Preventive effect of herbs Rosemary and Thyme (Salvia Rosmarinus and Thymus Vulgaris) on Osteoporosis in female Rats

Dr. Dalia R. Hassan
Nutrition and Food Science, Home Economics Department, Faculty of Specific Education - Fayoum University

المجلة العلمية المحكمة لدراسات وبحوث التربية النوعية

المجلد التاسع– العدد الأول– مسلسل العدد (١٩)– يناير ٢٠٢٣م

رقم الإيداع بدار الكتب ٢٤٢٧٤ لسنة ٢٠١٦

ISSN-Print: 2356-8690 ISSN-Online: 2974-4423

موقع المجلة عبر بنك المعرفة المصري https://jsezu.journals.ekb.eg

البريد الإلكتروني للمجلة E-mail JSROSE@foe.zu.edu.eg

Preventive effect of herbs Rosemary and Thyme (Salvia Rosmarinus and Thymus Vulgaris) on Osteoporosis in female Rats.

Dalia R. Hassan
Nutrition and Food Science, Home Economics Department, Faculty of Specific Education - Fayoum University

Abstract

Osteoporosis is a worldwide disease characterized by reduction of bone mass, thus the aim of this study is to suppress glucocorticoid induced osteoporosis in female rats by the action of rosemary and thyme. The present study was carried out on thirty female rats. The rats were divided into six groups (five rats each). The first group was fed on a basal diet and represents the negative control, while the other five groups were injected subcutaneously with betamethasone at a dose of 4 mg/ kg BW three times a week. One group of them represents the positive control. The other four groups were fed on a basal diet containing 5% and 7.5% of rosemary and thyme for a period of eight weeks. The positive control group showed a significant decrease ($P<0.05$) in serum vitamin D3, Phosphorus (P), Calcium (Ca), levels and Estradiol (E2), Ca and P in femur bone with a substantial reduction in bone mineral density (BMD), and a significant increase in serum malondialdihyde (MDA),C–reactive protein (CRP),interleukin-6 (IL-6) and Alkaline phosphatase (ALP)compared with control group . On the other hand, all osteoporosis groups administrated with different levels at 5% and 7.5% of rosemary and thyme had a significant decrease in serum MDA,CRP,IL-6 and ALP and a significant increase at ($P<0.05$) in serum Vit D3, Ca, P ,E2 ,Ca, P in bone and BMD, compared with the positive control group. Conclusion rosemary and thyme herbs demonstrated bone protection in female rats against glucocorticoid-induced osteoporosis. Rosemary and thyme had a potent protective effect due to its content of essential oils.

Key words: Osteoporosis; Essential Oils; Thyme; Calcium; Phosphorus.

Introduction

Osteoporosis is a serious condition marked by the deterioration of bone microstructure and the loss of bone mass, which can increase the risk of fracture and therefore have an influence on morbidity, death rates, and the quality of life in afflicted women both **Golob and Laya (2015**). People all over the world are susceptible to osteoporosis, which is characterized by a loss of bone mass and structural changes in the bone that raise the risk of fracture and make the bone more brittle. It is projected that osteoporosis will increase in prevalence as the population ages (**Qassem et al., 2017**). Osteoblasts, which produce bone, make up a

portion of the bone. Osteoid makes up the bone matrix, and osteoclasts, which break down or resorb bone, are also present in the bone. The balance of cytokines controls the activity of bone cells; abnormalities cause osteoporosis (**Orr, 2019**).

Glucocorticoids are frequently used to inhibit immunological function or inflammation. Glucocorticoid-induced osteoporosis, a significant and frequent iatrogenic consequence, is brought on by high dosages and prolonged usage of glucocorticoids in a significant part of patients (**Chotiyarnwong and McCloskey, 2020**). The WHO classifies osteoporosis as having a T-score less than -2.5 SD and osteopenia as having a T-score between -1.0 and -2.5 SD and normal BMD as a T-score exceeding 1 standard deviations (SD) (**Akkawi and Zmmerly, 2018).** Sage, thyme extract, rosemary, and other herbs were shown to increase bone density in animals, according to a study, although additional research is required in people (**Ahmed et al., 2016).**

Rosemary (*Rosmarinus officinalis L.*), one of the most extensively used plant extracts that has been shown to exhibit antioxidant activity, is utilized as a herb for flavour as well as an antioxidant in processed foods and cosmetics (**Wright et al., 2014**). Thyme (*thymus vulgaris L.*), a popular medicinal plant used all over the world and thought to offer possible preventive effects against bone loss, is a rich source of volatile chemicals and essential oils (**Elbahnasawy et al.,2019**).

Thyme, or *Thymus vulgaris L.*, is a medicinal plant that is abundant in essential oils and other volatile compounds. It is widely utilized around the world and is thought to have a potential preventive effect against bone loss (**Zeyad et al., 2022**). When eaten, the essential oils have no negative side effects or digestive issues because they are biodegradable and harmless substances with antibacterial action. Thymol, carvacrol, α-terpinene, and caryophyllene are the primary components of thyme essential oil. Thyme, or *Thymus vulgaris L.*, is a medicinal plant that is abundant in essential oils and other volatile compounds. It is extensively used worldwide and is believed to have a possible bone loss prevention effect (**Sienkiewicz et al., 2017 and Nieto, 2020).**

In light of these results, the goal of the current research was to evaluate the potential protective benefits of two herbs (rosemary and thyme), that may assist to avoid developing osteoporosis in female rats caused by glucocorticoids, as well as their impact on bone tissues.

Materials and Methods

Plant Materials

Thyme, (*Thymus vulgaris L.*) and rosemary, (*Rosmarinus officinalis L.*) fresh dried herbs were purchased at the National Research Centre in Cairo, Egypt's Medicinal and Aromatic Plant Unit. To create a

powder, the dry herbs were finely pulverized. Gama Trade Company provided the kits that were used to measure the levels of estradiol hormone, serum total calcium, serum phosphorus, serum calcium, and serum alkaline phosphatase. The betamethasone (Dexaglobe Ampoules) was purchased from Pharmacy.

Rats

Thirty albino adult female rats non-pregnant (weighing about aged 8-9 weeks 180±10g /BW) were purchased from the animal house, Helwan, Cairo, Egypt.

Biological study:

Diet Standard diet was prepared from fine ingredients per 100 gm diet according to **(Reeves et al., 1993**). Biological study

Experimental design:

Rats were kept in separate housing in wire cages and were fed on a basal diet for one week to adapt. After 1 week of adaptation, thirty rats were divided into six groups (five rats each). The first group was fed on a standard diet and represented as negative control, while the other five groups (2^{nd},3^{rd},4^{th},5^{th} and 6^{th})were injected subcutaneously with betamethasone at a dose of 4 mg/ kg BW three times a week for eight weeks (**Zhang, 2014**). One group of them was fed on standard diet and represents the positive control. The other four groups were fed on a standard diet containing 5%, 7.5% both (*Salvia Rosmarinus and Thymus Vulgaris*) for a period of eight weeks.

Isolation and Examination of Rosemary and Thyme Aromatic Substances:

By using Clevenger-style equipment, rosemary and thyme were hydrodistilled for three hours to get the fresh herbs' essential oil. Each sample's resulting oil was dried over anhydrous sodium sulphate (**El-Zaeddi et al., 2016**). Thymol was identified as the primary constituent of thyme using capillary electrochromatography linked to diode array detection (CEC-DAD) and column chromatographic spectrometry (LC-MS/MS) (**Micucci et al., 2020**).

Biochemical analysis:

Serum analysis:

The rats were slaughtered under anesthesia at the conclusion of the study period (8 weeks), and blood samples from the hepatic portal vein were collected, then the serum was extracted using centrifuge tubes. Without using any anticoagulants, Simple tubes were used to collect and allow blood samples to clot. Centrifuged blood samples for 10 minutes at room temperature at 3000 rpm to get clear serum. At -18^{o}C, serum was kept frozen till testing. The left femurs were weighted and put in foil paper and kept in a deep freezer until analyzed.

Calcium and phosphorus levels in the separated serum were determined using the technique of **(Burtis et al., 2012**), Vitamin D3 was determined according to **(wacker and Holick, 2013**), The amount of malondialdehyde (MDA) was measured using the **(Draper and Hadley, 1990**) methods ,inflammatory biomarkers C-Reactive Protein and interleukine -6 (CRP and IL-6) concentrations were determined according to **(Yaser etal., 2020).** Estradiol(E2) by enzyme-linked immune sorbent assay (ELISA) according to (**Saldanha et al., 2011**) and alkaline phosphatase assay (ALP) according to the method by **(Bergmeyer et al., 1986).**

Bone analysis:

In order to assess the bone mineral density of animals, the Medical Service Unit of the National Research Center in Dokki, Egypt, used a Norland XR 46, version 3.9.6/2.3.1 hardware and software for Dual Energy X-Ray Absorptiometry (DEXA).The determination of bone mineral density (BMD) was measured in the right femur of each animal. With this method, a computerised measurement of the right femur's proximal and middle. The left femur's calcium and phosphorus levels were measured in accordance with (**Qin et al., 2021**). One gramme of bone powder was precisely weighed and placed in digesting tubes.

Statistical analysis

One-way analysis of variance (ANOVA) was used to examine the data using the SPSS programme, and the least significant difference (L.S.D.) test was then used to compare the results across groups. Results were shown as mean standard deviation (SD), with $P<0.05$ being used to determine significance (**Armitage and Berr, 1987)**

Results and discussion:

The volatile substances in the essential oils of thyme and rosemary were discovered and their respective area percentages were given in (Table 1). Among the elements found in rosemary, (1,8-cineole) was the major component (45.89%) followed by camphor (25.78%), α-pinene (6.91%), and α-terpineol (4.93%). The thymol was the most abundant constituent (72.91%), Carvacrol comes next. (6.27%), and p-cymene (5.16%), among the isolated volatile compounds of thyme. The primary volatile chemicals of rosemary (*Rosmarinus officinalis L.*), which has several medicinal uses, include 1, 8-cineole, camphor, α-pinene, and α-Terpinene. In the culinary industry, rosemary is frequently utilized as a flavoring ingredient (**Sienkiewicz et al., 2013 and Hamdoon et al., 2020**). Thyme that is still one of the highest amounts of antioxidants and is abundant in vitamins and minerals that is vital for good health (**Dauqan and Abdullah, 2017**).

About 628 g/g of volatile chemicals built up the rosemary extract's fragrant quality. A rosemary mixture of fresh leaves, branches, and stems has a total volatile amount of 135 g/kg (135,000 g/g), according to (**Szumny et al., 2010).**These findings were different from those obtained by(**Szumny et al.,2010**).Thymol was identified as the primary constituent of the essential oil of thyme during our examination of herbs in search of active chemicals for bone metabolism and health. According to several investigations, the two main constituents in thyme essential oil were p-cymene and thymol **(Pirbalouti et al., 2013 and Boruga et al., 2014**). Thymol's powerful antioxidant activity helped to minimize oxidative damage **(Wei et al., 2016**). Thymol has been demonstrated to reduce apoptosis and lipid peroxidation (**Meeran et al., 2016**).

Muhlbauer et al. (2003) evaluated the monoterpenes present in the essential oils of thyme and rosemary, including eucalyptol (1,8-cineole), camphor, borneol, thymol, α-pinene, and bornyl acetate, which prevent bone resorption when added to the diet of rats. They proposed that the monoterpenes camphor, borneol, and thymol, which are direct inhibitors of osteoclast resorption, prevent bone loss by directly influencing bone cells, altering calciotropic hormones, or increasing intestinal calcium absorption.

Sapkota et al. (2018) examined how thymol affected osteoclastogenesis and bone loss in mice and found that it inhibited osteoclast activity, decreased demineralization, and offered protection from cytokines that induce inflammation and bone loss. By significantly reducing inflammatory bone loss, thymol applications were found to be effective treatments for bone serious diseases.

Table (1) Essential oil content of Salvia Rosmarinus and Thymus Vulgaris

Rosmary	Area %	Thyme	Area %
α-Pinene	6.91	α-Terpinene	0.46
Camphen	2.13	p-Cymene	5.16
Camphor	25.78	c-Terpinene	2.49
α-Terpineol	4.93	Carvacrol	6.27
β-Phellandrene	1.89	Terpinen-4-ol	0.41
Limonene	1.34	α-Terpineol	0.18
1,8-Cineole	45.89	Thymol	72.91
Terpinolene	1.39	thymol methyl ether	0.33

Data in Table (2) showed supplementation with thyme and rosemary especially at 7.5% significantly increased serum phosphorus and calcium levels at ($P<0.05$) in both rosemary and thyme groups (3.99±0.43, 3.87±0.56 and5.83±0.48 , 6.62±0.75)in comparison to the positive group (2.84±0.34,3.61±0.56); On the other hand, rosemary and thyme substantially raised the vitD3 levels in all treatment groups at

(P<0.05) compared to the positive group (0.040±0.006), Nevertheless, these levels were considerably (P<0.05) lower than those seen in the normal control group (0.068±0.006).

Insulin-like growth factor I expression is directly and indirectly inhibited by glucocorticoids, reducing the function of the remaining osteoblasts. The first loss of bone following glucocorticoid exposure is probably caused by the activation of bone resorption. In time, the reduction in bone remodeling and ongoing rise in fracture risk will result from the suppression of bone production (**Peng et al., 2021**) The serum Ca and P levels were returned to normal after a treatment plan with rosemary and thyme. Sage, rosemary, and thyme contain phytoestrogenic compounds that resemble estrogen in structure and have the ability to bind to estrogen receptors, perhaps promoting Calcium uptake in intestinal cells via the estrogen receptor (**Magda and Fahmy, 2015).**

According **to Rudin et al. (2019**) 's theory that The body aims to replenish appropriate calcium levels when there is a calcium deficit in the blood, the study indicated a significantly higher level of vitamin D3 following treatment with herbs compared to positive group of rats. PTH levels in the blood commonly rise as a result of the parathyroid glands' that need to enhance PTH production in order to elevate blood calcium levels by absorbing it from bones. In those with low blood calcium levels and high PTH levels, secondary hyperparathyroidism may arise, indicating that the greater level of PTH is a normal response.

In male Sprague-Dawley rats with low body calcium levels, **Elbahnasawy et al., (2019**) assessed the preventive benefits of Thymus vulgaris and Rosmarinus essential oils (EOs) against osteoporosis. The inhibition of bone loss, elevations in plasma calcium, and vitamin D3 levels show that essential oils treatment is helpful in preventing bone resorption and osteoporosis, improvements in bone mineral density, and reduction of inflammation and oxidative stress.

Table (2) effect of Salvia Rosmarinus and Thymus Vulgaris on serum vitamin D ,P,Ca in osteoporotic rats (Mean±S.D)

Groups	Vit D3 ng/ml	P mmol/l	Ca mmol/l
Negative	0.068 ± 0.006^{a}	5.19 ± 0.57^{a}	7.82 ± 0.93^{a}
Positive osteo	0.040 ± 0.006^{c}	2.84 ± 0.34^{c}	3.61 ± 0.56^{d}
Osteo Ros 5%	0.052 ± 0.005^{b}	3.63 ± 0.55^{b}	4.93 ± 0.46^{c}
Osteo Thy 5%	0.051 ± 0.004^{b}	3.57 ± 0.49^{b}	4.66 ± 0.48^{c}
Osteo Ros7.5%	0.055 ± 0.002^{b}	3.99 ± 0.43^{b}	5.83 ± 0.48^{c}
Osteo Thy7.5%	0.053 ± 0.003^{b}	3.87 ± 0.56^{b}	6.62 ± 0.75^{b}

The value in each column with different superscript are significant at (P<0.05). P: phosphorus Ca: calcium

Lipid peroxidation and oxidative stress were assayed in (Table 3), Malondialdehyde MDA levels were substantially higher in the positive

group (10.29±01.17) in comparison to the control group (5.16±0.19) at (P<0.05); otherwise, herb therapy effectively reduced MDA levels. (P<0.05) in Rosemary and Thyme groups at 5% and 7.5% levels (8.30±0.86, 8.17±0.89, 6.16±0.93 and 5.92±1.27, respectively).From data in same table showed interleukin -6 (IL-6) of positive groups increased significantly (P<0.05) (20.18±2.05) than control group but, there were an improvement when rosemary and thyme added at levels (5,7.5 %) (16.07±1.72, 14.01±1.59, 11.62±1.69 and 8.58±0.22 respectively) and the results enhanced with increased levels of two herbs. Concomitantly, the inflammatory biomarker CRP was also estimated as shown in Table (3). The positive control group's CRP inflammation level was noticeably elevated(P<0.05) when compared with normal control (2.18± 0.16 and 0.91±0.86).There were a significant decrease at(P<0.05) of CRP in comparison to positive group in both Rosemary and Thyme groups at levels 5% and7.5% (1.74±0.25, 1.30±0.09, 0.97±0.06 and 0.92±0.10), respectively.

In healthy pre- and postmenopausal women, the production of IL-6 and tumor necrosis factor alpha TNF-α by peripheral blood monocytes is strongly correlated with bone resorption or bone loss (**Bolaji et al., 2019**). IL-1, IL-6, and TNF-α regulate the liver's synthesis of C-reactive protein (CRP), which is regarded as an indicator of extensive inflammation (**Xuehong et al., 2022**). In several inflammatory disorders as well as in healthy people, there is evidence that there is a correlation between subclinical systemic inflammation and bone loss, with the amount of CRP being correlated with bone mineral density (**Ganesan et al., 2005).** Results revealed that thyme and rosemary are effective against oxidative stress and inflammation, and thyme has been used for centuries for its antibacterial and antitumor effects. Thyme also has antioxidant effects (**Faraja et al., 2021**).

Malondialdehyde was seen to be significantly higher in the group of positive controls. in the current investigation, which suggests that the calcium-deficient rats were experiencing elevated oxidative stress. Malondialdehyde levels were found to be greater in cases of bone resorption; this is an indication of serious oxidative stress and free radical production caused by calcium deficiency (**Lian et al., 2010).**

In cases of osteoporosis and osteomyelitis, several inflammatory indicators are frequently related with inflammation. TNF-α and IL-6, two pro-osteoclastic cytokines, are increased under these circumstances. IL-1, IL-6, and TNF-α controlled the synthesis of liver C-reactive protein, which is regarded as a sensitive inflammatory biomarker. Bone mass and CRP levels have been linked in both healthy people and people with inflammatory diseases, demonstrating a connection between

inflammatory responses and bone resorption. The results of the study demonstrated that thyme has an effective anti-inflammatory and anti-oxidative stress impact (**Zeyadi and Khalifa, 2022).**

Table (3) Effect of Salvia Rosmarinus and Thymus Vulgaris on serum MDA, CRP and IL-6 in osteoporotic rats (Mean±S.D).

Groups	MDA(µmol/l)	CRP mg/dl	IL-6 pg/ml
Negative	5.16±0.19 ^{c}	0.91± 0.86 ^{c}	5.94±0.31 ^{e}
Positive osteo	10.29±01.17^{a}	2.18± 0.16 ^{a}	20.18±2.05 ^{a}
Osteo Ros 5%	8.30±0.86 ^{b}	1.74±0.25 ^{b}	16.07±1.72 ^{b}
Osteo Thy 5%	8.17±0.89 ^{b}	1.30± 0.09 ^{b}	14.01±1.59 ^{b}
Osteo Ros7.5%	6.16±0.93 ^{c}	0.97± 0.06 ^{c}	11.62±1.69 ^{c}
Osteo Thy7.5%	5.92±1.27 ^{c}	0.92± 0.10 ^{c}	8.58±0.22 ^{de}

The value in each column with different superscript are significant at ($p<0.05$).
MDA: Malondialdehyde CRP: C- reactive protein IL-6: interleukin -6

From table 4 could be noticed that E2 level decreased significantly in positive group due to glucocorticoid injection when compared to control group (16.14±1.24 and 44.69±2.91) while all groups administered rosemary and thyme at 5,7.5 % levels were significant increased at ($P<0.05$) and the improvement was observed by increasing the levels of herbs. On the other hand, serum ALP positive group showed a highly significant increased at ($P<0.05$) as compared with negative group (138.8±5.01 vs 65.84±3.99) while, all groups administered rosemary and thyme at 5, 7.5% levels were significant decreased at ($P<0.05$) in serum ALP when compared with negative group and the rate of decrease achieved by increased the level of herbs.

According to the current study, increased ALP activity potentially induce elevated bone turnover rates, which are marked by increases in bone formation and resorption, while bone resorption is greater than bone creation, leading to bone loss (**Elwakf et al., 2014).**This shows a rise in osteoblastic and osteoclastic activity, causing a net loss of bone overall and an increase in the excretion of urine hydroxyproline, a bone turnover marker (**Wu et al., 2008**).

The observed improvement of bone metabolic indicators ALP also demonstrated to the beneficial effects of medicinal herbs supplemented diets (**Chiechi et al., 2002**). The benefits of phytoestrogenic herbs were equivalent across all measured parameters, suggesting that they virtually equally protect against bone loss **(Boulbaroud et al., 2008**). The current investigation found that the positive group's ALP activity was noticeably greater than that of the treatment groups. A vital biochemical indicator of bone development is

serum ALP.Increased levels of this enzyme are seen in metabolic diseases such as osteoporosis (**Devarshi et al., 2022**).

Malondialdehyde could be reduced and overall antioxidant capacity might be increased by rosmarinic acid (RO) in all treatment groups (P<0.05). On the other hand, levels of estradiol in the treated groups dramatically rose, and the ability of osteoblast to manufacture estradiol may have also had a contribution in this rise (**Aleih et al., 2011).**

Table (4) Effect of Salvia Rosmarinus and Thymus Vulgaris on serum E2 and ALP in osteoporotic rats (Mean±S.D).

Groups	E2pg/ml	ALP u/l
Negative	44.69±2.91 ^{a}	65.84±3.99 ^{d}
Positive osteo	16.14±1.24 ^{d}	138.8±5.01 ^{a}
Osteo Ros 5%	26.88±2.35 ^{c}	113.4±5.12 ^{b}
Osteo Thy 5%	28.19±2.53 ^{c}	120.4±5.15 ^{b}
Osteo Ros7.5%	34.97±2.16 ^{b}	86.6±3.12 ^{c}
Osteo Thy7.5%	37.38±2.81 ^{b}	86.8±4.13 ^{c}

The value in each column with different superscript are significant at (p<0.05).

Femur mass and bone mineral density(BMD) are shown in Table (5) positive group showed significant changes in femur mass and bon mineral density (2.17±0.27 and 0.07±0.00), respectively, compared to the negative group (3.98±0.31and 0.25±0.01). Otherwise, In comparison to the positive control, the herbal treatment increased significantly (P<0.05) the BMD and femur in all treatment groups, whereas bone phosphorus and calcium trended in the same direction..

The Glucocorticoid has deleterious effect on bone density, and led to suppression of bone formation by a decreasing the number and functioning of osteoblast and induced bone loss (**Sarkis et al., 2012 and Okafor et al., 2016)**. Rosemary and thyme-supplemented diets in the current study had a beneficial impact on BMD. The results are consistent with those of **Elbahnasawy et al., (2019),** who discovered that these botanicals' essential oils stopped bone deterioration. Monoterpenes and essential oil extracts have an immediate impact on bone cells via blocking the mevalonate pathway and prenylating minor G-proteins like Rac and Rho (coupled receptors and their control by cyclic nucleotides) to prevent bone resorption.

Additionally, investigations by **Magda and Fahmy, (2015**) and **Yue et al. (2022**) on the favorable flavonoids effects on bone growth and BMD discovered that the positive control group's femur mass was decreased as a result of a calcium shortage. Furthermore, **Hunt et al., (2008**) discovered that in developing rats, low amounts of calcium drastically decreased bone density and compromised the shape and biomechanical characteristics of bone.

The dietary components that help stop bone loss caused by oestrogen insufficiency have received a lot of attention recently. In the current study, BMD was positively impacted by feeding meals enriched with herbs (rosemary and thyme). According to **Zeyadi and Khalifa, (2022**) flavonoids, a family of phytoestrogens produced from plants, have been shown to stimulate bone morphogenetic protein 2 (BMP2) gene transcriptions when ingested through the stomach (**Wang et al., 2013**). Phenolic substances found in thyme and rosemary may be able to attach to the estrogen receptor (**Magda and Fahmy, 2015**).

These findings are consistent with **Arnold et al. (2015**) and may be explained by the calcium concentration level, which is thought to be the primary trigger for the release of calcitonin by C-cells. The trigger for the release of calcitonin is decreased when blood calcium levels are decreased.

The findings collected showed that thyme and rosemary supplementation were enhancing intestine calcium uptake, which might raise the low plasma calcium levels to normal levels and perhaps stop or lessen bone loss. These recommendations are consistent with the findings of both this study and **Banu et al. (2012**), who discovered that thyme enhanced calcium absorption from the gastrointestinal tract and had a variety of other effects that helped maintain calcium homeostasis.

Table (5) Effect of Salvia Rosmarinus and Thymus Vulgaris on bone P, Ca, femur and BMD in osteoporotic rats (Mean±S.D).

Groups	PB mg/g	CaB mg/g	femur m g/kg BW	BMD g/cm2
Negative	13.22±1.91 ^{a}	45.2±3.66 ^{a}	3.98±0.31 ^{a}	0.25±0.01 ^{a}
Positive osteo	7.69 ±0.86 ^{c}	16.6±2.32 ^{d}	2.17±0.27 ^{c}	0.07±0.00 ^{c}
Osteo Ros 5%	9.09±1.14 ^{b}	21.4±1.11 ^{c}	2.89±0.17 ^{b}	0.13±-.00 ^{b}
Osteo Thy 5%	9.24±1.62 ^{b}	22.4±2.07 ^{c}	3.07±0.29 ^{a}	0.14±0.01 ^{b}
Osteo Ros7.5%	12.88±1.12 ^{a}	35.0±3.72 ^{b}	3.18±0.27 ^{a}	0.19±0.02 ^{b}
Osteo Thy7.5%	13.36±1.70 ^{a}	34.0±3.21 ^{b}	3.18±0.35 ^{a}	0.21±0.02 ^{ab}

The value in each column with different superscript are significant at ($p<0.05$).

Conclusion:

Our findings shown that thyme and rosemary essential oils, that are commonly employed in a range of medical treatments and as food additives, are potent inhibitors of bone turnover and offer several advantages for bone formation and anti-inflammatory properties. However, thyme possesses a more potent anti-osteoporosis and anti-resorption effect than rosemary in several parameters. As a result, using rosemary and thyme as a natural remedy can aid in reducing bone loss linked to estrogen deficient situations.

References

Ahmed, R. A., Ali, M. A., and Qayssar, J. F., (2016): A Comparative Study of the Effect of Thyme and Calcium with Vitamin D3 in Treatment of postmenopausal Women with Osteoporosis. Inter.J. of Pharm.Tech .9, (5), 260-268.

Akkawi, I., and Zmmerly, H., (2018): Osteoporosis: Current Concepts, Review Article Joints, 6, (2), 122-127.

Aleih, G., Arash, K., Laya, F., Amir, A. K., Mehdi, M., Hamidreza, A. A., Bushra, A. H., Elham, G., Meisam, N. and Elaheh, O., (2011): Effect Of Rosmarinic Acid On Estrogen, FSH And LH In Female Diabetic Rats. African J. of Pharma. and Pharma., 5, (11), 1427-1431.

Armitage, P., and Berry, J., (1987): Statistical Methods in Medical Research. Blackwell, Oxford, UK,93-212.

Arnold, J. F., and Barton, S. L., (2015): Calcitonin, The Forgotten Hormone: Does It Deserve To Be Forgotten? Clin. Kidney. J., 8, (2), 180–187.

Banu, J., Varela, E., and Fernandes, G., (2012): Alternative therapies for the prevention and treatment of osteoporosis," Nutrition Reviews, 70, (1), 22–40.

Bergmeyer, H. U., Horder, M., and Rej, R. (1986): International Federation of Clinical Chemistry (Ifcc) 1, 2. J. clin. Chem. clin. Biochem., 24, (7), 481-495.

Bolaji, L.O., Linda, S., Barbara, K.S., Michelle, M. C, Sonya, M., Jane, C., Nicole, C. R. and Marlena, C.K., (2019): Inflammatory Markers And Bone Health In Postmenopausal Women: A Cross-Sectional Overview. Immu. and Age., 16, (15), 1-12.

Boruga, O., Jianu, C., Mis, C., Golet, I., Gruia, A. T., and Horhat, F. G., (2014): Thymus Vulgaris Essential Oil: Chemical Composition And Antimicrobial Activity, J. of Med. and Life, 7, (3), 56–60.

Boulbaroud, S., ArfaouI, A., Abdelkrim, C., Mesfioui, A., Ouichou, A., and EL Hessni A., (2008): Does flaxseed uptake reverse induced bone loss in ovariectomized rats? Int. J. Osteoporos. Metab. Disord., 1, (1), 24-30.

Burtis, C. A., Ashwood, E. R., and Bruns, D. E., (2012): Tietz Textbook of Clinical Chemistry and Molecular Diagnostics - E-Book. Elsevier Health Sciences. - Medical - 2256 pages.

Chiechi, L. M, Secreto, G., Vimercati, A., Greco, P., Venturelli, E., Pansini, M. Fanelli, F., Loizzi, P. and Selvaggi, L., (2002): The Effects Of A Soy Rich Diet On Serum Lipids, The Menfis Randomized Trial. Maturitas, 41, 97-104

Chotiyarnwong, P., and McCloskey, E. V., (2020): Pathogenesis Of Glucocorticoid-Induced Osteoporosis And Options For Treatment. Natu. Rev. Endocr., 16, (8),437-447.

Dauqan, E.M.A., Abdullah, A., (2017): Medicinal and Functional Values of Thyme (*Thymus vulgaris L*.) herb. J. App. Biol. Biotechnol., 5, 017–022

Devarshi, R., Nayank, G., Zeenat, A., Shah, W., and Rajeshwar, N. S., (2022): Prevalence of Abnormal Bone-Specific Alkaline Phosphatase in Orthopaedic Trauma Patients: A Cross-Sectional Study from a Tertiary Trauma Centre. Cureus.14, (4),e24264. doi: 10.7759/cureus.24264

Draper, H. H., and Hadley, M., (1990): Malondialdehyde Determination As Index Of Lipid Peroxidation. Meth. in Enzymo., 186, 421–431.

Elbahnasawy, S. A., Valeeva, E. R., El-Sayed, E. M., Rakhimov, I. I., (2019): The Impact of Thyme and Rosemary on Prevention of Osteoporosis in Rats. Journal of nutrition and metabolism, p 1-10.

El-Zaeddi, H. J. Mart´ınez, T., Cal ın, A. S, Burl ,´ F. O., and Carbonell, B. , (2016): "Volatile Composition Of Essential Oils From Different Aromatic Herbs Grown In Mediterranean Regions Of Spain," Foods, 5, (4), 41.

El Wakf, A. M., Hassan, H. A., and Gharib, N. S., (2014): Osteoprotective Effect Of Soybean And Sesame Oils In Ovariectomized Rats Via Estrogen-Like Mechanism.
Cytotechnology. 66, (2),335–343.

Faraja, D. G., Jiheng, L., Wenhua, M., Jinghu, X., Fedrick, C., Meiling, C. and Shaimaa, R. H., (2021): Antimicrobial Properties and Mechanism of Action of Some Plant Extracts against Food Pathogens and Spoilage Microorganisms. Front Microbial. 9, 1639.

Ganesan, K., Teklehaimanot, S., Tran, T. H., Asuncion, M., and Norris, K. (2005):"Relationship of C-reactive protein and bone mineral density in community-dwelling elderly females," J. of the Nation. Med. Associ, 97, 329–33.

Golob, A. L., and Laya, M.B., (2015): Osteoporosis: Screening, Prevention, and Management. The Med. Clin. of North Amer, 99, (3), 587–606.

Hamdoon, A. M., Mohsen, S. A., Salman, A. A. M, Mohamed S. A. A., Abdulmalik, N. A. A, and Riaz, A. K., (2020): Drying Induced Impact on Composition and Oil Quality of Rosemary Herb, *Rosmarinus Officinalis Linn*, Molecules, 25,(12), 2830.

Hunt, J. R, Hunt, C. D., Zito, C. A., Idso, J. P., and Johnson, L. K., (2008):Calcium Requirements Of Growing Rats Based On Bone Mass, Structure, Or Biomechanical Strength Are Similar, J. of .Nut., 138, 8, 1462–1468.

Lian, K. C., Chuang, J. J and Hsieh, C.W., (2010): Dual Mechanisms Of NF-Kb Inhibition In Carnosol-Treated Endothelial Cells," Toxico. and Appl.Pharm., 245, 1, pp. 21–35.

Magda, M. E., and Fahmy, G. E., (2015): Anti-Osteoporotic Effect Of Medical Herbs And Calcium Supplementation On Ovariectomized Rats. The J.of Bas. & Appl. Zoo. 72, 81-88.

Meeran, M. F. N, Jagadeesh, G. S., and Selvaraj, P., (2016): Thymol, A Dietary Monoterpene Phenol Abrogates Mitochondrial Dysfunction In B-Adrenergic Agonist Induced Myocardial Infarcted Rats By Inhibiting Oxidative Stress," Chemico-Bio. Interac., 244, 159–168,

Micucci, M., Protti, M., Aldini, R., Frosini, M., Corazza, I., Marzetti, C., Mattioli, L.B., Tocci, G., Chiarini, A. and Mercolini, L. (2020): *Thymus vulgaris L*. Essential Oil Solid Formulation: Chemical Profile and Spasmolytic and Antimicrobial Effects. Biomolec . 10, 860.

Muhlbauer, 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, 372–380.

Nieto, G., (2020): A Review On Applications And Used Of Thymus In The Food Industry. Plants, 9, 961.

Okafor, H. K., Odugbemi, A. I., and Omogo, S. E., (2016): A Comparative Study of the Ameliorative Effects of Skimmed Milk and Tuna Fish in the Dietary Management of Dexamethasone Sodium Phosphate Induced Osteoporotic Female Wister Rats. J.of Advanc. In Med. and Pharma .Sci., 1-13

Orr. S. (2019):"Pathophysiology of Osteoporosis", Nursing Student Class Projects (Formerly MSN). 385-395.

Peng, C. H., Lin, W.Y, Yeh, K.T., Chen, I.H, Wu, W.T, Lin, M.D., (2021): The Molecular Etiology And Treatment Of Glucocorticoid-Induced Osteoporosis. Tzu. Chi. Med .J. 33, (3), 212–23.

Pirbalouti, A. G., Hashemi, M., and Ghahfarokhi, F. T., (2013):"Essential oil and chemical compositions of wild and cultivated -ymus daenensis Celak and *ymus vulgaris L," Industrial Crops and Products, 48, 43–48.

Qaseem, A., Forciea, M. A., McLean, R. M., Denberg, T. D., (2017): Clinical Guidelines Committee of the American College of Physicians Treatment of low bone density or osteoporosis to prevent fractures in men and women: a clinical practice guideline update from the American College of Physicians, Ann .Intern .Med , 166, (11), 818–839.

Qin, X., Shen, Q., Guo, Y., Li. X., Liu, J., Ye, M., and Zhang. C., (2021): Physicochemical properties, digestibility and anti-osteoporosis effect of yak bone powder with different particle sizes. Food Res. Inter., 145: 110401.

Reeves, P. G., Nielsen, F. H. and Fahey, G. C. J.r (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. Nov; 123 (11):1939-51.

Rudin, A.V, McKenzie, T.J, Wermer, R.A, Thompson, G.B,and Lyden, M.L., (2019): Primary Hyperparathyroidism: Redefining Cure. Am Surg. 85,(2),214-218.

Saldanha, C. J., Remage-Healey, L., and Schlinger, B. A., (2011): Synaptocrine Signaling Steroid Synthesis And Action At The Synapse. Endocr. Rev., 32,(4), 532-549.

Sapkota, M., Li, L., Kim, S.W., and Soh, Y., (2018): Thymol inhibits RANKL-induced osteoclastogenesis in RAW264.7 and BMM cells and LPS-induced bone loss in mice." Food and Chem. Toxico., 120, 418–429.

Sarkis, K. S., de Medeiros, P., M., Szejnfeld, V. L and Martini, L. A., (2012): High Bone Density And Bone Health. Endocrinología y Nutrición (English Edition), 59, (3), 207-214.

Sienkiewicz, M., Tysakowska, M., Pastuszka, M., Bienias, W. and Kowalczyk, E., (2013): The Potential Of Use Basil And Rosemary Essential Oils As Effective Antibacterial Agents," Molecules. 18, (8), 9334–9351.

Sienkiewicz, M., Łysakowska, M., Kowalczyk, E., (2017):"The Ability Of Selected Plant Essential Oils To Enhance The Action Of Recommended Antibiotics Against Pathogenic Wound Bacteria," Burns, 03, 9, 310–317.

Szumny, A., Figiel, A., Gutiérrez-Ortíz, A. and Carbonell-Barrachina, A.A., (2010): Composition Of Rosemary Essential Oil (Rosmarinus Officinalis) As Affected By Drying .method. J. Food Eng, 97, 253–260.

Wacker, M., and Holick, M.F., (2013): Sunlight and Vitamin D: A global perspective for health. Deimaloendocrinol. 5,51-108.

Wang, Z. Q., Li J. L. and Sun, Y. L (2013):"Chinese Herbal Medicine For Osteoporosis: A Systematic Review Of Randomized Controlled Trails," Evidencebased Complementary and Alternative Medicine, vol 2013, 11. https://doi.org/10.1155/2013/356260

Wei, H. K., Xue, H. X., Zhou, Z. X., and Peng, J. (2016): A Carvacrolthymol Blend Decreased Intestinal Oxidative Stress And Influenced Selected Microbes Without Changing The Messenger RNA Levels Of Tight Junction Proteins In Jejunal Mucosa Of Weaning Piglets. Animal, 15, 1–9.

Wright, N. C., Looker, A. C., Saag, K. G., (2014):"The Recent Prevalence Of Osteoporosis And Low Bone Mass In The United States Based On Bone Mineral Density At The Femoral Neck Or Lumbar Spine," J. of Bone and Min. Res, 29, (11), 2520–2526.

Wu, T., Zhang, Y., Yuan, H., Wu, G., Li, B. and Cui, L. (2008): Preventive Effects Of Salvianolic Acid B On Osteopenia Of Rats Induced By Ovariectomy. Bone. 43,75–76. doi: 10.1016/j.bone.2008.08.105.

Xuehong, Z., Liang, C., Bin, C., Qinzhi, X., Yanqin, Z., Jinhua, Y., Qiulin, Y., (2021): Effect Of Chromium Supplementation On Hs-CRP, TNF-A And IL-6 As Risk Factor For Cardiovascular Diseases: A Meta-Analysis Of Randomized-Controlled Trials. Complem. Thera. in Clin. Prac. 42, 101291.

Yaser, G. Behnam, A., Mehrzad, J., Mohsen, G., Reza, V. and Behnam, S. (2020): Determination of Serum Levels of Interleukin-6, Interleukin-8, Interleukin-10, and Tumor Necrosis-Alpha and their Relationship with the Total Body Surface Area in Children. J .Burn Care .Res. , 2; 41,(3),539-543.

Yue, Z., Meng, L., Qiyao, J., Hanzhong, K., Qingchun, L., Ling-F, Z. and Jiansong, F., (2022): Evolving Roles of Natural Terpenoids from Traditional Chinese Medicine in the Treatment of Osteoporosis. Frontiers in Endocrinology, 13, 901545. doi: 10.3389/fendo.2022.901545.

Zeyadi, M. A., and Khalifa, F. K. ,(2022): The protective role of almond and thyme in carbonated beverage-induced osteoporosis in male albino rats. International Journal of Health Sciences, 6(S5), 880–890.

Zhang, Y., Wang, K., Zheng, J., Shan, H., Kou, J., Liu, R. and Wang, C. (2014): Glucocorticoids Activate The Local Renin–Angiotensin System In Bone: Possible Mechanism For Glucocorticoid-Induced Osteoporosis. Endocrine, 47, 598–608.

ملخص البحث:

هشاشة العظام هي مرض عالمي يتميز بانخفاض كتلة العظام ، وبالتالي فإن الهدف من هذه الدراسة هو تثبيط هشاشة العظام التي يسببها الجلوكورتيكويد في إناث الفئران عن طريق إكليل الجبل والزعتر. الطريقة: أجريت الدراسة الحالية على ثلاثين أنثى جرذ. تم تقسيم الجرذان إلى ست مجموعات (خمسة فئران لكل مجموعة). المجموعة الأولى تم إطعامها على الغذاء الاساسى وتمثل بالمجموعة الضابطة السلبية ، بينما تم حقن المجموعات الخمس الأخرى بالبيتاميثازون تحت الجلد بجرعة ٤ مجم / كجم من وزن الجسم ثلاث مرات في الأسبوع. مجموعة واحدة منهم تمثل المجموعة الضابطة الإيجابية. تم تغذية المجموعات الأربع الأخرى بنظام غذائي أساسي يحتوي على ٥٪ و ٧.٥٪ من إكليل الجبل والزعتر لمدة ثمانية أسابيع. النتائج: أظهرت المجموعة الضابطة الإيجابية انخفاضًا معنويًا في فيتامين د ٣ والفوسفور والكالسيوم ومستويات الاستراديول والكالسيوم والفوسفور في عظم الفخذ وانخفاض معنوي في معادن العظام. الكثافة ، وزيادة معنوية في المالون داى الدهيد ، والبروتين التفاعلي ، والإنترلوكين ٦ والفوسفاتاز القلوي بالمقارنة مع مجموعة الضابطة السلبية. من ناحية أخرى ، فإن جميع مجموعات هشاشة العظام التي تم تناولها بمستويات مختلفة عند ٥٪ و ٧.٥٪ من إكليل الجبل والزعتر كان لها انخفاض معنوي في مصل في المالون داى الدهيد ، والبروتين التفاعلي ، والإنترلوكين ٦ والفوسفاتاز القلوي وزيادة معنوية في فيتامين د٣، الكالسيوم ، الفوسفور ، الاستراديول ، والكالسيوم والفوسفور في عظم الفخذ و في معادن العظام ، مقارنة مع المجموعة الضابطة الإيجابية. الخلاصة أظهرت حماية الزعتر وأعشاب إكليل الجبل للعظام من هشاشة العظام التي يسببها الجلوكوكورتيكويد في إناث الجرذان.إكليل الجبل والزعتر كان لة تأثير وقائي قوي بسبب محتواه من الزيوت الأساسية.

الكلمات المفتاحية: هشاشة العظام، الزيوت الأساسية ، زعتر، الكالسيوم، الفوسفور.