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Effectiveness of Pectin on Serum Pancreatic Enzymes Activity of Type 2 Diabetes Mellitus

A Thesis

Submitted to the Council of the College of Medicine, University of Kerbala, as a Partial Fulfillment in the Requirements for the Degree of Master in Clinical Chemistry

By

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2022 A.D. **1444 A.H.**

بسم الله الرحمن الرحيم

﴿وَلَسَوْفَ يُعْطِيكَ رَبُّكَ فَتَرْضَىٰ(٢٦)﴾

صدق الله العلي العظيم

سورة الضحى

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Effectiveness of Pectin on Serum Pancreatic Enzymes Activity of Type 2 Diabetes Mellitus

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Dedication

To my family, who always picked me up on time and encouraged me to go on every adventure, especially this one.

Zahraa Raad

2022

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Zahraa Raad

2022

Summary

Background: Diabetes mellitus (DM) is a series of metabolic conditions that lead to hyperglycemia which is caused by impaired insulin secretion, poor insulin efficiency, or both. Type 2 diabetes mellitus (T2DM) is an islet paracrinopathy, in which the equal relationship between both the glucagon secreting alpha cell and the insulin-secreting beta cell is disrupted, resulting in hyperglucagonemia and hyperglycemia. Patients with T2DM are mostly characterized by being obese or having a higher body fat percentage, distributed pre-dominantly in the abdominal region. In this condition, adipose tissue promotes insulin resistance through various inflammatory mechanisms, including increased free fatty acid (FFA) release and adipokine deregulation. Pectin is a biocompatible polysaccharide polymer with intrinsic biological activity, which may exhibit different structures depending on its source or extraction method. Pectin is susceptible to physical, chemical, and/or enzymatic changes. Apple pomace and a smaller extent is the main source of commercial pectin. Pectin-modified nanomaterials has an antidiabetic agent in (T2DM) through decreasing blood glucose levels and/or insulin secretion after a sugar load, helps to reduce blood cholesterol, and reduces the risk of cardiovascular disease. Due to its high availability and low production cost, pectin can benefit treatments for weight reduction and obesity. Besides, as pectin is a weak acid, it resists dissociation in the gastric environment and binds covalently to the active sites of pancreatic lipase. Furthermore, it is highly desirable to study the effect of pectin-modified nanoparticles on the pancreatic enzymes in sera of (T2DM) patients by evaluating the lipase and amylase activities in the absence and presence of nanoparticles, evaluating of lipid profile, as well as ghrelin levels in sera of (T2DM) patients, and assess the relationship of pancreatic enzymes and studied biochemical parameters.

Method: It included a group of 39 patients with type 2 diabetes mellitus and 39 healthy people between the age of 35 to 60 years. Quantitative determination of α-amylase in serum was done by kinetic method (CNPG3) and the fasting serum glucose, lipase, total serum cholesterol, serum triglycerides, and HDL were determined by the colorimetric method. Additionally, LDL was calculated by the indirect method and VLDL was calculated by dividing the triglycerides concentration by 5. The HbA1c determination was based on the turbid metric inhibition immunoassay (TINIA) for hemolyzed whole blood and the measurement of serum human insulin and serum human ghrelin were done by an Enzyme-Linked Immunosorbent Assay (ELISA). The preparation of pectin nanoparticles, and calcium carbonate nanoparticles ($CaCO_3$ NPs). As well, calcium pectin core-shell nanoparticles denoted as Ca@pectin NPs were synthesized coating of the surface of calcium carbonate nanoparticles with pectin polymer. The characterization of prepared nanomaterials was done by using field-emission scanning electron microscopy (SEM), X-ray diffraction (XRD), dynamic laser scattering (DLS), ultra violet visible spectrophotometer (UV-Vis-NIR), Zeta potential, and transmission electron microscopy (TEM).

Results: Results indicated a significant difference in FBS among groups, The mean levels of FBS for DM was (203.7 ± 72.3) mg/dL which was significantly higher than for the control group (88.6 ±7.64) mg/dL, ($p \leq 0.001$). The mean of HbA1c for DM (9.5 ±2.35) was significantly higher than for control (4.9 ±0.48), $p \leq 0.001$. The mean levels of the Amylase & Lipase activity in the DM patients group were (71.4 ±29.11) and (49.7 ±14.69) (U/L) respectively, while the mean levels in the control group were (72.7 ±27.19) and (42.0 ±7.35) (U/L). The mean levels of Lipase activity were shown a significant difference in T2DM patients compared to the healthy control group, ($p < 0.05$). Serum hormones were also

increased significantly ($P < 0.05$), suggesting that DM patients had an obvious insulin resistance. The mean levels of the serum Insulin & Ghrelin in the DM patients group were (18.3 ±6.04) and (3.3 ±1.01) (IU/L) respectively, while the mean levels in the control group were (14.5 ±5.41) and (2.6 ±0.71) (IU/L). Lipid abnormalities in patients with diabetes often termed “diabetic dyslipidemia”, are typically characterized by high total cholesterol (T-Chol), high triglycerides (TG), low range of high-density lipoprotein cholesterol (HDL-C), and massively increased levels of small dense (VLDL and LDL) particles. The morphology and structure of the prepared nanopectin were characterized by using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The average size of Ca@pectin NPs increased after coating with pectin polymer, indicating successful coating of pectin on the surface of $CaCO_3$ NPs. The particle sizes of the as-made nanomaterials were also further examined using dynamic laser scattering (DLS) measurements, demonstrating the successful pectin polymer coating on $CaCO_3$ NPs and the formation of $CaCO_3$@pectin core-shell NPs. The crystalline structure of $CaCO_3$@pectin was further identified by the X-ray diffraction (XRD) pattern. The results showed that the $CaCO_3$ NPs are highly crystalline while the Ca@pectin NPs are amorphous. These results clearly indicate the successful formation of Ca@pectin composite NPs. The optical absorption of $CaCO_3$@Pectin NPs has the characteristic peak of $CaCO_3$ which at near 400 nm is clear evidence for the formation of $CaCO_3$@Pectin NPs. The pool activity of serum Amylase in the presence of $CaCO_3$@Pectin NPs, calcium nanoparticle, and pectin polmer was 63.1< 82.2< 109.3 (U/L) respectively, while the pool activity of serum lipase was 12.9> 12.2< 20 (U/L). The spearman rank test analysis was used to analyse the response relationship between parameters. Mainly, serum FBS was positively related to the HbA1c, TG, and VLDL levels. In addition, TG levels were significantly associated with total cholesterol, VLDL, and Insulin levels. As a risk

factor for dyslipidemia in (T2DM), serum LDL was highly significantly and positively related to high levels of total cholesterol (all $P < 0.001$). The overall relationship between the parameters and study cases are also the individual Correlation Coefficient of the Ghrelin, Lipase, and Amylase.

Conclusion: $CaCO_3$@Pectin NPs have an inhibitory effect on the activity of the enzymes lipase and amylase, while the pectin polymer showed an activating effect on enzyme amylase. Therefore, it is possible to study the possibility of using pectin nanoparticles in various forms as a carrier of diabetes drugs according to the physiological state of the diabetic patient.

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List of abbreviations
BMI: Body mass index.
$CaCO_3$: Calcium carbonate.
$Cacl_2$: Calcium chloride.
CETP: Cholesteryl ester transports protein.
DM: Diabetes mellitus.
DE: De-esterification.
EC: Enzyme commission number.
EDENMs: Endocrine disrupting engineered nanomaterials.
ENMs: Engineered nanomaterials.
FFA: Free fatty acid.
FPG: Fasting plasma glucose.
GalA: D-galacturonic acid.
GH: Growth hormone.
GLUT: Glucose transporter.
HbA1c: Glycated haemoglobin.
HG: homogalacturonan.
HDL:High density lipoprotein.
IEC: International expert committee.
IR: Insulin resistance.
LDL:Low density lipoprotein.
LPL:Lipoprotein lipase.
MNPs: Metal nanoparticles.
min: Minute.
mL: Milliliter.
µg: Microgram.
µL: Microliter.
NPs: Nanoparticles.
ng: Nanogram.
PG: Plasma Glucose.
RG-I: rhamnogalacturonan I.
RG-II: rhamnogalacturonan II.
Rha: Rhamnose.
rpm: Rotation per minute.
TTAB: Tetra-decyltrimethylammouium.
T2DM: Type 2 diabetes mellitus.
TAG: Triacylglycerol.
VLDL:Very low density lipoprotein.
WHO: World health organization.

CHAPTER ONE

Introduction and literature review

1. Introduction

1.1. Type 2 diabetes mellitus (T2DM)

Diabetes mellitus (DM) is a series of metabolic conditions that lead to hyperglycemia which is caused by impaired insulin secretion, poor insulin efficiency, or both (1,2). DM is characterized by the existence of immune-mediated (Type 1 diabetes), insulin resistance (Type 2 diabetes), gestational or other chronic hyperglycemia; genetic, environmental abnormalities, infections, and some drugs or affects the beta cells of the islets of Langerhans (3–5). Type 2 diabetes mellitus (T2DM) is a major public health issue or threat in the twenty-first century while nowadays; no country escapes from the diabetes invasion (6). The estimated worldwide prevalence of T2DM is expected to increase to 6.4% in 2030 in contrast with the percentage of 4.6% in 2000.

The progression of the disease makes insulin secretion unable to maintain glucose homeostasis, producing hyperglycemia. Patients with T2DM are mostly characterized by being obese or having a higher body fat percentage, distributed pre-dominantly in the abdominal region. In this condition, adipose tissue promotes insulin resistance through various inflammatory mechanisms, including increased free fatty acid (FFA) release and adipokine deregulation (7).

The main drivers of the T2DM epidemic are the global rise in obesity, sedentary lifestyles, high caloric diets, and population aging, which have quadrupled the incidence and prevalence of T2DM (7, 8). Overall, the prevalence of diabetes is higher in men than in women, and most commonly in adults aged 40 years or older. In addition, the incidence of T2DM is increasing more rapidly in adolescents and young adults. India, China, the United States, Indonesia, and Japan are the top five countries for T2DM prevalence (9, 10).

The middle-east is highly affected by T2DM with a prevalence rate varying between 7 and 22%. (11) T2DM remains a leading cause of cardiovascular disorders, blindness, end-stage renal failure, amputation, cognitive decline, and chronic liver disease (12, 13).

1.1.2. Epidemiology of T2DM

The epidemiology of T2DM is affected both by genetics and the environment. Genetic factors exert their effect following exposure to an environment characterized by sedentary behavior and high-calorie intake. Common glycemic genetic variants for T2DM have been identified by genome-wide association studies, but these only accounts for 10% of total trait variance, suggesting that rare variants are important (14). People of different ethnic origins may have different specific phenotypes that increase predisposition to clusters of CVD risk factors, including hypertension, insulin resistance, and dyslipidemia (15).

1.1.3. Pathophysiology of type 2 diabetes mellitus

Diabetes mellitus type 2 is characterized by insulin insensitivity due to insulin resistance, consequent pancreatic β-cell failure, and reduced insulin output (16, 17).The primary events are believed to be an initial deficit in insulin secretion and, in many patients, relative insulin deficiency in association with peripheral insulin resistance (18, 19). Insulin resistance occurs due to a high rate of free fatty acids and proinflammatory cytokines in plasma contributing to a decrease in glucose transfer to the muscle tissue, increased production of hepatic glucose, and increased fat breakdown, high levels of free fatty acids induces resistance to insulin in the liver as well as muscle cells. It results in a reduction of insulin-mediated glucose release by muscle cells and increased gluconeogenesis in the liver, leading

to increased circulating glucose levels **Figure 1.1** (20). The role of impaired alpha cell activity has lately been demonstrated in type 2 DM pathophysiology (21, 22).

The majority of patients with this form of diabetes are obese, and obesity itself causes or aggravates insulin resistance (23, 24). Many of those who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region (25).In relative terms, the plasma insulin content (both fasting and meal stimulated) is normally increased, but "respective" to the austerity of insulin resistance, the plasma insulin concentration is not adequate to maintain natural glucose homeostasis (26). The function of surplus glucagon cannot be overlooked; however, type 2 diabetes is an islet paracrinopathy, in which the equal relationship between both the glucagon secreting alpha cell and the insulin-secreting beta cell is disrupted, resulting in hyperglucagonemia and hyperglycemia (27, 28). Ketoacidosis is infrequent in this type of diabetes; when seen it usually arises in association with the stress of another illness such as infection (29, 30).

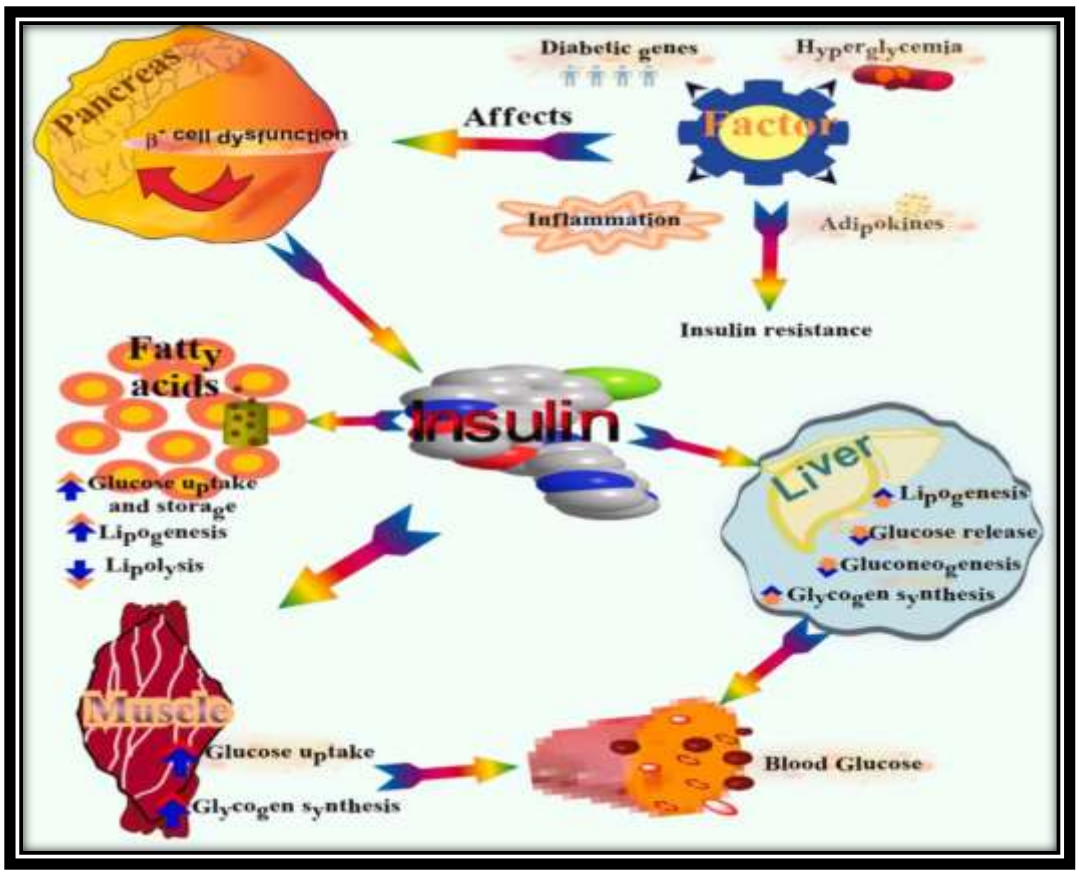


Figure 1.1: A basic outline of the pathophysiology of abnormal glucose metabolism in type 2 diabetes mellitus (20).

1.1.4. Complications of diabetes mellitus

Persistent hyperglycemia in uncontrolled diabetes mellitus can cause several complications, both acute and chronic.

1.1.4.1. Acute complications

Acute complications happened at any time and may lead to chronic, or long-term, complications, hypoglycemia, hyperglycemic diabetic coma, and hyperosmolar hyperglycemic state (HHS), which is a life-threatening emergency that only happens in people with type 2 diabetes. It's brought on by severe dehydration and very high blood sugar levels. Diabetic ketoacidosis (DKA) is a

life-threatening emergency where the lack of insulin and high blood sugar levels lead to a build-up of ketones (31, 32).

1.1.4.2. Chronic complications

These are long-term problems that can develop gradually, and can lead to serious damage if they go unchecked and untreated. Eye problems (retinopathy), some people with diabetes develop an eye disease called diabetic retinopathy which can affect their eyesight. If retinopathy is picked up usually from an eye screening test, it can be treated and sight loss prevented. Foot problems, diabetes foot problems are serious and can lead to amputation if untreated. Nerve damage can affect the feeling in feet and raised blood sugar can damage the circulation, making it slower for sores and cuts to heal (31, 32).

The patients with diabetes, high blood sugar for a long time can damage the blood vessels which may lead to heart attacks and stroke. Kidney problems (nephropathy) and diabetes may cause damage to the kidneys over a long time making it harder to clear extra fluid and waste from body. This is caused by high blood sugar levels and high blood pressure. It is known as diabetic nephropathy or kidney disease. Patients with diabetes may develop nerve damage (neuropathy), which is caused by complications of high blood sugar levels. This can make it harder for the nerves to carry messages between the brain and every part of our body so it can affect how we see, hear, feel and move .Hyperglycemia can lead to more sugar in saliva, causing gum disease or other mouth problems. This brings bacteria that produce acid which attacks the tooth enamel and damages gums. The blood vessels in gums can also become damaged, making gums more likely to get infected (31, 32).

1.1.5. Management of type 2 diabetes mellitus

The American diabetes association's standards of medical care in diabetes focus on diet and physical exercise for the management of diabetes (33). Lifestyle management and psychosocial care are the cornerstones of diabetes management, mostly in overweight and obese diabetic patients. However, there is poor application and maintenance of lifestyle modification actions. Despite the clear recommendations on a healthy diet and increased physical activity, the lack of relevant applications of these guidelines in the primary health care system has been highlighted (34,35). Diet therapy is important for the prevention as well as the treatment of all stages of type 2 diabetes but continues to remain poorly understood and highly controversial (36, 37). When obesity coexists with hyperglycemia, as seen in the majority of individuals with type 2 diabetes, weight reduction is the major goal of dietary therapy (38–41). The goal is the prevention of clinically significant glycosuria, water and electrolyte loss, infections, and the development of non-ketotic hyperosmolar coma, insulin is indicated for type 2 diabetic patients with insulinopenia whose hyperglycemia does not respond to diet therapy either alone or combined with oral hypoglycemic drugs (42). Controlling blood glucose with insulin has the potential to be the most effective blood glucose-lowering therapy (43). Many patients with type 2 diabetes eventually require insulin therapy, since type 2 diabetes is associated with insulin resistance, insulin requirements can exceed 1 unit/kg/day (44). Medications used to manage blood glucose in patients with type 2 diabetes mellitus (Sulfonylureas, Metformin, Acarbose, Miglitol, Pramlintide, Pioglitazone, Nateglinide, Glyburide, Chlorpropamide) (45).

1.1.6. Diagnosis of diabetes mellitus

To be diagnosed as diabetic, one's blood glucose level needs to be equal to or above a certain value. According to the American diabetes association (ADA), there are four methods for the diagnosis of diabetes, and the same methods are used for the screening of pre-diabetes in patients as shown in **Table 1.1**. These methods are:

(1) Fasting plasma glucose test (FPG): where fasting refers to the absence of food and drink intake, apart from water, for at least 8 h before the test.

(2) Oral glucose tolerance test (OGTT): where a patient consumes a glucose syrup solution containing 75 g of glucose before which a blood test is carried out to determine 2 h plasma glucose (PG).

(3) A1C (glycated hemoglobin or hemoglobin bounded to glucose) levels *via* a laboratory test.

(4) Random PG of more than or equal to 200 mg/dL or 11.1 mmol/L in patients displayed symptoms of hyperglycemia or hyperglycemic crisis (46).

Table 1.1: A comparison among normal, pre-diabetes, and diabetes based on three diagnosis methods (46).

State	FPG	Plasma glucose in OGTT	HbA1C
Normal	<100 mg/dL or 5.5 mmol/L	<140 mg/dL or 7.8 mmol/L	<5.7 % or 39 mmol/mol
Pre-diabetes	≥100 mg/dL or 5.5 mmol/L	≥140 mg/dL or7.8mmol/L	≥ 5.7 % or 39 mmol/mol
Diabetes	≥126 mg/dL or 7.0 mmol/L	≥200 mg/dL or 11.1mmol/L	≥ 6.5% or 48 mmol/mol

The glycated hemoglobin (HbA1c) and fructosamine are also still useful for determining blood sugar control over time. However, practicing physicians frequently employ other measures in addition to those recommended. In July 2009, the international expert committee of the Oman medical specialty board (IEC) recommended the additional diagnostic criteria of an HbA1c result ≥6.5% for DM. This committee suggested that the use of the term pre-diabetes may be phased out but identified the range of HbA1c levels ≥6.0% and <6.5% to identify those at high risk of developing diabetes mellitus (47). As with the glucose-based tests, there is no definite threshold of HbA1c at which normality ends and DM begins (48). The IEC has elected to recommend a cut-off point for DM diagnosis that emphasizes specificity, commenting that this balanced the stigma and cost of mistakenly identifying individuals as diabetic against the minimal clinical consequences of delaying the diagnosis in a patient with an HbA1c level <6.5% (49).

1.2. Pectin

Pectin is a biocompatible polysaccharide with intrinsic biological activity, which may exhibit different structures depending on its source or extraction method. The extraction of pectin from various industrial by-products presents itself as a green option for the valorization of agro-industrial residues by producing a high commercial value product. Pectin is susceptible to physical, chemical, and/or enzymatic changes (50). To date, apple pomace (14%), and to a smaller extent, is the main source of commercial pectin. However, the prolonged commercial success of pectin has shown the importance of using large volumes of fruit and vegetable by-products as raw materials to produce value-added products. The pectin extraction could be carried out applying conventional methods or with the combination of emerging technologies such as ultrasound, microwaves, and enzymatic extraction (54).

1.2.1. Structure of pectin

The structure of pectin **Figure 1.2** is very difficult to be determined because pectin can change during isolation from plants, storage, and processing of plant material (50). In addition, impurities can accompany the main components. At present, pectins are a group of polysaccharides rich in D-galacturonic acid (GalA). The pectin family consists of three pectic polymers: homogalacturonan (HG), rhamnogalacturonan I (RG-I), and rhamnogalacturonan II (RG-II) (51). HG is composed of (1, 4)-linked α-D-GalA residues and is called a "smooth region". Some of the carbonyl groups of HG are methyl esterified or acetylated. RG-I (so-called "hairy" region) is composed of alternating GalA and rhamnose (Rha) units. Galactose (Gal) and arabinose (Ara) may be linked as side chains of RG-I associated with Rha units. Rhamnogalacturonan (HG) may also be covalently crosslinked to RG-I *via* Rha units (51). RG-II is highly branched and very complex compound that may be cross-linked by borate diesters (52). The RG-II backbone is similar to HG, however, it is decorated with complex side chains, including also xylogalacturonan and apiogalacturonan (53). It is thought that these three polysaccharide domains can be covalently linked to form a pectic network throughout the primary cell wall matrix and middle lamellae. This network has considerable potential for modulation of its structure by the action of cell wall-based enzymes (54).

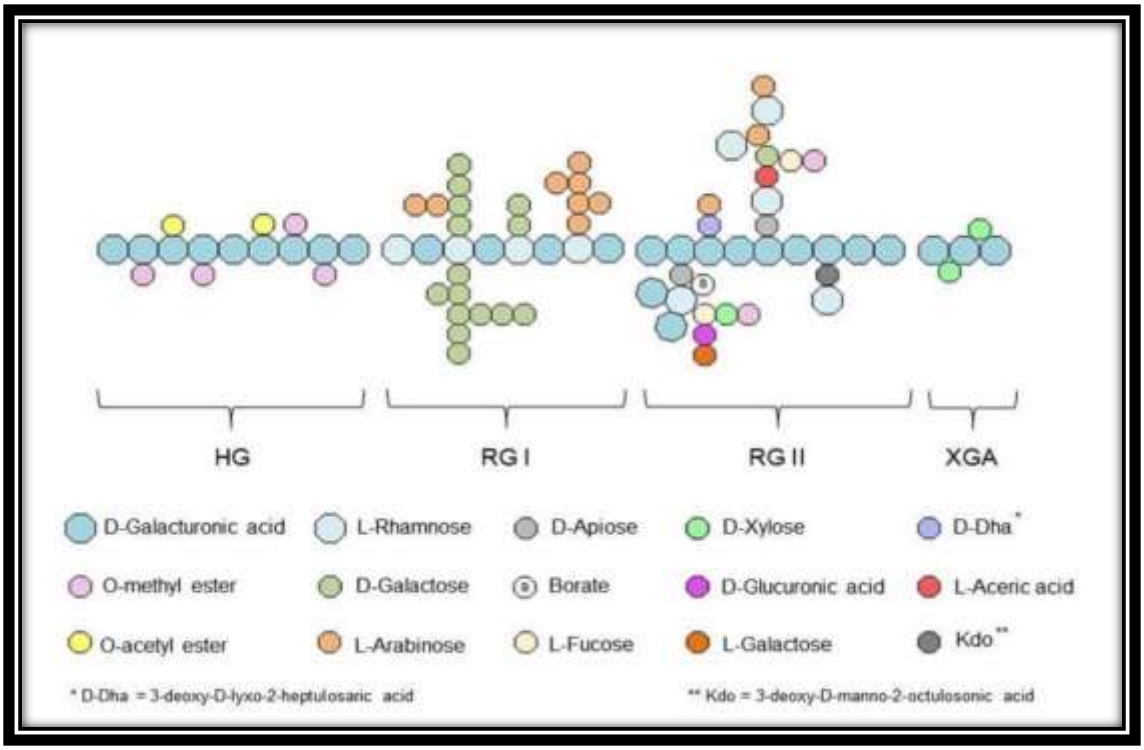


Figure 1.2: Chemical structure of the pectin molecule (55).

1.2.2. Medical uses of pectin

Pectin has applications in the pharmaceutical industry. Pectin favorably influences cholesterol levels in the blood. It has been reported to help reduce blood cholesterol in a wide variety of subjects and experimental conditions as comprehensively reviewed (56). Consumption of at least 6 g/day of pectin is necessary to have a significant effect on cholesterol reduction. Amounts less than 6 g/day of pectin are not effective (57). Pectins have various advantages as additives in food and pharmaceutical preparations: (a) Pectins have a good gel-forming ability in presence of divalent cations which makes them suitable carriers for delivering bioactive agents and used for delivering drugs via oral, nasal, and vaginal routes and it has been well accepted by many patients (58). (b) Pectins have a long-standing reputation of being non-toxic and have high availability and low production cost. Due to the ability of pectin to form gels in acidic media, there

is an improvement in the contact time of medications for obesity (59). The ability of gels to swell under acidic conditions can benefit treatments for weight reduction and obesity. This is because when the gels reach the aqueous environment of gastric fluids, they swell and stick to the walls of the stomach before digestion, providing a feeling of satiety and lack of appetite (60). In addition, diets rich in soluble fiber, including pectin, increase the excretion of bile acids and, consequently, result in the reduction of cholesterol, having a favorable impact on reducing the risk of cardiovascular disease (61, 62). The cholesterol-lowering properties of pectin are related to physicochemical properties, including viscosity, molecular weight, de-esterification (DE), and the presence of acetylation or amidation. Studies indicate that high molecular weight pectin lowers cholesterol levels more effectively than low molecular weight pectin (61).The inhibition performed by pectin results from competitive inhibition with the substrate (oil/fat), entering the scene with the formation of pectin–lipase complexes. As pectin is a weak acid, it resists dissociation in the gastric environment and binds covalently to the active sites of pancreatic lipase (62).

1.2.3. Pectin as an antidiabetic agent in type 2 diabetes mellitus

Numerous studies have shown that soluble dietary fibers such as pectin and guar gum decrease blood glucose levels and/or insulin secretion after a sugar load (63-68). It has also been reported that soluble fibers improve glucose tolerance by decreasing the peak of postprandial glycemia and/or by preventing late hypoglycemia in normal subjects (69). And diabetic patients (70, 71). Dietary fiber plays, in particular, a role in managing the risk of this chronic disease. Fiber-rich foods generally take longer to chew, which may increase sensory satiety and reduce meal size (72–74). Furthermore, fibers may decrease intestinal passage rates, leading to more gradual nutrient absorption and prolonged feelings of satiety

(75). They may also decrease energy absorption by lowering the bioavailability of fatty acids and proteins (76, 77). Finally, dietary fibers can be fermented in the colon, which increases the concentration of short-chain fatty acids, which may enhance satiety via various mechanisms (78).

1.3. Insulin

Insulin is a peptide hormone secreted by the β- cells of the pancreatic islets of Langerhans and it maintains normal blood glucose levels by facilitating cellular glucose uptake, regulating carbohydrate, lipid, and protein metabolism, and promoting cell division and growth through its mitogenic effects (79). Insulin was found to be a polypeptide in 1928 with its amino acid sequence identified in 1952. Insulin is a dipeptide, containing A and B chains respectively, linked by disulfide bridges, and containing 51 amino acids, with a molecular weight of 5802. Its isoelectric point is pH 5.5 (80). The A chain comprises 21 amino acids and the B comprises chain 30 amino acids. The A chain has an N-terminal helix linked to an anti-parallel C-terminal helix; the B chain has a central helical segment as shown in **Figure 1.3**. The two chains are joined by 2 disulfide bonds, which join the N- and C-terminal helices of the A chain to the central helix of the B chain. In pro-insulin, a connecting peptide links the N-terminus of the A chain to the C-terminus of the B chain (81).

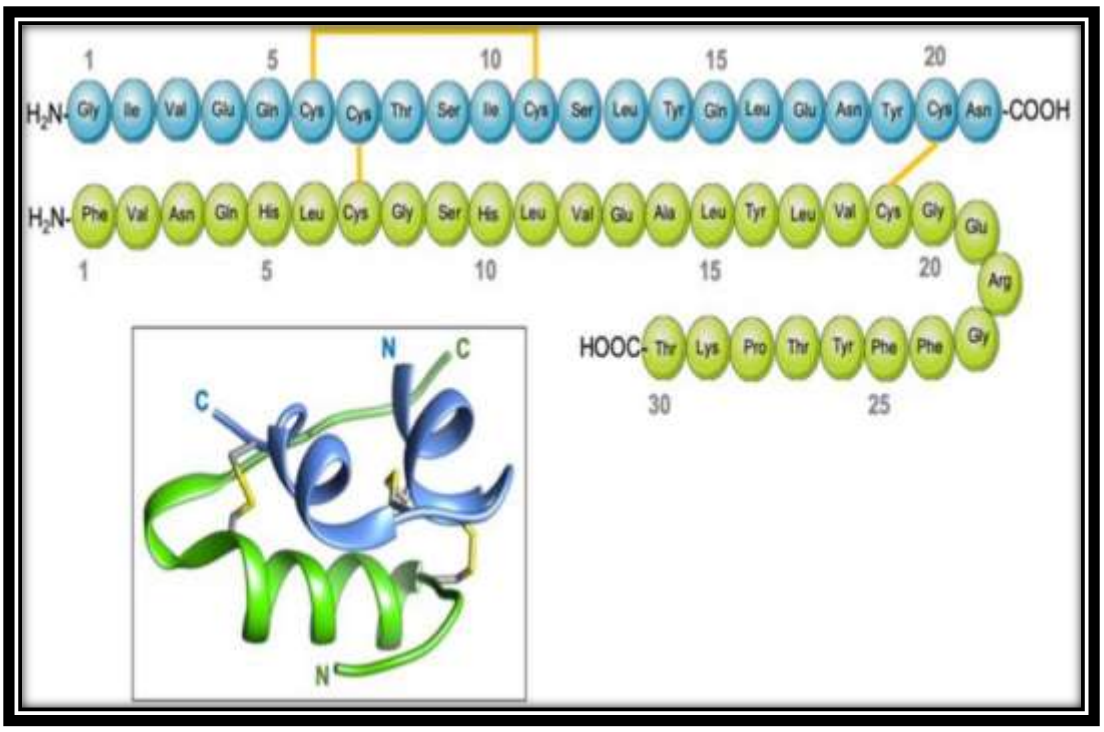


Figure 1.3: Comparative analysis of different expression systems used for the production of human insulin (82).

1.3.1. Function of insulin

Insulin in mammals induces cells to take up glucose and convert it to glycogen, inhibit glycogen breakdown and gluconeogenesis, and generally shift from catabolic to anabolic lipid and protein metabolism (83).

1.4. Ghrelin

Ghrelin is an acylated 28-amino acid peptide, Ghrelin is a containing an *n*-octanoyl group on the serine in position 3. Ghrelin is the only known peptide hormone modified by a fatty acid (84). Ghrelin is synthesized by the endocrine X/A-like cells of the fundus mucosa representing about 20% of gastric mucosal cells in humans as shown in **Figure 1.4** that was identified in 1999 from human and rat stomachs as the endogenous ligand, for the growth hormone (GH)

secretagogue-receptor (GHS-R), circulating ghrelin is produced predominantly in the stomach, ghrelin is a potent stimulator of GH release, feeding, and adiposity, as well as exhibiting positive cardiovascular effects, from the early stages of ghrelin research. It was recognized that the plasma ghrelin level correlates inversely with body weight and that low plasma ghrelin levels are associated with elevated fasting insulin levels (85).

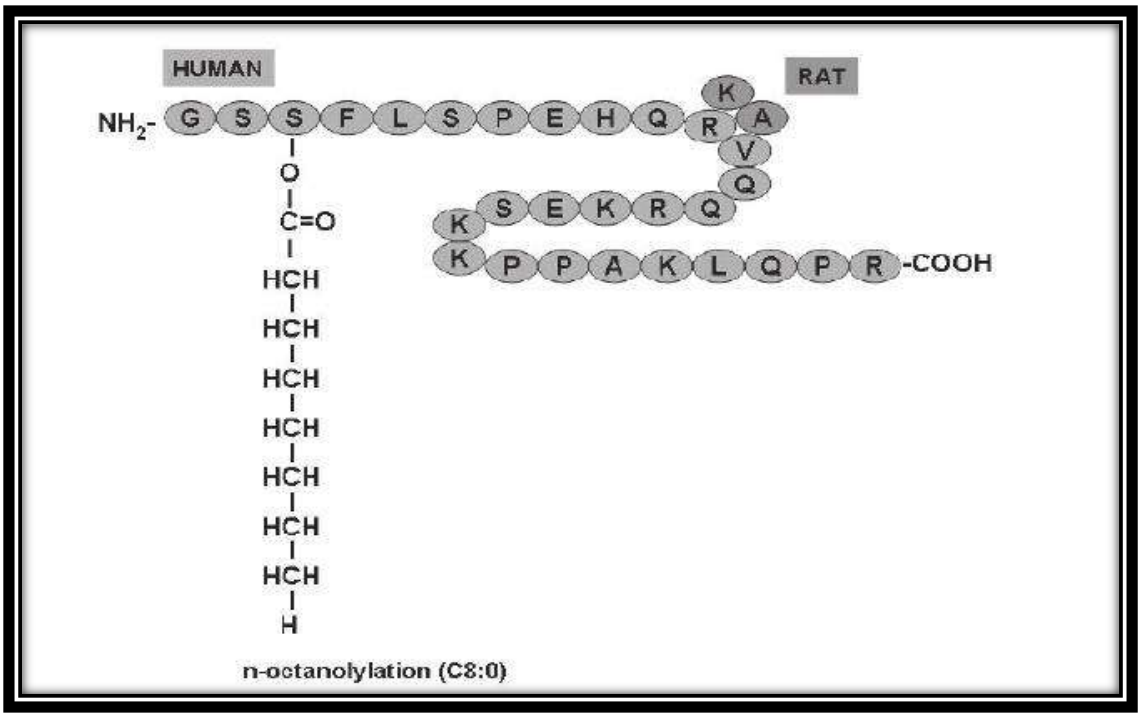


Figure 1.4: the structure of human ghrelin (86).

1.4.1. Function of ghrelin

The importance of ghrelin in body weight regulation was strengthened with the observation that circulating ghrelin levels show rapid as well as long-term changes: fasting increases circulating ghrelin levels, which drop after food intake, while lean subjects have higher ghrelin levels than obese subjects (87).

1.4.2. Ghrelin and type 2 diabetes mellitus

The key pathogenic feature of type 2 DM is insulin resistance leading to compensatory hyper secretion of insulin, ultimately leading to β-cell dysfunction. Type 2 diabetes, which is far more common than type 1 diabetes, is tightly associated with obesity, making it difficult to distinguish between the effects of diabetes alone, or in conjunction with obesity. Obese subjects have decreased circulating ghrelin levels (88). Several studies have found that low ghrelin levels are associated with elevated fasting insulin concentrations and the prevalence of type 2 diabetes and insulin resistance (89). Indeed, total plasma ghrelin, as well as unacylated ghrelin, concentrations were found to be lower in insulin-resistant obese adults relative to equally obese insulin-sensitive controls, indicating a link between ghrelin and insulin sensitivity (90).

1.5. Lipase

Lipase is an enzyme that breaks down triglycerides into free fatty acids and glycerol as shown in **Figure 1.5**. Lipases (triacylglycerol acyl hydrolases, enzyme commission number (EC) 3.1.1.3) (91). Lipases are present in pancreatic secretions and are responsible for fat digestion. There are many different types of lipases; for example, hepatic lipases are in the liver, hormone-sensitive lipases are in adipocytes, lipoprotein lipase is in the vascular endothelial surface, and pancreatic lipase is in the small intestine. Understanding lipase is crucial for understanding the pathophysiology of fat necrosis and is clinically significant in the understanding of acute and chronic pancreatitis. The role of lipase is also crucial in the mechanism of some medications indicated for lowering cholesterol (92).

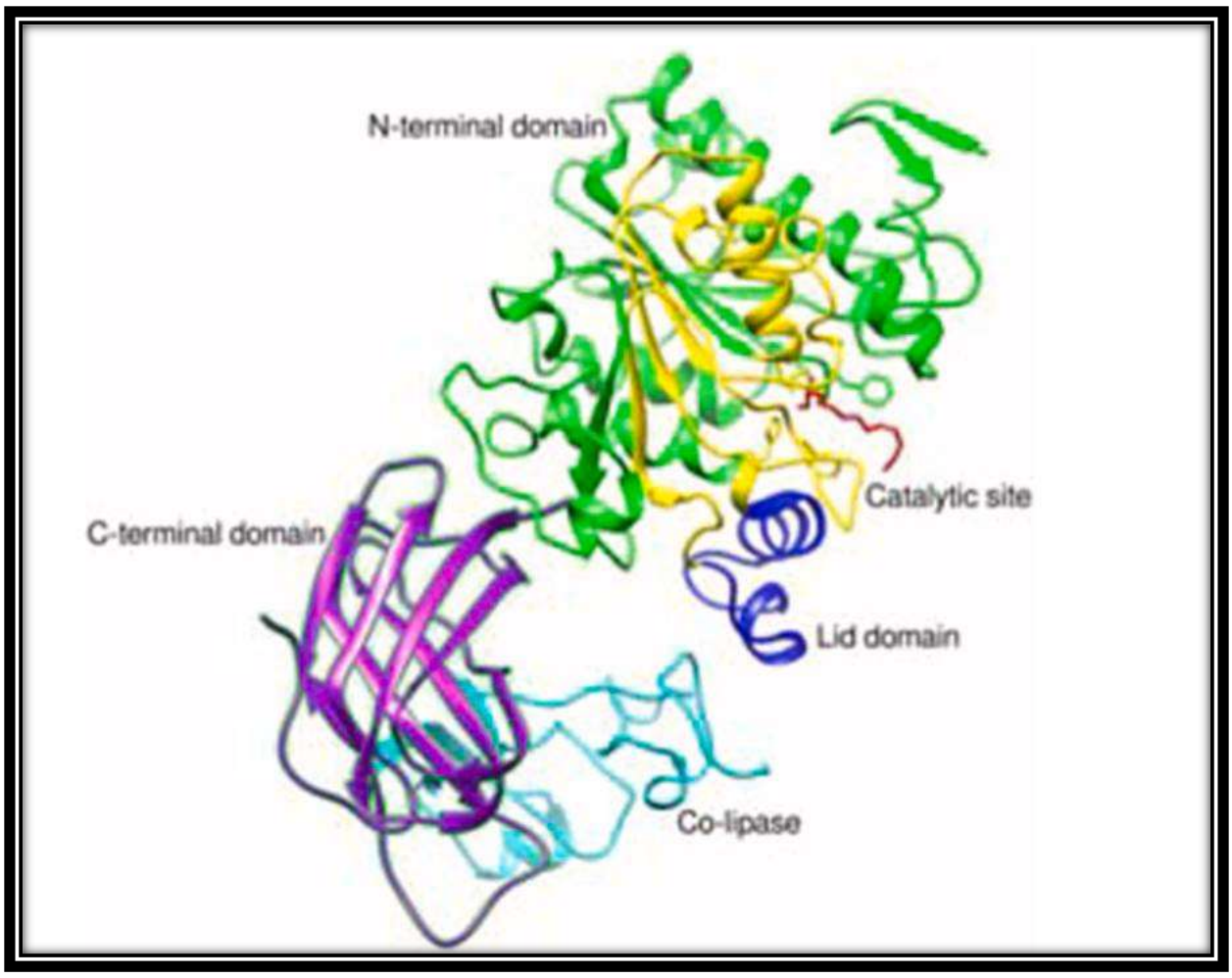


Figure 1.5: Shows tertiary structure of human pancreatic lipase enzyme (PDB.1LPB). Green color for the N-terminal domain, Yellow color for the catalytic site, Purple color for the C-terminal domain, turquoise color for Co-lipase, blue color for lid domain, and co-crystallized MUPA is shown by red color. (93).

1.5.1. The functions of lipase

Pancreatic lipase is found within the small intestine and is responsible for degrading dietary triglycerides (94). Pancreatic lipase is secreted in an active form, but its activity is enhanced by bile salts. Bile salts enhance the efficiency of lipolysis by increasing the surface area of oil-water interfaces at which water-

soluble lipase is effective; colipase is a small protein, synthesized in the pancreas, which allows pancreatic lipase to function despite micellar concentrations of conjugated bile salts. Bile salts by themselves hinder lipase adsorption onto triglycerides by covering the whole water-substrate interface. Colipase tends to prevent this and acts as an anchor for lipase adsorption, thus allowing lipase to hydrolyze substrate (95). Calcium may be required for the activation of lipase (96). Pancreatic lipase exhibits optimal activity under alkaline conditions and hydrolyzes triglycerides to fatty acids and glycerol, but mono- and diglycerides are also final products. Pancreatic lipase has greater activity against short-chain than long-chain triglycerides (97). A study investigating the effect of hyperglycemia on pancreatic exocrine functions in type 2 diabetic patients reported that serum amylase and lipase levels significantly increased with the regulation of glycemic control, but were still lower as compared with control. They found a significant negative correlation between serum amylase and lipase with basal FBG and HbA1c (98).

1.5.2. Lipase and diabetes mellitus

A recent study showed that serum pancreatic enzyme concentrations are often diminished in disease processes associated with subclinical diminution of exocrine_ pancreatic function, e.g. “diabetes mellitus" (99). Pancreatic lipase is the most important enzyme responsible for digestion of dietary fat, slowing down the deposition of fat into adipose tissue, and suppression of weight gain, which is of beneficial effects on overweight and obesity, which are common in diabetes, pancreatic lipase is a key enzyme responsible for the digestion and absorption of triglycerides, which represent 90 to 95% of the ingested fats. If somehow this initial movement of triglycerides from the intestinal lumen is blocked, hyperlipidemia can be prevented, the application of a lipase inhibitor was examined earlier as a treatment for obesity. Orlistat, a hydrogenated derivative of

lipstatin derived from Streptomyces toxitricini, a potent inhibitor of gastric, pancreatic, and carboxyl ester lipase, has proved to be effective for the treatment of obesity, as a major factor in the development of type 2 diabetes (100). A study has shown that there is a significant correlation between pancreatic enzyme activities and C peptide concentration (101). This difference may reflect the fact that in the exocrine pancreatic injury associated with human diabetes, the sequence of enzyme loss begins with trypsin and that lipase is the last enzyme to be lost (102).

1.6. Amylase

Amylase is an enzyme secreted by the pancreas as shown in **Figure 1.6**. Various other tissues including salivary glands, small intestines, ovaries, adipose tissue, and skeletal muscles secrete amylase. There are two major isoforms of amylase - pancreatic amylase and salivary amylase (103). EC 3.2.1.1 is the enzyme commission number (104). Amylase is the primary enzyme responsible to cleave starch into maltose, maltotriose, and α-limit dextrins in the process of digestion.

Figure 1.6: the structure of amylase (105).

1.6.1. Function of amylase

Amylases are predominant hydrolase enzymes that hydrolyze glycosidic bonds present in starch molecules and produce dextrins and oligosaccharides (106). Amylases are a member of the glycoside hydrolase family 13 (GH-13). (107) In starch, α-1,4-glycosidic linkages are hydrolyzed by amylases, so known as glycoside hydrolases. The first amylase was isolated, in the year 1833, by Anselme (108). There are two types of amylases, exo-amylases, and endo-amylases. Exo-amylases hydrolyze the nonreducing end of starch. Endo-amylases hydrolyze glycosidic linkages within the starch molecule (109). α-Amylase has a three-dimensional structure that helps it in binding to the substrate and makes it highly specific in its activity (110). α-Amylase is made up of a single oligosaccharide chain that contains 512 amino acids (57.6 kDa molecular weight) (111).

1.6.2. Amylase and diabetes mellitus

In diabetes mellitus, impaired pancreatic endocrine activity may affect its exocrine function causing maldigestion and malnutrition, majority of the studies focused the metabolic derangement due to impaired insulin action and persistent hyperglycemia. Therefore, the present study has been designed for evaluating exocrine pancreatic function by measuring serum amylase and lipase in type 2 diabetic subjects (112). In diabetes mellitus, hyperglycemia and/or insulin inactivity due to hypoinsulinemia or insulin resistance may induce pancreatic exocrine dysfunction and the development of pancreatic exocrine insufficiency (113).

1.7. The effect of calcium on pancreatic enzymes

The effect of calcium on the digestion of triglycerides by pancreatic lipase has been studied extensively (114-116). Calcium has been found to enhance the

hydrolysis of triglycerides in emulsion systems (116). In an *in vitro* model, the rate of fatty acid release increased with the increasing addition of $CaCl_2$ to emulsions during lipolysis by pancreatic lipase. However, this enhancement of lipid hydrolysis by $CaCl_2$ was dependent on the emulsifier used to stabilize the lipid droplets and the addition of other food components, such as calcium-chelating agents and polysaccharides (114). The effect of calcium on lipolysis by pancreatic lipase is attributed to calcium complexing with free fatty acids produced from the lipolysis of triglycerides to form insoluble calcium soap, thereby removing free fatty acids from the droplet surface, the free fatty acids inhibit the lipolysis of triglycerides by hindering the access of pancreatic lipase to the surfaces of lipid droplets (115). Calcium ions may play roles in the lipid digestion process, which impact both the rate and extent of lipid hydrolysis. Lipid digestion of emulsified lipids can be inhibited by the accumulation of long-chain free fatty acids (FFA) at the droplet surfaces since this restricts the access of the lipase to the triacylglycerol (117). Calcium is known to precipitate these accumulated free fatty acids, thereby removing them from the lipid droplet surface and allowing the lipase to access the emulsified lipids (118,119). Calcium ions can increase the rate and extent of lipolysis by this mechanism (120,121).

1.8. Nanotechnology and nanomedicine

Nanotechnology is defined as the understanding and control of matter at dimensions between 1 and 100 nm where unique phenomena enable novel applications (122). Nanotechnology is a huge project aimed at reducing substances, instruments, and tools. This technique not only reduced the size of nanomaterial produced but also greatly sought to enhance the properties of nanomaterial and the invention of new nanomaterial with unique properties (123). The development of nanoparticles has attracted the interest of many scientists in the field of drug

delivery systems as well as in tissue engineering, gene delivery, and imaging studies since the 1980s (124). The widespread use of polymers as biomaterials in the past decade, owing to their unique properties such as good biocompatibility and easy design and preparation, has led to a variety of structures and interesting biomimetic characters being demonstrated from the perspective of nanomedicine (125). Nanoparticles have outstanding advantages, for example, they can pass through the smallest capillary vessels because of their ultra-tiny volume and avoid rapid clearance by phagocytes so that their duration in the bloodstream is greatly prolonged. They can also penetrate cells and tissue gaps to arrive at target organs such as the liver, spleen, lungs, spinal cord, lymph, and colon. They show controlled-release properties due to the biodegradability, pH, ion, and/or temperature sensibility of the materials. Last but not least, they can improve the utility of drugs and reduce the toxic side effects (126).

Nanomedicine is a field with continuous progress, introducing novel applications in many health care areas. The underlying motivation is the improvement of quality of life with economic and social benefits. Some of the most promising areas are the following (127,128). Nanodiagnostics (molecular diagnostics, imaging using NPbased contrast materials, nanobiosensors) , nanopharmaceuticals (targeted drug delivery, nanotechnology-based drugs, implanted nanopumps, nanocoated stents), Reconstructive surgery (tissue engineering, implantation of rejection resistant artificial tissues and organs), Nanorobotics (vascular surgery, detection, and destruction of cancer), Nanosurgery (nanolasers, nanosensors implanted in catheters)Regenerative medicine (tissue repair), Ultrafast DNA sequencing (127,128).

1.8.1. Nanoparticles and their physiochemical properties

Nanoparticles are solid dispersion particulates in the size range of 10-1000 nm (129).They cause enhancement of particle mobility, diffusion, thermal stability, storage capacity, and greater surface area and also modulate the catalytic activity of attached enzymes (130). Toxicity, bioavailability, and solubility of various drugs (131). Nanoparticles are very widely used in making biosensors, solar cells, photo-detectors, ceramics, and Nano generators (132). They may possess different topographies and shapes like nanotubes, nanorods, nanorings, nanowires, etc. (133).Nanoparticles have a size equivalent to that of proteins and nucleic acids present in biosystems and they have an impact on them, a huge variety of modified nanostructures are being produced in the association of nanoparticles with biomolecules (134). Nanomaterials have unique properties and characteristics relative to bulk materials (e.g., high surface-area-to-volume ratio) that may endow them with unique mechanisms of toxicity from xenobiotics. Particularly, toxicity has been thought to originate from nanomaterial size and surface area, shape, and composition.

The three features—size, surface, and shape—discussed below, either independently or in combination, may ultimately be shown in the future to predict the toxicity of NPs (135).

1. Size Owing to their small size, NPs can cross cell membranes and penetrate blood vessel walls and the blood-brain barrier *via* passive and active diffusion, eventually interfering with cellular functions.

2. Surface for the same mass of any particular material, the combined surface area of a particle is inversely proportional to particle size. If the toxic properties of particles are determined by interactions occurring at the interface between particles

and biological systems, toxic responses should correlate with the total surface area of particles. It was observed in animal studies that the inflammatory response to inhaled TiO_2 particulates of different sizes, including the Nano scale range, varied as a function of the surface area.

3. Shape One of the benefits of nanotechnology is the ability to control the material structure with atomic precision. This control of materials on a Nano scale results in our ability to generate an immense number of engineered NPs with different shapes. Examples of the simplest engineered NPs are spheres, tubes, wires, rods, belts, and flakes. Examples of more complex engineered NPs are tripods, flowers, and brushes. Finally, the most complex NPs have three-dimensional structures such as multifunctional Nano scale particles, for example, functionalized liposomes, virosomes, and dendrimers (135).

Nearly, 25% of the major pharmaceutical compounds and their derivatives available today are obtained from natural resources (136). Natural compounds with different molecular backgrounds present a basis for the discovery of novel drugs. A recent trend in natural product-based drug discovery has been the interest in designing synthetically amenable lead molecules, which mimic their counterpart's chemistry. Natural products exhibit remarkable characteristics such as extraordinary chemical diversity, chemical and biological properties with macromolecular specificity, and less toxicity. These make them favorable and lead to the discovery of novel drugs (136). By using various types of nanoparticles for the delivery of the accurate amount of drug to the affected cells such as cancer/tumor cells, without disturbing the physiology of the normal cells, the application of Nano medicine and Nano-drug delivery system is certainly the trend that will remain to be the future arena of research and development for decades to come (137).

1.8.2. Green nanomaterials in the disease treatment

Plants have been used as natural remedies for curing many physiological disorders in traditional eastern medicine, particularly in Indian and Chinese. The 'Green' synthesis of copper (Cu), gold (Au), nickel (Ni), platinum (Pt), titanium (Ti), selenium (Se), silver (Ag), and zinc nanoparticles (Zn NPs) using plant resources had been previously reported in literatures. Pectin from different sources provide different gelling abilities, due to variations in molecular size and chemical composition. Like other natural polymers (138). The plant-based metal nanoparticles (MNPs) showed excellent antimicrobial, anticancer, antidiabetic, anti-inflammatory, antioxidant, and immunomodulatory activities. Recently, the synthesis of MNPs using different plant extracts (139). Most of the previous reports confirm that the presence of phytochemicals such as alkaloids, flavonoids, phenols, terpenoids, alcohols, sugars, and proteins in plant materials are involved in the reduction and stabilization of metal ions. Although the synthesis of MNPs using a single active substance from plant extract, it will be helpful for the purification of nanoparticles, further study on such MNPs in the biomedical sector is needed to treat specific diseases. The fact that flavonoids widely exist in plant extract contributes a major role in the bio reduction of metal ionic into nanoparticle formation (139).

1.8.3. Diabetes mellitus and nanomedicine

Nanomedicine in the management of diabetes Worldwide 285 million people are suffering from pervasive, chronic, and often insidious diabetes caused by the inability of the pancreas to control the blood glucose concentration (140). The preferred approach to insulin intake in the past decades is *via* the subcutaneous route, which, nonetheless, often fails to mimic the glucose homeostasis observed in

normal subjects because in this approach insulin is delivered to the peripheral circulation rather than to the portal circulation and directly into the liver, which is the physiological route in normal individuals. Furthermore, multiple daily injections of insulin referred for poor patient compliance are associated with subcutaneous route treatment. Therefore, many studies were done to find out the better and safer route of insulin administration, in this regard application of nanotechnology in medicine revealed a solution to overcome this problem (140).

Conventional diagnostic techniques for diabetes include analyzing fasting glucose levels, hemoglobin A1c levels, or oral glucose tolerance tests. In the setting of clinical trials, the measurements of autoantibodies are often used as a diagnostic test to identify individuals at high risk for the development of diabetes, and in the clinical setting, autoantibodies are sometimes used to distinguish those with type 1 diabetes when the nature of diabetes is unclear (141).These methods are deemed painful by some patients and rely on glucose measurements or antibody titers, which can vary based on many factors, including age, time of testing, and other physiological conditions (142). Moreover, the manifestation of disease symptoms such as hyperglycemia, often does not become clinically evident until years after disease onset (143). Which precludes early intervention. To address the weaknesses of traditional diagnostic tools, different types of nanotechnologies have been developed to potentially enable earlier and non-invasive detection of diabetes. The intermittent nature of conventional monitoring methods implies that a patient can miss potentially dangerous glycemic fluctuations between tests, putting patients at risk for severe complications.

Over the past three decades, various attempts have been made toward developing a hassle-free method for GM. With the eventual development of implantable biosensors, this idea became feasible and resulted in continuous GM

systems, which can provide constant GM for up to 10 days. The first generation of these devices included amperometric sensors that are implanted subcutaneously (144). These sensors emit a detectable electric current as a function of glucose concentration. Nanotechnology-based biosensors possess capabilities that can potentially circumvent these limitations (146). Nano medicine has been proven to be able to effectively improve the oral delivery efficacy of compounds by circumventing various delivery restrictions (145). It has been reported that the uptake of nanostructures is 15~250 times greater than that of microparticles (146). Besides, nanostructures are always employed to sustain the release of encapsulated compounds to reduce dose and dosing frequency, thereby increasing patient compliance and minimizing side effects (147). At present, a comprehensive review covering various oral Nano delivery systems of phytocompounds for type 2 diabetes mellitus treatment in the preclinical stage is not available.

1.8.4. The role of nanotechnology in the management of type 2 diabetic mellitus

Evidence suggesting the role of EDENM exposure in the pathogenesis of T2DM is gradually emerging, but a comprehensive understanding of a wide range of nanomaterials and their effect on candidate gene pathways and other causal factors involved in T2DM remains to be completely deciphered. The fact that ENM can disrupt the endocrine system, which may eventually lead to T2DM, has gathered support from several in vitro, in vivo, and epidemiological studies. Altered glucose metabolism through various molecular mechanisms including insulin resistance, decreased insulin sensitivity, induction of oxidative stress pathways, and altered homeostasis have been reported by numerous laboratory studies that examined the effect of EDENM exposures (148). On the other hand, a few studies also report a contrasting effect of the same EDENM on T2DM-related

molecules. Many research groups have come up with a safer application of ENMs by illustrating their ability to work as therapeutics. Particularly, some of the biologically synthesized ENMs (149, 150). Were found to possess therapeutic potential against T2DM (151–153). The biosynthesized AgNPs from bark methanol/water extract of E. polystachya contain bioactive compounds such as chalcones, flavonoids, and dihydrochalcones, which play a determinant role in the Phyto-fabrication of the AgNPs. EP/AgNPs promote pancreatic β-cell survival, insulin secretion-enhanced hyperglycemia, and hyperlipidemia in glucose-induced diabetic zebrafish. In addition, EP/AgNPs restore insulin secretion from INS-1 cells stimulated by H_2 O_2, suggesting that this could result from cytoprotection against oxidative injury. These findings suggest that EP/AgNPs could be used as therapeutic nanoparticles to prevent the development of diabetes (151). The function of gold nanoparticles as antidiabetic nanomaterial is achieved and the gold nanoparticle-based drugs will be more active to interact with the targets if subjected to further studies (152). Chitosan nanoparticles loaded with SR-B1 siRNA could be used as a possible therapeutic alternative to prevent HCV entry into liver cells in the disease's early stages (153).

Other non-metallic and metallic NMs for similar applications are also reported **Table 1.2**. In an interesting study, an increase in cell viability, ATP/ADP ratio, and secretion of insulin in response to glucose stimuli in the isolated pancreatic islets when treated with metallic nanoparticles prepared from cerium oxide (CeO_2- NPs) at a concentration of 100 nmol/L, either alone or in combination with 30 nmol/L sodium selenite, was reported. (154) These findings could be ascribed to the anti-oxidant potential of CeO_2^{-2}NPs, which may exert a different effect on insulin release. In a similar study, the effect of zinc oxide nanoparticles (ZON) on oxidative stress-mediated pancreatic β-cell death was

investigated in rats (RIN5f). The cellular levels of antioxidant factors and the rate of apoptosis were assessed in correlation with ZON uptake. RIN5f cell treatment with ZON (30 and 100µg/ml) resulted in cytotoxicity, oxidative stress, and apoptosis. In contrast, the sub-cytotoxic concentrations (1, 3, 10µg/ml) protected RIN5f cells from hydrogen peroxide (H_2O_2)-induced oxidative stress by reducing the cellular levels of ROS, increased SOD activity, and GSH, and reduced cell death (155). The findings reported in the above-mentioned studies indicated that nanomaterial type and size dependencies on the associated cellular toxicity are not precisely explored. Incomplete characterization of ENM may underlie discrepancies observed in available scientific reports (156). Confusion created from such incomplete explorations demands the development of a common understanding in the area of ENM characteristics (shape, size, surface area, mass concentration, or a combination of these), which should be a prerequisite to toxic effect determination. This broadens the scope of research to particularly define the physicochemical properties of ENM for their sustainable bio-medical applications. Considering this, ENMs for vital bio-medical, pharmaceutical, agricultural, and environmental applications are required to be well characterized for their uptake to various cell/ tissue types, interaction with cellular organelles, and cell mechanistic aspects within the intracellular environment (156). Results obtained from such experiments need to be replicated through *in vivo* studies conducted in different model animals. Additionally, *in vivo* studies that address the potential effects of EDENM on the development of T2DM at a large scale such as a population, a community, or an ecosystem with sufficient power are necessarily required. Further, most of the *in vivo* studies have been conducted on small rodents, specifically rats, mice, and hamsters, which may not be optimum for studying the long-term toxic effects of nanomaterial and makes it difficult to extrapolate the observed results to humans. Experiments by including other model systems, which

are more closely related to human systems, like Danio rerio, Daphnia magna, and Caenorhabditis elegans need to be conducted in more numbers. Furthermore, pre-clinical in vitro studies, such as those using blood samples from a control population, can also be conducted for impact-assessment of EDENMs (157). Additional support to the candidate mechanisms underlying EDENM mediated T2DM and identification of novel pathways can be achieved through the application of the "-omics" approach, which at present is virtually lacking. Also, developing high-throughput pre-screening (HTPS) and quantitative structure-activity relationship (QSAR) methods for in silico screening and prediction would be extremely important to comprehend the effect of EDENM (158). The importance of research on the safety aspect of ENMs, which would minimize the uncertainties regarding the health and environmental issues surrounding these advance materials and help in the development of safe applications of nanotechnologies.

Table 1.2: Different categories of ENM used in therapeutics against T2DM.

Type	Application	Study system	References
Non-metallic ENM			
1) PEG-b-PLGA–biodegradable Polyethylene glycol and Poly (lactic-co-glycolic acid) copolymer (PEG-b-PLGA) Based cationic lipid-assisted nanoparticles (clans)	Anti-inflammatory action	Diet-induced type 2 diabetes mice	(159)
2) Nanoparticles from dextran, poly (α-1,6 glucose), physically cross-linked with the tetra functional glucose-binding	Controlled delivery of insulin	*in vitro* studies	(160)

protein, Con A			
3) Injectable insulin nano-particles	Insulin delivery	Subcutaneous administration in diabetic mice	(161)
4) PLGA as the carrier to prepare recombinant human epidermal growth factor (rhEGF) nanoparticles	Diabetic wound healing	Diabetic rats	(162)
5) Insulin encapsulated in polyalkylcyanoacrylate nanocapsules	Hypoglycemia effect	Diabetic rats	(163)
Biosynthisized ENM			
1) Eysenhardtia polystachya-loaded silver nanoparticles (EP/AgNPs)	Antidiabetic activity	Pancreatic β cells, INS-1 cells, and Danio rerio	(151)
2) Gold nanoparticles (AuNPs) synthesized using Cassia auriculata plant extract	Increasing plasma insulin activity	Alloxan induced albino rats	(152)
Metallic ENM			
1) Insulin-coated gold nanoparticles (INS-GNPs)	For controlled and prolonged glucose regulation was reported	Intravenous and subcutaneous administration to diabetic mouse model	(164)
2) Selenium nanoparticles (SeNPs)	Oral delivery of insulin to enhance the antidiabetic effect	Normal (Sprague-Dawley, SD) and type II DM (Goto-Kakizaki, GK) rats	(165)

1.9. Aim of the study

The present study aims to study the impact of natural nanoparticles on the pancreatic enzymes in sera of patient's type 2 diabetes mellitus through the following steps:

1. Synthesis and characterization of pectin-modified calcium nanoparticles.
2. Evaluation of the Lipase and amylase activities in sera of type 2 diabetes mellitus patients in the absence and presence of nanoparticles.
3. Evaluation of lipid profile, as well as ghrelin levels in sera of type 2 diabetes mellitus patients.
4. Assessment of the relationship of pancreatic enzymes and studying biochemical parameters.

CHAPTER TWO

Materials and Methods

2. Methodology

2.1. Study design

The present work is a case-control study at the Center for Diabetes and Endocrinology of Hilla District, Babylon, Iraq from December 2021 to March 2022 It includes a group of 39 patients with type 2 diabetes mellitus and 39 healthy people between the age of 35 to 60 years. The sociodemographic aspects were collected through the self-reported technique (study questionnaire) in Appendix including age, gender, and BMI.

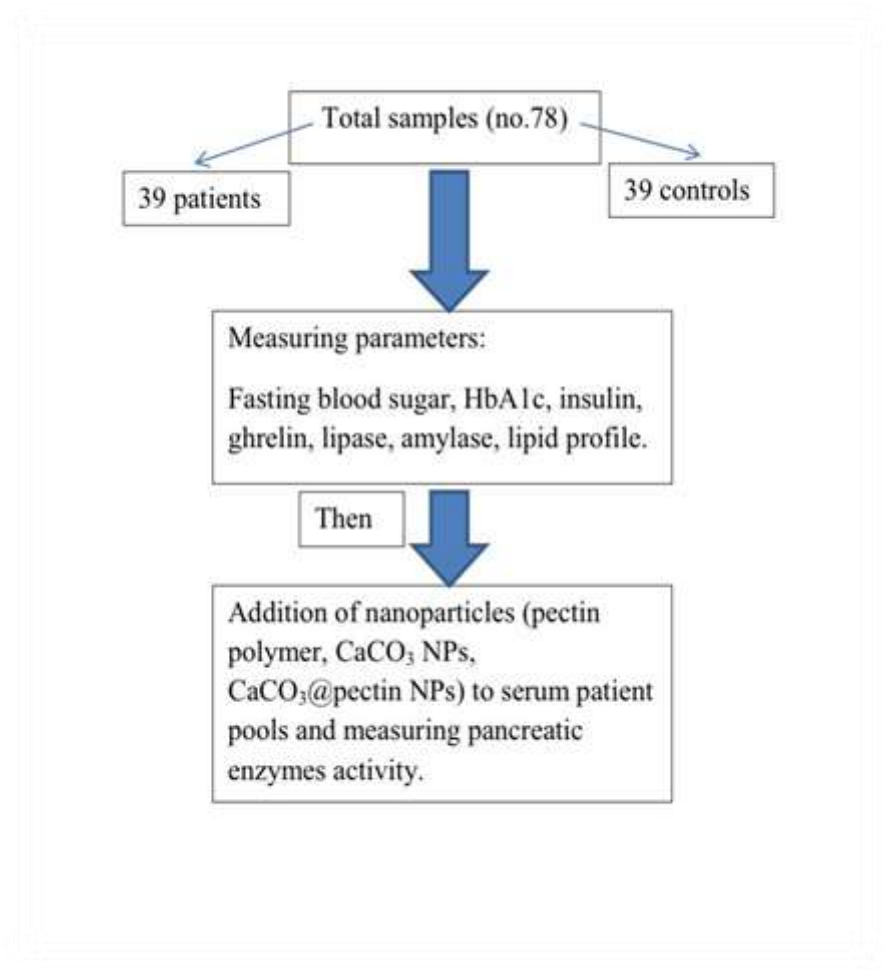


Figure 2.1: Study design of the research work.

2.2. Materials and instruments

2.2.1. Materials

The materials, kits, and tools with their suppliers which used in the study are listed in **Table 2.1** below.

Table 2.1: The materials and kits of the study.

No.	Material	Supplier
1	Alpha amylase SL kit	GIESSE DIAGNOSTICS, Italy
2	Calcium chloride ($CaCl_2$)	Sigma-Aldrich, Germany
3	Fasting blood glucose kit	ROCHE Cobas kit, Switzerland
4	HbA1c kit	ROCHE kit, Switzerland
5	HDL-cholesterol	Randox, United Kingdom
6	Human Ghrelin ELISA Kit	Bioassay technology laboratory/China
7	Human Insulin ELISA Kit	Bioassay technology laboratory/China
8	Hydrochloric acid (HCl)	Sigma-Aldrich, Germany
9	Lipase kit	GIESSE DIAGNOSTICS, Italy
10	Total cholesterol kit	Randox, United Kingdom
11	Triglyceride kit	Randox, United Kingdom
12	Sodium hydroxide (NaOH)	Sigma-Aldrich, Germany

2.2.2. Instruments

All the instruments and tools which used in the study are shown in **Table 2.2** below.

Table 2.2: the instruments and tools of the study.

No.	Instrument	Company supplied
1	Automatic chemistry analyzer	Geno TEK, Germany
2	Cooling centrifuge	Hettich, Germany
3	Deep freezer	Kryolab, Italy
4	Eppendorf tubes centrifuge	Hettich, Germany
5	Eppendorf tubes	Mheco, China
6	ELISA system (washer, printer, and reader)	Bio Tek, United States
7	Electric balance	Precisa, Switzerland
8	FT-IR spectrophotometer	Shimadzu, Japan
9	Field-Emission Scanning Electron Microscope (FE-SEM)	TESCAN, Japan
10	Gel tube	Mheco, China
11	Gilson Tips, 1000µl (blue)	Mheco, China
12	Gilson Micro-tips, 100µl	Mheco, China
13	Hot- plate stirrer	Lab Tech, Korea
14	Micropipette variable volumes	HUMAN Humapette , Germany
15	Oven	LEGEND, Germany
16	pH-meter	Ino Lab, Germany
17	Plane tubes	Mheco, China
18	Transmission Electron Microscope (TEM)	Leo, Germany
19	Teflon-lined stainless steel autoclave	ZZKD, China
20	UV-Visible spectrophotometer, Double beam, and single beam.	Shimadzu, Japan

21	Ultra-sonic path	Lab Tech, Korea
22	Ultrasonication bath	Labtek, Korea
23	X-Ray Diffraction (XRD)	Bruker, Germany

2.3. Study group

A total of 78 subjects of patients with type 2 diabetes mellitus and healthy people were studied. The protocol of the study was approved by the ethical research committee, College of Medicine, University of Kerbala, and the Center of Diabetes and Endocrinology of Hilla District, Babylon, Iraq.

2.3.1. Patient criteria

A 50% of the patient group was involved patients with type 2 diabetes mellitus, with age of (35-60) years.

2.3.2. Control criteria

The Control group involved (50%) approximately healthy subjects. All the subjects were not appeared with any signs and symptoms of the disease. Any patient with the following problems was excluded from the study:

a. Heart diseases and hypertension.
b. Insulin drug dependency.
c. Chronic liver disease.
d. Smokers, Pregnant, and Type 1 diabetic.

2.4. Approval of Ethical Committee

The study protocol was approved by the Ethical Committee of Kerbala Medical College, and the Committee of Endocrinology unit at the Center of

Diabetes and Endocrinology of Hilla District, Babylon, Iraq. Samples were obtained after consent from patients or the patient's relatives, as written in the questionnaire Appendix A.

2.5. Methods

2.5.1. Collection and storage of samples

Disposable syringes and needles were used for blood collection.Blood samples (5 ml) were obtained from patients and the control group. Blood samples were allowed to clot and then centrifuged at 4500×g for 10 minutes. Sera were separated and divided into a fraction in Eppendrof tubes then frozen at -20˚C until use.

2.5.2. Determination of body mass index

The body mass index (BMI) was calculated in all subjects according to a ratio depending on weight and height obtained by applying a mathematical equation, in which the weight in kilogram was divided by the square height in meters, and the results were considered as the following (166).

$$\textbf{BMI (kg/m}^2\textbf{) = weight (kg) / height (m}^2\textbf{)}$$

The ranges of (BMI) were categorized into groups, see **Table 2.3**

Table 2.3: Body mass index

Weight status	**BMI (kg/m^2)**
Underweight	≤ 18.5
Normal weight	18.5 - 24.9
Overweight	25-29.9
Obese	≥ 30

2.5.3. Determination of fasting blood glucose (FBG)

2.5.3.1. Principle

The colorimetric method for quantitative *in vitro* diagnostic measurement using the Glucose kit was used to assess the fasting serum glucose.

Glucose was oxidized by glucose-oxidase (GOD) to gluconate and hydrogen peroxide according to the following equation (167).

$$(Glucose + O_2 + H_2O \xrightarrow{GOD} H_2O_2 + Gluconate$$

$$2H_2O_2 + Phenol + 4\ Amino_{antipyrine} \xrightarrow{peroxidase} 4H_2O + Quinonimine$$

2.5.3.2. Reagents

a. Reagent 1 (Buffer):- Consist of 100 mmol/L of phosphate buffer pH7.5 and 0.75 mmol /L of Phenol.

b. Reagent 2 (Enzymes):- Consist of ≥15 KU/L of glucose oxidase, ≥1.5 KU/L of peroxidase, and 0.25mmol/L of 4-amino-antipyrine.

c. Reagent 3 (Standard):- Consist of 100 mg/dLor 5.55 mmol/L of glucose.

2.5.3.3. Preparation of the reagents

Working reagents were prepared by adding the substance containing reagent 2 in the vial (enzymes) to the vial of reagent 1 containing reagent 2 in the vial (Buffer). To complete the dissolving of all components, the mixture was mixed gently. **Table 2.4** describes the procedure.

2.5.3.4. Procedure

Table 2.4: The reagents and volumes of the fasting blood glucose method.

Tubes / Reagent	Blank	Standard	Sample
Working reagent	1 mL	1 Ml	1 mL
Standard (STD)	---------	0.01 mL	---------
Sample	---------	---------	0.01 mL

The tube was permitted to stand at a temperature (37 ºC) for 5 minutes after addition, and then the absorbance was measured at 500 nm, using a 1 cm light path cuvette.

2.5.3.5. Calculation

The concentrations of glucose in samples were calculated according to the equation below.

$$\boldsymbol{Glucose}\left(\frac{\boldsymbol{mg}}{\boldsymbol{L}}\right) = \frac{\boldsymbol{A.test}}{\boldsymbol{A.STD}} * \boldsymbol{Conc.of\ STD}\left(\frac{\boldsymbol{100\ mg}}{\boldsymbol{dL}}\right)$$

Where:

A= the absorbance of standard.

The normal value of fasting serum glucose concentration according to this method was around 75-115 mg/dL.

2.5.4. Measurement of serum HbA1c

2.5.4.1. Principle

The method used Tetradecyltrimethylammonium bromide (TTAB) as the detergent in the hemolyzing reagent to eliminate interference from leukocytes (TTAB does not lyse leukocytes). All hemoglobin variants which are glycated at the ß - chain N - terminus and which have antibody - recognizable regions identical to that of HbA1c are determined by the assay. The HbA1c determination was based on the turbid metric inhibition immunoassay (TINIA) for hemolyzed whole blood.

1. Sample and addition of R1 (buffer/antibody):

Glycohemoglobin (HbA1c) in the sample reacted with anti-HbA1c antibody to form soluble antigen-antibody complexes. Since the specific HbA1c antibody site was present only once on the HbA1c molecule, the formation of insoluble complexes did not take place.

2. Addition of R3 (buffer/polyhapten) and the start of reaction:

The polyhaptens reacted with excess anti-HbA1c antibodies to form an insoluble antibody-polyhapten complex. Then the complex was determined turbidimetrically.

Liberated hemoglobin in the hemolyzed sample was converted to a derivative having a characteristic absorption spectrum which was measured bichromatically during the preincubation phase (sample + R1) of the above immunological reaction.

2.5.4.2. Reagents

2.5.4.3. Preparation of reagents

Reagents - working solutions

Antibody Reagent (R1):

MES buffer: 0.025 mol/ L; TRIS buffer: 0.015 mol/ L, pH 6.2; HbA1c antibody (ovine serum): ≥ 0.5 mg / mL; detergent; stabilizers; preservatives

Polyhapten Reagent (R3):

MES buffer: 0.025 mol/L; TRIS buffer: 0.015 mol /L, pH 6.2; HbA1c polyhapten: ≥8 µg /mL; detergent; stabilizers; preservatives

R1 was in position A and R3 was in position C. Position B contained H_2O for technical reasons.

2.5.4.4. Calculation

The final result was expressed as % HbA1c and was calculated from the HbA1c/ Hb ratio as follows :

Protocol (% HbA1c acc . to DCCT/NGSP):

HbA1c (%)= (HbA1c / Hb)x 91.5 +2.15

2.5.5. Determination of lipid profile levels

2.5.5.1. Determination of serum total cholesterol

The total serum cholesterol was determined by the colorimetric method for the quantitative *in vitro* diagnostic measurement using a kit. (168)

A. Principle

The method was for the measurement of the total serum cholesterol, which involved the use of three enzymes: cholesterol esterase (CE), cholesterol oxidase (CO), and peroxidase (POD). In the presence of the former, the mixture of phenol

and 4-aminoantipyrine (4AA) was condensed by hydrogen peroxide to form quinoneimine dye proportional to the concentration of cholesterol in the sample. The serum cholesterol was measured using the enzymatic method that was were based on the following reactions:

$$\boldsymbol{Cholesterol\ ester + H_2O \xrightarrow{CE} Cholesterol + Free\ Fatty\ acids}$$

$$\boldsymbol{Cholesterol + O_2 \xrightarrow{CO} Cholesterol - 3 - one + H_2O_2}$$

$$\boldsymbol{2H_2o_2 + Phenol + 4 - amino - antipyrine \xrightarrow{POD} Quinoneimine(pink) + 4H_2o}$$

Where:

CE = Cholesterol esterase

CO = Cholesterol oxidase

POD = Peroxidase

B. Reagents

The components of the total serum cholesterol kit were used in this procedure was were presented in **Table 2.5**.

Table 2.5: The reagents, content, and concentrations of the total serum cholesterol kit.

Reagent	Content	Concentration
Reagent 1 buffer	Phosphate buffer pH (6.5) Phenol	30 mmol/L 5 mmol/L

Reagent 2 enzymes	4-amino-antipyrine Cholesterol esterase Cholesterol oxidase Peroxidase	0.3 mmol/L ≥ 150 U/L ≥ 100 U/L ≥ 5 KU/L
Reagent 3 standard	Cholesterol	200 mg/Dl

C. Preparation of reagents

The three sets of tubes (sample, standard, and blank) were prepared as the decelerated in **Table 2.6** below.

Table 2.6: The reagents and volumes of total serum cholesterol procedure.

Reagent	**Blank**	**Standard**	
Working reagent	1Ml	1mL	1mL
Standard(STD)	-------	10 µL	-------
Sample	-------	-------	10 µL

After addition, the solutions were mixed and incubated at least for 5 min at 37 ºC and the absorbance of samples and standards was measured at wavelength 500 nm against blank.

D. Calculation

The concentrations of total cholesterol in samples that were calculated according to the equation were shown below.

Conc. (mg/dL) = Abs. of sample /Abs. of standard × Conc. St (200 mg/dL).

According to the reference values of this method, the total cholesterol concentration in serum was < 200 mg/dL.

2.5.5.2. Determination of serum triglycerides (TGs)

Serum triglycerides were determined by the colorimetric method for the quantitative *in vitro* diagnostic measurement using a kit.

A. The principle of the method

The method was based on the enzymatic hydrolysis of serum or plasma triglyceride to glycerol and free fatty acids (FFA) by lipoprotein lipase (LPL). The glycerol was phosphorylated by adenosine triphosphate (ATP) in the presence of glycerol kinase (GK) to form glycerol-3-phosphate (G-3-P) and adenosine diphosphate (ADP). G-3-P was oxidized by glycerol-phosphate oxidase (GPO) to form dihydroxy acetone phosphate (DHAP) and hydrogen peroxide.

A red chromogen was produced by peroxidase (POD) catalyzed coupling of 4-aminoantipyrine (4-AA) and phenol with hydrogen peroxide (H_2O_2) proportional to the concentration of triacylglycerol in the sample. The serum triacylglycerol was measured using an enzymatic method which based on the following reactions:

$$\boldsymbol{Triglycerides \xrightarrow{Lipase} Glycerol + free\ fatty\ acids}$$

$$\boldsymbol{Glycerol + ATP \overset{GK}{\leftrightharpoons} Glycerol\ 3\ phosphate + ADP}$$

$$\boldsymbol{Glycerol - 3 - phosphate + O_2 \overset{GDP}{\leftrightharpoons} Dihydroxyacetone\ phosphate + H_2o_2}$$

$$\boldsymbol{H_2O_2 + 4\ aminoantipyrine \xrightarrow{POD} Quinoneimine(pink) + H_20 + HCL + 4Chlorophenol}$$

Where:

GK = Glycerol kinase

GPO = Glycerol-3-phosphate oxidase

POD = Peroxidase

The absorbance of the colored complex (quinoneimine), proportional to the amount of TGs in the serum, was measured at 500 nm.

B. Reagents

The components of the serum triglycerides kit which were used in this procedure are shown in **Table 2.7**.

Table 2.7: The reagents, content, and their concentrations of serum triglycerides kit.

Reagent	Content	Concentration
Reagent 1 Buffer	PIPES (pH 7.5) Magnesium chloride 4-Chlorophenol	50 mmol/L 4.5 mmol/L 5 mmol/L
Reagent 2 Enzymes	4-amino-antipyrine Lipases glycerol Kinase (GK) Peroxidase (POD) Glycerol -3- phosphate oxidase (GPO) Adenosine triphosphate Na (ATP)	0.25 mmol/L ≥ 1300U/L ≥ 400U/L ≥ 5 KU/L ≥1500U/L 2 mmol/L
Reagent 3 Standard	Triglyceride	200 mg/dL

C. Preparation of reagents

The preparation of the tubes (working reagent, distilled water, standard, and sample) is shown in **Table 2.8** below.

Table 2.8: The reagents and volumes of total serum triglycerides procedure.

Tubes / Solutions	Blank	Standard	Sample
Working reagent	1 mL	1 mL	1 Ml
Distilled water	10 μL	-------	-------
Standard	--------	10 μL	-------
Serum	--------	-------	10 μL

All tubes were mixed well, after that incubation for 5 minutes at 37 C^{o}, and then the absorbance of the sample and standard was measured against the blank at wavelength 500 nm (169).

D. Calculation

The concentrations of triglycerides in samples were calculated according to the equation below.

TG in (mg/dL) = A (Sample)/ A (Standard) x Standard Conc. 200 mg/dL.

According to the reference values of this method, the concentration of triglyceride in serum was between 35- 160 mg/dL.

2.5.5.3. Determination of high-density lipoprotein-cholesterol (HDL-C) concentration

A. Principle

Low-density lipoprotein-cholesterol (LDL-C), very low-density lipoprotein-cholesterol (VLDL-C), and chylomicrons were precipitated from specimens by

Phosphotungstic acid and $MgCl_2$. After centrifugation of the specimens, it was found that supernatant contains HDL-C then treated as total cholesterol. (170, 171)

B. Reagents

The components of the serum HDL-C kit used in this procedure were presented in **Table 2.9**.

Table 2.9: The reagents, content and concentrations of serum HDL-C kit.

Reagents	Compounds	Concentration
Reagent 1 Buffer	Phosphotungstic acid Magnesium chloride	0.55 mmol/L 25 mmol/L
Reagent 2 Standard	HDL- Cholesterol	50 mg/dL

C. Preparation of reagents

The sets of tubes (working reagent, distilled water, standard, and sample) were arranged are shown in **Table 2.10** below.

Table 2.10: The reagents and volumes of total serum HDL-C procedure.

Tubes / Solutions	Blank	Standard	Sample
Working reagent	1 mL	1 mL	1 mL
Distilled water	100 µL	--------	-------
Standard	--------	100 µL	-------
Supernatant	--------	--------	100 µL

All tubes were mixed well and incubated for five minutes at 37 ºC, and then the absorbance of the sample and the standard were measured against the blank at wavelength 500 nm.

D. Calculation

The concentrations of HDL-C in samples were calculated according to the equation below.

HDL-C (mg/dL) = A (Sample) / A (Standard) × Standard Conc. (50mg/dL)

According to the reference values of this method, the concentration of HDL-HDL-C in serum was between 35-55 mg/dL.

2.2.5.4. Assessment of very-low-density lipoprotein- cholesterol

Very low-density lipoprotein- cholesterol (VLDL-C) was calculated by dividing the triglycerides concentration by 5 and it was characterized by the concentration in milligram per deciliter. (172)

2.2.5.5. Assessment of low-density lipoprotein- cholesterol

Low-density lipoprotein- cholesterol (LDL-C) was calculated by the indirect method. (173)

LDL-C= total cholesterol – (HDL-cholesterol + VLDL cholesterol). LDL-C = total cholesterol - (HDL-cholesterol + TG/5).

According to the reference values, the normal value of LDL-cholesterol was less than 100 mg/dL.

2.2.6. Measurement of serum human insulin

A. Principle

The method was an Enzyme-Linked Immunosorbent Assay (ELISA). The plate was pre-coated with a human INS antibody. INS present in the sample was added and bound to antibodies that coated the wells. Then biotinylated human INS Antibody was added and bound to INS in the sample. Then streptavidin-HRP was added and bound to the Biotinylated INS antibody. After incubation, unbound Streptavidin-HRP has washed away during a washing step. The substrate solution was then added and color developed in proportion to the amount of human INS. The reaction was terminated by the addition of acidic stop solution and absorbance was measured at 450 nm.

B. Reagent provided

The components of the ELISA kit that were used in this procedure are presented in **Table 2.11**.

Table 2.11: The components of the ELISA kit.

Components	Quantity
Standard solution (80 mIU/L)	0.5 mL x1
Pre-coated ELISA Plate	12 * 8 well strips x1
Standard diluent	3 mL x 1
Streptavidin-HRP	6 mL x 1
Stop solution	6 mL x 1
Substrate solution A	6 mL x1
Substrate solution B	6 mL x1
Wash buffer concentrate (25x)	20 mL x1

Biotinylated human INS antibody	1 mL x1
User instruction	1
Plate sealer	2 pics
Zipper bag	1 pi

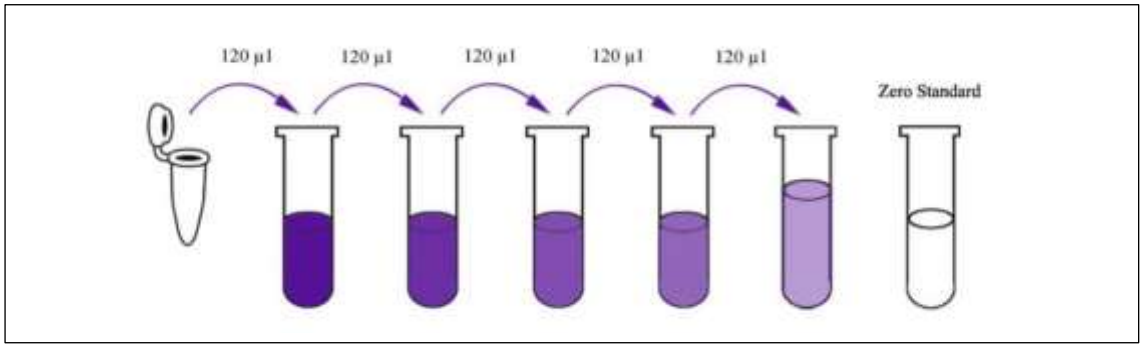
C. Reagent preparation

All reagents were brought to room temperature before use. Standard was reconstituted the 120 *μL* of the standard (80 mIU/L) with 120 µl of standard diluent to generate a 40 mIU/L standard stock solution. Allow the standard to sit for 15 mins with gentle agitation before making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (40 mIU/L) 1:2 with standard diluent to produce 20 mIU/L, 10 mIU/L, 5 mIU/L, and 2.5 mIU/L solutions. Standard diluent serves as the zero standards (0 mIU/L). every remaining solution should be frozen at -20 °C and used within one month. Dilutions of standard solutions suggested were as follows in **Table 2.12.**

Table 2.12: The concentrations and compositions of standards in ELISA protocol.

Standard concentration	Standard No.	Standard composition
40 mIU/L	Standard No.5	120 µL original standard + 120 µL standard Diluent
20 mIU/L	Standard No.4	120 µL standard No.5 + 120 µL standard diluent
10 mIU/L	Standard No.3	120 µL standard No.4 + 120 µL standard diluent

5 mIU/L	Standard No.2	120 µL standard No.3 + 120 µL standard diluent
2.5 mIU/L	Standard No.1	120 µL standard No.2 + 120 µL standard diluent



Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
80mIU/L	40mIU/L	20mIU/L	10mIU/L	5mIU/L	2.5mIU/L

Wash Buffer

Dilute 20 mL of wash buffer concentrate 25 x into deionized or distilled water to yield 500 mL of 1x wash buffer. When crystals formed in the concentrate, they were mixed gently until the crystals were completely dissolved.

D. Preparing the standard curve

This standard curve was only for demonstration purposes. A standard curve should be generated with each assay as shown in **Figure 2.1**.

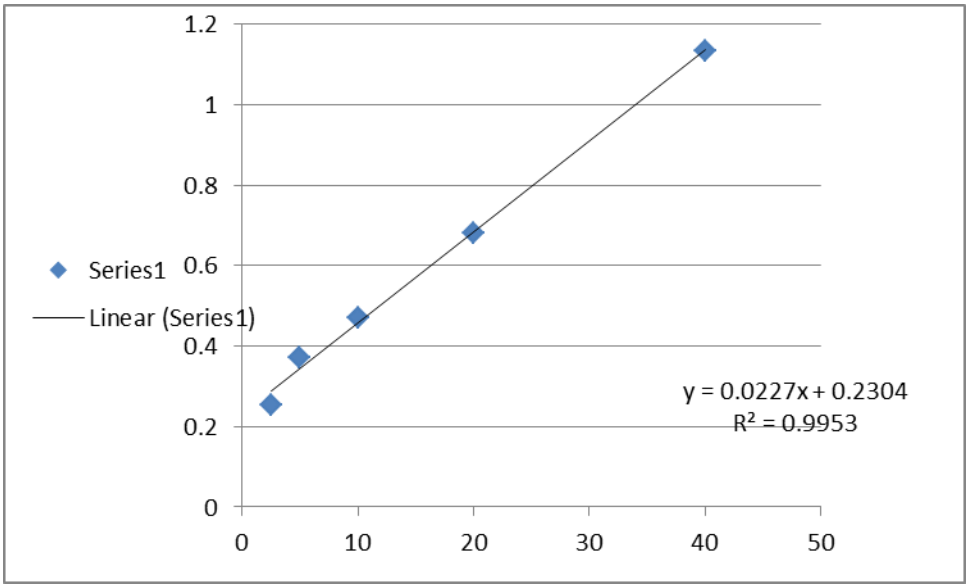


Figure 2.2: the standard curve of the insulin ELISA procedure.

E. Procedure

The procedure of the ELISA technique was done as the following:

a. All reagents, standard solutions, and samples were prepared as instructed. All reagents were brought to room temperature before use. The assay was performed at room temperature.
b. Strips were inserted in the frames for use.
c. Standard 50 µl was added to the standard well.
d. 40µl sample was added to sample wells and then 10µl anti-INS antibody was added to sample wells, and then 50µl streptavidin-HRP was added to sample wells and standard wells. Well was mixed and the plate was covered with a sealer. Then it was incubated for 60 minutes at 37°C.
e. Automated washing was done by aspirating all wells and washing 5 times with wash buffer, wells were overfilled with wash buffer. The plate was blotted onto paper towels.

f. 50µl substrate solution A was added to each well and then 50µl substrate solution B was added to each well. The plate which was covered with a new sealer was incubated for 10 minutes at 37°C in the dark.
g. 50µl Stop Solution was added to each well, then the blue color changed into yellow immediately.
h. The optical density (OD value) of each well was determined immediately by using a microplate reader which was set to 450 nm within 10 minutes after adding the stop solution.

2.2.7. Measurement of serum human ghrelin

A. The principle of the method

A sandwich kit was used for the accurate quantitative detection of human ghrelin (also known as GHRL) in serum. The method was an Enzyme-Linked Immunosorbent Assay (ELISA). The plate was pre-coated with a Human GHRL antibody. GHRL present in the sample was added and bound to antibodies coated on the wells. Then biotinylated Human GHRL Antibody was added and bound to GHRL in the sample. Later Streptavidin-HRP was added and bound to the Biotinylated GHRL antibody. After incubation unbound Streptavidin-HRP had washed away during a washing step. The substrate solution was then added and color developed in proportion to the amount of Human GHRL. The reaction was terminated by the addition of acidic stop solution and absorbance was measured at 450 nm.

B. Reagents

The components of serum human ghrelin by ELISA kit that were used in the procedure are presented in **Table 2.13**.

Table 2.13: The components of the human ghrelin ELISA kit.

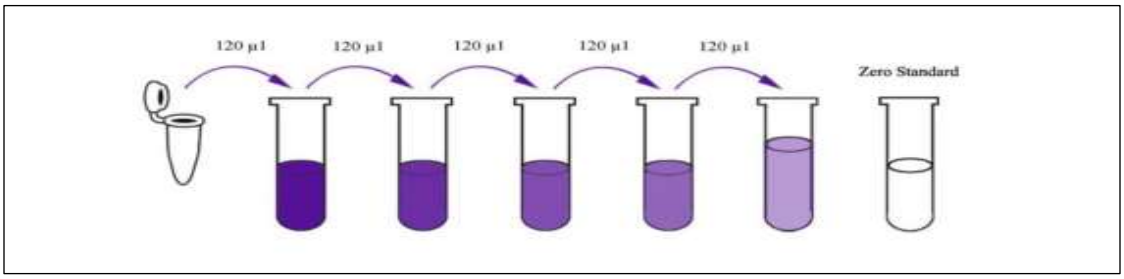
Components	Quantity (96T)	Quantity (48T)
Standard Solution (12.8ng/mL)	0.5mL x1	0.5mL x1
Pre-coated ELISA plate	12 * 8 well strips x1	12 * 4 well strips x1
Standard diluent	3 mL x1	3 mL x1
Streptavidin-HRP	6 mL x1	3 mL x1
Stop solution	6 mL x1	3 mL x1
Substrate solution A	6 mL x1	3 mL x1
Substrate solution B	6 mL x1	3 mL x1
Wash buffer concentrate (25x)	20 mL x1	20 mL x1
Biotinylated human GHRL antibody	1 mL x1	1 mL x1
User instruction	1	1
Plate sealer	2 pics	2 pics
Zipper bag	1 pic	1 pic

C. Preparation of the reagents

All reagents were brought to room temperature before their use. Standard was reconstituted the 120µL of the standard (12.8ng/mL) with 120µL of standard diluent to generate a 6.4ng/mL standard stock solution. The standard was allowed to sit for 15 mins with gentle agitation before making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (6.4ng/mL) 1:2 with standard diluent to produce3.2ng/mL, 1.6ng/mL, and 0.8ng/mL and0.4ng/mL solutions. Standard diluent serves as the zero standards (0 ng/mL). Any remaining

solution should be frozen at -20°C and used within one month. Dilutions of standard solutions that were suggested are shown in **Table 2.14** below.

Standard Concentration	Standard No.	Standard composition
6.4ng/mL	Standard No.5	120µl Original Standard + 120µl Standard Diluent
3.2ng/mL	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
1.6ng/mL	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
0.8ng/mL	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
0.4ng/ mL	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent



Standard Concentration	Standard No.5	Standard No.5	Standard No.5	Standard No.5	Standard No.5
12.8ng/mL	6.4ng/mL	3.2ng/mL	1.6ng/ mL	0.8ng/Ml	0.4ng/mL

Wash Buffer

Dilute 20 mL of wash buffer concentrate 25x into deionized or distilled water to yield 500mL of 1x wash buffer.

D. Preparing the standard curve

This standard curve was only for demonstration purposes. A standard curve was generated with each assay as shown in **Figure 2.2** below.

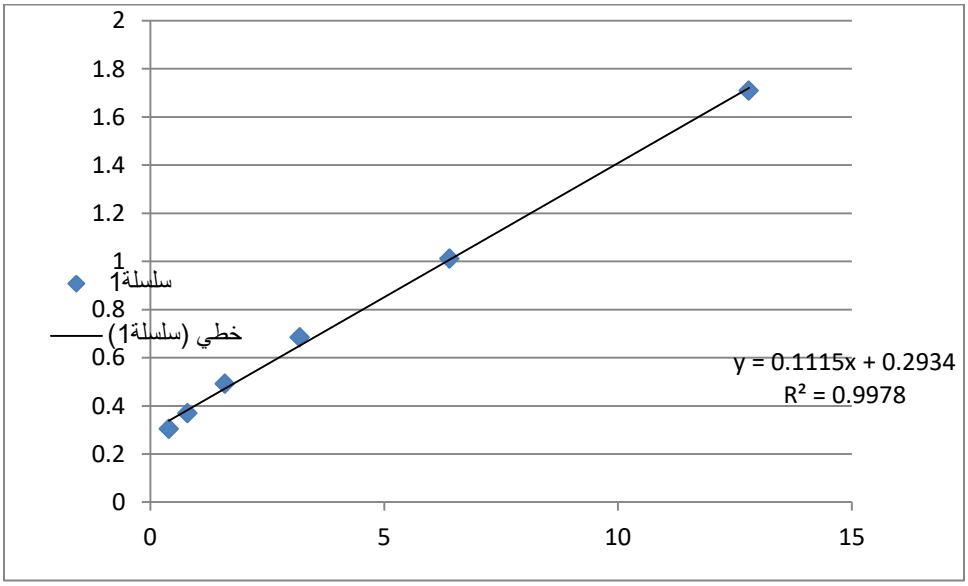


Figure 2.3: the standard curve of the ghrelin ELISA procedure.

E. The procedure

The procedure of the ELISA technique was done as the following:

a. All reagents, standard solutions, and samples were prepared as instructed. All reagents were brought to room temperature before use. The assay was performed at room temperature.
b. Strips were inserted in the frames for use.
c. 50 µl standard was added to the standard well.
d. 40µl sample was added to sample wells and then 10µl anti-GHRL antibody was added to sample wells, and then 50µl streptavidin-HRP was added to sample wells and standard wells. Well was mixed and the plate was covered with a sealer. Then it was incubated for 60 minutes at 37°C.

e. Automated washing was done by aspirating all wells and washing 5 times with wash buffer, wells were overfilled with wash buffer. The plate was blotted onto paper towels.
f. 50µl substrate solution A was added to each well and then 50µl substrate solution B was added to each well. The plate which was covered with a new sealer was incubated for 10 minutes at 37°C in the dark.
g. 50µl Stop Solution was added to each well, then the blue color changed into yellow immediately.
h. The optical density (OD value) of each well was determined immediately by using a microplate reader which setted to 450 nm within 10 minutes after adding the stop solution.

2.2.8. Determination of lipase by colorimetric method.

A. The principle of the method

The colorimetric substrate, 1,2-*ortho*-Dilauryl-rac-glycero-3-glutaric acid-(6'methyl-resorufin)-ester, was cleaved by pancreatic lipase and the resulting dicarboxylic acid ester was hydrolyzed under the alkaline test conditions to yield the chromophore methylresorufin.The formed methylresorufin, photometrically measured, was proportional to the amount of lipase in the sample.

B. Kit components

The components of the kit are presented in **Table 2.15** below.

Table 2.15: The components of the human lipase kit

Reagent (A) Volume = 10 mL	Good's buffer pH 8.0, colipase >2 mg/l, desoxycolate>1.0 mM, calcium

	ions >1 mM, detergent and preservative.
Reagent (B) Volume = 10 mL	Tartrate buffer pH 4.0, lipase substrate >0.1 mM, stabilizer, and preservative.

C. Calculation: Automated method.

D. Reagent preparation and procedure

The liquid reagents in the method were directly used while reagent (B) was mixed gently before had used. The reagents were added according to **Table 2.16** below

Table 2.16: The procedure and experimental conditions of the lipase estimation

Wavelength	580nm(570-590nm)
Light path	1 cm
Temperature	37°C
Reading	Against distilled water

E. Procedure

Pipette	Blank	Sample	Calibrator
Reagent(A)	1000 µL	1000 µL	1000 µL
Water	20 µL		
Sample		20 µL	
Calibrator			20 µL

Mix carefully; incubate at 37°C for 5 minutes.

Reagent (B)	250 µL	250 µL	250 µL

The reagent components were mixed, executing the first reading of absorbance after 2 minutes, incubating at 37°C. Then the other two readings were performed at 60 seconds intervals, after that the ΔA/min was calculated.

2.2.9. Measurement of alpha-amylase

A. Principle

Quantitative determination of α-amylase in serum was done by kinetic method (CNPG3). The α-amylase hydrolyzes 2-chloro-4-nitrophenyl-α-D-maltotrioside (CNPG3) into 2-chloro-4-nitrophenyl-α-D-maltoside (CNPG2), maltotriose (G3), glucose and 2-chloronitrophenol. The absorbance was changed in unit time measured at 405 nm is proportional to the enzyme activity in the sample.

B. Kit components

The kit used in the procedure included the reagents that were described in **Table 2.17.**

Table 2.17: The reagents, volumes and concentrations of the α-amylase kit.

Reagent (A) AMY Volume =10/50 ml	Good buffer pH 6 CNPG3 Sodium chloride Calcium acetate Sodium azide	100 mmol/L 3.1 mmol/L 10 mmol/L 1 mmol/L 15 mmol/L

C. Calculation: Automated method.

D. Reagents preparation and procedure

The procedure of the method was prepared according to the amounts of the reagents described in **Table 2.18**.

Table 2.18: The reagents, volumes and concentrations of the α-amylase kit.

Wavelength	405 nm
Light path	1 cm
Temperature	37°C
Reading	Against distilled water
Method	Increasing Kinetic
Sample/Reagent	1/50
Pipette	
Reagent (A)	1000 µL
Sample	20 µL

The reagents were mixed well and incubated at 37 °C for 1 minute, and then the initial absorbance was calculated against water. 3 readings at 60 seconds intervals were performed. The average value of the absorbance variations was calculated per minute (ΔA/min).

2.2.10. Preparation of serum pools and addition of nanomaterials

Pools were taken from the patients' serum and divided into three groups, each group to which nanomaterials were added (pectin polymer, $CaCO_3$ nanoparticles, and CaCO3@pectin nanoparticles). A one mg of nanoparticles was dissolved in 1 mL of distilled water. After that 20 µL of pectin polymer was added to 100 µL of serum, 20 µL of $CaCO_3$ nanoparticles was added to 100 µL of serum and 20 µL of $CaCO_3$@pectin nanoparticles was added to 100 µL of serum, then measured the activities of each enzyme (lipase and amylase).

2. 2.11. Synthesis and characterization of nanomaterials

2.2.11.1. Synthesis of calcium carbonate nanoparticles

Calcium carbonate nanoparticles ($CaCO_3$ NPs) were synthesized according to the previous literature method with a little modification. (174) Briefly, an aqueous solution of calcium chloride ($CaCl_2$) with concentration (0.33 M, 615 µL) was added to the solution containing 1.5 mL of sodium carboxymethyl cellulose solution (1% w/v) under stirring for 20 min. Then, 615 µl of 0.33 M of sodium carbonate (Na_2CO_3) was added under vigorous stirring for 60 min at room temperature. Then the product was centrifuged. Then, the precipitate was separated from the supernatant and then was washed twice with distilled water to remove unreacted species.

2.2.11.2. Preparation of pectin polymer

Pectin polymer was extracted from dried apple pomace powder and washed with distilled water (solid to liquid ratio of 1:20) for 15 min under constant mechanical stirring at 600 rpm and, then centrifuged to remove the supernatant (175). Briefly, pectin was extracted using deionized water (solid to liquid ratio of 1:20). The pH value was adjusted to 1.5−2.0 using 2 M HCl, and the mixture was then heated to 90−95 °C in a water bath and maintained for 60 min under constant mechanical stirring at 600 rpm. After extraction, the soluble portion was recovered *via* filtration. The filtrate was collected with alcohol and cooled to room temperature. Subsequently, 1.5 times the volume of 95% ethanol was added to the filtrate. After 4 h, the precipitate was washed three times with 5% (v/v) HCl in 95% ethanol. Thereafter, the precipitate was freeze-dried.

To decrease the length of polymer chains, the pectin chain was degraded by autoclaving at121°C and for 30 minutes, and the final product was collected and stored in the refrigerator for next used.

2.2.11.3. Preparation of calcium carbonate@pectin nanoparticles

The preparation of calcium pectin core-shell nanoparticles (denoted as Ca@pectin NPs was performed using a coating of pectin on the surface of calcium carbonate nanoparticles. Firstly, the as-prepared calcium carbonate nanoparticles were dissolved in distilled water (1 mg/mL, 50 mL) under vigorous stirring until complete dissolving. Pectin solution was also prepared with the concentration of (1 mg/mL) and drowsily was added to the $CaCO_3$ NPs solution with the molar ration of (1:0.4) of $CaCO_3$: pectin under string and heating at 50 ºC. The prepared solutions were then left under continuous stirring for 24 h at room temperature. The final product was separated by centrifugation at 10,000 rpm for 10 min. and dried in an oven at 60 ºC.

2.2.11.4. Characterization of nanoparticles

The morphology and structure of the Ca@pectin core-shell NPs were measured by scanning electron microscopy (FE-SEM, JSM-6601F; ARYA Electron Optic) operated at an accelerating voltage of 20 kV, and transmission electron microscopic (TEM, 912AB, Leo, Germany) at an accelerating voltage of 200 kV. The UV-Vis-NIR optical absorption spectra were recorded on a spectrophotometer (1900, SHIMADZU, Japan). FTIR spectra were recorded using the FTIR instrument (Shimadzu. 1800, Japan). The hydrodynamic diameters and Zeta potentials of the as-prepared samples were measured on Zeta potential analyzer (ZEN3600; Germany). X-ray powder diffraction (XRD) was tested by (PW1730, Philips, Holanda). The structure of the Ca@pectin core-shell NPs were

observed by transmission electron microscopy (TEM) on 912AB (Germany) with an accelerating voltage of 200 kV. The UV-vis-NIR optical absorption spectra of samples were recorded on spectrophotometer 1900, SHIMADZU (Japan). The hydrodynamic diameters and Zeta-potentials of samples were measured on the zeta potential analyzer (ZEN3600; Germany). X-ray powder diffraction (XRD) was tested by (PW1730, Philips, Holanda**).**

2.2.12. Statistical Analysis

Information from the questionnaire and all test results from study groups samples were entered a data sheet. The data analysis for this work was generated using the Statistical Package for the Social Sciences software, version 28.0 (IBM, SPSS, Chicago, Illinois, USA) and the Real Statistics Resource Pack software for Mac (Release 7.2) of the resource pack for Excel 2016. Copyright (2013 – 2020).

Descriptive statistics was performed on the data of each group. Values were illustrated by n (%) for categorical; Scale variables were presented by mean ± standard deviation for normal data while non-normal data, The distribution of the data was checked using Shapiro-Wilk test as numerical means of assessing normality. Biomarkers were compared using *spearman rank* test to evaluate the relationship within the case study. Significant differences in categorical variables among the parameters were confirmed through analytical statistical tests. Results of all hypothesis tests with p-values ≤ 0.05 (two-side) were considered to be statistically significant.

CHAPTER THREE

Results and discussion

3. Results and discussion

The occurrence of diabetes mellitus (DM) is increasing rapidly at an accelerating rate worldwide. The status of diabetes has changed over the last three generations; whereas before it was deemed a minor disease in older people but currently it is now one of the leading causes of morbidity and mortality among middle-aged and young people. High blood glucose-mediated functional loss, insulin sensitivity, and insulin deficiency lead to chronic disorders. Nanotechnology in diabetes studies has encouraged the development of wide modalities for glucose and insulin regulation that might hold the potential to improve the quality of life of diabetics (176).

3.1. Age

For DM, the age had a mean of 52.3± 5.9 years and for control, with an average of 47.4± 4.8 years. The results of the t-test indicated there were no significant differences (p =0.06) in age among the groups. The means and standard deviations are presented in **Table 3.1** and **Figure 3.1.**

Table 3.1: Summary statistics for age, BMI, and diabetic duration in DM patients compared to the control group.

Variables		Mean± SD	SE_M	Mini	Max	Mdn	P value
Age (Year)	DM patients group	52.3± 5.9	0.95	36	59	54	0.06
	Control group	47.4± 4.8	1.35	31	58	51	
BMI(kg/m^2)	DM patients group	30.9±4.9	0.79	18.08	43	31	0.87
	Control group	25.1±3.8	0.63	10.96	28.72	25.8	
Duration of DM (year)		9.6± 5.4	0.86	5	27	7	/

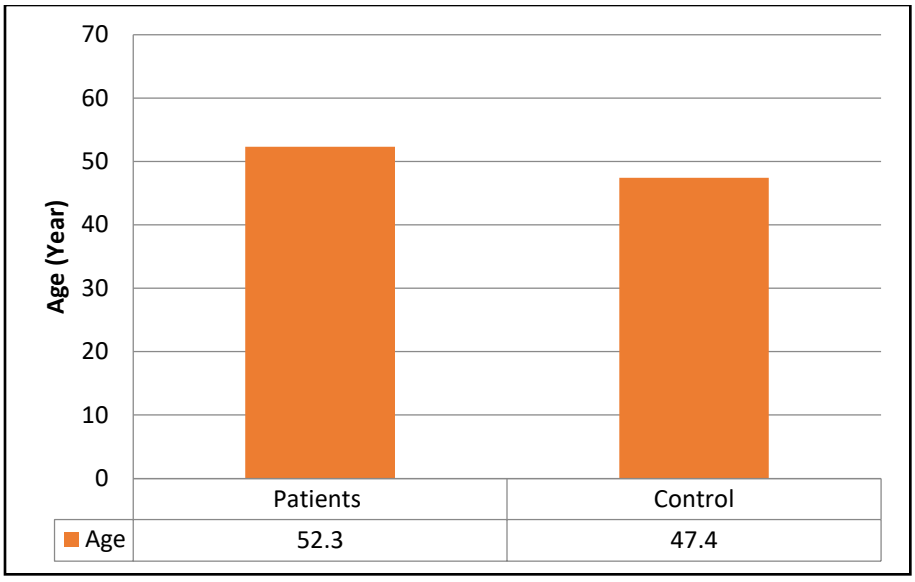


Figure 3.1: Baseline characteristics of age among type II diabetes mellitus patients compared to the healthy control group.

3.2. Sex

The gender distribution was nearly similar in both groups; there was no significant difference in sex among the groups (P≥ 0.05). Frequencies and percentages are presented in **Table 3.2** and **Figure 3.2**.

Table 3.2: Frequencies and percentages of age categories comparisons among the study groups.

Gender		Patients Group	Healthy Control Group
Sex (No.)	Male	20	19
	Female	19	20

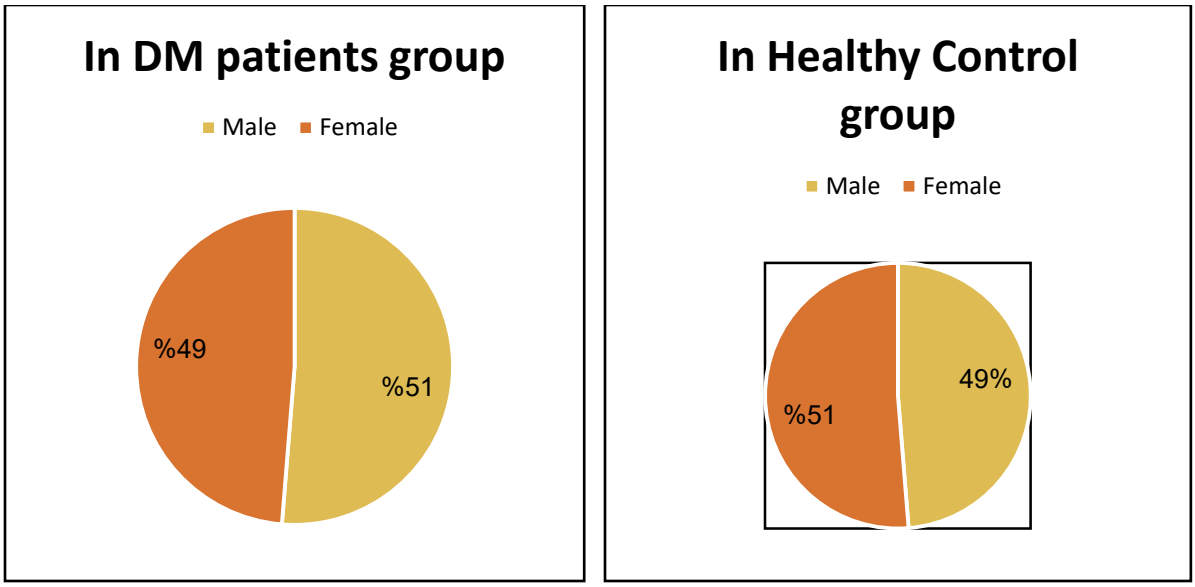


Figure 3.2: Baseline characteristics of age among type II diabetes mellitus patients compared to the healthy control group.

3.3. Body mass index

The results of the t-test indicated no significant differences in BMI among the studied groups, as shown in **Table 3.1**. The mean BMI for Control was (25.1±3.8) kg/m^2, while in the DM group was (30.9±4.9) kg/m^2($p \geq 0.05$). based on the BMI subgroups, results were revealed that about 61.5% of the DM group were classified as an obese group. The frequency of the BMI subgroup was presented in **Table 3.3** and **Figure 3.3.**

Table 3.3: Frequencies of BMI subgroups categories comparisons among the study groups.

BMI subgroups	Patients Group	Healthy Control Group
Non-obese	15	39
Obese	24	0

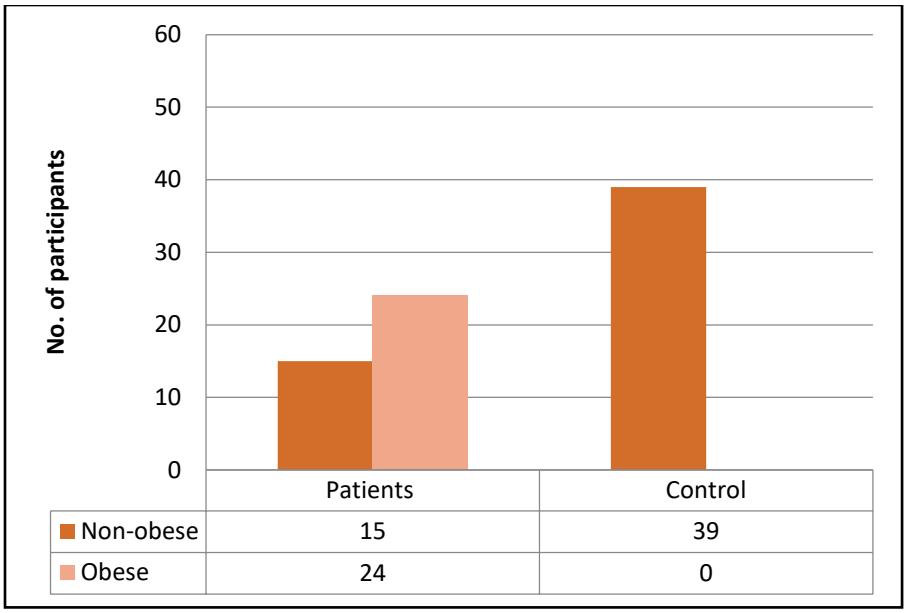


Figure 3.3: Baseline characteristics of BMI subgroups among type II diabetes mellitus patients compared to the healthy control group.

The results of age and sex that are present in **Table 3.1** and **Figure 3.1** showed no significant differences ($p = 0.06$) between the studied groups which confirms the match of age and sex between the compared groups. The BMI results were demonstrated in **Table 3.1**, although there was a difference in the mean levels of BMI among the study groups, the p-value was >0.05. DM is multifactorial where obesity and abnormal basal metabolic rate are considered most critical. of people diagnosed with T2DM, about 80% are obese. It is also reported that obese individuals have an increased odds of developing T2DM which is estimated to increase the incidence by two-fold (177). Obesity leads to various metabolic disorders which majorly include diabetes (178). Obesity-induced metabolic disability in susceptible individuals will promote IR on the one side and progressive β-cell dysfunction on the other. The nutrient excess problem can also be aggravated by increasing circulating glucose sugar, FFAs, & other nutritious

components. However, not all overweight or obese people experience hyperglycemia (179).

3.4. Fasting blood sugar

Generally, patients with type II diabetes mellitus patients were shown an increasing range level of FBS when compared to the healthy control groups. Results indicated a significant difference in FBS among groups, The means, and standard deviations were presented in **Table 3.4**. The mean level of FBS for DM was (203.7 ± 72.3) mg/dL which was significantly higher than for control group (88.6 ±7.64) mg/dL, ($p < 0.001$). The Distribution of serum levels of fasting blood sugar in type II diabetes mellitus patients compared to healthy control group was presented in **Figure 3.4**.

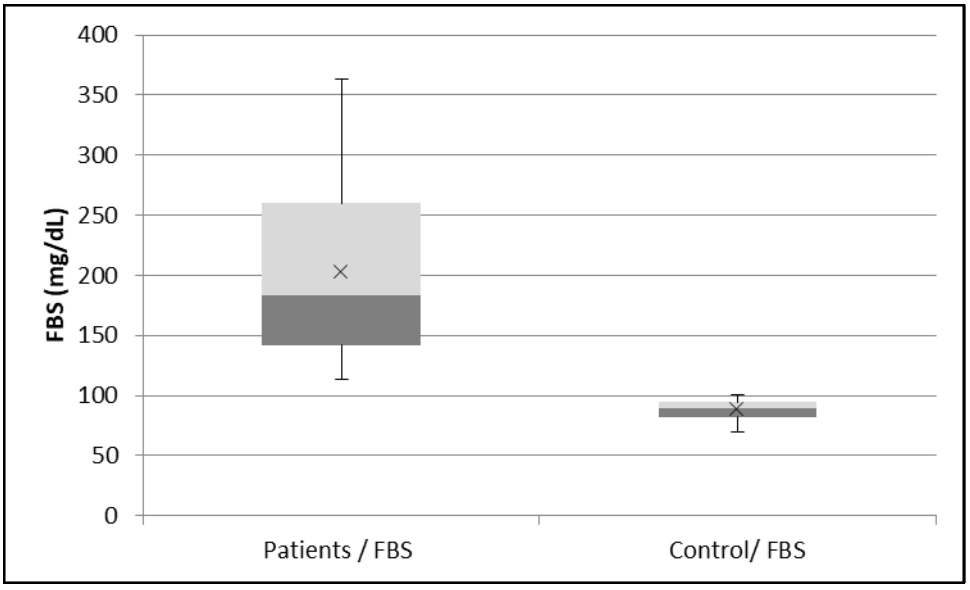


Figure 3.4: Boxplot of the distribution of serum level of fasting blood sugar in type II diabetes mellitus patients compared to the healthy control group.

Table 3.4: Descriptive statistics and mean difference of biomarkers level in type II diabetes mellitus patients compared to the healthy control group.

Variables		Mean± SD	SE_M	Mini	Max	Mdn	P value
FBS (mg/dL)	DM patients group	203.7 ± 72.3	11.88	113	363.6	186.8	<0.001
	Control group	88.6 ±7.64	1.26	70	101	90	
Amylase (IU/L)	DM patients group	71.4 ±29.11	4.77	30	146	67	0.52
	Control group	72.7 ±27.19	4.42	26	117	74.5	
Lipase (IU/L)	DM patients group	49.7 ±14.69	2.41	22.17	92.14	49.95	0.023
	Control group	42.0 ±7.35	1.12	29.99	62.14	39.93	
Insulin (IU/L)	DM patients group	18.3 ±6.04	0.99	2.9	42.4	17.65	0.001
	Control group	14.5 ±5.41	0.83	2.6	21.9	15.8	
Ghrelin (IU/L)	DM patients group	3.3 ±1.01	0.15	0.5	6	3.3	0.002
	Control group	2.6 ±0.71	0.12	0.4	3.7	2.6	
HbA1c %	DM patients group	9.5 ±2.35	0.39	5.9	15	8.9	<0.001
	Control group	4.9 ±0.48	0.08	4.1	5.9	5	
TG (mg/dL)	DM patients group	169.2 ±66.08	10.86	70.4	338	147.8	0.0004
	Control	104.3 ±34.78	4.95	93	193	149.5	

	group						
Cholesterol (mg/dL)	DM patients group	202.4 ±78.75	12.94	97.5	401.7	179.4	0.003
	Control group	149.3 ±30.13	5.72	39	170	107	
HDL (mg/dL)	DM patients group	52.2 ±13.8	2.27	19.3	79.4	41.87	0.001
	Control group	43.2 ±8.41	1.38	39	80	53	
VLDL (mg/dL)	DM patients group	33.8 ±12.21	2.17	14.08	67.6	29.56	<0.001
	Control group	20.8 ±6.95	1.14	7.8	34	21.4	
LDL (mg/dL)	DM patients group	128.1 ±70.85	11.64	32.04	331.35	98.84	0.002
	Control group	76.2 ±27.57	4.52	22.4	121.2	81.45	

3.5. Hemoglobin A1C

The hemoglobin A1c (HbA1c) was also examined. The mean levels of serum HbA1c in DM patients were significantly higher than those of healthy individuals ($P < 0.05$), as illustrated in **Figure 3.5** and **Table 3.4**. The mean of HBA1C for DM (9.5 ±2.35) was significantly higher than for Control (4.9 ±0.48), $p \leq 0.001$.

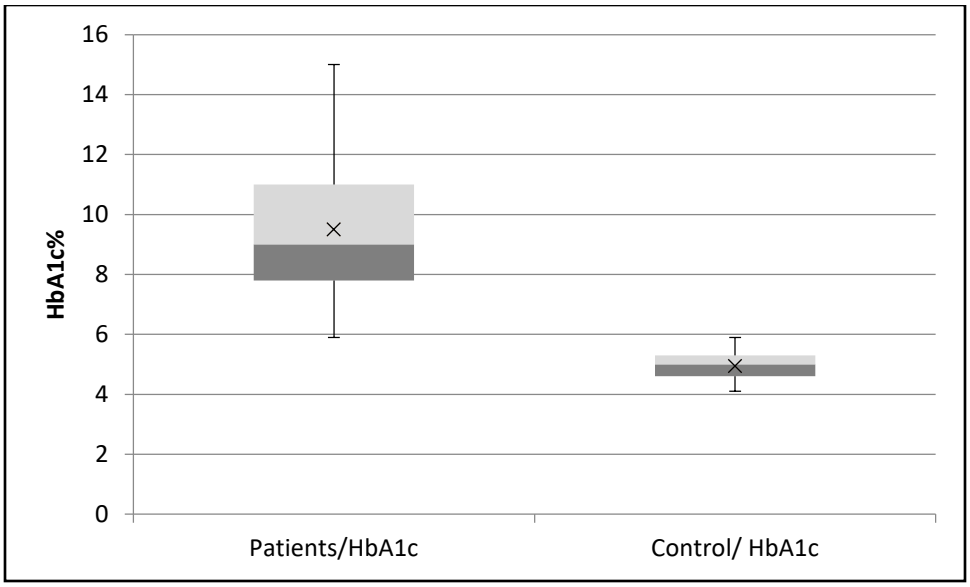


Figure 3.5: Boxplot of the distribution of serum level of HbA1c% in TypeII Diabetes Mellitus patients compared to the healthy control group.

The results for FBS and HbA1c analysis were significant ($p < 0.001$) and indicated there were significant differences in FBS among the group **Figure 3.4** and **Table 3.4.** Results indicated that diabetic patients (DM) have a significantly higher sugar profile than the control group. Also, the mean levels of serum HbA1c in DM patients were significantly higher than those of healthy individuals ($P < 0.05$), as illustrated in **Figure 3.5**.

Diabetes is characterized by chronic hyperglycemia and causes long-term complications. Since glycated hemoglobin considers a reflection of integrated glycaemia over the entire 120-day lifespan of the red blood cell, the levels of HbA1c are strongly correlated with FBS. HbA1c provides a reliable measure of chronic glycemia and correlates well with the risk of long-term diabetes, it's an ideal tool for monitoring and chronic management of diabetes (180). Among diabetics, the blood glucose levels increase in the blood and the glucose attaches to the hemoglobin molecule in a concentration-dependent manner. The glucose-

bound (glycated) hemoglobin or HbA1c provides the average glucose levels in an individual's blood as it becomes glycated with the hemoglobin. It is important to note that the HbA1c levels are directly proportional to the blood glucose levels (181). Type 2 diabetes mellitus (DM) manifests itself in terms of hyperglycemia due to compromised insulin production (182). The significance of the HbA1c test lies in the diagnosis and the prognosis of diabetes patients, which lends it to a detailed understanding of insulin and insulin resistance. There is a direct correlation between HbA1c and insulin resistance, where HbA1c is more strongly associated with insulin sensitivity in healthy subjects with normal glucose tolerance (183).

3.6. Serum insulin Levels

Serum insulin levels also increased significantly ($P < 0.05$), suggesting that DM patients had obvious insulin resistance. The distribution of Serum hormone levels was demonstrated in **Figure 3.6.** The means and standard deviations were presented in **Table 3.4**. The mean levels of serum insulin in the DM patients group were (18.3 ±6.04) (IU/L) respectively, while the mean levels in control group were (14.5 ±5.41) (IU/L).

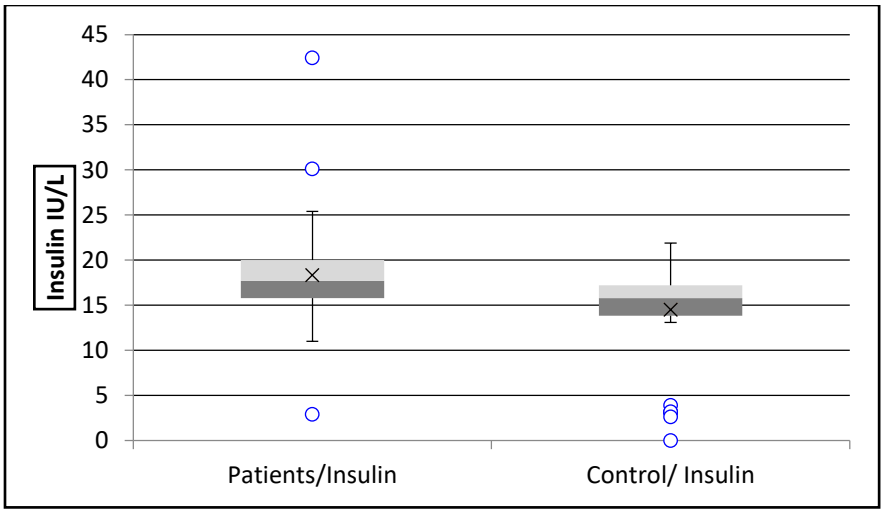


Figure 3.6: Boxplot of the distribution of serum level of insulin IU/L hormone in type II diabetes mellitus patients compared to the healthy control group.

Increased levels of insulin were reported to be a characteristic feature of T2DM. A progressive inability of the β cells to compensate for the prevailing insulin resistance by sufficient hyperinsulinaemia heralds the clinical onset of this disorder (184). The primary defects in insulin action appear to be in muscle cells and adipocytes, with impaired (glucose transporter-4 (GLUT4)) translocation resulting in impaired insulin-mediated glucose transports (185). Insulin resistance is a condition of reduced insulin sensitivity. Insulin sensitivity is commonly described as the ability of insulin to lower plasma glucose levels, which it by suppressing hepatic glucose production and stimulating glucose utilization in skeletal muscle and adipose tissue.

Both inherited and acquired influences contribute to the development of insulin resistance. The inherited defects responsible for the more common forms of insulin resistance, which are typically seen in individuals with type 2 diabetes, are largely unidentified. Although mutations in insulin receptors, glucose transporters, and signaling proteins have been identified, these are relatively rare. The acquired

causes of insulin resistance include inactivity, overeating, aging, hyperglycemia, increased levels of free fatty acids (FFAs), and the effects of some medications (186).

The etiology of hyperinsulinaemia is not yet fully elucidated. Although there are several theories, further research will likely show a multimodal pathology. The most common physiological principle of that might be the long-term action of increasing glucose levels therefore, healthy cells could be subjected to acute hyperglycaemia. Although many cells can absorb glucose without using insulin (glucose transporter-1 (GLUT1)) hyperglycaemia causes insulin to be released from pancreatic cells to facilitate absorption, especially in muscle and adipose cells (GLUT4) (187). Insulin binds to cellular insulin receptors and facilitates the translocation of GLUT4 to the cellular surface. During this process, the insulin and its receptor are absorbed into the cell to be replaced by the internal pool of insulin receptors (188).

Other has suggested that acute insulin resistance has no consequence as long as the cell has viable GLUT4 on the cellular surface. However, GLUT4 have a relatively short half- life. (189). During this period where the cells are replacing their insulin receptors, moderately elevated blood glucose levels may need slightly higher than normal insulin levels to restore normoglycaemia. This moderate hyperinsulinaemia may delay the return to normal insulin receptor function (acute insulin resistance).This state of insulin resistance due to down-regulated insulin receptors is reversible should the person not be subjected to further episodes of hyperglycaemia. It does not matter whether this is via high, but acute, blood glucose elevations, or moderately elevated glucose levels over a prolonged period. Prolonged impaired insulin signaling impedes GLUT4 translocation to the cellular surface thus causing impaired glucose uptake and prolonging hyperglycaemia, causing a positive feedback cycle. This will both aggravate and prolong the insulin

resistance, potentially turning it from a transitory state to a persistent or chronic state (190).

3.7. Serum pancreatic enzyme amylase activity (U/L)

The distribution of serum pancreatic enzymes (amylase) was examined as demonstrated in **Figure 3.7**. Generally, patients with Type II Diabetes Mellitus were shown an increasing range level of pancreatic enzymes when compared with that of the healthy control group. The mean levels of amylase activity in the DM patients group were (71.4 ±29.11) (U/L) respectively, while the mean levels in the control group were (72.7 ±27.19) (U/L). Lipase activity showed a significant difference in type II diabetes mellitus patients compared to healthy control group, ($p < 0.05$).

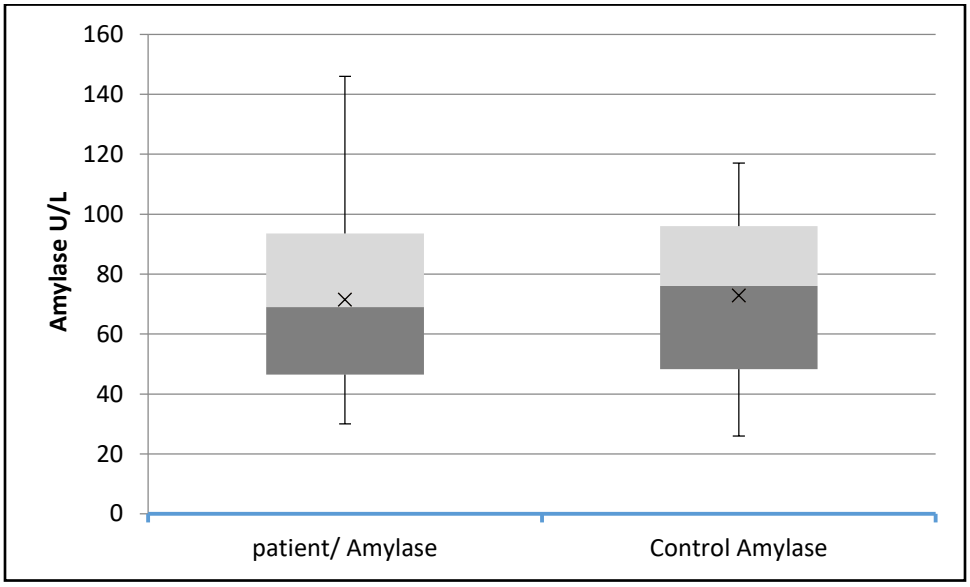


Figure 3.7: Boxplot of the distribution of serum level of serum pancreatic enzymes (amylase U/L) in type II diabetes mellitus patients compared to the healthy control group.

There are very few human studies that tried to probe the biochemical features and the underlying mechanisms to link the endocrine islet cells and the exocrine acinar cells (191).

Diabetes mellitus (DM), a metabolic disorder characterized by hyperglycaemia, associated with deficiency or resistance to insulin indicates an endocrinal abnormality of the pancreas. Amylase and lipase are enzymes secreted by the exocrine portion of the pancreas. Endocrinal derangement observed in diabetes may interfere with the exocrine function of the pancreas. Also, the low serum amylase levels in diabetes were associated with increased blood glucose levels (negative correlation) due to impaired insulin action either because of insulin resistance and/or inadequate insulin secretion (191). Patel R *et al*., in their study, they explained that reduced amylase secretion in the diabetic pancreas may be due to reduced cytosolic free calcium concentration (Ca^{2+}) and gene expression for amylase and not to the gene expression of cholecystokinin (CCK) a receptor in pancreatic acinar cells (192).

Long-standing type 2 diabetes mellitus (T2D) is associated with exocrine pancreatic failure and patient with T2D is at higher risk for developing acute or chronic pancreatitis. Otherwise, longstanding T2D and poor glycemic control are the major risk factors for Diabetic complications, such as retinopathy, nephropathy, neuropathy, and so on (193). Another study has demonstrated the elevated serum pancreatic enzymes (amylase and or lipase) activity that indicated an increased incidence of pancreatitis (194).

3.8. Serum pancreatic enzymes lipase activity (U/L)

The distribution of serum pancreatic enzymes (lipase) was examined as demonstrated in **Figure 3.8**. Generally, patients with type II diabetes mellitus were

shown an increasing range level of pancreatic enzymes when compared with that of the healthy control group. The mean levels of lipase activity in the DM patients group were (49.7 ±14.69) (U/L) respectively, while the mean levels in the control group were (42.0 ±7.35) (U/L).

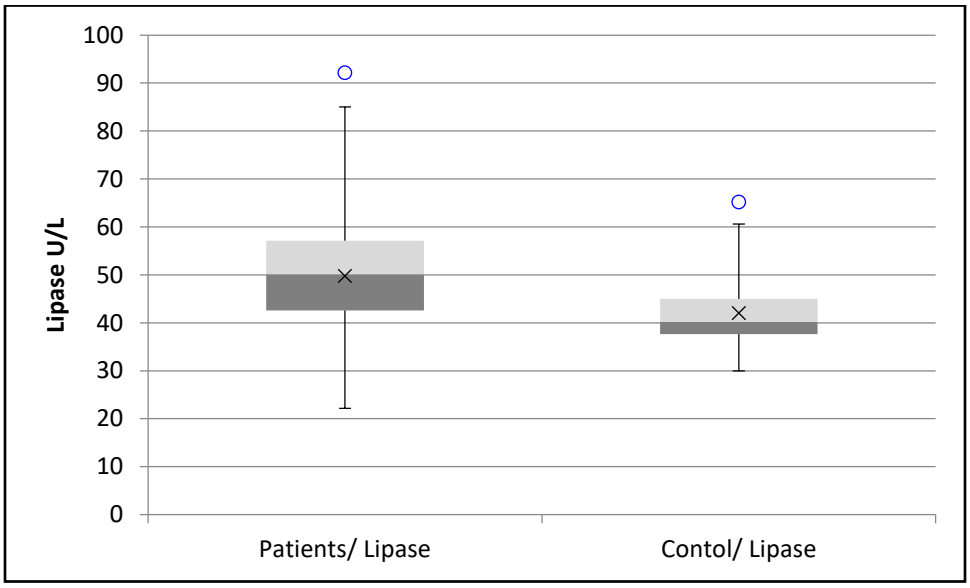


Figure 3.8: Boxplot of the distribution of serum level of serum pancreatic enzymes (lipase U/L) in type II diabetes mellitus patients compared to the healthy control group.

Abnormally high levels of serum lipase activity can be compounded in diseases other than pancreatitides, such as kidney disease, hyperglycaemia, and ketoacidosis (195). Although the incidence of pancreatitis was associated with T2DM, the pathogenic mechanism is still unclear (194). The high serum lipase activity in diabetes and prediabetes groups can interfere with the pancreas exocrine-endocrine interactions. Because insulin stimulates enzyme synthesis and releases in the exocrine pancreas, the high activity of lipase both in diabetes and prediabetes groups are associated with impaired insulin action due to insulin

resistance and inadequate insulin secretion (196). The increasing activity of lipase in T2D indicates pancreatic exocrine inflammation, named Pancreatitis (197). In a previous study, it has shown that the elevation activity of lipase correlates with long duration of diabetes, decreasing level of insulin, increased level of FPG, HbA1c, creatinine, insulin resistance, and dyslipidaemia profile (increasing level of total cholesterol, triglyceride, and small dense LDL particles). The hypothetical structural model analysis shows that there is a significant positive correlation between the increased glucose level and lipase activity, but not significantly contribute to glucose elevation. Thus, we predict that pancreatitis or the high value of serum lipase correlates with glycaemic control impairment or the duration of Indonesian T2D. Uncontrolled glucose levels can stimulate the level of triglyceride, and it will stimulate the lipase secretion to degrade triglyceride into free fatty acid (198).

3.9. Serum hormone ghrelin Level

The distribution of serum ghrelin hormone levels was demonstrated in **Figure 3.9**.The means and standard deviations were presented in **Table 3.4**. The mean levels of serum ghrelin in the DM patients group were (3.3 ±1.01) (IU/L), while the mean levels in the control group were (2.6 ±0.71) (IU/L).

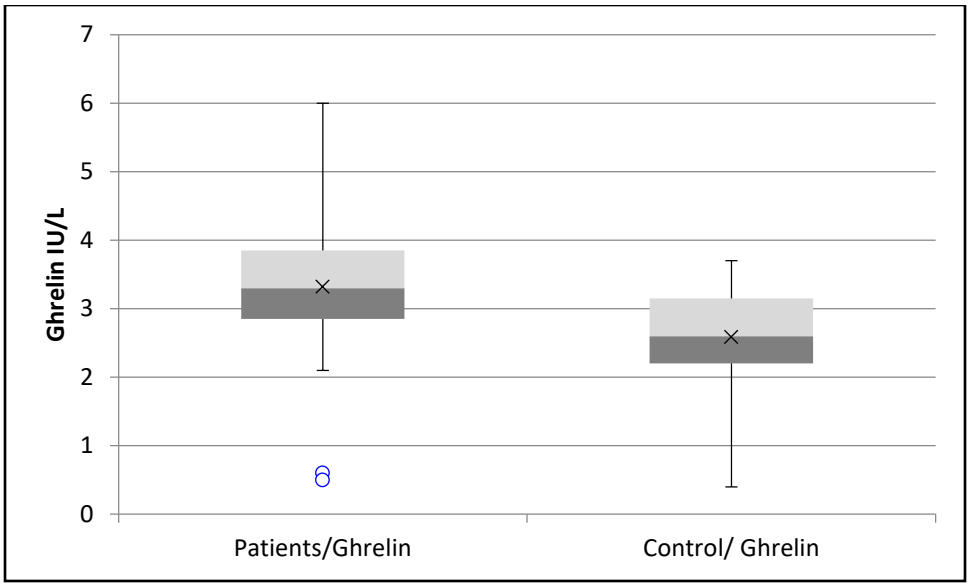


Figure 3.9: Boxplot of the distribution of serum level of ghrelin IU/L hormone in type II diabetes mellitus patients compared to the healthy control group.

Ghrelin was elevated significantly in type two diabetes mellitus. This hormonal protein is a crucial controller of the balance of energy and breakdown of fat in the central nervous system as well as a range of peripheral organs, including the pancreas and adipose tissue (199). Gastrointestinal hormone (ghrelin) regulates lipid metabolism and acts as connective tissue between the brain and the rest of the body (200). Ghrelin levels may be affected by a variety of variables, including food composition, activity, climate, and way of life (201). A rise in insulin, glucagon, and leptin levels in the blood is associated with ghrelin, according to Skuratovskaia *et al* (202). Uncontrolled diabetes is characterized by marked behavioral and metabolic perturbations that arise as a consequence of profound insulin deficiency, including severe hyperglycemia, Ghrelin has been reported to affect insulin signaling system implicating peripheral actions of ghrelin in glucose homeostasis (203). Also ghrelin secretion is increased in conditions of negative energy balance (204).Which might result in inducing obesity. These energy-related

effects may suggest a ghrelin-insulin or gut-pancreas axis in the control of body weight and energy metabolism (205).

3.10. Lipid profile

3.10.1. Total cholesterol

Lipid abnormalities in patients with diabetes often termed "diabetic dyslipidemia", are typically characterized by high total cholesterol (T-Chol), high triglycerides (TG), low range of high-density lipoprotein cholesterol (HDL-C), and massively increased levels of small dense (VLDL and LDL) particles. Results indicated a significant difference($P < 0.05$) in cholesterol levels in type II diabetes mellitus patients (202.4 ±78.75 mg/dL) compared to the healthy control group(149.3 ±30.13mg/dL), as shown in **Figure 3.10**, **Table 3.4**.

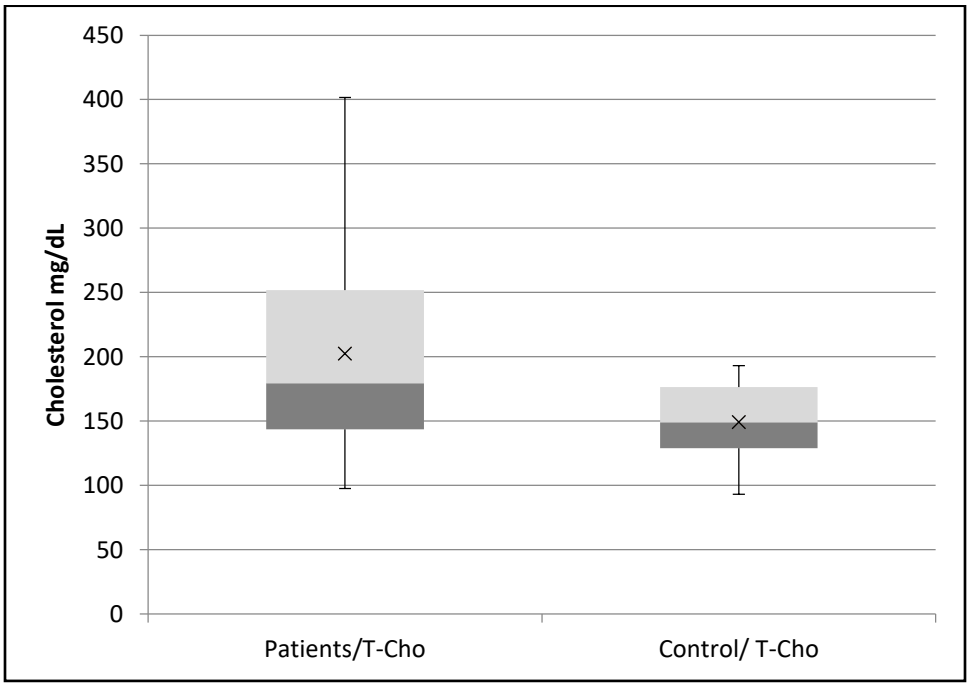


Figure 3.10: Boxplot of the distribution of serum level of cholesterol mg/dL in type II diabetes mellitus patients compared to the healthy control group.

3.10.2. Triglyceride

An analysis of differences in the mean levels of TG was conducted, and the results indicated that there was a significant increase in the TG in DM patients compared to the healthy control group, $p < 0.001$.The mean of TG for control (104.3 ±34.78) mg/dL was significantly lower than for DM (169.2 ±66.08). The means and standard deviations are presented in **Table 3.4** and **Figure 3.11.**

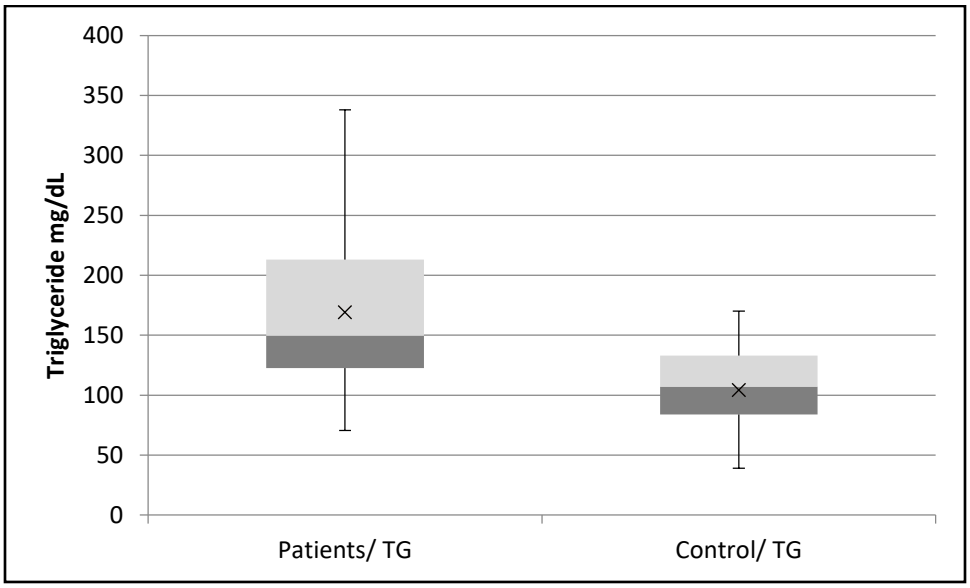


Figure 3.11: Boxplot of the distribution of serum level of triglyceride mg/dL in type II diabetes mellitus patients compared to the healthy control group.

3.10.3. High-density lipoprotein

Regarding of high-density lipoprotein, results indicated that there was a significant increase in the HDL in DM patients compared to the healthy control group, $p < 0.001$. The mean of HDL for Control (43.2 ±8.41) mg/dL was significantly lower than for DM (52.2 ±13.8), The means and standard deviations are presented in **Table 3.4** and **Figure 3.12**.

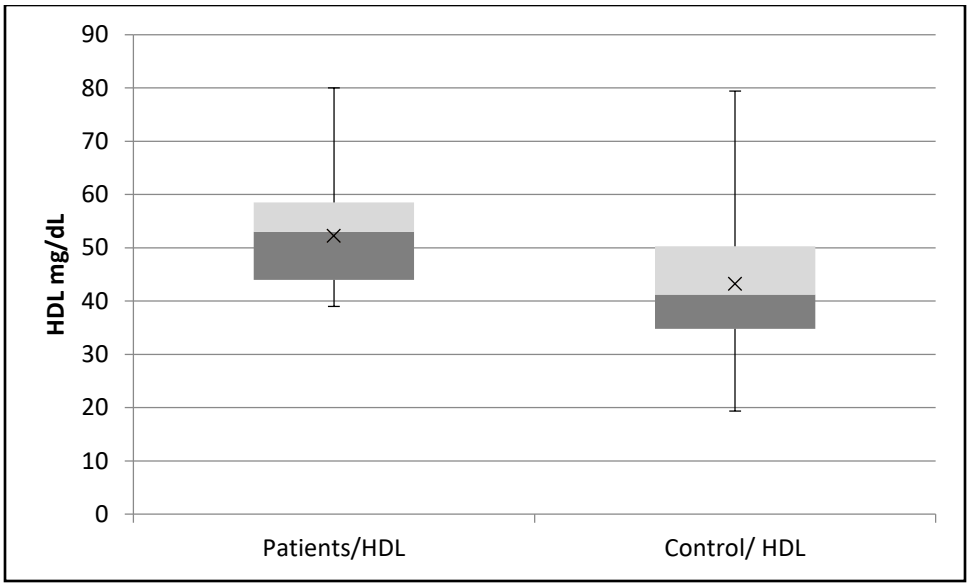


Figure 3.12: Boxplot of the distribution of serum level of HDL mg/dL in type II diabetes mellitus patients compared to the healthy control group.

3.10.4. Very low-density lipoprotein & low-density lipoprotein

The means and standard deviations of serum VLDL & LDL are presented in **Table 3.4**. The mean levels of the LDL in the DM patients group (128.1 ±70.85 mg/dL) were significantly higher ($p < 0.05$) than that of the control group (76.2 ±27.57 mg/dL) **Figures 3.13**.The mean levels of the VLDL in the DM patients group (33.8 ±12.21 mg/dL) were significantly higher ($p < 0.05$) than that of the control group (20.8 ±6.95mg/dL) **Figures 3.14**.

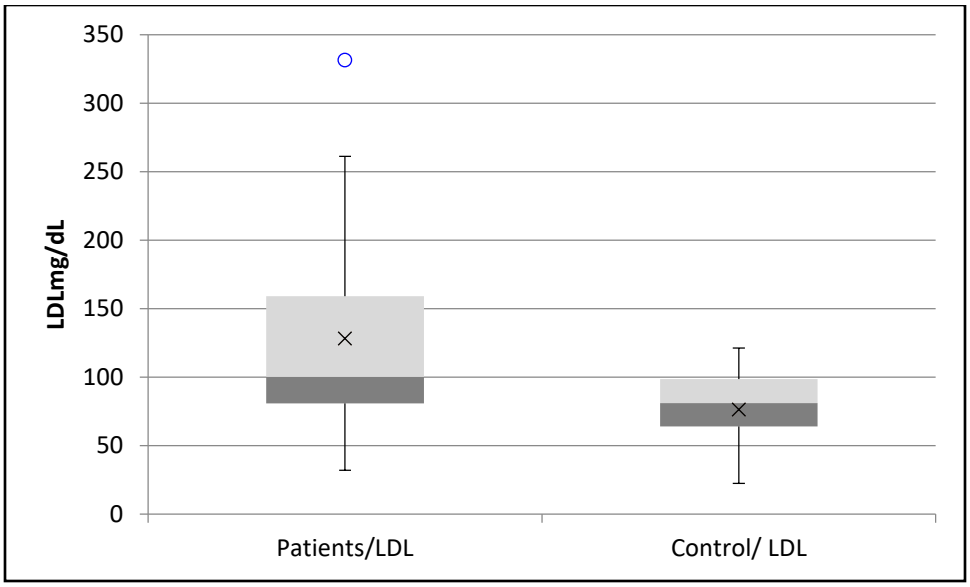


Figure 3.13: Boxplot of the distribution of serum level of LDL mg/dL in type II diabetes mellitus patients compared to the healthy control group.

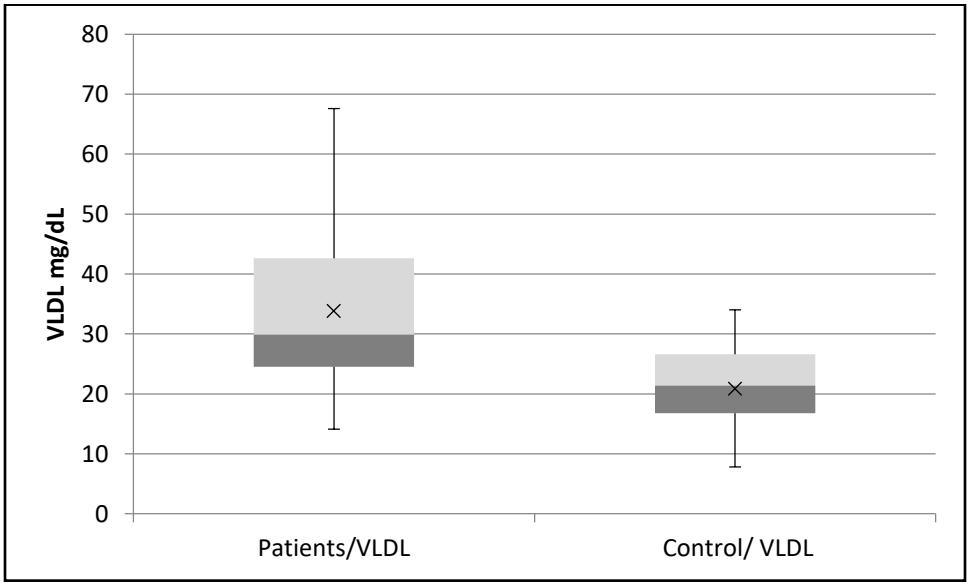


Figure 3.14: Boxplot of the distribution of serum level of VLDL mg/dL in type II diabetes mellitus patients compared to the healthy control group.

Lipid abnormalities in patients with diabetes often termed "diabetic dyslipidemia", are typically characterized by high total cholesterol (T-Chol), high triglycerides (TG), low range of high-density lipoprotein cholesterol (HDL-C), and massively increased levels of small dense (VLDL and LDL) particles. Results indicated a significant range difference in type II diabetes mellitus patients compared to the healthy control group as shown in **Figure 3.10**.

One of the common metabolic abnormalities in DM individuals is disturbed lipid metabolism and several forms of dyslipidemias have been observed in these patients. As many as 60% of diabetics exhibit some degree of hyperlipidemia and lipid metabolism appears to be related to the severity and duration of hyperglycaemic state3 and the degree of its control (206).

Epidemiological studies have shown an increased prevalence of lipid disorders in T2DM (207,208). combined high TG and LDL-C status, also known as "atherogenic dyslipidemia", were more likely to be present in T2DM individuals. It is recognized that dyslipidemia is an independent risk factor for cardiovascular disease. Elevated blood glucose level combined with dyslipidemia increases atherosclerosis-related inflammation and makes it more extensive (209). others have also observed the association of dyslipidemia with microvascular complications related to T2DM namely diabetic retinopathy, diabetic nephropathy, and diabetic neuropathy (210).

Several factors are related to diabetic dyslipidemia including insulin effects on liver apoprotein production, regulation of lipoprotein lipase, actions of cholesteryl ester transfer protein (CETP), and peripheral actions of insulin on adipose and muscle tissue (211). In an insulin-resistant state, hypertriglyceridemia is primarily due to increased hepatic production of very low-density lipoprotein (VLDL) particles, postprandial hyperlipidemia, and low lipoprotein lipase (LPL) levels. This hypertriglyceridemia enhances the CETP-mediated interchange of TG

from TG-rich lipoproteins to HDL-L/HDL-VL and the subsequent TG enrichment of HDL-C. Hepatic lipase has greater activity against TG and will, thus, convert large HDL particles too small HDL particles, which are also cleared more rapidly from the circulation by the kidney (212).

3.11. Correlation analysis

Considering the important role of the measured biomarkers in type II diabetes mellitus patients, the spearman rank test analysis was used to analyze the response relationship between parameters.

Mainly, Serum FBS was positively related to the HbA1c, TG, and VLDL levels. In addition, TG levels were significantly associated with total cholesterol, VLDL, and Insulin levels. As a risk factor of dyslipidemia in type II diabetes mellitus, serum LDL was highly significant positively related to high levels of total cholesterol (all $P < 0.001$). The overall relationship between the parameters and study cases was presented in **Figure 3.15**, also the individual Correlation Coefficients of the ghrelin, lipase, and amylase were presented in **Table 3.5**.

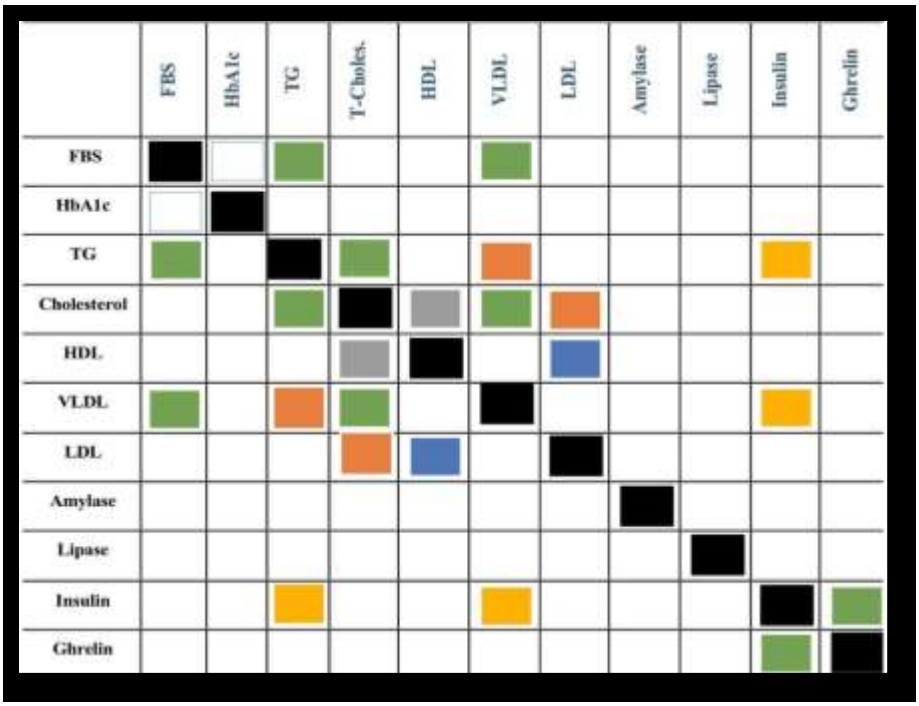


Figure 3.15: Heatmap of the spearman rank test analysis of type II diabetes mellitus patient. white boxes indicate a lack of correlation ($p>0.05$) while colored boxes reported statistically significant direct and indirect correlations, respectively. The intensity of the colour indicates the following relation: Yellow colour (r=0.3);green (r=0.4); blue (r=0.5); silver (r=0.6); light blue (r=0.7); orange(r=0.9).

Table 3.5: Correlation coefficients of ghrelin, lipase, and amylase and the measured biomarkers in DM patients.

Variables		Correlation Coefficient	P value
Ghrelin	FBS	-0.2	0.98
	HbA1c	-0.36	0.82
	TG	-0.28	0.77
	T-Choles.	0.25	0.64
	HDL	0.33	0.84
	VLDL	-0.28	0.77
	LDL	-0.2	0.94
	Insulin	0.47*	0.019
	Amylase	-0.2	0.45
	Lipase	0.21	0.3

Lipase	FBS	-0.238	0.14
	HbA1c	0.29	0.76
	TG	-0.11	0.48
	T-Choles.	0.287	0.6
	HDL	-0.276	0.4
	VLDL	-0.11	0.65
	LDL	0.3	0.16
	Insulin	0.23	0.18
	Amylase	0.23	0.2
	Ghrelin	0.2	0.31
Amylase	FBS	-0.328*	0.041
	HbA1c	-0.221	0.1
	TG	-0.28	0.084
	T-Choles.	-0.303	0.061
	HDL	-0.238	0.14
	VLDL	-0.28	0.08
	LDL	-0.216	0.18
	Insulin	-0.2	0.8
	Lipase	0.23	0.45
	Ghrelin	-0.12	0.16

Considering the important role of the measured biomarkers in type II diabetes mellitus patients, the spearman rank test analysis was used to analyze the response relationship between parameters. Correlation coefficients of ghrelin, lipase, and amylase were presented in **Table 3.5**.

Diabetes mellitus (DM), a metabolic disorder characterized by hyperglycaemia, associated with deficiency or resistance to insulin indicates an endocrinal abnormality of the pancreas. Amylase and lipase are enzymes secreted by the exocrine portion of the pancreas. Endocrinal derangement observed in diabetes may interfere with the exocrine function of the pancreas. low serum amylase levels in diabetes were associated with increased blood glucose levels (negative correlation) due to impaired insulin action either because of insulin resistance and/or inadequate insulin secretion. The decrease in amylase level was due to the insufficiency of pancreatic exocrine acinar cells (213).

Ghrelin, the orexigenic peptide hormone also affects glucose metabolism. Circulating levels of ghrelin rise before and fall after a meal, thereby contributing to appetite and weight gain (214).

Also, in **Figure 3.15,** Serum FBS was positively related to the HbA1c, TG, and VLDL levels. In addition, TG levels were significantly associated with total cholesterol, VLDL, and Insulin levels. As a risk factor of dyslipidemia in type II diabetes mellitus, serum LDL was highly significant positively related to high levels of total cholesterol (all $P < 0.001$).

One of the common association metabolic abnormalities in DM patients is disturbed lipid metabolism and several forms of dyslipidemias have been observed in these patients. As many as 60% of diabetics exhibit some degree of hyperlipidemia and lipid metabolism appears to be related to the severity and duration of the hyperglycaemic state and the degree of its control (215).

Lipid abnormalities are prevalent in DM patients because of IR which affects key enzymes and pathways in lipid metabolism: Apo protein production, regulation of lipoprotein lipase, the action of cholesterol ester transfer proteins, and hepatic and peripheral actions of insulin (216).

Hyperglycemia and the high level of IR associated with T2DM have multiple effect on fat metabolism which results in the production of atherogenic dyslipidemia characterized by lipoprotein abnormalities: elevated very low-density lipoprotein cholesterol (VLDL) elevated low-density lipoprotein cholesterol (LDL-c), elevated triacylglycerol (TAG) and decreased high-density lipoprotein cholesterol (HDL-c) (217). The main cause of lipid abnormalities in T2DM patients is impaired secretion of insulin which affects the liver apolipoprotein production and regulates the enzymatic activity of lipoprotein lipase (LpL) and cholesterol ester transport protein (CETP). Moreover, its deficiency reduces the

activity of hepatic lipase; therefore, several steps involved in the production of biologically active LpL might be altered in T2DM compared to controls (215).

3.12. Characterization of nanoparticles profile

The nanoparticles of Ca@pectin, calcium nanoparticles, and pectin nanoparticles were characterized by Philips XL30 SFEG scanning electron microscope (SEM). Energy Dispersive X-ray (EDX) microanalysis was also used identification of the elemental composition of all prepared nanoparticles in order to identification of elemental composition. The zeta potentials of NPs were examined by a micrometrics Zeta/Nano Particle Analyzer (Nano Plus-3). The UV-vis spectra of the samples were attained via UV-Vis Spectrophotometer (Shimadzu UV-2600, Japan).

3.13. Synthesis and characterization of Ca@pectin nanoparticles

Initially, Calcium carbonate nanoparticles ($CaCO_3$ NPs) were first synthesized by the co-precipitation method in the presence of surfactant by the mixing of the two precursors salts ($CaCl_2$ and Na_2CO_3) (174).

Pectin polymer with a large molecular weight was prepared by extraction from apple pomace in the presence of deionized water and acidic media. Then, the extracted polymer was degraded by hydrothermal method using autoclave at 121 °C to decrease the length of polymer chains.

Then, the calcium pectin core-shell nanoparticles (Ca@pectin) NPs were prepared by coating the pectin polymer on the surface of calcium carbonate nanoparticles under stirring in an aqueous solution. The synthesis procedures of Ca@pectin NPs are illustrated in **Figure 3.16** below.

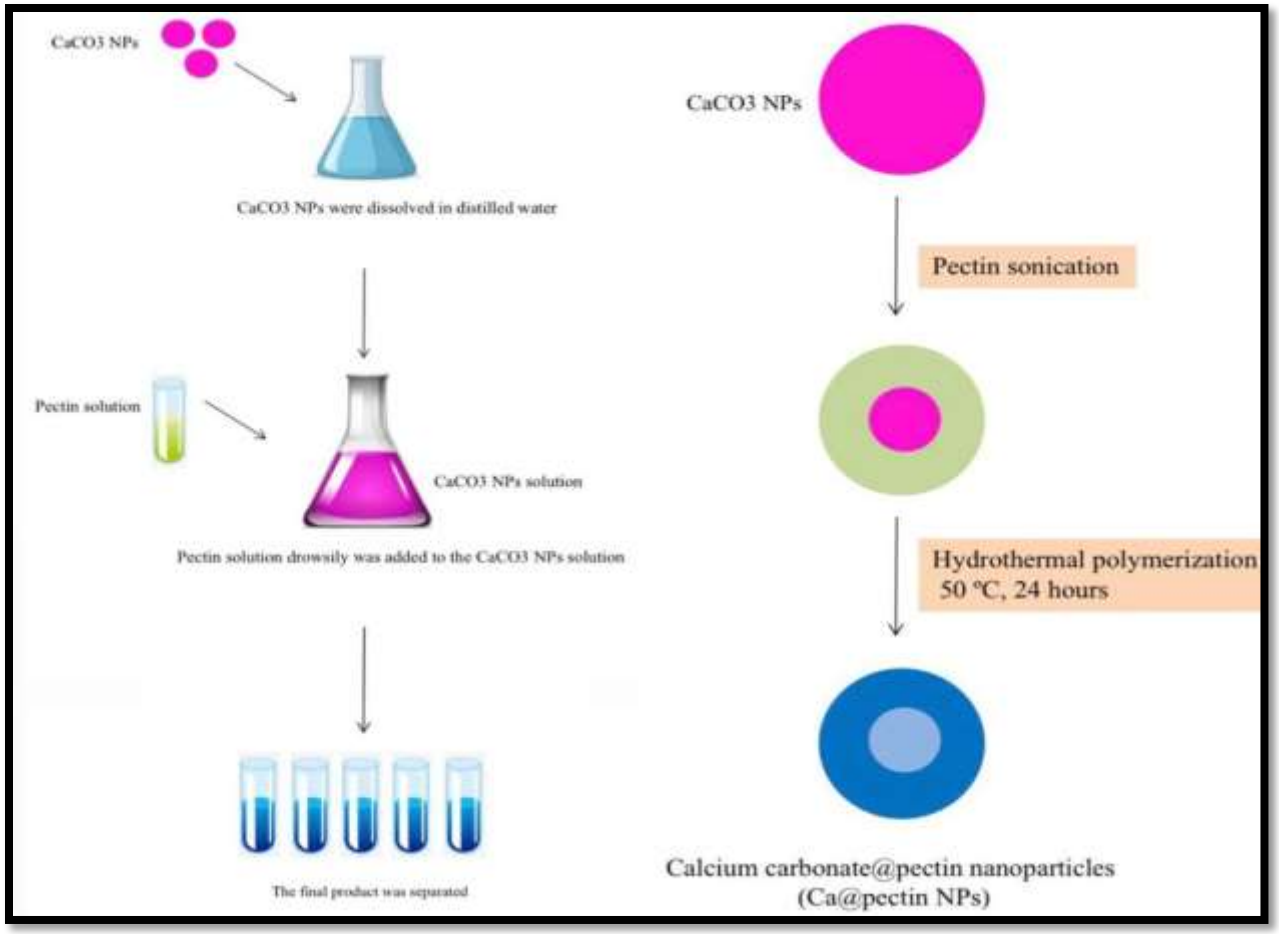


Figure 3.16: The synthesis procedures of Ca@pectin NPs.

The morphology and structure of the prepared samples were characterized by using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). As shown from SEM images **Figure 3.17**, the as-prepared $CaCO_3$ NPs appeared to be nanospheres with a particles size of about 20-50 nm, while the average size of Ca@pectin NPs was increased after coating with pectin polymer, indicating the successful coating of pectin on the surface of $CaCO_3$ NPs.

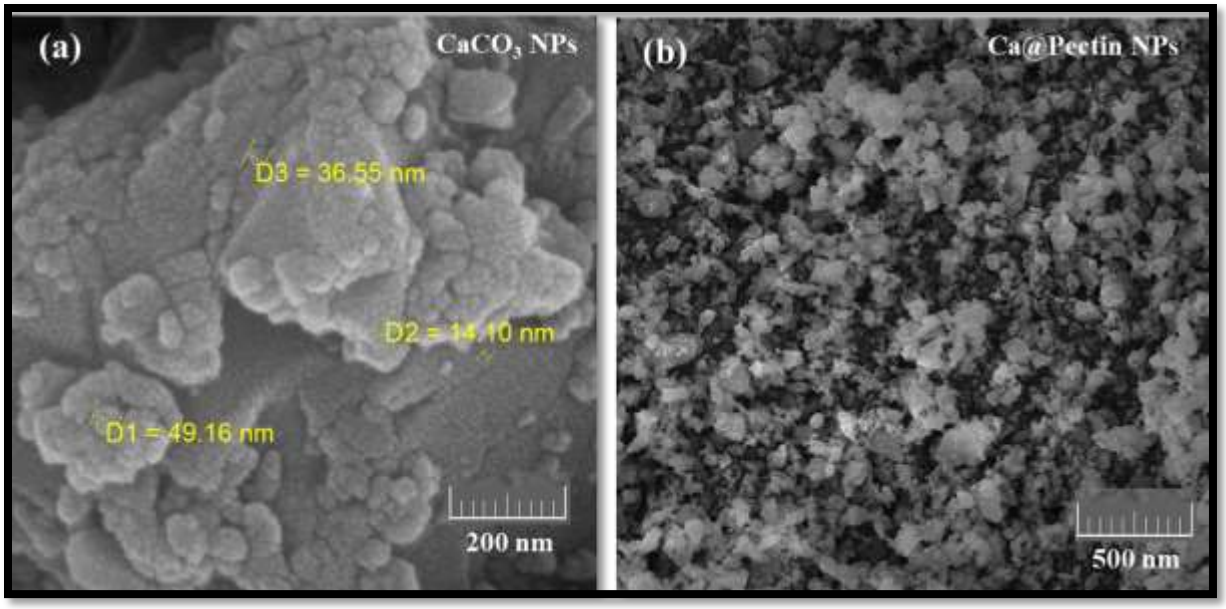


Figure 3.17: SEM images of (a) $CaCO_3$ NPs, (b) Ca@pectin NPs.

The pectin shells are well encapsulated on the surface of $CaCO_3$ NPs with a thickness of about 10-20 nm, forming Ca@pectin core-shell NPs. The average size of $CaCO_3$ NPs were increased after coating with pectin to ~80 nm, indicating the successful formation of Ca@pectin core-shell NPs. It is clearly seen that $CaCO_3$ NPs cores appeared in the dark contrast, while the outer bright coatings on each $CaCO_3$ NPs nanosphere correspond to the pectin polymer shells **Figure 3.18**. The $CaCO_3$ NPs core is crystalline while the pectin polymer shell is amorphous in nature, in good agreement with XRD measurements.

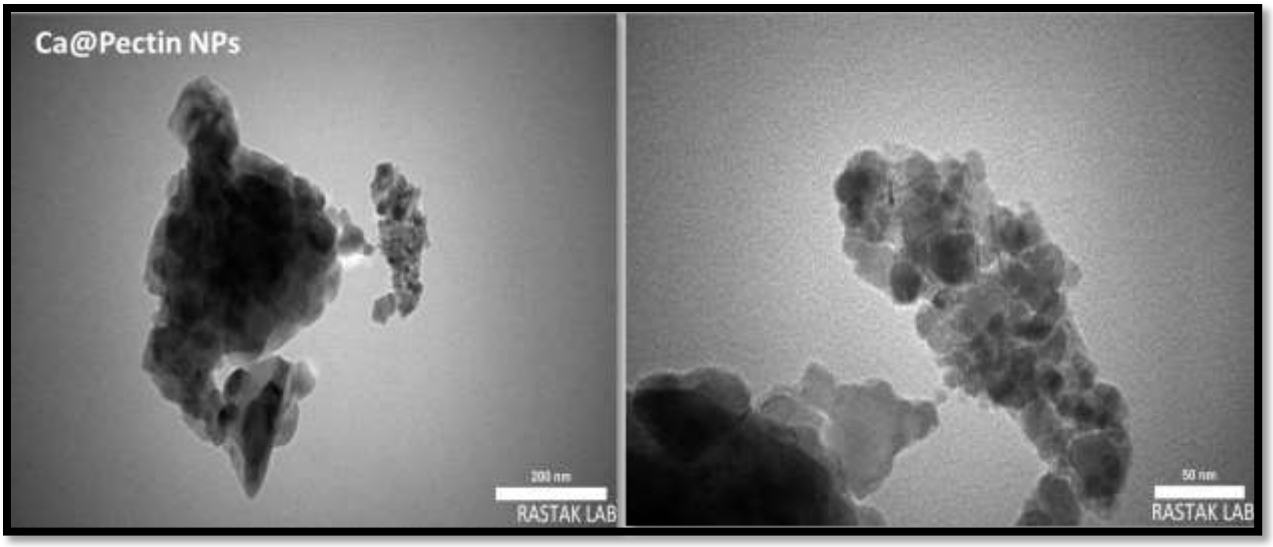


Figure 3.18: TEM images of Ca@pectin NPs.

The elemental distribution of the as-prepared $CaCO_3$@pectin NPs was examined by Energy-Dispersive Spectroscopy (EDS) of SEM technique, as clearly shown in **Figure 3.19** the elemental mapping clearly demonstrated the presence of all the elements in the $CaCO_3$@ pectin structure, which is the further evidence for the formation of $CaCO_3$@ pectin nanoparticles.

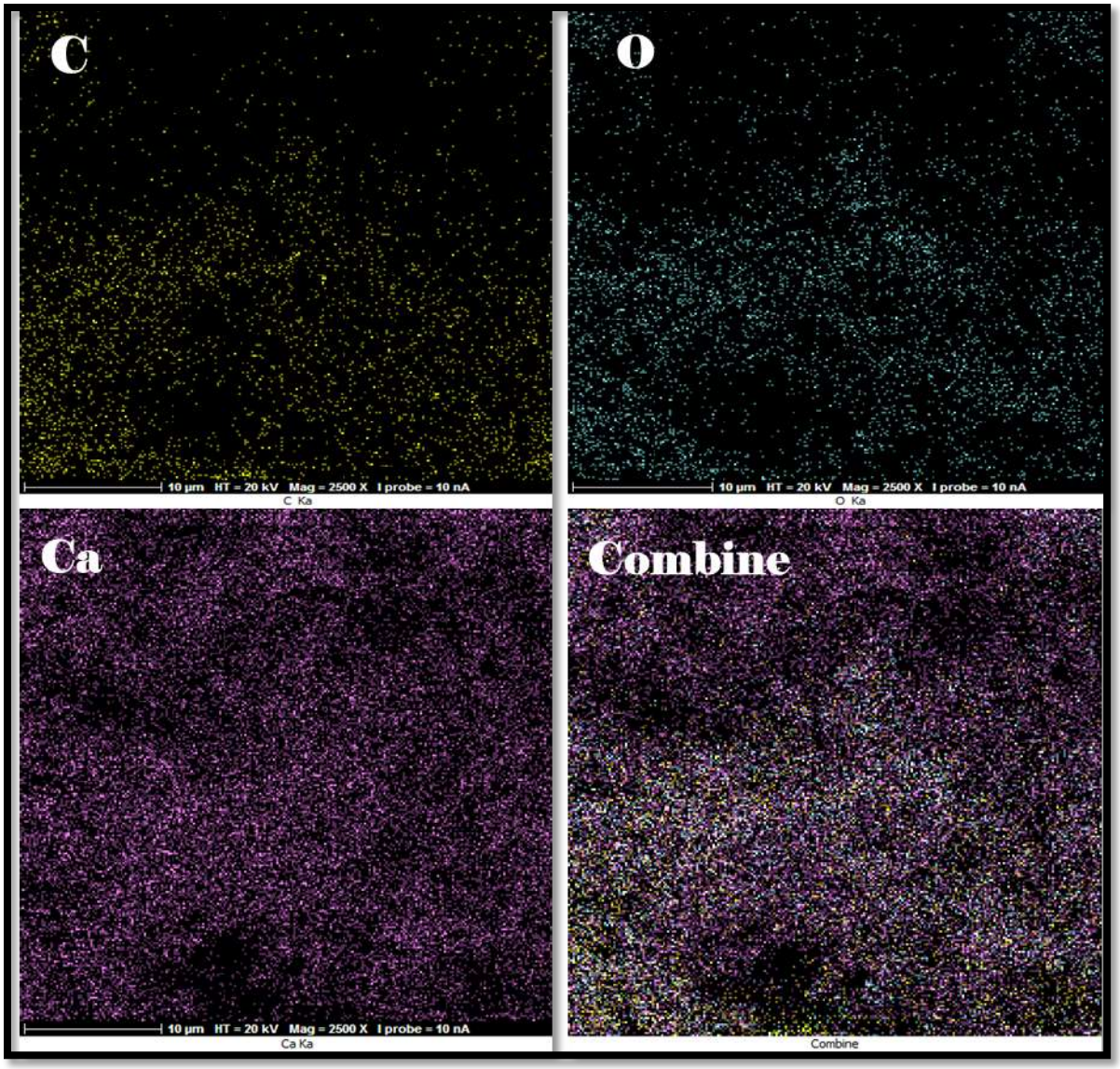


Figure 3.19: EDS-SEM of the $CaCO_3$@ pectin nanoparticles

The particle sizes of the as-made nanomaterials were also further examined using dynamic light scattering (DLS) measurements, where it appears to be larger than those determined by TEM. The diameters of $CaCO_3$ NPs were ~100 nm while, after coating with the pectin polymer layer, an increase in the average size from ~100 nm to ~170 nm was observed, as shown in **Figure 3.20**, demonstrating the successful pectin polymer coating on $CaCO_3$ NPs and the formation of $CaCO_3$@ pectin core-shell NPs.

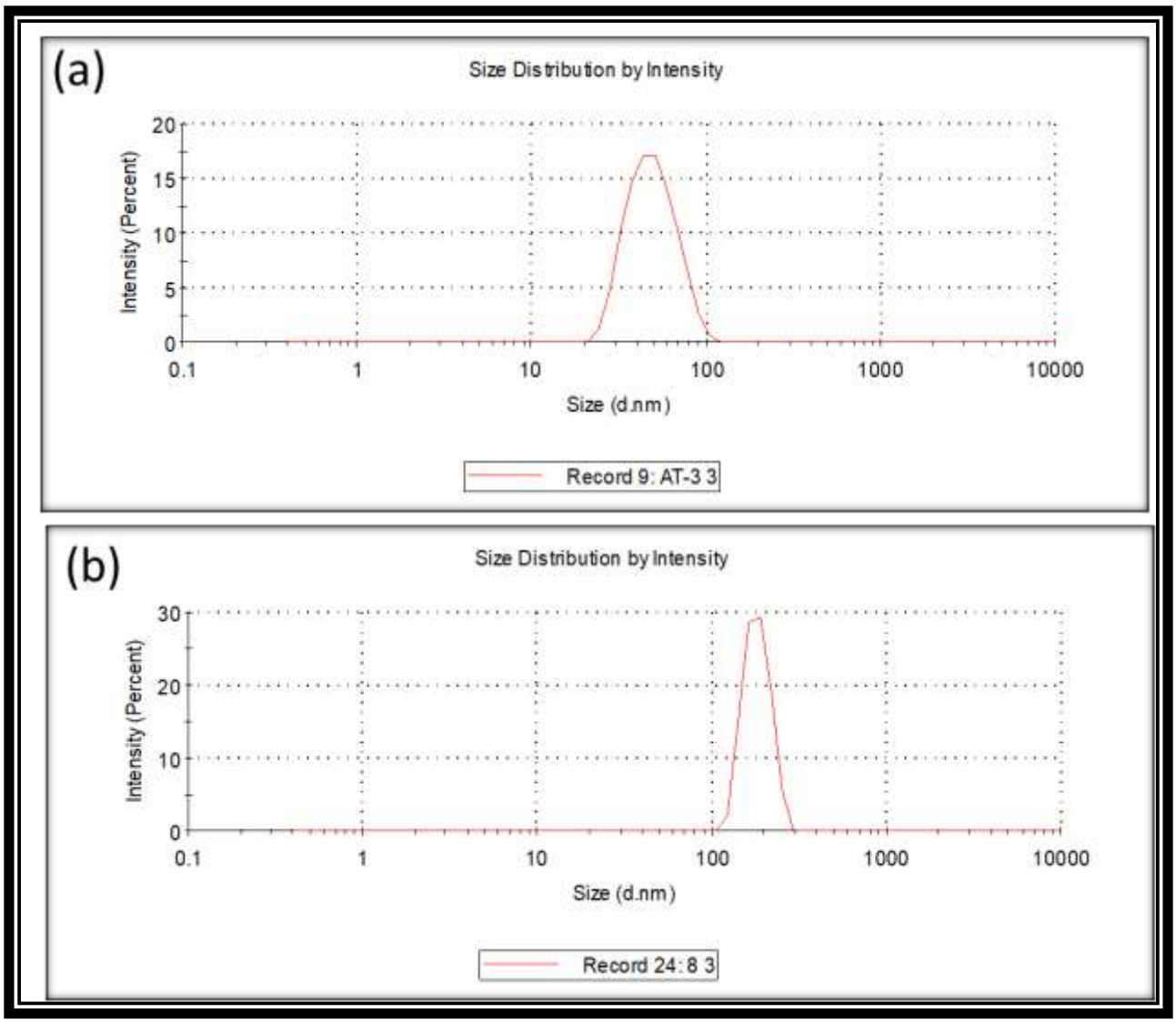


Figure 3.20: DLS results of (a) $CaCO_3$ NPs, (b) $CaCO_3$@pectin NPs

The crystalline structure of $CaCO_3$@pectin was further identified by the X-ray diffraction (XRD) pattern, as shown in **Figure 3.21**. The results showed that the $CaCO_3$ NPs are highly crystalline and all the peaks match very well with the diffraction peaks of the standard pattern calcite $CaCO_3$ (JCPDS card number 47-1743) (218).

The spectrum of Ca@pectin NPs demonstrated that the Ca@pectin NPs are amorphous in nature and the appearance of the sharp characteristic diffraction peak

at 29.64° which indexed to the (104) plane of $CaCO_3$, clearly indicating the successful formation of Ca@pectin composite NPs.

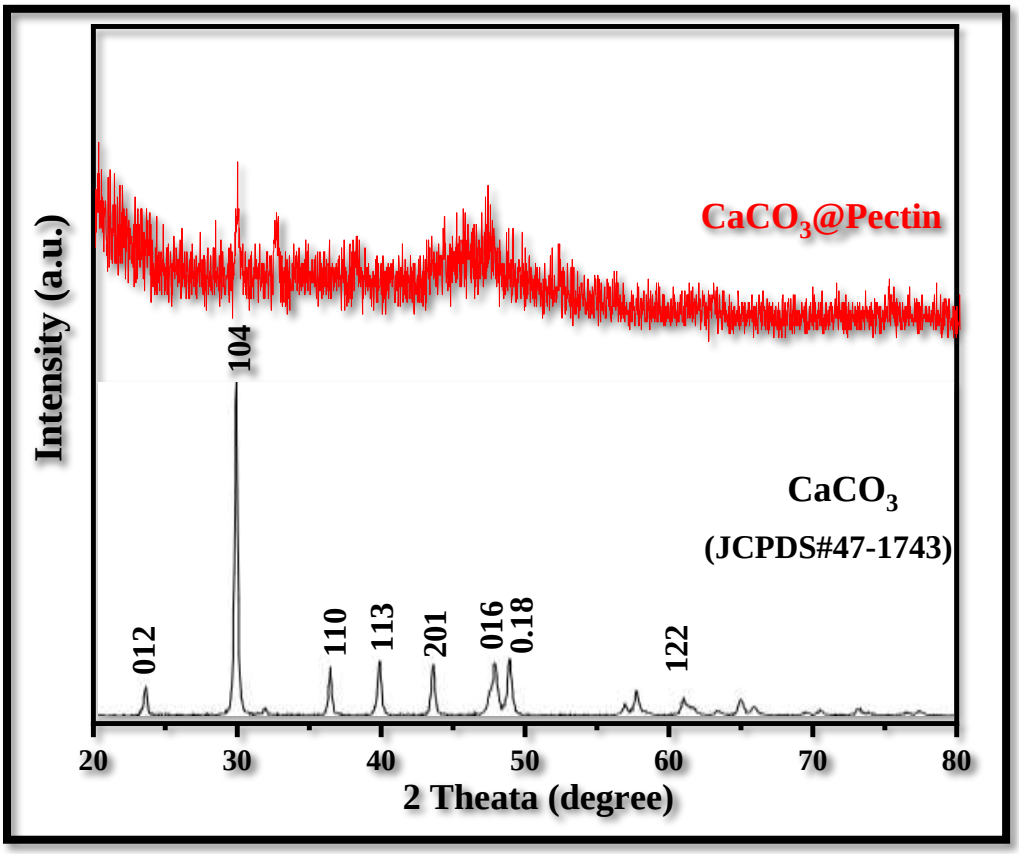


Figure 3.21: XRD results of (a) $CaCO_3$ NPs, (b) $CaCO_3$@pectin NPs.

Furthermore, the optical absorption properties of the as-prepared samples were investigated by UV−Vis-NIR spectra, as shown in **Figure 3.22** $CaCO_3$@pectin NPs have the characteristic peak of $CaCO_3$ which is near 400 nm (219). Which is clear evidence for the formation of $CaCO_3$@Pectin NPs.

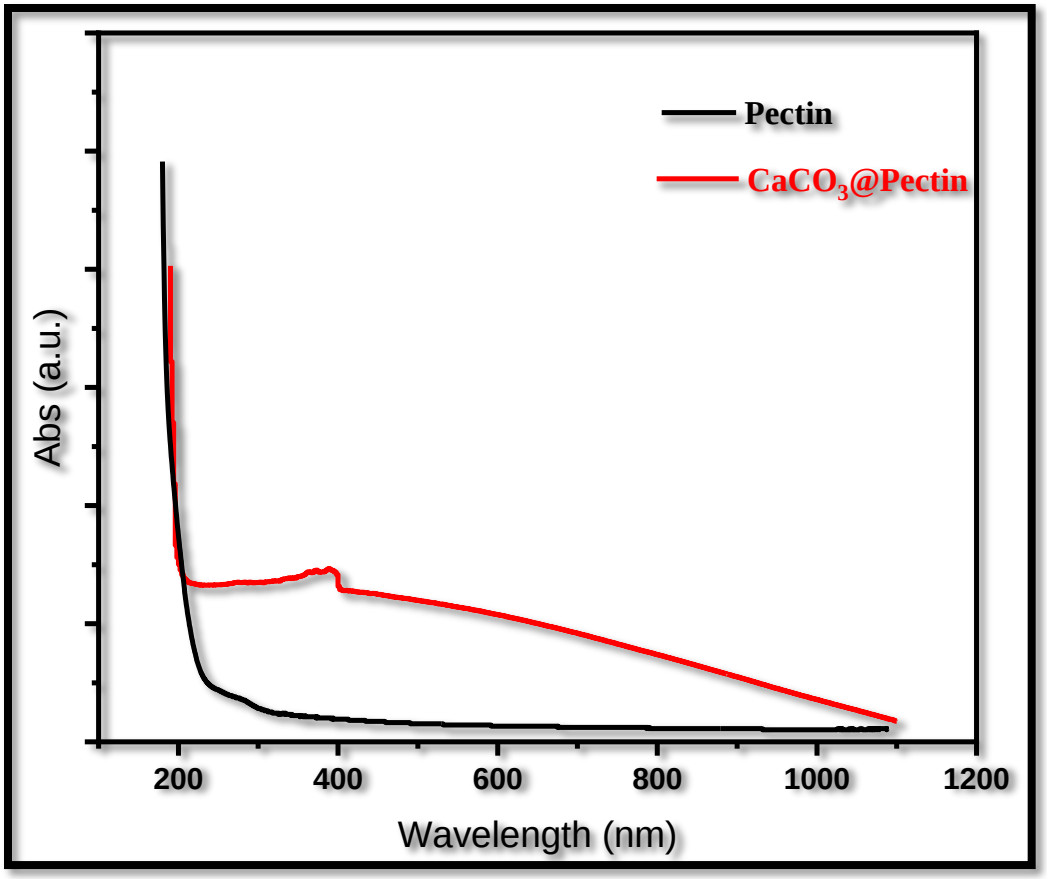


Figure 3.22: UV−Vis-NIR spectra of (a) Pectin, (b) $CaCO_3$@pectin NPs.

3.14. Effects of nanoparticles on pancreatic enzymes activity

Estimation of mean levels of determination pool activity of serum Amylase and lipase enzymes with and without the prepared nanoparticles ($CaCO_3$@pectin NPs; calcium nanoparticle and pectin polymer) in type II diabetes mellitus patients was illustrated in **Figures 3.23 & 3.24**.

$CaCO_3$@Pectin NPs have an inhibitory effect on the activity of the enzymes lipase and amylase. The pool activity of serum amylase in the presence of $CaCO_3$@pectin NPs; calcium nanoparticle and pectin polymer was 63.1< 82.2<109.3 (U/L) respectively, while the pool activity of serum Lipase was 12.9> 12.2< 20 (U/L).

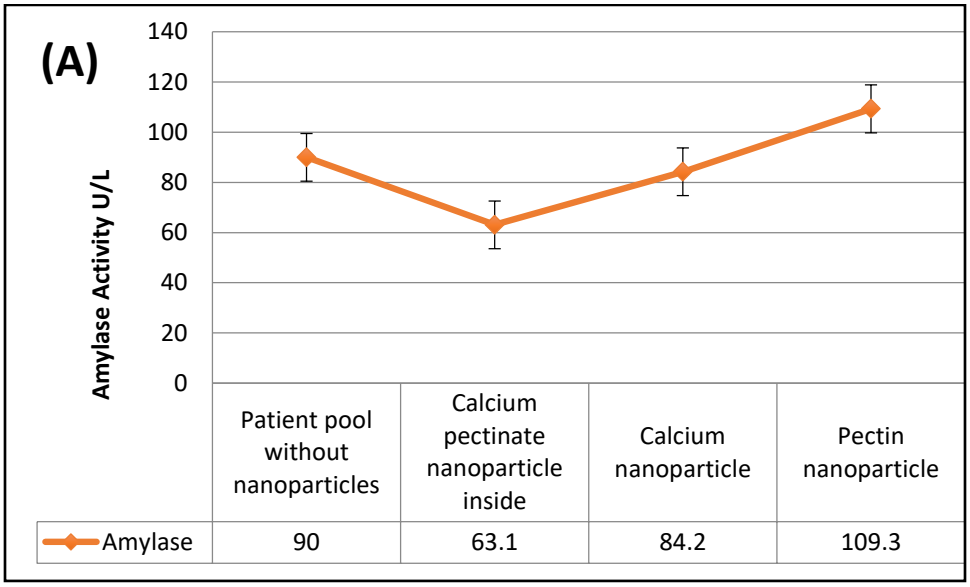


Figure 3.23: Estimation plot of determination pool activity of serum amylase enzyme with and without prepared nanoparticles ($CaCO_3$@pectin NPs; calcium nanoparticle and pectin polymer) in type II diabetes mellitus patients

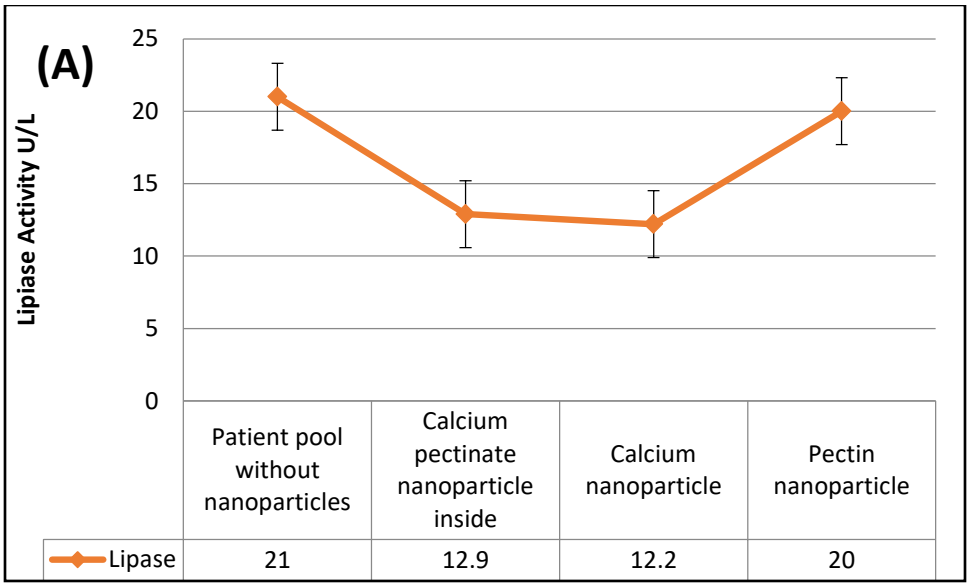


Figure 3.24: Estimation plot of determination pool activity of serum lipase enzyme with and without prepared nanoparticles ($CaCO_3$@pectin NPs; calcium nanoparticle and pectin polymer) in type II diabetes mellitus patients.

Table 3.6: Activation & inhibition % of the prepared nanoparticles in the serum amylase & lipase enzymes pool.

Nanoparticles	Amylase Activity		Lipase Activity	
	Activation %	Inhibition %	Activation %	Inhibition %
Calcium pectinate nanoparticle inside	-------	29.89	-------	38.57
Calcium nanoparticle	-------	6.44	-------	41.90
Pectin polymer	21.44	------	-------	4.76

CHAPTER FOUR

Conclusion and future work

Conclusion and future work

A) Conclusion:

High blood glucose-mediated functional loss, insulin sensitivity, and insulin deficiency lead to chronic disorders. Nanotechnology in diabetes studies has encouraged the development of wide modalities for glucose and insulin regulation that might hold the potential to improve the quality of life of diabetics. The results of this study were concluded:

1. Generally, patients with type II diabetes mellitus were shown an increasing range level of pancreatic enzymes when compared with that of the healthy group. Only the mean levels of the Lipase activity was shown a significant difference in type II diabetes mellitus patients compared to the healthy control group.

2. Serum hormones were also increased significantly, suggesting that DM patients had an obvious insulin resistance.

3. Results indicated a significant difference in LDL, VLDL, and cholesterol levels in Type II Diabetes Mellitus patients compared to the healthy control group. There was a significant increase in HDL and TG in DM patients compared to the healthy control group.

4. The $CaCO_3$ NPs core is crystalline while the pectin polymer shell is amorphous in nature, in good agreement with XRD measurements.

5. $CaCO_3$@Pectin NPs have an inhibitory effect on the activity of the enzymes lipase and amylase, while the pectin nanoparticles showed an activating effect on enzyme amylase.

6. Serum FBS was positively related to the HbA1c, TG, and VLDL levels. In addition, TG levels were significantly associated with total cholesterol, VLDL, and Insulin levels. As a risk factor of dyslipidemia in type II diabetes mellitus, serum LDL was highly significant positively related to high levels of total cholesterol.

B) Future work:

Pectin can be tailormade to generate new applications, as structural changes result in different functions and greater bioactivities. Still, it stands out that pectin can be extracted from the most varied sources, being the by-products of the food industry a green solution (due to the valorization of agro-industrial residues), which is associated with more environmentally friendly methods, allows a sustainable extraction and an environmentally friendlier product. Among the future challenges, the extraction of pectin without solvent or with neoteric can be highlighted to meet the fifth principle of green chemistry: Safer solvents and auxiliaries.

CHAPTER FIVE

References

References

1. Egan AM, Dinneen SF (2019) What is diabetes? Medicine (United Kingdom). 47:1–4

2. Punthakee Z, Goldenberg R, Katz P. (2018) Defnition, classifcation and diagnosis of diabetes , prediabetes and metabolic syndrome diabetes Canada clinical practice guidelines expert committee. Can J Diabetes 42:S10–5. Available from: https:// doi.org/https://doi.org/10.1016/j.jcjd.2017.10.003

3. H.W. Baynest, Classification, Pathophysiology, Diagnosis and Management of Diabetes Mellitus, J. Diabetes Metabol. 6 (5) (2015).

4.Centers for Disease Control and Prevention. National Diabetes Statistics Report. Atlanta, GA: Centers for Disease Control and Prevention, US Department of Health and Human Services; 2020. https://www.cdc.gov/diabetes/data/statistics/2014statisticsreport.html. (Accessed June 23, 2020.(

5. M. Nair, Diabetes Mellitus, Part 1: Physiology and Complications, Brit. J. Nurs. 16 (3) (2007) 184–188.

6. Zimmet P (2003) The burden of type 2 diabetes: are we doing enough? Diabetes Metab 29(4): 6S9–6S18.

7. Chatterjee, S.; Khunti, K.; Davies, M.J. Type 2 diabetes. Lancet 2017, 389, 2239–2251.

8. NCD Risk Factor Collaboration. Worldwide trends in diabetes since 1980: A pooled analysis of 751 population-based studies with 4.4 million participants. Lancet 2016, 387, 1513–1530.

9. Virally M, Blicklé JF, Girard J, Halimi S, Simon D, Guillausseau PJ (2007) Type 2 diabetes mellitus: epidemiology, pathophysiology, unmet needs and therapeutical perspectives. Diabetes Metab 33(4):231–244 Fig. 3 Funnel plot of the odds ratios of cross-sectional studies European Journal of Nutrition 1 3.

10. Rathmann W, Giani G (2004) Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 27(10):2568–2569.

11. Mansour AA, Wanoose HL, Hani I, Abed-Alzahrea A: Diabetes screening in Basrah, Iraq: a population-based cross-sectional study. Diabetes Res Clin Pract. 2008, 79 (1): 147-150. 10.1016/j.diabres.2007.07.016.

12. Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M et al (2012) Management of hyperglycaemia in type 2 diabetes: a patient-centered approach. Position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetologia 55(6):1577–1596.

13. Essien UR, Shahid NN, Berkowitz SA (2016) Food insecurity and diabetes in developed societies. Curr Diabetes Rep 16(9):79.

14. Grarup, N.; Sandholt, C.H.; Hansen, T.; Pedersen, O. Genetic susceptibility to type 2 diabetes and obesity: From genome-wide association studies to rare variants and beyond. Diabetologia 2014, 57, 1528–1541.

15. Wong, N.D.; Zhao, Y.; Patel, R.; Patao, C.; Malik, S.; Bertoni, A.G.; Correa, A.; Folsom, A.R.; Kachroo, S.; Mukherjee, J.; et al. Cardiovascular Risk Factor Targets and Cardiovascular Disease Event Risk in Diabetes: A Pooling Project of the Atherosclerosis Risk in Communities Study, Multi-Ethnic Study of Atherosclerosis, and Jackson Heart Study. Diabetes Care 2016, 39, 668–676.

16. R.P. Robertson, Antagonist: diabetes and insulin resistance - philosophy, science, and the multiplier hypothesis, J. Lab. Clin. Med. 125 (5) (1995) 560–564.

17. C. Kahn, Insulin Action, Diabetogenes, And the Cause of Type II Diabetes, Diabetes 43 (8) (1994) 1066–1085.

18. Reaven GM. 1998 The role of insulin resistance in human disease. Diabetes. 37:1595–1607.

19. Olefsky JM. 1989 Pathogenesis of non-insulin dependent diabetes (type 2). In: DeGroot LJ, Besser GM, Cahill JC, eds. Endocrinology, 2nd ed. Philadelphia: Saunders; 1369–1388.

20. MANAITHIYA, Ajay, et al. GPR119 agonists: Novel therapeutic agents for type 2 diabetes mellitus. Bioorganic Chemistry, 2021, 113: 104998.

21. K. Fujioka, Pathophysiology of Type 2 Diabetes and The Role of Incretin Hormones and Beta-Cell Dysfunction, J. Am. Acad. Phys. Assist. 20 (12) (2007) 3–8.

22. E. Hackett, N. Jacques, Type 2 diabetes: pathophysiology and clinical features, Pharm. J. Clin. Pharmacist 1 (2009) 475.

23. Campbell PJ, Carlson MG. Impact of obesity on insulin action in NIDDM. Diabetes 1993; 42: 405–10.

24. Bogardus C, Lillioja S, Mott DM, Hollenbeck C, Reaven G. Relationship between degree of obesity and in vivo insulin action in man. Am J Physiol 1985; 248: E286– E291.

25. Kissebah AH, Vydelingum N, Murray R, Evans DF, Hartz AJ, Kalkhoff RK et al. Relationship of body fat distribution to metabolic complications of obesity. J Clin Endocrinol Metab 1982; 54: 254–60.

26. P.J. Kumar, M. Clark, Textbook of Clinical Medicine [online], Saunders, London, 2002 https://www.scirp.org/(S(351jmbntvnsjt1aadkposzje))/reference/ReferencesPapers.aspx?ReferenceID=1601105.

27. R. Unger, L. Orci, Paracrinology of Islets and The Paracrinopathy Of Diabetes, Proc. Nat. Acad, Sci. 107 (37) (2010) 16009-–16012.

28. R. Mahler, M. Adler, Type 2 Diabetes Mellitus: Update on Diagnosis, Pathophysiology, And Treatmen, J. Clin. Endocrinol. Metabol. 84 (4) (1999) 1165–1171.

29. Banerji MA, Chaiken RI, Huey H, Tuomi T, Norin AJ, MacKay IR et al. GAD antibody negative NIDDM in adult black subjects with diabetic ketoacidosis and increased frequency of human leukocyte antigen DR3 and DR4: flatbush diabetes. Diabetes 1994; 43: 741– 45.

30. Umpierrez GE, Casals MMC, Gebhardt SSP, Mixon PS, Clark WS, Phillips LS. Diabetic ketoacidosis in obese African–Americans. Diabetes 1995; 44: 790–95.

31.Patoulias D, Papadopoulos C, Stavropoulos K, Zografou I, Doumas M, Karagiannis A. Prognostic value of arterial stiffness measurements in cardiovascular disease, diabetes, and its complications: The potential role of sodium-glucose co-transporter-2 inhibitors. J Clin Hypertens (Greenwich). 2020 Apr;22(4):562-571.

32. Liakopoulos V, Franzén S, Svensson AM, Miftaraj M, Ottosson J, Näslund I, Gudbjörnsdottir S, Eliasson B. Pros and cons of gastric bypass surgery in individuals with obesity and type 2 diabetes: nationwide, matched, observational cohort study. BMJ Open. 2019 Jan 15;9(1):e023882.

33. American Diabetes Association (2019) Standards of medical care in diabetes. Diabetes Care 42:13–28.

34. Orozco-Beltrán D, Gil-Guillen VF, Quirce F, Navarro-Perez J, Pineda M, Gomez-De-La-Cámara A et al (2007) Control of diabetes and cardiovascular risk factors in patients with type 2 diabetes in primary care. The gap between guidelines and reality in Spain. Int J Clin Pract 61(6):909–915.

35. Hermans MP, Brotons C, Elisaf M, Michel G, Muls E, Nobels F (2013) Optimal type 2 diabetes mellitus management: the randomised controlled OPTIMISE benchmarking study: baseline results from six European countries. Eur J Prev Cardiol 20(6):1095–1105.

36. Henry RR, Griver K. 1998 Diet therapy of type 2 diabetes mellitus. In: DeFronzo R, ed. Current therapy of diabetes mellitus. Chicago: Mosby-Yearbook; 79.

37. Grundy SM. 1991 Diet therapy in diabetes mellitus: is there a single best diet? Diabetes Care. 14:796–821.

38. Wing RR. 1995 Use of very low-calorie diet in the treatment of obese persons with non-insulin dependent diabetes mellitus. J Am Diet Assoc. 95:569–572.

39. Henry RR, Gumbiner B. 1991 Benefits and limitations of very low calorie diet therapy in obese NIDDM. Diabetes Care. 14:802–823.

40. Hanson RL, Narayaan KM, McConce DR, et al. 1995 Rate of weight gain, weight fluctuation, and incidence of NIDDM. Diabetes. 44:261–266.

41. Albu J, Konnarides C, Pi-Sunyer FX. 1995 Weight control: metabolic and cardiovascular effects. Diabetes Rev. 3:335–347

42. Hermansen K, Mortensen LS, Hermansen ML (2008) Combining insulins with oral antidiabetic agents: effect on hyperglycemic control, markers of cardiovascular risk and disease. Vasc Health Risk Manag 4: 561-574.

43. Kroon L, Goldfine ID, Tanyolac S (2010) Insulin - Pharmacology, Types of Regimens, and Adjustments. Insulin -"Pharmacology, Types of Regimens, and Adjustments.

44. Riddle MC (2005) Glycemic management of type 2 diabetes: an emerging strategy with oral agents, insulins, and combinations. Endocrinol Metab Clin North Am 34: 77-98.

45. RIPSIN, Cynthia M.; KANG, Helen; URBAN, Randall J. Management of blood glucose in type 2 diabetes mellitus. *American family physician*, 2009, 79.1: 29-36.

46. Khan, R.M.M., Chua, Z.J.Y., Tan, J.C., Yang, Y., Liao, Z. and Zhao, Y., 2019. From pre-diabetes to diabetes: diagnosis, treatments and translational research. Medicina, 55(9), p.546.

47. International Expert Committee. International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. Diabetes Care 2009;32:1-8 .

48. Cox EM, Elelman D. Test for screening and diagnosis of type 2 diabetes. Clin Diabetes 2009;4(27):132-138 .

49. Novosel'skaya, I.L., et al. (2000). Trends in the science and applications of pectins. Chemistry of Natural Compounds, 36, pp. 1-10.

50.FREITAS, Cariny Maria Polesca, et al. Structure and applications of pectin in food, biomedical, and pharmaceutical industry: A review. Coatings, 2021, 11.8: 922.

51.Willats, G. T., McCartney, L., Mackie, W., & Knoxl, J. P. (2001). Pectin: cell biology and prospects for functional analysis. Plant Molecular Biology, 47, 9-27.

52. Ishii, T., Matsunaga, T., Pellerin, P., O'Neill, M. A., Darvill, A., & Albersheim, P. (1999). The plant cell wall polysaccharide rhamnogalacturonan II self-assembles into a covalently cross-linked dimer. Journal of Biological Chemistry, 274: 13098-13104.

53. Caffall, K. H., & Mohnen, D. (2009). The structure, function, and biosynthesis of plant cell wall pectic polysaccharides. Carbohydrate Research, 344, 1879-1900.

54. Picot-Allain, M. C. N., Ramasawmy, B., & Emmambux, M. N. (2020). Extraction, Characterisation, and 28 Application of Pectin from Tropical and Sub-Tropical fruits: A Review. Food Reviews International, DOI: 10.1080/87559129.2020.1733008.

55. Marenda, F.R.B.; Mattioda, F.; Demiate, I.M.; de Francisco, A.; de Oliveira Petkowicz, C.L.; Canteri, M.H.G.; de Mello Castanho Amboni, R.D. Advances in studies using vegetable wastes to obtain pectic substances: A review. J. Polym. Environ. 2019, 27, 549–560.

56. Sriamornsak, P. (2001-2002). Pectin: The role in health. Journal of Silpakorn University, 21-22, pp. 60-77.

57. Ginter, E., et al. (1979). Natural hypocholesterolemic agent: pectin plus ascorbic acid. International Journal of Viticulture and Natural Resource, 49, pp. 406-408.

58. Vandana Tyagi, Pramod Kumar Sharma, Rishabha Malviya, Pectins and Their Role in Food and Pharmaceutical Industry: A Review, Journal of Chronotherapy and Drug Delivery, 2015; 6(3): 65- 77.

59. Sriamornsak, P.; Wattanakorn, N.; Takeuchi, H. Study on the mucoadhesion mechanism of pectin by atomic force microscopy and mucin-particle method. Carbohydr. Polym. 2010, 79, 54–59.

60. Martau, G.A.; Mihai, M.; Vodnar, D.C. The use of chitosan, alginate, and pectin in the biomedical and food sector-biocompatibility, bioadhesiveness, and biodegradability. Polymers 2019, 11, 1837.

61. Wicker, L.; Kim, Y.; Kim, M.J.; Thirkield, B.; Lin, Z.; Jung, J. Pectin as a bioactive polysaccharide extracting tailored function from less. Food Hydrocoll. 2014, 42, 251–259.

62. Naqash, F.; Masoodi, F.A.; Rather, S.A.; Wani, S.M.; Gani, A. Emerging concepts in the nutraceutical and functional properties of pectin—A Review. Carbohydr. Polym. 2017, 168, 227–239.

63. Giacco R, Clemente G, Riccardi G. Dietary fibre in treatment of diabetes: myth or reality? Dig Liver Dis 2002;34(suppl 2):S140 – 4.

64. Kabir M, Oppert J-M, Vidal H, Bruzzo F, Fiquet C, Wursch P, et al. Four-week low-glycemic index breakfast with a modest amount of soluble fibers in type 2 diabetic men. Metabolism 2002;51:819 –26.

65. Ou S, Kwok K, Li Y, Fu L. In vitro study of possible role of dietary fiber in lowering postprandial serum glucose. J Agric Food Chem 2001;49:1026 –9.

66. Kalkwarf HJ, Bell RC, Khoury JC, Gouge AL, Miodovnik M. Dietary fiber intakes and insulin requirements in pregnant women with type 1 diabetes. J Am Diet Assoc 2001;101:305–10.

67. Jenkins DJA, Kendall CWC, Axelsen M, Augustin LS, Vuksan V. Viscous and nonviscous fibres, nonabsorbable and low glycaemic index carbohydrates, blood lipids and coronary heart disease. Curr Opin Lipidol 2000;11:49 –56.

68. Wursh P, Pi-Sunyer FX. The role of viscous soluble fiber in the metabolic control of diabetes. A review with special emphasis on cereals rich in beta-glucan. Diabetes Care 1997;20:1774 – 80. 376 M. Kim / Nutrition 21 (2005) 372–377

69. Jenkins DJA, Leeds AR, Gassull MA, Cochet B, Alberti GM. Decrease in postprandial insulin and glucose concentrations by guar and pectin. Ann Intern Med 1977;86:20 –3.

70. McIntosh M, Miller C. A diet containing food rich in soluble and insoluble fiber improves glycemic control and reduces hyperlipidemia among patients with type 2 diabetes mellitus. Nutr Rev 2001;59:52–5.

71. Jenkins DJA, Goff DV, Leeds AR, Alberti KG, Wolever TM, Hockaday TD, et al. Unabsorbable carbohydrates and diabetes: decreased postprandial hyperglycemia. Lancet 1976;2:172– 4.

72. Pereira MA, Ludwig DS. Dietary fiber and body-weight regulation: observations and mechanisms. Pediatr Clin North Am 2001; 48: 969–980.

73. Zijlstra N, de Wijk RA, Mars M, Stafleu A, de Graaf C. Effect of bite size and oral processing time of a semisolid food on satiation. Am J Clin Nutr 2009; 90: 269–275.

74. Zijlstra N, Mars M, De Wijk RA, Westerterp-Plantenga MS, De Graaf C. The effect of viscosity on ad libitum food intake. Int J Obes 2008; 32: 676–683.

75. Dikeman CL, Fahey JGC. Viscosity as related to dietary fiber: a review. Crit Rev Food Sci Nutr 2006; 46: 649–663.

76. Baer DJ, Rumpler WV, Miles CW, Fahey GC Jr. Dietary fiber decreases the metabolizable energy content and nutrient digestibility of mixed diets fed to humans. J Nutr 1997; 127: 579–586.

77. Kristensen M, Damgaard TW, Sorensen AD, Raben A, Lindelov TS, Thomsen AD et al. Whole flaxseeds but not sunflower seeds in rye bread reduce apparent digestibility of fat in healthy volunteers. Eur J Clin Nutr 2008; 62: 961–967.

78. Sleeth ML, Thompson EL, Ford HE, Zac-Varghese SE, Frost G. Free fatty acid receptor 2 and nutrient sensing: a proposed role for fibre, fermentable carbohydrates and short-chain fatty acids in appetite regulation. Nutr Res Rev 2010; 23: 135–145.

79.Cefalu WT. Insulin resistance: cellular and clinical concepts. Exp Biol Med (Maywood). 2001;226:13-26.

80. Home PD. Insulin therapy. In: Alberti KGMM, Zimmet P, Defronzo RA editors & Keen H (Hon) editor International Textbook of Diabetes Mellitus (2nd ed) John Wiley & Sons, New York; 1997 p. 899-928.

81. Dodson G, Steiner D. The role of assembly in insulin's biosynthesis. Curr Opin Struct Biol. 1998;8:189-94.

82.ALYAS, Jessica, et al. Human Insulin: History, Recent Advances, and Expression Systems for Mass Production. *Biomedical Research and Therapy*, 2021, 8.9: 4540-4561.

83. WU, Qi; BROWN, Mark R. Signaling and function of insulin-like peptides in insects. Annu. Rev. Entomol., 2006, 51: 1-24.

84. DELPORTE, Christine. Structure and physiological actions of ghrelin. *Scientifica*, 2013, 2013.

85. YADA, T., et al. Ghrelin signalling in β-cells regulates insulin secretion and blood glucose. *Diabetes, Obesity and Metabolism*, 2014, 16.S1: 111-117.

86.SATO, Takahiro, et al. Structure, regulation and function of ghrelin. *The Journal of Biochemistry*, 2012, 151.2: 119-128.

87. KORBONITS, Márta, et al. Ghrelin—a hormone with multiple functions. *Frontiers in neuroendocrinology*, 2004, 25.1: 27-68.

88. Tschöp M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E, Heiman ML. Circulating ghrelin levels are decreased in human obesity. Diabetes 2001; 50: 707-709 .

89. 119 Ikezaki A, Hosoda H, Ito K, Iwama S, Miura N, Matsuoka H, Kondo C, Kojima M, Kangawa K, Sugihara S. Fasting plasma ghrelin levels are negatively correlated with insulin resistance and PAI-1, but not with leptin, in obese children and adolescents. Diabetes 2002; 51: 3408-3411.

90. McLaughlin T, Abbasi F, Lamendola C, Frayo RS, Cummings DE. Plasma ghrelin concentrations are decreased in insulin-resistant obese adults relative to equally obese insulin-sensitive controls. J Clin Endocrinol Metab 2004; 89: 1630-1635.

91.WANG, Huifang; WU, Yanyan; WANG, Yueqi. Whole-Genome Sequencing of a Potential Ester-Synthesizing Bacterium Isolated from Fermented Golden Pomfret and Identification of Its Lipase Encoding Genes. *Foods*, 2022, 11.13: 1954.

92. Cerk IK, Wechselberger L, Oberer M. Adipose Triglyceride Lipase Regulation: An Overview. Curr Protein Pept Sci. 2018;19(2):221-233.

93. KUMAR, Ashwani; CHAUHAN, Shilpi. Pancreatic lipase inhibitors: The road voyaged and successes. *Life Sciences*, 2021, 271: 119115.

94. Waldmann E, Parhofer KG. Apheresis for severe hypercholesterolaemia and elevated lipoprotein(a). Pathology. 2019 Feb;51(2):227-232.

95. VANDERMEERS, André, et al. A Calcium-Dependent Protein Activator of Guanosine 3′: 5′-Monophosphate Phosphodiesterase in Bovine and Rat Pancreas: Isolation, Properties and Levels in vivo. *European journal of biochemistry*, 1977, 81.2: 379-386.

96. JANOWITZ, Morris. Content analysis and the study of sociopolitical change. *Journal of Communication*, 1976, 26.4: 10-21.

97. COHEN, D. A. N. Maximizing final yield when growth is limited by time or by limiting resources. *Journal of theoretical biology*, 1971, 33.2: 299-307.

98. Ata N, Dal K, Kucukazman M, Yeniova AO, Karakaya S, Unsal O et al. The effect of glycemic control on CEA, CA 19-9, amylase and lipase levels. Open Med. 2015; 10: 8-13.

99.Dandona P, Elias E, Becket AG. Serum trypsin concentrations in diabetes mellitus. Br Med J 1978; 2: 1125-6.

100. SAVYCH, Alona; MARCHYSHYN, Svitlana. Inhibition of pancreatic lipase by water extracts of some herbal mixtures. *Methods*, 2021, 20: 21.

101. DANDONA, P., et al. Exocrine pancreatic function in diabetes mellitus. *Journal of clinical pathology*, 1984, 37.3: 302-306.

102. JUNGLEE, D., et al. Low pancreatic lipase in insulin-dependent diabetics. *Journal of Clinical Pathology*, 1983, 36.2: 200-202.

103. Vissers RJ, Abu-Laban RB, McHugh DF. Amylase and lipase in the emergency department evaluation of acute pancreatitis. The Journal of Emergency Medicine 1999;17(6): 1027–37.

104.FAROOQ, Muhammad Adeel, et al. Biosynthesis and industrial applications of α-amylase: A review. *Archives of Microbiology*, 2021, 203.4: 1281-1292.

105. Magalhães, O.P. and Souza, M.P., 2010. Application of microbial amylase in industry–A review. *Brazilian Journal of Microbiology*, *41*, pp.850-861.

106. Sundarram A, Pandurangappa T, Murthy K (2014) α-Amylase production and applications: a review. J Appl Environ Microbiol 2:166–175 (Sundarram et al. 2014).

107. Kumar V (2011) Identifcation of the sequence motif of glycoside hydrolase 13 family members. Bioinformation 6:61–63 (Kumar 2011).

108. Payen Sharma AK, Sharma V, Saxena J, Chandra R, Alam A, Prakash A (2015) Isolation and screening of amylolytic bacteria from soil. Int J Sci Res Agric Sci 2:159–165 (Sharma et al. 2015).

109. Kamon M, Sumitani JI, Tani S, Kawaguchi T, Kamon M, Sumitani J, Kawaguchi T (2015) Characterization and gene cloning of a maltotriose-forming exo-amylase from Kitasatospora sp. MK-1785. Appl Microbiol Biotechnol 99:4743–4753 (Kamon et al. 2015).

110. Ofen WA, Viksoe-Nielsen A, Borchert TV, Wilson KS, Davies GJ (2015) Three-dimensional structure of a variant termamyl-like Geobacillus stearothermophilus α-amylase at 1.9 Å resolution. Acta Crystallogr Sect F Struct Biol Comm 71:66–70 (Ofen et al. 2015).

111. Sahni TK, Goel A (2015) Microbial enzymes with special reference to α-amylase. Bio Evol 2:19–25 (Sahni and Goel 2015).

112. TANVI, Noor E. Jannat, et al. Serum amylase and lipase levels in type 2 diabetes mellitus. *Journal of Bangladesh society of physiologist*, 2017, 12.2: 52-56.

113. Nakajima K, Oshida H, Muneyuki T, Kakei M. Pancrelipase: an evidence-based review of its use for treating pancreatic exocrine insufficiency. Core Evid. 2012; 7: 77–91.

114. Hu, M., Li, Y., Decker, E. A., & McClements, D. J. (2010). Role of calcium and calciumbinding agents on the lipase digestibility of emulsified lipids using an in vitro digestion model. Food Hydrocolloids, 24, 719–725.

115. Scow, R. O. (1988). Effect of sodium taurodeoxycholate, CaCl2 and albumin on the action of pancreatic lipase on droplets of trioleoylglycerol and the release of lipolytic products into aqueous media. Biochimie, 70, 1251–1261.

116. Zangenberg, N. H., Müllertz, A., Kristensen, H. G., & Hovgaard, L. (2001). A dynamic in vitro lypolysis model: I. Controlling the rate of hydrolysis by continuous addition of calcium. European Journal of Pharmaceutical Sciences, 14, 115–122.

117. Fave, G., Coste, T. C., & Armand, M. (2004). Physicochemical properties of lipids: new strategies to manage fatty acid bioavailability. Cellular and Molecular Biology, 50 (7), 815e831.

118. Patton, J. S., & Carey, M. C. (1979). Watching fat digestion. Science, 204(4389), 145e148.

119. Patton, J. S., Vetter, R. D., Hamosh, M., Borgstrom, B., Lindstrom, M., & Carey, M. C. (1985). The light-microscopy of triglyceride digestion. Food Microstructure, 4(1), 29e41..

120. Hwang, S., Lee, S., Ahn, I. S., & Yung, J. K. (2009). Highly efficient production of monoglycerides by the continuous removal of fatty acids from lipase-catalyzed oil hydrolysis. Biocatalysis and Biotransformation, 27(5e6), 290e295.

121. 64. Zangenberg, N. H., Mullertz, A., Kristensen, H. G., & Hovgaard, L. (2001b). A dynamic in vitro lipolysis model II: evaluation of the model. European Journal of Pharmaceutical Sciences, 14(3), 237e244.

122. National Nanotechnology Initiative (NNI). National Science and Technology Council. Committee on Technology, Subcommittee on Nanoscale Science, National Technology Initiative Strategic Plan, www.nano.gov (2011, accessed 25 August 2015).

123. Zhang, W., Zhang, D. and Liang, Y. (2019). Nanotechnology in remediation of water contaminated by poly-and perfluoroalkyl substances: A review. Environmental Pollution, 247, 266-276.

124. Dmour I, Taha MO. Natural and semisynthetic polymers in pharmaceutical nanotechnology. Organic materials as smart nanocarriers for drug delivery. Elsevier Inc; 2018.

125. Bennet D, Kim S. Polymer nanoparticles for smart drug delivery. Application of nanotechnology in drug delivery. InTech; 2014.

126. Liu Z, et al. Polysaccharides-based nanoparticles as drug delivery systems. Adv Drug Deliv Rev 2008;60(15):165062.

127. Jain KK (2008) Nanomedicine: application of nanobiotechnology in medical practice. Med Princ Pract 17:89-101.

128. Surendiran A, Sandhiya S, Pradhan SC, Adithan C (2009) Novel applications of nanotechnology in medicine. Indian J Med Res 130: 689-701.

129. Y.Z. Chen, C.T. Yang, C.B. Ching, and R. Xu, Immobilization of lipases on hydrophobilized zirconia nanoparticles: highly enantioselective and reusable biocatalysts. Langmuir 24 (2008) 8877-8884.

130. G. Zhou, K.K. Fung, L.W. Wong, Y. Chen, R. Renneberg, and S. Yang, Immobilization of glucose oxidase on rod-like and vesicle-like mesoporous silica for enhancing current responses of glucose biosensors. Talanta 84 (2011) 659-665.

131. S. Bhatia, Nanoparticles Types, Classification, Characterization, Fabrication Methods and Drug Delivery Applications, Natural Polymer Drug Delivery Systems, Springer (2016) 33- 93.

132. A.B. Moghaddam, T. Nazari, J. Badraghi, and M. Kazemzad, Synthesis of ZnO nanoparticles and electrodeposition of polypyrrole/ZnO nanocomposite film. Int. J. Electrochem. Sci. 4 (2009) 247-257.

133. M. Ali, and M. Winterer, ZnO nanocrystals: surprisingly â€˜aliveâ€™. Chem. Mater. 22 (2009) 85-91.

134. I. Yamashita, Fabrication of a two-dimensional array of nano-particles using ferritin molecule. Thin Solid Films 393 (2001) 12-18.

135. PATEL, Jayvadan; CHAMPAVAT, Vibha. Toxicity of nanomaterials on the gastrointestinal tract. *Biointeractions of nanomaterials*, 2014, 3: 259.

136. PATRA, Jayanta Kumar, et al. Nano based drug delivery systems: recent developments and future prospects. *Journal of nanobiotechnology*, 2018, 16.1: 1-33.

137. Pandit A, Zeugolis DI. Twenty-fve years of nano-bio-materials: have we revolutionized healthcare? Fut Med. 2016;11(9):985–7.

138. SRIAMORNSAK, Pornsak. Application of pectin in oral drug delivery. *Expert opinion on drug delivery*, 2011, 8.8: 1009-1023.

139. SATHISHKUMAR, Palanivel, et al. Flavonoids mediated ‘Green’nanomaterials: A novel nanomedicine system to treat various diseases–Current trends and future perspective. *Materials letters*, 2018, 210: 26-30.

140. RAHIMAN, Shaik; TANTRY, Bilal Ahmad. Nanomedicine current trends in diabetes management. *J. Nanomed. Nanotechol*, 2012, 3.5: 1000137.

141. Regnell, S.E. and Lernmark, Å. (2017) Early prediction of autoimmune (type 1) diabetes. Diabetologia 60, 1370–1381

142. American Diabetes Association (2019) 2. Classification and diagnosis of diabetes: standards of medical care in diabetes – 2019. Diabetes Care 42, S13–S28

143. Bleich, D. et al. (1990) Analysis of metabolic progression to type I diabetes in ICA+ relatives of patients with type I diabetes. Diabetes Care 13, 111–118.

144. LEMMERMAN, Luke R., et al. Nanomedicine-based strategies for diabetes: diagnostics, monitoring, and treatment. *Trends in Endocrinology & Metabolism*, 2020, 31.6: 448-458.

145. Dening TJ, Rao S, Thomas N, Prestidge CA. Oral nanomedicine approaches for the treatment of psychiatric illnesses. J Control Release. 2016;223:137–156.

146. Ochubiojo M, Chinwude I, Ibanga E, Ifianyi S. Nanotechnology in Drug Delivery. Recent Advances in Novel Drug Carrier Systems. 2012.

147. Gutierrez RMP, Mendez JVM, Vazquez IA. Chapter 2 - A novel approach to the oral delivery of bionanostructures for systemic disease. In: Andronescu E, Grumezescu AM, editors. Nanostructures for Oral Medicine. Elsevier; 2017:27–59.

148. PRIYAM, Ayushi; SINGH, Pushplata Prasad; GEHLOUT, Shweta. Role of endocrine-disrupting engineered nanomaterials in the pathogenesis of type 2 diabetes mellitus. Frontiers in endocrinology, 2018, 9: 704.

149. Singh P, Kim YJ, Zhang D, Yang DC. Biological synthesis of nanoparticles from plants and microorganisms. Trends Biotechnol. (2016) 34:588–99. doi: 10.1016/j.tibtech.2016.02.006.

150. Ingale AG, Chaudhari AN. Biogenic synthesis of nanoparticles and potential applications: an eco- friendly approach. J Nanomed Nanotechnol. (2013) 4:165. doi: 10.4172/2157-7439.1000165.

151. Campoy AHG, Gutierrez RMP, Manriquez-Alvirde G, Ramirez AM. Protection of silver nanoparticles using Eysenhardtia polystachya in peroxide-induced pancreatic β-cell damage and their antidiabetic properties in zebrafish. Int J Nanomed. (2018) 13:2601. doi: 10.2147/IJN.S1 63714.

152. Venkatachalam M, Govindaraju K, Mohamed Sadiq A, Tamilselvan S, Ganesh Kumar V, Singaravelu G. Functionalization of gold nanoparticles as antidiabetic nanomaterial. Spectroch Acta Part A (2013) 116:331–8. doi: 10.1016/j.saa.2013.07.038.

153. Karthick V, Kumar VG, Dhas TS, Singaravelu G, Sadiq AM, Govindaraju K. Effect of biologically synthesized gold nanoparticles on alloxan-induced diabetic rats—An in vivo approach. Coll Surf B Biointerfaces (2014) 122:505–11. doi: 10.1016/j.colsurfb.2014. 07.022.

154. Pourkhalili N, Hosseini A, Nili-Ahmadabadi A, Rahimifard M, NavaeiNigjeh M, Hassani S, et al. Improvement of isolated rat pancreatic islets function by combination of cerium oxide nanoparticles/sodium selenite through reduction of oxidative stress. Toxicol Mech Methods. (2012) 22:476– 82. doi: 10.3109/15376516.2012.673093.

155. Asani SC, Umrani RD, Paknikar KM. Differential dose-dependent effects of zinc oxide nanoparticles on oxidative stress-mediated pancreatic β-cell death. Nanomedicine (2017) 12:745–59. doi: 10.2217/nnm-2016-0426.

156. Septiadi D, Crippa F, Moore TL, Rothen-Rutishauser B, Petri-Fink A. Nanoparticle–cell interaction: a cell mechanics perspective. Adv Mater. (2018) 30:1704463. doi: 10.1002/adma.201704463.

157. Wan HT, Leung PY, Zhao YG, Wei X, Wong MH, Wong CK. Blood plasma concentrations of endocrine disrupting chemicals in Hong Kong populations. J Hazard Mater (2013) 261:763–9. doi: 10.1016/j.jhazmat.2013.01.034.

158. Lai RWS, Yeung KWY, Yung MMN, Djurisic AB, Giesy JP, Leung KMY. Regulation of engineered nanomaterials: current challenges, insights and future directions. Environ Sci Pollut Res Int. (2018) 25:3060–77. doi: 10.1007/s11356-017-9489-0.

159. Liu Y, Cao ZT, Xu CF, Lu ZD, Luo YL, Wang J. Optimization of lipidassisted nanoparticle for disturbing neutrophils-related inflammation. Biomaterials (2018) 172:92–104. doi: 10.1016/j.biomaterials.2018. 04.052.

160. Zion TC, Tsang HH, Ying JY. Glucose-sensitive nanoparticles for controlled insulin delivery. Int J Nanomed. (2003) 12:4037–57. Available online at: http://hdl.handle.net/1721.1/3783.

161. Gu Z, Aimetti AA, Wang Q, Dang TT, Zhang Y, Veiseh O, et al. Injectable nano-network for glucose-mediated insulin delivery. ACS Nano (2013) 7:4194–201. doi: 10.1021/nn400630x.

162. Yuejie C, Demin Y, Penghua W, Jun X, Daiqing L, Min D. Nanotechnology promotes the full-thickness diabetic wound healing effect of recombinant human epidermal growth factor in diabetic rats. Wound Repair Regener. (2010) 18:499–505. doi: 10.1111/j.1524-475X.2010.00612.x.

163. Damge C, Michel C, Aprahamian M, Couvreur P. New approach for oral administration of insulin with polyalkylcyanoacrylate nanocapsules as drug carrier. Diabetes (1988) 37:246–51. doi: 10.2337/diab.37.2.246.

164. Shilo M, Berenstein P, Dreifuss T, Nash Y, Goldsmith G, Kazimirsky G, et al. Insulin-coated gold nanoparticles as a new concept for personalized and adjustable glucose regulation. Nanoscale (2015) 7:20489– 96. doi: 10.1039/C5NR04881H.

165. Deng W, Xie Q, Wang H, Ma Z, Wu B, Zhang X. Selenium nanoparticles as versatile carriers for oral delivery of insulin: insight into the synergic antidiabetic effect and mechanism. Nanomed Nanotechnol Biol Med. (2017) 13:1965–74. doi: 10.1016/j.nano.2017.05.002.

166. Zhou, Bin et al. 2017. ―Worldwide Trends in Blood Pressure from 1975 to 2015: A Pooled Analysis of 1479 Population-Based Measurement Studies with 19·1 Million Participants.‖ The Lancet 389(10064): 37–55.

167. Barham, Denise, and P. Trinder. 1972. ―An Improved Colour Reagent for the Determination of Blood Glucose by the Oxidase System.‖ The Analyst 97(1151): 142–45.

168.Perk, J. et al. 2012. ―Erratum: ‗European Guidelines on Cardiovascular Disease Prevention in Clinical Practice (Version 2012)' the Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (Constit.‖ European Heart Journal 33(17): 2126.

169.Rouffy, J., and J. Jaillard. 1986. ―Effects of Two Antihypertensive Agents on Lipids, Lipoproteins, and Apoproteins A and B: Comparison of Prazosin and Atenolol.‖ The American Journal of Medicine 80(2 SUPPL. 1): 100– 103.

170.Jung, Un Ju, and Myung-Sook Choi. 2014a. ―Obesity and Its Metabolic Complications: The Role of Adipokines and the Relationship between Obesity, Inflammation, Insulin Resistance, Dyslipidemia and Nonalcoholic Fatty Liver Disease.‖ OPEN ACCESS Int. J. Mol. Sci 15: 15. www.mdpi.com/journal/ijms.

171. Jung, Un Ju, and Myung Sook Choi. 2014b. ―Obesity and Its Metabolic Complications: The Role of Adipokines and the Relationship between Obesity, Inflammation, Insulin Resistance, Dyslipidemia and Nonalcoholic Fatty Liver Disease.‖ International Journal of Molecular Sciences 15(4): 6184–6223.

172. ABBAS, Ameer Athab Abdulkadhim. The Effect of Obesity on Lipocalin-2 and Adipolin Levels in Women withType II Diabetes Mellitus in Babylon Governorate. PhD Thesis. University of Babylon.

173.FRIEDEWALD, William T.; LEVY, Robert I.; FREDRICKSON, Donald S. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical chemistry, 1972, 18.6: 499-502.

174. ELBAZ, Nancy M., et al. Controlled synthesis of calcium carbonate nanoparticles and stimuli-responsive multi-layered nanocapsules for oral drug delivery. *International Journal of Pharmaceutics*, 2020, 574: 118866.

175. ZAINUDIN, Badrul Hisyam; WONG, Tin Wui; HAMDAN, Halimaton. Design of low molecular weight pectin and its nanoparticles through combination treatment of pectin by microwave and inorganic salts. *Polymer Degradation and Stability*, 2018, 147: 35-40.

176. Mishra, V.; Nayak, P.; Sharma, M.; Albutti, A.; Alwashmi, A.S.S.; Aljasir, M.A.; Alsowayeh, N.; Tambuwala, M.M. Emerging Treatment Strategies for

Diabetes Mellitus and Associated Complications: An Update. Pharmaceutics 2021, 13, 1568.

177. Tyagi K, Agarwal NB, Kapur P, Kohli S, Jalali RK. Evaluation of Stress and Associated Biochemical Changes in Patients with Type 2 Diabetes Mellitus and Obesity. Diabetes Metab Syndr Obes. 2021 Feb 16 and 14:705-717. 2021.

178. Sachin Kumar, Tapan Behl, Monika Sachdeva, Aayush Sehgal, Shilpa Kumari, Arun Kumar, Gagandeep Kaur, Harlokesh Narayan Yadav, Simona Bungau, Implicating the effect of ketogenic diet as a preventive measure to obesity and diabetes mellitus, Life Sciences. 2021.

179. M.A. Exley, L. Hand, D. O'Shea, L. Lynch, Interplay between the immune system and adipose tissue in obesity, J. Endocrinol. 223 (2014) R41–R48. 2014.

180. HermanW.H., FajansS.S. Hemoglobin A1c for the diagnosis of diabetes: practical considerations. Pol Arch Med Wewn. 2010 and 37–40., 120:. 2010.

181. NathanD.M., DavidsonM.B., DeFronzoR.A. Impaired fasting glucose and impaired glucose tolerance: implications for care. Diabetes Care. 2007 and 753–9., 30:. 2007.

182. BuseJ.B., PolonskyK.S., BurantC.F. Chapter 31: Type 2 diabetes mellitus. In: MelmedS., PolonskyK.S., LarsenP.R., KronenbergH.M., eds. Williams Textbook of Endocrinology. 12th ed. Philadelphia, PA: Saunders Elsevier and 1386–450., 2011:. 2011.

183. LinJ.D., ChangJ.B., WuC.Z. Identification of insulin resistance in subjects with normal glucose tolerance. Ann Acad Med Singapore. 2014 and 113–9., 43:. 2014.

184. Reaven G., The metabolic syndrome or the insulin resistance syndrome? Different names, different concepts, and different goals. Endocrinol Metab Clin North Am. 2004 and 33:283-303. 2004.

185. Hunter SJ, Garvey WT. Insulin action and insulin resistance: diseases involving defects in insulin receptors, signal transduction, and the glucose transport effector system. Am J Med. 1998 and 105:331-45. 1998.

186. Paul S. Jellinger, Metabolic consequences of hyperglycemia and insulin resistance, Insulin, Volume 4, Issue 1, 2009, Pages 2-14,. 2009.

187. Wilcox, G. (2005). Insulin and insulin resistance. Clinical Biochemist Reviews, 26(2), 19-39. 2005.

188. Huang S, Czech MP. The GLUT4 glucose transporter. Cell Metab. 2007 Apr and 5(4):237-52. 2007.

189. Schnurr, T. M., Reynolds, A. J., Komac, A. M., Duffy, L. K., & Dunlap, K. L. (2015). The effect of acute exercise on GLUT4 levels in peripheral blood mononuclear cells of sled dogs. Biochemistry and Biophysics Reports, 2, 45-49. 2015.

190. Crofts, C., Zinn, C., Wheldon, M., & Schofield, G. (2015). Hyperinsulinemia: A unifying theory of chronic disease? Diabesity, 1(4), 34-43. doi:10.15562/diabesity.2015.19. 2015.

191. Madole MB, Iyer CM, Madivalar MT, Wadde SK, Howale DS. Evaluation of Biochemical Markers Serum Amylase and Serum Lipase for the Assessment of Pancreatic Exocrine Function in Diabetes Mellitus. J Clin Diagn Res. 2016 Nov and 10(11):BC01-BC04. 2016.

192. Patel R, Shervington A, Pariente JA, Martinez-Burgos MA, Salido GM, Adeghate E, et al. Mechanism of exocrine pancreatic insufficiency in streptozotocin-induced type 1 diabetes mellitus. Ann N Y Acad Sci. 2006 and 1084:71–88. 2006.

193. Ewald N, PD Hardt. Diagnosis and treatment of dia- betes mellitus in chronic pancreatitis. World J Gastro- enterol 2013 and 7276–7281., 19(42):. 2013.

194. Srihardyastutie, A., Soeatmadji, D.W. and Fatchiyah, A.A., 2015. Relation of elevated serum lipase to Indonesian type 2 diabetes mellitus progression. Biomed Res, 26(2), pp.293-8. 2015.

195. Amandeep Kaur, N Verma. Proteomics : A hallmark tool for identification of biomarker (LIPASE) in type I & II diabetes mellitus patients. Adv Appl Sci Res 2012 and 1842–1847., 3(3):. 2012.

196. Ake Andren-Sandberg, PD Hardt. Workshop report: Giessen international workshop on interactions of ex- ocrine and endocrine pancreatic diseases. J Pancreas 2005 and 382–405., 6(4):. 2005.

197. JM Beauregard, JA Lyon, C Slovis. Using the litera- ture to evaluate diagnostic tests : amylase or lipase for diagnosing acute pancreatitis ?. J Med Libr Assoc 2007 and 121–126., 95(2):. 2007.

198. A Srihardyastutie, DW Soeadmadji, F Fatchiyah, A Aulanniam. Lipase/amylase ratio as the indication of pancreatic exocrine inflammation and the correlation with insulin resistance in type 2 diabetes melli- tus. (BASIC) 2014 and 33–37. 2014.

199. StoicaL, GadeaR,et al Plasma ghrelin, adiponectin and leptin levels in obese rats with type 2 diabetes mellitus after sleeve gastrectomy and gastric plication. Experimental and Therapeutic Medicine. 2021 Mar 1 and 21(3):1-. 2021.

200. Jakubowicz D, Wainstein J, Landau Z, Ahren B, Barnea M, Bar-Dayan Y, Froy O. High-energy breakfast based on whey protein reduces body weight, postprandial glycemia and HbA1C in Type 2 diabetes. The Journal of nutritional biochemistry. 2017 Nov 1 and 49:1-7. 2017.

201. Giezenaar C, Trahair LG,et al . Effects of randomized whey-protein loads on energy intake, appetite, gastric emptying, and plasma gut-hormone concentrations in older men and women. The American journal of clinical nutrition. 2017 Sep 1 and 106(3):865-77. 2017.

202. Skuratovskaia D, Vulf M, Chasovskikh N, Komar A, Kirienkova E, Shunkin E, Zatolokin P, Litvinova L. The links of ghrelin to incretins, insulin, glucagon, and leptin after bariatric surgery. Frontiers in genetics. 2021 and 12. 2021.

203. Murata, M., Okimura, Y., Iida, K., Matsumoto, M., Sowa, H., Kaji, H., Kojima, M., Kangawa, K. and Chihara, K. (2002) J. Biol. Chem., 277, 5667-5674. 2002.

204. Shiiya T, Nakazato M, Mizuta M, Date Y, Mondal MS, Tanaka M, Nozoe SI, Hosoda H, Kangawa K, Matsukura S. Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. J Clin Endocrinol Metab 2002 and 240–244., 87:. 2002.

205. Adeghate, E. and Ponery, A.S., 2002. Ghrelin stimulates insulin secretion from the pancreas of normal and diabetic rats. Journal of neuroendocrinology, 14(7), pp.555-560. 2002.

206. Dave M., Gupta, A.K. and Patel, P., Correlation between fasting blood sugar level, HbA1C level and serum lipid levels in Type 2 diabetes mellitus patients. 2019.

207. Islam N., Rahman Z., Choudhury S., Afrin L., Rahman S., Aftabuddin M. Prevalence of Dyslipidemia and Associated Factors among the Sub-Urban Bangladeshi Population. Univ. Heart J. 2012 and 8:15–19. 2012.

208. Bhowmik B., Munir S.B., et. al. Prevalence of type 2 diabetes and impaired glucose regulation with associated cardiometabolic risk factors and depression in

an urbanizing rural community in Bangladesh. Diabetes Metab. J. 2012 and 36:422–432. 2012.

209. Taskinen M.R., Borén J. New insights into the pathophysiology of dyslipidemia in type 2 diabetes. Atherosclerosis. 2015 and 239:483–495. 2015.

210. Al-Ani F.S., Al-Nimer M.S., Ali F.S. Dyslipidemia as a contributory factor in etiopathogenesis of diabetic neuropathy. Indian J. Endocrinol. Metab. 2011 and 15:110–114. 2011.

211. Goldberg I.J. Diabetic Dyslipidemia: Causes and Consequences. JCEM. and 86:965–971. 2001.

212. Badimón J.J., Santos-Gallego C.G., Badimón L. Importance of HDL cholesterol in atherothrombosis: How did we get here? Where are we going? Rev. Esp. Cardiol. 2010 and 63:20–35. 2010.

213. Madole MB, Iyer CM, Madivalar MT, Wadde SK, Howale DS. Evaluation of Biochemical Markers Serum Amylase and Serum Lipase for the Assessment of Pancreatic Exocrine Function in Diabetes Mellitus. J Clin Diagn Res. 2016 Nov and 10(11):BC01-BC04. 2016.

214. Dezaki K., Sone H., Yada T. Ghrelin is a physiological regulator of insulin release in pancreatic islets and glucose homeostasis. Pharmacology and Therapeutics. 2008 and 118(2):239–249. 2015.

215. Biadgo B, Abebe SM, Baynes HW, Yesuf M, Alemu A, Abebe M. Correlation between Serum Lipid Profile with Anthropometric and Clinical Variables in Patients with Type 2 Diabetes Mellitus. Ethiop J Health Sci. 2017 May and 27(3):215-226. 2017.

216. Frank B, Stampfer J, Steven M. Elevated risk of cardiovascular disease prior to clinical diagnosis of type 2 diabetes. Diabetes Care.2002; 25:1129-34.

217. Mullugeta Y, Chawla R , Kebede T , Worku Y. Dyslipidemia Associated with Poor Glycemic Control in Type 2 Diabetes Mellitus and the Protective Effect of Metformin Supplementation. Ind J ClinBiochem. 2012; 27(4):363–369.

218. RENDER, Diane, et al. Biomaterial-derived calcium carbonate nanoparticles for enteric drug delivery. Journal of nanomaterials, 2016, 2016.

219. VARGHESE, Neethumol; HARIFARAN, Manjusha; CHERIAN, A. Benny. SYNTHESIS OF CALCITE NANOPARTICLES FROM BIOGENIC WASTE USING GELATIN AND ITS SPECTRAL CHARACTERIZATION. 2015.

Appendix (A)

Diabetes Mellitus Type 2 Questionnaire:

Patient information:

Name:		Age:
Height:	Weight:	BMI:
Gender: Male: ☐ Female: ☐		Family history:
Smoking: Yes ☐ No ☐		Duration of diseases:
Treatment:		

Chronic diseases:

Hypertension:	Autoimmune diseases:	Other diseases:

Biochemical Tests:

FBS	
HbA1c	
Insulin	
Ghrelin	
Amylase	
Lipase	
Triglycerides	
Cholesterol	
HDL	
VLDL	
LDL	

Other Notes:

الملخص

الخلفية: داء السكري (DM) هو سلسلة من حالات التمثيل الغذائي التي تؤدي إلى ارتفاع السكر في الدم الناجم عن خلل إفراز الأنسولين أوخلل كفاءة الأنسولين أو كليهما. داء السكري من النوع 2 (T2DM) هو اعتلال في جزرباراكرينوباثي ، حيث تتعطل العلاقة المتساوية بين كل من خلية ألفا التي تفرز الجلوكاجون وخلية بيتا التي تفرز الأنسولين ، مما يؤدي إلى فرط جلوكاجون الدم وارتفاع السكر في الدم. يتميز مرضى T2DM في الغالب بأنهم يعانون من السمنة أو لديهم نسبة دهون أعلى في الجسم ، موزعة بشكل سائد في منطقة البطن. في هذه الحالة ، تعزز الأنسجة الدهنية مقاومة الأنسولين من خلال آليات التهابية مختلفة ، بما في ذلك زيادة إفراز الأحماض الدهنية الحرة (FFA) وتحرير الدهون.البكتين هو بوليمر متعدد السكاريد متوافق حيوياً مع نشاط بيولوجي ، والذي قد يظهر هياكل مختلفة اعتمادًا على مصدره أو طريقة الاستخراج. البكتين عرضة للتغيرات الفيزيائية والكيميائية و / أو الأنزيمية. Apple pomace وبدرجة أقل هو المصدر الرئيسي للبكتين التجاري. تحتوي المواد النانوية المعدلة بالبكتين على عامل مضاد لمرض السكر في (T2DM) من خلال خفض مستويات الجلوكوز في الدم و / أو إفراز الأنسولين بعد تحميل السكر ، ويساعد على تقليل نسبة الكوليسترول في الدم ، ويقلل من خطر الإصابة بأمراض القلب والأوعية الدموية.نظرًا لتوافره العالي وتكلفة إنتاجه المنخفضة ، يمكن أن يستفيد البكتين من علاجات إنقاص الوزن والسمنة. إلى جانب ذلك ، نظرًا لأن البكتين حمض ضعيف ، فإنه يقاوم التفكك في بيئة المعدة ويرتبط تساهميًا بالمواقع النشطة lipase البنكرياس. علاوة على ذلك ، من المستحسن للغاية دراسة تأثير الجسيمات النانوية المعدلة من البكتين على إنزيمات البنكرياس في مصل مرضى (T2DM) من خلال قياس أنشطة lipase و amylase في غياب ووجود الجسيمات النانوية ، وقياس الدهون ، وكذلك مستويات ghrelin في مصل مرضى (T2DM) ، وتقييم العلاقة بين أنزيمات البنكرياس والماركرات البيوكيميائية المدروسة.

المواد وطرق العمل: وشملت الدراسة مجموعة من 39 مريضا يعانون من داء السكري من النوع 2 و 39 شخصا أصحاء تتراوح أعمارهم بين 35 إلى 60 عاما. تم التحديد الكمي لـ α-amylase في مصل الدم بالطريقة الحركية (CNPG3) وتم تحديد مستوى glucose ، lipase ، الكوليسترول الكلي في الدم ، الدهون الثلاثية في الدم ، HDL بواسطة طريقة القياس اللوني. بالإضافة إلى ذلك ، تم حساب LDL بالطريقة غير المباشرة وتم حساب VLDL عن طريق قسمة تركيز الدهون الثلاثية على 5. استند تحديد HbA1c إلى (TINIA) للدم الكامل المنحل وقياس insulin في الدم ومصل ghrelin تم إجراؤها بواسطة (ELISA).

تحضير جزيئات البكتين النانوية وجسيمات كربونات الكالسيوم النانوية (CaCO3 NPs). بالإضافة إلى ذلك ، فإن الجسيمات النانوية ذات القشرة الأساسية من البكتين الكالسيوم والمشار إليها باسم $CaCO_3$@ pectin NPs كانت عبارة عن طلاء مركب لسطح جسيمات كربونات الكالسيوم النانوية مع بوليمر البكتين. تم توصيف المواد النانوية المحضرة باستخدام المجهر الإلكتروني لمسح الانبعاث الميداني (SEM) ، حيود الأشعة السينية (XRD) ، تشتت الضوء الديناميكي (DLS) ، مقياس الطيف المرئي فوق البنفسجي (UV-Vis-NIR) ، إمكانات زيتا ، و المجهر الإلكتروني النافذ (TEM).

النتائج: أشارت النتائج إلى وجود فرق معنوي في FBS بين المجموعات ، كان متوسط مستويات FBS لـ DM (203.7 ± 72.3) ملجم / ديسيلتر وهو أعلى بكثير من المجموعة السيطرة (88.6 ± 7.64) مجم / ديسيلتر ، (p 0.001)). كان متوسط HbA1c لـ 2.35 ± 9.5) DM) أعلى بكثير من الاصحاء (4.9 ± 0.48) ، p 0.001. كان متوسط مستويات نشاط amylase وlipase في مجموعة مرضى ± 71.4) DM 29.11) و (49.7 ± 14.69) (U / L) على التوالي ، بينما كان المتوسط في المجموعة السيطرة (72.7 ± 27.19) و (42.0) ± 7.35) (U / L). أظهر متوسط مستويات نشاط lipase اختلافًا كبيرًا في مرضى T2DM مقارنة بمجموعة السيطرة (الاصحاء) ، (P <0.05).كما ان زيادة هرمونات المصل بشكل كبير (P <0.05) ، مما يشير إلى أن مرضى DM لديهم مقاومة واضحة للأنسولين. كان متوسط مستويات insulin وghrelin في مصل الدم في مجموعة مرضى 6.04 ± 18.3) DM) و (3.3 ± 1.01) (IU / L) على التوالي ، بينما كان المتوسط في المجموعة السيطرة (14.5 ± 5.41) و (2.6). ± 0.71) (IU / L).غالبًا ما يُطلق على التشوهات الدهنية في مرضى السكري اسم "عسر شحميات الدم السكري" ، وتتميز عادةً بارتفاع الكوليسترول الكلي (T-Chol) ، وارتفاع نسبة الدهون الثلاثية (TG) ، وانخفاض مستوى كوليسترول البروتين الدهني عالي الكثافة (HDL-C) ، ومستويات متزايدة بشكل كبير من جزيئات صغيرة كثيفة (VLDL و LDL). تم تمييز شكل وتركيب النانوبكتين المحضر باستخدام الفحص المجهري الإلكتروني (SEM) والمجهر الإلكتروني النافذ (TEM). زاد متوسط حجم $CaCO_3$@ pectin NPs بعد التغليف ببوليمر البكتين ، مما يشير إلى نجاح تغليف البكتين على سطح CaCO3 NPs. تم أيضًا فحص أحجام الجسيمات للمواد النانوية المصنوعة باستخدام قياسات تشتت الضوء الديناميكي (DLS) ، مما يدل على نجاح تغليف بوليمر البكتين على CaCO3 NPs وتشكيل CaCO3 @ pectin core-shell NPs.

تم تحديد البنية البلورية لـ CaCO3 @ pectin بشكل أكبر من خلال نمط حيود الأشعة السينية (XRD). أظهرت النتائج أن CaCO3 NPs شديدة البلورية بينما $CaCO_3$@ pectin NPs غير متبلور. تشير هذه النتائج بوضوح إلى التكوين الناجح لـ $CaCO_3$@ pectin NPs المركب. الامتصاص البصري لـ CaCO3

Pectin NPs @ له CaCO3 والذي ما يقارب من 400 نانومتر دليل واضح على تكوين CaCO3 @ Pectin NPs. كان نشاط تجمع بمصل amylase في وجود CaCO3 @ Pectin NPs وجسيمات الكالسيوم النانوية وجزيئات البكتين النانوية 63.1 <82.2 <109.3 (U / L) على التوالي ، بينما كان نشاط تجمع lipase المصل 12.9 > 12.2 <20 (U / L). تم استخدام تحليل اختبار سبيرمان لتحليل علاقة الاستجابة بين الماركرات. بشكل أساسي ، كان FBS المصل مرتبطًا بشكل إيجابي بمستويات HbA1c و TG و VLDL. بالإضافة إلى ذلك ، ارتبطت مستويات TG بشكل كبير مع مستويات الكوليسترول الكلي ، VLDL ، ومستويات الأنسولين. كعامل خطر لخلل شحميات الدم في (T2DM) ، كان LDL في الدم مرتبطًا بشكل كبير وإيجابي بمستويات عالية من الكوليسترول الكلي (الكل P <0.001). العلاقة الكلية بين الماركرات وحالات الدراسة هي أيضًا معامل الارتباط لـ Ghrelin و Lipase و Amylase.

الخلاصة: CaCO3 @ Pectin NPs لها تأثير مثبط على نشاط إنزيمات lipase وamylase، بينما أظهرت جزيئات البكتين النانوية تأثيرًا منشطًا على إنزيم amylase . لذلك ، من الممكن دراسة إمكانية استخدام جزيئات البكتين النانوية بأشكال مختلفة كناقل لأدوية السكري حسب الحالة الفسيولوجية لمريض السكري.

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تأثير البكتين على فعالية انزيمات البنكرياس المصلية لمرضى السكري من النوع الثاني

رسالة ماجستير

إلى مجلس كلية الطب/ فرع الكيمياء و الكيمياء الحياتية/ جامعة كربلاء كجزء من متطلبات نيل درجة الماجستير في الكيمياء السريرية

من قبل

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