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جامعة عين شمس، كلية التربية النوعية، القاهرة، مصر

تحية طيبة وبعد،،،

يسر **معامل التأثير والاستشهادات المرجعية للمجلات العلمية العربية (ارسيف – ARCIF)**، أحد مبادرات قاعدة بيانات **"معرفة"** للإنتاج والمحتوى العلمي، إعلامكم بأنه قد **أطلق التقرير السنوي التاسع للمجلات للعام 2024**.

ويسرنا تهنئتكم وإعلامكم بأن **المجلة المصرية للدراسات المتخصصة** الصادرة عن **جامعة عين شمس، كلية التربية النوعية، القاهرة، مصر، قد نجحت في تحقيق معايير اعتماد معامل "ارسيف Arcif"** المتوافقة مع المعايير العالمية، والتي يبلغ عددها **(32) معياراً**، وللاطلاع على هذه المعايير يمكنكم الدخول إلى الرابط التالي: http://e-marefa.net/arcif/criteria/

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كما صُنفت مجلتكم في **تخصص العلوم التربوية** من **إجمالي عدد المجلات (127)** على المستوى العربي ضمن الفئة **(Q3)** وهي الفئة الوسطى ، مع العلم أن **متوسط معامل "ارسيف" لهذا التخصص** كان **(0.649)**.

وبإمكانكم الإعلان عن هذه النتيجة سواء على موقعكم الإلكتروني، أو على مواقع التواصل الاجتماعي، وكذلك الإشارة في النسخة الورقية لمجلتكم إلى معامل **"ارسيف Arcif"** الخاص بمجلتكم.

ختاماً، نرجو في حال رغبتكم الحصول على شهادة رسمية إلكترونية خاصة بنجاحكم في معامل " **ارسيف** "، التواصل معنا مشكورين.

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محتويات العدد

الجزء الثانى :

أولاً : بحوث علمية محكمة باللغة العربية :

The effect of different mixtures rich in (Vitamin D, calcium, Phosphors) on Vitamin D and bone health in female experimental rats

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Abstract

This study aimed to determine the effect of a different mixture rich in vitamin D, calcium, and phosphorus on bone health in female rats hat were intentionally induced into menopause. Five groups of mice were used. Negative group: They were fed the basal on a diet only, while the rest of the mice were given VCD doses for menopause in female mice. Positive group: fed only on diet without any treatments. While the third, fourth and fifth groups were fed a mixture of nutritional ingredients that affect bone health. The results indicated that consuming the mixture led to a significant increase ($P<0.05$) in vitamin D.

Keywords: Rats, Vitamin D, DEXA scans and Histopathology of bone.

ملخص:

العنوان : تأثير خلطات مختلفة غنية بفيتامين د والكالسيوم والفوسفور على نسبة فيتامين د وصحة العظام في إناث فئران التجارب

المؤلفون : أسامة السيد مصطفى ، عبور محمد محمد عبد الرحمن ، صفاء مصطفي عبد الفتاح فايد ، سلمى شاكر غنيم محمد

تهدف هذه الدراسة إلى قياس مدى تأثير خلاطات غنيه بفيتامين د والكالسيوم والفوسفور على صحة العظام لدى إناث الفئران التي تم وصولها عمداً لسن اليأس باستخدام VCD. حيث تم استخدام خمس مجموعات من الفئران. المجموعة السلبية : تم تغذيتها بالنظام الغذائي الأساسي فقط، بينما أعطيت بقية الفئران جرعات لايحداث انقطاع الطمث لدى إناث الفئران. المجموعه الضابطه الموجبه تم تغذيتها على الغذاء الاساسيفقط دون أي علاجات. بينما تمت تغذية المجموعة الثالثة والرابعة والخامسة بخليط من المكونات الغذائية التي تؤثر علي صحة العظام. حيث أشارت النتائج إلى أن تناول الخليط أدى إلى زيادة معنوية ($P<0.05$) في فيتامين د.

الكلمات الدالة : الفئران، فيتامين د، ديكسا و التشريح المرضي للعظام.

INTRODUCTION

Vitamin D level has gained rowing interest as it is known to have an important role in the overall human body health and protect against many diseases such as osteomalacia, osteoporosis, cancer and cardiovascular diseases. Most of the determinants of vitamin D deficiency are modifiable and can be prevented by lifestyle improvement (**Sizar, *et al.*,2018).**

A skeleton depends on the continuous renewal and maintenance of the bone tissue. The process of bone remodeling is highly controlled and consists of a fine-tuned balance between bone formation and bone resorption. Biochemical markers of bone turnover are already in use for monitoring diseases and treatments involving the skeletal system, but novel biomarkers reflecting specific biological processes in bone and interacting tissues may prove useful for diagnostic, prognostic, and monitoring purposes. The Wnt-signaling pathway is one of the most important pathways controlling bone metabolism and consequently, inhibitors of the pathway such as sclerostin and Dickkopf-related protein 1 (DKK1) have crucial roles in controlling bone formation and resorption. Thus, they might be potential markers for clinical use as they reflect a number of physiological and pathophysiological events in bone and in the cross-talk with other tissues in the human body **(Li *et al.*, 2018).**

Calcium (Ca) is an essential and critical component for human health. This element takes part in various biological processes, such as nerve conduction, muscle contraction, bone structure, signal transduction, regulation of hormonal secretion, and vascular activities **(Li *et al.*, 2018).** About ninety-nine percent of the calcium contained in the human body is stored in the skeleton in the form of hydroxyapatite and other calcium salts. Calcium intake is of utmost importance during the early stages of life, since the increase in bone mass takes place up to the age of 30 **(Włodarek *et al.*, 2014).** Calcium is a mineral involved in a large number of vital functions **(WHO.2004, &Ross, *et al.*,2011).**

Vitamin D is a fat-soluble vitamin that is naturally present in a few foods, added to others, and available as a dietary supplement. It is also produced endogenously when ultraviolet (UV) rays from sunlight strike the skin and trigger vitamin D synthesis. Vitamin D obtained from sun exposure, foods, and supplements is biologically inert and must undergo two hydroxylations in the body for activation. The first hydroxylation, which occurs in the liver, converts vitamin D to 25-hydroxyvitamin D [25(OH) D], also known as "calcidiol." The second hydroxylation occurs primarily in the kidney and forms the physiologically active 1, 25-dihydroxyvitamin D [1,25(OH)2D], also known as "calcitriol" **(Institue of Medicine, 2010).**

The objective of this work was to study the effect of different mixtures rich in (Vitamin D, calcium, Phosphors) on vitamin D and bone health in female experimental rats

Materials and methods:

Materials:

1- Basel diet ingredients were purchased from good-quality materials (Al - Gomhouria Company for Trading Drugs, chemicals, Medical Instruments, Cairo, Egypt). The basel diet of rats was prepared according to **(AOAC, 2020).**

2- Animals: Forty female albino rats weighing 180 ± 5 g were obtained from the animal house colony of the National Research Center, Dokki, and Cairo, Egypt. The animals were divided into five groups of eight rats in Each group

3- Kefir milk and whale liver oil was obtained from Emosha 's oraganic food company.

4- Eggs (white egss). Soy milk, yogurt almarai and orange juice were purchased from Carrefour Market in Egypt.

5- The experimental rats were injected with Injected with (VCD) 4-vinylcyclohexene dioxide was obtained from Nile

Pharmace Company in Cairo, Egypt. The expermentah rats injected by ((160 mg/kg/day in sesame oil, i.p.) for 15 days.

6- The experimental rats were injected with Injected with (VCD) 4-vinylcyclohexene dioxide was obtained from Nile Pharmace Company in Cairo, Egypt. The expermentah rats injected by ((160 mg/kg/day in sesame oil, i.p.) for 15 days.

Methods:

Biological experiment design:

The VCD mouse model of menopause requires repeated daily injections of VCD (160 mg/kg/day in sesame oil, i.p.) to cause selective loss of primordial and primary follicles in the ovary via the direct inhibition of auto phosphorylation of the survival receptor c-kit, located on the plasma membrane of the oocyte **(Mark-Kappeler,2011).** Within 15 days after the cessation of daily dosing, VCD had depleted all primordial follicles. During this time frame of impending ovarian failure, there is an increase in cycle length, estrogen levels fluctuate until they reach very low levels, and FSH levels increase as the inhibitory effects of estrogen are removed, thus mimicking per menopause in humans**.**

Forty adult female albino rats weighing 180 ± 5 g will be used in the current investigations. Animals were maintained under standard conditions according to **(El Okda *et al.*, 2016),** Rats were acclimatized to the diet provided for one week before starting the experiment, and then the rats were divided into five groups of forty animals eight rats each as follows:

Group 1: control (-): Feed on a basal diet only.

Group 2: control (+): fed on a basal diet, and injected with VCD (160 mg/kg/day in sesame oil, ip)

Group 3: Fed on a basal diet, and injected with VCD (160 mg/kg/day in sesame oil, i.p.) and administered (yogurt 5 g, eggshell 6 g, orange juice 200 ml, Cod liver oil 80 microliters per day and tap water to reach 1000 ml) daily for 6 weeks.

Group 4: Fed on a basal diet, and injected with VCD (160 mg/kg/day in sesame oil, i.p.), and administered (kefir 10 g, eggshell 6 g, orange juice 200 ml, cod liver oil 80 microliters per day and tap water to reach 1000 ml) daily for 6 weeks.

Group 5: Fed on a basal diet, and injected with VCD (160 mg/kg/day in sesame oil, i.p.), and administered (soy milk 20ml , eggshell 6 g, orange juice 200 ml, cod liver oil 80 microliters per day and tap water to reach 1000 ml) daily for 6 weeks.

In the first 2 weeks of the experiment, animals from groups (2), (3), (4) and (5) followed the protocol to induce menopause in rats.

After 6 weeks of the experiment, rats were sacrificed after overnight fasting. Blood samples were collected as practically as possible in clean and dry tubes from the portal vein and left to clot at room temperature (26-27º C). Blood samples were then centrifuged at 3000 rpm for 15 min. The serum was carefully separated and kept at - 20º C until analysis.

Biological analysis:

The daily feed intake (FI) per group was calculated throughout the experimental period (8 weeks). The biological value of different diets was assessed by determining of body weight gain percent according to the method of **Chapman et al. (1959)** using the following equations.

- **Body weight gain ratio BWG = [Final weight (g) – initial weight (g)/ Initial weight]**
- **Feed efficiency ratio (FER) = weight gain (g) / Feed intake (g)**

At the end of the experimental period (8 weeks), the rats were fasted overnight and then sacrificed after anesthesia by using ethyl ether. Blood samples were immediately collected in clean and dry tubes from the portal vein of each rat and then centrifuged at 3000 r.p.m. for 15 min. to obtain the serum. Serum

samples were separated and stored at – 10 ºC until further determinations of the tested parameters.

Blood samples:

1- Biochemical analysis

Determination of serum vitamin D: vitamin D was determined according the method of **(Insert, 2017).**

2- Bone Density:

Determination of bone mineral density in the femur bones

Measurement of BMD (bone mineral density) has been carried out by techniques such as dual photon absorptiometry, quantitative computed scanning tomography, ultrasound and dual X-ray absorptiometry (DXA), the latter of which is widely used to measure BMD in humans. It applies a calibration process to the system using phantoms, which are commonly made of hydroxyapatite (HAp) with the L1–L4 lumbar vertebrae shape**. (Aerssena *et al.*, 1998).** Finally, the experimental data for diagnosis are compared with standard error. All results were evaluated as mean and LSD to ascertain the significance among means of the treatment. Duncan's multiple range tests at a significant level of ($P < 0.05$) was applied, using the SPSS statistical program **(SAS, 1996).** One way ANOVA followed by a post-Duncan test **(Snedecor and Cochran, 1989).**

DXA, microarchitecture, and FEA measurements were compiled in Excel and the data were analyzed using the Statistical Analysis System (SAS version 9.1). The Generalized Linear Model (GLM) procedure was followed by the Fisher's least significant difference test for mean separation. The significance level was set at $p<0.05$.

Histopathological Analysis

Bone samples were collected kept in 10% neutral buffered formalin solution, routinely processed, sectioned at 5µm

thickness, and stained with Hematoxylin and Eosin (H&E) for subsequent histopathological examination (Bancroft. 2013). Tissue slides were examined by Olympus BX43 light microscope and captured using Olympus DP27 camera linked to Cellsens dimensions software (Olympus).

Results and discussion:

Table (1). Effect of Yogurt, kefir milk and soy milk on initial body weight, final body weight, weight gain and feed efficiency ratio in female l rats

Groups	IBW	FBW	BWG	FER %
Control (-)	177.50 ± 7.6[a]	225.63 ±6.8[a]	48.13 ±0.74[a]	6.80 ± 0.03[a]
Control (+)	180.00 ±6.6[a]	212.50 ±7.9[a]	32.50 ±14.5[a]	4.99 ±0.57[a]
Yogurt	179.38 ±5.8[a]	220.63 ±4.2[a]	41.25 ±10.0[a]	5.85 ± 0.35[a]
Kefir milk	183.75 ±5.5[a]	220.63 ±6.2[a]	36.88 ±11.7[a]	4.89 ±0.39[a]
Soya milk	183.75 ±6.4[a]	220.63 ±6.2[a]	36.88 ±0.18[a]	5.03 ±0.01[a]

*Values are expressed as means ± SE.
* Values at the same column with different letters are significant at P ≤ 0.05
*BWG= Body weight again

The data in the table (1) Showed, non-significant changes in IBW between all groups. It was found that The groups that were treated with yogurt, soy milk, and kefir milk demonstrated a notable increase in final body weight compared to the positive control group, while simultaneously displaying a significant decrease in final body weight compared to the negative control group, this results agreement with (**Malkawi *et al.* 2018),** The data on the initial weights of the rats revealed statistically significant differences (P<0.05) among the osteoporotic rat groups. However, by the end of the experiment, no statistically significant differences were observed. Notably, the group fed yogurt exhibited higher body weight gain, aligning with our findings. (**Baburao *et al.*, 2019),** reported that yogurt is a good source of zinc, calcium, phosphorus, folate, niacin, magnesium and protein, as well as vitamins B2, B1, and B12. It provides protein of high biological value, while milk and dairy products, especially yogurt, contain bioavailable vitamins and minerals. Yogurt and other dairy products improve the overall quality of the

meal and increase the likelihood that it meets guidelines Nutritional content. **Deguchi and Mutai (1985)** documented alterations in vitamin levels throughout the fermentation process.

Data in the same table (1) showed that the positive group recorded a significant decrease in final weight, change in weight, weight gain. On the other hand, weight gain, feed intake, and feed efficiency ratio improved with rats that were fed yogurt, followed by the group fed with soy milk and kefir milk, compared with the control group **(Zhou *et al.* 2008).** The same result was indicated by **(Sartang *et al*. 2015)** who reported that body weight increased significantly with mice fed fermented soy milk .**(Samanta *et al.* 2014)** Probiotic treatment in rats led to a rise in their final body weight, though the exact reason behind this increase remains unclear

Result showed that there were no significant differences ($P<0.05$) in initial body weight, Improved final body weight and body weight gain among the experimental rat groups throughout the study period. This is in agreement with the results obtained by the author **(Kruger, *et al.*, 2009)** who found similar results when growing male and female rats were fed yogurt and soy yogurt.

Table (2). Effect of Yogurt, kefir milk and soya milk on vitamin D

Groups	Vitamin D (ng/ml)
Control (-)	14.40 ± 1.19a
Control (+)	2.34 ±0.58d
Yogurt Control	8.66 ±0.88c
Kefir milk	9.24 ±1.2b,c
Soya milk	10.16 ±0.96 b

*Values are expressed as means ± SE.
* Values at the same column with different letters are significant at $P \leq 0.05$

The result in Table (2) showed the vitamin D level (ng/ml) in the tested groups. The group fed on soy milk recorded the highest value of vitamin D and reached 10.16 ng/mL. This aligns with the findings of **El-Zeiny *et al.* (2023)**, who investigated serum vitamin D levels in control negative, control positive, and osteoporosis groups fed various soy-based products including

soybean milk, yogurt, and fruity soy milk yogurt.(**El-Zeiny *et al.*,2023)** This aligns with the findings of **El-Zeiny *et al*. (2023),** who investigated serum vitamin D levels in control negative, control positive, and osteoporosis groups fed various soy-based products including soybean milk, yogurt, and fruity soy milk yogurt.

Table(3). Effect of Yogurt, kefir milk and soya milk on bone mineral density

Groups	BMD(g/ cm2)	BMC (g)	Area (cm2)
Control (-)	0.26 ± 0.07a	0.08 ± 0.02a	0.31 ±0.05a
Control (+)	0.08 ±0.04c	0.02 ±0.01d	0.25 ±0.07c
Yogurt	0.13 ±0.01b	0.03 ±0.01c	0.23 ±0.01d
Kefir milk	0.15 ±0.09b	0.03 ±0.02c	0.20 ±0.04e
Soya milk	0.17 ±0.02b	0.05 ±0.05b	0.29 ±0.04b

*Values are expressed as means ± SE.

* Values at the same column with different letters are significant at $P \leq 0.0$

Table (3) displays the impact of different diets on BMD and BMC of female rats, including those treated with yogurt, kefir milk, and soy milk. The data in this Table revealed that, BMD and BMC of the positive control group fed on basal diet decreased significantly, as compared to the negative control group, on the other hand treated rats with yogurt, kefir milk, and soya milk induced significant increase in these parameters, as compared to the positive control group, especially soy milk.

The result showed in table (3) showed that bone mineral area (BMA), bone mineral content (BMC), and bone mineral density (BMD). The results recorded significantly decreased in rats which did not have menstruation, in contrast to treatment with milk products, where bone mineral content increased. The bone mineral density in the group of rats fed with soy milk, and the bone mineral content in both the yogurt and kefir milk groups were equal (0.03) and the difference in bone mineral density between them was slight. The bone mineral area in the bones was significantly increased after giving soy milk. This results in general agreement with **(Abdel-Mobdy , *et al.*, 2021)** whose results showed that the yogurt treatment was significantly

superior to other treatments in increasing bone mineral density (27%).

Histopathology

Bone

Histopathological examination of bone tissue from the control group (**Fig. 1**) revealed normal histological structure of bone trabeculae that appeared to be of a normal thickness. The trabeculae were connecting with relatively small marrow spaces. The cement lines were regular and continuous. On the contrary, the PC group (**Fig. 2**) showed marked thinning in the bone trabeculae with wide marrow spaces in-between. The trabeculae exhibited markedly defective calcification with irregular cement lines. G3 group (**Fig. 3**) showed mild improvement as most of the examined sections showed trabeculae of moderate thickness and relatively wide marrow spaces. The trabeculae showed better calcification. Some markedly improved individuals showed trabeculae of apparently normal thickness but with irregular cement lines. Moderate improvement was detected in bone sections from G4 group (**Fig. 4**), the thickness of bone trabeculae was increased, and the degree of calcification was improved as well with existence of few thin trabeculae and wide marrow spaces. Marked improvement was noticed in G5 group (**Fig. 5**), as the trabeculae were of normal thickness and well-connected creating small marrow spaces. The calcification lines were regular and obvious.

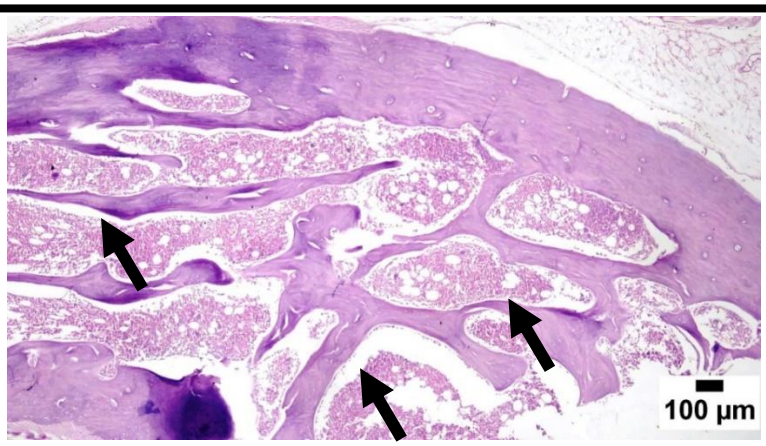

photo. (1) Photomicrograph of bone, control group showing normal bony trabeculae (arrows) (H&E).

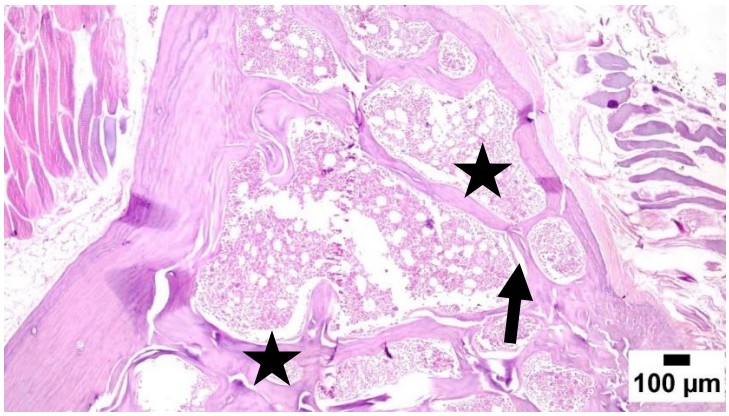

Photo. (2) Photomicrograph of bone, PC group showing thin less connected trabeculae (arrow) and wide marrow spaces (stars) (H&E).

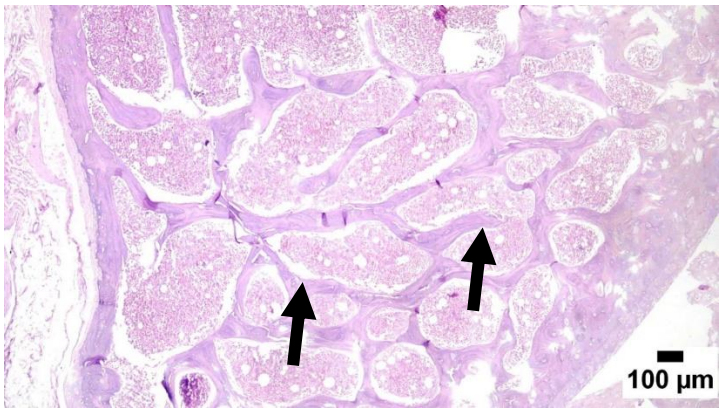

Photo. (3) Photomicrograph of bone, G3 group showing thin trabeculae (arrows) with more connection and relatively wide marrow spaces (H&E).

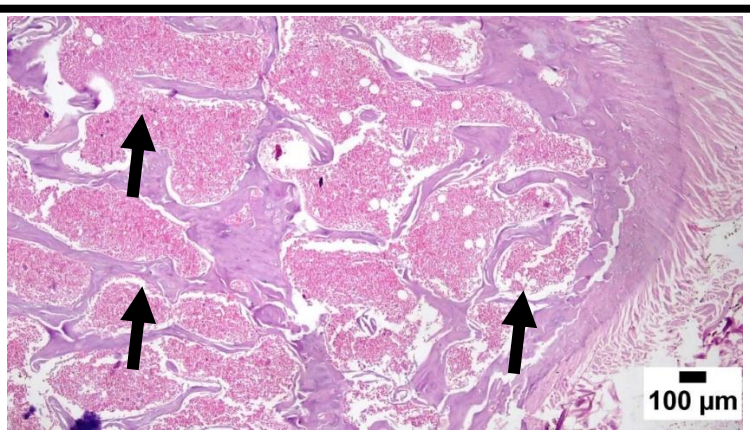

Photo. (4) Photomicrograph of bone, G4 group showing apparently normal trabeculae with few thin trabeculae (arrow) (H&E).

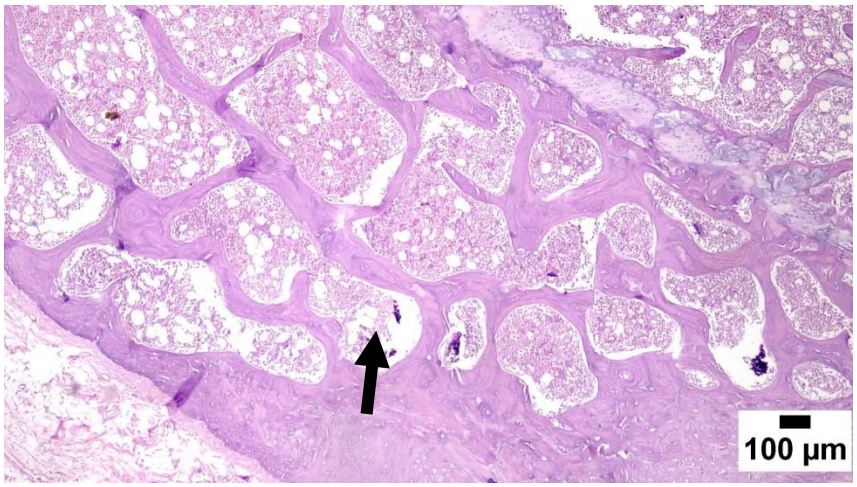

Photo. (5) Photomicrograph of bone, G5 group showing apparently normal trabeculae (H&E).

Conclusion:

Osteoporosis is among the major health issues, specifically in the elderly. It is one of the main causes of morbidity and mortality in this group of people. In addition, it imposes a profound financial burden on the healthcare system every year. Today, because of the rapid growth of the elderly population, osteoporosis has become an epidemic in some societies. According to the definition of the World Health Organization, osteoporosis is a systematic skeletal disease, characterized by the loss of bone mass density (BMD) and damage to the

microstructure of bone tissue, leading to increased bone vulnerability and risk of fracture. Ovariectomized (OVX) female rats show significantly reduced hormone concentrations.

Endogenous estrogens stimulate bone remodeling abnormalities that increase bone loss and increase the risk of osteoporosis. Menopause was deliberately caused by using 4-phenylcyclohexene dioxide (VCD) on female experimental rats. Five groups of rats were used (8 rats in each group): The first group: which is the negative control group, which was fed only the control diet, while the rest were given Rats were given two-week doses of VCD (160 mg/kg/day in sesame oil) to induce amenorrhea in female rats. The second group: It is the positive control, and the experiment period continued without any treatments, while the third, fourth, and fifth groups ate the control diet with a mixture of (egg shells, orange juice, and cod liver oil), then the control diet with yogurt, then the control diet with kefir milk, then the control diet. Control with soy milk.

Finally, rats were fasted overnight and then sacrificed with ethyl ether. Blood samples were immediately collected from the portal vein of each mouse and then centrifuged at 3000 p.m. For 15 minutes. To get the serum. Serum samples were separated and stored at −10°C until further parameter tested, such as vitamin D and DEXA.

Therefore, Vitamin D, calcium, Phosphors in different mixtures is very benefit for bone health.

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