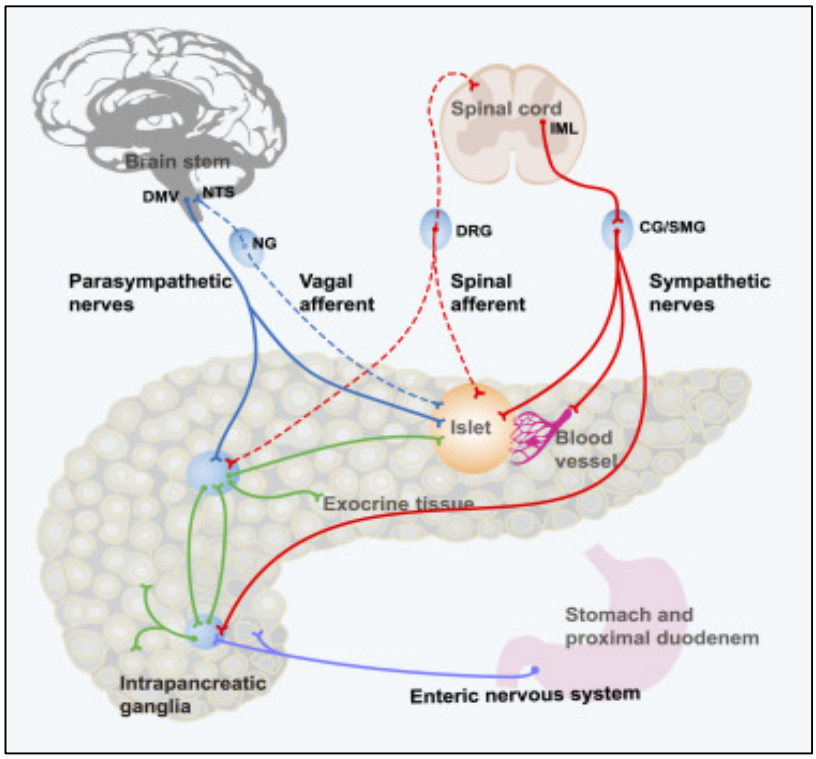


Figure 3: Innervation of the pancreas (**Ding *et al*., 2024**)

4. Histo-physiology of the pancreas

The pancreas is an unpaired, elongated, finely lobulated organ with a thin fibrous capsule, which has both endocrine and exocrine features. The lobules are comprised of acini cells with digestive secretions that classify the pancreas as an accessory exocrine digestive gland. These secretions travel through a ductal system into the duodenum. Between the acini lie the islets of Langerhans, which form the endocrine portion of the pancreas. The islets consist of four types of endocrine cells: alpha cells secreting glucagon, beta cell secreting insulin, delta cells secreting somatostatin, and pancreatic polypeptide cells secreting pancreatic polypeptide (**Coyle and Kulendran.,2024**). The pancreas is embedded in a connective tissue capsule, which delineates the lobules of the pancreas. The lobules comprise clusters of tubolo-acinar glands that are surrounded by connective tissue, blood vessels, pancreatic ducts and other supportive structures. In the histological structure of the pancreas, two basic elements are distinguished (**Karpińska and Czauderna, 2022**):

❖ The islets of Langerhans consist of five types of endocrine cell: α-cells (glucagon-producing), β-cell (insulin), δ-cells (somatostatin), ε-cells (ghrelin) and PP-cell (pancreatic polypeptide). The average islet diameter of the islets in humans and mice has been estimated to be 60–130 μm. Importantly, islet sizes are not normally distributed and the 10% largest islets account for 50% of the β-cell volume. In general β-cells locate to the core of the islet with α and δ-cells at the periphery, the distribution is more random in human islets.

❖ The acini are groups of secretory cells producing digestive enzymes. The acini constitute the rest of the organ and are responsible for the secretion of pancreatic juice and pancreatic enzymes. The **Figure 4** report the cellular heterogeneity of pancreas (the acini and islets of Langerhans cells)

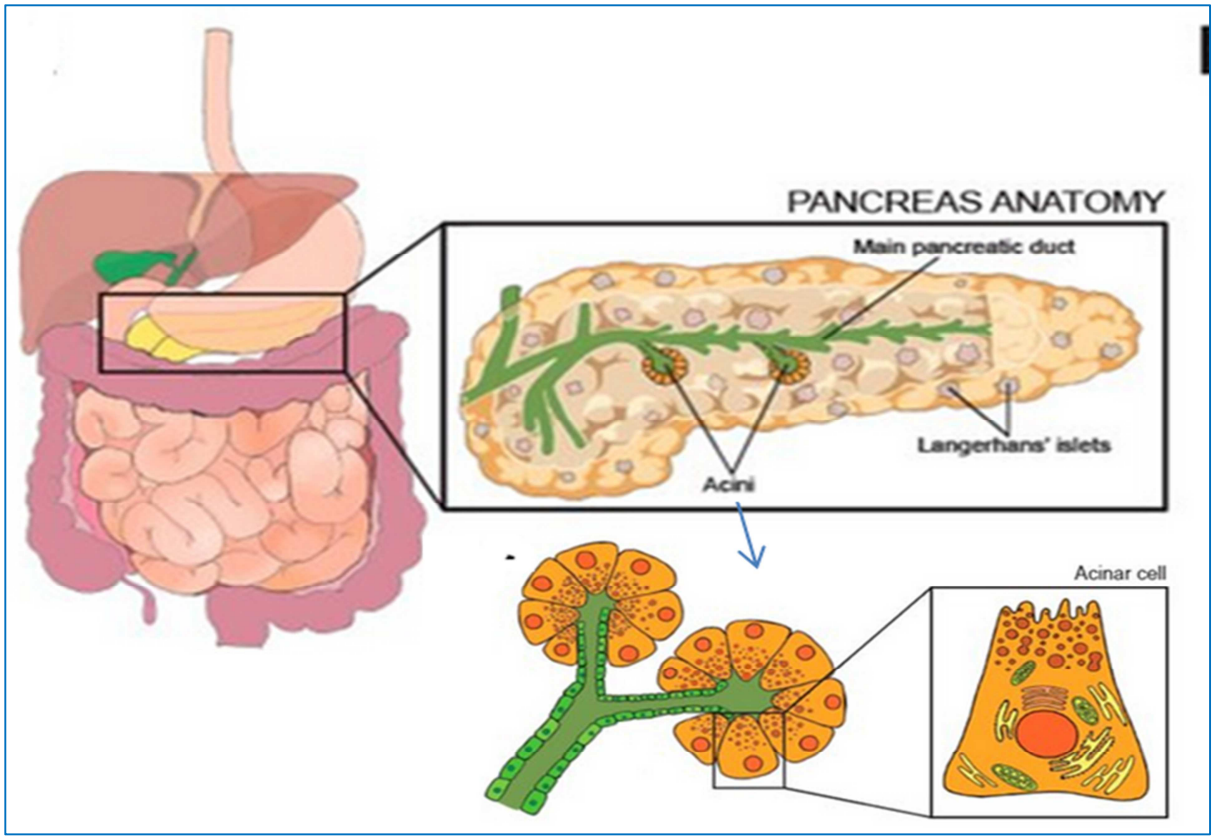


Figure 4. The cellular heterogeneity of the pancreas: exocrine (acini), endocrine (Langherans' islets) and stromal elements **(Paoli and Carrer , 2020).**

4.1. Endocrine function and insulin secretion

The endocrine pancreas is composed of clusters of cells formerly known as the islets of Langerhans, or more simply termed pancreatic islets, to which the secretion of a number of pancreatic peptide hormones for glucose homeostasis is attributed (**Leung and Leung., 2010).**There are approximately one million islets of Langerhans in the adult human pancreas **(Moldovan and Brunicardi 2001**). The human pancreas contains one to two million islets, primarily located in the body and tail, where they form the pancreatic endocrine portion. Each islet, about 300 μm in diameter, is surrounded by reticular fibres that penetrate the islet and encircle the network of capillaries [11]. Each islet of Langerhans contains five distinct cell types **(Nacher *et al*., 2017):**

- ✓ **α cells**, which produce the hyperglycemic hormone glucagon;
- ✓ **β cells**, which produce the hypoglycemic hormone insulin and ;
- ✓ **cells δ**, which produce somatostatin, a hormone which has an inhibitory effect on α and β cells;
- ✓ **PP cells**, which produce pancreatic polypeptide; and
- ✓ **ε cells**, which produce ghrelin.

Human islets have a lower percentage of **β cells** (55-75 %), a higher percentage of **α cells** (20-35 %) and a percentage of **δ** and **PP cells** around 10 %. These percentages illustrate that cellular composition varies significantly between different islets and different subjects. The cells of a pancreatic islet are distributed in such a way as to form a highly organized structure (**Ruiz-Cordero *et al.,* 2016)**.

In each islet, the β cells are arranged in the centre and are surrounded by α and δ cells at the periphery. Neurovascular bundles consisting of arterioles, sympathetic and parasympathetic nerves course through the center of each islet as shown in **Figure 5.** The arterioles form capillaries that pass between the cells to the peripheries and enter the portal system. This arrangement together with the presence of gap junctions allows hormonal products from one cell type to regulate the function of the surrounding different cell types. This type of local cell-to-cell regulation is also known as paracrine signalling. A good example in which insulin from β cells down-regulates glucagon secretion from α cells **(Lim *et al.,*2023).**

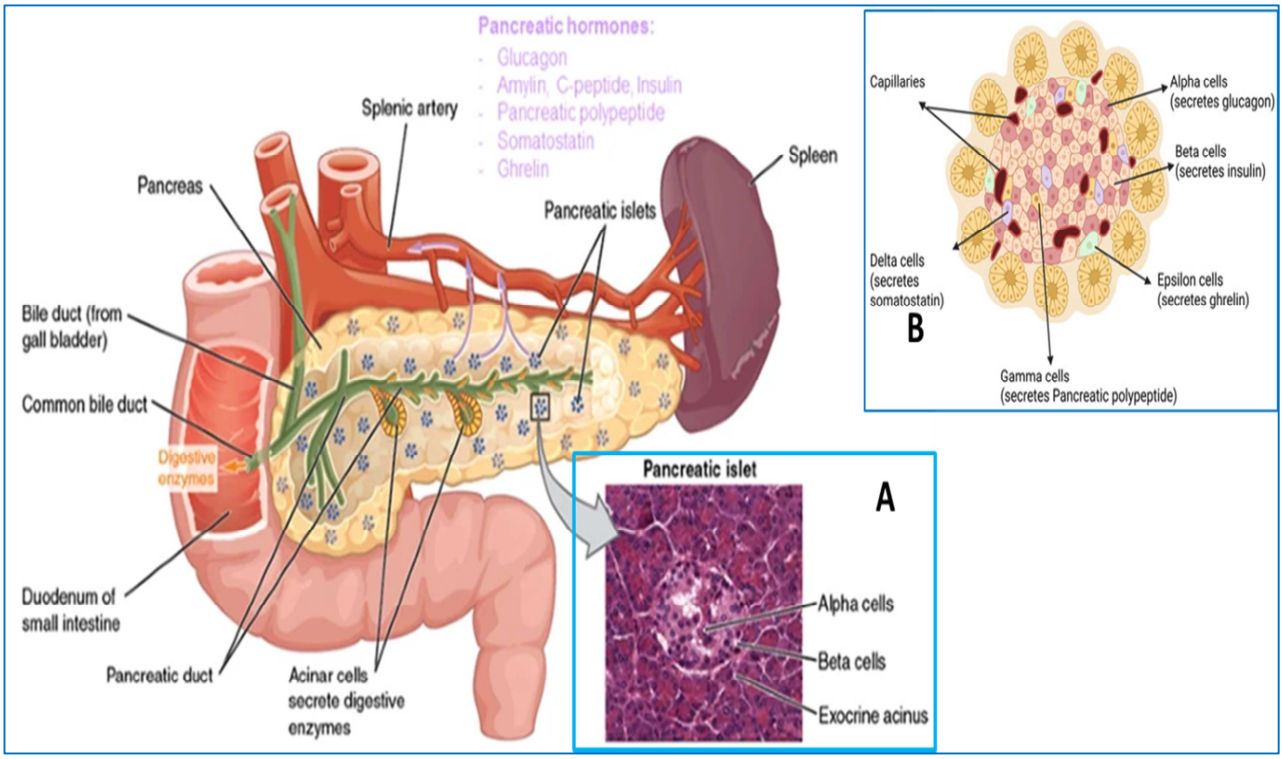


Figure 5. The micrograph shows the pancreatic islets **(A)** and A schematic representation of various cell types within the pancreatic islet**(B)** (**Carroll *et al*., 2024).**

4.1.1. β-cells and insulin secretion

➢ The β-cells are the principal component of the pancreatic islets in all species. They are polygonal cells, with an average diameter of 13–18 μm that possess ~10,000 secretory granules , each containing up to 8–9 fg insulin (**Rorsman and Renström 2003**). In an adult human being, beta cells release 30–70 U insulin per day (mainly depending on body weight), half of which is secreted after meals and the rest under basal conditions. The release of this hormone is regulated by a complex network of many different triggering, potentiating or inhibiting signals, which allows the supply of the hormone in amount, kinetics and adaptability to match the minute- by-minute variable needs of the body (**Marchetti *et al*.,2017).**

- Insulin, a peptide hormone composed of 51 amino acids is the most important hormone for energy metabolism such as glucose, fat and protein and for maintaining homeostasis (**Park *et al*., 2021**). Insulin secretion occurs from the B cell of the islets of Langerhans in the pancreas. It is stored in crystalline form in the secretory vesicles as a Zn_2-insulin complex and accounts for 5-10% of the total protein content of the β-cell, more than any other protein. It is released by regulated exocytosis. Only a small fraction of the secretory granules (<1%/h) undergo exocytosis even at high glucose concentrations. Human β-cells also contain lipofuscin bodies that can be used to estimate the age of the β-cells (**Rorsman and Ashcroft, 2018**).

- Exogenous glucose is taken up by GLUT2 and undergoes glycolysis inside the cell. Elevated ATP levels alter the ATP/ADP ratio, which in turn leads to the closure of ATP-sensitive K^+-channels (**Wendt and Eliasson, 2020**). The subsequent membrane depolarization opens voltage-dependent Ca^{2+}-channels in response to increasing intracellular calcium levels, which eventually trigger insulin secretion following vesicle fusion with the membrane (**Park *et al*., 2021**). To date, numerous soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) isoforms, including syntaxin-1, -3 and -4, SNAP-25 and -23, as well as syntaptobrevins 2 and 3 (VAMP2 and 3), have been shown to be involved in glucose-stimulated insulin secretion, whereas VAMP8, a non-essential SNARE protein for glucose-stimulated insulin secretion, has a role in the regulation of the glucagon-like peptide-1-potentiated insulin secretion. In addition to SNARE and SM proteins, a calcium sensor is required for the initiation of membrane fusion (**Arora *et al*.,2021).** Arginine is known as one of the strongest insulin secretagogues in a glucose-dependent manner, but major mechanism is unknown (**Umeda *et al*., 2015**). **Figure 6** shows the glucose-stimulated insulin release from a pancreatic β-cell.

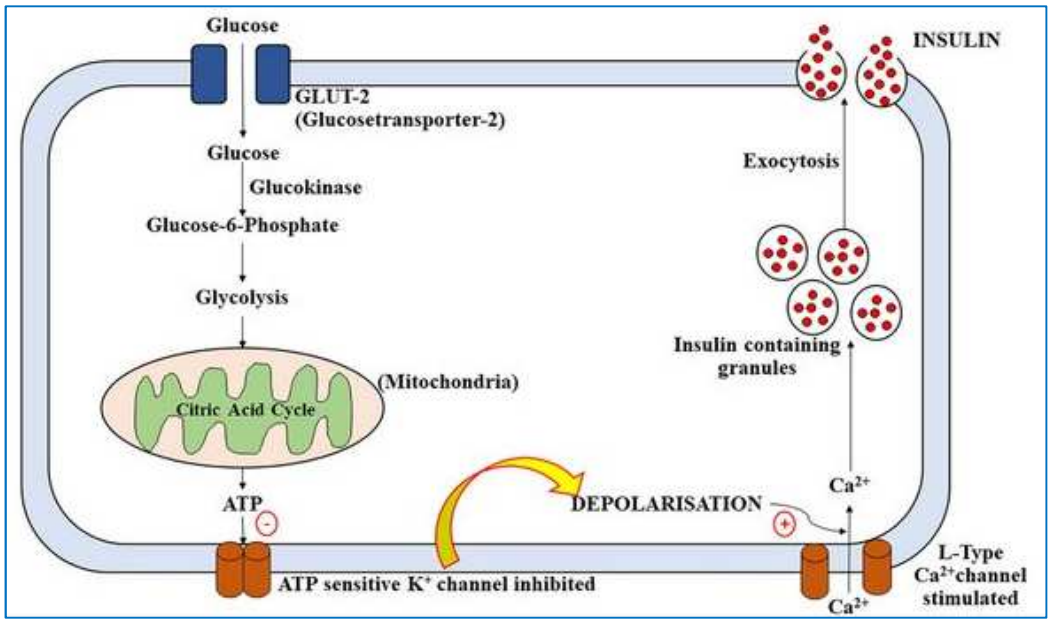


Figure 6. Mechanism of insulin secretion from beta cells of pancreas (**Arora *et al*.,2021**).

4.1.2. Maintenance of blood glucose levels by glucagon and insulin

Through its various hormones, particularly glucagon and insulin, the pancreas maintains blood glucose levels within a very narrow range of 4–6 mM (**Röder *et al*.,2016**). This preservation is accomplished by the opposing and balanced actions of glucagon and insulin, referred to as glucose homeostasis (**Figure 7).** During sleep or in between meals, when blood glucose levels are low, glucagon is released from α-cells to promote hepatic glycogenolysis (**Khan and Pessin, 2002).** In addition, glucagon drives hepatic and renal gluconeogenesis to increase endogenous blood glucose levels during prolonged fasting. In contrast, insulin secretion from β-cells is stimulated by elevated exogenous glucose levels, such as those occurring after a meal. After docking to its receptor on muscle and adipose tissue, insulin enables the insulin-dependent uptake of glucose into these tissues and hence lowers blood glucose levels by removing the exogenous glucose from the blood stream .Furthermore, insulin promotes glycogenesis, lipogenesis and the incorporation of amino acids into proteins; thus, it is an anabolic hormone, in contrast to the catabolic activity of glucagon **(Rorsman and Ashcroft , 2018).**

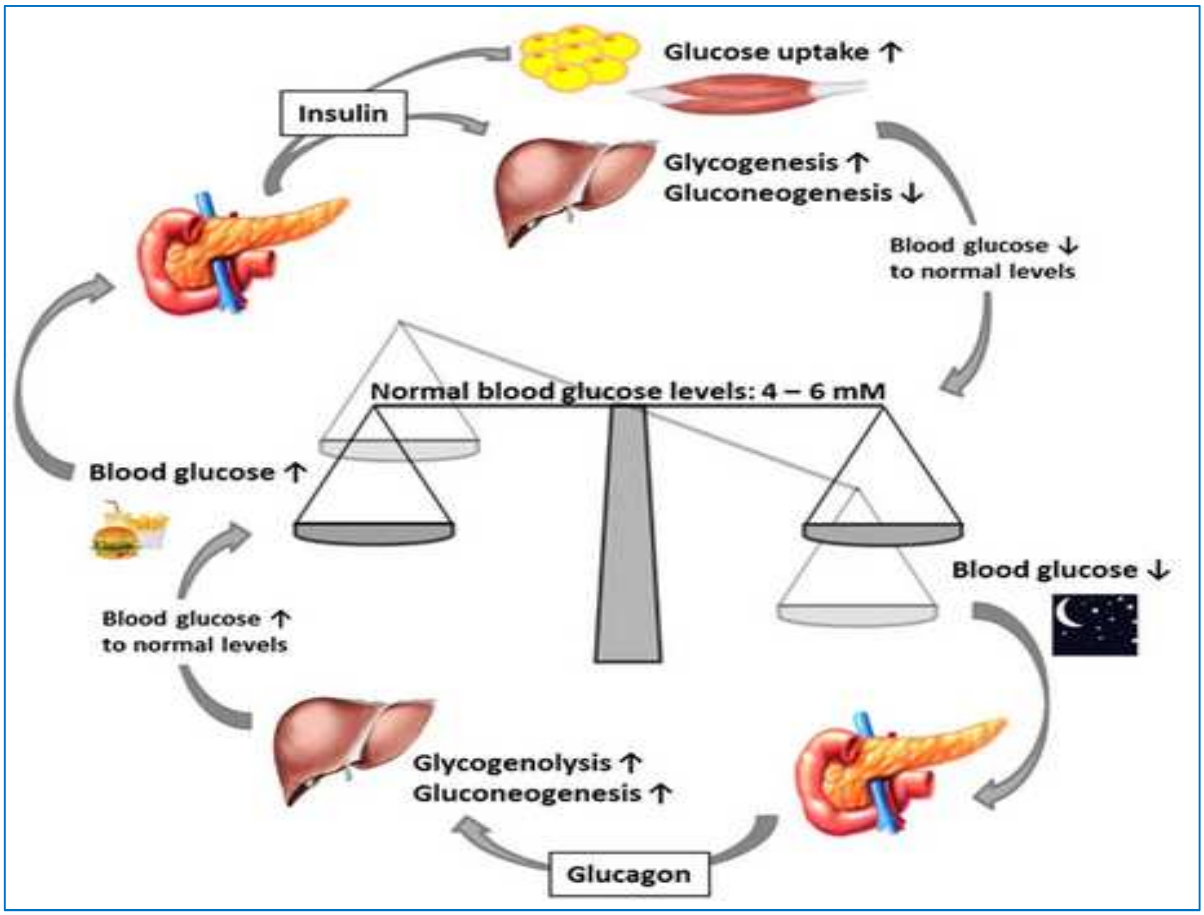


Figure 7. Maintenance of blood glucose levels by glucagon and insulin (**Röder *et al*.,2016).**

4.2. Exocrine function

The exocrine pancreas is an important organ because it is essential for the digestion of the food we consume **(Petersen *et al.,* 2021).** The exocrine part of the pancreas represents 90 percent of the pancreatic volume. It is constituted of acini, *i.e.* cell clusters that secrete pancreatic exocrine enzymes, and of ducts, which drain these enzymes into the duodenum **(Alexandre-Heymann *et al*., 2019)**. The presence of the acini, together with the rich excretory apparatus of the exocrine pancreas, allows its classification as a compound acinous (or alveolar) gland (**Motta *et al*.,1997**).

The digestive enzymes are stored in secretory granules which are concentrated near the apical membrane of the cell. The acini drain into the intercalated ducts, and groups of the intercalated ducts converge into larger intralobular ducts, which in turn drain into much larger extralobular ducts; the latter form a main collecting duct which empties into the duodenum **(Leung and Ip, 2006**). Acinar cells produce and store digestive (pro) enzymes, including proteases (trypsinogen, chymotrypsin, elastase, etc.), amylase, lipase, and nucleases, in zymogens. Fatty and amino acids in chyme stimulate intestinal release of cholecystokinin (CCK), a neuro-enteroendocrine hormone. CCK activates the vagus nerve leading to cholinergic stimulation of acinar cells and consequent release of zymogen granules **(Foster *et al*., 2020).**

1. Definition of diabetes

Diabetes is a chronic, metabolic disease characterized by elevated levels of blood glucose (or blood sugar), which leads over time to serious damage to the heart, blood vessels, eyes, kidneys, and nerves . There are various types of diabetes mellitus that include: Type1, Type 2, gestational, post-pancreatectomy, mature onset diabetes of the youth, neonatal diabetes, and medication-induced diabetes. Type1 contributes up to about 10 percent of the estimated 422 million diabetes cases worldwide **(Egan and Dinneen, 2019)**. The International Diabetes Federation estimates an overall rise in the prevalence of diabetes mellitus to 552 million by 2030 **(Alam *et al.*, 2014).** Diabetes mellitus involves a complete lack of insulin due to severe damage and death of beta cells. While the exact triggers are unknown, potential factors include viral infections, exposure to harmful chemicals, or autoimmune responses that target and destroy these cells **(Antar *et a*l., 2023).**

2. Etiologic classification of diabetes mellitus

Diabetes mellitus (DM) is broadly classified into three types by etiology and clinical presentation, type 1 diabetes, type 2 diabetes, and gestational diabetes. Some other less common types of diabetes include monogenic diabetes and secondary diabetes **(Figure 8).**

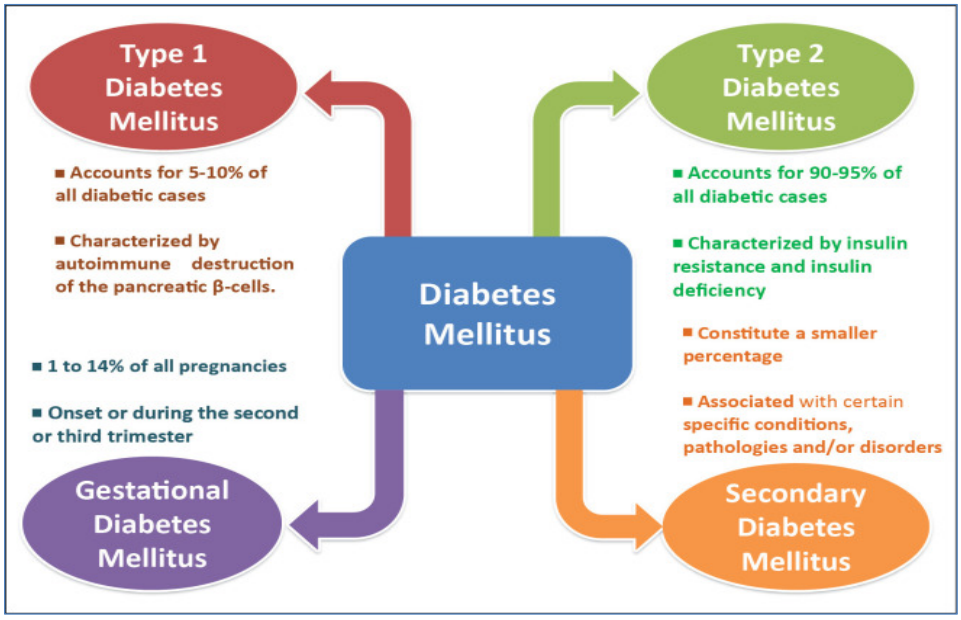


Figure 8. Classification of diabetes mellitus (**Banday *et al.*, 2020**)

2.1.Type 1 diabetes mellitus

Type 1 diabetes mellitus (T1DM) accounts for 5-10% of DM and is characterized by autoimmune destruction of insulin-producing β cells in the islets of the pancreas. As a result, there is an absolute deficiency of insulin. A combination of genetic susceptivity and environmental factors (viral infection, toxins, diet..etc) have been implicated as triggers for autoimmunity. T1DM is most commonly seen in children and adolescents though it can develop at any age (**American Diabetes Association, 2020**).

2.2.Type 2 diabetes mellitus

Type 2 diabetes mellitus (T2DM) affects 95% of the diabetic population. T2DM is characterized by a complicated process in which the fundamental issue is a balance between insulin production by β cells and insulin action, resulting in insulin resistance to insulin-stimulated glucose in the blood. Impaired glucose tolerance is the illness' intermediate stage that determines the risk of heart disease. Many individuals with T2DM are obese, indicating that obesity may induce some sort of insulin resistance. Because of the absence of apparent symptoms, T2DM is commonly undiagnosed. Most of the symptoms develop slowly and are frequently not severe enough to be detected **(Sanyaolu *et al*., 2023).**

2.3.Monogenic diabetes

A single genetic mutation in an autosomal dominant gene causes this type of diabetes. Around 1 to 5% of all diabetes cases are due to monogenic diabetes. MODY is a familial disorder and usually presents under the age of 25 years (**Bhattacharya *et al*., 2025**).

2.4. Secondary diabetes

Secondary diabetes is caused due to the complication of other diseases affecting the pancreas for example, pancreatitis. Certain drugs and toxins have the potential to interfere with insulin secretion or action, either on their own or by triggering diabetes in individuals who already have insulin resistance. For instance, drugs like nicotinic acid and glucocorticoids can impact insulin action **(Antar *et al.,* 2023).**

3. Factors and the risk of developing diabetes mellitus

Different pathological conditions perpetuating diabetes are depicted in **Figure 9.**

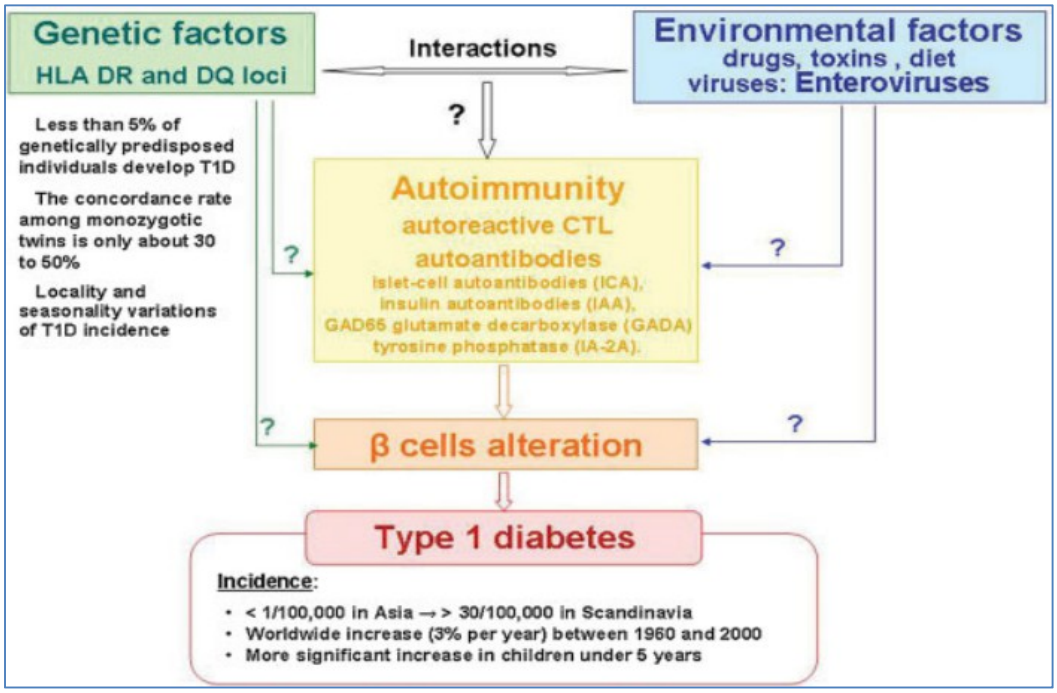


Figure 9. Genetic and environmental factors contributing to the development of diabetes (**Ja¨ıdane *et al*., 2010**).

3.1. Genetic Predisposition

Numerous suspect genes associated with type 1 diabetes mellitus have been identified, suggesting a need to focus on the disease's causal genes and mechanisms. At least 70 genes are suspected in the pathogenesis of type 1of diabetes. However, the most frequently implicated genes include human leukocyte antigen (HLA), insulin, cytotoxic T lymphocyte-associated antigen 4, and protein tyrosine phosphatase non-receptor type 22 . Mutations in these genes may lead to insulin insufficiency and, consequently, type 1of diabetes by tricking immune cells (T-cells and B-cells), into attacking self-antigens and triggering the autoimmunity of β cells (**Mittal *et al.,* 2024; Todd, 2024**) . Furthermore, this pathophysiology can be mediated through aberrant epigenetic modifications, including DNA methylation and histone post-translational modifications, in the mentioned genes (**Yahaya *et al*., 2024**). **Figure 10** showed the gene-

environment interaction can induce epigenetic modifications, initiating the autoimmune destruction of pancreatic β cells and consequently triggering the onset of Type 1 diabetes .

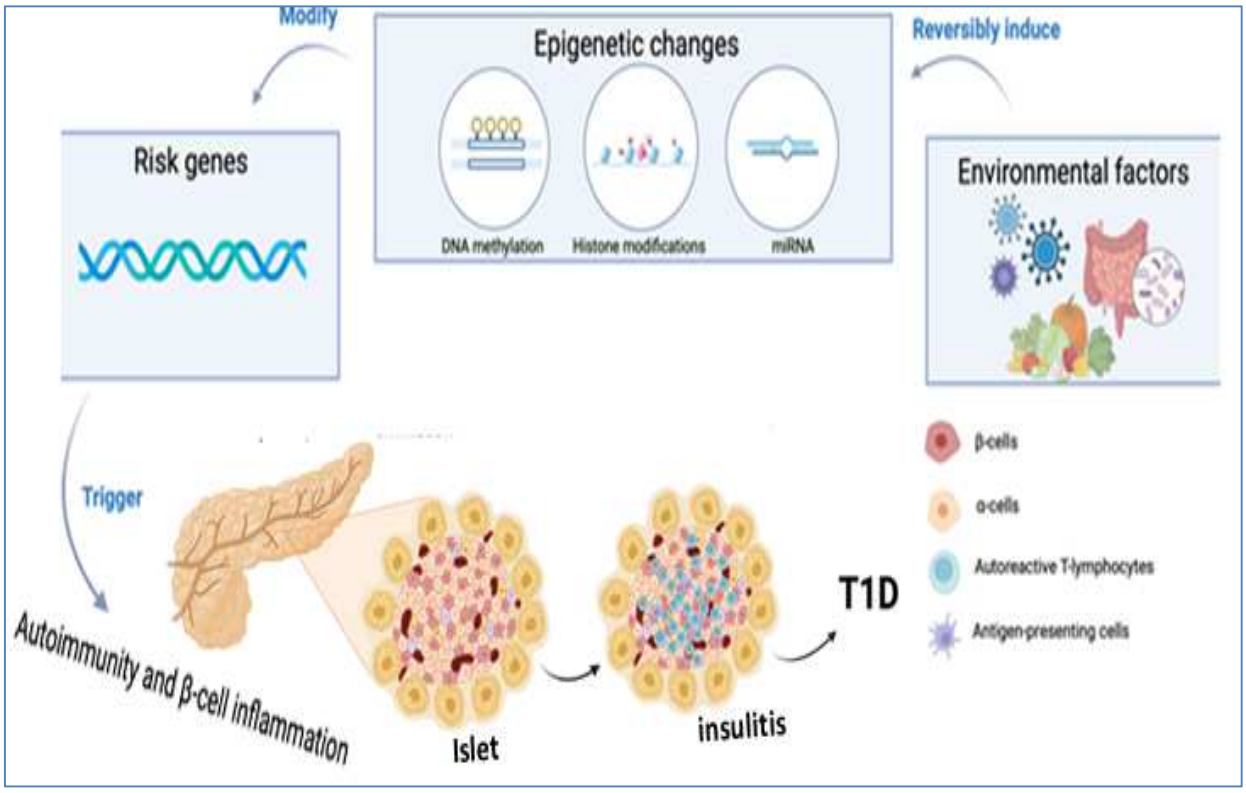


Figure 10. Gene-environment and the onset of Type 1 Diabetes **(Mittal *et al*., 2024).**

3.2. Environmental factors

Environmental factors play a role in etiopathogenesis of diabetes. Environmental factors include pollution, unhealthy diet, lack of physical activity, exposure to enteroviruses, and damage to immune cells.

3.2.1. Gut microbes

Gut microbiota–host interactions control energy homeostasis, glucose metabolism, and lipid metabolism. In addition to genetic and environmental factors, gut microbes may play an important role in the modulation of metabolic diseases **(Zheng *et al*., 2022**). Type-2 diabetes patients showed an increase in multiple pathogenic bacteria, such as *Clostridium hathewayi*,

Clostridium symbiosum and *Escherichia coli*, while healthy controls had a high abundance of butyrate-producing bacteria **(Keramat *et al*., 2013).**

3.2.2. Diet

Unhealthy food rich in saturated fatty acids, refined carbohydrates, and sweets cause obesity and DM. These nutrients in excessive quantities increase the risk of obesity and diabetes, especially if there is genetic risk (**Raman *et al.,* 2016**). These dietary factors increase inflammation and act as potential risk factors for diabetes.**(Kolb *et al*., 2017).** Exposure to unhealthy food retailers such as fast-food outlets has been associated with less healthy diets, obesity, increased insulin resistance, increased triacylglycerol concentration and type 2 diabetes, mainly in studies performed in the USA **(Duffey *et al.,* 2009).**

3.2.3. Viruses

Enteroviruses infect billions of people every year. Among them, coxsackievirus B (CVB) has been the most frequently associated with type 2 diabetes **(Tracy *et al*., 2002).** The CVB can cause acute inflammatory diseases like myocarditis, meningitis, and pancreatitis. Infection of isolated human islets with either CVB reduced their insulin content and glucose-stimulated insulin secretion **(Petzold *et al.,* 2015).** Prolonged inflammation due to viral persistence and antigenic stimulation has also been suggested as a potential mechanism leading to autoimmunity **(Chehadeh *et al.,* 2000).** Since 2020, from the beginning of the COVID-19 pandemic, more and more studies have been looking for a link between Severe Acute Respiratory Syndrome Coronavirus 2 and diabetes development **(Zorena *et al*., 2022).**

3.2.4. Physical inactivity

Sedentary life style with constant use of automobiles even for short distances, sitting long hours at office/study table, and more so in watching television and using computers have transformed the lives of most urbanites. These practices have increased the incidence of diabetes and heart disease (**Raman, 2016**). Furthermore, the built environment is hypothesised to be associated with type 2 diabetes incidence primarily through physical activity-related pathways. Indeed, meta-analyses consistently showed an established association of living in

neighbourhoods with high walkability and green space with a 10–20% lower risk of type 2 diabetes **(Beulens *et al*., 2022).**

3.2.5. Exposure to chemicals and drugs

Chemicals from water pollution and plastic bottles used for water-storing are implicated in immune dysfunction and type 2 diabetes **(Porta, 2006)**. Experimental chemical agents that induce diabetes are afloxan and streptozotocin. Alloxan selectively inhibits glucose-induced insulin secretion. Streptozotocin destroys the β cells, causing a state of insulin-dependent diabetes (**Lenzen *et al*.,2007)**. Drugs may induce hyperglycaemia through a variety of mechanisms, including alterations in insulin secretion and sensitivity, direct cytotoxic effects on pancreatic cells and increases in glucose production. Glycaemic adverse events occur more frequently with thiazide diuretics and inhibitors of the renin-angiotensin system **(Fathallah *et al.,*2015).** On a global level, glucocorticoid use is linked to 2% of new-onset diabetes mellitus (**Jain and Lai, 2024).**

4. Diagnostics of type 1 diabetes

The criteria for the diagnosis of diabetes mellitus for many years has required blood glucose measurements (**Kahanovitz *et al.*,2017**). The oral glucose tolerance test previously recommended by the National Diabetes Data Group has been replaced with the recommendation that the diagnosis of diabetes mellitus be based on: two fasting plasma glucose levels of 126 mg per dL or higher. Other options for diagnosis include two-hour postprandial plasma glucose (2hrPPG) readings of 200 mg per dL or higher after a glucose load of 75 g (**Mayfield, 1998**). Recently, professional organizations, including the American Diabetes Association have recommended the use of the hemoglobin A1c (HbA1c) for the diagnosis of diabetes. Chemically, HbA1c forms when glucose irreversibly binds to the N-terminal valine of haemoglobin's chain. Around 60 % of glucose attaches to the chain's valine, while the rest binds to the chain's valine or lysine residues. In healthy individuals, haemoglobin is composed of 97% HbA (adult haemoglobin), 2.5% HbA2, and 0.5% HbF (foetal haemoglobin). About 94% of HbA is non-glycated, while 6% is glycated primarily as HbA1c (5%), with minor fractions HbA1a and HbA1b (totaling 1%t) (**Kahanovitz *et al*., 2017**).

5. Physiopathology mechanisms of diabetes

Pathophysiology of diabetes is primarily driven by insulin resistance, and numerous studies have explored various contributing factors, including genetic and environmental influences, that lead to type 2 diabetes mellitus **(Figure 11)**.

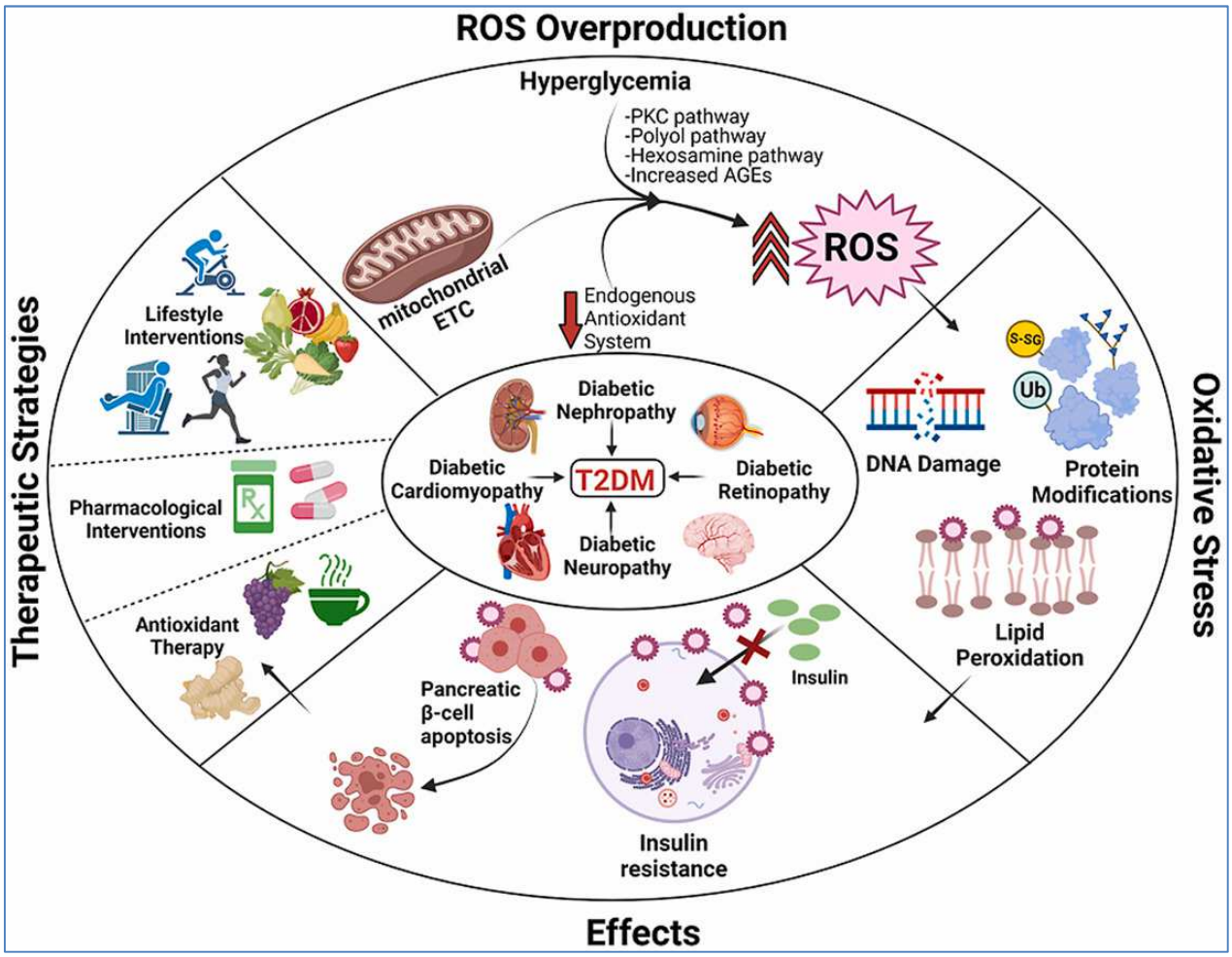


Figure 11. Physio-pathological mechanisms of diabetes (**Jasvinder *et al*.,2022).**

5.1. Insulin resistance

Insulin resistance is a situation when insulin does not show enough action particularly in reducing blood glucose level despite its sufficient concentration in blood. GLUT2 gene (liver and β cells) and GLUT4 gene (adipose tissue and skeletal muscle) are key target genes for the genetic susceptibility of diabetes (**Kohei *et al*., 2010; Sen *et al*., 2016).** Insulin

signaling starts when insulin binds to its receptors (IRs). Consequently, IRS 1/2 is phosphorylated in tyrosine, what leads to the downstream activation of PI3K and Akt. In skeletal muscle, Akt stimulates GLUT4 translocation to cell membranes thus allowing glucose to enter the cell (**Barbosa *et al.*,2014**). In liver, insulin stimulates glycogen production through phosphorylation of GSK-3 and consequently dephosphorylation of glycogen synthetase (**Ojo *et al.*,2023**). Signalling for insulin deficiency is triggered by several factors **(Figure 12),** including IRS or downstream-located effector molecules as well as posttranslational changes in mutations and in insulin receptors **(Lee *et al.*,2022).** The most changes in insulin resistance include (1) a reduction in the number of IRs and their catalytic activities; (2) enhanced Ser/Thr phosphorylation in the IRs and IRS; (3) enhanced Tyr phosphatase activity, in particular PTP-1B involving receptors and IRS dephosphorylation; (4) lowered PI3K and AKT kinase activity; and (5) defects in functions and expression of GLUT4 **(Pasupuleti *et al.*,2020).**

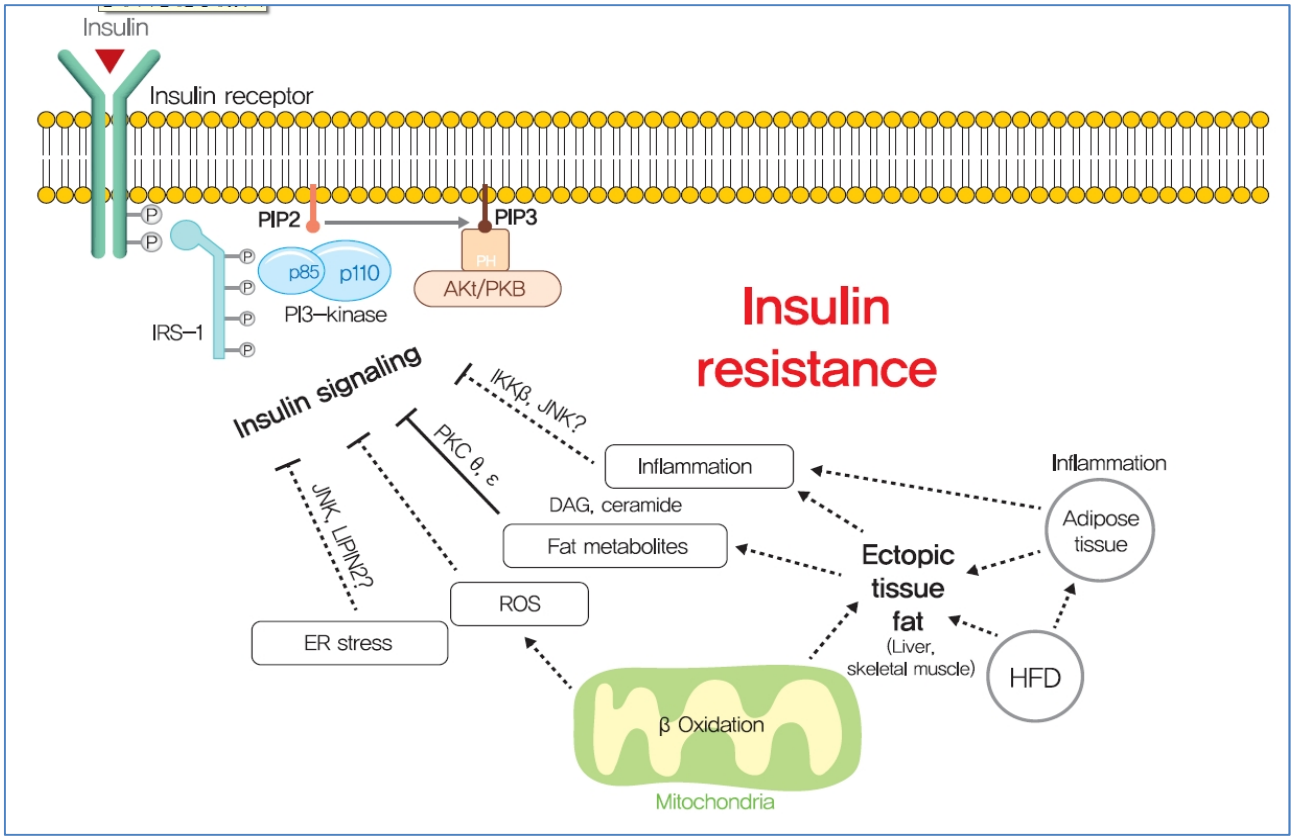


Figure 12. Potential mechanisms for insulin resistance **(Lee *et al.*,2022).**

5.2.Insulin secretion

Insulin secretion by pancreatic β is mainly controlled by blood glucose levels, gut hormones secreted in response to food intake have an important role in potentiating glucose-stimulated insulin secretion (**Vilsbøll and Holst, 2004**). These gluco-incretin hormones are GLP-1 (glucagon-like peptide- 1) and GIP (gluco-dependent insulinotropic polypeptide). Their action on pancreatic β cells depends on binding to specific G-coupled receptors linked to activation of the adenylyl cyclase pathway. In addition to their effect on insulin secretion both hormones also stimulate insulin production at the transcriptional and translational level and positively regulate β cell mass (**Thorens, 2003; Alsalim *et al*., 2023).** In patients with Type 2 diabetes, the incretin effect is either greatly impaired or absent, and it is assumed that this could contribute to the inability of these patients to adjust their insulin secretion to their needs **(Figure 13)**.

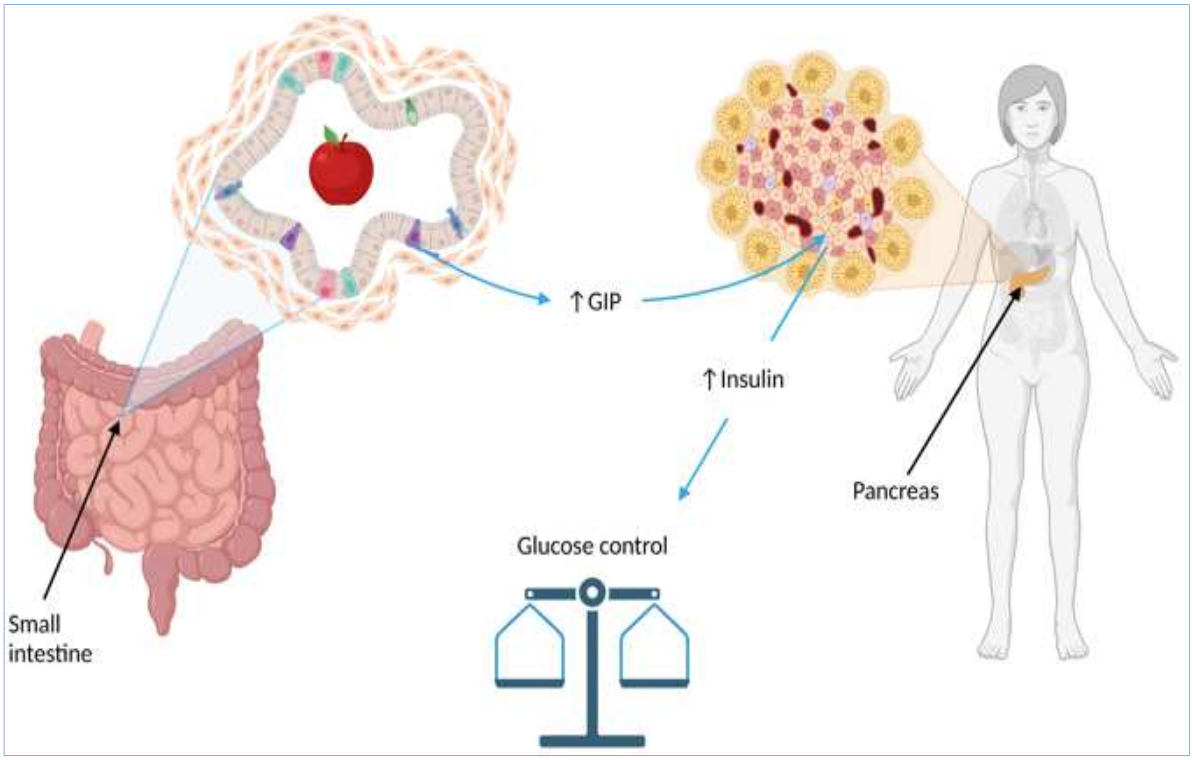


Figure 13. Gastro-intestinal effects of glucose-dependent insulinotropic peptide (GIP) in response to food **(Site 1).**

5.3 Increased hepatic glucose production

Hepatic glucose production accounts for 90% of endogenous glucose production, and it is crucial for systemic glucose homeostasis. The summation of fluxes from gluconeogenesis, glycogenolysis, glycogen synthesis, glycolysis and other pathways is referred to as Net hepatic glucose production (**Moore *et al.*, 2012**). When nutrients become scarce, insulin levels are decreased and glucagon is secreted from pancreatic cells to promote hepatic glucose production to meet brain and red blood cells energetic demands **(Petersen *et al*., 2017**). In type 2 diabetes, hepatic glucose production remains elevated after fasting and is inadequately suppressed by insulin, primarily due to increased gluconeogenesis rather than glycogen breakdown (**Rizza, 2010**). The rise in hepatic glucose production likely stems not only from worsening liver insulin resistance but also from declining β cell function (**Sharabi *et al*.,2015**). Insulin consistently overrides glucagon in suppressing hepatic glucose production, suggesting hyperglucagonemia alone cannot elevate hepatic glucose production without underlying insulin resistance **(Petersen *et al*., 2017).**

5.4. Diabetes, mitochondrial dysfunction and obesity

Diabetes mellitus, like other metabolic disorders, is linked to changes in mitochondria dysfunction (**Belosludtsev *et al.*, 2020**). Mitochondria play a role in reactive oxygen species (ROS) mediated signalling, apoptosis, calcium signalling, and steroid synthesis (**Montgomery, 2019**). Abnormal mitochondrial function results in lipid accumulation and insulin resistance, as cells require a balance between mitochondrial ATP synthesis through oxidative phosphorylation, and dissipation of the proton gradient to minimize damage from ROS **(Bournat and Brown, 2010).**

Free fatty acid metabolism is an important fuel source for skeletal muscle and correlate strongly with insulin sensitivity in obesity. Increased exogenous fat intake (obesity) and endogenous fat input leads to excessive lipid accumulation in insulin target tissues and β cells (**Adams *et al*.,2004**. The accumulation of toxic lipid metabolites *eg*, fatty acyl coenzyme-A (FACoA), diacylglycerol (DAG), and ceramide produce insulin resistance through serine kinase activation (**Abdul-Ghani and DeFronzo, 2008**). Furthermore, the impaired lipids of mitochondrial β-oxidation could be used as diabetic nephropathy progression markers **(Figure 14).** These studies suggest abnormal fatty acid β-

oxidation in the kidney during diabetic nephropathy (**Ming *et al*., 2022).**

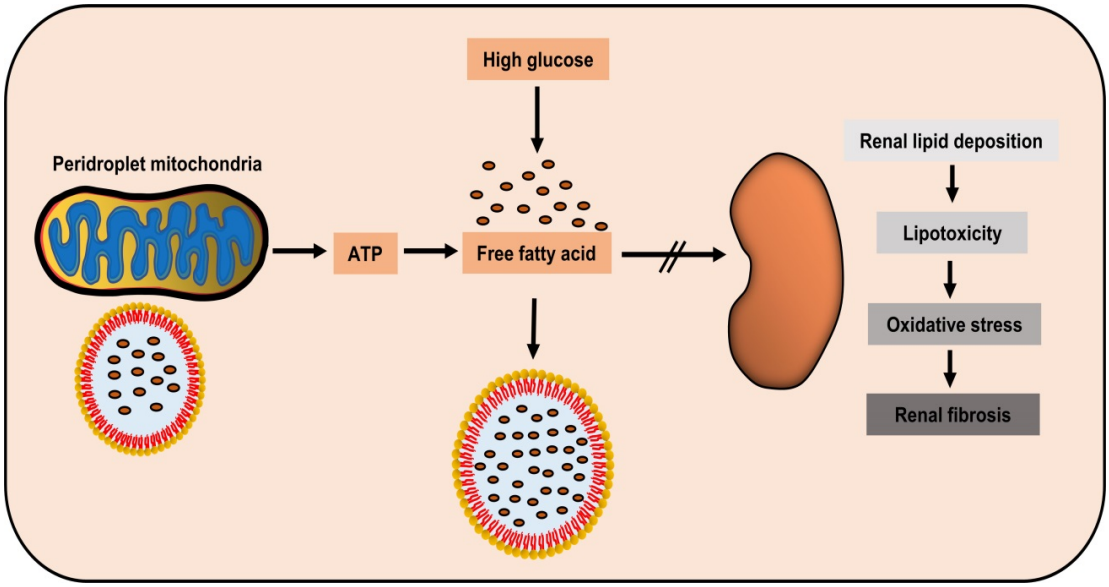


Figure 14. High glucose and mitochondria dysfunction increase free fatty acids levels in kidney (**Ming *et al*., 2022).**

5.5. Diabetes and oxidative stress

The hyperglycemia-induced ROS production contributes to micro- and macro-vascular diabetic complications. These ROS are produced in the endoplasmic reticulum, phagocytic cells and peroxisomes, with the mitochondrial electron transport chain playing a pivotal role **(Pasupuleti *et al*.,2020)**. A hyperglycaemic state can lead to an increase in the levels of oxidative stress-induced DNA damage markers such as 8-hydroxy-2'-deoxyguanosine; lipid-peroxidation; protein oxidation products such as nitrotyrosine and carbonyl levels and also lower the activity of antioxidant enzymes (**Oguntibeju, 2019).** It also modulates several intracellular signaling pathways that lead to insulin resistance and impairment of β-cell function **(Jasvinder *et al*.,2022; Pasupuleti *et al*., 2020)**. Additionally, oxidative stress activates protein kinase C (PKC) and accelerates the formation of advanced glycosylation end products (AGEs) as depicted in **Figure 15.** ROS may itself potentiate the generation of ROS along with other pro-inflammatory cytokines and chemokines around the β cells that disrupt the blood flow into the β-cells and abolish its function (**Bantayehu *et al*., 2024**).

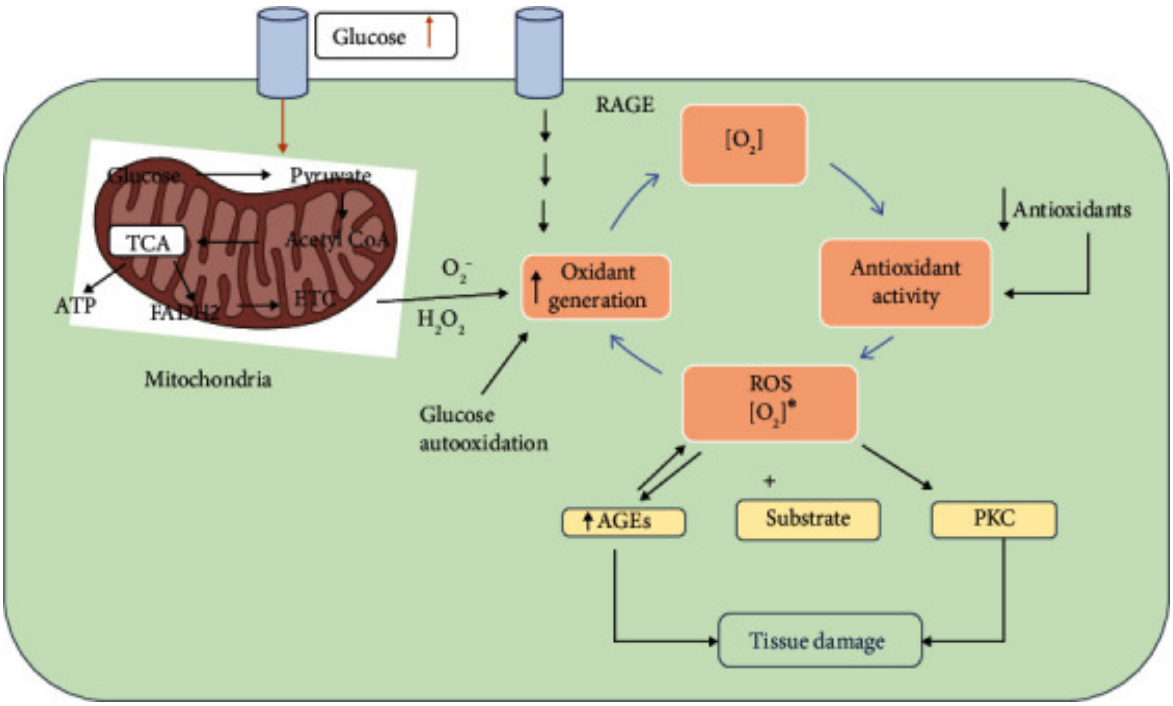


Figure 15. The role of ROS generation and oxidative damage in diabetes **(Pasupuleti *et al.*,2020)**. Glycosylation end products (AGEs) and activates protein kinase C (PKC)

5.6. Diabetes and inflammation

Both type 1 and type 2 diabetes show increased signs of systemic inflammation, with elevated levels of TNF-α and IL-6, which can directly contribute to insulin resistance including insulin regulation, ROS, lipoprotein lipase action and adipocyte function (**Graves *et al.*,2006**). These cytokines may not just indicate diabetes but could also play a role in causing type 2 diabetes. The heightened inflammation in diabetics has severe consequences, such as an 80 % risk of death from coronary artery disease in type 2 diabetes patients **(Navarro and Mora, 2005).** Type 1 diabetes patients with damaged β cells emit auto-antigens, to which the T-helper was exposed via antigen-presenting cells (APC). Active T-helper cells create cytokines that increase inflammation, which in turn causes ROS and Fas to be released, which cause β-cell death **(Bantayehu *et al.*, 2024). Figure16** show the common risk factor of inflammation for diabetes mellitus of both Types.

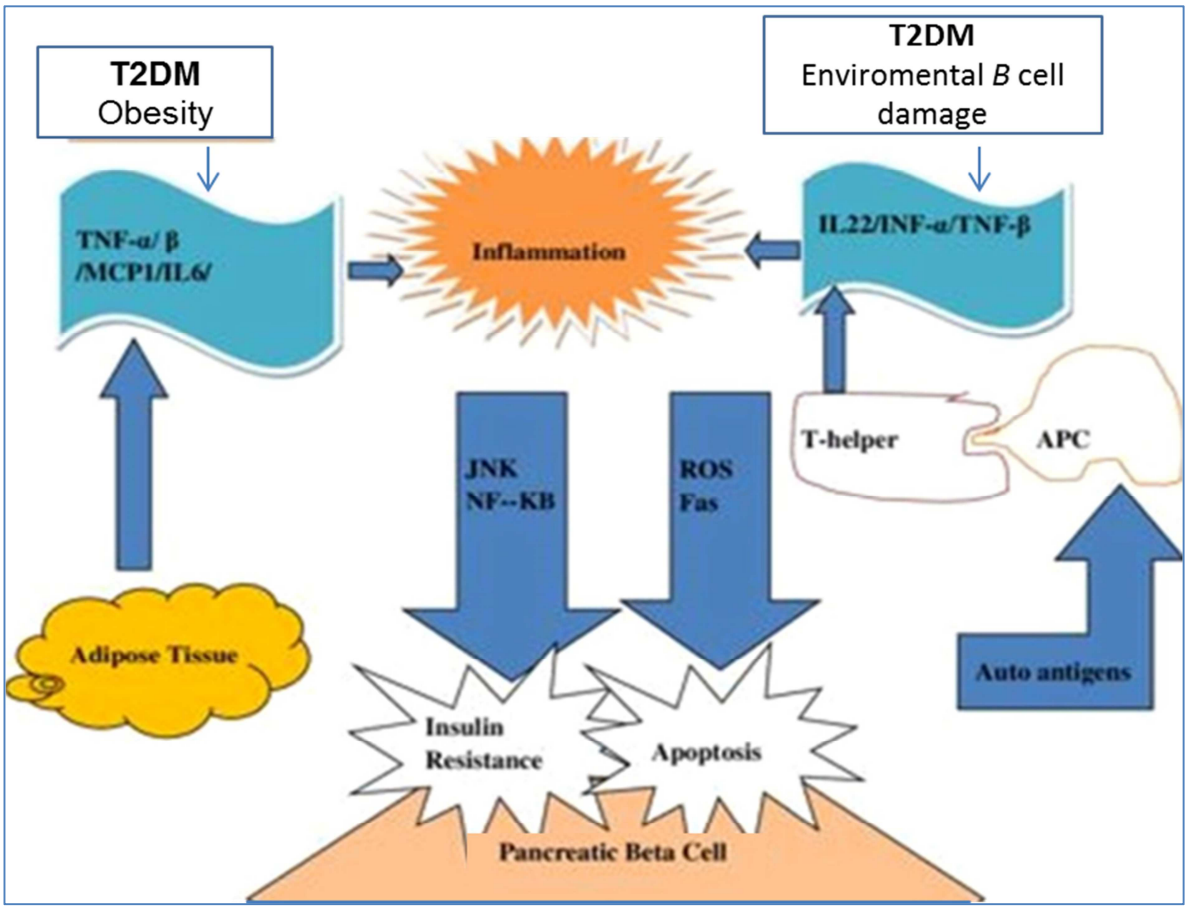


Figure 16. Common risk factor of inflammation for diabetes mellitus both types **(Bantayehu *et al*., 2024).**

6. Diabetes related complications

The high-glucose state of diabetes can contribute to diverse complications such as diabetic microangiopathy and peripheral neuropathy **(Figure 17).** Some of these diabetic microangiopathies involve retinopathy, nephropathy and heart disease, such as Retinopathy, Blind, Nephropathy, Proteinuria, Podocytes, Vascular and Cardiomyopathy. In contrast, peripheral neuropathy presents with Amputation and Schwann cells **(Papatheodorou *et al*,. 2015).**

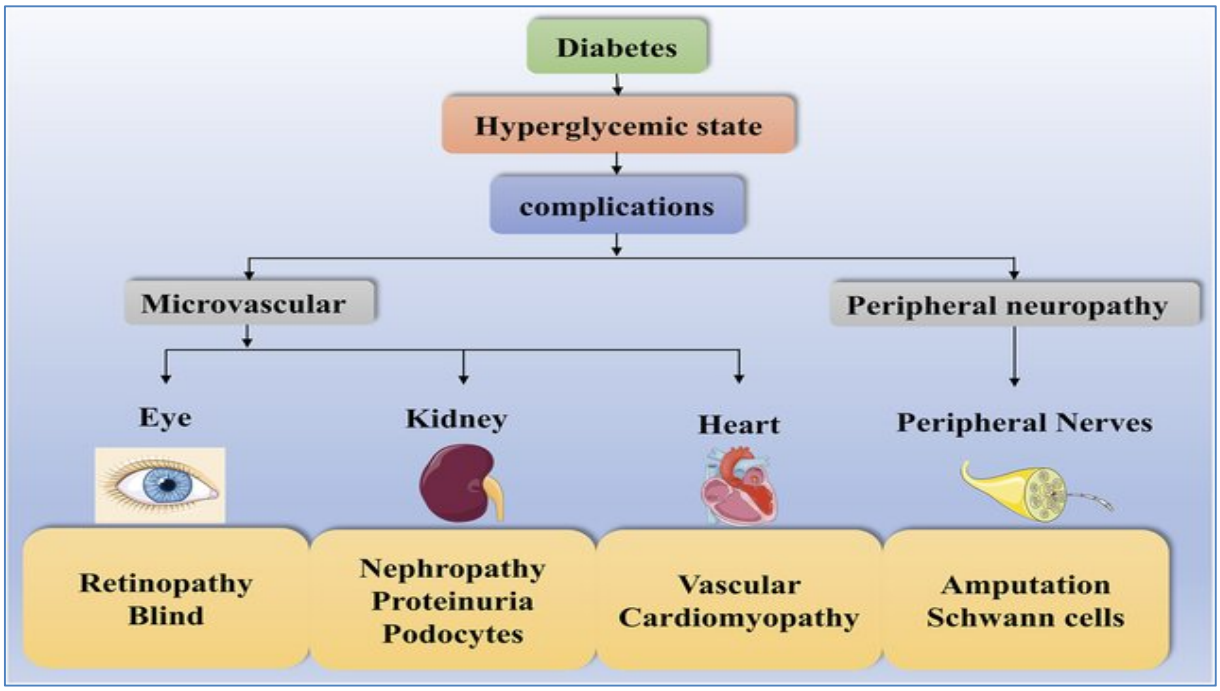


Figure 17. Diabetes complications (**Ge *et al.*, 2022**).

- **Renal system damage**: Clinically, diabetic kidney disease is characterised by increased urinary albumin output and a decline in the glomerular filtration rate, both reflecting a progressive deterioration of renal function (**Dronavalli *et al.*, 2008**).

- **Cardiovascular pathology:** Evidence suggests that hyperglycemia is an important contributor to the increased cardiovascular disease risk associated with diabetes. Hyperglycemia leads to glycation and peroxidation of proteins which cause arterial damage, and it also has direct toxic effects on arterial walls **(Marks and Raskin, 2000).**

- **Eye damage:** After years of clinically silent intraretinal changes, vascular tortuosity and retinal haemorrhage (**Wang and Lo (2018**). Fluid accumulation within the central neural retina, referred to as diabetic macula oedema, manifests as abnormal retinal thickening and is the most common cause of visual loss in individuals with diabetic retinopathy **(Nentwich and Ulbig , 2015).**

- **Nervous system damage:** In the central nervous system, diabetes may lead to cognitive impairment, leukoencephalopathy, and an increased risk of stroke and dementia (**Zochodne and Toth, 2014**). Various types of peripheral nerve disorders can develop, the most common being

distal symmetric polyneuropathy, affecting nerves of the extremities in a bilateral, symmetric pattern and progressing in a distal-to-proximal manner **(Eid *et al*.,2019).**

7. Therapia

The conventional treatment of T2DM involves use of oral medications such as thiazolidinediones, biguanides (metformin), meglitinides, dipeptidyl peptidase-4 (DPP-4) inhibitors, sulfonylureas, sodium/glucose cotransporter-2 (SGLT-2) inhibitors, incretin mimetics (GLP-1 receptor agonists), amylin mimetics and insulin analogues. The biguanides and thiazolidinediones come under the insulin sensitisers class, whereas sulfonylureas are the insulin secretagogues. The majorly used anti-diabetic agents are summarized below. Table 1 highlights various therapeutic interventions for the treatment of T2DM and its complications (**Jasvinder *et al*., 2022).**

1. Animals and treatment

Ten adult male, *Albino Wistar* rats, weighing 190± 10g were used. The rats used in the experiment were raised in the animal facility of Mentouri University of Constantine. Rats were maintained in sanitized polypropylene cages (5 per cage) under standard conditions of temperature (22 ± 2 °C), relative humidity (55 ± 10%), and photoperiod (12 h light and 12 h dark) with free access to food and water. Following an adaptation phase, the rats were divided into two homogeneous groups (5 rats/group):

- **Group1:** The control group received only saline 0.9% during 7 days of treatment (1 mL/kg)
- **Group 2:** The dexamethasone group received intraperitoneal injections of dexamethasone (10 mg/kg of the body weight) for 7 consecutive days **(Yashwant *et al*., 2011).**

Dexamethasone was used to induce liver function perturbation and diabetes mellitus. It is a synthetic corticosteroid with anti-inflammatory and anti-allergic action.

2. Specimens' collection

At day 8, rats were fasted overnight (12 h) and were then anaesthetized by chloroform and the abdomen was opened and the blood was collected from abdominal vein into heparinized tube and allowed to clot. Plasma was separated by centrifugation at 3500 rpm for 15 min and frozen until analysis. Livers were removed immediately, weighed and washed with a cold serum saline (0.9%). Liver was stored for the analysis of tissue oxidative stress markers.

3. Estimated Parameters

3.1. Body weight

Animals were weighed daily during investigation period.

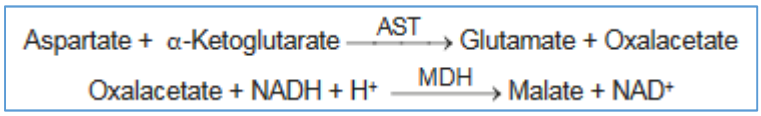
3.2. Biochemical Analysis

Blood transaminases (AST and ALT), cholesterol, triglycerides and glucose levels were measured colorimetrically using a kit supplied by Spinreact (Barcelona, Spain) and Shimadzu Uv-Vis1200 spectrophotometer. The detailed measurement protocols are placed in appendices section.

3.2.1. Aspartate aminotransferase

- ***Principle of the method***

Aspartate aminotransferase (AST) formerly called glutamate oxaloacetate (GOT) catalyses the reversible transfer of an amino group from aspartate to α-ketoglutarate forming glutamate and oxalacetate. The oxaloacetate produced is reduced to malate by malate dehydrogenase (MDH) and NADH:

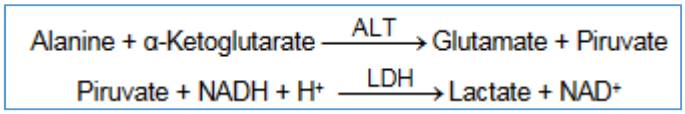


The rate of decrease in concentration of NADH, measured photometrically, is proportional to the catalytic concentration of AST present in the sample.

3.2.2. Alanine aminotransferase

- ***Principle of the method***

Alanine aminotranferase (ALT) formerly called Glutamate pyruvate transaminase (GPT) catalyses the reversible transfer of an amino group from alanine to α-ketoglutarate forming glutamate and piruvate. The piruvate produced is reduced to lactate by lactate dehydrogenase (LDH) and NADH:

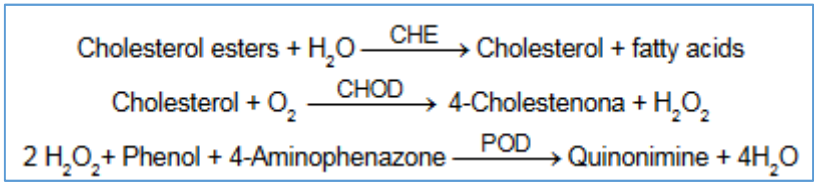


The rate of decrease in concentration of NADH, measured photometrically, is proportional to the catalytic concentration of ALT present in the sample.

3.2.3. Cholesterol

- ***Principle of the method***

The cholesterol present in the sample originates a coloured complex, according to the following reaction:

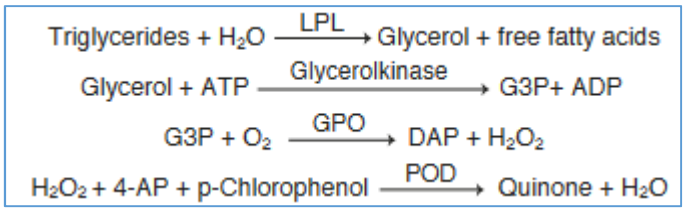


The intensity of the color formed is proportional to the cholesterol concentration in the sample.

3.2.4. Triglycerides

- ***Principle of the method***

Sample triglycerides incubated with lipoproteinlipase (LPL), liberate glycerol and free fatty acids. Glycerolis converted to glycerol-3-phosphate (G3P) and adenosine-5-diphosphate (ADP) by glycerol kinase (GK) and ATP. Glycerol-3- phosphate (G3P) is then converted by glycerol phosphate oxidase (GPO) to dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H_2O_2). In the last reaction, hydrogen peroxide (H_2O_2) reacts with 4-aminophenazone (4-AP) and *p*-chlorophenol in presence of peroxidase (POD) to give a red colored dye (Quinone). Reactions are summarized as fellow:

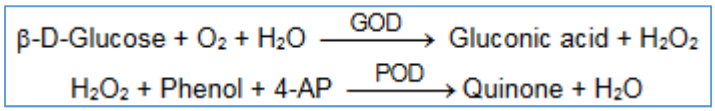


The intensity of the color formed is proportional to the triglycerides concentration in the sample.

3.2.5. Glucose

- ***Principle of the method***

Glucose oxidase (GOD) catalyses the oxidation of glucose to gluconic acid. The formed hydrogen peroxide (H_2O_2), is detected by a chromogenic oxygen acceptor, phenol, 4-aminophenazone (4-AP) in the presence of peroxidase (POD):



The intensity of the color formed is proportional to the glucose concentration in the sample.

4. Liver and kidney oxidative stress parameters

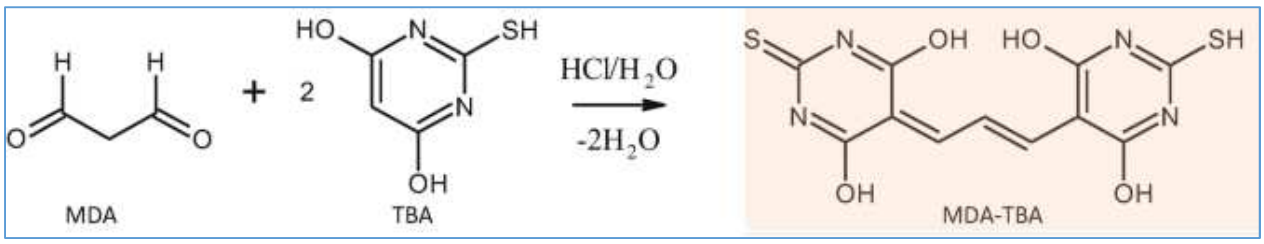
- ***Preparation of liver homogenate***

The liver samples were homogenized in potassium chloride solution (KCl: 1.15%) solution using IKA T18 Ultra Turrax homogenizer. Obtained homogenate was used for MDA and GSH estimation in liver.

4.1. Determination of lipid peroxidation level (MDA)

- ***Principle of the method***

The assay involves the reaction of lipid peroxidation products, primarily malondialdehyde (MDA), with thiobarbituric acid (TBA), which leads to the formation of MDA-TBA2 adducts called TBARS. TBARS yields a red-pink color that can be measured spectrophotometrically at 532 nm. The TBARS assay is performed under acidic conditions (pH = 4) and at 95 °C (**Ohkawa et *al*. (1979)** according to the following reaction equation:

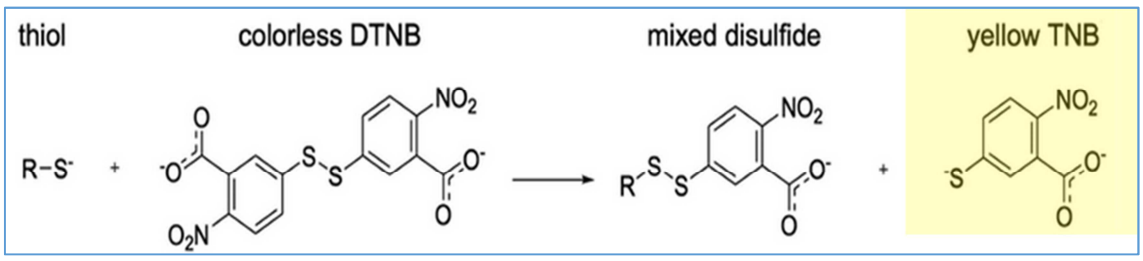


- For liver MDA estimation, 1 ml of homogenized tissue was incubated with 1 ml of TBA (0.37%) and 0.5 ml of trichloroacetic acid (TCA, 15%) for one hour at 95 °C. After cooling, the mixture was centrifuged and the absorbance of the supernatant was determined at a wavelength of 532 nm. The molar extinction coefficient of the MDA-TBA adduct (ε = 1.56 × 10^5 M^{-1} cm^{-1}) was used to determine the MDA concentration. The results are presented in nmol/g of tissue.

4.2. Determination of reduced glutathione (GSH)

- ***Principle of the method***

Ellman's assay is used to estimate free thiol groups. This reaction is based on the ability of the reagent 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) to react with the thiol to give the mixed disulfide and 2-nitro-5-thiobenzoate(TNB). In water (at neutral and alkaline pH conditions) TNB is in its anionic yellow form TNB^{2-}which is quantified by measuring the absorbance at 405 nm (**Ellman, 1959)** according to the following relation equation:



- For this assay, 0.8 ml of tissue homogenate is precipitated with 0.2 ml of trichloroacetic acid (10% TCA) for 10 minutes. Following centrifugation, 0.5 ml of the supernatant is incubated with 0.5 ml of DTNB (0.1 M) in Tris-EDTA buffer solution (0.02 M, pH 8.0). The absorbance of the mixture is evaluated at 412 nm against a blank prepared under the same conditions. The calculation of the GSH concentration is then done using the molar extinction coefficient of TNB ($\varepsilon = 13.6 \times 10^3\ M^{-1}cm^{-1}$), and the results are expressed in µM/mg of tissue.

5. Statistical Analysis

Results were expressed as mean ± SEM (Standard Error of the Mean). Statistical analysis was performed using Excel. The difference between the control group and the dexamethasone treated group was determined using the Student's *t*-test. All observed differences were considered significant at the 5% probability level ($p<0.05$).

1. Results

1.1. Effect of treatment on rat body weight change

Figure 18 shows the weight gain in the rats treated with intraperitoneal injection of dexamethasone (10mg/kg/day) for 7days. On the first day of experiment, no significant change in the body weight of dexamethasone group as compared to the control group.

By the end of the 7 days of treatment, there was a significant difference ($p<0.05$) in weight gain between the dexamethasone group and the control group. The body weight in dexamethasone group increased from 199.40±10g in day 1 to 229.80±5g in 7th day marking a significant increase compared to the control group.

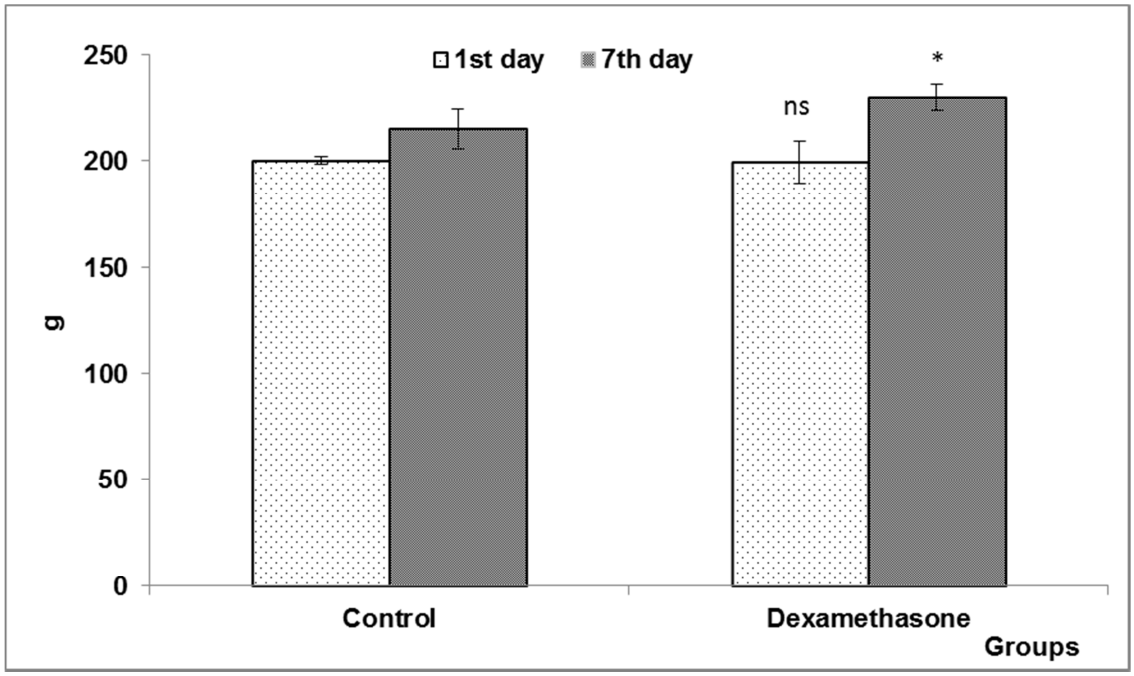


Figure 18. Body weight change

ns: non-significant difference;
*$p<0.05$: significant different between dexmethasone group and control.

1.2.Effect of treatment on plasma transaminases (AST and ALT) levels

Figure 19 represents liver enzymes AST (aspartate aminotransferase) and ALT (alanine aminotransferase) levels in blood of rats treated with intraperitoneal injection of dexamethasone (10mg/kg/day) for 7days. A significant increase in the activities of AST (126.7±6 U/L *vs* 99.7±11 U/L, $p<0.05$) and ALT (109.5±10 U/L *vs* 78.4±7 U/L, $p<0.001$) was observed in dexamethasone treated group when compared to the control group. The activities of theses liver enzymes increases due to various hepatotoxic xenobiotics substances.

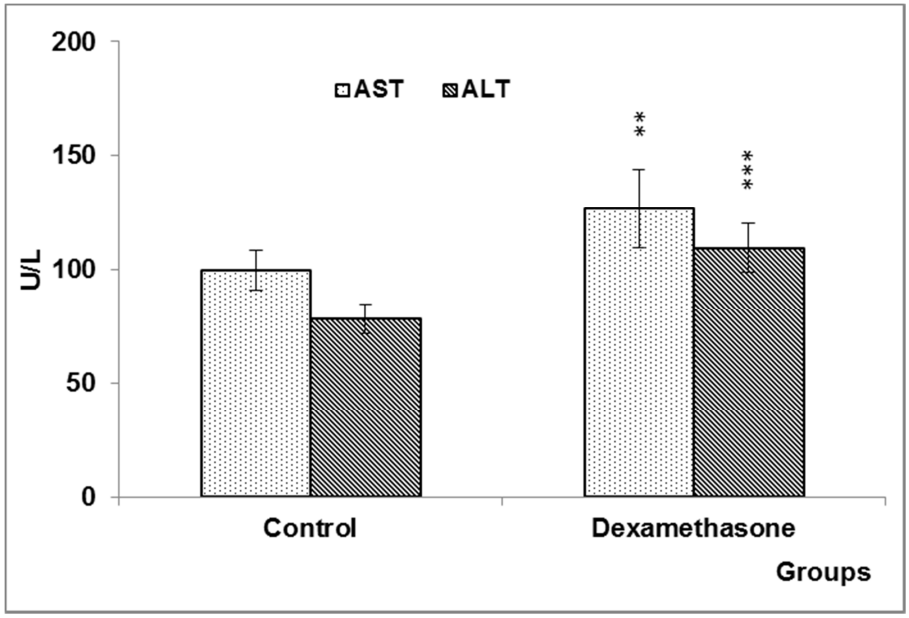


Figure 19. Plasma transaminases levels (ALT and AST)

**p<0.05 and ***p<0.001 : significant difference between dexmethasone group and control.

1.3. Effect of treatment on plasma triglycerides and cholesterol levels

Figure 20 illustrates the impacts of dexamethasone treatment (10mg/kg/day) for 7days on plasma triglycerides and cholesterol levels in comparison to the control group.

Triglyceride level in the control group was relatively low at approximately 58.86 ± 10 mg/dL. A highly significant increase was recorded in the dexamethasone group at levels approximately 94.29± 5mg/dL ($p < 0.001$).

On the other hand, plasma cholesterol levels registered in the control group was relatively low (91.27± 5mg/dL). Compared with those of control rats, the cholesterol level of rats treated with dexamethasone treatment (10mg/kg/day) for 7days were significantly higher (122.4mg/dL, $p < 0.05$).

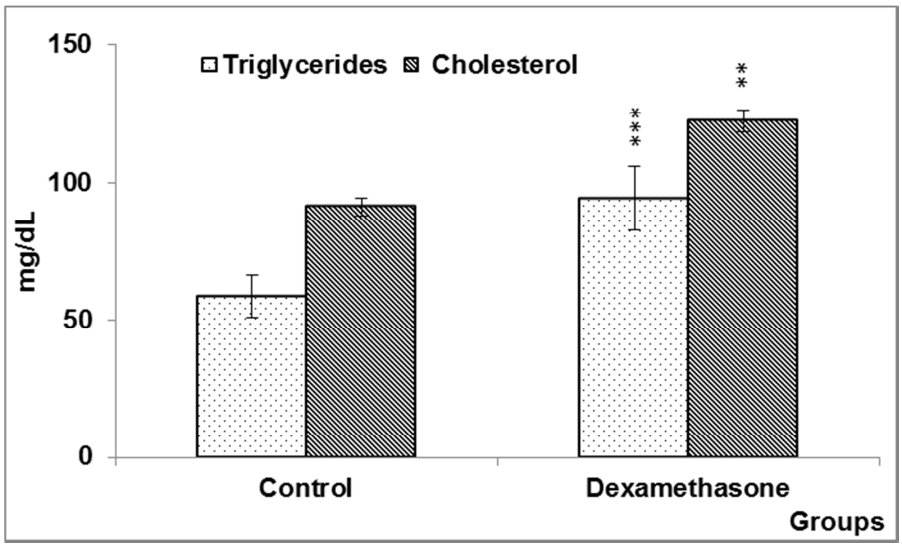


Figure 20. Plasma triglycerides and cholesterol levels

**p<0.05 and ***p<0.001: significant difference between dexamethasone group and control.

1.4. Effect of treatment on glycaemia levels

The results of the blood glucose levels following dexamethasone treatment (10mg/kg/day) for 7days are shown in the **Figure 21**. A significant increase in serum concentration of blood glucose level was noted in the group received dexamethasone (159.7±5 *vs* 92.3±9, P<0.01) when compared with control group. High glucose level in serum was also the outcome of dexamethasone toxicity.

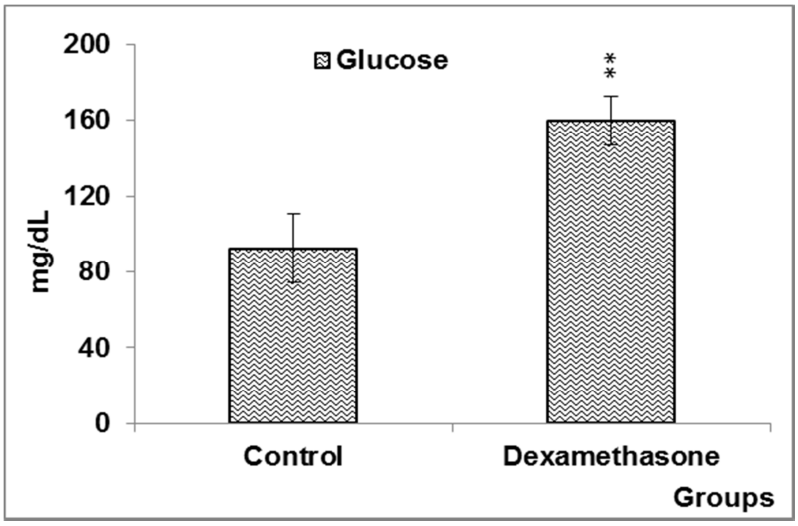


Figure 21. Glycaemia levels

**p<0.05: significant difference between dexmethasone group and control.

1.5. Effect of treatment on Liver oxidative stress markers (MDA and GSH)

In **Figure 22**, liver MDA levels were relatively low in the control groups. The level was recorded at 4.32±0.9 nM/g suggesting minimal lipid peroxidation and oxidative stress under normal physiological conditions. However, liver MDA levels rose significantly high in the dexamethasone group to 7.70 ±1.1 nM/g ($p < 0.001$).

In another hand, our results also showed high liver GSH levels (7.6 ±1.5 nM/g) in the control group compared to the dexamethasone group. The dexamethasone treatment (10mg/kg/day) for 7days induced a decline liver GSH levels (6.05 ±1.7 nM/g, $p< 0.01$).

Liver MDA and GSH results suggesting increased oxidative stress and lipid peroxidation in response to dexamethasone treatment.

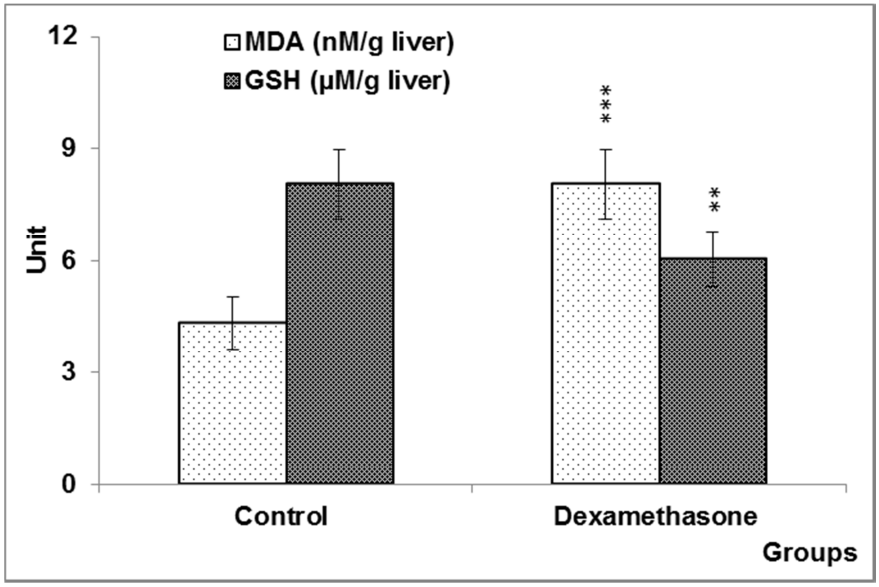


Figure 22. Liver oxidative stress markers (MDA and GSH)

**p<0.01 and ***p<0.001 : significant difference between dexmethasone group and control group.

Conclusion and Perspectives

Dexamethasone is among the most widely used artificial glucocorticoids. It has many properties like anti-inflammatory and immune suppressant. Dexamethasone is associated with vast range of diseases related to metabolism. Overtime, its use has been suggested to have several side effects, for example, hepatic injury, skeletal muscle atrophy, and insulin resistance. Dexamethasone increases the production of ROS, which in turn increases oxidative stress-mediated tissue damage.

Following our study, intraperitoneal administration of dexamethasone (10mg/kg/day for 7 days) induced significant metabolic associated hepatic injury *Albino Wistar* rats used , evidenced by elevated serum ALT and AST activity, the gold-standard biomarkers indicative of hepatocellular necrosis and loss of integrity. Concurrently, dexamethasone profoundly disrupted lipid metabolism, causing hypertriglyceridemia and hypercholesterolemia through the stimulation of adipocyte lipolysis probably *via* hormone-sensitive lipase up regulation and enhanced hepatic de novo lipogenesis. Dexamethasone treatment also induced hyperglycemia by disrupting glucose homeostasis through dual hepatic and pancreatic mechanisms: directly increasing hepatic glucose output *via* up regulation of gluconeogenic enzymes and inducing insulin resistance, while simultaneously impairing pancreatic β-cell function through inhibition of insulin synthesis and secretion, and promotion of apoptosis. Oxidative stress manifested by significantly elevated hepatic malondialdehyde (MDA), a key lipid peroxidation product reflecting ROS overproduction, and associated depletion of the critical antioxidant reduced glutathione (GSH). This redox imbalance signifies compromised antioxidant defences and directly contributes to cellular damage, intensifying hepatocellular injury as well as β-cell dysfunction, hence collectively demonstrating dexamethasone's liver and pancreas toxic potential.

Future research studies should prioritize developing new interventions such as specific antioxidants to counteract dexamethasone-induced oxidative stress. Also, glucocorticoids should be used with caution and their routine use should be avoided.

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GOT - ASAT
Kinetic Test. IFCC Without Pyridoxal phosphate
Quantitative determination of aspartate amino transferase activity

Reagent composition

Reagent 1 Buffer	Buffer Tris pH 7.8 à 30°C L- aspartate	80 mmol/l 200 mmol/l
Reagent 2 Substrate	NADH LDH MDH Oxoglutarate	0,18 mmo/l 800 UI/l 600 UI/l 12 mmol/l

Reagent Preparation

Working solution: Mix the substrate with 3 ml REF(10018) and REF(10032) or 10 ml REF (10025) of buffer R1.For REF (10049) reconstitute each R2 with one vial R1.

Procedure

Wavelength: 340 nm ;
Temperature : 25 – 30 or 37°C ;
Tank: 1 cm thick;
Adjust the spectrophotometer zero to air or distilled water.

Working solution	1 ml	3 ml
Pre-incubate at the selected temperature (25, 30 or37°C).		
Sample	100 µl	300 µl
Mix and incubate 1 minute. Measure the decrease in optical density per minute for 1 to 3 minutes.		

Calculation of ASAT concentration (at wavelength 340 nm) :

$$\Delta \text{ DO / min} \times 1750 = \text{IU/l}$$

GPT - ALAT
Kinetic Test. IFCC Without Pyridoxal phosphate
Quantitative determination of alanine amino transferase activity

Reagent composition

Reagent 1 Buffer	Buffer Tris pH 7.5 à 30°C Alanine	100 mmol/l 500 mmol/l
Reagent 2 Substrate	NADH LDH Oxoglutarate	0.18 mmo/l 1200 U/l 15 mmol/l

Reagent Preparation

Working solution: Mix the substrate with3 ml REF(11015) and REF(11039) or 10 ml REF(11022) of Buffer R1.For REF(11046) reconstitute each R2 with one vial R1.

Procedure

Wavelength: 340 nm ;
Temperature : 25 – 30 or 37°C ;
Tank: 1 cm thick;
Adjust the spectrophotometer zero to air or distilled water.

Working solution	1 ml	3 ml
Pre-incubate at the selected temperature (25, 30 or 37°C).		
Sample	100 µl	300 µl
Mix and incubate 1 minute. Measure the decrease in optical density per minute for 1 to 3 minutes.		

Calculation of ALAT concentration (at wavelength 340 nm):

$$\Delta \text{ DO / min} \times 1750 = \text{IU/l}$$

Cholesterol
Enzymatic Colorimetric test (CHOD- PAP)
Reagent for the quantitative determinationof Total Cholesterol

Reagent composition

Reagent 1 Buffer solution	Pipes pH 6.9 Phenol	90 mmol/l 26 mmol/l
Reagent 2 Enzymes	Cholesterol oxidase Peroxidase Cholesterol esterase Amino-4-antipyrine	300 U/l 1250 U/l 300 U/l 0.4 mmol/l
Reagent 3 Standard	Standard Cholesterol	200 mg/dl 2 g/l 5.17 mmol/l

Reagent Preparation

Working solution: Dissolve lyophilisate R2 with one vial of buffer R1

Procedure

Wavelength: 505 nm (500-550);
Temperature: 37°C ;
Tank: 1 cm thick;
Adjust the spectrophotometer zero on the reagent blank.

	Blank	Standard	Sample
Standard	- -	10 µl	- -
Sample	- -	- -	10 µl
Working solution	1 ml	1 ml	1 ml
Mix, read absorbances after incubation for 5 minutes at 37°C or 10 minutes at 20 - 25°C. Staining is stable 30 minutes.			

Calculation of Cholesterol concentration (mg/dl)

= (Abs. Sample/Abs. Standard) x n

n: Standard value , Abs: Absorbance

Triglycerides
Enzymatic colorimetric Method (GPO-PAP)

Reagent composition

Reagent 1 Buffer solution	Pipes buffer pH 7,2 Chloro-4-phenol	50 mmol/l 2 mmol/l
Reagent 2 Enzymes	Lipoprotein lipase Glycerokinase Glycerol 3-P-Oxidase Peroxidase Amino-4-antipyrine ATP	150000 U/l 800 U/l 4000 U/l 440 U/l 0,7 mmol/l 0,3 mmol/l
Reagent 3 Standard	Standard glycerol (trioleine)	200 mg/dl 2 g/l 2,28 mmol/l

Reagent Preparation

Working solution: Dissolve lyophilisate R2 with one vial of buffer R1

Procedure

Wavelength: 505 nm (490nm-550nm);
Temperature: 37°C;
Tank: 1 cm thick;
Adjust the spectrophotometer zero on the reagent blank

	Blank	Standard	Sample
Standard	- -	10 µl	- -
Sample	- -	- -	10 µl
Working solution	1 ml	1 ml	1 ml
Mix, read absorbances after incubation for 5 minutes at 37°C or 10 minutes at 20-25°C. Staining is stable 30 minutes.			

Calculation of Triglycerides concentration (mg/dl)

= (Abs. Sample/Abs. Standard) x n

n: Standard value , Abs: Absorbance

Glucose
Enzymatic Colorimetric Method (GOD- PAP)
Reagent for the quantitative determination of glucose in human plasma and cerebrospinal fluid (CSF)

Reagent composition

Reagent 1 Buffer	Buffer Tris pH= 7 Phenol	100 mmol/l 0,3 mmol/l
Reagent 2 Enzymes	Glucose oxidase Peroxidase Amino-4-antipyrine	10000 U/l 1000 U/l 2,6 mmol/l
Reagent 3 Standard	Standard Glucose	100 mg/dl 1 g/l 5,56 mmol/l

Reagent Preparation

Working solution: Dissolve lyophilisate R2 with contents of one vial Buffer R1.

Procedure

Wavelength: 505 nm (492 - 550);
Temperature: 37 °C;
Tank: 1 cm thick;
Adjusting the spectrophotometer zero with the reagent blank.

	Blank	Standard	Sample
Standard	- -	10 µl	- -
Sample	- -	- -	10 µl
Working solution	1 ml	1 ml	1 ml
Mix, read absorbances after 10 minutes incubation at 37 °C or 30 minutes at 20-25 °C. Staining is stable 30 minutes.			

Calculation of glucose concentration (mg/dl):

Glucose = (Abs. Sample/Abs. Standard) x n
n: Standard value , Abs: Absorbance

Academic year : 2024-2025	**Présenté par :** HASSAN Asha Ali

Study of Dexamethasone Toxicity in Rat Model

Thesis for obtaining the Master Degree Microbiology and Hospital Hygiene

Abstract

Diabetes mellitus, characterized by hyperglycemia and metabolic dysregulation, represents a global health crisis affecting 537 million adults, with projections identifying it as the 7th leading cause of mortality by 2030. Glucocorticoids like dexamethasone are widely used for immunosuppression. They induce severe metabolic complications, including steroid-induced diabetes. This study investigated dexamethasone induced diabetes and hepatic toxicity in a male *Albin wistar* rat model. Rats received intraperitoneal injection of dexamethasone (10 mg/kg/day) for 7 days to induce metabolic disturbance and toxicity.

Obtained results showed that dexamethasone induced an increase in rat's body weight, lipid profile parameters mainly; triglycerides and cholesterol and hyperglycaemia confirming the metabolic disturbance. Also, a rise in plasma transaminases (AST and ALT) was registered, indicating hepatic injury. Furthermore, obtained results showed an increase in hepatic malondialdehyde (MDA) and reduced glutathione (GSH), confirming redox imbalance and oxidative stress state flowing dexamethasone treatment.

This work establishes dexamethasone as a model for metabolic associated hepatic injury and underscores oxidative stress as a central mediator in glucocorticoid induced diabetes.

Keywords: *Dexamethasone, Glucocorticoid, Hepatotoxicity, Oxidative stress, Diabetes.*

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