University of Kerbala
College of Education for Pure Sciences
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Effectiveness of Calcium Carbonate@ Arabian Gum Nanoparticles on Sera of Diabetes mellitus Type II with Osteoarthritis Patients

A Thesis

submitted to the Council of the College of Education for Pure Sciences / University of Kerbala as part of the requirements for obtaining a Master's Degree in Chemistry

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Dedication

To my master, the owner of the time, may Allah hasten his honorable return

To the heroes of resistance throughout the Arab world

To the patient Mujahedeen in Gaza, glory

To the soul of my dear mother. Allah have mercy on her

To the person who provided me with generosity, strength, and dignity... my dear father

To my support, my pride, my joy, and my honor...my dear brothers

To all those from whom I have received advice, support and assistance

To everyone who wished me well

To all my friends and relatives, I dedicate my humble work to you.....

Acknowledgement

First of all, thanks to Allah for inspiring me with strength, patience, and guidance to perform this work.

I would like to express my thanks and gratitude to the patients for participating in this study, with my wishes for their health and wellness. I would like to express my deep and sincere gratitude to my supervisors for their inspiring guidance, help, and encouragement, which were the essential motivation to continue this work. I wish them a long life with my best wishes.

I would like to thank the staff of the department of Chemistry of the College of Education for pure sciences /University of Kerbala and Department of Chemistry and Biochemistry, College of Medicine/University of Kerbala for providing various requirements and equipment to accomplish this project.I would like to thank the team of Rehumatoidology clinic and the laboratory in AL-IMAM Al-Hassan Hospital in holy Karbala city for their assistance in conducting laboratory testing and their moral support.

Thanks for all…

Soaad ALqoraishy

Summary

Diabetes mellitus type2 and Osteoarthritis are the most common diseases particularly in Iraq and in the world. The present work was carried out in Rheumatology department in AL-IMAM Al- Hassan Hospital in holy Karbala city at 90 women (50 women with osteoarthritis and diabetes mellitus type2 as patients group and 40 women apparently healthy as a control group (all of them aged from 40-70). The aim of this study is to study the differences of blood glucose, hemoglobin A1C, high sensitivity C-reactive protein, serum calcium, vitamin D3, parathyroid hormone, serum albumin and alkaline phosphatase in patients of T2DM with OA compared to control group in Iraqi women by measuring those parameters and study the effect of the Calcium Carbonate@Arabian Gum Nanoparticles on sera of the patients group. The work included two parts. The first part was measuring the parameters that diagnose the two diseases, which were fasting blood sugar and hemoglobin $A1_C$ for diabetes mellitus while for osteoarthritis had been measured serum calcium, vitamin D3, parathyroid hormone, high sensitivity C-reactive protein, serum albumin and alkaline phosphatase. The second part included preparation Calcium Carbonate@Arabian gum Nanoparticles by Co-precipitation method and characterized by scanning electron microscopy, transmission electron microscopic, X-ray powder diffraction and investigated by UV−Vis-NIR spectra. Calcium Carbonate@Arabian gum Nanoparticles were added to1ml pool of sera of 15 patients in different concentrations and the results showed that there was a significant difference between patients group and controls group in blood glucose, hemoglobin A1c alkaline phosphatase, parathyroid hormone and high sensitivity C-reactive protein ($p<0.05$) .There were significant differences in Alb and calcium ($p<0.01$).

Albumin negatively significant with Parathyroid hormone ($P<0.05$). Significant differences in high sensitivity C-reactive protein and

phosphorus($P<0.05$). The results after adding $CaCO_3$@AGNPs and $CaCO_3$NPs showed a decrease in the levels of blood glucose, alkaline phosphatase, albumin, parathyroid hormone, and phosphorus in the blood. It was also observed that the level of calcium increased. It could be concluded that there is a high association between diabetes mellitus type tow and osteoarthritis disease and the Synthesized Calcium Carbonate@Arabian Gum NPs is very effective in reducing glucose levels. Calcium Carbonate NPs can provide the calcium easier than Carbonate@Arabian Gum NPs.

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Abbreviations	
ADA	American Diabetes Association
ACL	Anterior Cruciate Ligament
AG	Arabic Gum
Alb	Albumin
ALPs	Alkaline phosphatases
APCs	Antigen-presenting cells
BMI	Body mass index
$CaCO_3$	Calcium carbonate
$CaCO_3$NPs	Calcium carbonate nanoparticles
$CaCO_3$@AGNPs	Calcium carbonate@Arabian gum nanoparticles
Ca-SR	Calcium-sensing receptor
CircRNA	Circular RNA
CRP	C-reactive protein
DM	Diabetes mellitus
DLS	Dynamic laser scattering
ER	Endoplasmic reticulum
ESCs	Embryonic stem cells
ECM	Extracellular matrix
EDS	Energy-Dispersive Spectroscopy
FPG	Fasting plasma glucose
GA	Golgi apparatus
GPx	Glutathione peroxidase
GNP	Graphite nanoplatelets

HbA1C	Haemoglobin A1C
HsCRP	High sensitivity C-reactive protein
HDL	High density lipoprotein
HPG	Hyperbranched polyglycerol
IDF	International Diabetes Federation
IAPP	Islet amyloid polypeptide
IR	Insulin resistance
IPSCs	Induced pluripotent stem cells
JDS	Japan Diabetes Society
KGN	Kartogenin
LDL	Low density lipoprotein
MDA	Malondialdehyde
NPs	Nanoparticles
NAFLD	Non-alcoholic fatty liver disease
NLRP3	Nucleotide-binding oligomerization domain-like receptor protein 3
NF-κB	Nuclear factor kappa B
OA	Osteoarthritis
OGTT	Oral glucose tolerance test
Pi	Phosphorus
PTH	Parathyroid hormone
PTOA	Post-traumatic osteoarthritis
ROS	Reactive oxygen species
SiRNA	Small interfering RNA
SEVs	Small extracellular vesicles
SEM	Scanning electron microscopy
T2DM	Type 2 diabetes mellitus

TEM	Transmission electron microscopy
WHO	World Health Organization
XRD	X-ray powder diffraction

CHAPTER ONE

INTRODUCTION

&

LITERATURE REVIEW

1. Introduction

Type 2 diabetes mellitus (T2DM) and osteoarthritis (OA) are two common conditions whose prevalence is predicted to increase. OA and T2DM often coexist due to their high incidence and shared risk factors [1]. For instance, there is strong evidence linking OA to obesity and most T2DM patients have obesity. Additionally, one well-known risk factor for both OA and T2DM is aging [2]. Diabetes mellitus (DM) is defined by the American Diabetes Association (ADA) as a collection of metabolic illnesses marked by hyperglycemia brought on by abnormalities in insulin production, insulin action, or both [3]. DM is one part of the metabolic syndrome worldwide. About 11% of the general population has (DM), which can cause a number of problems [4]. T2DM results from the body's ineffective use of insulin and comprises 90% of people with DM worldwide. It is one of the top 10 causes of death worldwide causing 4 million deaths in 2017 [5]. According to a report by the International Diabetes Federation (IDF) in 2021 more than 1 in 10 adults now have diabetes globally and that the number of people with diabetes will continue to expand rapidly in the future. The total number of adults (20–79 years) with diabetes in 2045 will be 629 million from 425 million in 2017 (48% increase) [6].

Osteoarthritis (OA) is one of the most prevalent joint disorders, and it leads to diarthrodial articular cartilage deterioration with eventual disability in adults [7,8]. The prevalence, progression, and severity of the symptoms of OA can be affected by multiple factors such as comorbidities, lifestyle, diet, age, and genetics [9]. Recent studies have suggested phenotypically subcategorizing OA to better understand the pathogenesis and causes related to OA. These subtypes entail age-

related, post-traumatic event-related, and metabolic syndrome-related categories [10]. By classifying OA into categories, Further

evaluation of how other pre-existing conditions and differences in lifestyle influence the development of OA is possible. This issue is particularly important given the high prevalence of OA associated with metabolic syndrome [11]. The comorbidities and risk factors in OA pathogenesis share common features with those seen in T2DM [12]. Since the 1980s, it has been observed that those diagnosed with OA also have a preexisting DM diagnosis, and vice versa. Owing to their frequent coexistence, OA and DM have a substantial impact on the health burden on both the individual and the wider community [13].

1.1. Diabetes Mellitus Type II

Diabetes is a widespread and dangerous illness. According to estimates, diabetes affects 1 in 11 persons globally and accounts for 11% of all fatalities each year [14]. A deficiency in insulin action, secretion, or a combination of the two can cause DM, a common metabolic disorder. The inefficient utilization of insulin by the body causes T2DM, which affects 90% of diabetics globally. Over the past few decades, the number of persons with diabetes has quickly increased, from 108 million in 1980 to 422 million in 2014. It is projected that this number will more than double in the next 20 years. In addition, diabetes is predicted by the World Health Organization WHO to rank seventh among all causes of mortality by 2030 [15]. DM is a chronic illness that arises from insufficient insulin production by the pancreas or from the body's inability to utilize the insulin that is produced. One hormone that controls blood sugar is insulin. Uncontrolled diabetes frequently results in hyperglycemia, also known as elevated blood glucose or elevated blood sugar, which over time seriously damages numerous bodily systems, including the

blood vessels and neurons [16]. The WHO describes diabetes mellitus as a chronic metabolic disease marked by high blood glucose levels. Over time, this condition can cause damage to the heart, blood vessels, eyes, kidneys, and nerves. T2DM, which is characterized by insufficient insulin secretion by pancreatic islet β-cells, tissue insulin resistance IR, and insufficient compensatory insulin secretory response, accounts for more than 90% of cases of DM [17].

1.1.1. The Occurrence of Diabetes Mellitus Type II

Insulin resistance, the outcome of which is the disturbance of several cellular pathways, causes a reduction in the sensitivity or reactivity of cells in peripheral tissues, specifically the muscle, liver, and adipose tissue, to insulin. This leads to the occurrence of T2DM [18]. Early in the disease, β-cell hyperfunction is triggered by impaired insulin sensitivity, which leads to an increase in insulin production as a compensatory measure to maintain normoglycemia. Hyperinsulinemia, or elevated amounts of circulating insulin, thereby averts hyperglycemia. Over time, though, the β-cells' increased release of insulin becomes insufficient to offset the decline in insulin sensitivity [19]. Furthermore, β-cell function starts to deteriorate, and β-cell dysfunction ultimately results in insufficient amounts of insulin. Consequently, hyperglycemia develops when normoglycemia cannot be sustained [20].

1.1.2. Sign and Symptoms in Diabetes Mellitus Type II

Most early symptoms are from higher-than-normal levels of glucose, a kind of sugar, in the blood.

1.1.2. 1. Early signs of Diabetes Mellitus Type II

1. Fatigue and Hunger

The body breaks down food into glucose, which is used by the cells as fuel. However, for the cells to absorb glucose, insulin is required. The body cannot absorb glucose and becomes energy-depleted if it produces insufficient or no insulin, or if the cells reject the insulin that is produced. The patient may become more fatigued and hungry than normal as a result [21].

2. Having Increased Thirst and Frequency of Urination

The typical individual typically needs to urinate four to seven times in a 24-hour period, while those who have diabetes may urinate much more frequently. Normally, when glucose goes through the kidneys, it is reabsorbable by the body. However, the kidneys might not be able to fully reabsorb the glucose. When blood sugar levels raise. The body needs fluids to produce more pee as a result of this. It will need to go more frequently as a result. Additionally, the patient may urinate more. The patient may become extremely thirsty as a result of their frequent urination [22].

3. Xerostomias and Itchy skin.

(Xerostomias) dry mouth is a common symptom of DM. Sometimes dry mouth is the first noticeable symptom of diabetes. Itchy skin a result of poor circulation can make a sufferer uncomfortable.

4. Hazed vision.

The body's fluid levels fluctuating may cause the eye's lenses to enlarge. They become disoriented and morph [23].

5. Acanthosis Nigerians

The skin condition known as Acanthosis Nigerian causes velvety, light-brown to black spots to appear on the neck, under the breasts, in the groin, and the armpits [24].

1.1.2.2. Symptoms of Diabetes Mellitus Type II [25]

These Symptoms typically become apparent after a prolonged period of elevated glucose.

1. Infections with yeast

It can affect both men and women with diabetes. Since yeast is fed on glucose, an abundance of it is conducive to its growth. Any warm, moist fold of skin on the body might harbor infections, including between fingers and toes, under breasts and in or around sex organs.

2. Slow-healing sores or cuts

Over time, high blood sugar can affect the blood flow and cause nerve damage that makes it hard for the body to heal wounds.

3. Pain or numbness in the feet or legs

This is another result of nerve damage.

1.1.3. Pathogenesis of T2DM

A disorder of carbohydrate metabolism T2DM is characterized by hyperglycemia typically due to the interaction of insulin resistance and impaired beta cell function. T2DM is caused by predominant insulin resistance and relative insulin deficiency or a predominant disorder of insulin secretion with or without insulin

resistance [26] Pancreatic β-cells produce insulin in response to increased arterial glucose concentrations. Second-phase insulin release is slower, reaching a steady state at 2-3 hours after the initial rise in arterial levels of glucose, whereas first-phase insulin release peaks at 2-4 minutes after the initial rise in arterial levels of glucose and declines rapidly by 10–15 minutes [27]. β-cells are induced to release more insulin when they are insulin resistant compared to when they are insulin sensitive. Hyperglycemia and T2DM are ultimately caused by insufficient insulin production, particularly when insulin resistance and inflammation associated with obesity are present [28].

1.1.4. Risk Factors for Diabetes Mellitus Type II

As diabetes has become more common, the severity of the issue as it relates to public health has also increased. The disease itself begins when a number of risk factors come together. The main risk factors for prediabetes and DM are genetics, environment, loss of the first phase of insulin launch, sedentary lifestyle, lack of exercise, smoking, alcohol consumption, dyslipidemia, diminished β-cell sensitivity, hyperinsulinemia, and increased glucagon activity. These factors seem to be important in the development of insulin resistance or non-functionality, which leads to the progression of the disease [29].

1.1.4.1. Aging

As β cells age, they accumulate more Reactive oxygen species (ROS), unfolded proteins, DNA damage, Islet amyloid polypeptide IAPP, advanced glycation end-products AGEs and other forms of cellular stress. These alterations cause β cells to undergo cellular senescence and impair their capacity for proliferation and regeneration. The function of β cells in islet blood arteries may be disrupted by oxidative damage, inflammation, and fibrosis [30]. One of the extrinsic causes is

the aging-related decrease in insulin sensitivity in peripheral insulin-responsive tissues, which raises the need for insulin and eventually leads to β cell fatigue. When combined, the aging process's increasing insulin resistance and the β cells' lowered ability to secrete insulin cause the body to lose its ability to regulate glucose, which eventually results in the establishment of age-related diabetes [31].

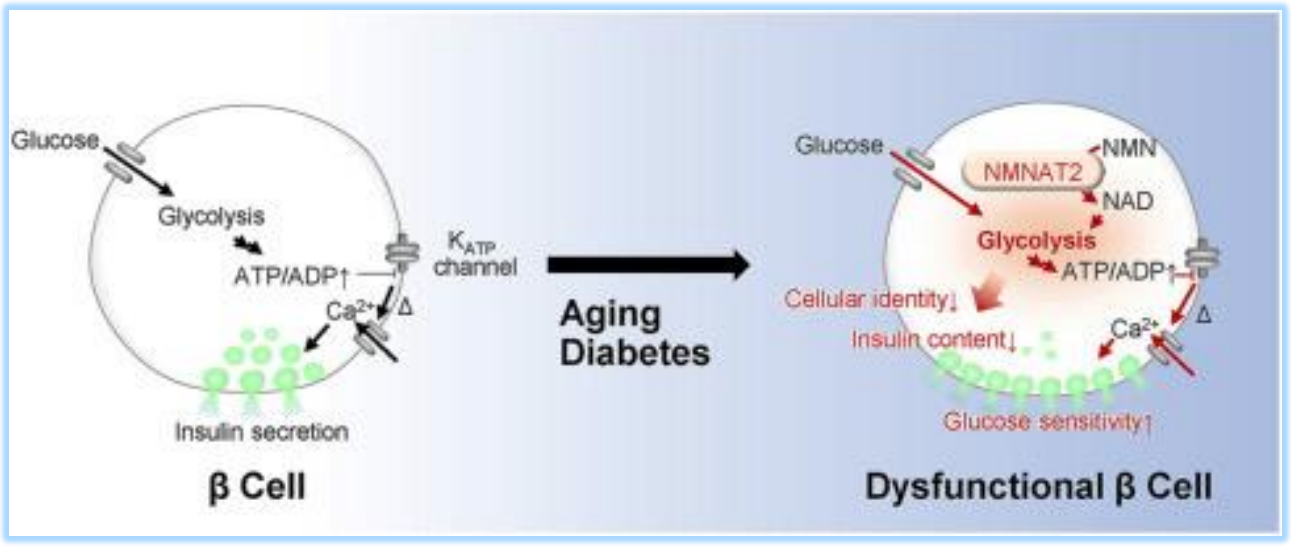


Figure (1.1) Effect of aging on beta cells [32].

1.1.4.2. Obesity

In clinical practice, obesity is typically determined by calculating the body mass index (BMI), which is calculated by dividing the body weight in kilograms by the height in square meters (kg/m2) [33]. Obesity is described as an excess of body fat that negatively impacts health [34].

An excessive amount of body fat accumulates and causes a variety of metabolic disorders and diseases, such as insulin resistance, non-alcoholic fatty liver disease (NAFLD), prediabetes, T2DM, and atherogenic dyslipidemia (high plasma triglyceride and low plasma HDL-cholesterol concentrations) [35]. T2DM risk

often rises in proportion to a steady increase in body mass index (BMI), which measures obesity [36].

1.1.4.3. Genetic

Diabetes Mellitus is becoming a major global cause of death due to its fast rising prevalence. Recent research indicates that genetic data significantly influences the ability to forecast the risk of diabetes and to provide tailored anti-diabetic medication. Thus far, over 70 susceptibility genes are found to be significantly linked across the entire genome to T2DM. All the genetic loci discovered to date, however, only contribute to approximately 10% of the total heritability of T2DM. Furthermore, little is known about the relationship between the pathophysiology of the illness and these unique susceptibility loci [37].

1.1.5. Complications of Diabetes Mellitus Type II

Over time, high blood sugar (hyperglycemia) in diabetics damages a variety of biological tissues in the body, increasing the risk of life-threatening and disability-causing medical consequences. effects of DM are: -

1. Eye disease, as a result of fluid imbalances, tissue edema, and injury to the blood vessels in the eyes [38].

2. Foot problem, caused by decreased blood supply to the foot and nerve damage [39].

3.Gum disease and other dental issues as dangerous germs are encouraged to proliferate in the mouth by a high blood sugar level in saliva. Food particles and bacteria interact to create plaque, a soft, sticky film. Eating foods high in sugar or carbohydrates can also cause plaque. Certain kinds of plaque lead to foul breath and gum disease [40].

4. Heart disease and stroke, resulting from injury to the blood vessels and the nerves that regulate the heart and blood vessels [41].

5.Kidney disease because of harm to the kidneys' blood veins. Diabetes frequently results in elevated blood pressure. Kidney damage can also result from that [42].

6.Nerve problems (diabetic neuropathy), a condition brought on by injury to the nerves and the tiny blood arteries that supply the nerves with nutrition and oxygen [43].

7.Sexual and bladder problems, resulting from nerve injury and decreased blood supply to the bladder and genitalia [44].

8. Skin conditions, Some of which result from diminished circulation and modifications to the tiny blood vessels. It is also more common for people with diabetes to get infections, particularly skin infections [45].

1.2. Osteoarthritis

The most prevalent musculoskeletal disorder that poses serious health and societal issues globally is OA. Its precise cause is yet unknown. OA affects over 10% of the global population with clinical symptoms, primarily affecting those over 65 [46]. OA is a dangerous illness with a significant global medical burden, particularly in view of the recent improvements in life expectancy [47]. The degeneration of hyaline articular cartilage, ligaments, menisci in the knee, hypertrophic alterations in the joint capsule, subchondral bone remodeling, osteophyte formation, and synovial inflammation are all components of OA, a chronic, systemic disease that progresses slowly. Pain, lower extremity

dysfunction, and joint abnormalities accompany it [48]. OA is characterized by degenerative changes in the articular cartilage. In the initial phases of the illness, the cartilage's water content rises while the quantity of proteoglycans falls. Moreover, the collagen network is weakened by a decrease in the production of freshly deposited collagen type 2 and an increase in its breakdown. A decline in the number of functionally active chondrocytes is also linked to the amplification of cartilage apoptosis [49]. The cartilage loses some of its elasticity and compressive strength as a result of the alterations discussed above [50]. The chondrocytes found in the deeper layers of articular cartilage multiply in response to degenerative processes, generating new collagen and proteoglycans that initiate the repair process [51].

1.2.1. Signs and Symptoms

Osteoarthritis symptoms include the following.

• Severe, painful joint pain made worse by prolonged use [52].

• The main signs of the illness Crepitus and a reduced range of motion are often observed [53].

• Stiffness during repose (gelling). This condition can occur and often lasts shorter than 30 minutes in the morning [54].

1.2.2. Pathogenesis of Osteoarthritis.

1.2.2.1 Aging

The biggest risk factor for OA is aging, which is defined as the steady loss of tissue and organ function over time [55]. According to the Framingham Osteoarthritis study, the frequency of radiographically visible OA, or joint space

narrowing, rises with age, starting at 12.9% in those between the ages of 30 and 40 and reaching 43.7% in those over 80 [56]. Numerous processes have been proposed to explain cellular aging. One well-known mechanism is the gradual buildup of random, unrepaired molecular damage to proteins, lipids, and DNA, which eventually causes cellular abnormalities and tissue malfunction and increases the risk of age-related illnesses and frailty [57]. Reactive oxygen and nitrogen species generated by mitochondria and cellular stress responses, respectively, are the main causes of this damage. These modifications have the immediate consequence of decreasing chondrocytes' capacity to maintain cartilage homeostasis and lowering the threshold of damage-inducing load [58].

1.2.2.2. Obesity

A Body Mass Index (BMI) of more than 30 kg/m2 is considered obese. This condition has epidemic proportions and is a global concern [59]. Because walking applies a force three to six times the body weight to the knee, carrying more weight increases the pressures placed on the joints [60]. Losing weight dramatically reduced the likelihood of developing knee OA [61]. A reduction in body mass index of two units or more (equivalent to a weight loss of roughly 5.1 kg) during the ten years preceding the specified test reduced the odds of getting osteoarthritis by more than fifty percent, per a Framingham research conducted on women [62].

1.2.2.3. Trauma

Post-traumatic osteoarthritis (PTOA) is frequently brought on by joint instability or intra-articular fractures resulting from traumatic injury. Unusual loading vectors and elevated contact stresses brought on by joint damage are known to harm articular cartilage [63]. PTOA accounts for approximately 12% of all OA

[64], and the most vulnerable joints are those that bear weight. For instance, trauma to the knee elements, such as meniscal resection and anterior cruciate ligament (ACL) tears, causes radiographic OA to grow earlier in life [65]. According to estimates, 21% of individuals with ACL transection injuries experience PTOA this number rises to 48% in patients with concurrent meniscal injuries [66]. In contrast, post-traumatic origin accounts for 70–80% of cases of radiographic ankle OA, and the majority of patients are younger than those with primary ankle OA [67].

1.2.2.4. Genetic

Genetic factors account for 39–65% of instances of OA in the general population, according to twin epidemiological research [68]. Due to rare mutations in type II, IX, or XI collagen, which are prevalent collagens found in articular cartilage, there are hereditary forms of OA that induce premature OA, which can start as early as puberty and create a severe, debilitating form of arthritis that affects different joints. But compared to OA of the hands, the data linking hereditary factors to OA of the lower extremity joints such as the knee or hip is less compelling [69].

1.2.2.5. Chronic Overuse/Overloading on joints

Physiologic mechanical loading is a constant condition for chondrocytes and is necessary to preserve a homeostatic balance between the catabolic and anabolic processes [70]. This balance is mediated by anti-inflammatory signaling, a decrease in the activity of matrix-degrading enzymes, and the suppression of pro-inflammatory cytokines and inflammatory mediators [71]. However, supraphysiological loading has been shown to tip this balance in favor of catabolic processes that cause bone marrow lesions, subchondral sclerosis, cartilage

thinning, and the onset of OA. Furthermore, the region that experiences greater cartilage loss is frequently linked to higher mechanical loading [72].

1.2.2.6. Gender

Women are more likely than men to have hip, knee, and hand (OA), and the incidence rises around menopause [73]. Numerous writers have hypothesized that hormonal variables may play a part in the emergence of OA. Results, however, are contradictory [74] and other factors (such as decreased cartilage volume, bone loss, or weak muscles) may account for the differences between men and women [75].

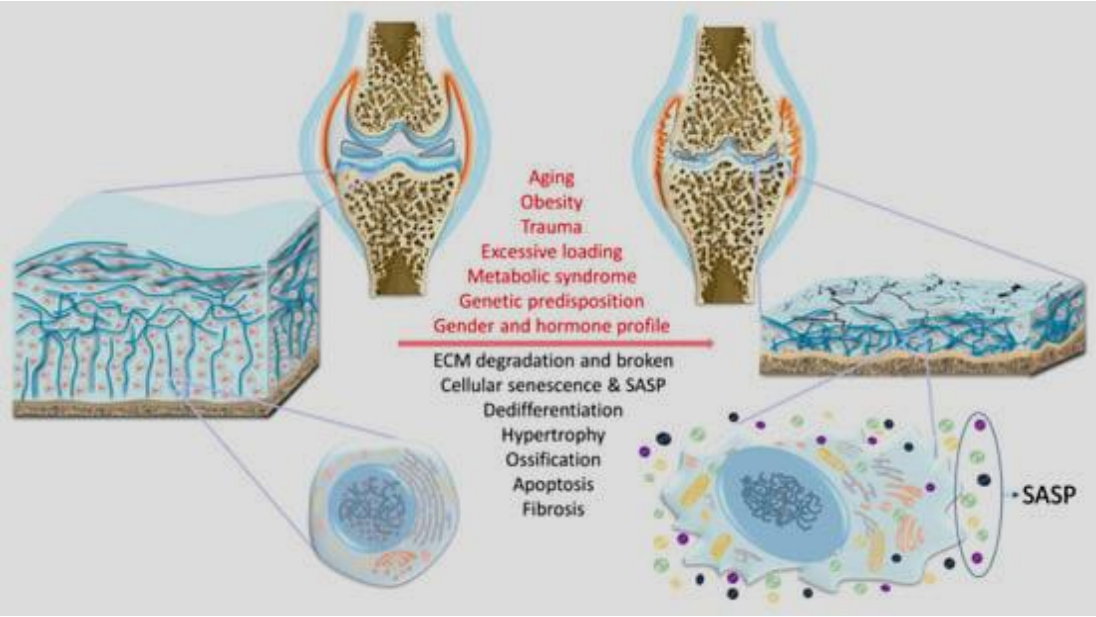


Figure (1.2) Risk factors and structural alterations in OA [76].

1.3. Routine Tests to Diagnose Diabetes Mellitus Type II and Osteoarthritis

1.3.1. Diagnosis of Diabetes Mellitus Type II

Diabetes can be diagnosed by plasma glucose criteria, either the fasting plasma glucose (FPG) value, Oral glucose tolerance test (OGTT) or Heamoglobin A_1C (HbA_1c) criteria [77].

1.3.1.1. Fasting blood glucose

Normal human physiology is dependent on a tight control of the fasting blood glucose (FBG) levels and prolonged increase in FGB levels are hallmark of all types of diabetes. Many types of glucose tests can be used to measure FBG levels [78]. FBG tests can reveal temporary or long-term hyperglycemia or hypoglycemia. The standard, automated glucose oxidase methods are used in clinical laboratories to obtain FBG levels in plasma or serum. Sugar is measured after eight hours of fasting, and the normal level of sugar ranges from 80-120 mg /dl [79].

1.3.1.2. Oral glucose tolerance test

when a blood test is performed to measure the patient's plasma glucose (PG) levels two hours after the patient takes a glucose syrup solution containing 75 g of glucose [80].

1.3.1.3. HemoglobinA1c

The Japan Diabetes Society (JDS) published revised diagnostic guidelines for diabetes mellitus, which included using HbA1c as a diagnostic tool and defining diabetes mellitus in epidemiological research based only on an HbA1c test result of $\geq 6.5\%$ [81]. While diagnosing diabetes solely based on HbA1c values has a

number of disadvantages, it is a popular method for epidemiological research because it eliminates the need for a fasting blood sample or glucose tolerance test, allows for the detection of chronic hyperglycemia with a single measurement, and reduces the possibility of misclassification due to the low variability of HbA1c. The HbA1c is used value-based definition of diabetes for this purpose [82]. The fraction of hemoglobin molecules that have glucose attached to them is measured by HbA1c. HbA1c is positively correlated with increased glucose concentrations throughout the preceding two to three months.

Table (1.1) Diagnostic standard for HbA1c in diabetes [83].

HbA1C	**<5.7%**	**5.7–6.4%**	**> 6.4%**
Diagnosis	**Normal**	**Prediabetes**	**Diabetes**

1.3.2. Diagnosis of Osteoarthritis

1.3.2.1. High sensitivity C-reactive protein

High sensitivity C-reactive protein hsCRP its chemical structure (L-lysyl-L-Prolyl-L-glutaminyl-L-leucyl-L-tryptophyl-L-proline) as shown in figure (1.3) is a C-reactive protein measured by a highly sensitive assay [84]. An acute-phase protein called C-reactive protein (CRP) is created in the liver as a byproduct of the body's normal inflammatory reaction [85]. When inflammatory circumstances exist, it is produced in higher quantities, and high amounts signify a more serious form of systemic inflammation in the patient. hsCRP tests are a helpful non-specific sign of inflammation and for spotting infection since they evaluate the amount of CRP in blood plasma [86]. hsCRP gauges the amount of CRP in blood

plasma, helps identify infection, and serves as a non-specific biomarker of inflammation. Diabetes patients have higher CRP levels, and having higher CRP concentrations increases the chance of getting T2DM. The impacts of the gut microbiota on immunological responses and metabolism might influence chronic inflammation, which can change CRP levels and contribute to the onset and maintenance of dysglycemia. The normal value of hsCRP is less than 5mg/L [87].

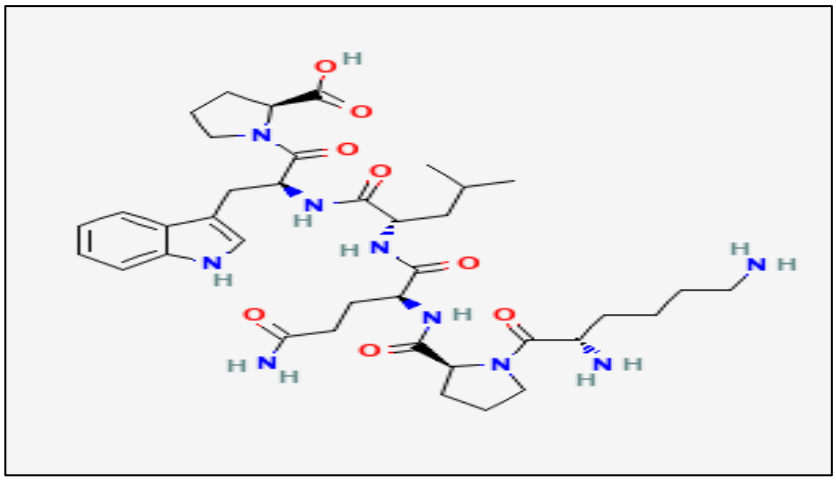


Figure (1.3) Chemical structure of hsCRP [88].

1.3.2.2. Parathyroid hormone

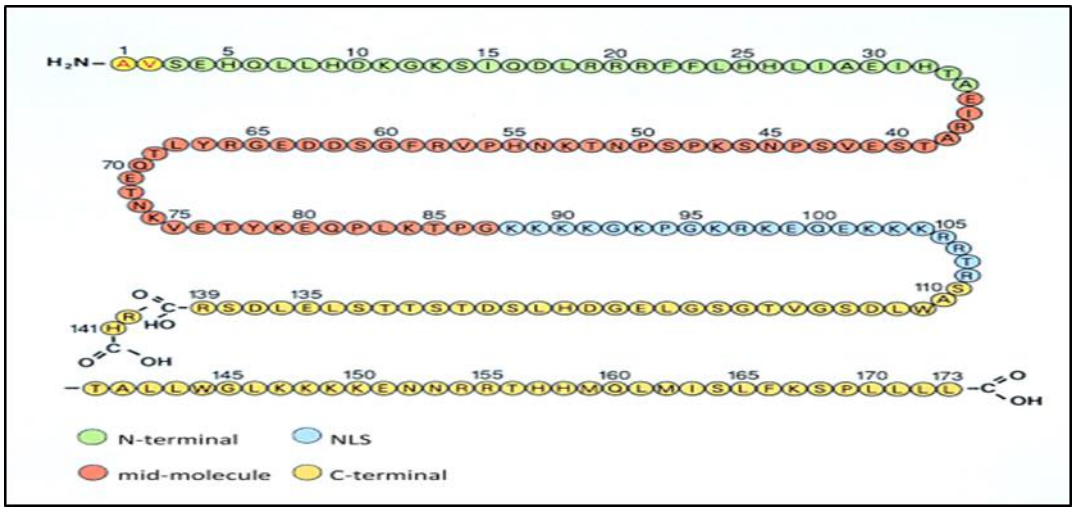


Figure (1.4) Structure of PTH [89]

Parathyroid hormone after being cleaved from preproparathyroid hormone (115 amino acids) to proparathyroid hormone (90 amino acids), parathyroid hormone (PTH), a 9400 D molecular weight peptide, is released as shown in figure (1.4). The complete PTH (1–84) is the active biological form. Its half-life in circulation is less than three minutes, and it is primarily cleared by the liver (60–70%) and kidney (20–30%) [90]. Changes in extracellular calcium induce PTH production through a feedback loop mostly mediated by the calcium-sensing receptor (Ca-SR). This G protein-coupled receptor on parathyroid cells controls PTH production that is controlled by calcium. While high levels of ionized calcium boost calcitonin secretion and restrict PTH release, a decrease in ionized calcium stimulates PTH secretion [91]. It also activates the renal enzyme D-hydroxylase, which activates the synthesis of 1-25-D vitamin D3, which is necessary to enhance the absorption

of calcium from food [92].

The ages	**conc.of PTH**
Birth – <1 year.	6 – 88 pg/ml
1 – <9 years.	15 – 65 pg/ml
9 – <17 years.	22 – 88 pg/ml
17 years	15 – 65 pg/mL

Table (1.2) The normal range of PTH. [93]

1.3.2.3. Albumin

Albumin is the most abundant protein in human plasma. Alb is globular protein commonly found in blood plasma [94]. It is synthesized in the liver as a long peptide of 585-amino acid, with a half-life of approximately 25 days [95]. It has a number of physiological functions that regulates acid-base physiology, binds vital substances in the bloodstream (hormones, fatty acids, bilirubin, Calcium) and transport them to organs as shown in Figure (1.5) and inhibits platelet function, vascular permeability, and colloid-osmotic pressure. Its characteristics of trapping free radicals and antioxidant activity are also mentioned. Maintenance of colloidal osmotic pressure [96]. Normal albumin range is (3.4 - 5.4) g/dL [97].

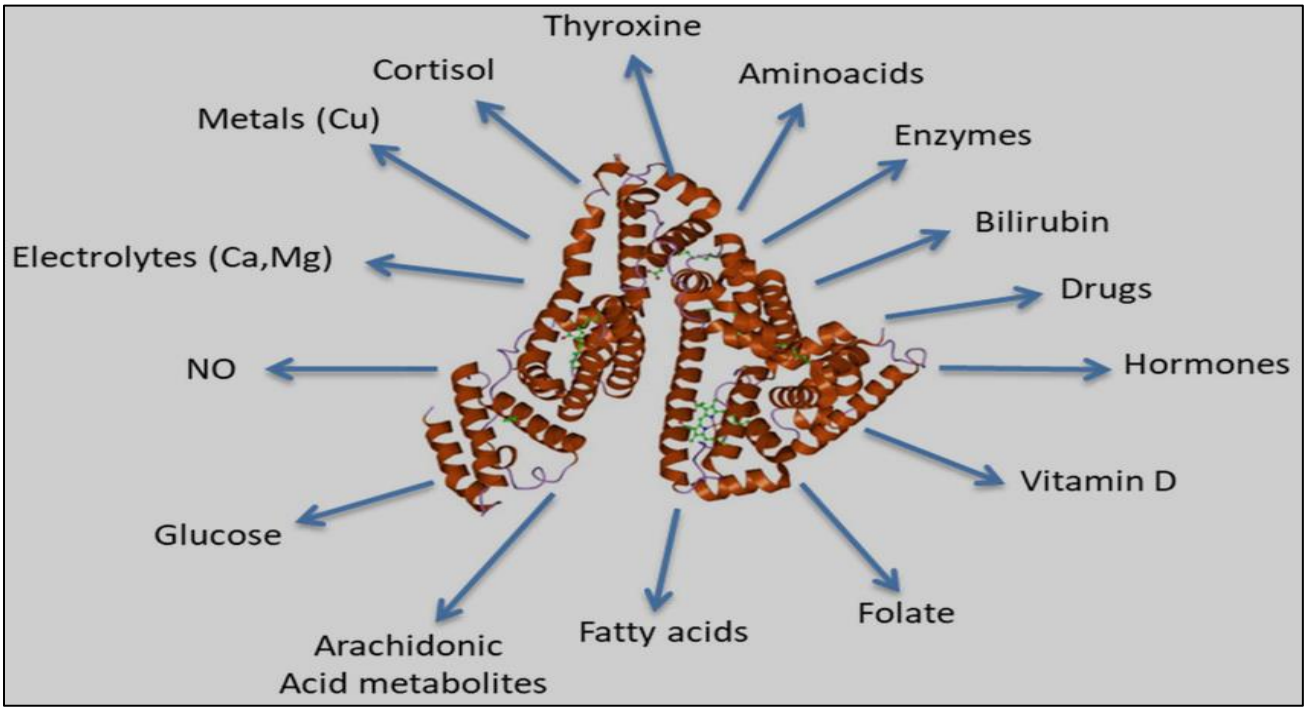


Figure (1. 5) Albumin and substances transported by it [98].

1.3.2.4. Vitamin D3

Vitamin D3 is a member of a group of fat-soluble secosteroids that have biological effects and improve the lower intestine's absorption of calcium,

magnesium, and phosphate. The two most significant substances in humans are vitamin D3 (also known as cholecalciferol) and vitamin D2 (also known as ergocalciferol) [99]. Prohormone vitamin D is produced from cholesterol and is fat soluble. There are two ways to get vitamin D3. either from sunshine or artificial UV-B radiation, or from food such a fish, eggs, milk, and cereals, or from supplements containing vitamin D3. One of the main ways to obtain vitamin D is by exposure to UVB radiation, and skin production of vitamin D3 can be adversely affected by insufficient UVB radiation exposure [100].

1.3.2.4.1. Activation of vitamin D3

Skin production or dietary sources of vitamin D are not physiologically active. Two stages of protein enzyme hydroxylation one in the liver and one in the kidneys activate it [101]. Since most mammals can manufacture enough of it if they receive enough sunlight, vitamin D is not necessary and isn't even considered a vitamin. Rather, it may be regarded as a hormone [102]. with calcitriol, the active form of vitamin D produced through activation of the pro-hormone, acting on several nuclear receptors to cause effects. In the liver, ergocalciferol becomes 25-hydroxy ergocalciferol while cholecalciferol becomes calcifediol (25-hydroxy cholecalciferol). To assess a person's vitamin D level, their serum is tested for these two vitamin D metabolites, often known as 25-hydroxy vitamin D or 25(OH)D. The kidneys and certain immune system cells further hydroxylate calcifediol to create calcitriol, or 1,25-dihydroxycholecalciferol, which is the physiologically active form of vitamin D [103].

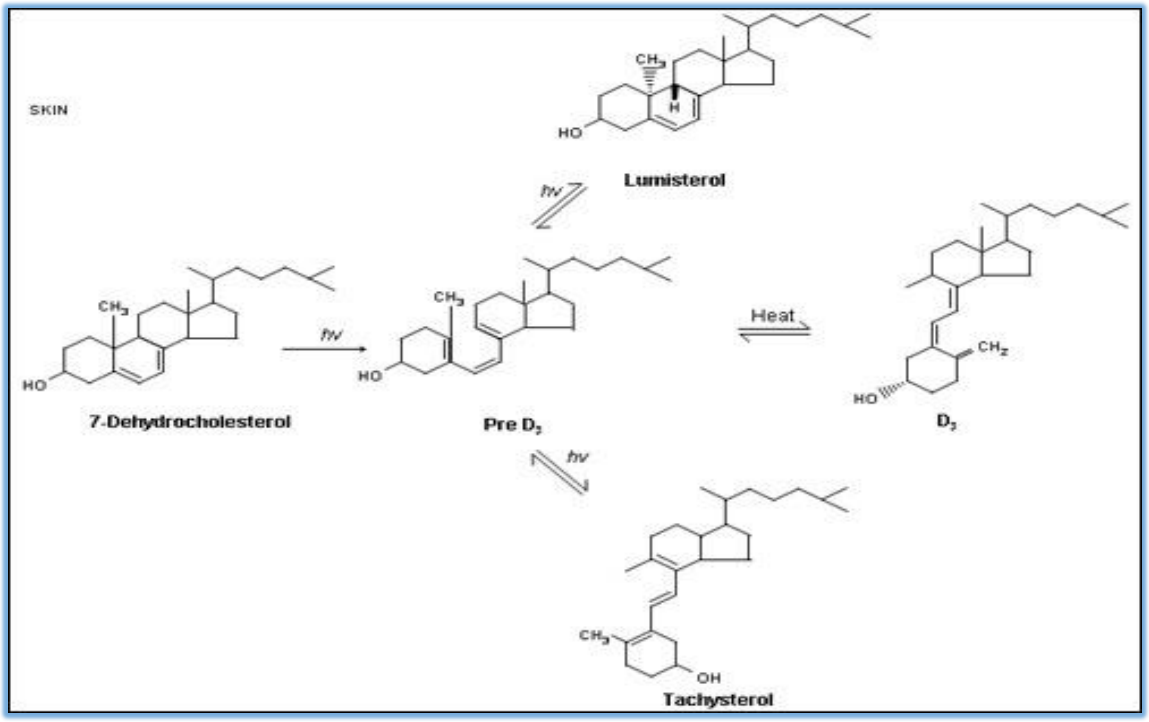


Figure (1.6) Activation of vitamin D

7-Dehydrocholesterol is converted by the skin to vitamin D3. When the B ring of the cholesterol structure is broken by the ultraviolet B component of sunlight, pre-D3 is produced. After then, pre-D3 is changed into D3 by heat-induced rearrangement. Further radiation exposure to pre-D3 can result in the reversible synthesis of lumisterol3 and tachysterol3, which, in the absence of light, can return to pre-D3 [104].

1.3.2.4.2. The major roles of Vitamin D3

1-Controlling the amount of phosphate and calcium in the body.

2-Encouraging the normal development and remodeling of bone.

3. Calcitriol also affects immunological, neuromuscular, and cell proliferation in addition to reducing inflammation.

4- The metabolism and equilibrium of calcium are significantly influenced by vitamin D. There is conflicting data about the additional health benefits of vitamin

D supplementation in people with low vitamin D levels. With the exception of shielding high-risk populations against osteomalacia and rickets [105].

Table (1.3) Vitamin D3 status

Vitamin D3 status	25(OH)D Concentration in blood	
Deficiency	**< 10ng/ml**	**< 25nmol/L**
Insufficiency	**10-30 ng/ml**	**25-75 nmol/L**
Sufficiency	**30-100 ng/ml**	**75-250nmol/L**
Toxicity	**> 100 ng/ml**	**> 250 nmol/L**

Conversion factor. ng/ml = 2.5 x nmol/L Normal serum concentrations [106].

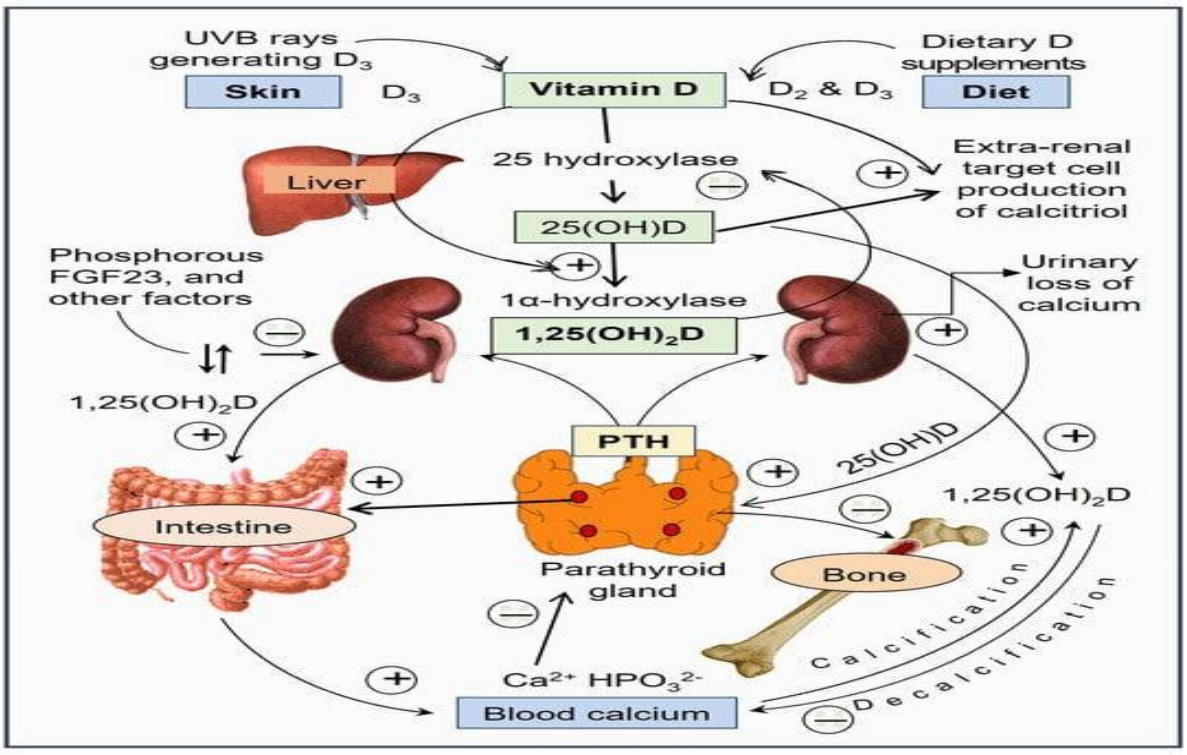


Figure (1.7) Schematic illustration of generation of vitamin D and its calcium/mineral regulatory functions of calcitriol in conjunction with PTH and fibroblast growth factor-23 (FGF-23). The **Figure** also depicts sites of activation of vitamin D—25-hydroxylation in the liver and 1α-hydroxylation in renal tubular cells and peripheral target cells. 1α-hydroxylation of 25(OH)D in proximal renal tubular cells generates the circulatory, hormonal form of calcitriol that controls calcium homeostasis [107].

1.3.2.4.3. Vitamin D3 and Osteoarthritis

As a prohormone, vitamin D aids in controlling the body's calcium levels for healthy bones and muscles. It possesses anti-inflammatory and immunity-modifying properties. It is crucial to understand the relationship between serum vitamin D levels and the severity of arthritis and how it affects treatment [108]. The increased phagocytic and antibacterial activity of macrophages caused by the active form of vitamin D may account for the immunomodulatory effect.

Preventing B-lymphocyte and T-cell differentiation and the subsequent generation of cytokines, as well as downregulating antigen-presenting cells (APCs). The inflammatory activity of vitamin D is explained by the reduction of prostaglandin by vitamin D, inhibition of p38 stress kinase signaling, creation of pro-inflammatory cytokines as a result, and inhibition of nuclear factor kappa B (NF-κB) signaling [109].

1.3.2.4.5. Vitamin D3 and diabetes mellitus

Low vitamin D3 has been linked to T2DM, with two primary explanations proposed. first, vitamin D3 stimulates pancreatic b cells to secrete insulin; thus, vitamin D3 deficiency is linked to insulin resistance. Secondly, low levels of vitamin D3 lead to inflammation and elevated markers of inflammation. Additionally, it is linked to the onset of metabolic syndrome. Furthermore, vitamin D3 genetic variation may result in poor glycemic control [110].

1.3.2.5. Alkaline phosphatase

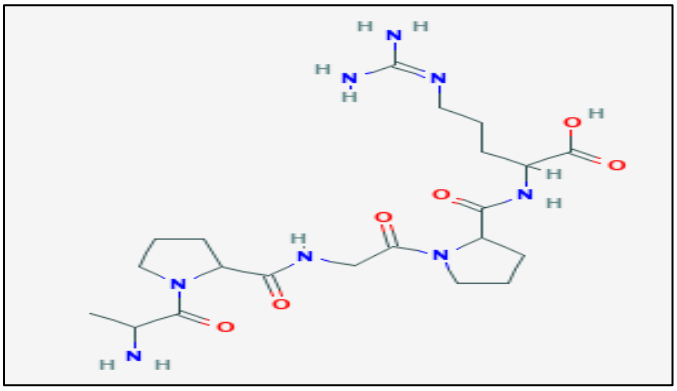


Figure (1.8) chemical structure of ALP [111].

Alkaline phosphatases (ALPs) (DL-alanyl-DL-prolyl-glycyl-DL-prolyl-DL-arginine) shown in figure (1.8) are a class of isoenzymes that are present on the outer layer of the cell membrane. They accelerate the hydrolysis of organic

phosphate esters in the extracellular space. Zinc and magnesium are required cofactors for this enzyme. ALPs are classified as true isozymes even if their physiochemical characteristics differ and their distribution across tissues varies, as they can catalyze the same process. ALP is present in the cytoplasm of liver cells as well as the canalicular membranes of hepatocytes [112]. An increasing number of organs, including the kidney, liver, ileal mucosa, placenta, and bone, have decreasing amounts of ALP. The liver and bone account for more than 80% of the ALP in serum, with the colon contributing quite slightly. Despite being found in many different bodily tissues [113]. For the majority of individuals with increased enzyme levels, the liver is the main source. Increased osteoblast activity, which is seen in bone abnormalities or typically during growth phases, is the next most likely contribution. Furthermore, the influx of placental ALP in the late third trimester considerably raises the levels in pregnant women [114]. ALP screening is helpful in identifying several conditions, such as bone disease and liver illness or injury. Research has demonstrated that ALP serves as a dependable indicator of cardiovascular events and death, including stroke in individuals with hypertension [115]. and spontaneous cerebral hemorrhage, [116] as well as being associated with inflammatory diseases such osteoarthritis of the knee [117]. ALP normal range in serum ranges from 20 to 140 IU/L which can vary from lab to lab [118].

1.3.2.5. 1. Diabetes Mellitus Type II and Alkaline phposphatase

The level of the hepatic alkaline phosphatase increases significantly in diabetics. Where revealed number of studies that ALP is correlated with glucose metabolism, insulin resistance, and metabolic syndrome due to its role as a hepatobiliary marker. In a recent study, insulin stimulated the proliferation and differentiation of osteoblasts, and resulted in an increase in bone-specific ALP levels in vitro [119]. Serum ALP activity was independently and positively

associated with severe knee osteoarthritis, implying that serum ALP level might be a valuable additional surrogate biomarker in the evaluation of severe knee osteoarthritis [120].

1.3.2.6. Phosphorus

The sixth most prevalent element in the human body is phosphorus (Pi), a vital nutrient. Phosphorus ions, also known as phosphates, are negatively charged and highly reactive when exposed to oxygen [121]. The vast majority (85%) of PI in the body exists as a component of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] in the extracellular matrix of bone and teeth and form ester bonds with other molecules like proteins and peptides. Phosphorus phosphorylates kinases and dephosphorylates phosphatases in order to phosphorylate a variety of macromolecules and form complexes with other ions. In contrast, intracellular phosphorus accounts for 14% of total body phosphorus [122]. In healthy adults**,** the range of phosphate content in serum is closely maintained between 2.5 and 4.5 mg/dL (0.80 and 1.45 mmol/L) [123].

1.3.2.7. Calcium

Calcium is the fifth most abundant element in the human body and is essential for life. It has a key role in many physiological processes including skeletal mineralization, muscle contraction, nerve impulse transmission, blood clotting, and hormone secretion. More than 99% of calcium in the body is stored in the skeleton as hydroxyapatite, [124] which provides skeletal strength and is a source of calcium for the multiple calcium-mediated functions as well as for the maintenance of serum calcium within the normal range (8–10 mg/dL). Less than 1% of calcium is located in the blood, soft tissues, and extracellular fluid. Serum calcium is either protein-bound (~40%), notably by albumin, bound as a complex

to small anions (for example, phosphate or citrate; ~9%) or in the free or ionized state (~51%) [125]. It is the ionized calcium that is available to enter cells and result in the activation of essential physiological processes. Calcium is only available to the body through dietary intake. In the elderly, there is inadequate intestinal absorption of calcium combined with an age-related hormonal decline, which results in adverse effects on bone health [126].

1.3.2.7. 1. Calcium and insulin secretion

β-cells are responsible for insulin production, which is synthesized as pre-proinsulin. In the maturation process, pre-proinsulin undergoes a conformational modification carried out with the help of several proteins in the endoplasmic reticulum (ER) to yield proinsulin. Afterward, proinsulin is translocated from the ER to the Golgi apparatus (GA), entering into immature secretory vesicles and being cleaved into C-peptide and insulin [127]. Once matured, insulin is stored in granules until insulin release is triggered. Insulin release is primarily triggered by a response to high glucose concentrations. It is worth noting that some other factors can also induce insulin release such as amino acids, fatty acids and hormones [128]. When circulating glucose levels increase, β-cells take in glucose mainly through the glucose transporter 2 (GLUT2), a solute carrier protein that also works as a glucose sensor for β-cells. Once glucose enters, glucose catabolism is activated, increasing the intracellular ATP/ADP ratio, which induces the closing of ATP-dependent potassium channels in the plasma membrane. This leads to membrane depolarization and opening of the voltage dependent Ca^{2+} channels, enabling Ca^{2+} to enter the cell. The rise in the intracellular Ca^{2+} concentration triggers the priming and fusion of the secretory insulin-containing granules to the plasma membrane, resulting in insulin exocytosis [129].

1.4. Calcium carbonate $CaCO_3$

Chemically known as $CaCO_3$, Calcium Carbonate is an inorganic salt that is resonance-stabilized and utilized therapeutically as a phosphate binder, antacid, food additive, and nutritional supplement. One of the most prevalent minerals in the earth's crust is $CaCO_3$, which is typically found in organic materials like egg and oyster shells, the exoskeletons of crustaceans, and dark leafy greens like kale and broccoli [130].

1.4.1. The medical importance of Calcium carbonate

1. Nutritional benefits.

a-It is used to treat low serum calcium conditions like osteoporosis, osteomalacia, hypothyroidism, hypoparathyroidism, pseudo hypoparathyroi- dism,George syndrome, kidney dysfunction, pancreatitis, rheumatoid arthritis, Fanconi syndrome, pregnancy, nursing mothers, postmenopausal women [131].

b-While taking certain medications such as anti-seizure medication (phenytoin and phenobarbital), calcitonin, corticosteroids, bisphosphonate alendronate, ibandronate, zoledronic acid, and risedronate), Rifampin (antibiotic), Chloroquine, Plicamycin and Citrate are used during apheresis (anticoagulant) [132].

2. Anti-acidic

In the states of stress gastritis, exocrine pancreatic insufficiency, bile acid-mediated diarrhea, non-ulcer dyspepsia, duodenal and gastric ulcers, NSAID upper gastrointestinal mucosal damage, biliary reflux, and bile acid-mediated diarrhea, calcium carbonate is recommended as an antacid [133]. Similar to

magnesium and aluminum salts, calcium carbonate has been shown to improve gastrointestinal motility and be used to treat constipation [134].

3. Phosphate binder for chronic kidney disease

In addition, calcium carbonate is approved for use as a phosphate binder, a treatment for hyperphosphatemia associated with chronic kidney disease, a means of treating overdose by producing urinary alkalization, and a preventive measure in pregnant women before delivery to avoid aspiration pneumonitis [135].

4. Cancer treatment

According to recent studies, the use of calcium supplements to treat colorectal adenomas and rare-earth-doped $CaCO_3$ with cerium to destroy tumor cells via X-ray-induced photodynamic therapy may also be used as $CaCO_3$ indications [136].

1.5. Arabian gum

Arabian gum is an item obtained from the dried discharges of sticky stems and branches of *Acacia senegal*. It consists of a water-soluble dietary fiber digestible only in the intestines. AG is a sticky substance that is extracted from the umbrella-shaped branches of Acacia seyal and Acacia Senegal [137]. AG is a compound of arabinogalactan and protein. The magnesium, calcium, and potassium salts of Arabic acid make up this complex. 1-3-linked β-D-galactopyranosyl units make up the Arabic acid structure, and branches with two to five β-D-galactopyranosyl residues coupled by 1,3-ether linkages and 1,6-linkages to the fundamental β-D-galactopyranosyl chain complete the structure [138].

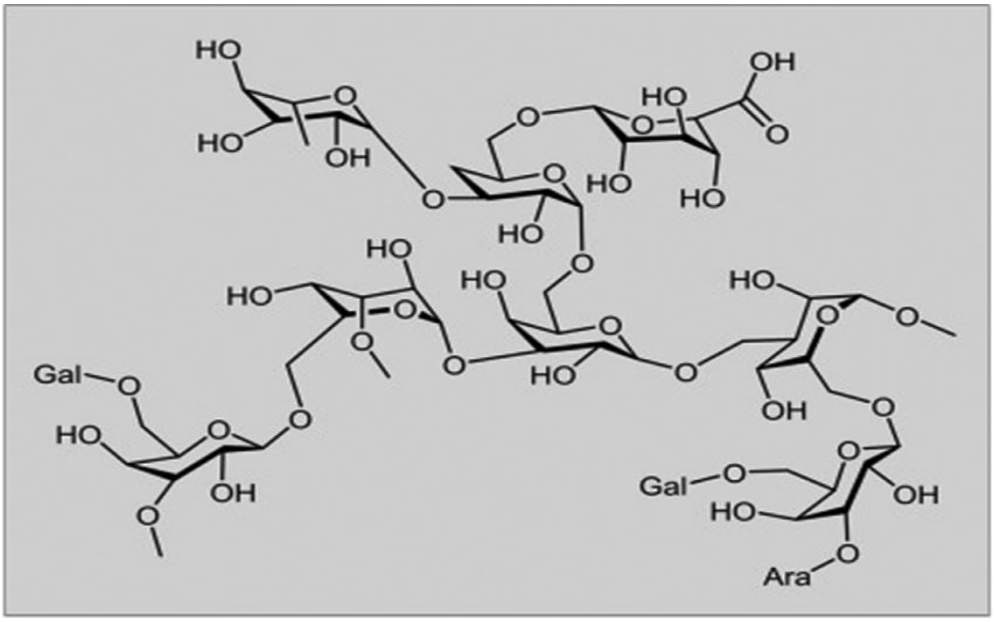


Figure (1. 9) AG general chemical structure [139].

1.5.1. Health benefits of Arabian gum

Arabian gum is used to treat a variety of illnesses, including rheumatoid arthritis, gastrointestinal issues, sickle cell disease, periodontitis, and metabolic problems [140]. Improved absorption of calcium from the gastrointestinal tract therefore its intake is associated with many useful health effects These health benefits include.

- Anti-obesity (AG lowers the body mass index and body fat percentage)
- Lipid lowering potential (AG decreases total cholesterol, LDL, and triglyceride)
- As antioxidant
- Kidney and liver support

- Immune function *via* modulating the release of some inflammatory mediators
- Prebiotics improve the intestinal barrier function, prevent colon cancer, and alleviating symptoms of irritable bowel diseases [141,142].

1.5.2. Arabian Gum as Anti-diabetic

Arabian Gum has a preventive role in preventing T2DM, as eating AG that contains a lot of fiber affects fat and glucose metabolism. A study revealed that eating 30 grams of AG daily after six weeks causes a decrease in the BMI and fat percentage. It also gives A feeling of fullness and reduces calories, thus preventing obesity, which is the main cause of T2DM [143]. In prediabetic and diabetic small experimental rats, food supplementation with GA lowers blood sugar levels and HbA1C through its hypolipidemic, anti-inflammatory, and antioxidant enzyme-boosting effects. Furthermore, GA proposed a hypoglycemic impact by stimulating insulin secretion. Furthermore, randomized clinical trials showed that supplementing with GA significantly improved serum glucose levels [144]. The effect of AG does not stop at reducing glucose levels and HbA1C only, but also reduces the complications of DM. Where it protects the diabetic liver *via* enhancement of antioxidant enzymes' overexpression. Hepatic SOD and glutathione peroxidase (GPx) were considerably overexpressed and malondialdehyde MDA level was remarkably reduced in the hepatic tissue of diabetic animals [145,146].

1.6. Nanotechnology

A relatively new and developing branch of technology, nanotechnology works at the nanoscale, or less than 100 nm. Since Richard Feynman's ground breaking presentation on "There's Plenty at the Bottom" in 1959, it has been an area of study since the turn of the century [147]. Nanotechnology is a synthesis of science and technology that integrates the fields of biology, chemistry, engineering, and physics. Manipulation of matter with a minimum dimension between one and one hundred. Nanometres is its definition [148]. Owing to the special properties of nanomaterials, nanotechnology has a wealth of intriguing potential that has

sparked the creation of a wide range of applications meant to improve human well-being [149].

1.6.1. Nanomaterials

Materials with at least one dimension between 1 and 100 nm are referred to as nanomaterials. These materials' small size results in some unique functional qualities that make them appropriate for use as food additives, treatments, or packaging materials [150,151]. Enhanced optical clarity (when dimensions <50 nm), enhanced resistance to creaming or sedimentation, enhanced stability to aggregation, novel textural features, elevated bioavailability, and tailored release qualities are some of these novel functional attributes. Certain nanomaterials are produced by processing techniques (like Nanoemulsions), while others are found naturally (like casein micelles) or are extracted from nature (like Nanocellulose or Nanochitin) [152]. Depending on their forms, nanomaterials can be categorized as 0-, 1-, 2-, or 3-dimensional objects. For example, bulk nanocomposites are 3-D materials, whereas tiny particles are 0-D, fibers are 1-D, and sheets are 2-D. Nanoparticles (NPs) are the most prevalent form of Nanomaterial utilized in the food business [153]. Based on the materials they are made of; nanoparticles can be classified into two primary categories. inorganic and organic. Nanoparticles can be broadly categorized into two groups. inorganic and organic, depending on the materials they are formed of. The primary constituents of organic nanoparticles are proteins (zeinNPs and casein micelles), carbohydrates (nanochitin and nanocellulose nanogels), phospholipids, or lipids (liposomes). Inorganic nanoparticles are typically composed of metals or metal salts, such as copper, gold, silicon dioxide, titanium dioxide, or zinc oxide [154].

1.6.2. Nanomedicine

Nanomedicine is a specialty branch of medicine that applies the concepts of nanotechnology to treat and/or prevent a broad range of diseases [155]. Since many organic mechanisms found inside the human body are similar in length to a nanometer, nanoparticles and nanomaterials may be able to cross physical barriers to reach new transport sites and interact at different levels with small proteins or DNA in blood or within organs, tissues, or cells [156].

1.6.3. Medical applications of Nanotechnology

In the medical field, nanotechnology is widely used as Nanomedicine. Certain nanoparticles may find use in tissue engineering, pharmaceuticals, targeted medications, biomedical implants, new diagnostic tools, imaging, and techniques. Today, high-toxicity medicines, such as chemotherapy drugs for cancer, can be provided with greater safety because of nanotechnology. Furthermore, wearable technology can identify real-world infections, cancer cell states, and significant changes in vital signs [157].

1.6.4. Nanotechnology in drug delivery

It aims to use nanotechnology in this field to develop the therapeutic effect of molecules, as drug delivery methods are of great importance in medicine, which requires precise communication during their processes throughout the body and are targeted even greater if these molecules and their interactions are controlled with other cells, in addition to controlling the formation of these molecules and their surface chemistry [158]. Nano-drug systems primarily focus on improving the bioavailability of specific tissue delivery, extending injectable medicines' half-life, and orally giving medicinal products. Nano drugs are administered at lower levels, with remarkable improvements in their pharmacological effects and a

reduced danger to health and adverse effects [159]. NPs, including polymeric, magnetic, liposomal, carbon nanotube, quantum dots, dendrimers, metallic, and polymeric NPs, have revolutionized drug delivery and the medical services industry as a whole. Through the use of nanotechnology, some important knowledge on the molecular causes of diseases has been found. Scientists are able to resolve a number of troubling issues, including safety, bioethical concerns, toxicity threats, and physiological and pharmacological challenges [160].

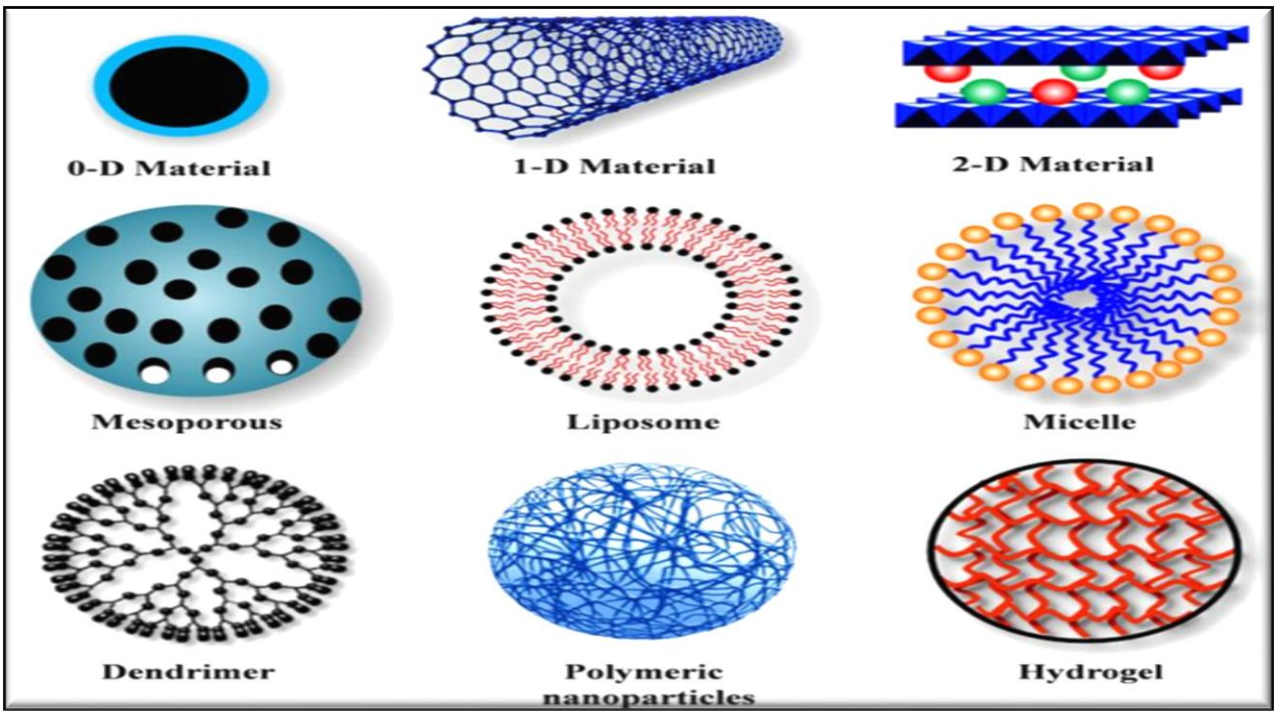


Figure (1.10) Shapes of nanoparticles [161].

1.6.5. Nanotechnology in diagnosing Diabetes Mellitus

Sensing technologies for precise medical information, such as diabetes detection, can be made possible by Nanotechnology. Patients with DM must independently monitor their blood sugar levels regularly to reduce the chance that it will drop to dangerously low levels. It can occasionally be uncomfortable and difficult to

maintain this pattern, especially for the young and the elderly [162]. The development of wearable and implanted sensor devices that can provide continuous and reliable medical information is made possible by nanotechnology [163]. The two most popular methods for utilizing nanotechnology in diabetes diagnosis are implantable sensors and microphysiometers. Multiwalled carbon nanotubes, which are electrically conductive, are used to construct the microphysiometer. Because the current at the electrode and the concentration of insulin in the chamber are intimately correlated, the nanotubes are fully functional at pH levels that are typical of living cells [164]. The conventional detection methods typically measure insulin production at intervals, by collecting and measuring small samples, periodically. The microphysiometer can detect insulin levels continuously and indirectly, by estimating the transfer of electrons which are produced when insulin molecules get oxidize, by the glucose. Fundamentally, when the cells produce more insulin molecules, the current which is generated inside the sensor, increases and vice versa, allowing real time monitoring insulin concentrations in. Nanostructured implantable sensors use polyethylene glycol beads, coated with fluorescent molecules in order to monitor diabetes blood sugar levels. The beads are injected under the skin, staying in the interstitial fluid [165]. If the glucose in the interstitial fluid falls to dangerous levels, glucose displaces the fluorescent molecules and creates a glow, which is seen on a tattoo placed on the arm. This method is considered as very effective. However, sensor microchip is another alternative, which is being developed to continuously monitor crucial body parameters such as pulse, blood glucose and temperature. In these applications, the microchip is implanted under the skin, transmitting a signal that could be monitored continuously. Recently a microchip based test to distinguish between the two main forms of diabetes mellitus, allowing differential diagnosis

has been developed [166] This cheap, portable, microchip-based test can diagnose type-1 diabetes. Traditional methods for detecting diabetes are expensive, quite slow and they are available only in well-equipped health-care centers. The proposed test applies fluorescence to detect the antibodies. The glass plates which are formed the base of each microchip are coated with gold nanoparticle-sized, allowing the amplification of the fluorescent signal in order to obtain reliable antibody detection. The gold NPs ensure the creation of nanogaps, supporting the enhanced electric field. This technology is expected to improve patient care, assisting in a better understanding of the disease [167].

1.6.5.1. The treatment of Diabetes mellitus by nanomaterials

Currently being researched are several kinds of nanomaterials for the administration of insulin in the treatment of diabetes. The most promising of the ones suggested include ceramic nanoparticles, liposomes, dendrimers, polymeric micelles, and polymeric biodegradable nanoparticles. Each of these groups of nanomaterials accrues benefits contingent upon the mode of administration [168].

1.6.5.1.1. Oral administration

Insulin is loaded onto carrier materials to create oral insulin delivery nanosystems. To preserve medication stability and increase bioavailability, the ideal materials should have the following properties. pH responsiveness, bioadhesion, biocompatibility, biodegradability, modifiability, and ease of processing. Oral delivery nanosystems have traditionally been built using a range of polymers. Depending on the source, they might be categorized as synthetic or natural polymers. Common natural polymer carrier materials are starch, bile acid, hyaluronic acid, proteins, chitosan, and sodium alginate [169,170].

1.6.5.1.2. Inhalation

It is common for diabetic patients to need insulin therapy, which is administered parenterally to regulate blood glucose levels. In addition to being painful, the patients find the 2-3 daily insulin injections to be uncomfortable and annoying. This resulted in the development of other insulin delivery methods, including transdermal, oral, and pulmonary. The hydrophilic nature of the medication, low skin permeability, and potential for skin irritation and rashes owing to patches are the drawbacks of the transdermal method [171]. Among the main drawbacks of oral delivery are peptide breakdown, which results in reduced bioavailability at the target site, and decreased intestinal epithelial permeability [172]. Because of the lungs' high permeability and huge surface area for absorption, the pulmonary route seems more promising than the other two and may be suitable for both local and systemic delivery of medicines [173]. The medications can be administered by intratracheal or intranasal administration via the pulmonary route as aerosol or inhalation particles. This pathway offers rapid blood flow and defense against deterioration, deposition, and penetration in alveoli [174]. Since ancient times, solid lipids have been utilized in the pharmaceutical industry to prepare a variety of compositions. In order to combine the characteristics of nanoparticles and biodegradable lipid carriers, the phrase solid lipid nanoparticle SLN was proposed in the 1990s. It is well known that the SLN are stable carriers and that their matrix permits controlled drug release. Large surface area, reduced cellular toxicity, enhanced absorption, and excellent solubility and bioavailability are some of the benefits that SLNs offer. Lipid components in the SLN make it simple to create hydrophilic and lipophilic therapies [175]. Protein encapsulation into solid lipid nanoparticles has been shown to improve bioavailability and distribution at the target site. In order to improve therapeutic benefits and reduce the need for

repeated dosages, the SLN can be designed for sustained and regulated release [176].

1.6.5.1.3. Gene therapy

Gene therapy is a strategy for mitigating the effects of a faulty gene [177]. Gene therapy include gene editing and regulation in addition to gene addition. Gene therapy is regarded as a novel method of treating illnesses. Instead of traditional medications, oligonucleotides like DNA, RNA, or small interfering RNA (siRNA) give their therapeutic action. Such therapeutic oligonucleotides are anticipated to enter target cells after injection and either boost or suppress malfunctioning gene expression. While siRNA silences the mRNA of faulty genes, DNA or RNA restores target protein transduction that has been compromised by defective genes. In diabetes, gene therapy has been widely studied for restoring insulin production in pancreatic cells or transfecting insulin genes into other cells, such as the liver, adipocytes, and muscles [178,179] with the aim of inducing production of insulin. In gene therapy for insulin production, vectors can be administered via local injection into the pancreas, muscle, or adipose tissue, or injected into the portal vein to reach the liver. Intravenous injection is also possible. In this case, a different biodistribution of nanoparticles compared to viral vectors can make them more active. Indeed, circulating NPs have been demonstrated to selectively accumulate in body compartments such as tumour and inflamed tissues due to extravasation through pathological discontinuities in their capillaries and in the liver, spleen, and bone marrow [180]. Accumulation in the liver is particularly advantageous for the transfection of liver cells to induce insulin production. Viral vectors, in contrast, do not provide selective accumulations due to their ability to cross blood vessels and distribute throughout the body. This uncontrolled distribution can cause off-target gene delivery with unwanted side effects. In

addition to the interest in gene therapy for insulin production, numerous studies have been conducted on the genes responsible for the evolution of T1DM and T2DM-related genes as possible treatment targets. Approximately 75 independent genetic loci for T2DM have been identified through genetic studies, and several new therapeutic targets have been determined [181]. Genetic loci could significantly impact drug responses and disease incidence and development. It has been found that gene therapy could inhibit the production of nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3). The inhibition of NLRP3 mitigates inflammation, protecting against pancreatic-cell apoptosis and preventing the development of T2DM in mice [182]. The administration of naked oligonucleotides would be highly ineffective in gene therapy, as they are rapidly eliminated by endonucleases present in blood and bodily fluids, are phagocytosed by circulating macrophages, and are unable to enter the target cells due to electrostatic repulsion with the negatively charged biologic membranes. Viral vectors such as adenovirus, adeno-associated virus, and lentivirus, or non-viral vectors such as liposomes or nanocapsules, are used to protect the therapeutic oligonucleotides from the environmental conditions and allow their penetration into the target cells [183].

1.6.5.1. 4. Nanoparticles for Cell Therapy

1.6.5.1. 4.1. Pancreatic cells

Transplanting live pancreatic cells into the diabetic patient's pancreas is known as cell treatment for diabetes. The Food and Drug Administration

approved Lantidra (Donislecel) in June 2023. This marked the first approval of a pancreatic islet cell treatment utilizing pancreatic cells from deceased donors to treat type 1 diabetes. If percutaneous or transvenous transhepatic access are not

practical, laparoscopic or open surgery can be performed to inject lanternida into the hepatic portal vein [184].

1.6.5.1. 4.2. Stem Cells

Using pluripotent or multipotent stem cells to produce surrogate β-cells for transplantation is less cumbersome. To this aim, various types of stem cells can be exploited, including induced pluripotent stem cells (iPSCs), embryonic stem cells (ESCs), and adult stem cells [185]. Technological advancement has facilitated the development of stem cells using different kinds of tissue sources, such as adipose tissue, skin, bone marrow, umbilical cord blood, periosteum, and dental pulp. In searching for promising stem cells, the pancreas is the first organ of choice. Studies with animal models have indicated that a small number of pancreatic tissues, when made available, could restore the optimum pancreatic β-cell mass [186].

1.6.5.1. 5.Artificial pancreas

For diabetic patients, the creation of an artificial pancreas system consisting of a glucose meter, continuous glucose monitor, and insulin infusion pump for calibrating the monitor may be the long-term solution. Using a tiny silicon box filled with animal-derived pancreatic beta cells is an additional strategy. This strategy has been utilized to shield transplanted cells from the immune system and enable enough oxygen, glucose, insulin, and other nutrients to diffuse throughout the cells. The "smart" insulin patch that is currently being developed has made it possible to distribute insulin experimentally. "Smart" because it adjusts insulin release based on the demands of the body. It has over 100 microneedles that are loaded with glucose-sensing enzyme and insulin [187].

1.7. Nanotechnology and Osteoarthritis

1.7.1. Diagnostic of Osteoarthritis

Nanoparticles (NPs) are the most innovative biomaterials for potential diagnosis and management of OA. Nanomaterials such as liposomes, micelles, carbon nanoallotropes, and quantum dots are described as particles with sizes in the range of 1–100 nm. One of the important benefits of nanomedicine is the capability to design special NPs for detection of early osteoarthritic changes in cartilage tissue, e.g, using a liposome containing an antibody to type II collagen, which when combined with a dye emitting near-infrared light enables detection with in vivo optical imaging techniques [188].

1.7.2. Treatment of Osteoarthritis

Therapeutic compounds like medications and growth factors can be precisely engineered at the nanoscale using nanotechnology, resulting in the controlled release and encapsulation of these molecules. This characteristic lowers systemic adverse effects and improves therapeutic efficacy by facilitating prolonged and focused medication administration to particular body locations. With targeted drug administration, OA symptoms can be reduced and tissue healing can be encouraged by directly delivering anti-inflammatory or disease-modifying medications to the impacted joint tissues. Additionally, biomaterials enhanced by nanotechnology can be made to resemble the original tissue milieu, improving their biocompatibility and lowering the possibility of negative reactions or immunological responses [189]. This quality is essential for the long-term functionality and successful integration of biomaterials in biological applications. Furthermore, through inducing biological reactions and encouraging tissue growth, nanomaterials can aid in tissue regeneration [190]. Biomaterials enhanced

by nanotechnology can help regenerate and repair cartilage in the context of OA, thereby delaying the disease's progression and enhancing joint function [191]. Nanotechnology further enables the customization of biomaterials with a wide range of physical, chemical, and biological properties. Because of their adaptability, multifunctional biomaterials—like those used in medication administration, imaging, and tissue regeneration may be created that can carry out several functions at once. All of these benefits add up to their promise as novel approaches to solving a range of biomedical problems and enhancing patient outcomes [192].

1.7.2.1. Nano lubricant

Nano-lubricant is the term for the lubricant suspension that has been supplemented with NPs. To improve the lubricating effect of the lubricant, NPs can be stabilized in the lubricant [193]. Graphite nanoplatelets (GNPs) can enhance the lubricant's lubricating capabilities. It was discovered that there is a critical GNPs concentration for lubrication promotion, and that concentration is the point at which the promotion peaks. The fact that there is a minimum contact area at the peak between the items further suggests that adding NPs will help fill in the rough areas of the contact surface [194]. 1,3,9 malondialdehyde semi-dendritic hyperbranched polyglycerol (Mega HPG) is a novel lubricant that has been proposed in recent years. This is a single polymer particle at the nanoscale. The benefits of Mega HPG include low intrinsic viscosity, hydration, specific viscosity, compactness, and high water solubility—all of which can lower the friction coefficient between soft and hard surfaces. In a size-dependent manner, Mega HPG reduces the coefficient of friction between hard and soft natural surfaces by acting as interposed
single molecule ball bearings [195].

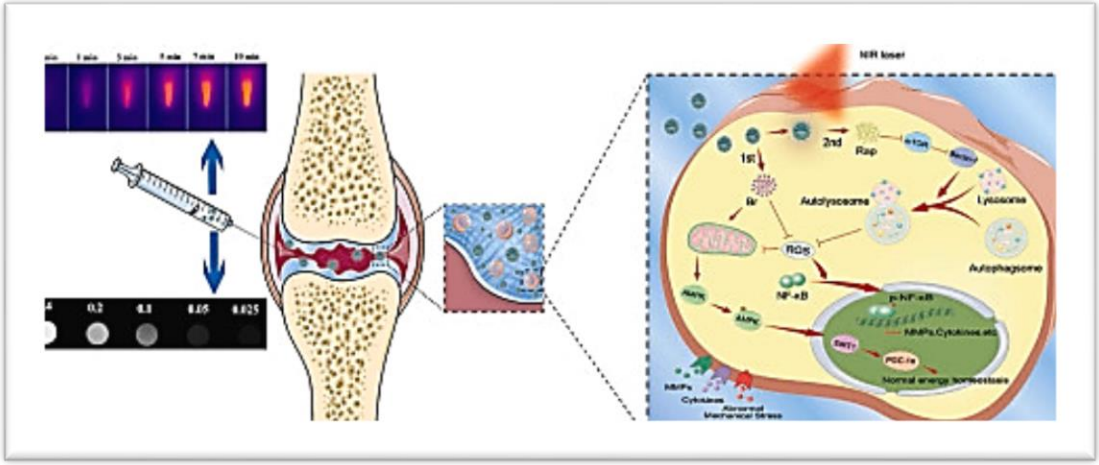


Figure (1.11) Lubricant drug–loaded NPs [196]

1.7.2.2. Osteocartilage regeneration scaffold

The direct creation of nanofiber scaffolds and the adding of NPs to traditional scaffolds to create nanoscale characteristics are two examples of how nanotechnology is used in scaffolds. Bone is composed of calcified connective tissue in the form of nanofibers. Nano scaffolds have the potential to aid in osteogenesis induction, cartilage repair, and support. Although bone marrow mesenchymal stem cells are the main focus of cartilage repair research at the moment, these cells are unable to produce effective repair results on their own. When it comes to applying tailored therapy, the combination of scaffolds and bone marrow mesenchymal stem cells especially those made using 3D printing may be a more viable approach [197].

1.7.2. 3.Gene delivery system

Nucleic acids can be transferred to chondrocytes via exosomes made by synovial mesenchymal stem cells, which will encourage chondrocyte proliferation. Sleep-related circular RNA (circRNA) cartilage healing was first screened out using

melatonin treatment and small extracellular vesicles (sEVs) carrying sleep-related circRNA (circRNA3503) were created because sleep has been demonstrated to be favorable to cartilage repair. The carrier of sEVs was a poly (D, L-lactide)-b-polyethylene glycol-b-poly (D, L-lactide) triblock copolymer gel. This technique is a useful treatment to stop the advancement of OA since in vitro investigations have demonstrated that it can encourage chondrocyte regeneration and lessen the progressive loss of chondrocytes [198].

1.7.2. 4. Nanoparticle polymers

Because of their extracellular matrix (ECM) resembling that of human tissue, their ability to alter cell adhesion to suppress an immune response, and their rapid breakdown by enzymatic or chemical processes, natural polymers are biocompatible. However, natural polymers lack mechanical strength, which makes using them in the medical industry challenging [199].

The sole naturally occurring basic polysaccharide is chitosan. Due to its high amino group content, chitosan can interact with the anionic system to produce both chemical and physical changes. Chitosan and glycosaminoglycan share structural similarities that can aid in the development of cartilage. Kartogenin (KGN) is a tiny chemical that is injected into the joint cavity along with chitosan NPs (KGN conjugated chitosan) to promote the development of human bone marrow mesenchymal stem cells into chondrocytes [200].

1.7.2. 5. NPs Micelles and liposomes

Amphiphilic polymers that self-assemble in aqueous solutions and have diameters ranging from 20 to 200 nm make up the micelles. Hydrophilic drugs can be utilized to repair the surface, while hydrophobic drugs are wrapped around the

micelle's core. Micelles exhibit preferential uptake when certain ligands such as polypeptides, antibodies, or other bind to them. Polyethylene glycol is the best to protect the micelles from being phagocytosed. For the treatment of OA, micellar drug delivery methods are frequently used.

Typical intra-particularly injectable lubricating drug-loaded nanoparticles for the treatment of osteoarthritis are constructed. Among these dual-purpose nanoparticles are: -

- Polymer brush-grafted MSNs that contain medication encapsulation.
- Drug-encapsulated polymer-based Nanospheres that self-lubricate.
- Drug-loaded liposomes [201].

1.8. Aims of the study

The present study aims to: -

1-Study the differences of fasting blood glucose, hemoglobin A1C, high sensitivity C-reactive protein, serum calcium, vitamin D3, parathyroid hormone, serum albumin and alkaline phosphatase in patient of T2DM with OA compared to control group in Iraqi women.

2-Study effects of the $CaCO_3$@AGNPs on level of each of blood glucose, calcium, alkaline phosphatase, albumin, parathyroid hormone and phosphorus in patients group.

CHAPTER TWO

MATERIALS & METHODS

2.Methodology

2.1. Study Design

The present work is a case –control study carried out on T2DM and OA patients in Rheumatology Unit in AL-IMAM Al- Hassan Hospital in holy Karbala city (50 women aged 40-70 years) and 40 women apparently healthy as a control group to study the effect of $CaCO_3$ NPs and $CaCO_3$@AGNPs on the serum of the patients group. The sample collection continued from mid-June 2023 to February 2024. During period of data collection, patients interviewed by questionnaire from those who visited the Rheumatology Unit. The questionnaire included number, age, weight, length, duration of DM, treatment of DM, treatment of OA, and additive to other diseases and other treatments.

Figure (2.1) Study design of the research work

Total samples no (90)

(50) patients **(40) Controls**

Measuring parameters: blood glucose, HbA1C, hsCRP, ALP, Alb, Ca^{+2}, PTH, VIT D3 and Pi

Measuring parameters blood glucose, ALP, Alb, Ca^{+2}, PTH, and Pi after 1-adding $CaCO_3$NPs 2-adding $CaCO_3$@AGNPs

2.2. Materials and instruments

2.2.1. Materials

The materials and their suppliers used in the study are listed in table 2.1 below

No	Materials	Supplier
1	Hemoglobin A1C kit	Boditech med ink, Korea
2	Blood glucose kit	GIESSE DIAGNOSTIC, Italy
3	PTH Kit	Abbott GmbH&Co.KG, Germany
4	Alkaline phosphatase	GIESSE DIAGNOSTIC, Italy
5	Serum calcium	GIESSE DIAGNOSTIC, Italy
6	Phosphorus	GIESSE DIAGNOSTIC, Italy
7	Serum albumin	GIESSE DIAGNOSTIC, Italy
8	Vitamin D3	Boditech med ink, Korea
9	HsCRP	SENTINEL CH.SPA,Italy
10	Calcium chloride	Sigma-Aldrich , Germany
11	Sodium carbonate	Sigma-Aldrich , Germany
12	Triton 100x	Sigma-Aldrich , Germany

2.2.2. Instruments

The instrument used in the study are

No	Instrument	Company supplied
1	UV-Visible spectrophotometer, Double beam, and single beam.	Shimadzu, Japan
2	Field-Emission Scanning Electron Microscope (FE-SEM)	TESCAN, Japan

3	Transmission Electron Microscope (TEM)	Leo, Germany
4	X-Ray Diffraction (XRD)	Bruker, Germany
5	Deep freezer	Kryolab, Italy
6	Micropipette variable volumes	HUMAN Humapette ,Germany
7	ELISA system (washer, printer, and reader)	Bio Tek, United States
8	Plane tubes	Mheco, China
9	Eppendorf tubes	Mheco, China
10	Gilson Tips, 1000µl (blue)	Mheco, China
11	Gilson Micro-tips, 100µl	Mheco, China
12	Gel tube	Mheco, China
13	Eppendorf tubes centrifuge	Hettich, Germany
14	Ultra-sonic path	Lab Tech, Korea
15	Hot- plate stirrer	Lab Tech, Korea
16	Automatic chemistry analyzer	Geno TEK, Germany
17	Oven	LEGEND, Germany
18	Heparinized tube	Mheco,China
19	Teflon-lined stainless steel autoclave	ZZKD, China

Patients with T2DM and OA and healthy people were studied. The protocol of study was approved by the ethical research committee, College of Education, University of Kerbela and Romatoidology clinic in AL-IMAM Al- Hassan Hospital in holy Karbala city. The duration of the study continued from mid- to February 2024.

1.The patients group included 50 women aged (40-70) suffer from diabetes mellitus type two and osteoarthritis

2. The control group included 40 women aged (40-70) apparently healthy

2.3.1. Methods Collection and Storage of Samples

Disposable syringes and needles were used for blood collection. Blood samples 5ML were obtained from patients and control groups .1ML of the blood was placed in a heparinized tube to conduct hemoglobin A1C. the rest of the samples were allowed to clot and then placed in the centrifuged at 5000xg for 10 minutes. Sera were separated and divided into fractions in Eppendorf tubes and frozen at -20℃ in deep freeze until use .

2.4. Routine tests to diagnose Diabetes Mellitus and Osteoarthritis

2.4.1. Blood Glucose

Principle

The fasting serum glucose was measured using the colorimetric method for quantitative in vitro diagnostic measurement using the glucose kit.

The glucose oxidase (GOD) oxidizes glucose to gluconic acid and forms hydrogen peroxide which, in the presence of peroxidase (POD), reacts with 4-AAP and phenol and produces a colored complex, whose color intensity is directly proportional to glucose concentration in the serum.

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

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

Reagents

1.Reagent A consists of buffer 100mmol/L, glucose oxidase (GOD)10000 U/L, Peroxidase (POD) 2000 U/L, 4 AAP(1mmol/L) and phenol (10 m mol/L)

2-Standard. - Glucose (100mg/dl)

Reagents are ready to be used

Procedure

Pipette	Blank	Sample	Standard
Reagent(A)	1000μL	1000μL	1000μL
Water	10μL		
Sample		10μL	
Standard			10μL

The reactants were mixed and incubated for 10 min at 37 °C then the absorbance were read of sample (Ax) and (As) against blank at 510 nm and light path 1 cm.

Calculation

Blood glucose = Ax÷As ×100mg/dL

Normal value (70-105 mg/dL)

Conversion Factor. expected values Serum/plasma.

Mmol/L×18=mg/dL [202]

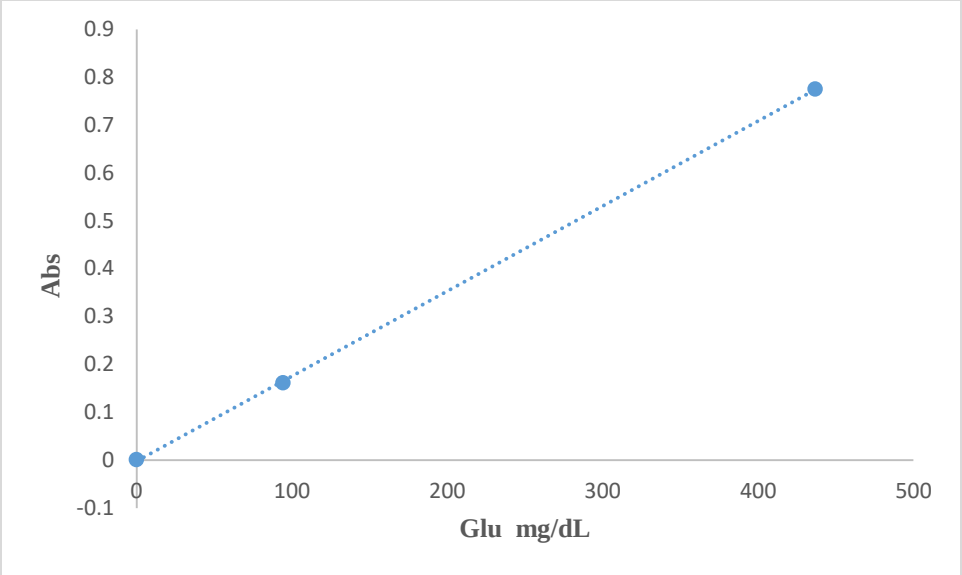


Figure (2.2) Calibration curve for glucose

2.4.2. Hemoglobin A1C (HbA1C)

The test determines the quantitative level of HbA1C in human whole blood using the sandwich immunoassay technique, also known as fluorescence immunoassay. It helps people with diabetes mellitus control and keep an eye on their long-term glycemic condition, only for use as an in vitro diagnostic [203].

The principle

Antibody in buffer binds to antigen in sample, forming antigen-antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized-antibody on test strip. The more antigen in sample forms the more antigen-antibody complex and leads to stronger intensity of fluorescence signal on detector antibody. Instrument for I-Chroma tests displays the content of glycated hemoglobin in terms of percent of the total hemoglobin in blood [204].

The Components

AFIAS HbA1c Neo consists of sealed aluminum pouch contains two cartridges and cartridge packaged in an aluminum pouch has four components including: -

1. The cartridge part contains: -

a. The membrane called a test strip which has streptavidin at the test line
b. Chicken IgY at the control line.

2. The detector part has a granule containing: -

a. Anti-Hemoglobin A0-fluorescence conjugate
b. Biotin- anti-HbA1c antibody conjugate
c. Anti-chicken IgY-fluorescence conjugate
d. Sodium azide as a preservative in phosphate buffered saline (PBS)

3. A hemolysis buffer part contains: -

a. Tween 20 as a detergent.
b. Sodium azide as a preservative in PBS.

4. Diluent part contains sodium azide as a preservative in PBS.

Procedure

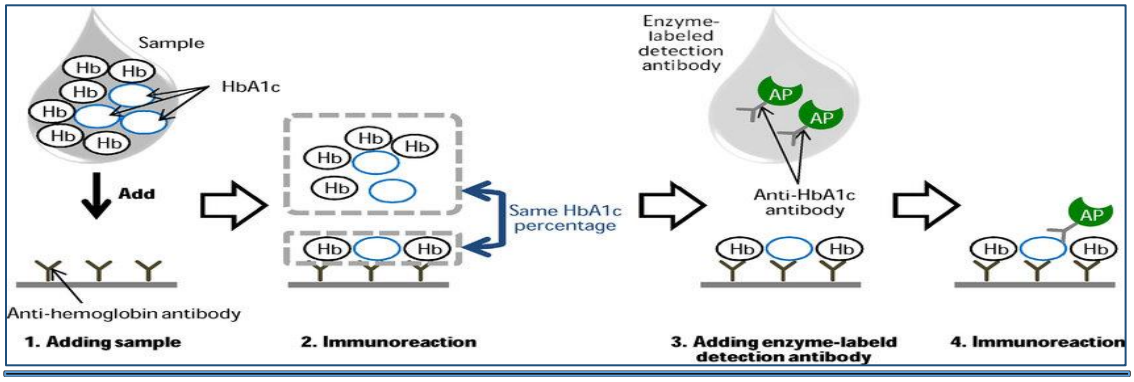


Figure (2.3) Scheme illustrates the procedure for direct detection of ratio of HbA1c to total hemoglobin [205].

1.It has been withdrawn 100 μL of heamolysis buffer and transferred into detection buffer tube.

2.It has been withdrawn 5 μL of tube blood using 5 μL capillary tube and put the capillary tube into the detection buffer tube.

3. Lid of the detection buffer tube has been closed and mixed the sample thoroughly by shaking it about 15 times.

4. Half of the cartridge has been coming out of the device.

5.75 μL of the sample mixture was Pipetted out and loaded into a sample well in the test cartridge.

6. The sample mixture has been waited until flow appeared in the windows. (about 10 seconds).

7. The cartridge into i-Chamber slot was inserted.

8. The cartridge in i-Chamber was left for (12 min) to scan the sample-loaded cartridge into the cartridge holder of the instrument for I-Chroma tests.

9.It has been ensured proper orientation of the cartridge before pushing it inside the cartridge holder.

10.An arrow has been marked on the cartridge especially for this purpose.

11.It has been pressed the ‘Select’ button on the instrument for ichroma tests to start the scanning process.

12. The Instrument for ichroma tests was started scanning the sample loaded cartridge.

13. The test result on the display screen of the instrument for ichroma tests was read.

Instrument for ichroma tests calculates the test result automatically.

The reference range: 4.5-6.5 % [206].

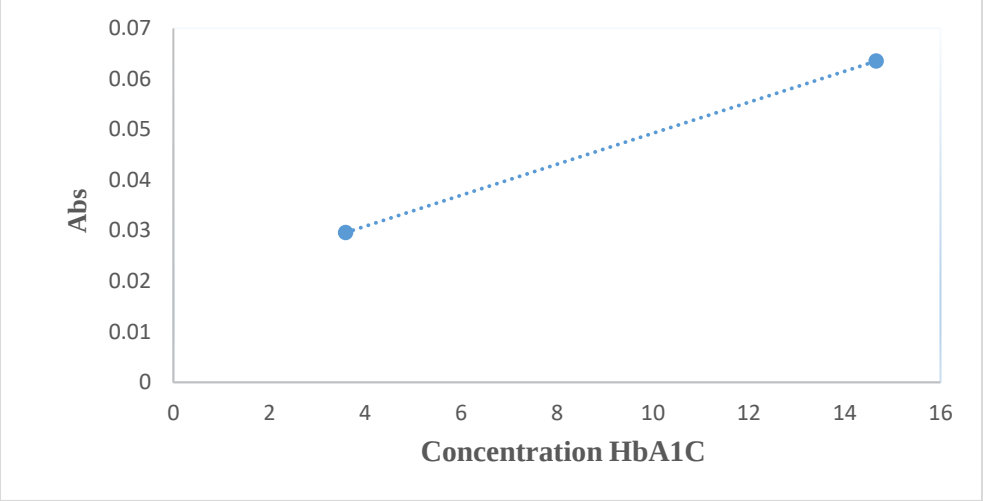


Figure (2.4) Calibration curve for HbA1C

2.4.3. High sensitivity C-reactive protein (hsCRP)

Principle

Multiagents CRP Vario is a latex Immunoassay developed to accurately and reproducibly measure blood CRP levels in serum and plasma. When a ntigen-antibody reaction occurs between CRP in a sample and anti-CRP antibody, which has been adsorbed to latex particles, agglutination results. This agglutination is detected as an absorbance change (572 nm), with the rate of change being proportional to the quantity of CRP in the sample. The method was used High Sensitivity (CRP16) [208].

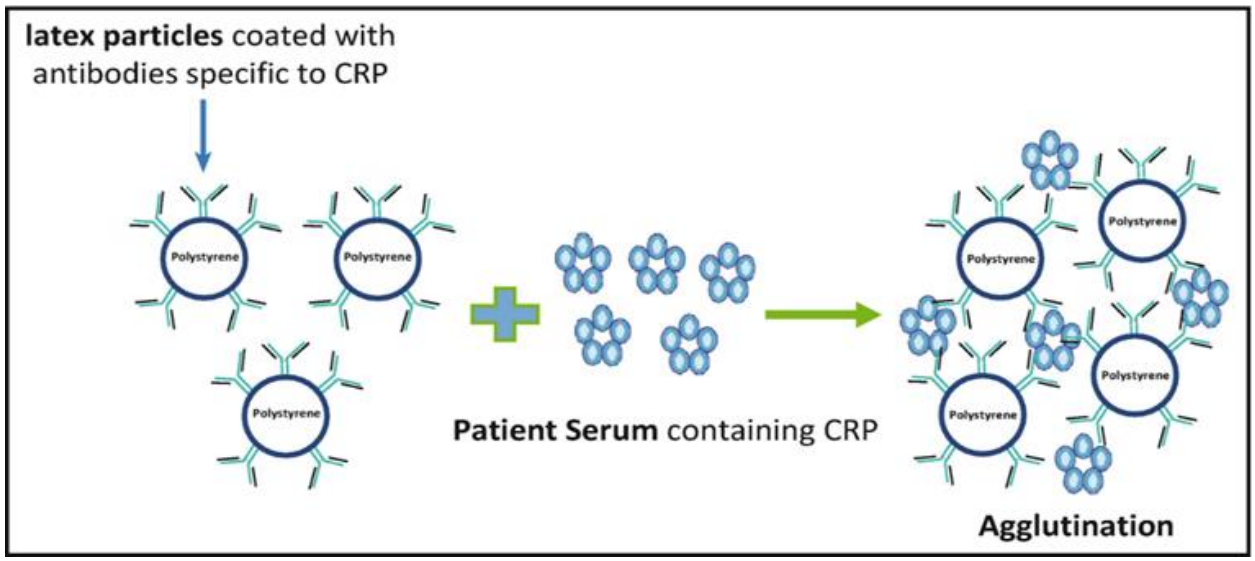


Figure (2.5) Scheme illustrates antigen-antibody reaction between CRP in a sample and anti-CRP antibody [207].

The Reagents: - Kit of CRP Vairo is supplied as a two-reagent kit which contains.

1- Reactive Ingredients

Reagents	Components of reagents	Concentration
R1	Glycine buffer (PH 7)	1.28 %
R2	Anti-CRP polyclonal antibodies (rabbit)	0.2%

2.Nonreactive nonreactive Ingredients

Reagents	Components of reagents	
R1	bovine albumin (≤1%)	sodium azide (<0.1%)

R2	bovine albumin (≤ 0.1%)	sodium azide (<0.1%)

***The reagents are ready to be used**

Procedure

Wels of reaction	Reactive Ingredients	Nonreactive Ingredients
High sensitivity	600	2.192
Standard	600	2,192
Wide range	500	1,836

Calculation is based on the minimum reagent fill volume per kit.

Normal value ≤0.5mg/dl or ≤5mg/L

[209]

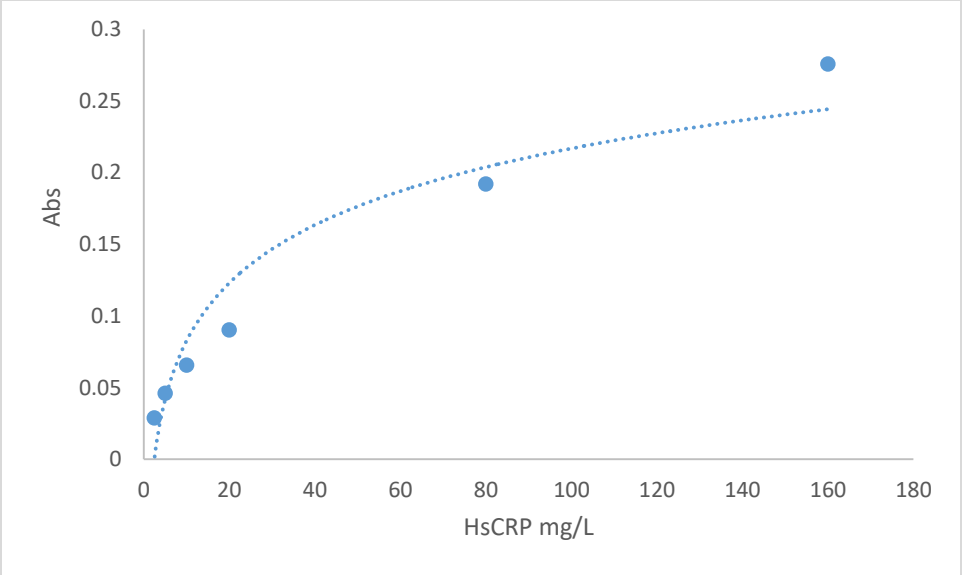


Figure (2.6) Calibration curve for hsCRP

2.4.4. Alkaline phosphatase

Principle

P-nitrophenylphosphate (4-NPP) is hydrolyzed by the enzyme alkaline phosphatase to produce *p*-nitrophenol (4-NP), the rate of which can be detected by spectrophotometer at 405 nm to determine the amount of enzyme activity in the sample.

Reagents

A. Consist of ALP Volume (40/80 ml), Buffer DEA (1mol/L) Magnesium chloride(0.5mmol/L)

B. Reagent (B) ALP (Volume 10 ml), p-nitrophenylphosphate (10 mmol/L)

Reagent preparation

Working reagents were prepared by adding 1 part of reagent B to 4 parts of reagent A and mixed gently. The working reagent was used as a monoreagent

Procedure

Pipette	Tube
Working reagent(A+B)	1000 ML
Sample	20 ML

The samples were mixed and incubated at 37°C for 1 minute, then were read the initial absorbance against water. 3 reading s were read at a distance of 60 seconds. The absorbance was measured at 405nm at 37°C, and the light path1cm.

Calculations

Activity of ALP in U/L= AA/min ×

The average value of the absorbance variations per minute(AA/min) was calculated by equation below

The normal value of the activity of ALP in adults between 98-279 U/L [210]

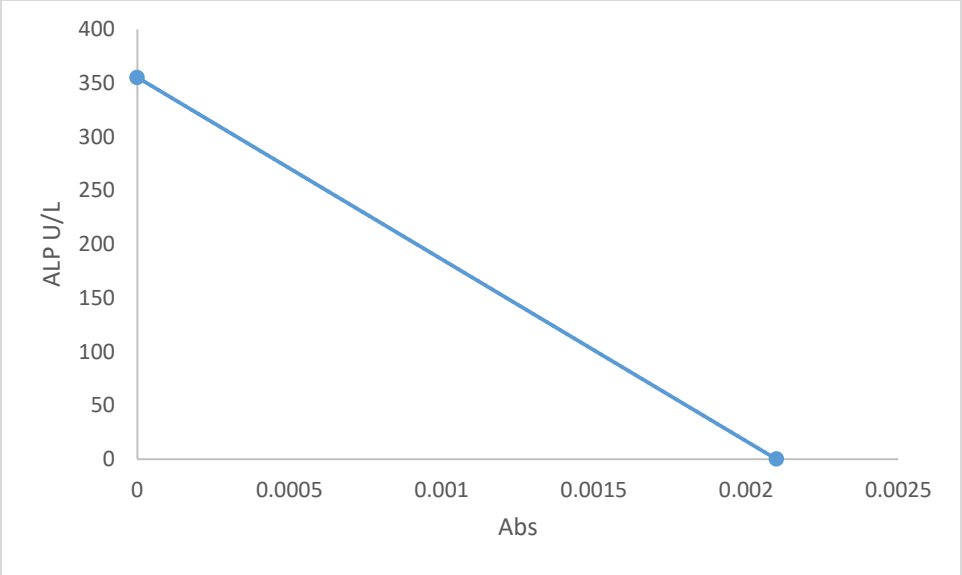


Figure (2.7) Calibration curve of ALP

2.4.5. Parathyroid hormone test (PTH)

Principle

The ARCHITECT Intact PTH assay is a two-step sandwich immunoassay for the quantitative determination of intact PTH in human serum and plasma using CMIA technology with flexible assay protocols [211].

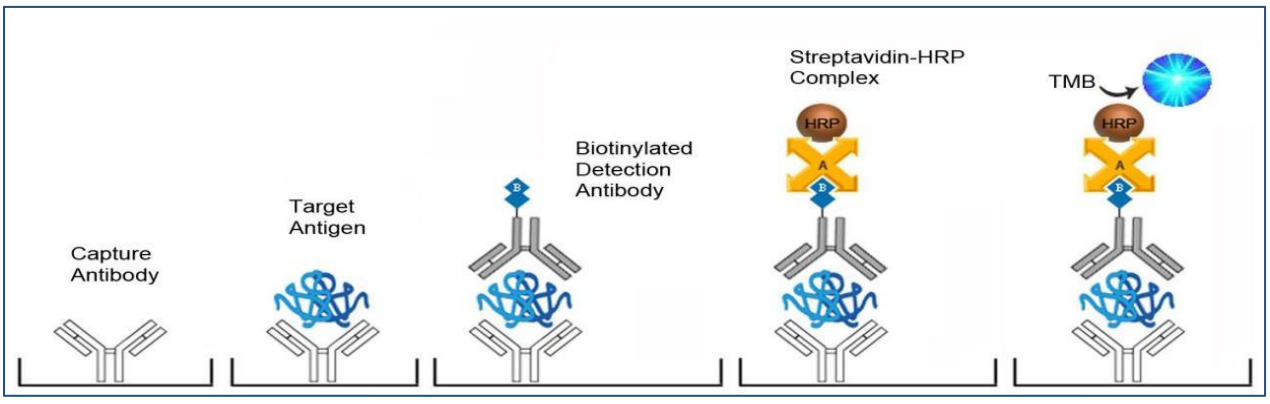


Figure (2.8) Scheme illustrates quantitative detection of Human PTH / in samples of Plasma and Serum. It is based on a Sandwich assay principle

The first step

Reagents of the first step

1. Assay diluent containing phosphate buffered saline solution. Preservative. antimicrobial agent.

2.Anti-PTH (goat, polyclonal) coated microparticles in tris-buffer. Preservative. sodium azide.

3. Anti-PTH (goat, polyclonal) acridinium labeled conjugate in MES buffer with protein (bovine, goat) stabilizer. Preservative. sodium azide.

The serum, assay diluent, and anti-PTH coated paramagnetic microparticles are combined. Intact PTH present in the sample binds to the anti-PTH coated microparticles. After washing, anti-PTH acridinium labeled conjugate is added to create a reaction mixture.

The second step

Reagents of the second step

1.Wash Buffer containing phosphate buffered saline solution. Preservative. antimicrobial agent.

2.Pre-Trigger Solution containing 1.32% (w/v) hydrogen peroxide.

3.Trigger Solution containing 0.35 N sodium hydroxide.

Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs).

A direct relationship exists between the amount of intact PTH in the sample and the RLUs detected by the ARCHITECT i System optics [212].

Normal value of PTH (10 to 65 Pg/ml)

Conversion formula
(Concentration in Pg/mL) x (0.106) = Pmol/L [213].

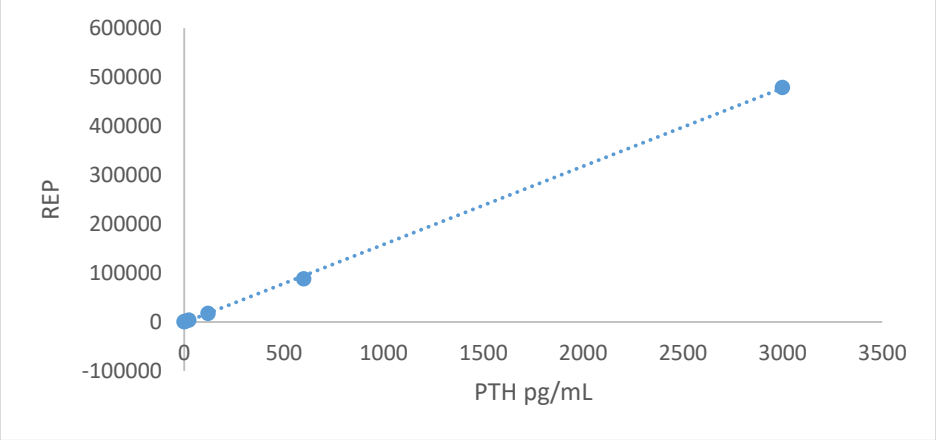


Figure (2.9) Calibration curve for PTH pg/mL

2.4.6. Serum albumin

Principle: - The serum albumin was measured quantitatively in vitro using the albumin kit and the colorimetric technique.

The albumin in the sample reacts with bromocresol green (BCG) in a pH 3.8 buffered solution to alter color. The intensity of color is correlated with the serum's albumin concentration.

Albumin in the sample is stable 1 week at 15-25°C and over 1month at 2 8°C avoiding bacterial contamination. The samples with presence of fibrin should be centrifuged.

Reagents

1-Reagent (A) Alb include Buffer pH 3.8(100 mmol), BCG (7 mmol)

2-Standard Alb include Bovine Albumin (3g/dl)

Reagents preparation

Reagent (A) Alb ready to use

Procedure

Pipette	Blank	Sample	Standard
Reagent A	1500*M*l	1500 μL	1500 μL
Water	10 *M*l		
Sample		10 μL	
Standard			10 μL

The reactants were mixed and incubated 2 min at room temperature then they were read the absorbance of sample (Ax) and (As)against blank at 628 nm and light path 1cm.

Calculation

Albumin g/dL= Ax/As × 3 (standard value)

Normal value (3.5-5.5 g/dl)

Conversion Factor. expected values Serum.

g/dL * 144.9 = μmol [214]

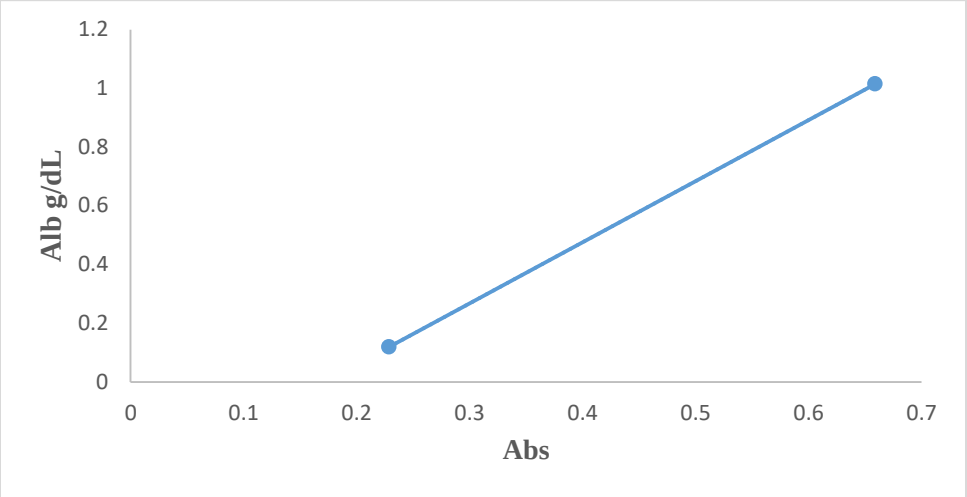


Figure (2.10) Calibration curve for Alb

2.4.7. Vitamin D3

Principle: -Sandwich immunodetection is the methodology of test for the other immobilized streptavidin on a test strip to collect the antigen-antibody complexes that the detector antibodies in buffer have formed in response to the antigens in the sample, they must migrate onto nitrocellulose matrix. A higher fluorescence signal from detector antibody-fluorescence, which is processed by the instrument for ichroma tests to demonstrate Total 25-OH Vitamin D (D2/D3) concentration in the sample, results from the presence of more antigens in the sample [215].

Ichroma Vitamin D Neo consists of

1.The cartridge contains the membrane called a test strip which has streptavidin at the test line and chicken IgY at the control line. All cartridges are individually

sealed in an aluminum foil pouch containing a desiccant, and they are further packaged in a box.

2.The detector tube has 2 granules antibody-fluorescence conjugate, antibody-biotin conjugate, anti-chicken IgY-fluorescence conjugate, sodium azide as a preservative in PBS. All detector tubes are packed in a pouch.

3.The extraction buffer contains sodium azide as a preservative in phosphate buffered saline buffer (PBS), and it is pre-dispensed in a vial. The extraction buffer is packed in a box.

Procedure

1- 150 μL of extraction buffer has been taken by a pipette and dispensed to the detector tube containing granules. When the granule form is completely dissolved in the tube, it becomes detection buffer. (The detection buffer must be used immediately. Do not exceed 30 seconds.)

2- Take 30 μL of serum using a pipette and dispense it to the detector tube.

If 30 μL capillary tube was used to collect the whole blood, put it directly into the detector tube.

3- Close the lid of the detector tube and mix the sample thoroughly by shaking it about 10 times. (The sample mixture must be used immediately. Do not exceed 30 seconds.)

4- Take 75 μL of the sample mixture and dispense it into the sample well of the cartridge.

5- The sample-loaded cartridge is inserted into the slot of the i-Chamber or an incubator (35°C).

6- Leave the sample-loaded cartridge in the i-Chamber or an incubator for 12 min

The instrument for ichroma tests calculates the test result automatically and displays Total 25-OH Vitamin D (D2/D3) concentration of the test sample in terms of ng/ml [216].

Working range (5 - 100 ng/ml).

Normal value 30-100 ng /ml

Conversion factor: 1 ng/ml = 2.5 nmol/l [217]

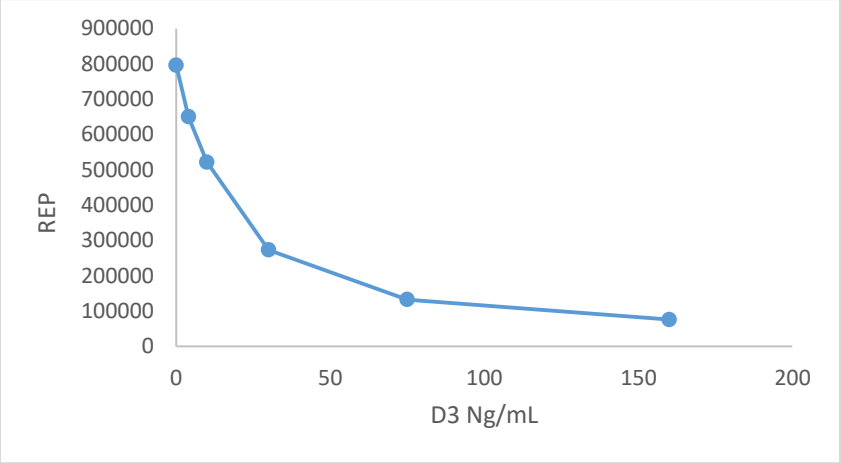


Figure (2.11) Calibration curve for D3

2.4.8. Serum Calcium

Principle: -To Evaluate the serum calcium, the calcium kit's colorimetric method for quantitative in vitro diagnostic measurement was used. Calcium and Arsenazo (III) combine to generate a stable blue-violet complex in PH-neutral media. The amount of calcium present in the sample is directly proportional with the color intensity.

Reagents

1.Reagent (A) Ca Volume (50/100 ml) includes Buffer (100 Mmol/l) and Arsenazo III(0.13Mmol/l)

2.Standard (Volume 10 ml) includes Calcium 10 mg/dL (2.5 Mmol/l)

and Sodium azide (5 Mmol/l)

***Reagents ready to use**

Procedure

Pipette	**Blank**	**Sample**	**Standard**
Reagent A	1000 μl	1000 μl	1000 μl
Water	10 μl		
Sample		10 μl	
Standard			10 μl

The reactants were mixed and incubated for 2 min at 37 °C then the absorbance were read of sample (Ax) and (As) against blank at 600 nm and light path 1 cm [218].

Calculation

Calcium mg/dL= Ax/As × 10 (standard value)

Conversion Factor. expected values Serum Mmol/l*18=mg/dl

Normal value 8.6-10.3mg/dL[218]

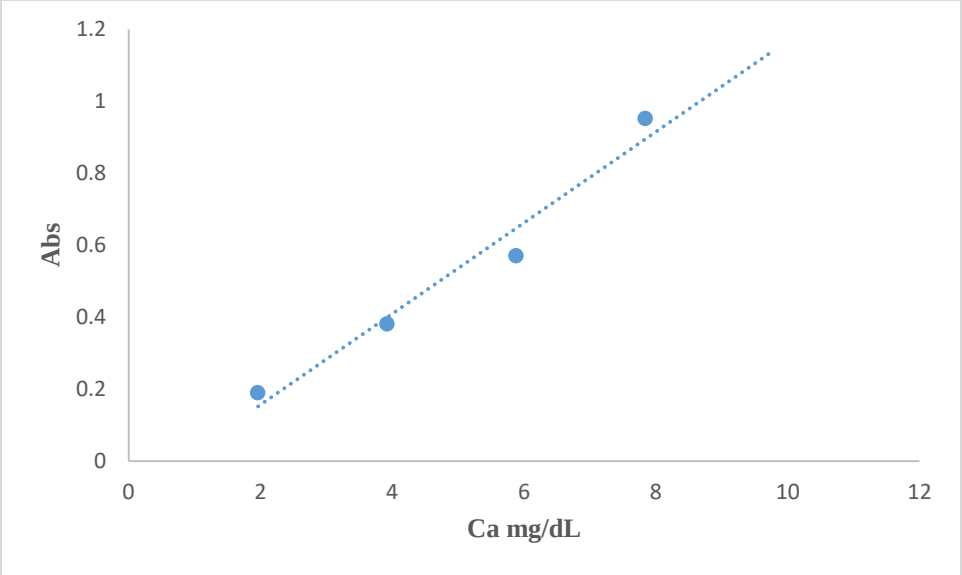


Figure (2.12) Calibration curve for Ca^{2+} mg/dL

2.4.9. Phosphorus

Principle

The serum Phosphorus was evaluated using the colorimetric method for quantitative in *vitro* diagnostic testing using the Phosphorus kit. A phosphomolybdate complex is created when phosphate ions combine with ammonium molybdate; the absorbance of complex at 340 nm indicates how much phosphorus is present in the sample. To produce complexes, a pH of acid is required.

Reagents

1.Reagent A includes Ammonium molybdate (0.5 Mmol/L) and Sulfuric acid 96%(150mmol/L)

2.Reagent B include Standard Phosphorus5 mg/dl (1.615 Mmol/L) and Sodium azide 4 Mmol/L

***Reagents ready to use**

Procedure

Pipette	Blank	Sample	Standard
Reagent A	1000 μl	1000 μl	1000 μl
Water	10 μl		
Sample		10 μl	
Standard	1000 μl		10 μl

The reactants were mixed and incubated for 5 min at 37 °C then the absorbance were read of sample (Ax) and (As)against blank at 340 nm and light path 1 cm.

Calculation

Phosphorus mg/dL= Ax÷As × 5 (standard value)

Conversion factor Mmol/L×18=mg/dL

Normal value in adults are about (0.2 - 0.3) mg/dL[219]

2.5. Synthesis and characterization of $CaCO_3$NPs, AGNPs and $CaCO_3$@AGNPs

2.5.1. Synthesis of $CaCO_3$NPs

$CaCO_3$NPs were synthesized according to the previous literature method with a little modification. Where 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. Next, 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 [220].

$$Na_2CO_3 + CaCl_2 \longrightarrow 2NaCl + CaCO_3$$

Equation: - preparing Calcium Carbonate Nanoparticles

2.5.2. Preparation of Arabian Gum polymer

1- The AG was ground well using an electric grinder and sieved to give a fine powder <250 μm.

2- 1 g of sifted gum was taken and dissolved in 100 ml of distilled water and sonicated by ultrasonic for 3min [221].

2.5.3. Preparation $CaCO_3$@AGNPs

$CaCO_3$NPs were synthesized by the co-precipitation method by the mixing of the two precursors salts ($CaCl_2$ and Na_2CO_3). And preparation AGNPs polymer Then, the calcium Arabian gum core-shell nanoparticles $CaCO_3$@AGNPs were prepared by coating the AG NPs polymer on the surface of $CaCO_3$ NPs under stirring in aqueous solution. The synthesis procedures of $CaCO_3$@AGNPs are illustrated in Scheme below [222].

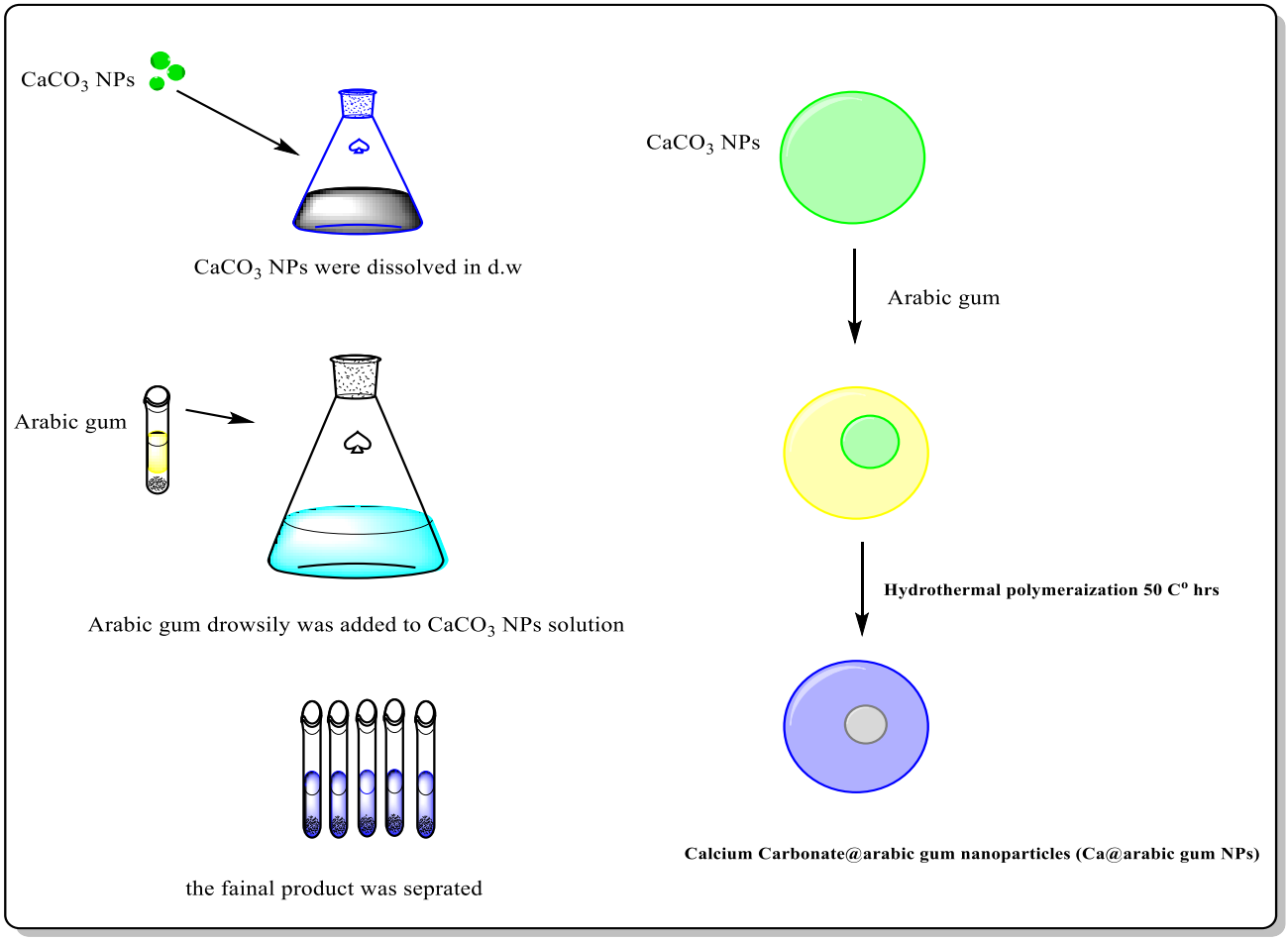


Figure (2.13) Scheme illustrates the preparation of $CaCO_3$@AGNPs

2.5.4. Effect of prepared nanomaterials $CaCO_3$NPs and$CaCO_3$@AGNPs on patients' serum pool

Many scientists specializing in diseases have sought ways to treat many diseases that pose a problem for medicine, as diabetes and arthritis are considered among the most common diseases, and science has not yet reached a definitive treatment for them. Among the attempts is this study, which included the preparation of $CaCO_3$NPs and $CaCO_3$@AGNPs. It is being tested on sera of patients with T2DM and OA.

Procedure

The procedure included the following steps

First step: - Preparation of serum pool consisting of 15 sera of patients by mixing 500 μL of each of serum of these 15 sera for one hour in shaker water bath at 37°C.

Second step: - Measurement of the (FBG, Phosphorus, ALP, Alb, Ca^{2+}, PTH) before adding $CaCO_3$NPs and $CaCO_3$@AGNPs

Third step: - Preparation 500 mg/ml solution of $CaCO_3$NPs and $CaCO_3$@AGNPs by solving 0.0025 g of in 50 ml deionized water as stock solution.

Fourth step: - Adding 30μL of)100 μg/mL, 250 μg/mL, 500 μg/mL) of $CaCO_3$NPs and $CaCO_3$@AGNPs to 1mL of sera of patients group and measuring (FBG, Pi, ALP, Alb, Ca^{2+}, PTH) after adding $CaCO_3$NPs and $CaCO_3$@AGNPs [223].

2.6. Haemolytic assay

Hemolysis is one of the major limiting factors of red cell concentrate and quality must be measured as a quality-monitoring requirement. According to international quality standards, percentage hemolysis must be monitored in 1.0% of red cell concentrates produced monthly and maintained under 0.8% [224].

Procedure

This study included healthy non-smoking female aged 25 years old. Blood was collected via venipuncture into test tube containing heparinized human blood. Different concentrations (100,200,300,400 and 500 μg/ml respectively) of prepared compounds $CaCO_3$NPs which was labeled A1, A2, A3, A4 and A5 and $CaCO_3$@AGNPs was labeled B1, B2, B3, B4, and B5 (respectively) added to 0.2 ml of the blood specimen. The mixture was then thoroughly stirred for 10

seconds. To avoid severe hemolysis, 10 ml of normal saline (0.9%) was added. For 10 minutes, the resulting mixture was centrifuged at 4000xg. The absorbance of mixture was measured at 540 nm. Blood has been diluted 100-fold in distilled water to achieve complete hemolysis (100%) of the sample. Normal saline was utilized as the negative control and Triton X-100 as the positive control [225].

Calculations

After the determination of absorbance, the percentage of hemolysis was calculated using the equation

$$\% \text{ of Hemolysis} = [(AT - AS) / (A\ 100\% - AS)] * 100\ \%$$

Where. AT=Absorption of the test sample.

AS= Absorption of control (normal saline solution.)

A100% =Absorption of 100 percent hemolysis [226].

2.7. Statistic

Information from the questionnaire from all participants was entered into a data sheet and were assigned a serial identifier number. Multiple entries were used to avoid errors. The data analysis for this work was generated using The Statistical Package for the Social Sciences software, version 26.0 (IBM, SPSS). Descriptive statistics were performed on the participants' data of each group. Values were illustrated by Mean for the continuous variable. Significant differences in categorical variables among the parameters were confirmed through analytical statistical tests. The results of all hypothesis tests with p-values <0.05 and $p<0.01$ were considered to be statistically significant. While the correlation coefficient was used to evaluate how the different biomarkers were related to each other.

CHAPTER THREE

RESULTS

&

DISCUSSION

3.Results and Discussion

Many researchers in the field of medicine and those interested in health dealt study osteoarthritis and type 2 diabetes, and the results of the research have varied in their conclusions, as some studies have shown that these two diseases often affect the elderly and have the same causes. The study entitled (The Association Between Diabetes Mellitus and Osteoarthritis: Does Diabetes Mellitus Play a Role in the Severity of Pain in Osteoarthritis?) showed that the chronic hyperglycemic states can increase inflammation in the microcellular environments of joints. Although the complete pathogenesis is not mapped out, advanced glycation end-products (AGEs) have a central role in perpetuating inflammatory processes in every anatomical part of the joint. This increased inflammation can then exponentiate the breakdown of cells and tissues, [227], while another study entitled (Association between diabetes mellitus, hypertension, and knee osteoarthritis in secondary referral hospitals in Indonesia with retrospective cross-sectional study. Annals of Medicine & Surgery) appeared that there were no significant association between diabetes mellitus with the osteoarthritis [228].

In this chapter, the results of the study covered (50 women) who suffer all from T2DM with OA diseases as patients group and (40 women) apparently healthy as the control group aged (40-70) years. The dependent variable for this study was the type of group while The independent variables of this study included (age, BMI, HbA1C, blood glucose, ALP, Alb, Ca^{2+}, PTH, hsCRP, phosphorus, and vitamin D3). Statistical analysis was carried out using SPSS version 26. Analysis of variance (T test) was used to compare means between two groups.

3.1. Age and body mass index

Table 3.1) Descriptive table Comparison between patient group and control group in age and BMI

Subject	Type of group	No	Mean	Std. Deviation	Range	P- value
Age years	Control	40	50.87	8.60	70-40	p>0.05
	Patient	50	53.36	8.02	70-40	
BMI(Kg/m^2)	Control	40	26.81	3.37	33.3-20.8	p>0.05
	Patient	50	29.70	5.90	46.67-16.8	

The results of the t-test showed no significant differences in age and BMI among the studied groups, as shown in **(Table 3.1)**. The mean of age for control was (50.87) years, while in the patient's group was (53.81) years and ($p \geq 0.05$). The mean BMI for control was (26.81) kg/m^2, while in the patient's group was (29.70) kg/m2 and ($p \geq 0.05$).

3.2. Fasting Blood glucose and HbA1C

For diabetes mellitus type 2

- The mean of HbA1C for patients group was 9.78±1.99786 and 5.0875±0.69363 for control group. The t-test shown that there was a significant difference between control group and patients group and p<0.05.

- The mean of FBG for patients group was 212.16±78.78 and 101.52±12.84 for control group. The t-test appeared that there is a significant difference p<0.05.

Table (3.2) Serum level of HbA1C and FBG

Parameters	Type of group	No	Mean ±SD	Range	P- value
HbA1C	Control	40	5.1±0.69	3	p<0.05
	Patient	50	9.78±1.99	6.5	
FBG mg/dL	Control	40	101.5250 ±12.84	52	p<0.05
	Patient	50	212.16±78.78	259	

Table (3.2) showed that there were significant differences between control group and patients group in HbA1C and FBS and p<0.05, because glycated hemoglobin is a measure of integrated glycaemia over the course of a red blood cell's 120-day life [229]. HbA1c is a useful tool for long-term diabetes care and monitoring since it is a trustworthy indicator of chronic glycaemia and has a strong correlation with the risk of developing the disease [230].

3.3. Serum level of phosphorus, ALP, Alb, Ca^{2+}, PTH, hsCRP and D3

Table (3.3) Serum level of phosphorus, ALP, Alb, Ca^{2+}, PTH, hsCRP and D3

Parameters	Type of group	No	Mean ±SD	Range	P- value
PI mg/dL	Control	40	3.29±o.62	4.5-2	p>0.05
	Patient	50	3.60±0.66	6.24-2.66	
ALP U/L	Control	40	535.65±524.03	2755-20	P<0.05
	Patient	50	1088.18±1312.70	5662-41	
Alb g/dL	Control	40	3.93±0.37	4.7-2.99	p>0.05
	Patient	50	3.88±0.41	4.5-2.4	

Ca^{2+} mg/dL	Control	40	8.33±0.93	11-6.5	p>0.05
	Patient	50	8.36±0.97	11.1-5.8	
PTH Pg/mL	Control	40	33.62±14.68	72.1-11	P<0.05
	Patient	50	28.72±12.78	56.9-10	
hsCRP mg/L	Control	40	3.33±2.25	13-0.61	P<0.05
	Patient	50	13.717±16.51294	62.41-0.5	
D3 ng/mL	Control	40	12.5915±4.58294	25.21-6.27	p>0.05
	Patient	50	15.05±7.10373	62.41-7.11	

Table (3.3) Showed that there was a significant difference between patient group and control group in ALP, PTH and hsCRP and (p<0.05). Abnormal ALP levels in the blood are linked to problems with the liver, gall bladder, and bones. On the other hand, new studies point to the serum ALP level as an inflammatory mediator linked to cardiometabolic disorders, such as dyslipidemia, hypertension, metabolic syndrome, and T2DM. Furthermore, elevated blood ALP levels were associated with a rise in other inflammatory indicators that predict osteoarthritis, including leukocyte count and CRP level [231].

Parameter	Correlation Coefficient	Age	FBG	Pi	Alb	Ca	PTH	hsCRP	BMI
Age year	r	1	-0.067	0.413**	0.314*	0.355*	-0.151	0.193	-0.181
	P-value		0.642	0.003	0.026	0.011	0.295	0.178	0.208
FBG mg/dL	r	-0.067	1	0.403**	0.146	0.128	-0.163	0.327*	-0.089
	P-value	0.642		0.004	0.312	0.377	0.258	0.020	0.540
Pi mg/dL	r	0.413**	0.403**	1	0.207	0.238	-0.193	0.291*	-0.283*
	p-value	0.003	0.004		0.150	0.096	0.178	0.041	0.046
Alb g/dL	r	0.314*	0.146	0.207	1	0.571**	-0.343*	0.049	0.047
	p-value	0.026	0.312	0.150		0.000	0.015	0.735	0.744
Ca^{2+} mg/dL	r	0.355*	0.128	0.238	0.571**	1	-0.393**	0.179	-0.028
	p-value	0.011	0.377	0.096	0.000		0.005	0.214	0.846

PTH pg/mL	r	-0.151	-0.163	-0.193	-0.343*	-0.393**	1	-0.015	0.146
	p-value	0.295	0.258	0.178	0.015	0.005		0.920	0.312
hsCRP mg/L	r	0.193	0.327*	0.291*	0.049	0.179	-0.015	1	-0.255
	p-value	0.178	0.020	0.041	0.735	0.214	0.920		0.074
BMI Kg/m^2	r	-0.181	-0.089	-0.283*	0.047	-0.028	0.146	-0.255	1
	p-value	0.208	0.540	0.046	0.744	0.846	0.312	0.074	

Table (3.4) The Correlations between Age, FBG, Pi, Alb, Ca^{2+}, PTH, hsCR and BMI.

* correlation is significant at the level 0.05

** correlation is significant at the level 0.01

Table (3.4) showed: -

- Age highly significant correlated with pi and $p < 0.01$ and with (Alb, Ca) and $p < 0.05$
- Significant correlation between Alb and calcium ($p<0.01$) (increase level of Alb with increased level of Ca^{2+}) and inversely significant with PTH ($P<0.05$).

Ca^{2+} is tightly regulated by PTH. As albumin is the primary protein that binds calcium, increased in serum albumin will result in increased in bound calcium and increase in total calcium (and vice versa). In reaction to a decrease in serum calcium, the active hormone is released [232].

- significant relation between phosphorus and hsCRP ($p<0.05$) (increase level of phosphorus with increased level of hsCRP)

Some studies have provided evidence that experimental evidence that hsCRP level was causally related to diabetes nephropathy. These findings suggest that the elevated hsCRP may be a causal risk factor for diabetes nephropathy in patients with T2 DM [233].

It has been demonstrated that a high phosphate load reduces the risk of cardiometabolic illnesses and inhibits pancreatic insulin production, which in turn affects glucose metabolism [234].

3.5. Characterization of $CaCO_3$@AG Nanoparticles

Using transmission electron microscopy (TEM) and scanning electron microscopy (SEM), the shape and structure of the produced samples were examined. The generated $CaCO_3$ NPs seemed to be Nanospheres with a particle size of roughly (20–50) nm, as demonstrated by SEM images in Figure (3.3). However, the average size of $CaCO_3$@AGNPs increased after coating with Arabian gum polymer, showing the successful coating of AG on the surface of $CaCO_3$NPs

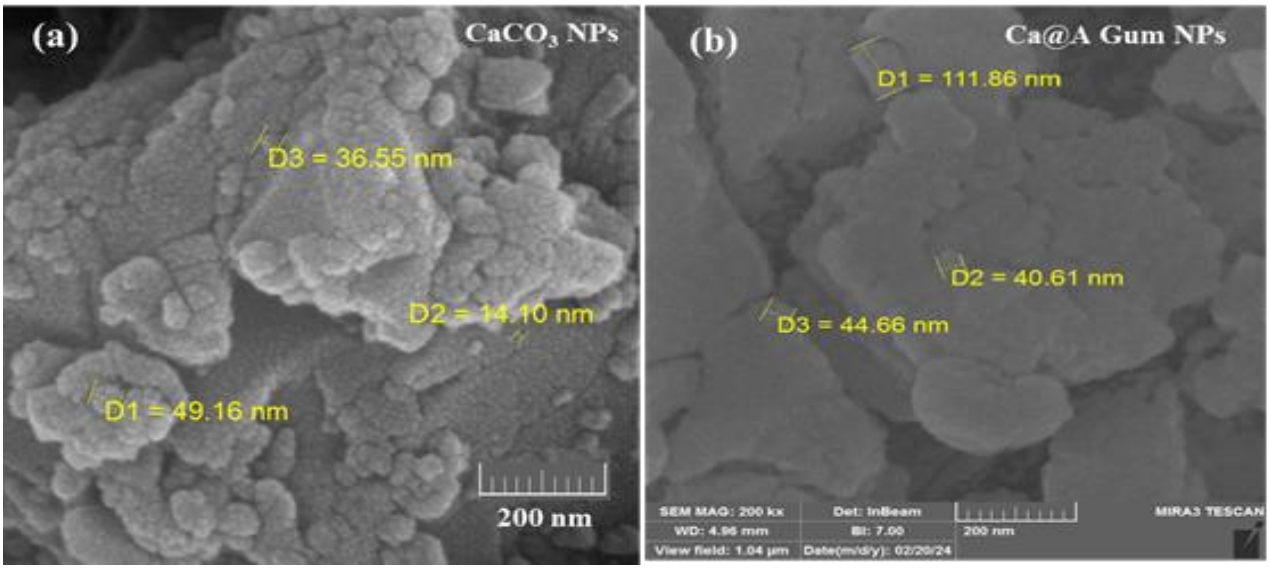


Figure (3.3) SEM images of (a) $CaCO_3$NPs, (b) $CaCO_3$@AGNPs.

$CaCO_3$@AG core-shell NPs are formed when the AG shells are firmly encased on the surface of $CaCO_3$NPs with a thickness of roughly 10–40 nm. Following coating with AG, the average size of $CaCO_3$NPs rose to 50–120 nm, demonstrating the effective production of $CaCO_3$@AG core-shell NPs. It is evident that the $CaCO_3$NPs cores were visible in the dark contrast, and that each

$CaCO_3$NPs Nanosphere's exterior bright coating matched the AG polymer shells Figure (3.4).

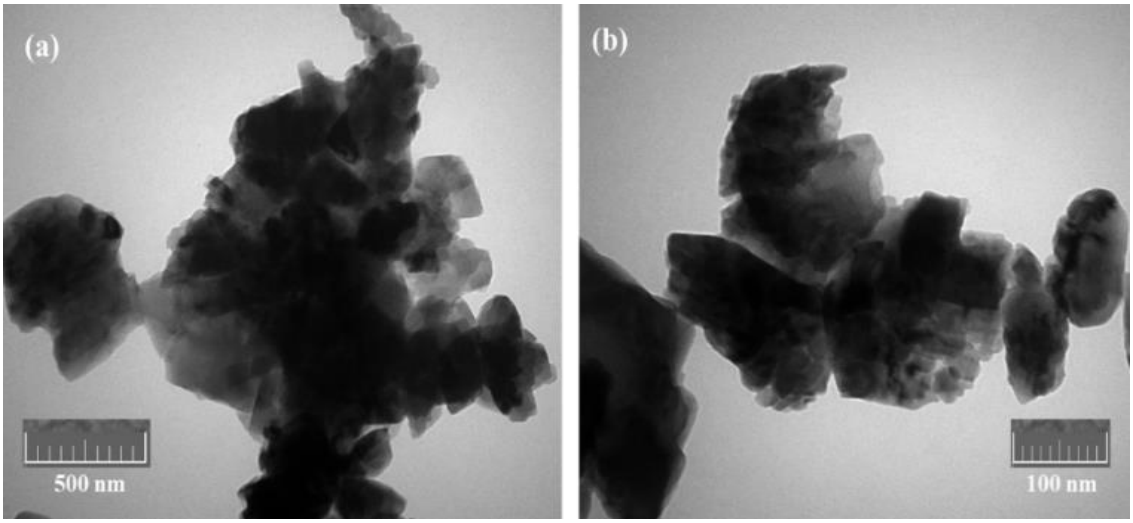


Figure (3.4) TEM images of $CaCO_3$@AGNPs (a) with the scale 500 nm, (b) with the scale 100 nm.

In good accord with XRD results, the AG polymer shell is amorphous and the $CaCO_3$NPs core is crystalline.

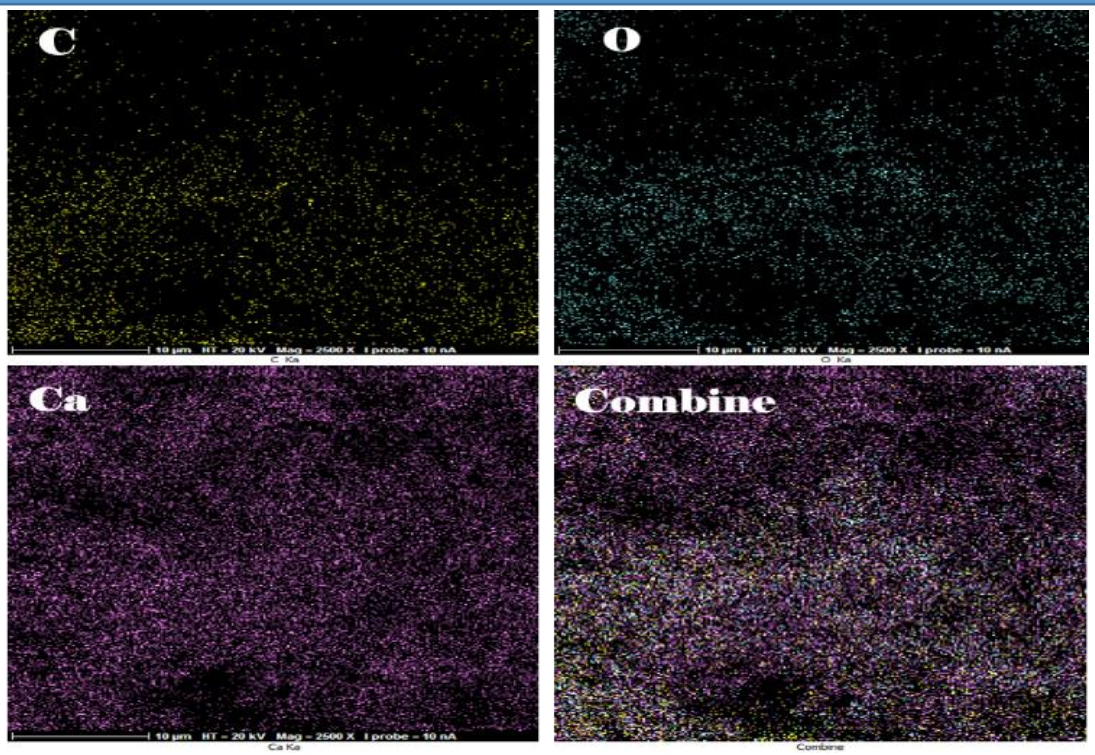


Figure (3.5) EDS-SEM of the $CaCO_3$@AGNPs

Energy-Dispersive Spectroscopy (EDS) of the SEM technique was used to analyze the elemental distribution of the as-prepared $CaCO_3$@AGNPs [235]. As can be seen in Figure (3.5), the elemental mapping clearly demonstrated the presence of all the elements in the $CaCO_3$@AG structure, which is additional evidence of the formation of $CaCO_3$@AGNPs [236].

Dynamic laser scattering (DLS) measurements were used to further investigate the particle sizes of the as-made nanomaterials, and the results suggest that the sizes are bigger than those found by TEM. The average size of the $CaCO_3$NPs increased from around 100 nm to approximately 455 nm following the application of the AG polymer layer, as depicted in Figure. This indicates that the AG polymer coating on the $CaCO_3$NPs was effective, and $CaCO_3$@AG core-shell NPs were formed.

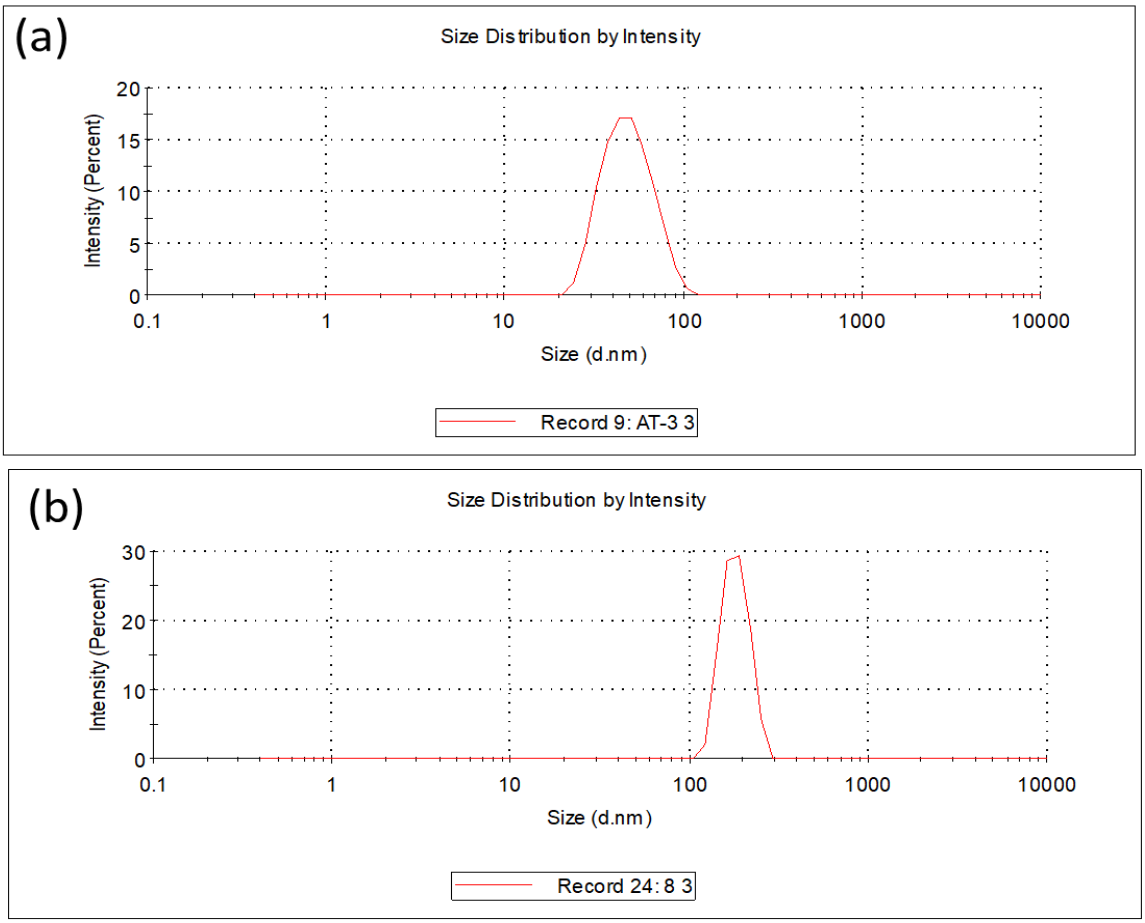


Figure (3.6). DLS results of (a) $CaCO_3$NPs, (b) $CaCO_3$@AGNPs

The results demonstrated that the $CaCO_3$@arabian gum NPs are highly crystalline and all of the peaks match very well with the diffraction peaks of the standard pattern calcite $CaCO_3$ (JCPDS card number 47-1743) [237]. The X-ray diffraction (XRD) pattern was used to further identify the crystalline structure of $CaCO_3$@AGNPs, as shown in Figure (3.7) The $CaCO_3$@AG NPs' spectra showed that they are also crystalline in nature, and the emergence of a distinct diffraction peak at 29.64°, which corresponded to the $CaCO_3$'s plane, amply confirmed that the $CaCO_3$@AGNPs were successfully formed.

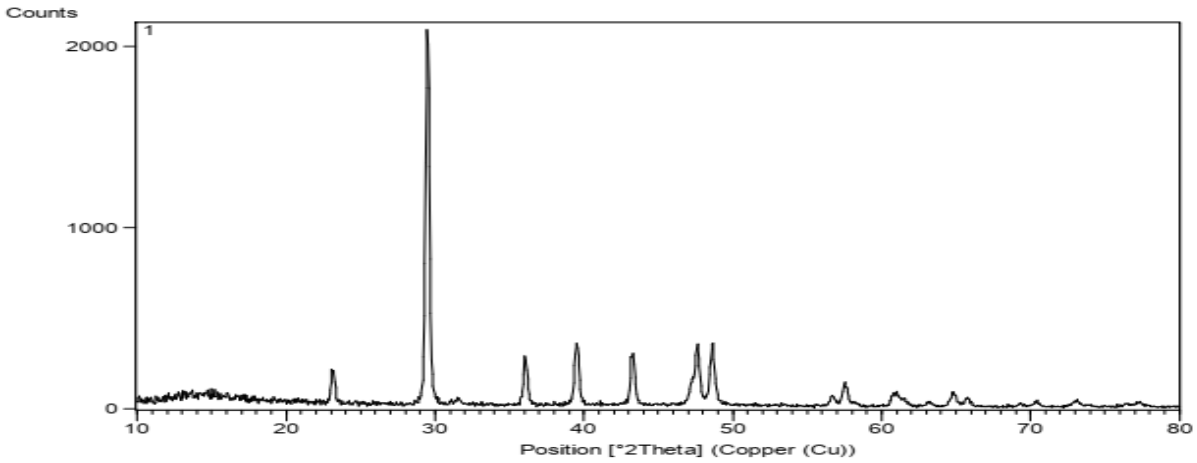


Figure (3.7). XRD results of $CaCO_3$@AGNPs

Furthermore, the optical absorption properties of the as-prepared samples were investigated by UV−Vis-NIR spectra, as shown in Figure (3.8) $CaCO_3$@AGNPs have the characteristic beak of $CaCO_3$ which is near 000 nm [238]. Which is clear evidence for the formation of $CaCO_3$@AGNPs

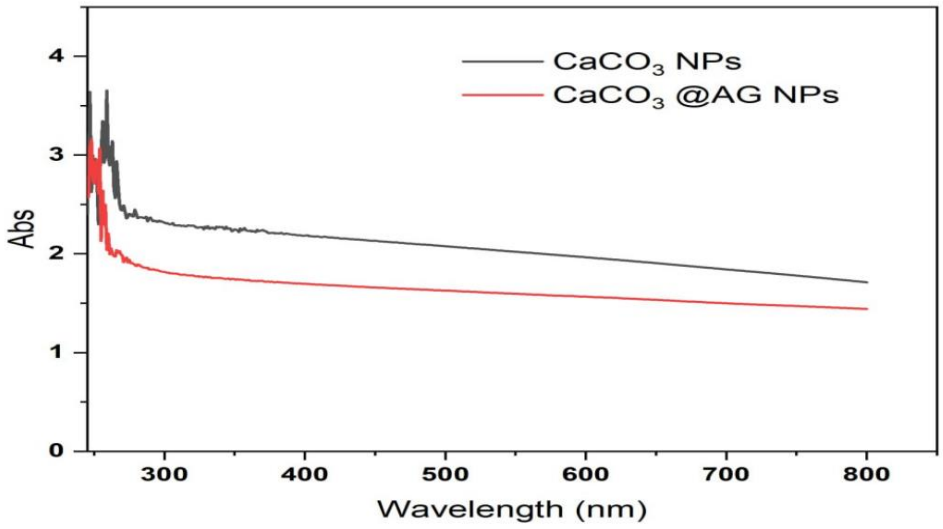


Figure (3.8) UV−Vis spectra of (a) $CaCO_3$NPs (b) $CaCO_3$@AGNPs

3.6. Effect of prepared nanocomposites on serum pool

Serum of 15 patients group were mixed (500µL of each of serum)

A-Firstly, effect of $CaCO_3$NPs

1ml of serum of patients group was mixed with

1-100 μL of (1mg /mL $CaCO_3$NPs)

2- 250 μL of (1mg /mL $CaCO_3$NPs)

3- 500μL of (1mg /mL $CaCO_3$NPs)

B-Secondly, effect of $CaCO_3$@AGNPs

1mL of sera of patients group was mixed with

1-100 μL of (1mg /mL $CaCO_3$@AGNPs)

2- 250 μL of (1mg /mL $CaCO_3$@AGNPs)

3- 500μL of (1mg /mL$CaCO_3$@AGNPs)

The results were

Parameters	0.00 μg/mL	100 μg/mL	250 μg/mL	500 μg/mL
Glucose mg/dL	209.68	188	161	136
Pi mg/dL	3.601	3.64	3.2	2.97
ALP U/L	1187.18	1450	1578	1883
Alb g/dL	3.896	3.9	3.5	3
Ca mg/dL	8.24	10	10.7	11.6
PTH pg/mL	287.198	56.2	49.9	41.1

Table (3.5) showed that the levels of each of glucose, phosphorus, PTH and albumin are decreased with increased concentrations of $CaCO_3$NPs while concentrations of calcium and activities of ALPs are increased with increased concentrations of $CaCO_3$NPs.

Table (3.6) Concentrations of $CaCO_3$@AGNPs

Parameters	**0.00 µg/mL**	**100 µg/mL**	**250 µg/mL**	**500 µg/mL**
Glucose mg/dL	209.68	189	160	133
Pi mg/dL	3.601	3.33	3.07	2.74
ALP U/L	1187.18	2093	11187	1079
Alb g/dL	3.896	3.4	3.3	3.1
Ca mg/dL	8.24	8.5	8.6	8.6
PTH pg/mL	287.198	48.3	48.9	48.9

Table (3.6) showed that the concentrations of each of glucose, phosphorus and PTH are decreased with increased concentrations of concentrations of $CaCO_3$@AGNPs

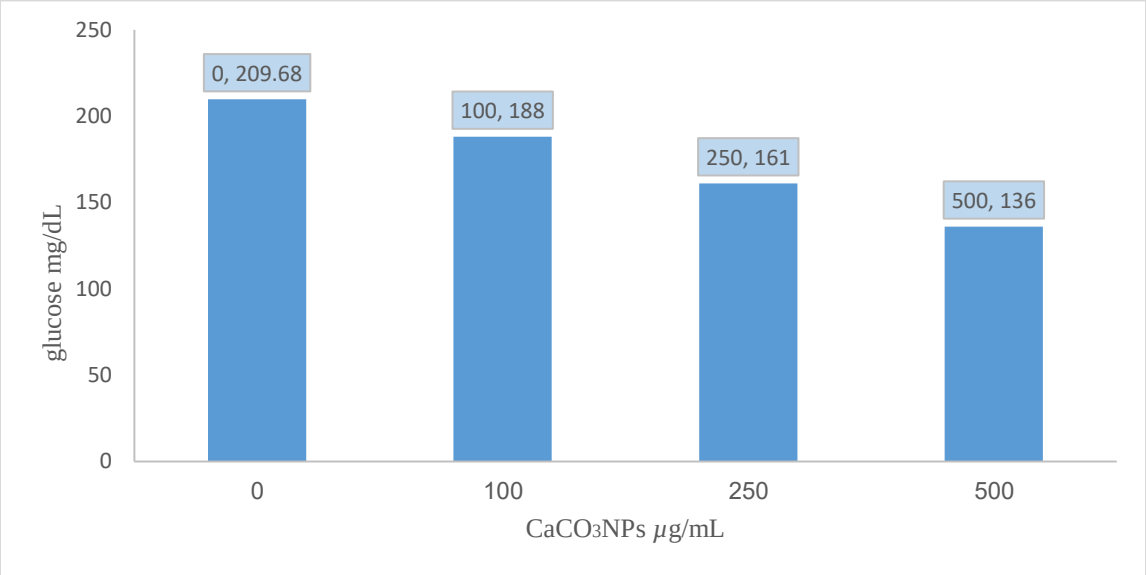


Figure (3.9): - Relation between levels of glucose and concentrations of $CaCO_3$NPs

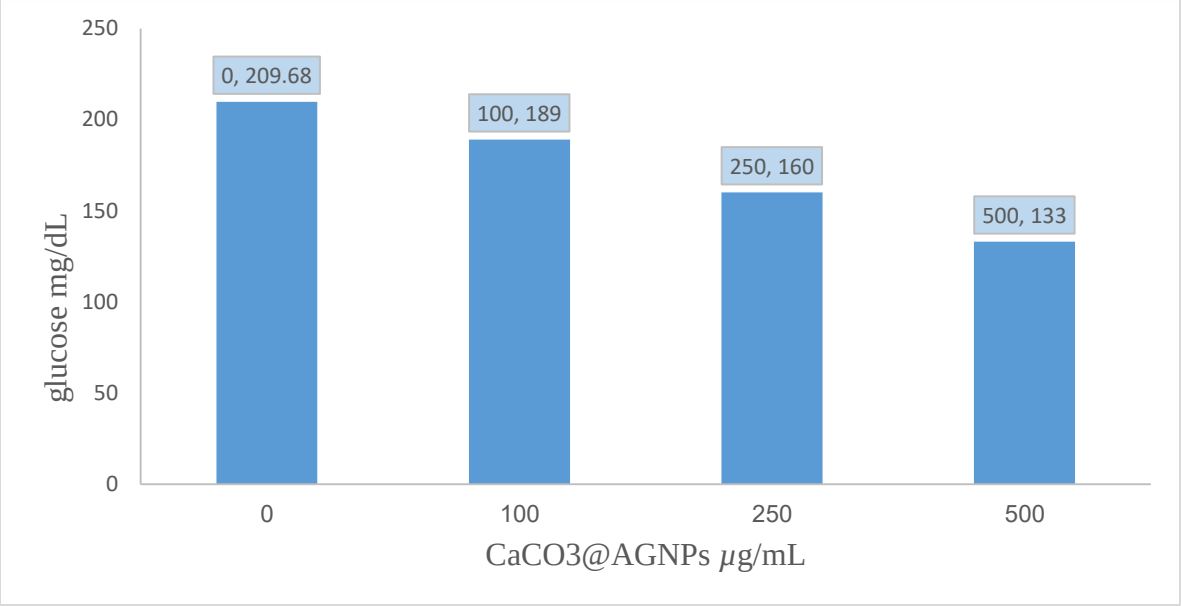


Figure (3.10) Relation between levels of glucose and concentrations of $CaCO_3$@AGNPs.

Decrease levels of glucose with increase the adding amounts of $CaCO_3$NPs and $CaCO_3$@AGNPs as shown in Figures (3.9) and (3.10). The rise in the intracellular Ca^{2+} concentration triggers the priming and fusion of the secretory insulin-containing granules to the plasma membrane, resulting in insulin exocytosis which acts on glucose metabolism [239]. It is noted from the two Figures above and from the two tables (3.5) and (3.6) that the levels of glucose decreased with the addition of the two nanocompounds, but the percentage of its decrease after adding $CaCO_3$@AGNPs is greater. The reason is due to AG, which contributes to lowering the level of sugar in the blood by decrease insulin resistance, in addition to the role of calcium carbonate [240].

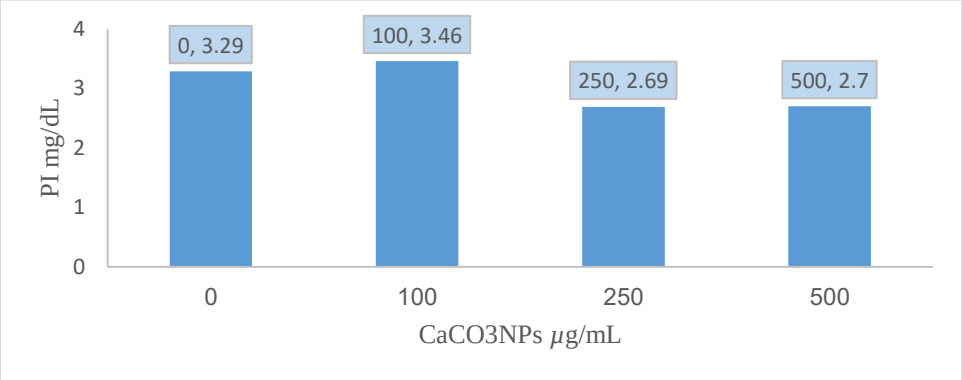


Figure (3.11): - Relation between levels of phosphorus and concentrations of $CaCO_3$NPs

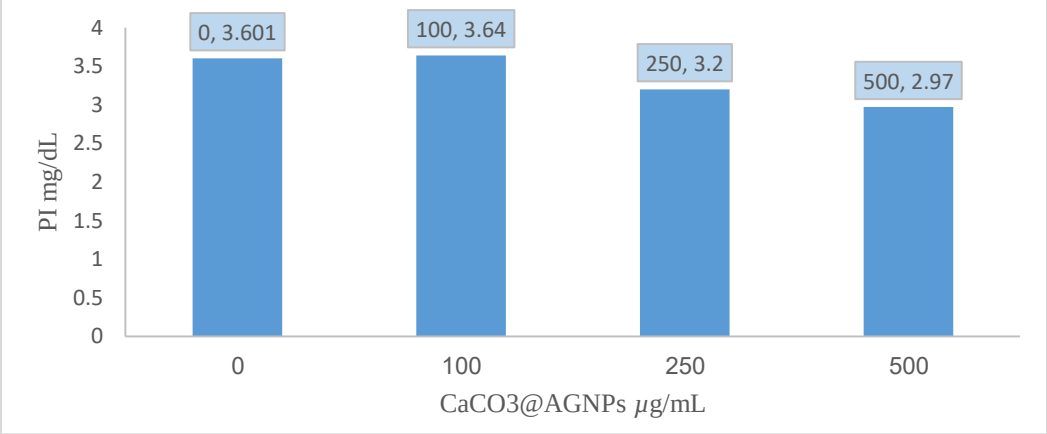


Figure (3.12) Relation between levels of phosphorus and amounts of $CaCO_3$@AGNPs.

Decrease levels of Phosphorus, with increase the adding amounts of $CaCO_3$NPs and $CaCO_3$@AGNPs as shown in Figures (3.11) and (3.12) because phosphorus is consumed in metabolism of glucose (glycolysis) [241]

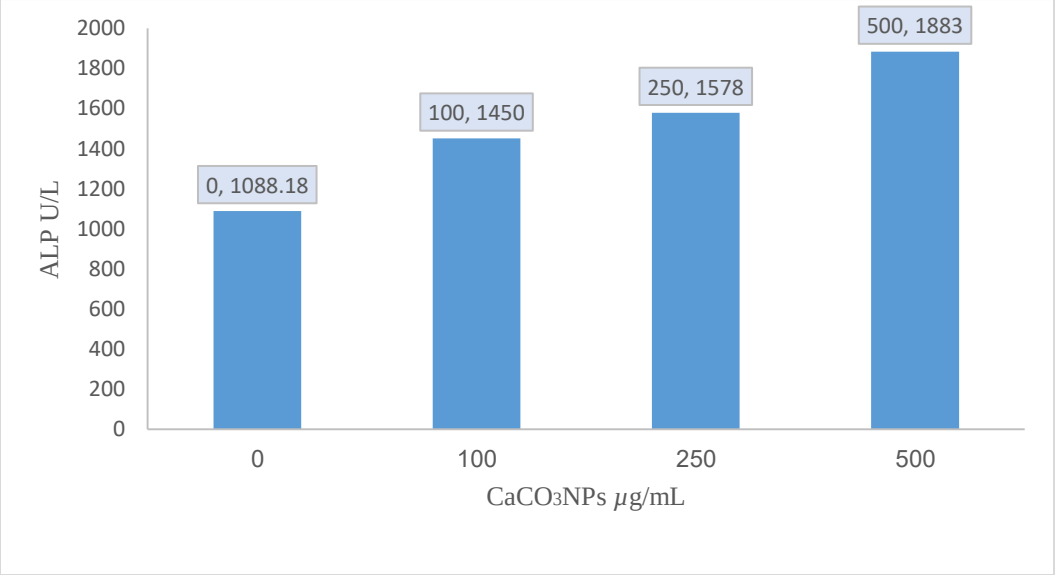


Figure (3.13) Relation between levels of alkaline phosphatase and concentrations of $CaCO_3$NPs

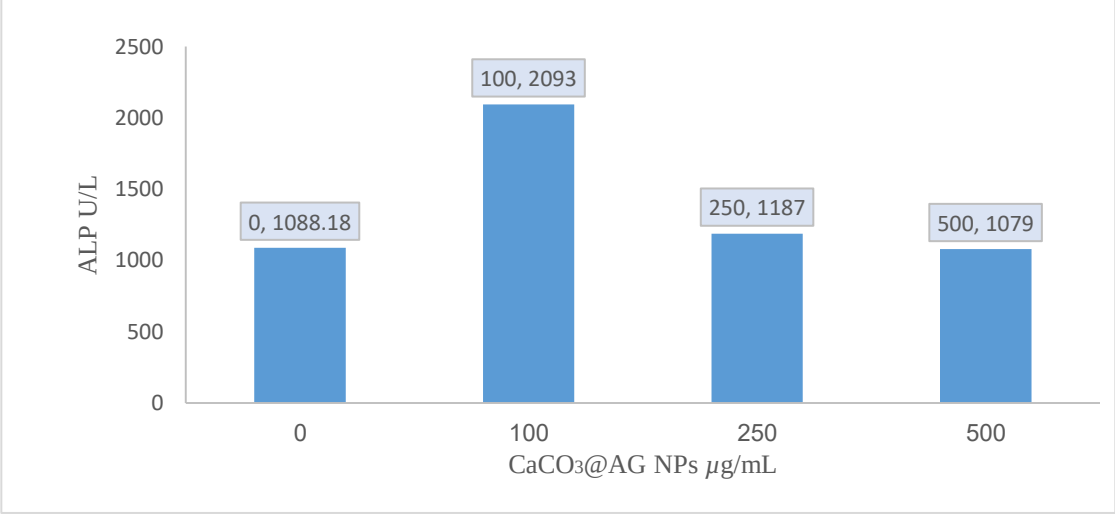


Figure (3.14) Relation between levels of alkaline phosphatase and concentrations of $CaCO_3$@AGNPs

Decrease activities of alkaline phosphatase with increase the adding amounts of $CaCO_3$NPs and $CaCO_3$@AGNPs as shown in Figures (3.13) and (3.14).

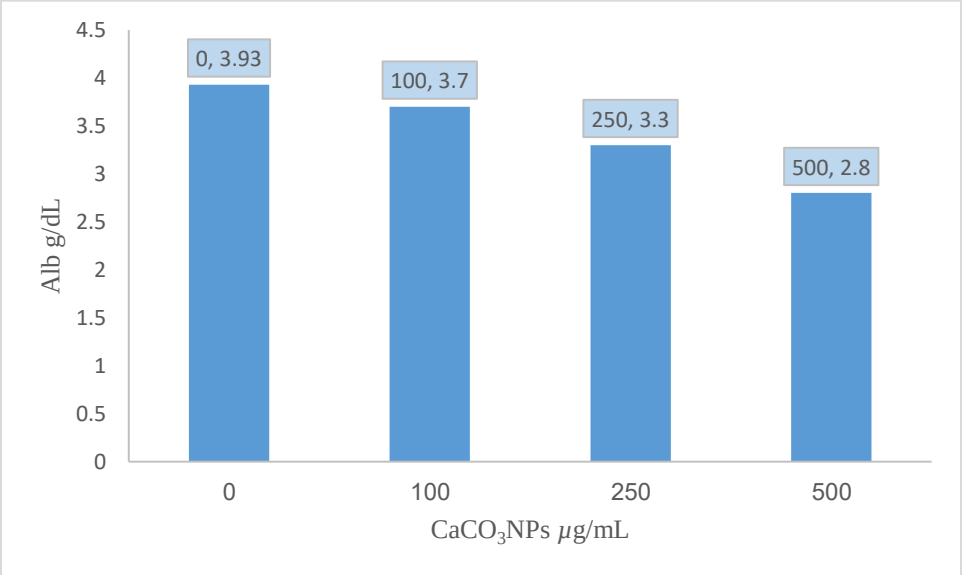


Figure (3.15) Relation between levels of albumin and concentrations of $CaCO_3$NPs

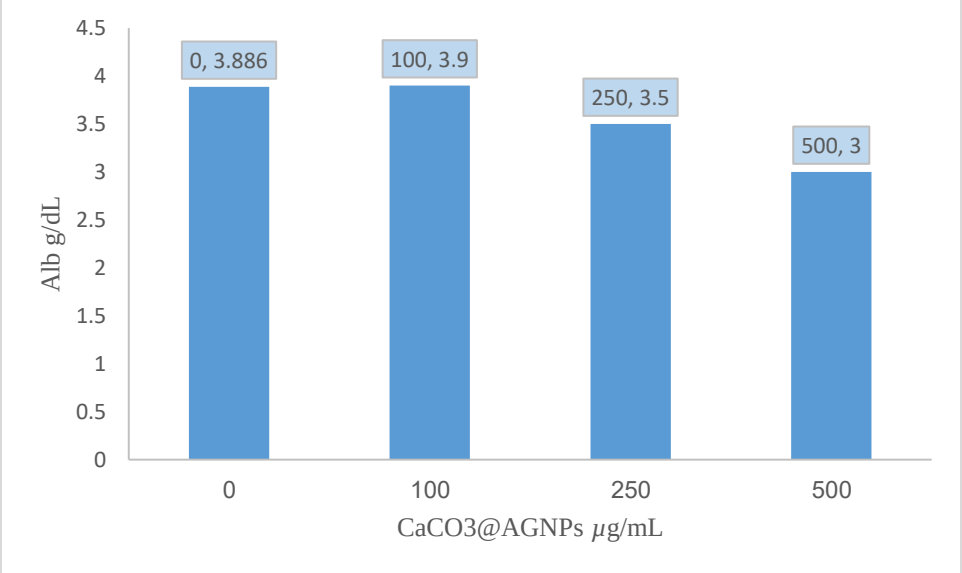


Figure (3.16) Relation between levels of albumin and concentrations of $CaCO_3$@AGNPs

Decrease levels of albumin with increase the adding amounts of $CaCO_3$NPs as shown in Figures (3.15) and (3.16)

Figure (3.17) Relation between levels of parathyroid hormone and amounts of $CaCO_3$NPs

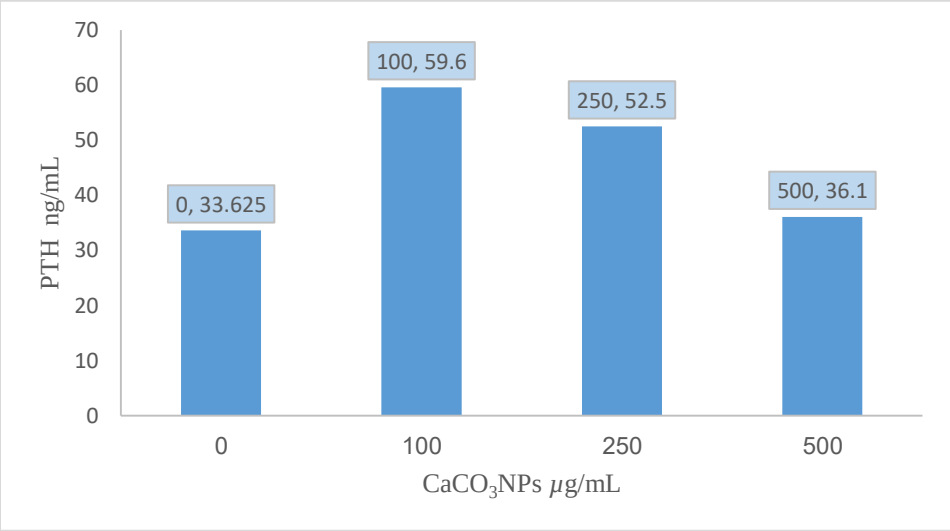
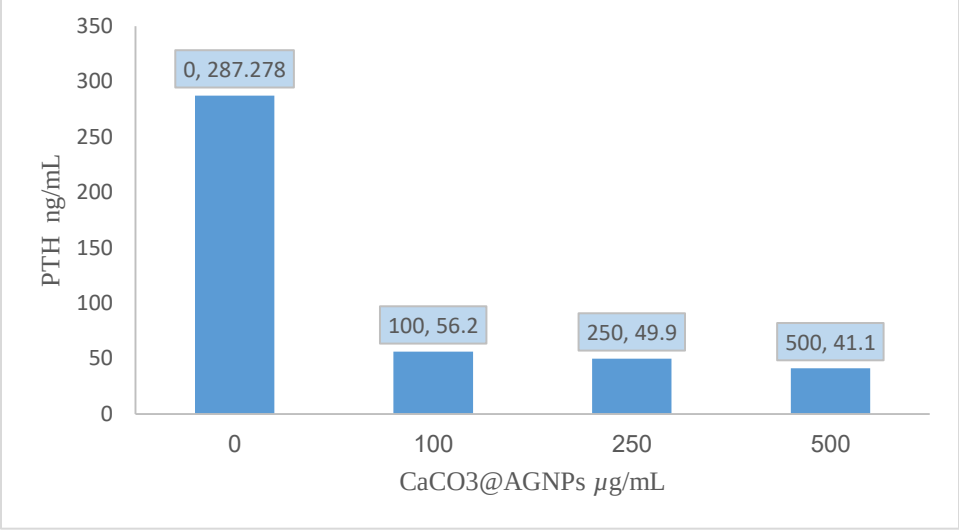


Figure (3.18) Relation between levels of parathyroid hormone and amounts of $CaCO_3$@AGNPs



From Figures (3.17) and (3.18) it is noted that the Nanocompounds have the same effect on parathyroid hormone (decrease levels of the hormone)

Decreasing levels of parathyroid hormone with increased levels of calcium [242].

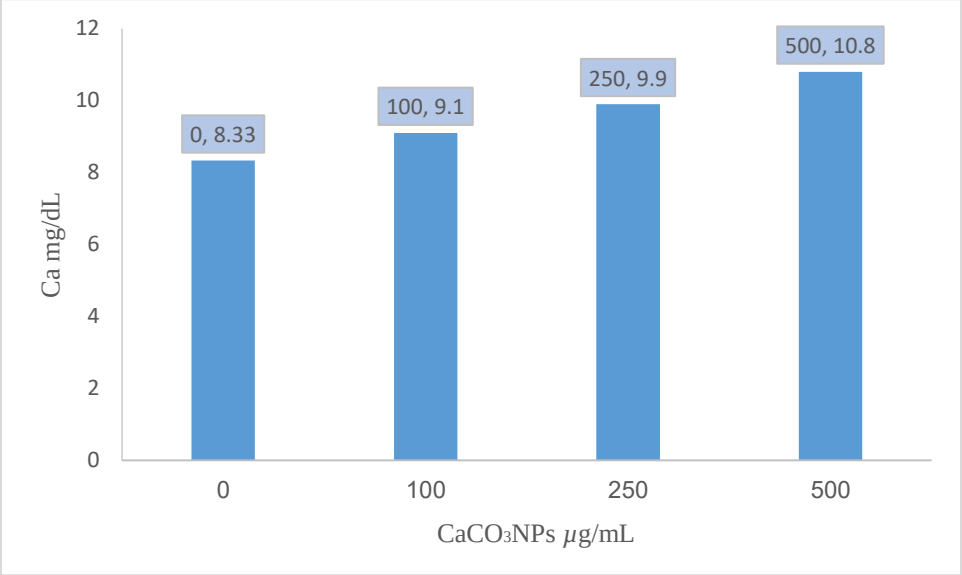


Figure (3.19). - Relation between levels of Ca^{2+} and amounts of $CaCO_3$NPs

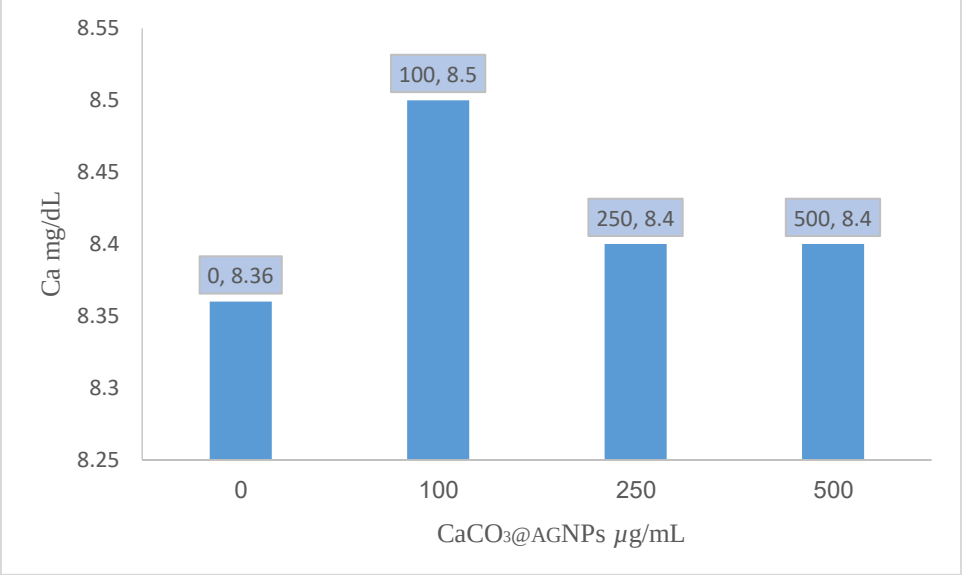


Figure (3.20) Relation between levels of Ca^{2+} and amounts of $CaCO_3$@AGNPs

From Figure (3.19), it is observed that calcium levels increase as the amounts of $CaCO_3$ NPs increases, while the increase of $CaCO_3$@AGNPs, the second compound, the calcium level does not increase much until it reaches a stable level, as appears clear in Figure (3.20). This is because the calcium in the second compound is restricted by Arabian gum.

3.7. Assay to exam hemolytic effect of nanoparticles. - `

This study included healthy non-smoking female aged 25 years old. Blood was collected via venipuncture into test tube containing heparinized human blood. Different concentrations (100,200,300,400 and 500 µg/ML respectively) of prepared compounds $CaCO_3$NPs which was labeled A1, A2, A3, A4and A5 and $CaCO_3$@AGNPs was labeled B1, B2, B3, B4, and B5(respectively) added to 0.2 mL of the blood specimen. The mixture was then thoroughly stirred for 10 seconds. To avoid severe hemolysis, 10 ml of normal saline (0.9%) was added. The resulting mixture was centrifuged at 4000xg for 10 minutes. The mixture's absorbance was measured at 540 nm. Blood has been diluted 100-fold in distilled water to achieve complete hemolysis (100%) of the sample. Normal saline was utilized as the negative control and Triton X-100 as the positive control [243].

Calculation

After the determination of absorbance, the percentage of hemolysis was calculated using the equation

% of Hemolysis = [(AT- AS) / (A 100% -AS)] * 100 %

Where. AT=Absorption of the test sample.

AS= Absorption of control (normal saline solution).

Table (3.7)

Amounts of NPs	$CaCO_3$ NPs	$CaCO_3$@AG NPs
100 μg/mL	%0.00	%0.2162
200 μg/mL	%2.405	%4.902
300 μg/mL	%3.607	%5.563
400 μg/mL	%4.432	%5.642
500 μg/mL	%5.272	%7.0305

Percentages of hemolysis

Figure 3.21 illustrates the dose-dependent hemolytic effect of $CaCO_3$NPs on red blood cells. As the concentration of $CaCO_3$NPs increased, the percentage of

hemolysis also increased. The highest hemolytic activity was observed at a concentration of 500 µg/mL, with a hemolysis percentage of 5.272%.

Notably, at lower concentrations (100-300 µg/mL), the hemolytic effect was relatively low, indicating that $CaCO_3$NPs may be biocompatible at these concentrations. However, at higher concentrations, the hemolytic effect becomes more pronounced, suggesting that caution should be exercised when using these nanoparticles in biological systems.

These findings highlight the importance of optimizing the concentration and formulation of $CaCO_3$NPs to minimize their hemolytic potential and ensure their safe and effective use.

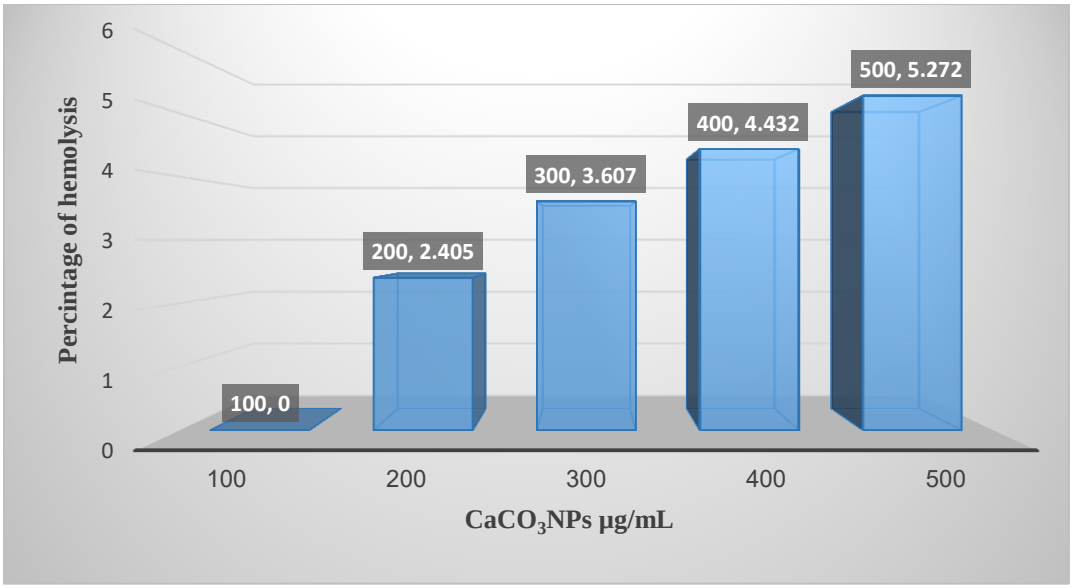


Figure (3.21): -Hemolytic effect of $CaCO_3$NPs

Figure (3.22) illustrates the dose-dependent hemolytic effect of $CaCO_3$@AGNPs on red blood cells. As the concentration of $CaCO_3$@AGNPs increased, the percentage of hemolysis also increased. The highest hemolytic activity was

observed at a concentration of 500 μg/mL, with a hemolysis percentage of 7.03%. The observed dose-dependent hemolytic effect suggests that $CaCO_3$@AGNPs can induce hemolysis at higher concentrations. This may be due to the interaction of the nanoparticles with the red blood cell membrane, leading to cell lysis and the release of hemoglobin. However, at lower concentrations, the hemolytic effect is minimal, indicating that these nanoparticles may be biocompatible and suitable for certain biomedical applications.

It is important to note that the hemolytic potential of nanoparticles can be influenced by various factors, including their size, shape, surface charge, and surface functionalization. Further studies are needed to investigate the underlying mechanisms of hemolysis induced by $CaCO_3$@AGNPs and to optimize their design for safe and effective use.

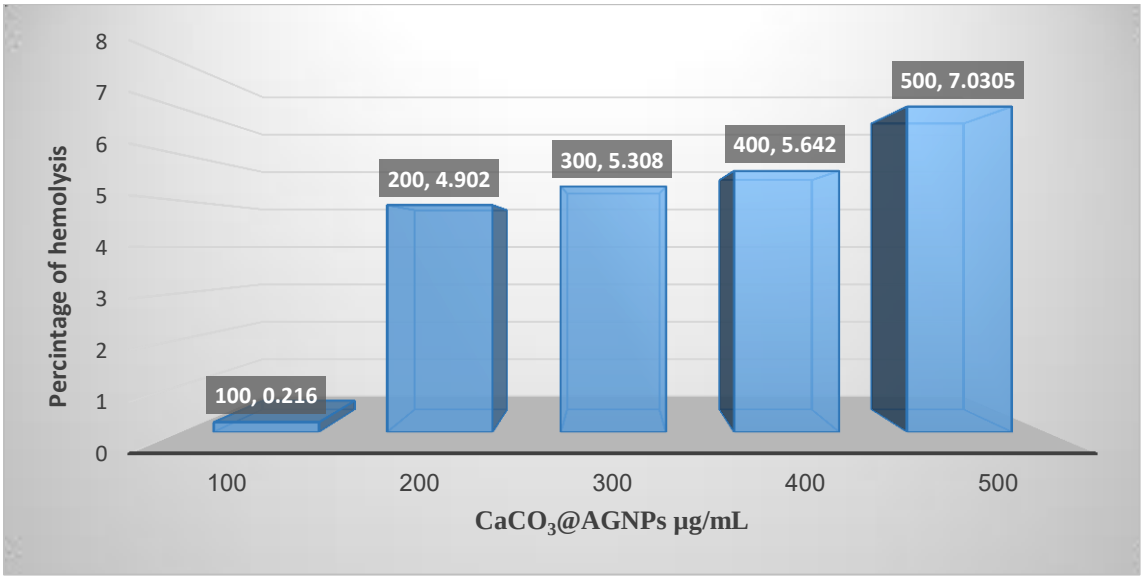


Figure (3.22) Hemolytic effect of $CaCO_3$@AGNPs

CHAPTER FOUR

CONCLUSIONS AND RECOMMENDATIONS

4.Conclusions

From all results of different variables in the current study, it could be concluded that:

1. There is a high association between and heavy metals like aluminum, lead, and cadmium, as well as essential metals like copper and zinc. This may lead to the onset and progression of neurodegenerative dementia.
2. The Synthesized Calcium Carbonate@Arabian GumNPs is very effective in reducing glucose levels. calcium carbonateNPs can provides the calcium easier than Calcium Carbonate@Arabian GumNPs**.**

5. Recommendations

1. **Further Research:** Conduct further studies to investigate the long-term effects and potential side effects of $CaCO_3$@AGNPs and $CaCO_3$NPs.
2. **Optimized Formulation:** Optimize the formulation of these nanoparticles to enhance their therapeutic efficacy and minimize potential side effects.
3. **Combination Therapy:** Explore the potential of combining $CaCO_3$@AGNPs and $CaCO_3$NPs with other therapeutic agents to achieve synergistic effects.
4. **Regulatory Approval:** Seek regulatory approval for the use of these compounds as therapeutic agents.

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الخلاصة

يعتبر مرض السكري من النوع الثاني والتهاب المفاصل العظمي من أكثر الأمراض شيوعا في العراق بشكل خاص وفي العالم بشكل عام. وهناك عوامل كثيرة تؤدي إلى الإصابة بهذين المرضين اللذين يعتبران مشكلة العصر. وتعتبر السمنة والشيخوخة من الأسباب الرئيسية لهذين المرضين بالإضافة إلى العديد من الأسباب الأخرى مثل نمط الحياة الخاطئ، وقلة ممارسة الرياضة وغيرها من الأسباب، وقد تم قياس بعض الاختبارات المتعلقة بالمرضين لتحديد مدى العلاقة بينهما تم تنفيذ الدراسة الحالية على مجموعة من الأمصال لنساء تتراوح أعمارهن بين (40 إلى 70) سنة يعانين من مرض السكري من النوع الثاني وهشاشة العظام في استشارية المفاصل في مستشفى الإمام الحسن في مدينة كربلاء المقدسة (50 امرأة مجموعة مرضى و40 امرأة يبدون بصحة جيدة كمجموعة ضابطة .الهدف من هذه الدراسة هو تقييم العلاقة بين مرض السكري من النوع الثاني والتهاب المفاصل العظمي لدى النساء العراقيات من خلال قياس بعض التحاليل الخاصة بالمرضين ودراسة تأثير كربونات الكالسيوم @ جزيئات الصمغ العربي النانوية على مصول المجموعتين. لقد تم تحضير كربونات الكالسيوم @ الصمغ العربي النانوي بطريقة الترسيب المشترك وتوصيفها عن طريق المجهر الإلكتروني المسح، وإمكانات زيتا المجهرية الإلكترونية النافذة، وحيود الأشعة السينية والتحقيق فيها بواسطة أطياف .UV−Vis-NIR

بعد قياس الاختبارت الخاصة بالمرضين (السكر النوع الثاني والتهاب المفاصل العظمي) فقد أظهرت النتائج أن هناك فرق معنوي بين مجموعة المرضى ومجموعة السيطرة في مستويات كل من الجلوكوز والسكر التراكمي وانزيم الفوسفاتيز القلوي وهرمون جار الغدة الدرقية والبروتين التفاعلي عالي الحساسية ($P<0.05$)كما وجدت فروق معنوية بين الالبومين والكالسيوم($P<0.01$) ، الالبومين ومعنوية سالبة مع هرمون جار الغدة الدرقية. وجد ان هناك اختلافات كبيرة في حساسية البروتين سي التفاعلي والفوسفورز ($P <0.05$)اما بالنسبة لتأثير كربونات الكالسيوم @ الصمغ العربي النانوية على مستوى كل من جلوكوز الدم، الكالسيوم، انزيم الفوسفاتيز القلوي، الألبومين، هرمون جار الغدة الدرقية والفوسفات حيث أظهرت النتائج انخفاض في كل من مستويات الجلوكوز وانزيم الفوسفاتيز القلوي والألبومين وهرمون جار الغدة الدرقية والفوسفات. كما لوحظ ارتفاع مستوى الكالسيوم ايضا.

Questionnaire

Number

Name

Date of sampling

Age weight Height

Duration of diabetes

Treatment of diabetes

Treatment of osteoarthritis

Other diseases

Other treatments

Test	The value
FBG	
HbA1C	
HsCRP	
Serum calcium	
Vit D_3	
ALP	
Alb	
Pi	
PTH	

Other information

جامعة كربلاء

كلية التربية للعلوم الصرفة

قسم الكيمياء

تأثير المركب النانوي كاربونات الكالسيوم @العلك العربي على امصال مرضى السكر النوع الثاني والتهاب المفاصل العظمي

رسالة مقدمة إلى مجلس كلية التربية للعلوم الصرفة / جامعة كربلاء وهي جزء من متطلبات نيل درجة الماجستير في الكيمياء

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جامعة كربلاء

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