

Ex vivo study on the combination of *Sophora japonica* fruit extract and a highly soluble form of curcumin on the skeletal system of rats with estrogen deficiency

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Abstract

The present study aimed to investigate the effects of Fructus *Sophorae* extract (FSE) combined with a highly soluble form of curcumin on the skeletal system of rats with estrogen deficiency induced by subcutaneous implantation of a gonadotropin-releasing hormone (GnRH, LH-RH) analogue, goserelin (GR). FSE and curcumin demonstrated significant bone-protective effects in GR-induced estrogen-deficient rats by maintaining calcium and phosphorus homeostasis that augmented markers of bone formation and bone mineral density. The administration of estradiol as a positive control and the combination of FSE and curcumin increased osteocalcin levels by 43% and 23%, respectively, above those in castrated animals and brought them closer to control levels. In the GR-treated group, β -CTx increased by 27%; in the group treated with estradiol, by 17%; and in the groups treated with FSE and the combination of FSE and curcumin, respectively, by about 15%.

These findings provide information about the bone-protective effects of FSE and curcumin against GR-induced bone loss.

Keywords

bone turnover markers, curcumin, goserelin, gonadotropin-releasing hormone, osteoporosis, *Sophora japonica*

Introduction

The use of gonadotropin-releasing hormone (GnRH) or luteinizing hormone-releasing hormone (LH-RH) agonists for the treatment of hormone-dependent malignancies, such as prostate, ovarian, and some breast cancers, is

becoming common practice. This form of endocrine therapy reduces sex hormone concentrations to near-castration levels by reducing the secretion of gonadotropins, and in particular LH-RH, from the pituitary gland, which in turn inhibits the growth and proliferation of malignantly degenerated cells. Although LH-RH agonist therapy can be an

effective option for the treatment of some cancers, it is also associated with numerous musculoskeletal side effects. Decreases in bone mineral density have been observed in both men and women receiving LH-RH agonists. The low levels of sex hormones that occur in postmenopausal women and in elderly men negatively affect the skeletal system. However, the decreased levels of sex hormones resulting from the natural aging process (i.e., a gradual decline in estrogen or testosterone over time) are different from the conditions experienced by patients receiving LH-RH agonist therapy (i.e., in individuals with normal estrogen or testosterone levels and a dramatic decline over a short period of time). This type of dramatic cessation of sex hormone synthesis has been shown to have a more serious impact on bone architecture, especially after surgical orchiectomy and oophorectomy (Hydock et al. 2008). Hormonal agents, such as GnRH agonists (goserelin, buserelin), inhibit gonadotropin secretion, resulting in a hypogonadal state that mimics menopause in women and chemical castration in men. Bone turnover, as indicated by circulating osteoblast and osteoclast biochemical markers, increases progressively with GnRH agonist use. This results in a 5–10-fold higher rate of bone loss and a 6.5%–17% lower bone mass in patients treated with GnRH agonists compared with patients not treated with these drugs (Patel and Ganti 2025).

This gives an opportunity for some authors to use this model of chemical castration in experimental animals to study the pathophysiology of hormone-dependent osteoporosis, as well as to conduct studies on new drugs and plant molecules for prevention, treatment, or adjuvant therapy in the fight against osteoporosis (Goulding and Gold 1990; Hydock et al. 2008; Mohamad et al. 2018; Yutong et al. 2023).

Many author teams are investigating the bone-protective effect of natural compounds of plant origin. Among them, curcumin shows promising therapeutic effects on osteoporosis (Fan et al. 2022; Li et al. 2024). Several studies have shown that curcumin improves bone microarchitecture in glucocorticoid-induced secondary osteoporosis by regulating the activation of microRNA-365 through MMP-9 (Li et al. 2015). Curcumin also affects dexamethasone-induced osteoporosis by increasing serum osteocalcin and reducing markers of bone resorption (Li et al. 2015). In vitro studies have shown that curcumin affects the differentiation, activity, and lifespan of osteoblasts and osteoclasts (Cho et al. 2013). It exhibits antioxidant and bone metabolism-regulating effects. Studies have shown that curcumin can improve bone microarchitecture and increase bone mineral density (BMD) in APP/PS1 transgenic mice (Yang et al. 2011) and has demonstrated bone-protective effects in animal models and patients with postmenopausal osteoporosis (Bose et al. 2018; Khanizadeh et al. 2018; Bukhari et al. 2019; Jiang et al. 2021). More importantly, recent studies have also established the therapeutic value of curcumin in diabetes-induced osteoporosis (Liang et al. 2020; Deng et al. 2021). The benefits of curcumin on bone formation and regeneration are due to its ability to reduce H₂O₂-stimulated apoptosis of osteoblasts (Li et al. 2020), improve mitochondrial function of osteoblasts (Dai et al. 2017), and re-

store impaired osteogenic differentiation of osteoblasts and bone marrow stromal cells (BMSCs) (He et al. 2020; Chen et al. 2021). It reduces the expression of inflammatory cytokines, including TNF, IL-1, IL-6, IL-8, and chemokines (Sadeghi et al. 2023). The resorption and bioavailability of curcumin remain the main problems.

Bone-protective effects of FSE have already been investigated by our team and published (Chakuleska et al. 2022).

All these data gave us a reason to study the anti-osteoporotic effect of the combination of FSE with a highly soluble form of curcumin (Cursol®) on goserelin (GR)-induced estrogen deficiency in female rats.

Materials and methods

Plant materials

The extract from fruits of *Sophora japonica* (Fabaceae) was kindly donated by Alexander Shkondrov, Assoc. Prof. in the Department of Pharmacognosy, Faculty of Pharmacy, Medical University of Sofia, Bulgaria. The phytochemical investigation of FSE was described previously in detail (Chakuleska et al. 2022).

Animals

Twenty-eight 3-month-old female Wistar rats were purchased from the National Breeding Centre, Sofia, Bulgaria, and randomly divided into seven groups of four animals per group (n = 4). Four rats constituted the control group. In the remaining 24 animals, chemical castration and estrogen deficiency were induced by subcutaneous implantation of goserelin (GR), a GnRH analogue primarily of LH-RH. Rats received a total of two doses of 3.6 mg goserelin depot formulation (goserelin acetate, contained in a biodegradable cylinder measuring 5 mm in length × 1 mm in width) implanted subcutaneously in the animal's neck. One 3.6 mg goserelin acetate depot has been shown to effectively reduce estrogen levels in female rats to castration levels for 28 days. Animals were implanted on days 1 and 29, thereby blocking estrogen production for a total of 56 days (8 weeks) (Hydock et al. 2008). The study design was approved by the Bulgarian Agency of Food Safety (permission № 251, issued on 20 November 2019), and the principles stated in the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS 123) (Council of Europe 2007) were strictly followed throughout the experiment.

Experimental design

The animals were divided into seven groups as follows:

Group 1: Control group;

Group 2: Rats with estrogen deficiency (ED), induced by subcutaneous (s.c.) implantation of two doses of goserelin depot (on days 1 and 29) (Hydock et al. 2008);

Group 3: Rats with ED, treated subcutaneously with estradiol as a positive control (EST) (40 µg/kg/day, 45 days) (Abe et al. 1993);

Group 4: ED rats treated orally with FSE (100 mg/kg/day, 45 days);

Group 5: ED rats treated orally with FSE (200 mg/kg/day, 45 days);

Group 6: ED rats treated with the combination of FSE (100 mg/kg/day) and Cursol® (2 g/kg/day) for 45 days (Cho et al. 2013).

Group 7: ED rats treated with the combination of FSE (200 mg/kg/day) and Cursol® (2 g/kg/day) for 45 days.

Periodically, on the 1st, 2nd, 4th, and 7th weeks after chemical castration, blood was taken from the tail vein (after warming and local anesthesia) for analysis of the biochemical parameters calcium, phosphorus, alkaline phosphatase, and acid phosphatase in serum. During these analyses, throughout the experimental period, no differences in biochemistry were noted between groups 4 and 5, as well as between groups 6 and 7; therefore, further studies were carried out only with the higher dose of FSE (200 mg/kg). On the 21st and 46th days, biochemical measurements of the pro-inflammatory markers CRP and IL-6 were also made. At the end of the experiment, on day 46, after 24 hours of fasting, the animals were anesthetized and killed by decapitation. Liver, bones, and small intestines were taken to assess the oxidative stress markers MDA and GSH, and blood was collected to assess bone metabolism and the inflammatory markers CRP and IL-6.

Markers of calcium homeostasis

Blood from the experimental animals was collected in tubes containing a clot activator. After centrifugation at 3000 × g for 10 min, serum was separated and stored at –20 °C for further evaluation. Calcium (Ca) and phosphorus (P) were measured on the 1st, 2nd, 4th, and 7th weeks after GR implantation using commercially available kits for the biochemical analyzer Mindray-BP 120 (China). 25-OH Vit. D was analyzed at the end of the experimental period using the electrochemiluminescence immunoassay (ECLIA) method with kits for the immunoassay analyzer (Cobas-Roche, Basel, Switzerland) in the hospital “Alexandrovskia.”

Markers of bone turnover

Markers of bone formation—osteocalcin (OC) and alkaline phosphatase (ALP)—and markers of bone resorption—Beta-CrossLaps (β-CTX) and acid phosphatase (ACP)—were analyzed with commercially available kits. ALP and ACP were measured on the 1st, 2nd, 4th, and 7th weeks using the biochemical analyzer Mindray-BP 120. OC and β-CTX were analyzed at the end of the experiment using ECLIA with kits for an immunoassay analyzer (Cobas-Roche, Basel, Switzerland) in the hospital “Alexandrovskia.”

Markers of oxidative stress

For the following experiments, the excised right femurs, livers, and intestines were washed with cold (4 °C) saline solution, blotted dry, weighed, and homogenized with appropriate buffers. Femurs were first frozen at –80 °C, then crushed in a porcelain mortar and ground in a laboratory mill (disintegrator type). Lipid peroxidation in livers, intestines, and bones was determined by measuring the rate of production of thiobarbituric acid reactive substances (expressed as MDA equivalents) using the method described by Héctor Polizio and Peña (2005). GSH was assessed in the same organs by measuring non-protein sulfhydryls after precipitation of proteins with trichloroacetic acid (TCA), using the method of Bump et al. (1983).

Statistical analysis

“MEDCALC” software was used for the statistical analysis of the results. Data are expressed as mean ± SD of four rats in a group. The significance of the data was assessed using the Mann–Whitney *U* test. Values of $p \leq 0.05$ were considered statistically significant.

Results

Parameters of calcium-phosphorus homeostasis

One week after goserelin implantation, calcium (Ca²⁺) levels were higher ($p < 0.05$) in all chemically castrated rats treated with natural substances compared to the control group and remained relatively higher until the end of the experimental period. In the groups treated with the combinations FSE 100 + CCN and FSE 200 + CCN, after the fourth week of the experiment, calcium levels decreased significantly by about 45% ($p < 0.05$) compared to the castrated group, and at the end of the experiment, on day 46, the calcium level in these groups was comparable to its level in the control group (Fig. 1). The groups treated with estradiol alone and both doses of FSE at the end of the experiment showed significantly lower calcium levels by about 10–15% compared to untreated estrogen-deficient rats, but still very high, by about 100%, compared to the control group (Fig. 1).

The phosphorus (P) level remained lower ($p < 0.05$) throughout the entire period of the experiment in all castrated rats treated with the studied substances compared to the control group (Fig. 2). In the groups treated with the combinations, by the end of the experimental period, the phosphorus level reached the control values. In the groups treated with FSE alone, the phosphorus level increased by 16% at the end of the experiment, a statistically significant difference compared to the estrogen-deficient group. Still, it did not reach the control serum concentrations.

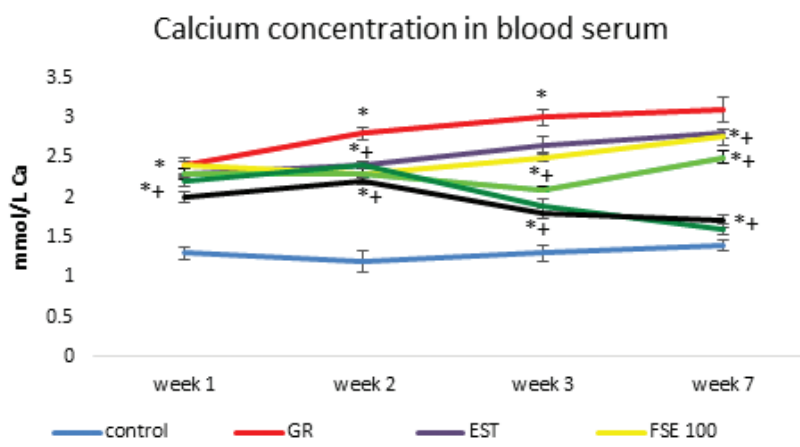


Figure 1. Calcium level in blood serum; * $p < 0.05$ compared to the control group; + $p < 0.05$ compared to the estrogen-deficient group.

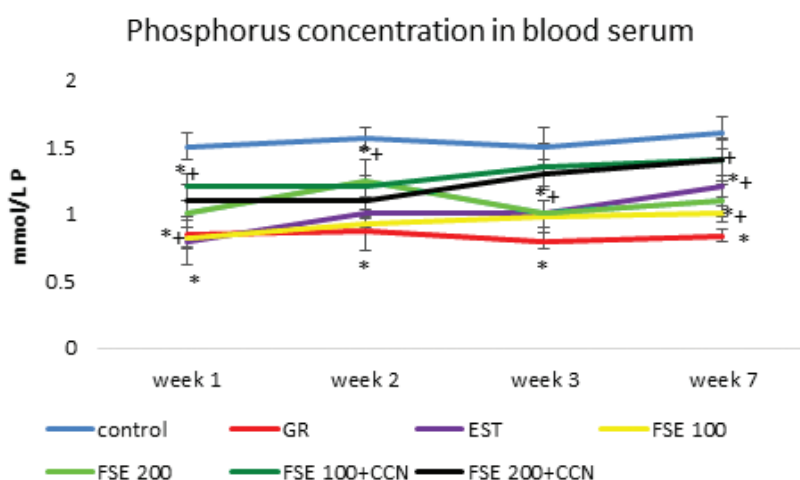


Figure 2. Phosphorus level (measured as phosphates) in blood serum; * $p < 0.05$ versus control group; + $p < 0.05$ versus GnRH group.

Bone metabolism parameters

Chemical castration led to a statistically significant increase in the activity of ALP and ACP (Figs 3, 4) compared to the control during the entire experimental period, which is also a sign of increased bone turnover. The individual administration of the substances did not statistically significantly affect the activity of ALP but reduced ($p < 0.05$) the activity of ACP compared to the castrated group, which is a marker for slowing the rate of bone resorption. The best effect was observed after the application of the combinations, which practically normalized the activity of the studied enzymes and brought them closer to the control values.

From Fig. 5, it is evident that animals with chemically induced gonadectomy and estrogen deficiency had a lower level of vit. D compared to the control group. Treatment with estradiol and the studied plant substances increased the level of vit. D compared to the pathological group, as in the group treated with the combination of FSE and curcumin, the level of vit. D is practically the same as that of the control animals.

Osteocalcin levels were significantly reduced in animals with ED and in those treated with FSE by 25% and 29%, respectively, compared to the control. The adminis-

tration of estradiol and the combination of FSE and curcumin increased osteocalcin levels by 43% and 23%, respectively, above those in GR-treated animals and brought them closer to control levels (Fig. 6).

Fig. 7 presents the results of the analysis of β -CrossLaps in the serum of the experimental animals. Subcutaneous implantation of an LH-RH analogue led to a statistically significant increase in the level of the studied parameter compared to the control group. In the GR-treated group, β -CTx increased by 27%; in the group treated with estradiol, by 17%; and in the groups treated with FSE and the combination of FSE and curcumin, respectively, by about 15%.

Markers of oxidative stress and inflammation

Fig. 8 shows the values from the study on the lipid peroxidation marker MDA in the livers, intestines, and bones. It is noteworthy that the estrogen deficiency induced by LH-RH agonist implantation led to a significant increase in the MDA level by 18%, 10%, and 13%, respectively, in the investigated organs. The administration of estradiol, the studied extract, and its combination with curcumin normalized the levels of lipid peroxidation.

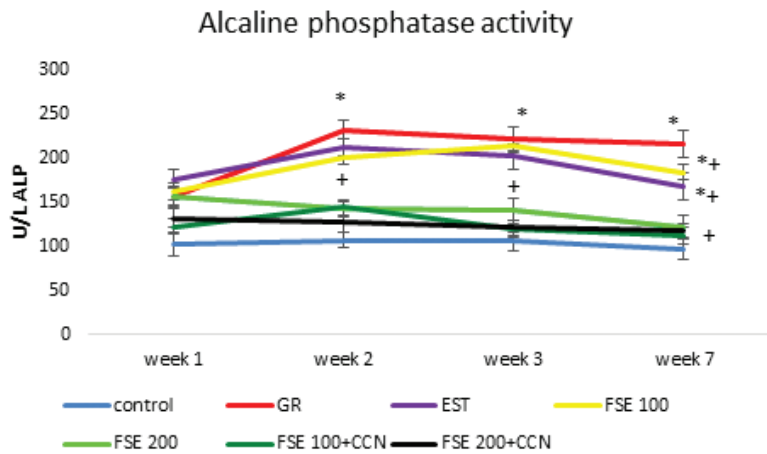


Figure 3. ALP activity in serum from experimental animals; *p < 0.05 compared to the control group; +p < 0.05 compared to the GnRH group.

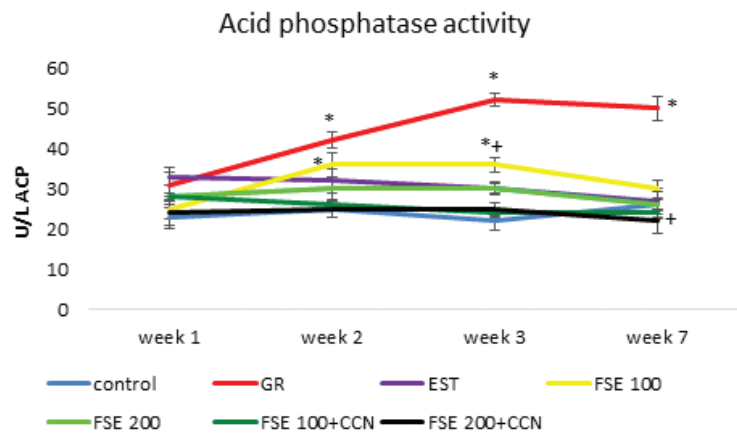


Figure 4. ACP activity in serum from experimental animals; *p < 0.05 compared to the control group; +p < 0.05 compared to the GnRH group.

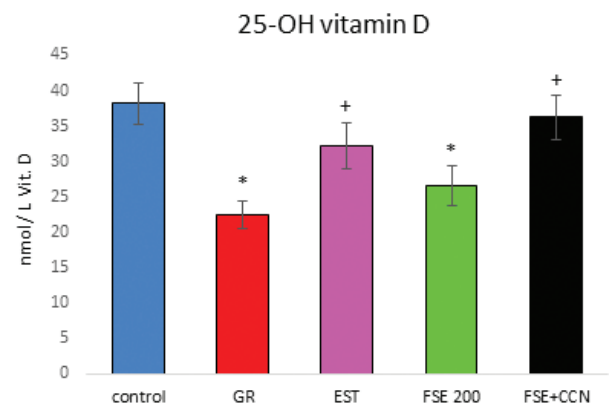


Figure 5. Concentration of vitamin D in blood serum; *p < 0.05 compared to the control group; +p < 0.05 compared to the GnRH group with estrogen deficiency.

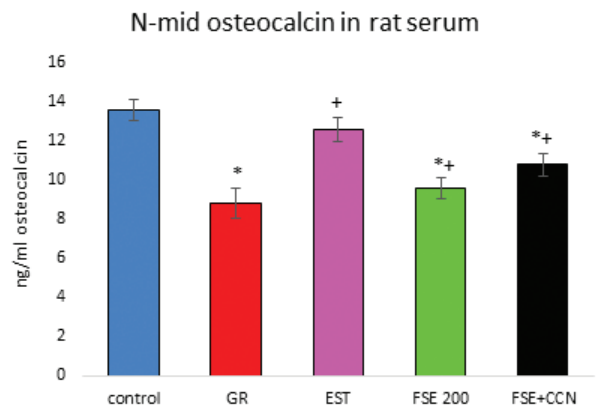


Figure 6. Osteocalcin level in serum of the studied animals; *p < 0.05 compared to the control; +p < 0.05 compared to the GnRH group with estrogen deficiency.

Estrogen deficiency induced a significant depletion of the levels of GSH (Fig. 9). In the liver, GSH decreased by 20%, and in the intestines and bones by 17% and 21%, respectively, compared to control animals. Treatment of deficient rats with estradiol, FSE, and the combination of FSE and curcumin maintained the GSH level close to the

control level, with the most pronounced antioxidant effect being exhibited by the FSE + CCN combination, in which the GSH level increased by 17%, 21%, and 30% in the liver, intestines, and bones, respectively (Fig. 9).

IL-6 measurement (Fig. 10) in the serum of experimental animals on day 21 showed that estrogen defi-

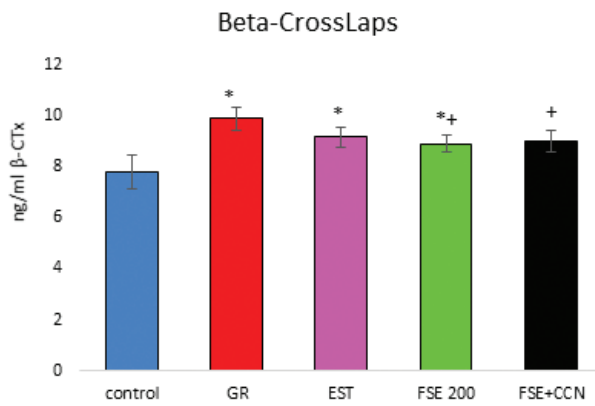


Figure 7. Level of beta cross-laps (β -CTx) in serum of the studied animals; * $p < 0.05$ compared to the control; + $p < 0.05$ compared to the GnRH group with estrogen deficiency.

ciency was associated with a significant increase in this pro-inflammatory marker by 144% compared to the control level. The administration of both estradiol and the studied natural compounds reduced the level of IL-6 by 32% and 41%, respectively, for the extract and its combination with curcumin.

On day 46 of the experiment, the level of IL-6 in the group with chemically induced estrogen deficiency was 100% higher than that of the controls. It is noteworthy that the level of the measured marker on day 46 was lower in the group with estrogen deficiency by 27% compared to the levels measured in the same group on day 21. The administration of estradiol and the studied biologically active substances did not lead to a statistically significant change in the levels of this pro-inflammatory marker compared to the group treated with goserelin, despite the tendency for its decrease.

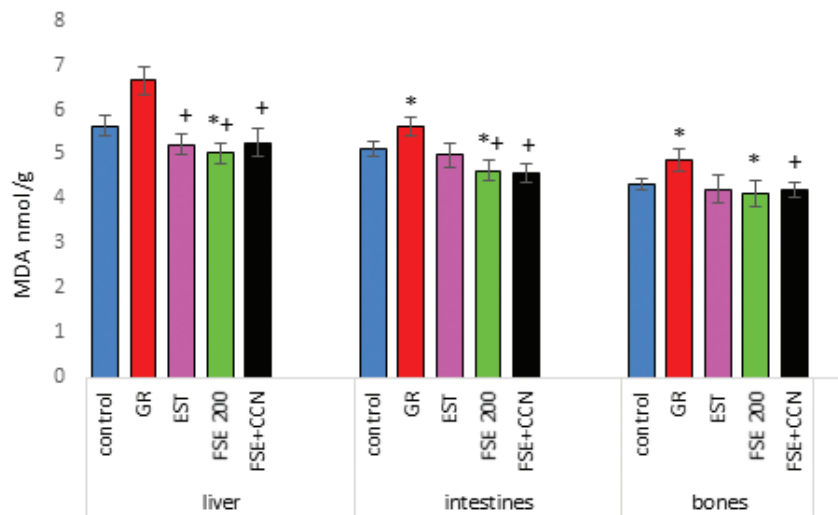


Figure 8. MDA levels in liver, small intestines, and bones of chemically castrated rats; * $p < 0.05$ compared to the control group; + $p < 0.05$ compared to the GnRH group.

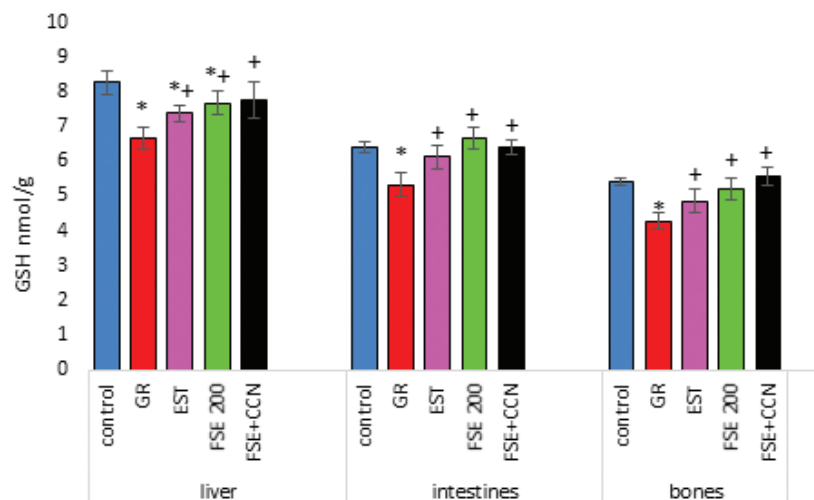


Figure 9. GSH levels in livers, small intestines, and bones of chemically castrated rats; * $p < 0.05$ versus control group; + $p < 0.05$ versus GnRH group.

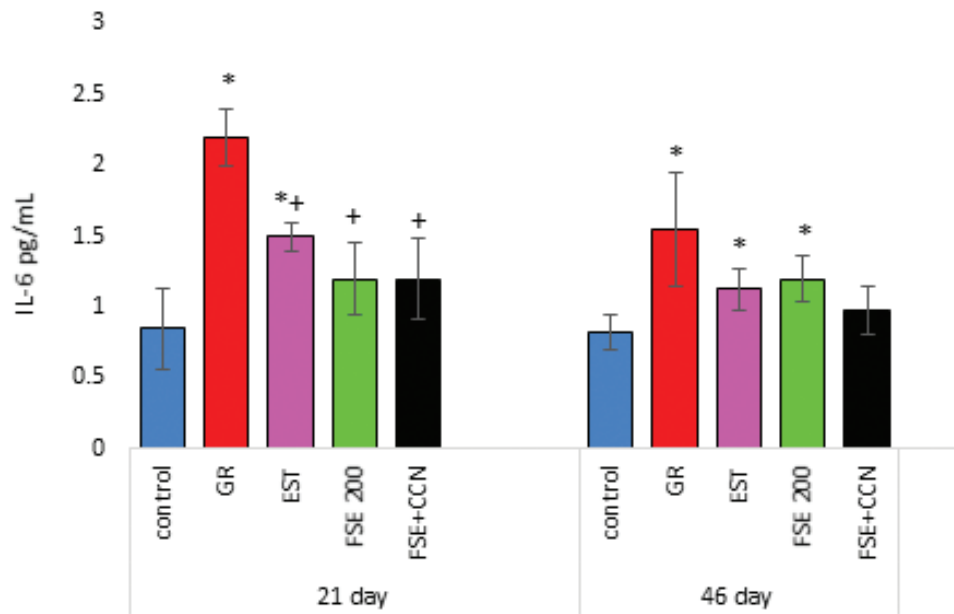


Figure 10. Serum IL-6 concentrations measured on days 21 and 46; * $p < 0.05$ versus control; + $p < 0.05$ versus GnRH group with estrogen deficiency.

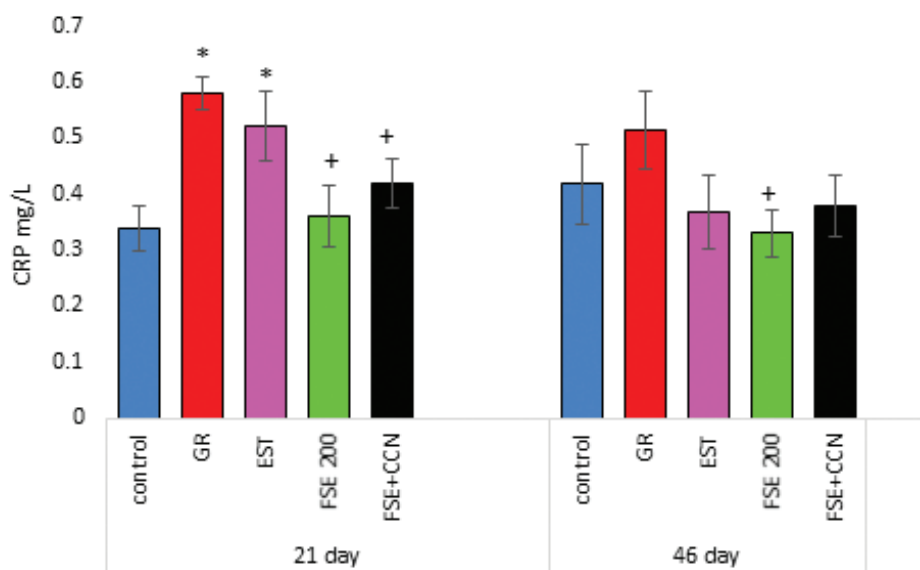


Figure 11. Serum CRP concentrations measured on days 21 and 46; * $p < 0.05$ compared to the control; + $p < 0.05$ compared to the GnRH group with estrogen deficiency.

Data from the measurement of the other pro-inflammatory marker, C-reactive protein (CRP), are presented in Fig. 11. Here, as with the measurement of IL-6, estrogen deficiency was associated with a 71% increase compared to the control group on day 21. The administration of FSE and its combination with curcumin reduced the level of CRP significantly by 40% and 33%, respectively, compared to the group with chemical castration.

On day 46, no statistically significant changes were observed in the levels of this marker, with the exception of the group treated with FSE, in which CRP decreased by 30% compared to the estrogen-deficient group.

Discussion

Estrogen deficiency is associated with reduced bone density and osteoporosis (OP) (Liang and Wang 2025). However, long-term use of modern anti-osteoporotic drugs can cause serious side effects, including increased cardiovascular risk and the risk of developing various types of tumors (Chen et al. 2024; Kwon et al. 2025).

A large number of natural compounds present in dietary foods and medicinal plants are effective in the prevention and treatment of OP. *Sophora japonica* L. (Fabaceae) is a source of natural isoflavones such as genistein

and daidzein, which may reduce the risk of fractures in patients with osteoporosis (Chakuleska et al. 2022).

The present study aimed to investigate the effects of *S. japonica* fruit extract (FSE) alone and in combination with a highly soluble form of curcumin (Cursol®) on bone architecture during chemical gonadectomy induced by subcutaneous implantation of the GnRH analogue goserelin (GR).

In the present study, markers of calcium homeostasis and bone turnover were significantly altered in chemically castrated rats. These changes were not as pronounced when estrogen-deficient rats were treated with the combination of FSE and curcumin.

Bone mineral is composed of calcium phosphate, and phosphorus is as important as calcium in helping to increase bone density and maintain bone strength. Although typical adult diets contain abundant phosphorus, 10% to 15% of adult women consume less than 70% of the recommended daily doses. When these women take high doses of calcium supplements consisting of carbonate or citrate salts, all of their dietary phosphorus may be bound and therefore unavailable for absorption. The current generation of anabolic agents for the treatment of osteoporosis requires a positive phosphorus balance of up to 90 mg/day. Attention to the nutritional adequacy of the diets of such patients is essential to realize the full potential of such therapies (Serna and Bergwitz 2020). Calcium plays a vital role in the development, growth, and maintenance of bone health. About 99% of the calcium in the body is found in the bones. Calcium in the blood is maintained within very narrow limits and a stable concentration. Apparently, increased bone turnover after estrogen reduction following goserelin implantation is one of the causes of hypercalcemia (Cheng et al. 2022). According to some authors, rapid bone loss is observed immediately after castration (Ryu et al. 2016), which is associated with increased plasma levels of this element. After this period, calcium homeostasis is maintained within very narrow limits with the participation of PTH and 1,25-OH Vit. D. Due to the faster rate of bone turnover in rats (Bauer et al. 2015), in the present study, calcium levels in all castrated animals were higher and phosphorus levels were lower than in the control group. By the end of the experiment (seventh week), in rats treated with the combination of FSE and curcumin, calcium and phosphorus were restored almost to control levels. Calcium decreased significantly by about 45% in the last week of the experiment, and phosphorus increased in the combined treatment by 67% compared to the group treated with the gonadorelin agonist goserelin (Figs 1, 2).

The results of the study show that the increased bone turnover occurring after administration of goserelin is characterized by increased activity of ALP and ACP. This, in turn, is associated with activation of bone remodeling processes, resorption, and bone formation, but the process of activated bone resorption certainly prevails, which leads to loss of bone density (Cheng et al. 2022). This indicates an increase in both osteoblastic and osteoclast activity, which leads to an overall net loss of bone. The protective role of FSE, especially in combination with curcumin, was established by the observed improvement

in bone metabolic markers ALP and ACP (Figs 3, 4). The best effect on the activity of the studied enzymes was observed after the application of the combinations, which practically normalized the activity of the studied enzymes and brought them closer to the control levels. The combined treatment led to a reduction in the increased enzyme activity, which is an indicator of slowing down bone turnover and, in particular, bone resorption. Compared to the castrated group, the combined treatment led to a statistically significant decrease in the activity of ALP and ACP by about 45% and 50%, respectively (Figs 3, 4).

The application of estradiol and FSE improved all the studied biochemical markers of bone turnover and calcium-phosphorus metabolism, but the changes were significantly more pronounced when they were combined with curcumin. The application of the extract, especially in combination with curcumin, slowed down bone resorption.

The beneficial effects of FSE found in this study may be due to the presence of the phenolic compounds daidzein and genistein and their potential to bind to and modulate alpha and beta estrogen receptors (Chakuleska et al. 2022). This is probably also related to the antioxidant effect of the studied substances. The antioxidant effects of curcumin (Jakubczyk et al. 2020; Dehzad et al. 2023; Nicoliche et al. 2024), daidzein (Li et al. 2022; Singh et al. 2023; Ahmad et al. 2024), and genistein (Burhan et al. 2020) have been described in a large number of literature sources.

Oxidative stress as a pathophysiological mechanism is known to be involved in osteoporosis, particularly in the reduction of calcium ion resorption and utilization (Kimball et al. 2021; Zhang et al. 2023). Estrogen deficiency causes bone loss and postmenopausal osteoporosis, the pathogenesis of which involves oxidative stress, inflammatory processes, and dysregulation of microRNA (miRNA) expression, which controls gene expression at posttranscriptional levels. Oxidative stress, due to increased reactive oxygen species (ROS), proinflammatory mediators, and altered miRNA levels, enhances osteoclastogenesis and reduces osteoblastogenesis through mechanisms involving the activation of MAPK and transcription factors (Iantomasi et al. 2023).

The antioxidant, anti-inflammatory, and protective effects of estradiol in premenopausal women have long been established. Estradiol exhibits antioxidant and anti-inflammatory properties that protect against osteoporosis by reducing oxidative stress and suppressing pro-inflammatory cytokines. In postmenopausal women, estrogen deficiency impairs antioxidant defenses, leading to increased reactive oxygen species (ROS) and enhanced inflammatory signals that promote bone resorption. The ability of estradiol to counteract these detrimental effects by improving the GSH/GSSG ratio and inhibiting inflammatory mediators helps maintain bone health. Such effects were also observed in our study, where estradiol reduced the levels of the pro-inflammatory markers IL-6 and CRP (Figs 10, 11) and increased the level of GSH (Fig. 9). These antioxidant and bone-protective effects were also found after treatment of chemically castrated animals with the studied bioactive substances, FSE and curcumin.

Conclusion

These findings provide information about the bone-protective effects of FSE and curcumin against GR-induced bone loss. The structural similarity between estradiol, daidzein, and genistein explains the bone-protective effects of FSE and indicates that *S. japonica* may be a useful source for the development of effective natural prophylactic/therapeutic products for maintaining adequate bone structure in age-related or secondary, drug-induced osteoporosis.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

The authors declared that no clinical trials were used in the present study.

The authors declared that no experiments on humans or human tissues were performed for the present study.

The authors declared that no informed consent was obtained from the humans, donors or donors' representatives participating in the study.

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- Experiments on animals: Bulgarian Agency of Food Safety (permission № 251, issued on 20.11.2019).
- The authors declared that no commercially available immortalised human and animal cell lines were used in the present study.
- ### Use of AI
- No use of AI was reported.
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- ### Author contributions
- RS: conceptualisation, experimental design, writing the manuscript draft. AS: technical support, plant extract preparation and characterisation. GM: project manager.
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- ### Data availability
- All of the data that support the findings of this study are available in the main text.
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