

Biological and Biochemical Studies of Therapeutic Effect of Thyme (*Thymus vulgaris L.*) - on Osteoporosis in Male Rats.

Prof. Dr . Amal Z. Nasef

Associate Professor of Nutrition and Food
Science
Faculty of Home Economics
Menoufia University, Shebin El-Kom,
Egypt

Youmna S. Flefel

Master's researcher of Nutrition and Food
Science
Faculty of Home Economics,
Menoufia University

Dr . Mohamed R. Elkabary

lecturer of Nutrition and Food Science
Faculty of Home Economics
Menoufia University, Shebin El-Kom,
Egypt

Abstract

A prevalent health concern, osteoporosis is characterized by mass loss and structural degradation of bone structure., Thus, the aim of this study is repressing glucocorticoid stimulated osteoporosis in male rats by using different doses of thyme powder (TP) .Thirty male albino rats were separated into five groups (6 rats each):G1, control negative fed balanced diet; G2, control positive fed balanced diet and injected with prednisolone (10mg/kg per daily); G3, G4, and G5 were also injected with prednisolone (10mg/kg daily) throughout the experiment in addition to being given different doses of TP 2.5, 5 and 7.5%, respectively of the basal diet weight. After 28-day, Blood samples were taken at the conclusion of the experiment to evaluate bone femur status and blood osteoporosis markers. The findings showed that TP accelerated the increase in body weight. Different doses of TP raised significantly the serum Ca, Mg, OC, and DPD, while the serum P, interleukin 6, PTH, and CRP significantly decreased. The femur's bone ash contents, primarily in calcium and phosphorus, and bone mineral density both showed significant improvements.

In conclusion, the moderate use of thyme improves bone growth and serum indicators of osteoporosis, it may be helpful for preventing osteoporosis in rats.

Keywords: osteoporosis, prednisolone, calcium, iL-6, thyme, , rats, bone.

دراسات بيولوجية وبيوكيميائية للتأثير العلاجي لنبات الزعتر (*Thymus vulgaris L.*) علي هشاشه العظام في ذكور الفئران

الملخص

تعتبر هشاشه العظام من المشاكل الصحيه الشائعه التي تتميز بانخفاض كتله العظام والتدهور الهيكلي للانسجه العظميه ، وبالتالي فإن الهدف من هذه الدراسه التأثير الوقائي لمسحوق الزعتر بجرعات مختلفه علي هشاشه العظام المحفزه باستخدام الجلوكوكورتيكويد في ذكور الجرذان . تم تقسيمهم الي ثلاثين فأرا من ذكور الألبينو إلي خمس مجموعات (٦ فئران لكل مجموعه) : المجموعه الأولى، مجموعه ضابطه سالبه (سليمه) و المجموعه الثانيه مجموعه ضابطه موجبه ؛ تتغذي علي الغذاء الأساسي مع حقنها بالبريدنيزولون (١٠ ملجم/كجم يوميا)؛ تم حقن المجموعه الثالثه والرابعه والخامسه ايضا بالبريدنيزولون (١٠ ملجم/كجم يوميا) طوال فتره التجربه بالإضافة إلي إعطائهم جرعات مختلفه من الزعتر ٢,٥ و ٥ و ٧,٥ % علي التوالي من وزن النظام الغذائي الأساسي . بعد ٢٨ يوما ، تم أخذ عينات الدم في ختام التجربه لتقييم علامات مصل هشاشه العظام وحاله عظم الفخذ . وقد أظهرت النتائج أن تناول الزعتر أدي إلي تغيرات بيولوجيه مثل زياده وزن الجسم. كما أدت الجرعات المختلفه من الزعتر إلي زياده ملحوظه في نسبه الكالسيوم ، والمغنسيوم ، الاوسيتوكالسين ، الديوكسيبيريدينولين في المصل ، في حين انخفضت بشكل ملحوظ مستويات الفوسفور ، الانترولكين ٦ ، هرمون الغده الدرقيه (الباراثورمون) والبروتين التفاعلي في المصل . كما كان هناك تحسن كبير في كثافه المعادن في عظم الفخذ ومحتويات رماد العظام وخاصه في الكالسيوم والفوسفور .

الخلاصه : الإستخدام المعتدل للزعتر يحسن نمو العظام ومؤشرات مصل هشاشه العظام ، وقد يكون مفيدا للوقايه من هشاشه العظام في الفئران.

الكلمات الافتتاحيه : هشاشه العظام ، بريدنيزولون ، الكالسيوم ، الانترولكين ٦ ، الزعتر ، الفئران ، العظام.

1. Introduction

Osteoporosis, a metabolic bone disease, is defined by a widespread reduction in bone mass (BM) and strength below the threshold required for mechanical support of normal activity and a higher risk of non-traumatic fractures. A disorder called osteoporosis causes the bones to gradually weaken and fracture more easily (Nayaket *et al.*, 2016).

Numerous inflammatory and autoimmune disorders are treated using glucocorticoids. On the other hand, a harmful systemic effect is a detrimental effect on bone that can result in glucocorticoid-induced osteoporosis. This condition is marked by a sudden and sharp rise in fracture risk and bone resorption, and it can intensify within three months of starting oral glucocorticoids (Soo-Kyung and Yoon-Kyung, 2021).

The medium- and long-term usage of glucocorticoids (GC) results in several significant adverse effects, the primary one being a decrease in bone mineral density (BMD). (Jose and Sergio, 2021)

Among the most significant lifestyle factors that affect bone density are physical activity, exercise, dietary habits, alcohol and cigarette use, and excessive dosages of medications, such as prednisolone, which may be corrected and are therefore seen as significant (Wong *et al.*, 2019). Glucocorticoids could cause profound reductions in bone formation. About 30% to 50% of patients receiving chronic glucocorticoid therapy suffered vertebral or hip fractures, which were often asymptomatic. Vertebral fractures occurred early after exposure to glucocorticoids (Payer *et al.*, 2010). Unhealthy eating habits can impede healing and raise the chance of breaking a bone (Nieves, 2013). A healthy diet is essential

for maintaining bone mass, which helps prevent and treat bone disorders (Cooper *et al.*, 2015).

In addition to several other micronutrients, calcium, phosphate, and vitamin D are necessary for healthy bone development and function. Between sodium and calcium (Ca), the human body has the most inorganic elements (Spiechowicz *et al.*, 2003). On the other hand, some medicinal herbs and nuts, like thyme, can treat bone resorption and prevent osteoporosis (Wang *et al.*, 2013 and He *et al.*, 2017)

Thyme (*Thymus vulgaris L.*) is a lamiaceae family medicinal plant that is grown all over the world for usage in cooking, cosmetics, and medicine (Hassan *et al.*, 2020). It is a medicinal herb that is high in essential oils and other volatile components, and it is thought to have a possible preventive effect on bone loss (Mühlbauer *et al.*, 2003). The essential oils are safe, biodegradable substances with antibacterial properties that don't cause adverse reactions or gastrointestinal problems after eating (Rajkowska *et al.*, 2014). Among the primary ingredients of thyme essential oil are carvacrol, terpinene, thymol, and caryophyllene (Sienkiewicz *et al.*, 2017). The primary biochemical actions of *Thymus vulgaris* are attributed to the presence of phenolic chemicals, specifically thymol and carvacrol (Newton, 2000). Thymol could be a useful therapeutic agent for the prevention of bone destructive diseases (Mahesh *et al.*, 2018). El-Masry *et al.* (2022) concluded that thyme powder could be promising foods for controlling liver cirrhosis because it has high total phenolic content and high antioxidant activity.

Generally, several studies confirmed that plants have healthy aspects as antioxidant activity (Elhassaneen *et al.*,2023; Elsaid *et al.*, 2022 ; Elgawish, *et al.*, 2019 and Omar and Kabil, 2018).

In addition, supplementing with thyme dramatically decreased inflammation and oxidative stress in addition to the development of osteoporosis (Mustafa and Fares,2022).All herbs, including thyme, garden cress, and other plants, contribute to the antioxidant defense system that protects against the production of free radicals, rheumatism, inflammation, and muscle soreness, so enhancing human health (Nasef and Khateib,2021)

Therefore, the main objective of this study was assessed the potential therapeutic effects of Thyme powder (TP) (*Thymus vulgaris L.*) on osteoporosis induced by glucocorticoids in male rats.

2. Material and Methods

2.1. Material:

2.1.1. plants:Thyme (*Thymus vulgaris,L.*) will be acquired in Haraz, Cairo, Egypt, for the trade of herbs.

2.1.2. Chemicals:El-Gomhoriya Company was the source of all chemicals obtained for the trading of drugs, chemicals, and medical equipment. We bought Disprelone-OD from a neighborhood drugstore.

2.1.3. Diet: The basal diet was prepared according to the method developed by (AIN.,1993). As for the vitamin mixture and salts mixture used were added according to (Reeves *et al.*,1993). Diet

contents were obtained from El-Gomhoriya Company for Trading Drugs, Chemicals and Medical instruments.

2.1.4. Rats: Thirty male type albino rats (Sprague Dawley Strain) weighing an ranges between (140-150 \pm 10g) were obtained farm Helwan farm, Cairo, Egypt.

2.2. Methods

2.2.1. Preparation of thyme powder (TP).

Thyme leaves were dried at 50⁰ C in a vacuum oven (GoldStar, Egypt), then milled (Moulinex Egypt, Al-ArabyCo.,Egypt) and storied in plastic bag according to (AOAC, 2005) .

2.2.2. Induction of osteoporosis:

Osteoporosis was induced by Oral injection into operationally with Prednisone as sours of Glucocorticoid (GC) purchased from some pharmacy in Cairo, Egypt, and used as a dose of 10 mg/kg body weight per daily (**Llonaet al., 2012**).

2.2.3. Experimental groups.

Thirty male albino rats were used in the study and divided into fivegroups (6rats/each) as follows:G1: control (ve-) was fed standard balanced diet ;G2: control (ve+) was fed balanced diet and injected by prednisolone (10mg/kg per daily) according to (**Llonaet al ., 2012**); G3, G4, and G5 were also injected with prednisolone (10mg/kg daily) throughout the experiment in addition to being given different doses of TP 2.5, 5 and 7.5%, respectively of the basal diet weight.

2.2.4. Ethical approval.

The scientific Research Ethics Committee (Animals Care and Use), Faculty of Home Economics, Menoufia University, Shebin El-kom, Egypt, approved the

study's biological experiments as ethically acceptable. endorsed the study protocol #23-SREC-8-2022.

2.2.5. Biological evaluation:

During the experimental period (28days), the diets consumed, and body weights were recorded once weekly. The body weight gain (BWG), feed efficiency ratio (FER), and organ /body weight were determined according to (**AbdElmeged and Alghamdi, 2021**), using the following equations:

$$\text{BWG}\% = (\text{Final Weight} - \text{Initial weight}) / \text{Initial weight} \times 100$$

$$\text{FER} = \text{BWG (g)} / \text{Gram's feed intake}$$

2.2.6. Blood sampling and Bones:

At the conclusion of the trial, blood samples were taken from the abdominal aorta through which the rats were slain while sedated with ether. This was done after a 12-hour fast. Blood samples were placed into sterile, dry centrifuge tubes, allowed to clot at room temperature, and then the serum was separated by centrifuging the tubes for ten minutes at 4000 rpm. The serum was thoroughly aspirated, then placed in sterile cuvette tubes and frozen at -20°C in preparation for analysis.

In the same time, The bones were removed and the flesh was removed from them, washed and stored in formalin solution 10% for histopathological testing according to the method mentioned by **Parasuramanet al.(2010)**.

2.2.7. Biochemical analysis.

After a heart puncture, blood samples were taken, allowed to clot for ten minutes, and then centrifuged for fifteen minutes at 12,000 rpm to extract the serum, which was then frozen at -80°C until biochemical analysis. Ca concentrations in serum (**Gindler and King,2015**).Magnesium and phosphorus

(Goodwin ,1970). Assessed colorimetrically using UV spectrophotometers and particular diagnostic reagent kits (BioMérieux, France).serum alkaline phosphate specific to bones (Nawawi and Girgis,2002).Serum measurements of osteocalcin (OC), interleukin- 6 (IL-6), and parathyroid hormone (PTH) concentrations were performed using quantitative noncompetitive sandwich ELISA assay kits (Market, San Jose, CA) as described by Norazlina *et al.* (2010).According to James *et al.*(1990), the assay of urine deoxypyridinoline (DPD) level was measured by high-performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA). A quantitative determination of C-reactive protein (CRP) was made using the CRP Turbilatex agglutination assay kit (Spinreact, Girona, Spain) as the reagent.

Bone analysis.

The amount of calcium and phosphorus in the right femur were determined by atomic absorption spectrophotometer according to Sutlovic *et al.*(2016) and(Demirel *et al.*,2012). The amount of Magnesium in the right femur was determined by the solution scanometric method according to Shokrollah *et al.*(2016).

2.3. Statistical analysis:

A completely randomized factorial design (SAS., 1988) was used to examine the data, and the Newman-Keuls Test was used to separate the means when a significant main effect was found. The SPSS program was used to determine whether differences between treatments of ($P \leq 0.05$) were significant. The biological outcomes were evaluated using One Way ANOVA.

3. Results and Discussion.

3.1. Effect of thyme powder on feed intake, feed efficiency ratio and body weight gain % of osteoporotic rats.

FI, FER and BWG% of osteoporotic rats were found in table (1) such data reveal that the osteoporotic rats showed significantly ($p \leq 0.05$) decreased in feed intake compared to normal rats and increased in FER and BWG that's due to a Prednisone injection. These results agreed with **Morgan et al. (2020)** who reported that prednisone can make you gain weight because it increases fluid retention and appetite. Supplementation with TP diet at (2.5-5-7.5%) for 28 days increase the level of FI and decrease the levels of FER and BWG considerably ($p \leq 0.05$). In the same direction **El-Kholie et al. (2020)** reported that the consumed of thyme powder decrease the BWG compared to control +ve. Recently, **Nasef and Khateib (2021)** discovered that giving experimental rats plant sections containing bioactive substances like TP increased the decline in FER and BWG.

Table 1. Effect of thyme powder on feed intake, feed efficiency ratio and body weight gain percentage of osteoporotic rats.

Groups	FI	FER	BWG %
Control (-ve)	15.82±0.21 ^a	0.091±0.011 ^d	27.10±0.76 ^d
Control(+ve)	9.53±0.22 ^c	0.232±0.034 ^a	41.69±1.43 ^a
TP (2.5%)	10.94±0.42 ^d	0.172±0.051 ^b	35.72±0.63 ^b
TP (5%)	11.06±0.09 ^d	0.159±0.034 ^b	33.70±1.11 ^b
TP (7.5%)	11.52±0.21 ^b	0.144±0.023 ^c	31.95±0.45 ^c

Means in the same row with different superscript letters are significantly different ($p \leq 0.05$).

(FI) feed intake, (FER) feed efficiency ratio, (BWG%) body weight gain percentage and (TP) Thyme Powder.

3.2. Effect of thyme powder on Parathyroid hormone and serum calcium of osteoporotic rats.

Data in (Table 2) showed the effect of TP on Parathyroid hormone (PTH) and Serum calcium (Ca) of osteoporotic rats. The positive group significantly decrease ($P \leq 0.05$) in Ca and increase in PTH compared to the negative group. Supplementation with TP diet led to reduced PTH and raised Ca levels. The group which treated with 5% TP showed efficient significant in reducing the amount of PTH and increasing Ca than groups treated with 2.5% TP. The best results observed in G5 (7.5%) TP which recorded significant decrease of PTH and significant increase in Ca compared to positive group. The results agree with **Amret *al.* (2019)** who claimed that rats fed thyme, the control positive group's PTH level increased dramatically, and when osteoporotic rats were given thyme supplementation, their plasma calcium levels increased significantly. Also, **Dalia (2023)** concluded that, all osteoporosis groups that administered with different doses of thyme showed substantial increase in serum calcium and P. Glucocorticoids also appear to potentiate parathyroid hormone (PTH) inhibition of collagen synthesis. **Rosen (2021)** reported that increased parathyroid hormone secretion and said PTH stimulates bone formation as well as its resorption and its intermittent administration stimulates bone formation than resorption. (**Chotiarnwong and McCloskey, 2020**) said glucocorticoid induces a negative balance by reducing calcium.

Table 2. Effect of thyme powder on Parathyroid hormone and serum calcium of osteoporotic rats.

Groups	PTH(Pg-ml)	Ca(mg-dl)
Control (-ve)	95.79±18.78 ^c	11.97±.61 ^a
Control (+ve)	583.25±101.69 ^a	5.92±.39 ^d
TP (2.5%)	309.00±59.00 ^b	7.24±.26 ^c
TP (5%)	223.50±131.74 ^{bc}	7.76±.99 ^{bc}
TP (7.5%)	163.62±60.82 ^c	8.80±.95 ^b

Means in the same row with different superscript letters are significantly different ($p \leq 0.05$). (PTH) Parathyroid hormone, (Ca) serum calcium and (TP) Thyme Powder.

3.3. Effect of thyme powder on serum phosphorus and serum magnesium of osteoporotic rats.

Table (3) indicated the effect of TP on P(mg-dl) and Mg (mmol-L) in serum of osteoporotic rats. The results showed there was significant increase in serum P (7.77mg-dl) compared to normal control (3.33 mg-dl). When 2.5, 5, and 7.5% of TP were added to the diet, there was a significant ($p \leq 0.05$) decrease in serum P when compared to the (+ve) group. On the other hand in the same table the level of serum Mg was decrease in osteoporotic rats by glucocorticoid was 1.29 (mmol-L) compared to normal rats (2.86 mmol-L). Supplementation 2.5, 5 and 7.5% of TP induced significant increase on the level of Mg compared to (+ve) group.

Amret al. (2019) revealed that, in comparison to the normal group, the prednisolone control group's plasma phosphorus levels had significantly decreased. And **Amret al. (2019)** found that, in comparison to the normal group, the prednisolone control group's plasma phosphorus levels had significantly decreased.

Table 3. Effect of thyme powder on serum phosphorus and serum magnesium on osteoporotic rats.

Groups	P(mg-dl)	Mg(mmol-L)
Control (-ve)	3.33±.55 ^c	2.86±.91 ^a
Control (+ve)	7.77±.86 ^a	1.29±.28 ^c
TP (2.5%)	5.28±.50 ^b	1.82±.30 ^{bc}
TP (5%)	4.96±.37 ^b	2.04±.39 ^{bc}
TP (7.5%)	4.31±1.94 ^{bc}	2.38±.44 ^{ab}

Means in the same row with different superscript letters are significantly different ($p \leq 0.05$).

(P) serum phosphorus , (Mg) serum magnesium and (TP) Thyme Powder .

3.4. Effect of thyme powder on Osteocalcin, Interleukin-6 and C-reactive protein of osteoporotic rats.

Data in Table (4) showed significant ($p \leq 0.05$) increase in serum level of IL-6 and CRP as compared to normal rats' groups and decreased serum levels of OC as compared to (-ve) control group. These results agree with **Avinashet et al., (2019)** who found that glucocorticoids reduced serum osteocalcin. Also, **Anna et al., (2012)** reported that glucocorticoid increase Interleukin-6. Finally, **John and Maria (2004)** who said that glucocorticoids reduce CRP concentrations via the anti-inflammatory.

Diets supplemented with TP (2.5-5 and 7.5 %) significantly ($P \leq 0.05$) lowered the high serum IL-6 and CRP when compared to the positive groups. Significant increase the level of serum OC as compared positive control group. The best results for improving the level of OC, IL-6 and CRP recorded for 7.5 % of TP groups.

(Dowd et al., 2003) found improvement when solving the three-dimensional structure of calcium bound osteocalcin. **Ammara et al. (2023, and Höferlet**

al., (2006) said that thyme is fill with calcium, and this grain has a positive effect on OC (Amirghofranet *al.*, 2016). Nagooret *al.* (2017) and Essawi and Srour (2000) found that the effect of tymolas anti-inflammatory properties and il-6 is a protein responsible for inflammation. Dalia (2023) said that all osteoporosis groups administrated with different levels of thyme had a significant decrease in serum CRP, IL-6 .

Table 4. Effect of thyme powder on Osteocalcin, Interleukin-6 and C-reactive protein of osteoporotic rats.

Groups	OC (Pg-ml)	IL-6(Pg-ml)	CRP(ng-ml)
Control (-ve)	859.50±74.46 ^a	96.41±25.79 ^f	10.06±1.80 ^d
Control (+ve)	222.25±25.46 ^c	620.92±136.56 ^a	104.16±5.58 ^a
TP (2.5%)	592.25±97.12 ^b	294.50±117.37 ^{cd}	53.01±16.53 ^b
TP (5%)	647.50±286.43 ^{ab}	238.12±76.21 ^{de}	39.83±15.30 ^{bc}
TP (7.5%)	745.50±145.63 ^{ab}	188.62±66.90 ^{def}	28.48±4.07 ^c

Means in the same row with different superscript letters are significantly different ($p \leq 0.05$). (OC) Osteocalcin, (IL-6) Interleukin-6, (CRP) C-reactive protein and (TP) Thyme Powder .

3.5. Effect of thyme powder on Urine deoxypyridinoline of osteoporotic rats.

The results at Table (5) indicated that the DPD of osteoporotic rats showed significant ($P \leq 0.05$) decrease as compared to negative control group. The mean value was (142.85ng-ml and 11.15ng-ml), respectively. Shang-Ian *et al.* (2012) reported a decrease in urinary deoxypyridinoline levels after treatment with glucocorticoid.

While supplemented diets with TP the significant ($P \leq 0.05$) increase as compared to positive group and the highest value recorded for 7.5 % group but the lowest value recorded for 2.5 % with significant ($P \leq 0.05$) differences. The mean value was (111.79ng-ml and 56.68ng-ml), respectively.

Deoxyypyridinoline (DPD) reflects systemic bone reabsorption, is considered useful for assessing the effects of osteoporosis treatment in the same line with **Kayoko *et al.*(2003)** who found the white sesame and thyme improve osteoporosis.

Table 5. Effect of thyme powder on Urine deoxyypyridinoline of osteoporotic rats.

Groups	DPD(ng-ml)
Control (-ve)	142.85±6.59 ^a
Control (+ve)	11.15±4.10 ^d
TP (2.5%)	56.68±25.40 ^c
TP (5%)	69.07±22.55 ^c
TP (7.5%)	111.79±15.04 ^b

Means in the same row with different superscript letters are significantly different ($p \leq 0.05$).
(DPD)Urine deoxyypyridinoline and (TP) Thyme Powder .

3.6. Effect of thyme powder on femur ash levels of calcium, phosphorus and magnesium of osteoporotic rats.

The effect of different supplemented levels of thyme on Ca ,P and Mg in bones are recorded in Tables (6). As for Ca, the findings showed that compared to the negative control group (healthy rats), the positive control group's mean value was considerably ($P \leq 0.05$) lower were 5.54(mg-dl) and 11.04 (mg-dl). the mean values of treated osteoporotic groups with TP(2.5-5 and 7.5%) showed significant increase ($P \leq 0.05$) when compared with positive control group, were 7.05, 7.87 and 8.88(mg-dl), respectively. So the best mean value was recorded for group (7.5% TP) .

With regards to the significance level (P), the data showed that the mean value of the positive control group was 2.92 (mg-dl) - 7.48 (mg-dl) respectively, and that the mean value of the negative control group was considerably lower ($P \leq 0.05$). The mean values of treated osteoporotic groups with TP(2.5-5 and 7.5%)

indicated significant decreases ($P \leq 0.05$), when compared to positive control group. The best result was recorded for the group (7.5% TP) when compared to the control positive group. (Manuel *et al.*, 2022) said that Long-term exposure to therapeutic doses of glucocorticoids decreases some minerals in bone and increases the risk of fractures

As regards the level of Mg, the data showed that the mean values of 2.18 (mmol-L) and 5.44 (mmol-L) for the positive control group and negative control rats, respectively, were considerably lower ($P \leq 0.05$). Compared to the positive control group, the mean values of the treated osteoporotic groups displayed significant changes ($P \leq 0.05$). But there are non-significant differences ($P \leq 0.05$) between groups (2,3 and 4). Numerically, the best result was recorded for group 5 which fed on 7.5% TP when compared to control positive group.

These results agree with Dalia., (2023) who said that all osteoporosis groups administrated with different levels of thyme had significant increase in Ca and P in bone. Dalal(2022) said that Thyme is loaded with potassium, calcium, iron, manganese, magnesium and selenium, so thyme improves Ca and Mg In bones.

Table 6. Effect of thyme powder on femur ash levels of calcium, phosphorus and magnesium of osteoporotic rats.

Groups	Ca(mg-dl)	P(mg-dl)	Mg (mmol-L)
Control (-ve)	11.04±1.73 ^a	2.92±.65 ^c	5.44±1.70 ^a
Control (+ve)	5.54±.62 ^c	7.48±.92 ^a	2.18±.67 ^b
TP (2.5%)	7.05±.99 ^{bc}	5.75±1.47 ^b	3.02±.87 ^b
TP (5%)	7.87±1.74 ^b	5.01±.62 ^b	3.37±1.08 ^b
TP (7.5%)	8.88±.90 ^b	4.50±.94 ^b	4.04±1.66 ^{ab}

Means in the same row with different superscript letters are significantly different ($p \leq 0.05$). (Ca) Bone calcium, (P) Bone phosphorus, (Mg) Bone magnesium and (TP) Thyme Powder.

4. Conclusion

Our findings obviously demonstrate that powdered thyme, which contains thymol, minerals, and vitamins, particularly calcium, is a powerful inhibitor of bone resorption and has various advantages for bone building and anti-inflammatory properties. Therefore, thyme has a preventive effect against osteoporosis and bone resorption.

5. References :

Abd-Elmeged L.S.M. and Alghamdi A.A.A. (2021) :The effect of consuming different proportions of hummer fish on biochemical and histopathological changes of hyperglycemic rats. Saudi J BiolSci.;29(1):140-147.

AIN.American Institute of Nutrition. (1993) :Purified diet for laboratory Rodent, Final report. Journal of Nutrition, 123:1939-1951.

Amirghofran Z. ;Ahmadi H. ; Karimi M.H. ; Kalantar F. ; Gholijani N. and Malek H.Z. (2016) : “In vitro inhibitory effects of thymol and carvacrol on dendritic cell activation and function,” Pharmaceutical Biology;54(7):1125-32.

Ammara A. ; Muhammad N. ; Baojun X. ; Roshina R. ; Gülden G. and Rana M. (2023): A comprehensive review of the bioactive components of sesame seeds and their impact on bone health issues in postmenopausal women. Food Funct.;14(11):4966-4980.

Amr S.E. ;Emiliya R.V. ; Eman M.E. and Natalya V.S. (2019) :Protective effect of dietary oils containing omega-3 fatty acids against glucocorticoid-induced osteoporosis ;Journal of Nutrition and Health52(4):323-331.

Amr S.E. ;Valeeva E.R. ; Eman M.E. andRakhimov I.I. (2019): The Impact of Thyme and Rosemary on Prevention of Osteoporosis in Rats , J NutrMetab.1431384.

Anna D. ; Christina K. ; Sara D.S. ; Christian E. ; Oliver B.H. ; Ute A. ; Johannes G.B. ; Christian T. and Fred S. (2012) : Glucocorticoids Increase Interleukin-6–Dependent Gene Induction by Interfering With the Expression of the Suppressor of Cytokine Signaling 3 Feedback Inhibitor ; ;55(1):256-66.

AOAC (2005): Official methods of the Association of official Analytical chemists .15th (ed.) Wilson boulevard arling, Virginia ; 22201,USA.

Avinash M.H. ;Nandakrishna B. ; Barkur A.S. ; Laxminarayana K.B. and Sudha V. (2019) : Effect of glucocorticoids exposure on serum osteocalcin levels ;American journal of veterinary research, 56(9), 1201–1205.

Chotiyarnwong P. and McCloskey E.V. (2020) :Pathogenesis of glucocorticoid- induce osteoporosisand options fortreatment; Nature reviews. Endocrinology, 16(8), 437–447.

Cooper C. ; Dawson-Hughes B. ; Gordon C.M. and Rizzoli R.(2015) : Healthy Nutrition, Healthy Bones: How Nutritional Factors Affect Musculoskeletal Health throughout Life, International Osteoporosis Foundation, Nyon, Switzerland.

Dalal H.H. ;Maha K. ; Sanaa K. and Samar Y. (2022): A Focused Insight into Thyme: Biological, Chemical, and Therapeutic Properties of an Indigenous Mediterranean Herb ;14(10):2104.

Dalia R.H. (2023) : Preventive effect of herbs Rosemary and Thyme (*Salvia Rosmarinus* and *Thymus Vulgaris*) on Osteoporosis in female Rats ;(19) 28;44.

Demirel G. ;Pekel A. ; Alp M. and Kocabağlı N. (2012) : Effects of dietary supplementation of citric acid, copper, and microbial phytase on growth performance and mineral retention in broiler chickens fed a low available phosphorus diet. *Journal of Applied Poultry Research*, 21, 335-347.

Dowd T.L. ; Rosen J.F. ; Li L. and Gundberg C.M. (2003) :The Three-Dimensional Structure of Bovine Calcium Ion-Bound Osteocalcin Using H NMR Spectroscopy; ;42(25):7769-79.

Elgawish, R.A. Abdel-Rahman, H. G. Helmy, S.A. Kabil, D.I.M. Abdelrazek, H. M. A. (2019), Alleviation of Lead-Induced Immunotoxicity by *Moringa oleifera* in Albino Rats. *Egyptian Academic Journal of Biological Science (Toxicology and Pest control)* , 11(1), 1-16.

Elhassaneen, Y. A.; Boraey, R. A., and Nasef, A. Z., (2023) : Biological Activities of Ashwagandha (*Withania somnifera L.*) Roots and their Effect on the Neurological Complications of Obesity in Rats. *American Journal of Food and Nutrition*; 11, (3) : 71-88.

El-Kholie E.M. ; El-Sheikh N.A. and Kasaab N.A. (2020) : Effect Of Basil (*Ocimum Basilicum*) And Thyme (*Thymus Vulgaris*) Leaves On Biological And Biochemical Changes In Induced Obese Rats. *Journal of Home Economics*, 30(4), 667-685.

El-Masry H.G. ;Ashkanani R.H. and Alhaifi A.R. (2022) : Potential Effects of Olive Oil and Thyme Powder on Oxidative Stress and Liver Functions of Cirrhotic Rats. *Journal of Home Economics*. 32(1): 58-75 .

Elsaid, K. S.; Hussien, S.; Alrashdi, B.; Mahmoud, H. A.; Ibrahim, M. A.; Elbakery, M.; El-Tantawy, H.; Kabil, D.A and EL-Naggar, S.A. (2022). Musa sp. Leaves Extract Ameliorates the Hepato-Renal Toxicities Induced by Cadmium in Mice *Molecules* , 27(2),559.

Essawi T. and Srouf M. (2000) :“Screening of some Palestinian medicinal plants for antibacterial activity,” *Journal of Ethnopharmacology*; 70(3):343-349.

Gindler E.M. and King J.D. (2015) : Rapid colorimetric determination of calcium in biologic fluids with methylthymol blue. *Am J Clin Pathol.*;58(4):376-82.

Goodwin J.F. (1970) :Quantification of serum inorganic phosphorus, phosphatase, and urinary phosphate without preliminary treatment. *ClinChem.*;16(9):776-80.

Hassan M.S. ;Naglaa H.M.H. and Mamdouh A.I.A. (2020) : Hepatoprotective activities of thyme (*Thymus vulgaris* L.) in rats suffering from obesity.. *Egyptian Journal of Chemistry*, 63(12), 5087-5101.

He J.B. ; Chen M.H. and Lin D.K. (2017) : “New insights into the tonifying kin herbs and formulas for the treatment of osteoporosis,” *Archives of Osteoporosis.*, 12, (1): 14.

Höferl M. ;Krist S. and Buchbauer G. (2006): Chirality influences the effects of linalool on physiological parameters of stress. *PlantaMed* ;72(13):1188-92.

James I.T. ;Perrett D. and Thompson P.W. (1990) :Rapid assay for hard tissue collagen cross-links using isocratic ion-pair reversed-phase liquid chromatography. *J Chromatogr.*;525(1):43-57.

John D. and Maria P. (2004):Relationship between C-reactive protein concentrations during glucocorticoid therapy and recurrent atrial fibrillation ;25(13):1100-7.

Jose R.V.D.C.J. and Sergio L.B. (2021) : Glucocorticoid-Induced Osteoporosis ; IntechOpen .doi: 10.57772/intechopen.97416.

Kayoko K. ; Kiyoshi N. ; Hiroshi N. ; Takami M. ; Hirotohi M. andYoshiki N. (2003) : Clinical usefulness of measurements of urinary deoxypyridinoline (DPD) in patients with postmenopausal osteoporosis receiving intermittent cyclical etidronate: advantage of free form of DPD over total DPD in predicting treatment efficacy ; ;21(4):217-24.

Llona K.S. ; Maria Z. ; Katarzyna R. and Lech S. (2012) : Effects of thalidomide on the development of bone damage caused by prednisolone in rats. PharmacolRep. ;64(2):386-95.

Mahesh S. ; Liang L. ; Se-Woong K. and Yunjo S. (2018) : Thymol inhibits RANKL-induced osteoclastogenesis in RAW264.7 and BMM cells and LPS-induced bone loss in mice. Food Chem Toxicol.;120:418-429.

Manuel G.; Ulrike B. ; Lorenz C. H.and Holger H. (2022) :Bad to the Bone: The Effects of Therapeutic Glucocorticoids on Osteoblasts and Osteocytes ; 13 : 835720.

Morgan C. ; Costello R.E. ; Ray D.W. and Dixon W.G. (2020) : How Do Glucocorticoids Used in Rheumatic Disease Affect Body Weight? A Narrative Review of the Evidence. Arthritis Care Res (Hoboken).;72(4):489-497.

Mühlbauer R.C. ; Lozano A. ; Palacio S. ; Reinli A. and Felix R. (2003) :

Common herbs, essential oils, and monoterpenes potently modulate bone metabolism. *Bone.*;32(4):372-80.

Mustafa A.Z. and Fares K.K (2022) :The protective role of almond and thyme in carbonated beverage-induced osteoporosis in male albino rats ; 6(S5):880-890

Nagoor M.F. ;Javed H. ; Al Tae H. ; Azimullah S. and Ojha S. (2017) : Pharmacological Properties and Molecular Mechanisms of Thymol: Prospects for Its Therapeutic Potential and Pharmaceutical Development. *Frontiers in Pharmacology, Pharmacol.*8:380.

Nasef A.N.Z. and Khateib B.R.M. (2021) : Study the Potential Therapeutic Effect of Garden Cress (*Lepidium sativum*) on Nephropathy Diabetic Rats: Biological and Biochemical Studies. *Alexandria Science Exchange Journal* ; 42(2):263-272.

Nawawi H. and Girgis S.I. (2002) :Serum levels of bone-specific alkaline phosphatase and procollagen type I carboxyterminal peptide in vitamin D deficiency. *Southeast Asian J Trop Med Public Health.*;33Suppl 2:124-30.

Nayak N.K. ;Khedkar C.C. ; Khedkar G.D. and Khedkar C.D. (2016) : Osteoporosis. In: Caballero B., Finglas P.M., Toldrá F., editors. *Encyclopedia of Food and Health*. Academic Press; Oxford, UK: pp. 181–185.

Newton B.M. (2000) :*Herbal Medicine, Expanded Commission Monographs*. Integrative Communications.

Nieves J. (2013) :effects of nutrients and nutraceuticals, beyond calcium and vitamin D. *Osteoporos Int.* ;24(3):771-86.

- Norazlina M. ;Hermizi H. ; Faizah O. ; Shuid A. N. ;Norliza M. and Ima-Nirwana S. (2010) :** Vitamin E reversed nicotine-induced toxic effects on bone biochemical markers in male rats. Arch Med Sci.;6(4):505-12.
- Omar, E. F. and Kabil, D.I. (2018).** Evaluation the efficacy of medium protein diet, turmeric and Arabic gum on chronic renal failure. Alexandria Science Exchange Journal, 39(3), 431-443.
- Parasuraman S. ;Raveendran R. and Kesavan R. (2010) :** Blood sample collection in small laboratory animals. J PharmacolPharmacother ;1(2):87-93.
- Payer J. ;Brazdilova K. and Jackuliak P.(2010):** Management of glucocorticoid-induced osteoporosis: prevalence, and emerging treatment options. Drug Healthc Patient Saf;2:49-59.
- Rajkowska K. ;Kunicka-Styczyńska A. ; Maroszyńska M. and Dabrowska M. (2014) :** The effect of thyme and tea tree oils on morphology and metabolism of *Candida albicans*. ActaBiochim Pol.;61(2):305-10.
- Reeves P. ; Nielsen F. and Fahmy G. (1993) :** AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr.;123(11):1939-51.
- Rosen H.N. (2021) :** Clinical features and evaluation of glucocorticoid-induced osteoporosis.UpToDate[Internet].Available/from: <https://www.uptodate.com/contents/clinical-features-and-evaluation-of-glucocorticoid-induced-osteoporosis?>
- SAS. (1988) :** SAS Users Guide: Statistics version5th Ed.,SAS.Institute Inc., Cary, NC.

Shang-Ian T. ; Gil Y. ; Yuin C.C. ; Sze H.C. ; Ee T.K. ; Yiong H.C. ; Susanna S.S.T. ; Ian Y.Y.T. and Suat H.T. (2012) : Prevention of Glucocorticoid-Induced Osteoporosis in Immunobullous Diseases With Alendronate ; ;148(3):307-14.

Shokrollahi A. ;Hemmatidoust K. and Zarghampour F.(2016) : Determination of magnesium by the solution scanometric method in a coloured titan yellow magnesi hydroxide complexform ;161-167.

Sienkiewicz M. ; Łysakowska M.SzymańskaG. , Kochan E. ; Krukowska J. ;Olszewski J. ; Zielińska-Bliźniewska H. andKowalczyk E. (2017):The ability of selected plant essential oils to enhance the action of recommended antibiotics against pathogenic wound bacteria. Burns.;43(2):310-317.

Soo-Kyung C. and Yoon-Kyung S. (2021):Update on Glucocorticoid Induced Osteoporosis.EndocrinolMetab (Seoul);36(3):536-543.

Spiechowicz U. ;Kokot F. and Wiecek A. (2003) :Markers of calcium-phosphate metabolism and bones alterations in long term kidney transplant patients. PrzeglLek. ;60(11):690-4.

Sutlovic D. ; Boric I. ; Sliskovic L. ; Popovic M. , Knezovic Z. and Nikolic I. (2016) : Bone mineral density of skeletal remains: discordant results between chemical analysis and DEXA method. LegalMedicine: 20:18-22.

Wang Z.Q. ; Li J.L. and Sun Y.L. (2013) Chinese herbal medicine for osteoporosis: a systematic review of randomized controlled trails. Evid Based Complement Alternat Med. 2013; 2013:356260.

Wong S.K. ;Mohamad N.V. ; Jayusman P.A. ; Shuid A.N. ; Ima-Nirwana S. and Chin K.Y. (2019):The use of selective estrogen receptor modulators on bone health in men. Aging Male.;22(2):89-101.