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Review

The Potential of Chickpea (*Cicer arietinum L.*) for Anti-Osteoporotic Agent in Post-Menopausal Women: A Systematic Review of *In Vivo* Study

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Abstract

This systematic review aimed to analyze the *in vivo* study of the anti-osteoporotic activity of chickpeas. A comprehensive search for English language papers published between January 2013 and December 2023 using the keywords chickpea or *Cicer arietinum*, anti-osteoporotic activity or anti-osteoporotic effect, chickpeas and anti-osteoporotic activity, *Cicer arietinum* and antiosteoporotic activity and their synonyms yielded 3566 results from PubMed, ScienceDirect, SpringerLink and Google Scholar. Articles were screened with titles, abstracts, full-text reviews based on inclusion/exclusion criteria, and evaluation of research quality using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE). Four articles included in this systematic review were displayed in the PRISMA 2020 flowchart. Descriptive data analysis was conducted by comparing findings from several publications. Chickpea

isoflavones affect bone metabolism by stimulating bone formation and inhibiting bone resorption *via* the RANK/RANKL/OPG pathway. Chickpeas might prevent osteoporosis caused by low estrogen levels in animal studies. In summary, further research is required to confirm the possibility of chickpeas as an anti-osteoporotic agent.

Keywords
Anti-osteoporotic; chickpea; isoflavones; OPG; RANK; RANKL

1. Introduction

Women's health is greatly influenced by estrogen after menopause. A progressive decrease in estrogen hormone levels causes several age-related illnesses in women, such as obesity, autoimmune diseases, and osteoporosis. Osteoporosis occurs widely in post-menopausal women and has become a public health concern, accounting for over 8.9 million fractures globally with consequences of the risk of fracture, increased morbidity, and mortality. These conditions will lower quality of life, including discomfort, incapacity, and a loss of autonomy. Consequently, osteoporosis prevention is crucial [1-4].

Estrogens are essential regulators of bone metabolism, and declining serum levels with age are related to lower Bone Mineral Density (BMD) levels. Hormone replacement therapy has been linked to major adverse consequences comprising endometrial and breast cancer, heart disease, and stroke [5-8]. In recent years, phytoestrogens have gained a considerable amount of attention because of their potential to prevent chronic conditions like cardiovascular disease (CVD), osteoporosis, and hormone-related malignancies. These phytochemicals exhibit a biological effect analogous to the action of estrogen and provide adult women with an effective and reliable alternative to hormone replacement therapy. They are classified as part of the class of flavonoids found in plant products, and chickpeas are one of the plant-based items with significant concentrations of phytoestrogens [9, 10].

Chickpea (*Cicer arietinum L.*) has been a familiar plant historically in Asia for many years because of its wide range of purposes. It is typically planted in about 14.5 mln ha, with an annual yield of 14.8 mln t and a productivity of approximately 1 t/ha in 2017, significantly lower than the estimated potential of 6 t/ha under ideal growth circumstances. India, Australia, Pakistan, Turkey, and Mexico are the world's top chickpea producers. It is third on the list of the most popular legume crops and plays a role in Xinjiang's traditional Chinese and folk medicine. Over the course of 15 years, chickpea consumption has more than doubled in the United States, from 1.9% in 2003 to 4.5% in 2018. Likewise, globally, the chickpea market is predicted to grow from USD 9.15 bln presently to 10.68 bln by 2028 [11-15]. In Indonesia, chickpeas are known as “kacang arab”, since they are usually brought as souvenirs by Hajj pilgrims from Arabia.

The chickpea has grown more and more recognized and investigated. They have a broad spectrum of biological effects, which include antibacterial, estrogenic, antioxidant, anti-fungal, anti-cancer, anti-inflammatory, anti-diabetic, anthelminthic, hypocholesterolaemia, antidiarrheal properties, promote healthy gut microbiota, control body weight [9, 11, 16-22]. It is additionally stated that chickpeas constitute a great source of carbohydrates (40-50 g/100 g), readily digestible proteins (21-

25 g/100 g), fats (4-6 g/100 g), minerals and vitamins, unsaturated fatty acids, soluble dietary fiber, a low glycemic index, and isoflavones. During germination, chickpeas may exhibit an almost 100-fold greater isoflavone concentration [14, 23].

Isoflavones are phenolic compounds that have a structural similarity to estradiol. They are typically found in several foods, but legumes such as soy, alfalfa, white and red clover, and beans are the most common. They are called phytoestrogens due to their molecular resemblance to 17β-estradiol, an estrogen-like compound. As a result, isoflavones can act as both estrogenic and anti-estrogen agents. Isoflavones, which resemble 17β-estradiol, bind to estrogen receptors and have estrogenic effects. Hence, they stimulate osteoblastic activity and inhibit osteoclastogenesis, finally increasing bone formation [24, 25]. Several animal studies have been conducted regarding the impact of chickpeas on bone metabolism as an anti-osteoporotic agent. This systematic review aimed to gain further insight into the mechanism of how the isoflavone in chickpeas affects bone metabolism by comparing results from several *in vivo* studies to provide a basis for further research.

2. Materials and Methods

2.1 Design

A systematic review was performed to identify *in vivo* studies concerning chickpeas' potential as an anti-osteoporotic agent from academic journals published from January 2013 to December 2023 using electronic PubMed, ScienceDirect, SpringerLink, and Google Scholar databases. The selection papers were chosen based on criteria such as primary peer-reviewed articles, animal studies, studies applying only chickpeas, and study outcomes measuring anti-osteoporotic activity. Articles written in non-English language and paid articles were excluded in this systematic review.

2.2 Data Collection

The research issue was how chickpeas affect bone metabolism in animal studies. PICOS framework was used to transform the concept of data, which consists of the following: 1. Population: Animals (rats or mice); 2. Intervention: Treatment with chickpea extracts; 3. Control: Treatment without chickpea extracts; 4. Outcome: anti-osteoporotic activity; 5. Study type: Experimental studies. The Boolean Operators (AND and OR) were used with the key-words chickpea OR *Cicer arietinum*, anti-osteoporotic activity OR anti-osteoporotic effect, chickpeas AND anti-osteoporotic activity, *Cicer arietinum* AND anti-osteoporotic activity and their synonyms.

2.3 Risk of Bias (RoB) for Animal Studies

The Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) was used to evaluate the quality of methodology in studies on animals [26]. The risk of bias in animal studies includes 10 entries dealing with six different types of bias, such as selection bias (sequence generation, baseline characteristics, and allocation concealment), performance bias (random housing and blinding); detection bias (random outcome assessment and blinding); attrition bias (incomplete outcome data), reporting bias (selective outcome reporting) and other biases. Two authors assessed the risk of bias (SD, DW). A "yes" decision suggests a low risk of bias, a "no" judgment suggests a high risk of bias, and the decision will be "unclear" if inadequate data is provided to correctly determine the risk of bias.

2.4 Data Analysis

This systematic review referred to the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) standards [27]. Articles that met the criteria for inclusion were included in the narrative synthesis. The selected articles were subsequently extracted and included in the table to assist in synthesizing data. In the results session, the data synthesis involved a descriptive analysis of the findings and a data summary.

3. Results

3.1 Selection Process

A total of 8570 articles were found using four electronic databases (filtering year of publication, English language, and open access, n = 3566). We used keywords ((Chickpea OR Chickpeas) OR (Chickpeas OR *Cicer arietinum*)) AND (Anti-osteoporotic agent OR anti-osteoporotic activity OR estrogenic activity OR osteoporosis OR bone) for PubMed, ScienceDirect, and Google Scholar databases while key words Chickpea OR Chickpeas OR *Cicer arietinum* for SpringerLink database. Four articles were ultimately selected through identification, screening, and resilience procedures. The study selection process flow chart, as suggested by the Preferred Reporting Items for Systematic Reviews (PRISMA) is depicted in Figure 1.

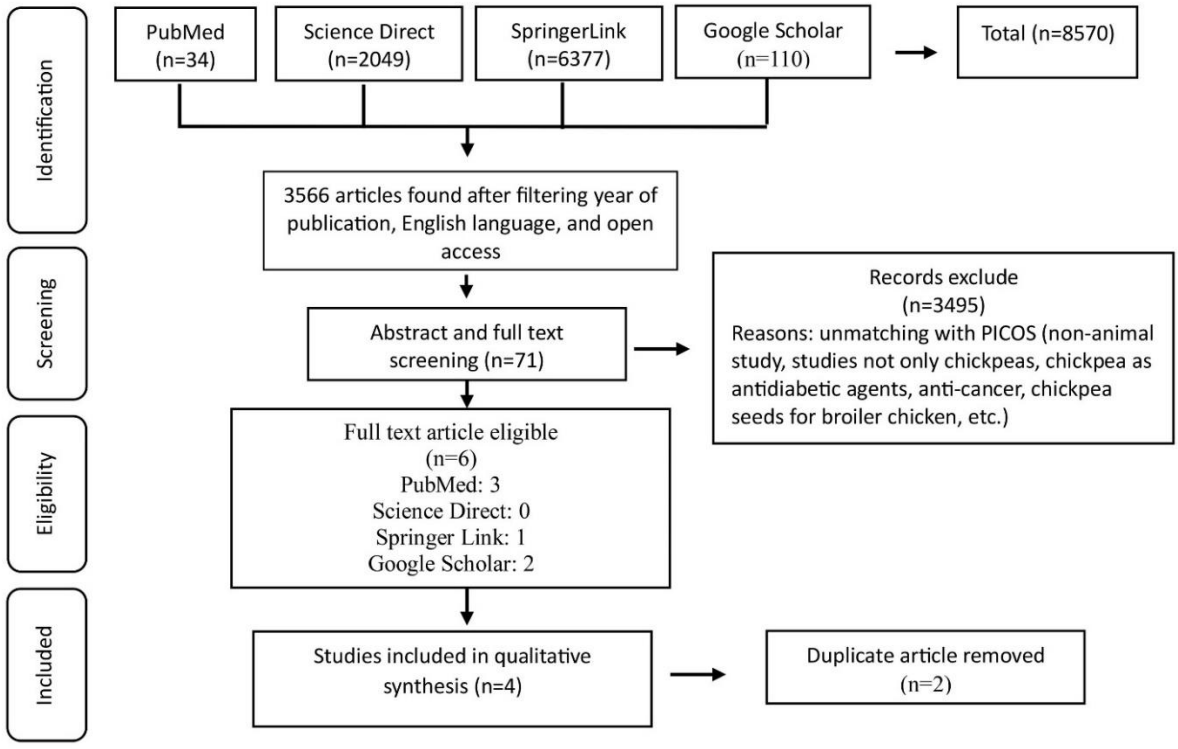

Figure 1 Flowchart of the *in vivo* study selection process.

3.2 Risk of Bias of Included Studies

Based on SYRCLE's RoB tool (Table 1), all studies have a low risk of bias regarding sequence generation since they randomized rats into a few groups before intervention (1). The baseline

characteristic of all studies was similar. The animals first had an ovariectomy (OVX) process, and then they received treatment. Therefore, they had a low risk of bias (2). Regarding allocation concealment, the risk was deemed unclear bias for all *in vivo* studies due to inadequate information about the method of concealment (3).

Table 1 Risk of bias *in vivo* studies according to SYRCLE's RoB tool of the four studies included in the systematic review.

Study	Selection bias			Performance bias		Detection bias		Attrition bias	Reporting bias	Other
	1	2	3	4	5	6	7	8	9	10
Ma HR et al. 2013 [28]	Y	Y	?	Y	N	?	?	Y	Y	Y
Fahmi et al. 2015 [29]	Y	Y	?	Y	N	?	?	Y	Y	Y
Sayed & Elfiky 2018 [30]	Y	Y	?	Y	N	?	?	Y	Y	Y
Huang et al. 2023 [14]	Y	Y	?	Y	N	?	?	Y	Y	Y

Y (YES) = low risk of bias; N (NO) = high risk of bias; ? = Unclear bias. Sequence generation (1), Baseline characteristics (2), Allocation concealment (3), Random housing (4), Blinding (5), Random outcome assessment (6), Blinding (7), Incomplete outcome (8), Selective outcome reporting (9), and other (10).

All of the studies have been classified as having a low risk of bias regarding random housing. Animals were kept in the baseline state before the commencement of the experiment, including temperature, lighting, and relative humidity. They were fed standard food and drink (4). However, as to blinding, there was no proof that the researchers manipulating the animals knew which group was the treatment group or the control group (5).

Concerning detection bias, which includes random outcome assessment (6) and blinding (7) has been characterized as unclear bias since it wasn't clarified in the primary studies whether the analysis of the outcomes was done randomly or whether those who performed the analysis were random. Each study showed a low risk of bias because there were no missing outcome data (8). For reporting bias, all of these studies have disclosed the study procedure and the expected outcomes. Thus, they have a low risk of bias. Regarding other variables of bias (10), every study was categorized as having a low risk of bias. The Animal Ethics Committee has approved all animal studies.

3.3 Details of Included Studies

We have tried to identify an *in vivo* study on chickpeas' anti-osteoporotic properties using four databases. Though there have been many studies concerning the impact of chickpeas on human health, we could only find four that discussed the anti-osteoporotic properties of chickpeas. The four articles were published between 2013 and 2023 in China (two studies) and Egypt (two studies), involving two types of rats, Sprague-Dawley and Wistar Albino rats. Two studies used isoflavones from chickpea sprouts with intragastric administration, whereas others used chickpea extract (Table 2).

Table 2 Details of included studies.

Author/ Year	Country	Type of chickpea extract	Route	Dose of chickpea	Control group	Intervention period (days)	Animal	Weight (g)	Age (days)	n/group	Group
Ma HR et al. 2013 [28]	China	ICS	Intragastric	20, 50, 100 mg/kg	17β-estradiol	36	Female Sprague-Dawley rats	220	70	8	6
Fahmi et al. 2015 [29]	Egypt	CAE	Oral	500, 1000 mg/kg	Alendronate, distilled water	70	Female Wistar albino rats	150-170	?	14	4
Sayed & Elfiky 2018 [30]	Egypt	CAE	Oral	500 mg/kg	Alendronate	70	Female Wistar albino rats	-	90-120	6	4
Huang et al. 2023 [14]	China	ICS	intragastric	100, 300 mg/kg	Raloxifene, saline	84	Female Sprague-Dawley rats	200-220	70	8	5

Isoflavone levels in chickpea sprouts were significantly higher than those in chickpea seeds. Various isoflavones occur in chickpea sprouts, and these compounds may have anti-osteoporosis properties through several different mechanisms. Biochanin A, formononetin, and biochanin A-7-O-β-D-glucoside constitute the three of these isoflavones that have been shown to have potent estrogenic actions, such as promoting uterine growth and inhibiting bone loss [14, 28].

Ma HR et al. [28] used 70% ethanolic isoflavone extract from chickpea sprouts (ICS), standardized to 72.3% isoflavone content. Four main isoflavones were found in ICS, 7,7% ononin, 13.2% of biochanin A-7-O-β-D glucoside, 19.5% of formononetin, and 31.9% of biochanin A. By contrast, Fahmi et al. [29] used *Cicer arietinum* extracts (CAE) with methanol as the solvent. The resultant extract was dried using a lyophilizer. Bioactive substances identified in the methanolic extract were daidzein, genistein, formononetin, and biochanin A. Similarly, Sayed and Elfiki [30] investigated the inhibitive impact of chickpea extract (CAE) on osteoclastogenesis using methanolic dissolving by Fahmi et al. [29]. The study concluded that CAE exhibits estrogenic properties and is a strong inhibitor of osteoclastogenesis as a result of the combined action of the phytoestrogens and the bone-protecting amino acids. Huang *et al.* [14] employed chickpea sprouts to examine the mechanism of isoflavones in relieving osteoporosis. The study identified eight bioactive chemicals that were essential molecules, including medicarpin, genistein, homoferreirin, maackiain, pratensein, daidzein, calycosin, and formonetin.

3.4 Outcome Assessment of Included Studies

There are several ways in which isoflavone phytoestrogens may regulate the metabolism of bone. Several bone biomarkers were used to show the anti-osteoporotic effect of chickpeas (Table 3).

Table 3 Outcome assessment of included studies.

Author/year	Outcome measurement	Anti-osteoporotic activity results	Conclusion
Ma HR et al. 2013 [28]	BMD, BV/TV, Tb.Th, Tb.Sp	ICS increased BMD, BV/TV, Tb.Th, and decreased Tb.Sp	ICS has the potential for osteoporosis treatment caused by estrogen deficiency
Fahmi et al. 2015 [29]	BMD, BMC, BALP, P, PTH, calcitonin levels, OPG, OPG/RANKL ratio	BMD values of all selected bone regions were found significantly higher ($P < 0.05$) than those of untreated OVX rats, CAE (500 mg and 1000 mg) increased the BMC, BALP activity, serum P, and decreased PTH. CAE does not affect calcitonin level.	CAE not only enhances osteoblast differentiation but also improves OPG and suppresses RANKL release in osteoblasts, hence preventing bone loss and osteoporosis.
Sayed & Elfiky 2018 [30]	OPG, RANKL, TRAP5b OPG/RANKL, serum estrogen (E2)	CAE increases E2, OPG, OPG/RANKL, and decreases RANKL and TRAP5b	CAE possesses estrogen-like properties and is an effective inhibitor of osteoclastogenesis.

Huang et al. 2023 [14]	BMD, BV/BT, BS/BV, Tb.N, Tb.Th, Tb.Sp, Tb.Pf	BMD, BV/BT, Tb.N, Tb.Th, were higher in the ICS treatment group. The BS/BV, Tb.Sp, and Tb.Pf were lower than those in untreated OVX rats.	ICS can reduce bone loss and improve trabecular structure in OVX model rats *in vivo*. ICS may play a major role in osteoporosis treatment *via* multiple factors, various targets, and different pathways.

BMD: bone mineral density, BV/TV: trabecular bone volume to tissue volume, Tb.Th: trabecular thickness, and Tb.Sp: trabecular separation, BMC: bone mineral content, BALP: bone-specific alkaline phosphatase, P: phosphorus, PTH: parathyroid hormone, E2: estrogen, OPG: osteoprotegerin, RANKL: receptor activator of nuclear factor- kappa β ligand, TRAP5b: tartrate-resistant acid phosphatase 5b, BS/BV: bone surface area to bone volume ratio, Tb.N: trabecular number, Tb.Pf: trabecular pattern factor.

4. Discussion

Chickpea consumption is typical in many parts of the world, including developing and developed countries, because of its high nutritional value. Furthermore, antioxidants in chickpeas, such as isoflavones and polyphenols, protect cells from free radical damage and lower the risk of hypertension, inflammation, diabetes, and metabolic syndrome. As a result, chickpeas are regarded as an excellent complete food that may improve general health [17, 31-33].

Previous studies indicated the positive effect of chickpeas on bone metabolism. An in vitro study conducted by Zakłos-Szyda et al. [25] suggests that fermented chickpea sprouts may inhibit bone resorption by reducing the secretion of proinflammatory cytokines (interleukin-6/IL-6 and tumor necrosis factor-α/TNF-α) and stimulate the mineralization of Saos-2 cells. However, further study is required to confirm this result. In this systematic review, we found and evaluated four *in vivo* studies that investigated the potential of chickpeas as an anti-osteoporotic substance. We concentrated on chickpeas in the form of chickpea extract (CAE) or chickpea sprouts (ICS) alone, as we intended to confirm that anti-osteoporotic activity was caused solely by chickpea bioactive components. The effect of phytoestrogen in chickpeas on bone metabolism as an alternative to estrogen in menopausal women was addressed in this systematic review.

Estrogen deficiency is the leading cause of osteoporosis in postmenopausal women. Bone loss begins around two years before menopause and peaks at 3-5% per year for the first three years after menopause. Beyond 5-10 years, a sluggish phase occurs continuously. The bone formation and resorption imbalance leads to osteoporosis [34, 35]. Estrogen is essential for maintaining bone mineral density in rats and humans. During menopause, estrogen levels drop, resulting in accelerated bone resorption and decreased bone mineral density. Antiresorptive medications and Hormone Replacement Therapy (HRT) are the therapy choices for postmenopausal osteoporosis. However, they have some side effects on long-term use, such as esophageal cancer, cardiovascular disease, breast and endometrial cancer. As a result, most female patients seek out natural biological products with an effective folk medicine foundation instead of traditional pharmaceuticals, which are utilized to prevent perimenopausal health problems [1, 5, 36].

Phytoestrogens, commonly known as “dietary estrogens,” are natural compounds with estrogen-like properties due to their similar molecular structure to 17-β-estradiol. They can bind to estrogen receptors, causing estrogenic or antiestrogenic actions. Specifically, they possess estrogenic benefits without causing common adverse effects such as increased risk of heart attacks and strokes and incidence of breast and endometrial cancer. The types of phytoestrogens are as follows: (1) isoflavones (genistein, daidzein, glycitein, biochanin A, and formononetin); (2) coumestans (coumestrol, wedelolactone, plicadin); and (3) lignans (plant lignans: pinoresinol, lariciresinol, secoisolariciresinol, matairesinol, and enterolignans: enterodiol, enterolactone) [37, 38].

Estrogen actions are regulated by two receptors such as estrogen receptor α (ERα) and β (ERβ). ERβ may neutralize the effects of ERα, thereby acting as a modulator of the latter's activity. The different biological functions of the two receptors refer to the yin/yang interaction. Induction of ERα leads to cell proliferation and promotes tumor growth, whereas ERβ activation balances this response. Generally, phytoestrogens bind to estrogen receptors (ERs) and function as mild agonists. Although their affinity to ERs is 1/100 to 1/10,000 that of 17-β-estradiol, they can achieve micromolar concentrations in the blood vessels, causing them to function as both agonists and antagonists [7, 25, 38].

Isoflavones are nonsteroidal plant chemicals that selectively modulate estrogen receptors. They bind to both ERα and ERβbut have greater affinity for ERβ and can selectively target bone cells without affecting other estrogen-sensitive organs like the breast or uterus. Isoflavones in chickpea beans are significant owing to their broad spectrum of biological activities, including antibacterial, antifungal, antioxidant, and estrogenic activities. The quantity and content of isoflavones were substantially improved by chickpea sprouting, and the isoflavone content of chickpea sprouts was significantly higher compared to that of soybeans. Its isoflavone components, particularly those of sprouted chickpea (*Cicer arietinum*), have been reported to help against conditions of cancer, hyperlipidemia, and osteoporosis. Given these benefits, isoflavones are receiving significant scientific interest and are being investigated as a possible substitute for hormone replacement therapy (HRT) in the management of osteoporosis [1, 11, 24, 39, 40].

Bone health results from the outcome of a balanced activity of bone modeling and remodeling. Osteoblasts (OBs) regulate long-term bone growth and mechanical adaptation, while osteoclasts (OCs) regulate bone replacement. The RANKL/RANK/OPG system has been discovered as a critical regulator of bone remodeling [41-44]. Osteoblasts and osteocytes produce the ligand for receptor activators of NF-kB (RANKL). It is essential for osteoclastogenesis through binding to the RANK or on the surface of osteoclast precursors, initiating the process of osteoporosis [43, 45]. RANK functions as the receptor for RANKL. Osteoprotegerin (OPG) has been identified as a secreted glycoprotein produced by various cells, particularly osteoblasts, lung and liver cells, and bone marrow B lymphocytes. OPG serves as a decoy for RANKL, binds to RANKL competitively, prevents RANKL-RANK interactions, and inhibits bone resorption. It is activated by estrogen, IL-4, or transforming growth factor beta (TGF-β). Once OPG binds to RANKL, it suppresses osteoclast development, survival, and function. As a result, bone formation occurs. High OPG concentrations prevent bones from resorption. To maintain healthy bone, the ratios of OPG and RANKL are tightly controlled. In pathological states like osteoporosis associated with menopause, lower estrogen levels lead to lower OPG, which in turn raises RANKL, which intensifies osteoclast activation and causes bone loss. This RANKL/RANK/OPG pathway could be a new therapy option for bone diseases [43, 46-50].

Chickpea isoflavones modulate the RANKL/RANK/OPG pathway by several mechanisms. Isoflavones in *Cicer arietinum* extract (daidzein and genistein) increase OPG production and decrease RANKL expression in osteoblasts, leading to a rise in the OPG/RANKL ratio and inhibiting osteoclast development. The findings indicated that the extract promotes osteoblast development, increases osteoblast OPG, and decreases osteoblast RANKL secretion, preventing bone loss. Genistein was also reported to bind to Estrogen Receptor to inhibit bone loss [18, 30].

Chickpea sprouts containing isoflavones used orally (100 and 300 mg/kg/day) diminish bone loss and enhance trabecular microarchitecture and biomechanical aspects of the fourth lumbar vertebra in OVX-induced osteoporotic rats, with a dose-dependent upward trend. Furthermore, ERα regulation of the OPG/RANKL pathway has been shown to improve osteogenic differentiation of bone marrow stem cells (BMSCs), raise OPG levels, and block osteoclastic resorption. Treatment with isoflavones derived from chickpea sprouts inhibited RANKL-induced osteoclastogenesis [40, 41].

The three primary isoflavonoids found in the *C. arietinum* sprouts were biochanin A, onionin, and formononetin. It has been demonstrated that biochanin A, a component of *C. arietinum* sprouts, may interact with RANKL to reduce osteoclast activation, osteoporosis, and mammary carcinogenesis. Biochanin A has received much interest recently due to its vast medicinal potential [25, 37]. The mechanism of chickpeas as an anti-osteoporotic is illustrated in Figure 2.

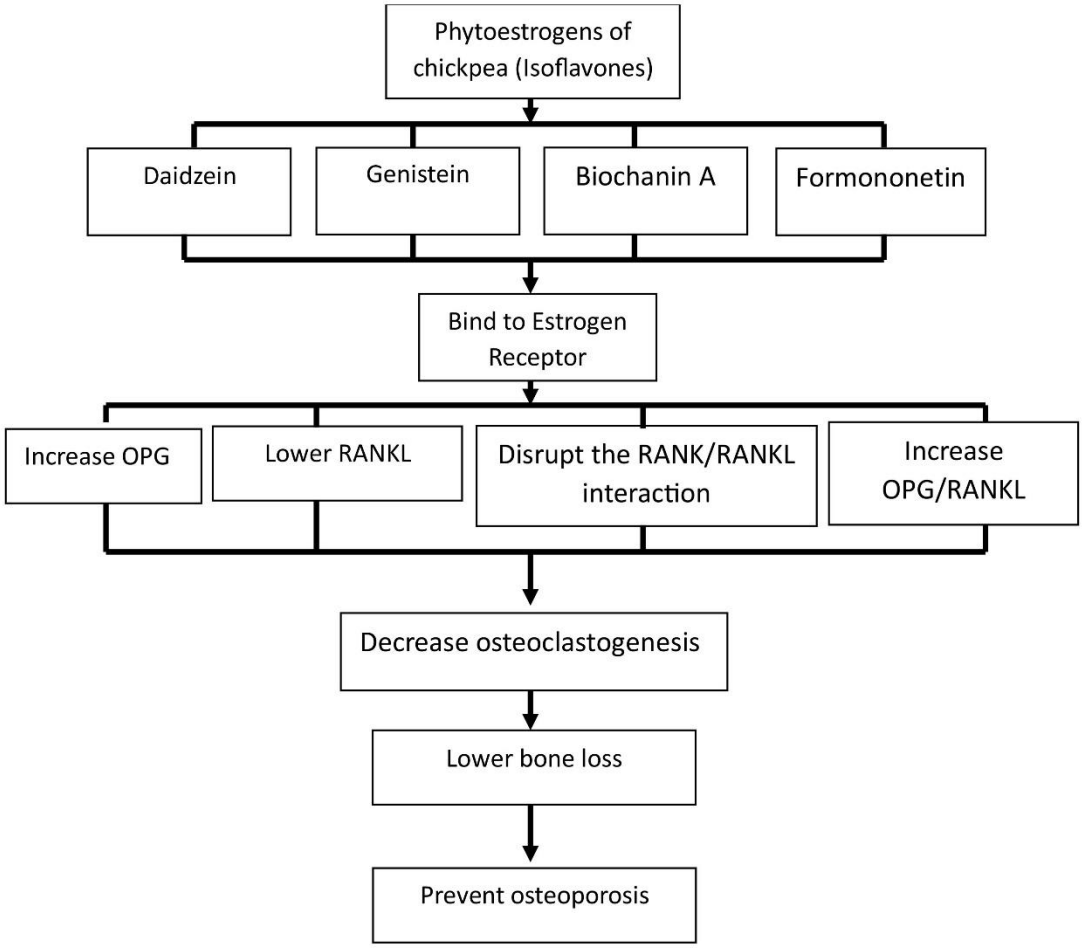

Figure 2 The mechanism of chickpeas as an anti-osteoporotic agent.

The *in vivo* study of Sayed & Elfiky [30] suggested that CAE reduces RANKL and TRAP5b while enhancing estrogen (E2), OPG, and OPG/RANKL. The resultant drop in the OPG/RANKL ratio and the increase in bone TRAP5b activity and RANKL align with cortical thinning and porosity. As the OPG/RANKL ratio indicates osteoformation and osteoresorption, CAE can inhibit osteoclastogenesis. By increasing the release of OPG, which binds to RANKL, osteoblastic cells may be stimulated by CAE to decrease the RANK/RANKL interaction and thereby minimize osteoclastogenesis. The phytoestrogen contents of CAE may be the cause of the up-regulation of OPG in the CAE group, as they bind to ERs α and β, resembling the function of estrogen in up-regulating OPG naturally.

An *in vivo* study by Fahmy et al. [29] showed that CAE enhances the OPG/RANKL ratio and decreases osteoclast differentiation by stimulating OPG synthesis and reducing RANKL expression in osteoblasts. According to these findings, CAE promotes osteoblast development, increases OPG, and decreases RANKL release in osteoblasts, which helps prevent osteoporosis and bone loss. Bone Mineral Density (BMD) levels rose after receiving 500 mg/kg body weight of CAE. However, this rise was only statistically significant ($P < 0.05$) in the case of the tibial bone. In the meantime, the BMD loss brought on by ovariectomy in all examined bones is considerably modulated ($P < 0.05$) by the administration of CAE (1000 mg/kg body weight). Conversely, following alendronate treatment, BMD values of all the chosen bone areas were considerably greater ($P < 0.05$) than those of the untreated OVX rats.

Ma HR et al. [28] found that interventions with E2 (OVX + E2) and ICS (OVX + ICS-50 and OVX + ICS-100) helped to reverse the bone loss induced due to OVX. ICS-50 and ICS-100 have potencies comparable to E2. When it came to healing the bone loss and structural alterations caused by the OVX, medium and high dosages of ICS had results that were similar to those of E2, but less potent. In a dose-responsive way, ICS reduced trabecular separation (Tb.Sp) while increasing BMD, trabecular bone volume to tissue volume (BV/TV), and trabecular thickness (Tb.Th). High doses of ICS had an effect equal to that of E2, indicating that ICS promoted bone development and elevated bone mineral content in the OVX rats' femurs. When OVX rats were given a high dose of ICS, osteoclast density was dramatically reduced, indicating that ICS had a beneficial effect on bone metabolism.

A study by Huang et al. [14] suggested that ICS markedly increased femoral bone mineral density and trabecular structure. In particular, compared to untreated OVX rats, the ICS therapy group had greater BMD, BV/TV, trabecular number (Tb.N), and Tb.Th values, while the bone surface area to bone volume ratio (BS/BV), Tb.Sp and trabecular pattern factor (Tb.Pf) values were lower. These results were consistent with raloxifene's previously reported effects. ICS can improve the trabecular structure and decrease bone loss. Isoflavones in ICS interact structurally with estrogen and have minor estrogenic effects. Numerous studies have shown that isoflavones bind to estrogen receptors and regulate bone metabolism.

Overall, studies with animals consistently indicate that the bioactive chemicals in chickpeas, or *Cicer arietinum*, modulate the metabolism of bone. Through the RANK/RANKL/OPG process, chickpea extract and sprouted chickpeas control bone formation and resorption. Chickpea isoflavones bind to estrogen receptors to induce OPG, stimulate OPG/RANKL, and block the RANK-RANKL interaction. By inhibiting osteoclastogenesis, they prevent osteoporosis.

This systematic review might not have included comparable studies from other nations because it was restricted to searching for publications from four databases and written in English. However, the review of the anti-osteoporotic properties of chickpeas is one of this paper's positive aspects.

Considering the current trend of growing demand for chickpeas as functional food, further study is required to corroborate information on the mechanism of chickpeas as anti-osteoporotic agents.

5. Conclusion

Chickpeas have anti-osteoporotic activity *via* the RANK/RANKL/OPG pathway. Chickpea isoflavones exhibit estrogenic action, including biochanin A, formononetin, and biochanin A-7-O-β-D-glucoside. They attach to ERs α and β, raise OPG, lower RANKL, disrupt the RANK/RANKL interaction, increase OPG/RANKL, and eventually decrease osteoclastogenesis. This systematic research shows chickpeas may have an anti-osteoporotic effect, preventing osteoporosis induced by low estrogen levels.

Given chickpeas' potential to prevent osteoporosis, further preclinical and clinical research with standardized techniques is required to completely comprehend chickpeas' anti-osteoporotic qualities.

Author Contributions

Conceptualization, S.D., G.B.; methodology, formal analysis, investigation, S.D., D.W.; writing-original draft preparation, S.D.; writing-review and editing, S.D., D.W., Y.Y., U.P., G.B. All authors have read and approved the published version of the manuscript.

Competing Interests

The authors have declared that no competing interests exist.

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