Impact of treadmill on calcium homeostasis regulators (PTH, Calcitonin, and Vitamin D3) in D-galactose-induced osteoporosis in Rats

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Abstract

Osteoporosis is characterized by bone loss density and deterioration of bone tissues, with osteoclasts typically being the only cells capable of resorbing bone. In this experiment, we aimed to investigate the effects of physical exercise, specifically treadmill use, on osteoclast activity. We used 24 male rats, randomly divided into three groups: the control group (C), the osteoporotic rats given daily 200mg/kg BW for 8 weeks in the D-gal group, and the rats in the third group given daily 200mg/kg BW with a Treadmill 25m/h daily for 5 days weekly in 8 weeks (D-gal + treadmill) group. Serum blood was drawn for biochemical analysis of PTH, Vit D, calcitonin, and minerals (Na, Ca, K, P). The results showed a significant increase of serum PTH, Vit D, calcitonin & Ca in the osteoporotic rats compared with other groups, while serum Na and K showed a significant decline in the osteoporotic rats group as compared with other groups. The impact of the treadmill was observed in a significant decrease in the level of serum PTH, Vit D, calcitonin & Ca compared with the control group. This suggests that the treadmill may have a regulatory effect on these calcium homeostasis regulators. The study concludes that D-gal affects the bone of the experimental anime by causing an imbalance in the hormones that are related to the bone and causing an imbalance in the serum mineral, which plays a critical role in Osteoporosis.

Keywords: osteoporosis, male rats, treadmill, PTH, Vit D, calcitonin.

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Introduction

Low bone density and micro architectural degradation of bone tissue cause osteoporosis, a systemic skeletal condition that increases the risk of fractures due to the brittleness of the bones [1]. There is a high rate of morbidity and mortality linked to osteoporosis fractures. The delicate balancing act of osteoblastic bone synthesis and osteoclastic bone resorption is essential for maintaining a healthy bone mass [2, 3]. When this equilibrium is disturbed, bone diseases such as osteoporosis can manifest [4]. Together, three crucial parameters—hormones (PTH, calcitonin, and vitamin D) and minerals (calcium, potassium, sodium, and phosphorus)—control the maturation and activity of osteoclasts. Along with vitamin D and parathyroid hormone (PTH), calcitonin is a key component in calcium regulation [5]. Bones are crucial organs because
they store minerals like calcium and phosphorus and give structural support and movement. Bone strength is influenced by both heredity and daily activity. Applying consistent mechanical pressure on bones is essential for their strength maintenance. Minerals and proteins are the main components of bones, and they play crucial roles in giving bones their characteristics. Bone would be too fragile if it were composed only of minerals, and too malleable and squishy if it were composed only of proteins. Collagen is a protein that bone cells make. It is in this matrix that the minerals calcium and phosphorus are deposited as hydroxyapatite crystals [6].

D-galactose is not commonly used as a direct food ingredient in the food industry, but it may be used indirectly in the manufacturing processes of some food products. For example, in Dairy Products: In the manufacturing processes of some dairy products, D-galactose may be used as an energy source for the bacteria used in fermentation, such as in yoghurt production. Another use in Pharmaceutical Products: D-galactose can be used in the manufacturing of some pharmaceutical products, such as dietary supplements or medicines containing sugar compounds, and in Specialty Food Products: Some speciality food products with specific nutritional compositions may use D-galactose as an ingredient, but this is not as common as other sugars such as sucrose [7].

A lot of animal studies have used D-galactose [D-Gal] to induce osteoporosis because of its effects on ageing and oxidative stress [7,8]. Overdosing on exogenous D-galactose might hasten the ageing process by triggering oxidative stress, apoptosis, and inflammation in various organs [9]. An increase in porosity along the periosteal surface, rather than the neocortical surface, causes a greater loss of strength in long bones [10]. This is why the bone cortex is more porous with age, leading to an increase in surface area but a decrease in strength.

The benefits of exercise and physical activity on bone formation, including increased size, density, and strength, are long-lasting [11]. Bone remodelling is an ongoing process that is accelerated by treadmill exercise. Bone remodeling is a collaborative effort between osteoblasts, which create new bone, and osteoclasts, which break down damaged or old bone[12]. This study uses a rigorous experimental design that incorporates D-Gal induction to simulate accelerated ageing and osteoporosis. Treadmill activity is considered as a possible moderator in this context. To better understand bone homeostasis and to find innovative ways to prevent and treat osteoporosis, it is necessary to comprehend the molecular mechanisms that underlie the effect of treadmill exercise on osteoclast activity [13].

Materials and Methods
Ethical approve
Under the reference number UOK.VET. PH.2023.075 This study was conducted at the Kerbala University/ College of Veterinary Medicines in Iraq's anatomical facility.
Methodology for an experiment

The current study utilized twenty-four male rats with an average body weight of 190g ± 210g. The rats were housed in clean, comfortable plastic cages and given enough ventilation. They were exposed to light for 12 hours per day and maintained a relative humidity of 50±5%. They were held for two weeks to acclimatise to the typical experimental setting.

Designing experiments

For sixty days, twenty-four rats were randomly assigned to one of three groups, with eight rats per group. The rats were treated as follows:

For the first group of rats, which served as a control, 0.2 ml of normal saline was injected intraperitoneally. The rats in the D-gal group received 200 mg/kg B.W. of D-galactose, while the rats in the D-gal+ treadmill group received the same injections plus the added benefit of running on a treadmill for 25 m/min, five days a week, for an hour, for eight weeks.

The process of drawing blood

The animals were given ketamine and xylazine to help them relax and regulate themselves before blood samples were taken, following an overnight starvation period. The experiment lasted sixty days. While the animal was lying on its back, a sterile medical syringe containing five millilitres of blood was inserted into its heart through a direct cardiac puncture. The serum was centrifuged at 3000 r/min for 5 minutes in a separate gel tube that did not include an anticoagulant. After transferring the serum to Eppendorf tubes, it was frozen at -20 °C until the measurements were finished.

Serum biomarker

Serum calcitonin, Serum PHT, serum Vit D & serum electrolyte were determined by use of a special Elisa kit from ELK biotechnology China.

Data analysis statistics

Statistical analysis of data for experiments in the present study was performed by prism V8.0 on the basis of one way, analysis of variance (ANOVA) using significant level of (P<0.05).

Results and Discussion

In the current study, there was a significant increase in the (PTH, Vit D & calcitonin) levels in the serum of the D-gal rats group as compared with the other groups. This result may occur due to Oxidative Stress-Induced DNA Damage; ROS generated during oxidative stress can inflict damage on cellular DNA. Parathyroid gland cells may be vulnerable to such genetic alterations, potentially impacting the expression of genes involved in PTH synthesis and release. Oxidative stress-induced changes in DNA
Integrity could lead to long-term consequences for PTH regulation, adding another layer to the complex relationship between oxidative stress and the endocrine system [14.15,16]. Figure 1 shows that the calcitonin levels in the d-gal group were significantly higher (739.58 ± 20.24) than in the control group (200.76 ± 3.33), with a p-value of less than 0.05. Nonetheless, as illustrated in figure [2], the d-gal group exhibited a significantly higher [PHT] at 201.55 ± 7.02 in comparison to the control group's [44.136 ± 3.66].

The serum vitamin D concentration in the d-gal group was found to be considerably higher (P<0.05) than in the control group (303.48 ± 4.12 vs. 140.5 ± 3.96), as shown in Figure (3) of the present study.

The third type of electrolyte is serum; as illustrated in Figure (4), the d-gal group had a significantly reduced serum Na concentration [81.05±5.46] compared to the control group [100.10±7.806] (P < 0.05). Nevertheless, as illustrated in Figure [5], the serum calcium concentration substantially rose in the d-gal group [21.66 ± 1.20] when contrasted with the control group [10.72 ± 0.52]. However, there was no statistically significant difference in serum P concentration between the d-gal and control groups (see Figure 6).

The serum K concentration in the d-gal group was found to be considerably lower [4.77 ± 0.31] compared to the control group [6.17 ± 0.26] (Figure 7, current study, P<0.05).

Figure (1): effect of 200mg/kg of D-gal and 25m/min for 1hr/5 days treadmill sport for 8 weeks on the serum Calcitonin concentration on male rats.

The effect of 200 mg/kg of D-gal and 25 m/min for 1 hour, five days of treadmill sport on the serum calcitonin concentration in male rats was observed in Figure (1) throughout an 8-week period. The serum calcitonin concentration was significantly different in the D-gal-treated group compared to the C group. Results are presented as
the average plus or minus the standard error (n=5/group), with a significance level of (****P < 0.05). Consider of C as the control group. For eight weeks, rats were given D-gal at a dosage of 200 mg/kg body weight daily. Rats were given 200 mg/kg B.W./day of D-gal and then subjected to a treadmill test for 8 weeks at a speed of 25 m/min, five days a week.

Figure (2): Effect of 200mg/kg of D-gal and 25m/min for 1hr/5 days treadmill sport for 8 weeks on the serum PTH concentration on male rats

The effect on serum PTH concentration of 200 mg/kg of D-gal and 25 m/min for 1 hour, five days of treadmill exercise for eight weeks in male rats. The serum PTH concentration was significantly different in the D-gal-treated group compared to the C group. The values are shown as the mean plus or minus the standard error, with n=5 in each group, and the significance level is ****< 0.05. Consider of C as the control group. For eight weeks, rats were given D-gal at a dosage of 200 mg/kg body weight daily. Rats were given 200 mg/kg B.W./day of D-gal and then subjected to a treadmill test for 8 weeks at a speed of 25 m/min, five days a week. Prolonged exposure to oxidative stress may contribute to parathyroid hyperplasia, a condition characterized by the enlargement of the parathyroid glands [17,18,19] This enlargement could be a compensatory response to oxidative damage, aiming to maintain adequate PTH production. However, the hypertrophic changes may lead to dysregulation of PTH secretion,
further complicating the relationship between oxidative stress and the endocrine function of the parathyroid glands [20, 21].

![Bar Chart](image)

**Figure (3): Effect of 200mg/kg of D-gal and 25m/min for 1hr/5 days treadmill sport for 8 weeks on the serum D3 concentration on male rats.**

This is the effect on male rats' serum vitamin D concentration after 8 weeks of treadmill exercise at 25 meters per minute for 1 hour, five days and 200 milligrams per kilogram of D-gal. The serum vitamin D concentration in the D-gal-treated group was significantly different from that of the C group. Results are presented as the average plus or minus the standard error (n=5/group), with a significance level of (**P < 0.05). Consider of C as the control group. For eight weeks, rats were given D-gal at a dosage of 200 mg/kg body weight daily. Rats were subjected to a combination of D-gal (200 mg/kg B.W./day) and treadmill sport (25 m/min, 5 days a week, for 8 weeks) in an experiment.
Figure (4): Effect of 200mg/kg of D-gal and 25m/min for 1hr/5 days treadmill sport for 8 weeks on the serum Na concentration in male rats

Compared to the control and D-gal + treadmill groups, the treated group D-gal had a significantly different serum Na concentration. All values are shown as the mean plus or minus the standard error, with n=5 per group, and **P < 0.05. Consider of C as the control group. For eight weeks, rats were given D-gal at a dosage of 200 mg/kg body weight daily. Rats were subjected to a combination of D-gal (200 mg/kg B.W./day) and treadmill sport (25 m/min, 5 days a week, for 8 weeks) in an experiment. Also, the current study shows a significant decrease in serum Na concentration in the treadmill group compared to the other group. This result may occur due to the effect of the treadmill to increase sweating in the rats, and the sweat contains an amount of Na in it and this leads to a decrease in the amount of serum Na. On the other hand, the d-gal group show a significant decrease in the Na as compared with the control group, this result may occur due to the ability of d-gal to increase of the oxidative stress in the kidney blood vessels that lead to decrease the Na reabsorption from the kidney [22,23] Also Mitochondrial dysfunction due to high oxidative stress in renal cells may impact sodium transport mechanisms. Oxidative stress can influence the redox state of proteins, including ion transporters in the kidney. Some sodium transporters are sensitive to changes in redox status, and alterations in their activity may affect sodium reabsorption [24,25,26].
Figure (5): Effect of 200mg/kg of D-gal and 25m/min for 1hr/5 days treadmill sport for 8 weeks on the serum Ca concentration in male rats.

The serum calcium concentration was significantly different in the D-gal treatment group compared to the C and D-gal + treadmill groups. Data are presented as the average plus or minus the standard error (n=5/group), and the significance level is p<0.0001. Consider of C as the control group. For eight weeks, rats were given D-gal at a dosage of 200 mg/kg body weight daily. Rats were subjected to a combination of D-gal (200 mg/kg B.W./day) and treadmill sport (25 m/min, 5 days a week, for 8 weeks) in an experiment. Calcium-sensing receptors (CaSR) play a crucial role in regulating PTH secretion by sensing changes in extracellular calcium levels. Oxidative stress has been linked to alterations in CaSR function. D-galactose-induced oxidative stress may modulate the sensitivity of CaSR in the parathyroid glands, affecting their ability to respond appropriately to changes in calcium concentrations. This, in turn, could impact the finely tuned regulation of PTH release in response to calcium homeostasis [17,18,19]. On the other hand, the serum Ca level of the d-gal group showed a significant increase as compared with the other group. This result may occur due to the imbalance of the Ca hormones in the body (PTH & calcitonin), and this leads to an increase Ca imbalance between the bone and the body, which leads to increased serum Ca[20,21] also the calcitonin hormone increase the activity of the osteoclast cells which lead to destruction of the osteocyte and this increase serum Ca level, inflammation due to increase the oxidative stress also case necrosis in the osteocyte that lead to release the Ca into the bloodstream which play a role in the increase the serum Ca consecration in the D-gal treated rats [22,23].
Figure (6): effect of 200mg/kg of D-gal and 25m/min for 1hr/5 days treadmill sport for 8 weeks on the serum p concentration on male rats

Comparing the D-gal treated group to the C and D-gal + treadmill groups, we found no statistically significant difference in serum p concentration. The results are shown as the average plus or minus the standard error (n=5/group). Consider of C as the control group. For eight weeks, rats were given D-gal at a dosage of 200 mg/kg body weight daily. Rats were given 200 mg/kg B.W./day of D-gal and then subjected to a treadmill test for 8 weeks at a speed of 25 m/min, five days a week.

Figure (7): Effect of 200mg/kg of D-gal and 25m/min for 1hr/5 days treadmill sport for 8 weeks on the serum k concentration on male rats
Notably, the serum K concentration differed significantly in the D-gal treatment group compared to C a. (*p<0.031), but there were no significant differences with the D-gal treadmill group. The results are shown as the average plus or minus the standard error (n=5/group). Consider of C as the control group. For eight weeks, rats were given D-gal at a dosage of 200 mg/kg body weight daily. In the 8-week study, rats were given 200 mg/kg B.W./day of D-gal and then subjected to a treadmill test at 25 m/min, five days a week. However, in contrast, serum concentration of K in the D-gal group shows a significant decrease as compared with the other group. The K concentration is insoulted with the Na, so when there is a decrease in the Na in the body due to disorder, the K level will also decrease. Since d-gal leads to damage to the blood vessels in the kidney, this leads to the excretion of minerals from the body, such as sodium and potassium, and this explains the reason for their deficiency in the process of complete reabsorption of the excreted materials [27,28].

The d-gal case imbalances the Ca-related hormones (PTH & calcitonin), which lead to imbalances in the calcium and minerals in the body.

Reference


