



Impact of hypercholesterolemic diet on the Ca-regulating hormones and some minerals in male rats

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Received: May. 02, 2024	Abstract Cholesterol is a fatty substance essential for the body. However, excessive amounts can lead to health problems. The study's objective was to investigate the potential effects of a diet high in cholesterol on the hormonal regulation of calcium metabolism and the balance of essential minerals in the body. Twenty male rats aged (1.5-2) months were divided as follows, 2 groups : (10) rats were fed a normal diet, and (10) rats were fed a high cholesterol diet(2%) for 8 weeks HCD group. Blood samples were collected after the end of the experiment for physiological and biomarker parameters calculation, as well as lipid profile, minerals, hormones, and antioxidants, after the end of the experiment (8 weeks). The results showed a significant elevation in serum of serum lipid profile (TG, TC, LDL) and decreased (HDL), an elevation in serum of Malondialdehyde and (MDA) and an increase in serum calcium levels. Calcitonin levels, parathyroid hormone, and Vit.D levels showed a significant decrease in serum Glutathione (GSH) levels in the cholesterol group compared to the control group. The study concludes that a hypercholesterolemic diet can lead to Dysregulation of Ca-regulating hormones, Dysregulation of parathyroid hormone (PTH), and Calcitonin, which may disrupt the delicate balance of calcium metabolism in the body. This Dysregulation could result in increased bone resorption, altered calcium absorption and excretion, and imbalances in other minerals involved in bone health and overall physiological processes. Keywords: Hypercholesterolemic diet; osteoporosis; Ca-regulating hormones; minerals.
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Introduction

Osteoporosis is a condition characterized by low bone mass and structural deterioration of bone tissue, leading to increased bone fragility and a higher risk of fractures [1]. Ca-regulating hormones play a crucial role in the maintenance of bone health, and their Dysregulation can contribute to the development or progression of osteoporosis [2]. The chronic metabolic bone disease osteoporosis causes a decrease in bone mass, deterioration of the bone structure, and fractures and heightened vulnerability to

fractures [3] High cholesterol levels can contribute to various physiological conditions, including fat accumulation, cardiovascular disease, and Alzheimer's disease, thereby causing damage to the body [4] and also Osteoporosis and osteopenia are associated with altered bone microstructure, leading to increased fracture risks in animals, for example, low in calcium and vitamin D and excessive dieting with cholesterol [5].

Hypercholesterolemia is a multifaceted disorder that arises from various factors, encompassing both lifestyle choices and genetic predisposition. Additionally, it is a contributing factor to cardiovascular diseases (CVDs), which account for 172 million fatalities annually [6]. There is a correlation between a decrease in bone mineral density (BMD) and a high-cholesterol diet. In general, animals who are fed a high-cholesterol diet have weight loss, even if there is no substantial variation in energy consumption among the experimental animals [7]. Recent research indicates that consuming a diet high in fat, known as a high-fat diet (HFD), not only causes obesity but also triggers metabolic irregularities and the absorption of bone. This leads to a decrease in bone mass and weakened bone strength, ultimately increasing the likelihood of both spontaneous and traumatic bone injuries [8]. This suggests that excess fat is detrimental to bone health in animals [9]. Elevated levels of cholesterol, specifically low-density lipoprotein (LDL) cholesterol, have the potential to penetrate the walls of arteries and undergo oxidative modification. This modified LDL can trigger an inflammatory response and attract immune cells, leading to the formation of fatty plaques in the arteries [10] Reactive oxygen species (ROS) produced during this process can worsen inflammation and contribute to the advancement of atherosclerosis [11]. Elevated levels of lipids in the bloodstream, specifically cholesterol, In addition, reactive oxygen species (ROS) are produced and significant factors in the progression of coronary artery disease and atherosclerosis. The human body has developed a sophisticated defence mechanism involving antioxidant enzymes to mitigate the detrimental impact of different oxidizing agents. The organism is safeguarded against oxidative damage through the combined action of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) [12].

In rats, hypercholesterolemia was found to be linked to a decrease in bone density, A decrease in bone synthesis and an increase in bone resorption. In vitro investigations also showed that free cholesterol inhibited osteoclast differentiation and growth, increased malondialdehyde (MDA) levels, and reduced glutathione activity. Moreover, hypercholesterolemia elevated osteoblast activity [13,14].

The endocrine system is responsible for producing and regulating hormones in the body. Hormones play a vital role in various bodily functions, including bone growth and maintenance; three important parameters, hormones (PTH, Calcitonin & Vit D), and minerals (Ca, K, Na, P), work together to regulate the development and activation of osteoclasts. calcitonin is a major calcium-regulating factor, along with parathyroid hormones (PTH) and vitamin D [15] These hormones regulate the balance between bone formation and resorption, while minerals provide the structural components necessary for bone strength. Adequate intake of minerals, vitamins, and a balanced diet,



along with hormonal balance, exercise, and a healthy lifestyle, are essential for maintaining optimal bone health [16]

Bone is a very important organ that not only provides structural support and mobility but also acts as a storehouse of minerals such as calcium and phosphorous. The strength of the bone is a factor of genetics as well as use. To keep bones strong, constant mechanical pressure has to be applied to bones. Bones are made up of minerals and proteins, primarily, both of which are important in conferring properties to bone. Minerals alone would make the bone too brittle, and proteins alone would make it too soft and flexible. The minerals, calcium and phosphorous, are deposited as crystals of hydroxyapatite in the matrix made up of collagen, and collagen is produced by bone cells [17]. There are direct links between parathyroid hormone (PTH) and cholesterol metabolism, as vitamin D (controlled by PTH) influences the creation of cholesterol. However, they do not have a direct correlation or impact on one another. PTH largely controls the levels of calcium and phosphate, although cholesterol has its own metabolic routes and regulatory mechanisms [18] Parathyroid hormone (PTH) is a hormone that is released by the parathyroid glands, which are tiny glands located in the neck. Parathyroid hormone (PTH) plays a crucial role in regulating the levels of calcium and phosphate in the body. It impacts the morphology of the skeletal system. Regulate optimal concentrations of calcium and phosphate in the circulatory system. Parathyroid hormone (PTH) facilitates the elevation of calcium levels by stimulating the release of calcium from bones [19].

Calcitonin is a hormone that is released by the thyroid gland. The main function is to regulate the levels of calcium and phosphate in the body. Calcitonin functions to lower blood calcium levels by suppressing bone calcium release and enhancing calcium excretion through the kidneys. Calcitonin also functions to suppress the activity of osteoclasts, which are the cells responsible for the degradation of bone tissue [20]. Vitamin D is essential for maintaining skeletal health since it affects the bone mineralization process and the balance of calcium and phosphate levels. Additionally, it aids in the regulation of the parathyroid hormone [21].

Vitamin D and cholesterol metabolism are intricately connected through the same biosynthetic pathway. Cholesterol is a lipid with multiple functions. Cell membranes require it as a vital component [22]. The study aimed to determine some physiological parameters, hormones, and minerals in a Hypercholesterolemic diet in male rats.

Materials and methods

A field experiment was carried out in the spring agricultural season 2023 on 17/3/2023 in Ibn Al-Bitar Vocational Preparatory School in the Al-Husseiniyah area in the Holy Karbala Governorate, within longitude 32 and latitude 44. The area of the experimental unit was 3 m², leaving 1m² of space between the replicates and leaving 1m² between the experimental units. The plant density reached 53,333 plants he⁻¹. This study aims to study the effect of spraying nano-mortaring moringa leaf extract on some growth characteristics and yield of four varieties of maize. The split-block experiment



was carried out in a randomized complete block design (RCBD) with three replications. The main plots included four concentrations of nano-moringa leaf extract (0, 200, 400, and 600 mg L⁻¹) sprayed every two weeks until physiological maturity; during this time, the nano-moringa leaf extract was prepared following the steps described by [10]. The subplots included four varieties of maize (NadH9055, NadH362, NadH386, and NadH315).

Five were sprayed with Moringa nano extract at two concentrations of 400 and 600 mg L⁻¹, which caused a significant increase in the number of grains per row, as they recorded averages of 28.02 and 30.15 grains row⁻¹, respectively, compared to the spraying treatment with water only, which gave an average of 23.79 grains row⁻¹, which did not differ significantly from the concentration of 200 mg L⁻¹. The increase in the number of grains in the row when increasing the concentration of the Moringa nano extract can be attributed to the role of organic extracts in increasing the height of the plant and thus increasing the leaf area and thus obtaining a high photosynthesis process and transporting its products to the downstream. At the same time, the height of the plant reduces the shading of the leaves above the ear, which is reflected in an increase in the rate of pollination and fertilization, and the number of grains increases [17].

The results are shown in Table 5 that the Maize varieties differed significantly in the number of rows in the ear, as the NadH362 and NadH9055 varieties gave the highest average number of grains per row, as the recorded averages reached 32.31 and 31.02 grains row⁻¹, respectively, while the NadH386 and NadH315 varieties gave the lowest averages, amounting to 20.70 and 22.35 grains row⁻¹, in succession. The decrease in the number of grains in a row may be attributed to the long period from emergence until 50% tasselling and silking, which coincided with high temperatures, and this, in turn, affects the formation of pollen grains with high vitality or leads to the pollen grains stiffening and dying and thus failing. The fertilization process is due to the inability to grow when it falls on the stigmas [18].

The results of Table 5 showed that there was a significant interaction between spraying treatments with Moringa nano extract and Maize varieties in terms of the number of grains per row. The spraying treatment with Moringa nano leaf extract at a concentration of 600 mg L⁻¹, when mixed with the variety NadH362, gave the highest average for this trait, amounting to 35.24 grains row⁻¹, while the comparison treatment with the NadH386 variety gave a lower average for the same trait, amounting to 14.51.

Materials and Methods

Ethical approved

The study was conducted at the anatomical facility of the College of Veterinary Medicine at Kerbala University in Iraq, under the reference number (UOK.VET.PH.2023.076).



Experimental protocol

Twenty white male rats weight ($100\text{g}\pm 75\text{g}$) were used in this study and came from the College of Pharmacy at the University of Kerbala in Iraq. They ranged in age from (4–6) weeks, and the animals were housed in clean, specialized plastic enclosures. To start the experimental design, the rats were placed in a clean box compartment. We utilized a 12-hour light cycle and a relative humidity of $50\pm 5\%$. The subjects were retained for two weeks so that they could adjust to the usual testing conditions. The experiment began on September 25 and ended on November 23. In this experiment, the temperature of the room was maintained at (23-26) C° by using a room thermostat. A continuous ventilation vacuum was used to keep the air in the room changing continuously, and the animal was fed on pellets of fresh ration prepared in advance.

Experimental Design

Two groups of twenty white male rats were randomly split and given the following treatments (8 weeks). One-half of this group of rats was fed a regular meal orally as the control group, and the other half was fed a cholesterol-rich meal for eight weeks, comprising 2% cholesterol (w/w) [23].

Collect of the blood samples:

During the trial, blood samples were obtained after 8 weeks of the trial, with the animals being controlled and comfortable with ketamine and xylazine before the blood was taken. The serum was extracted from the heart using the heart puncture method; it was centrifuged for 5 minutes at 4000 revolutions per minute using a specialized gel tube. Once the serum had been separated, it was promptly transferred to Eppendorf tubes for storage -10-C° .

Serum biomarker

Serum calcitonin, Serum PHT, serum Vit D, and serum electrolyte were determined using a special Elisa kit from ELK Biotechnology China. Serum GSH and MDA were measured using ELISA kits (Fine Bio, Wuhan/China), as were lipid profiles (TC, TAG, LDL, and HDL) in the blood.

Statistical analysis

GraphPad software Prism version (8.0) was used to conduct the statistical analysis. The standard of significance for the analysis was $P < 0.05$, and the data points were reported as mean and Standard Error.

Result and Discussion

This study showed a significant increase ($P < 0.05$) of lipid profile low-density lipoproteins (LDL) in the hypercholesterolemic diet groups of rats as (18.85 ± 1.58) mg/dl compared with control groups (15.98 ± 1.93), and decreased high-density lipoproteins (HDL) in the hypercholesterolemic diet groups of rats as (35.72 ± 2.79) mg/dl compare

with control groups (42.06 ± 3.86) mg/dl respectively figure 1. Hypercholesterolemia also can lead to the accumulation of lipids, including cholesterol, in bone tissue. As a result of this lipid accumulation within the bone, normal bone structure can be disrupted, compromising the strength of the bone. As a result, the bones can be more prone to fractures [24]. In addition to hypercholesterolemia being detrimental to bone health [25], high cholesterol diets also decrease bone marrow stromal cell proliferation and differentiation, which results in decreased osteoblast genesis. Under these circumstances, the functionality and equilibrium of osteoblasts are disrupted, leading to an increase in osteoclast activity and quantity, ultimately resulting in a decrease in bone mass [26]. Concurrently, dyslipidemia, characterized by Low-density lipoproteins (LDL) and total cholesterol (TC) are elevated, coupled with reduced levels of high-density lipoproteins (HDL), heightens the susceptibility to atherosclerosis and associated cardiovascular diseases [27]. The study showed a substantial rise in blood LDL levels in those on a high-cholesterol diet in comparison to the control group, as well as there was an important reduction in the levels of HDL in the bloodstream of individuals following a cholesterol-rich meal, compared to the control group. This result may occur due to the absorbed cholesterol getting into the circulation, which may be a factor in the rise in LDL cholesterol [28]. Some studies reverse the idea that dietary cholesterol is transported in the bloodstream within lipoprotein particles called chylomicrons. As these particles are broken down, chylomicron remnants are formed, releasing cholesterol into the bloodstream. This cholesterol can contribute to an increase in LDL cholesterol [29]. Also, studies have shown that chronic conception of a high-cholesterol diet increases serum LDL. Also, cholesterol has an impact on high-density lipoprotein cholesterol (HDL-C or "good" cholesterol) levels [30], HDL cholesterol is known for its protective role in cardiovascular health, as it helps transport cholesterol away from the arteries to the liver for excretion [31], Diets high in cholesterol may contribute to inflammation and oxidative stress in the body. These factors can impact the functionality of HDL cholesterol and may reduce its effectiveness in protecting against atherosclerosis [32].

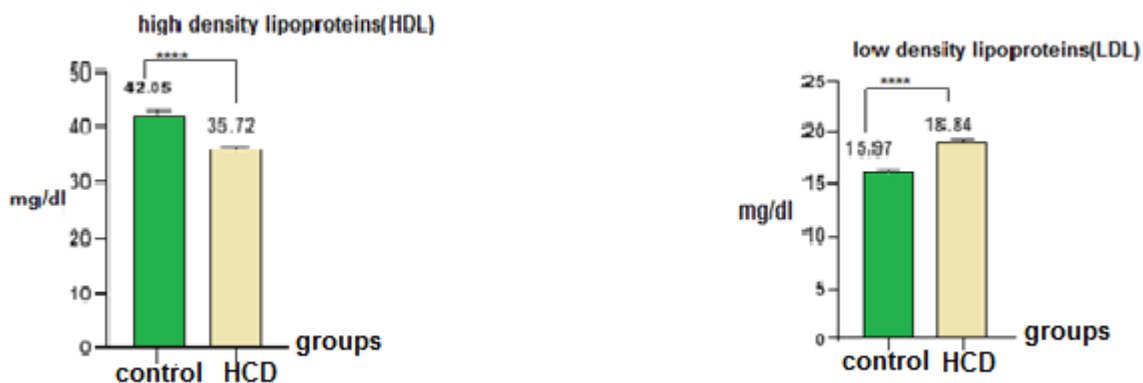


Figure (1): effect of hypercholesteremic diet on serum HDL and LDL concentrations in male rats. HDL concentration in mg/dl, LDL concentration in mg/dl; the

control group rats was a normal diet daily for 8 weeks, while hypercholesteremic group rats got 0.2 mg/kg BW of cholesterol daily for 8 weeks

There was a significant increase ($P < 0.05$) in lipid profile (TC and TAG) in hypercholesteremic diet groups of rats as compared with control groups (33.22 ± 2.45 , 38.71 ± 3.37), respectively, figure 2. In the current study, a diet high in cholesterol raised TC and TG compared to the control group. This result agrees with [33]. The study showed an increase of MDA concentration with a decrease of GSH concentration in the hypercholesteremic diet compared with control groups.

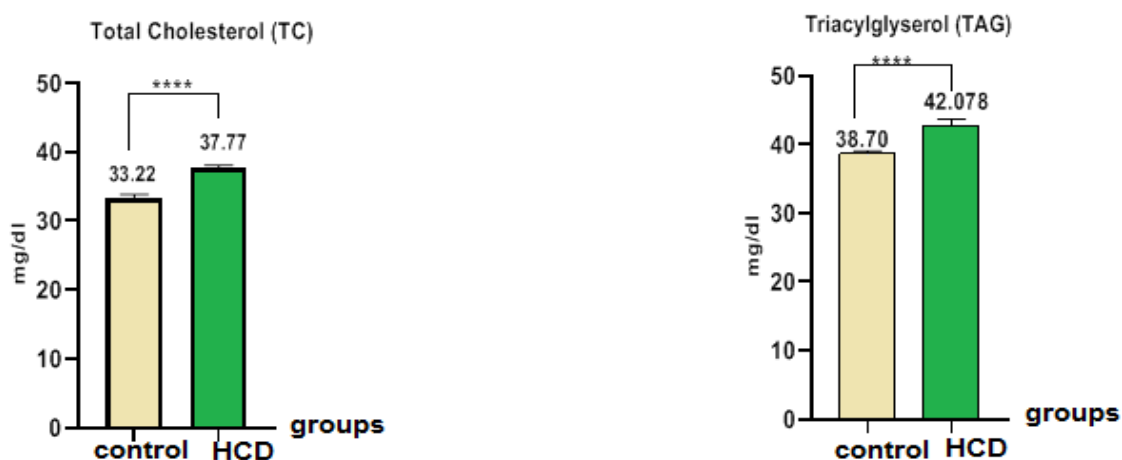


Figure (2): Effect of hypercholesteremic diet on serum TAG and TC concentrations in male rats. TAG concentration in mg/dl, TC concentration in mg/dl; the control group rats had a normal diet daily for 8 weeks, while hypercholesteremic group rats got 0.2 mg/kg BW of cholesterol daily for 8 weeks.

The study also illustrated the concentration of antioxidants in mice dosed with cholesterol was found a significant increase ($P < 0.05$) in the rate of MDA (0.5014 ± 0.083) concurrent with a significant decrease ($P < 0.05$) in the rate of GSH (19.67 ± 1.46) compare with control groups (0.3500 ± 0.046) and (23.03 ± 2.74) respectively figure (3). The study showed an increase in MDA concentration with a decrease in GSH concentration in the hypercholesteremic diet compared with control groups.

A study conducted by [34] found that GSH (glutathione) and MDA (Malondialdehyde) are both molecules associated with oxidative stress, it is associated with the onset and progression of osteoporosis [35]. Malondialdehyde has been implicated in various pathological conditions, including bone disorders. Studies have investigated the effects of MDA on bone health, particularly in the context of osteoporosis and bone metabolism [36].

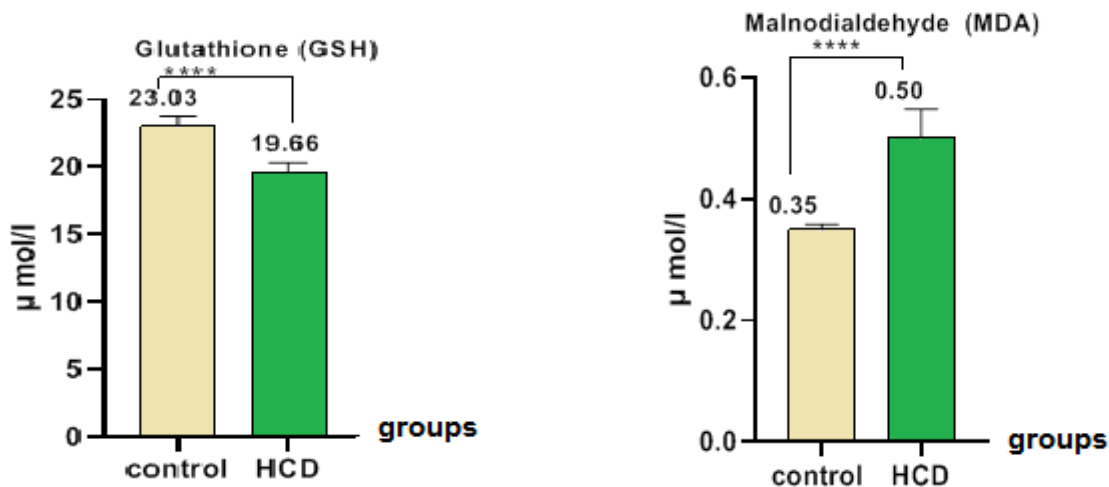


Figure (3): the effect of hypercholesteremic diet on the concentrations of malondialdehyde and glutathione peroxidase in the serum and Male rats were fed hypercholesteremic dietary treatment for 8 weeks, with both control and hypercholesteremic groups receiving 0.2 mg/kg BW cholesterol daily

The results of the current study showed no significant differences in the concentration of minerals, including potassium and sodium, in the blood of the rats under the experiment, except for calcium, where a significant increase ($P < 0.05$) was recorded (22.90 ± 1.402) mg/dl different from control groups (8.200 ± 0.57) mg/dl.

There was only a significant increase in calcium levels in rats fed with cholesterol compared to the control rats in the current study, but no significant differences were found in potassium, sodium, or phosphorus. Calcium is an essential mineral for maintaining healthy bones, and its deficiency can lead to bone loss and osteoporosis [37]. These electrolytes, such as sodium and potassium, are also important for overall health but have a lesser direct impact on bone metabolism, Sodium and potassium help maintain fluid balance and cellular function but do not have direct effects on bone mineralization [38].

The results from this study of the present examination revealed significant variations in the concentration of hormones, including parathyroid hormone and Calcitonin and vitamin D levels in the rats' blood.

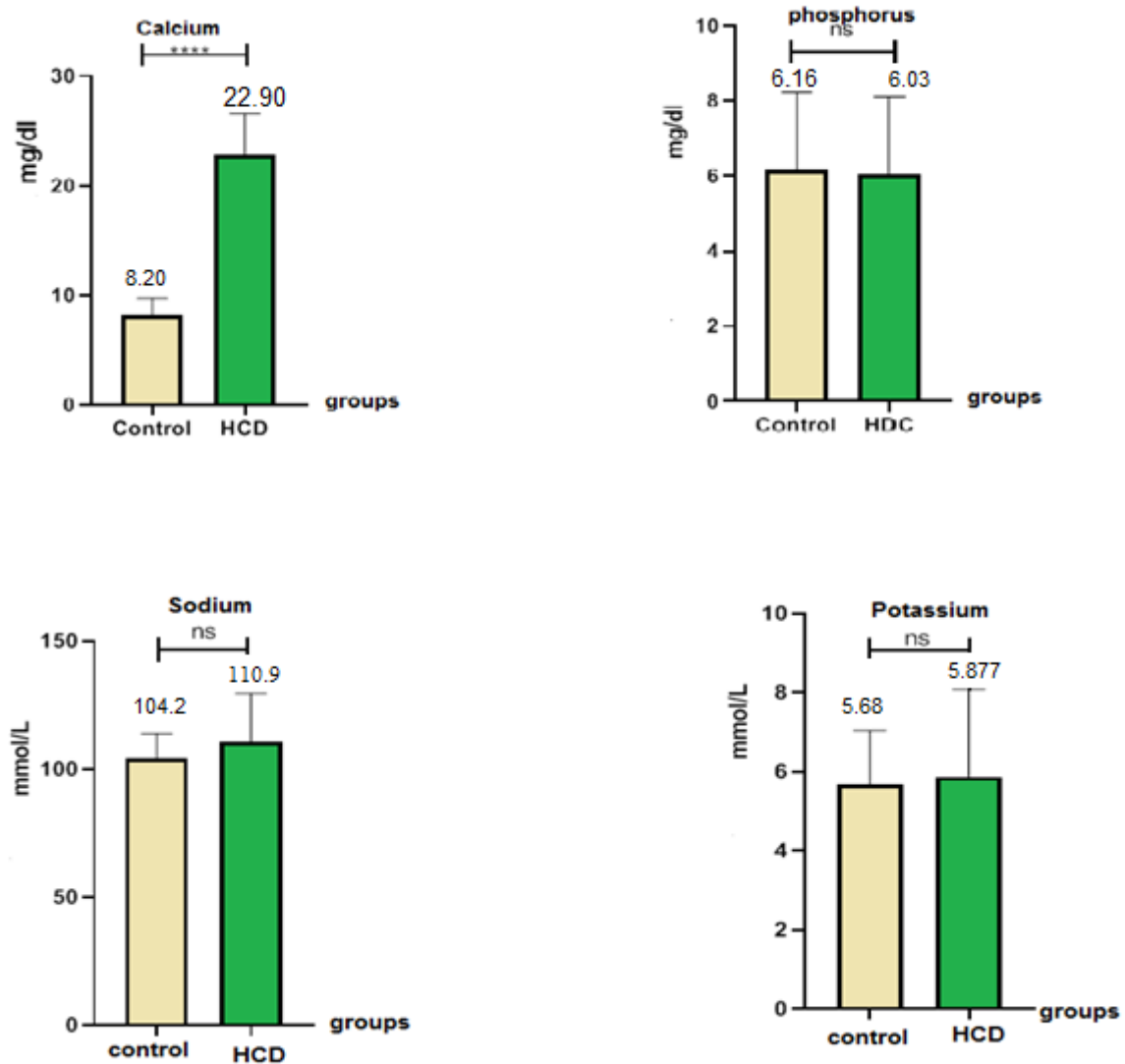


Figure (4): Sodium, calcium, and potassium calcium concentration in the serum of male rats after a hypercholesteremic diet. Control group rats were given a normal diet daily for 8 weeks. Hypercholesteremic group rats (rats received 0.2 mg/kg BW cholesterol) were given a normal diet daily for 8 weeks.

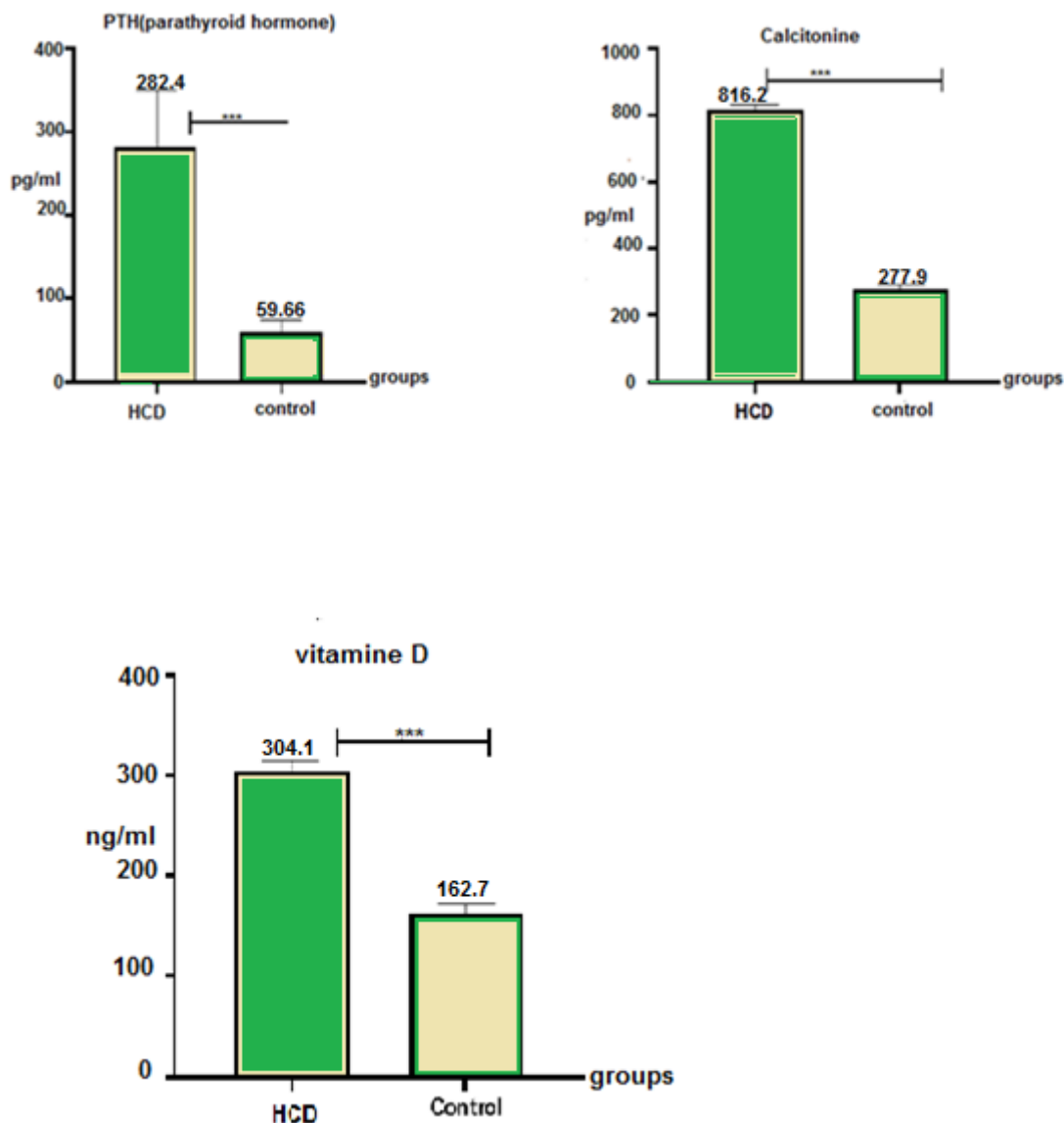


Figure (5): PTH and Calcitonin and Vit. D in male rats after hypercholesteremic diet, rats in the control group were fed normal diets daily for eight weeks, while rats in hypercholesteremic-diet (0.2 mg/kg BW cholesterol) were fed normal diets daily for eight weeks.

The results from this study of the present examination revealed significant variations in the concentration of hormones, including parathyroid hormone and Calcitonin and vitamin D levels in the rats' blood.

The present study observed a considerable elevation in the levels of parathyroid hormone (PTH), vitamin D, and Calcitonin in the cholesterol group compared to the control group. Calcium-sensing receptors (CaSR) have a vital function in controlling the release of PTH by detecting variations in the levels of calcium outside the cells and within the cells. Oxidative stress has been linked to alterations in the activity of the CaSR [39]. The CaSR is a G protein-coupled receptor that is essential for regulating

calcium balance in the body by detecting alterations in extracellular calcium concentrations. It is predominantly manifested in the parathyroid glands [40]. Oxidative stress can influence the levels of expression of the CaSR. The signalling of CaSR is regulated by intricate intracellular pathways, and the receptor's ability to convey signals in response to fluctuations in calcium levels may be impaired by oxidative stress syndrome [41]. Extended exposure to oxidative stress can potentially lead to parathyroid hyperplasia, a disorder defined by the excessive activation of the parathyroid glands. This activation may serve as a compensation reaction to oxidative damage, with the goal of maintaining sufficient production of PTH. Nevertheless, hypertrophic alterations can result in the disruption of PTH secretion, establishing a link between oxidative stress and the endocrine role of the parathyroid glands. This can disrupt the signaling cascades, resulting in an imbalance in the levels of PTH, Vitamin D, and Calcitonin in the cholesterol group compared to the control group[42].

hypercholesterolemic diet can lead to the deregulation of Ca-regulating hormones (PTH, Calcitonin, Vit.D) and minerals (Ca, Na, P, K); this Dysregulation could potentially result in increased bone resorption, altered calcium absorption and excretion, and imbalances in other minerals involved in bone health.

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