Review Article

The Effect of Selected Herbal Medicines on Bone Turnover Markers: A Systematic Review and Meta-Analysis

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Abstract

Objective: To evaluate systematically the therapeutic effects of five herbal medicines (*curcumin, black seed, ginger, cinnamon,* and *flaxseed oil*) on bone turnover markers as a primary outcome.

Materials and methods: A comprehensive systematic search of the literature was conducted in the electronic databases consisting of the Cochrane Library, MEDLINE, Web of Science, Scopus, Embase, ProQuest, and Google scholar, as well as SID, Magiran, and Irandoc for Persian literature up to December 2020. All Randomized controlled trials and quasi-experiments evaluated the impact of studied herbal medicines on bone turnovers of Bone Specific Alkaline Phosphatase (BSAP), osteocalcin, C-terminal Telopeptide type 1 Collagen (CTX-I), Deoxypyridinoline (DPD) were analyzed.

Results: Sixteen interventional studies comprised 968 participants included in systematic review. Ten of eligible studies with 603 participants included in meta-analysis. C*urcumin, black seed* and *flaxseed* did not have a significant effect on BSAP (SMD=-1.76, 95%CI: -6.85 to 3.33, p=0.50, I^2=0.99, 6 trials, 241 participants), CTx (SMD=-0.17ng/mL, 95%CI:-0.43 to 0.09, p=0.21, I^2=1.000, 5 trials, 216 participants), DPD (MD=0.82nmol/mmol, 95%CI:-0.05 to 1.68, p=0.06, I^2=0.000, 2 trials, 67 participants), osteocalcin (SMD=-2.02ng/mL, 95%CI:-4.49 to 0.45, p=0.11, I2=0.79, Six trials, 229 participants). As secondary outcomes, femoral neck Bone Mineral Density (BMD) increased significantly (p=0.03, I^2=0.12) but lumbar spine BMD didn't differ (p=0.28, I2=0.97). *Curcumin* significantly increased total hip BMD (p<0.001, I^2=0.12). QiangGuYin containing *cinnamon* as a combined Chinese medicine had significant effect on P1NP, β-CTx, and BMD.

Conclusion: Studied herbs except for QiangGuYin had no significant effects on bone turnover markers. Due to high heterogeneity between trials, further high-quality trials are suggested.

Keywords: Medicinal Plants; Bone Remodeling; Bone Density; Meta-Analysis; Systematic Review

Introduction

Bone remodeling and turnover are caused by the balancing between the two processes including new bone formation by osteoblast cells and bone resorption by osteoclast cells (1,2). In many bone disorders like osteoporosis, the decline of bone density, as well as down-regulation of bone mineral density is expected

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(3). In this regard, many therapeutic strategies have been applied to regulate the pointed processes to protect the normal bone structure. These strategies play a crucial role in maintaining bone mass content and preventing any uncontrolled decline of bone loss (4, 5). The natural compounds seem to overcome the side effects of chemical compounds with similar or even superior therapeutic effects (6).

Curcumin, derived from the curcumin longa-L plant, has been shown to have several beneficial biological effects such as anti-inflammatory, anti-infection, and chemo preventive effects (7). In some animal studies, the beneficial effects of *curcumin* on bone remodeling have been shown (8). It seems that the protective effects of *curcumin* are mediated by its inhibiting effects on osteoclast genesis, inhibition of osteoclasts proliferation (9-11).

Another herbal extract that has been used for the regulation of bone metabolism is *black seed* derived from the Ranunculaceae family. The extract of this herb is globally used as an antihypertensive, anti-diarrheal, analgesic, digestive, anti-diabetics, anti-cancer, immunomodulator, and even anti-bacterial (12-14). Most of the therapeutic properties of this plant are due to the presence of thymoquinone (TQ), which is a major active chemical component of the essential oil (15). In some recent studies, the beneficial and regulatory effects of *black seed* on osteoporosis and bone healing by activating turnover activating have also been revealed (16).

Ginger extract is another material that has been tested with many therapeutic effects (17,18). In some animal studies, it has been demonstrated that the sub-fractions of crude *ginger* extract, including essential oils and gingerols can inhibit osteoclast cell differentiation (19). Another herbal product that has been revealed to have therapeutic effects on bone health is *flaxseed oil* that is rich in a-linolenic acid (20). It has been also demonstrated that a-linolenic acid in *flaxseed oil* could be able to protect the bones by preventing alveolar bone loss (21-23).

Another beneficial herbal source, *cinnamon* has been traditionally used as a folk herbal extract for treating inflammation. In some experimental studies, the effects of *cinnamon* on metabolic and hormonal effects have been investigated (24). In this regard, its impact on increasing the estradiol level, triggering luteinizing hormone secretion, as well as progesterone section has been revealed (25). Recently, the researches on the animal model showed the ability of *cinnamon* to normalize bone turnover markers (BTMs) and bone mineral elements (26).

Overall, there are insufficient or contradictory trials in the scientific literature concerning the effectiveness of herbal extract on regulating bone metabolism and turnover. Hence, the present study aimed to assess the therapeutic effects of five common herbal compounds (*curcumin, black seed, ginger, cinnamon, and flaxseed oil*) on BTMs as primary and bone mineral density as secondary outcomes.

Materials and methods

Study endpoints: This article was designed as a systematic review and meta-analysis based on the Cochran Guide and the PRISMA Statement (5). Some bone turnover biomarkers and bone formation-related biomarkers including Bone Specific Alkaline Phosphatase (BSAP) and Osteocalcin (OC) and two biomarkers related to bone resorption, including C-terminal Telopeptide type 1 Collagen (CTX-I) and Deoxypyridinoline (DPD) were analyzed as primary endpoints. Secondary endpoints included three indicators of bone mineral density, including total hip Bone Mineral Density (BMD), femoral neck BMD, and lumbar spine BMD. Serum levels of three biomarkers related to bone formation, including Total Alkaline Phosphatase (ALP) and Procollagen Type 1 N-terminal Propeptide (P1NP), and Procollagen Type 1 C-terminal Propeptide (P1CP) and eight bone biomarkers related to Hydroxyproline (HYP), Hydroxylysine (HYL), Pyridinoline (PYD), Bone Sialoprotein (BSP), Osteopontin (OP), Tartrate-resistant acid Phosphatase 5b (TRAP 5b), N-terminal Telopeptide type 1 Collagen (NTX-I) and Cathepsin K (CTSK) were also considered as secondary endpoints that were systematically assessed and reported. The side effects reported in some reviewed articles were also reported as secondary results of the systematic review.

Inclusion and Exclusion Criteria

The studies included in this review consisted of all human clinical trials or quasi-experimental interventional studies aimed to assess the effects of selected medicinal plants or active ingredients including *turmeric* or *curcumin*, *black seed* (Nigella Sativa), *flaxseed*, *cinnamon* (Cinnamomum Verum), and *ginger* (Zingiber Officinale) on bone turnover and bone mineral density. As the exclusion criteria, review studies, animal studies, observational studies, study protocols, cellular-molecular studies, as well as studies involving children or adolescents (aged less than 18 years) were not analyzed systematically. The target population of this study included adults of all ages,

sex, and health conditions. The intervention included oral supplements such as pills, capsules, powders, syrups, or diets based on these herbs (Unlike most drugs, micronutrients alone do not work effectively but have synergistic effects in combination with the food matrix) (27,28), and no restrictions were placed on how long the supplement was used, as well as the dosage and intervals of supplementation. The control group included people receiving a placebo or a diet without these plants (the usual daily diet). The PICOS format (participants, interventions, comparison, outcomes, and study design) was applied to depict the study eligibility criteria (Table 1).

Search strategy: A large systematic search was conducted by three authors (AMI, MA & HKh) separately on all published manuscripts (without restrictions on publishing date or the language of articles) on article databases of PubMed, Scopus, Web of Science, Cochrane Central Register of Controlled Trials, and Embase, as well as SID, Magiran, Irandoc, and Iranmedex databases for Persian articles. The papers presented at the seminars and congresses were also reviewed. The keywords provided by the MeSH [("Curcumin" OR "Curcuma Longa" OR "Turmeric" OR "Nanocurcumin" OR "Curcuminoid")/("Black seed" OR "Black cumin" OR "Black caraway" OR "Kalongi" OR "Fennel flower seed" OR "Bunium Persicum seed" OR "Habbah Albarakah" OR "Siyah daneh" OR "Nutmeg flower" OR "Nigella Sativa)/("Ginger" OR "Zingiber officinale")/("Cinnamon" OR "Cinnamon Zeylanicum" OR "Cinnamomum" OR "Ceylon cinnamon" OR "True cinnamon")/("Flaxseed oil" OR "Flaxseed" OR "Common flax oil" OR "linseed oil) AND ("Osteocalcin" OR "OC" OR "Bone gamma-carboxyglutamic acid-containing protein" OR "BGLAP" OR"Bone γ-carboxyglutamic acid protein")/("Procollagen type 1 N-terminal propeptide" OR "Procollagen type 1 amino-terminal propeptide" OR "N-terminal propeptide of type 1 collagen" OR "P1NP")/("Carboxy-terminal collagen cross link" OR "Carboxy-terminal collagen cross link of type 1 collagen" OR "CTX 1" OR "Carboxy-terminal of type 1 collagen")/("Bone specific Alkaline phosphatase" OR "Bone Alkaline phosphatase" OR "Bone specific ALP" OR "BSALP" OR "BALP")] were used in combination with Boolean operators to search the pointed databases Endnote X8 software (Thomson Reuters, Philadelphia, PA) was used to manage the searched articles. Two researchers (MA & AMI) independently reviewed the title and summary of the articles and then reviewed the full text. During the process of evaluating the articles, the disputed cases between the researchers were finally decided after discussion with the third researcher (AFKh). ***Data extraction:*** An electronic form was designed to extract data from articles that included the following sections: author's name and year of publication, country of study, type of study, sample size, age and gender of participants, type of supplement prescribed, supplement dose, and course of treatment in the intervention and control groups, follow-up period, evaluated outcomes and how to measure them, study results and possible side effects that were extracted from the eligible studies by three authors (AMI, MA & HKh) (Table 2).

Table 1: PICOS criteria for inclusion and exclusion of studies

PICO	Eligibility criteria
Study participants	All adults receiving a dietary supplement or diet containing one of the studied plants curcumin (turmeric), Nigella Sativa (black seed), Flaxseed, cinnamon (Cinnamomum Verum) and ginger (Officinale Zingiber) No age, sex or health restrictions
Intervention	Oral therapy supplement in the form of tablets, capsules, powder, syrup or diet based on the studied plants (curcumin, black seed, flaxseed, cinnamon and ginger) No restrictions on the duration of supplement use, dosage and supplementation intervals
Comparison	Placebo or control
Outcomes	
Primary endpoints	Two cases of bone formation biomarkers include BSAP (Bone Specific Alkaline Phosphatase) and OC (Osteocalcin) and two cases of bone analysis biomarkers include CTX-I (C-terminal Telopeptide type 1 Collagen) and DPD (Deoxypyridinoline).
Secondary endpoints	Three cases of bone formation biomarkers include ALP (Total Alkaline Phosphatase), P1NP (Procollagen Type 1 N-terminal Propeptide) and P1CP (Procollagen Type 1 C-terminal Propeptide) and eight cases of bone biomarkers related to HYP (Hydroxyproline).), HYL (Hydroxylysine), PYD (Pyridinoline), BSP (Bone Sialoprotein), OP (Osteopontin), TRAP 5b (Tartrate-resistant Acid Phosphatase 5b), NTX-I (N-terminal Telopeptide type 1 Collagen), CTSK (Cathepsin K) and three indicators of bone marrow density include Total Hip BMD, Femoral Neck BMD, and Lumbar Spine BMD Side effects of supplements
Study design	Controlled Clinical Trials (RCTs) or quasi-experimental studies

Table 2: Characteristics of the included studies

Authors year	Type of study	Sampl size	Sex	Place	Age	ntervention (dosage)	Comparison (dosage)	Duration of therapy	Outcome measures	Health condition of participants	Side effects
Poonam Ashish Gupte et al. (2019) (5)	Pilot clinical study	Intervention Group: n=17 Control Group: n=25	Male=8 Female=34	India	40-65	SLCP 400 mg (80 mg curcumin) twice daily for	Ibuprofen 400 mg once in the morning + Dextrin in the evening for	90 days	PGE2, LTB4, IL-6, IL-1B, TNF-a, UCTX-II (ELISA method)	Monoclonal Gammopathy of Undefined Significance	Heartburn and nausea (n=2). rash and itching all over the body (n=1)
Masoud Hatefi et al. (2018) (7)	RCT	Intervention Group: n=50 Control Group: n=50	Male =73 Female =27	Iran	19-65	Curcumin 110 mg/kg/day for 6 months	Placebo	6 months	BMD of Lumbar Spine, Femoral Neck & Total Hip (DXA) BALP, sCTX, Osteocalcin & PINP	Postmenopausal Osteoporosis	Not Reported
Fatemeh Khanizah et al. (2018) (8)	RCT	Alendronate Group: n=20 Alendronate + Curcumin Group: n=20 Control Group: n=20	Female=60	Iran	55-65	Alendronate 5 mg/day Curcumin 110 mg/day + Alendronate 5 mg/day	Calcium Carbonate 1000-1500 mg/day	12 months	BMDs of the lumbar spine, femoral neck, total hip (DXA) BALP osteocalcin CTx	Postmenopausal Osteoporosis	Not Reported
Terry Golombick et al. (2009) (9)	Single-blind, cross-over pilot study	Group A: n=17 Group B (placebo): n=9	Male=16 Female=10	Australia	Over 45	Curcuminoid tablets 1g (900 mg of curcumin, 80 mg of desmethoxycurcumin, and 20 mg of bisdesmethoxycurcumin) two tablets twice daily & crossed over at 3 months after initiating therapy.	Placebo tablets 1 g (microcrystalline cellulose, dicalcium phosphate, PVPK 30, sodium starch glycolate, and magnesium stearate) two tablets twice daily & crossed over at 3 months.	6 months	Serum calcium, 25 (OH) D, BALP, Serum B2 microglobulin, Serum paraprotein & immunoglobulinelectrophoresis. uNTx	Postmenopausal Osteoporosis	Diarrhea and abdominal cramping (n=2)
Yves Henrotin et al. (2014) (10)	Exploratory non-controlled clinical trial	Study Group: Bio- n=22	Male=7 Female=15	Belgium	49-77	Bio-optimized curcumin: 42 mg curcumin + polysorbate: 3 caps in the morning & 3 cap in the evening	-	3 months	Coll2-1 & Coll2-1NO2 Fib3-1 & Fib3-2 MPO, hsCRP, U-CTX-II	Postmenopausal Osteoporosis	diarrhea & vomiting (n=2)

Table 2: Characteristics of the included studies (continue)

Authors year	Type of study	Sampl size	Sex	Place	Age	ntervention (dosage)	Comparison (dosage)	Duration of therapy	Outcome measures	Health condition of participants	Side effects
Shirin Hasani-ranjbar et al. (2015) (11)	randomized double blind clinical trial	Study Group: n=15 Placebo Group: n=15	Female=30	Iran	50-65	Nigella Sativa capsule: 600 mg nigella sativa in each capsule, twice a day	Placebo:600 mg placebo in each capsule, twice a day	6 months	CTX, 25-OH-vitamin D, osteocalcin and bone alkaline phosphatase	Postmenopausal Osteoporosis	No side effects due to NS supplementation were observed
Neda Valizadeh et al (2009) (12)	single-blind, placebo controlled, pilot study	Nigella sativa Group*:* n=5 Placebo Group: n=7	Female=12	Iran	48-74	3ml, 0.05 ml/kg/day of nigella sativa extract + 2 tablets of Calcium-D supplements per day	Placebo+2 tablets of Calcium-D supplements per day	3 months	BMD of the Lumbar spine and Total hip, Weight and Height, CBC diff, ALT- AST and ALP, BUN and Cr, Serum Calcium and Phosphorus, Osteocalcin, CTX and Bone-ALP.	Unknown	Not reported.
Neda Valizadeh et al (2009) (13)	single-blind, placebo controlled clinical trial	Nigella sativa Group*:* n=9 Placebo Group: (n=13)	Female =22	Iran	49-72	3ml, 0.05 ml/kg/day of nigella sativa extract + 1 tablet of Calcium-D supplement per day	3ml of placebo (Sunflower oil) +1 tablet of Calcium-D supplement per day	3 months	BMD of the Lumbar spine and Total hip, Weight and Height, CBC diff, ALT- AST and ALP, BUN and Cr, Serum Ca and P, Osteocalcin, CTX and Bone-ALP.	Obesity	No reports of adverse reactions were observed in the study
Zhen-Yu Shi et al (2017) (14)	Randomized, open-label, placebo-controlled study	Alendronate Group: n=80 QiangGuYin Group: n=80 Placebo Group: n=80	Female =240	China	45-70	Alendronate 70 mg/week QiangGuYin granules 20 gr/day	Placebo	12 months	BMD at the lumbar spine, total superior hip, femoral neck, and hip trochanter bone turnover markers of t-P1NP and serum β-CTX	Healthy	hypertension 2.5%, nausea 3.7%, diarrhea 2.5%, in QGY group
Edralin A. Lucas et al (2002) (15)	Randomized controlled double blind parallel study	Treatment Group: n= 29 Control Group: D: n=29	Female =58	USA	Postmenopausal women younger than 65 yr old	40 gr of ground whole flaxseed+ 1000 mg elemental calcium+ 400 IU vitamin D daily	40 gr of wheat-based regimen+ 1000 mg elemental calcium+ 400 IU vitamin D daily	3 months	Serum 17 estradiol, Estrone, FSH, SHBG, Serum IGF-I, IGFBP-3, Total Alkaline Phosphatase, Calcium, Tartrate-Resistant Acid Phosphatase activities and BSAP activity, TC, TG,HDL-C, Non HDL-C,apo A-1 and apo B. Urinary Cr and Dpd.	Renal failure	gastrointestinal problems, lack of palatability of regimen

Table 2: Characteristics of the included studies (continue)

Authors year	Type of study	Sampl size	Sex	Place	Age	ntervention (dosage)	Comparison (dosage)	Duration of therapy	Outcome measures	Health condition of participants	Side effects
Jennifer D Brooks et al (2004) (16)	randomized, double-blind, parallel, placebo-controlled study	Flaxseed Group: n=16 Soy Group: n=15 Placebo Group: n=15	Female =46	Canada	Not Reported	Flaxseed muffin: 25 gr ground flaxseed as a flaxseed muffin daily Soy muffin: 25 gr soy flour as a soy muffin daily	25 gr whole-wheat flour as a placebo muffin daily	16 weeks	Nutrient intake, Total urinary phytoestrogens excretion, Urinary estrogen metabolites 2-hydroxyestrone and 16 α-hydroxyestrone, Serum Estradiol, Estrone, and Estrone Sulfate, Serum BSAP and Urinary DPD.	Postmenopausal Osteoporosis	Not Reported
S. Dodin et al (2005) (17)	randomized, double-blind, placebo-controlled trial	Flaxseed Group: n=101 Placebo Group: n=98	Female=199	Canada	45-65	40 gr flaxseed daily, 20 gr flaxseed as two slices of bread+20 gr flaxseed as ground grains to add to cereal, juice, or yogurt,	40 gr wheat germ daily: 20 gr wheat germ as two slices of bread+20 gr wheat germ as ground grains to add to cereal, juice, or yogurt,	12 months	Dietary intake, Weight, Height, BMI, Systolic blood pressure, Diastolic blood pressure, Total cholesterol, LDL cholesterol, HDL cholesterol, Triglyceride, BMD at the lumbar spine and femoral neck, Quality of life, Vasomotor domain, Hot flushes and Night sweats.	Postmenopausal Osteoporosis	Digestive problems (10 women in flaxseed group and 5 women in placebo group) and difficulty with treatment intake (5 women in flaxseed group and 1 women in placebo group).
Amy E Griel et al (2007) (18)	randomized, double-blind, balanced order, three period crossover trial	Linoleic Acid Diet Group: n=23 α-Linolenic Acid Diet Group: n=23 Control Group: n=23	Male=20 Female=3	USA	Not Reported	Linoleic Acid (LA) Diet: high linoleic acid diet α-Linolenic Acid (ALA) Diet: high α-linolenic acid diet	Average American diet	24 weeks	Serum Fatty acid profile, Serum N-telopeptides of type I collagen (NTx), Serum bone-specific alkaline phosphatase, Serum TNF-α, IL-6, IL-4 and IL-1β.	Postmenopausal Osteoporosis	Not Reported
Carla Mora Aguilar et al (2017) (19)	RCT	Brown Flaxseed Group: n=9 Golden Flaxseed Group: n=11 Control Croup: n=10	Female=30	Brazil	40-55	BF Group: one pack of brown flaxseed in a day (40 gr/day) + a calorie-restricted diet of 250 kcal/day GF Group: one pack of golden flaxseed in a day (40 gr/day) + a calorie-restricted diet of 250 kcal/day	A calorie-restricted diet of 250 kcal/day for 12 weeks.	12 week	Dietary intake, Weight, Height, Waist Circumference, Lean Body Mass, Fat Body Mass, Serum TNF-α, IL-1β, IL-6 and IL-10, Serum 17β-oestradiol, 25 (OH) vitamin D3, Osteocalcin and NTx-I and Urinary Calcium.	Unknown	Not Reported

Table 2: Characteristics of the included studies (continue)

Authors year	Type of study	Sampl size	Sex	Place	Age	ntervention (dosage)	Comparison (dosage)	Duration of therapy	Outcome measures	Health condition of participants	Side effects
Sujatha Rajaram et al (2017) (20)	single-blind, randomized, crossover trial	Eicosapentaenoic acid/Docosahexaenoic acid diet: n=24 α-linolenic aci diet: n=24 Combination diet: n=24 Control diet:n=24	Male=9 Female=15	USA	20-70	EPA/DHA diet Group. ALA diet Group: (42–49 gr flaxseed oil/week + 10 gr walnuts, 3 times/week), Combination diet Group for 8 weeks and a 4 week washout between treatments	Diet with seven calorie levels (1500 – 3000 kcal/day) for 8 weeks and a 4 week washout between treatments	32 weeks	Serum CTX, Serum P1NP, Serum Osteocalcin, Serum Insulin-like growth factor- 1, Peroxisomal proliferator activated receptor-gamma (PPAR-γ) mRNA levels	Postmenopausal Osteoporosis	Not Reported
Maryam Mirfatahi et al (2018) (21)	parallel, randomized, doubleblinded, clinical trial	Flaxseed oil Group: n=17 Control Group: n=17	Male=22 Female=12	Iran	18 years and greater	6 gr/day of flaxseed oil (as one Iranian tablespoon) as a usual oil with salad at lunch or di	6 gr/day of MCT oil (as one Iranian tablespoon) as a usual oil with salad at lunch or dinner	8 weeks	Serum Osteocalcin, Osteoprotegerin, N-telopeptide and Receptor activator of nuclear factor kappa B ligand Dietary intake, Dialysis Adequacy, Serum Intact parathyroid hormone, Phosphorus and Calcium.	Unknown	No adverse events were reported.

SLCP:solid lipid curcumin particles, PGE2:prostaglandin E2, LTB4:leukotriene B4, IL-6:interleukin 6, IL-1B:interleukin 1 beta, TNF-a:tumor necrosis factor alpha, UCTX-II:urinary carboxy terminal telopeptides of type II collagen, OA:osteoarthritis, ELISA:enzyme-linked immunosorbent assay, RCT:*randomized controlled trial*, BMD:*bone mineral density*, DXA:dual-energy X-ray absorptiometry, BALP:bone Alkaline Phosphatase, SCTX:serum carboxy terminal telopeptides, PINP:procollagen type I N-terminal propeptide, SCI: spinal cord injuries, CTX:carboxy terminal telopeptides, UNTx:urinary N-telopeptide of type I collagen, MGUS:monoclonal gammopathy of undefined significance, MPO:myeloperoxidase, hsCRP:high sensitivity C-reactive protein, NS:nigella sativa, CBC diff:complete blood count with diffrential, ALT:alanine aminotransferase, AST:aspartate aminotransferase, ALP:alkaline phosphatase, BUN:blood urea nitrogen, Cr:creatinine, Ca:calcium, P:phosphorus, T-P1NP:total procollagen type I N-terminal propeptide, β-CTX:beta carboxy terminal telopeptides, QGY:qiangGuYin herbal formula, FSH:follicle-stimulating hormone, SHBG:sex hormone binding globulin, IGF-I:insulin-like growth factor I, IGFBP-3:insulin-like growth factor binding protein 3, BSAP:*bone* specific alkaline phosphatase, TC:total cholesterol, TG:triglyceride, HDL-C:high density lipoprotein cholesterol, apo A-1:apolipoprotein A1, apo B: apolipoprotein B, DPD:deoxypyridinoline, TRAP:tartrate-resistant acid phosphatase, BMI:body mass index, LDL-C:low density lipoprotein cholesterol, NTx:N-telopeptides of type I collagen, IL-4:interleukin 4, IL-10: interleukin10, EPA:eicosapentaenoic acid, DHA:docosahexaenoic acid, ALA:alpha linoleic acid, PPAR-γ:peroxisomal proliferator activated receptor-gamma, MCT:medium chain triglycerides, NFκB:nuclear factor kappa B, iPTH:intact parathyroid hormone.

Evaluation of the quality and risk of bias of the articles: The quality of the articles and the risk of bias were assessed through the Cochrane Booklet by two researchers (AMI & HKh). The following items were assessed: allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), and selective reporting (reporting bias). Disputes were also resolved through consultation with a third researcher (AFKh). The bias of the studies was demonstrated using Review Manager 5.3 (RevMan; The Cochrane Collaboration, Oxford, UK) software.

Meta-analysis and data synthesis: Data were synthesized through both quantitative and qualitative approaches. If there were adequate data for pooling, meta-analysis was done using Review Manager 5.3 statistical software. If there were no adequate data, the findings were reported as systematic review. In this study, all variables included in the meta-analyses were continuous, and the mean and standard deviation before and after the interventions were used for quantitative analysis. In cases of lack of access to the mean and standard deviation, these indicators were calculated using other central tendency and dispersion measurements such as median values and interquartile range which were been reported in the articles (29). In cases where the mean values and standard deviations were not mentioned in the text of the article, Universal Desktop Ruler 3.8 software was used to calculate these values from the relevant graphs. Also, in cases where the SE values were reported instead of SD, the SE values were converted to SD using the formula (SE =SD/√n). The units related to the serum levels of the evaluated factors, if variable, were identified by referring to the Internet address http://www.endmemo.com/sconvert/ng_mlppb.php and then analyzed. In order to calculate mean differences (MDs) with 95% confidence intervals (CIs), the mean changes and SDs of changes for all continuous variables were used. The standard deviation changes were also calculated using the following formula: In which the SD_b is the SD for baseline and the SD_f is the SD of follow-up values, and r represents the correlation between baseline and the follow-up values.

$$SD_{\text{change score}} = \sqrt{SD_b^2 + SD_f^2 - 2 * r * SD_b * SD_f}$$

For statistical evaluation of articles, a heterogeneity test was used which indicates the percentage of diversity between the studies, and if the I^2 value was more than 0.50, these studies were considered heterogeneous, and therefore their results were reported as Random Effect Meta-Analysis. The Fixed Effect model was also used for studies with the lowest levels of Heterogeneity (I^2<0.50). Forest Plot was also used to display the final results of this review study.

Results

Study search and selected articles: Details of the process of searching and selecting articles, as well as the reasons for excluding articles in the systematic review study and meta-analysis are presented in Figure 1. Out of 3307 identified articles in search of different databases, 1829 articles were removed from the study due to duplicity and the rest of the articles were evaluated to evaluate the entry criteria. Of the 1478 articles reviewed, 1284 were excluded due to the lack of relevance of the title to the purpose of the present study and 178 due to non-compliance with the aim of the present study or the uncertainty of the target plant (9 articles), no clinical trial or experimental studies (65 articles), animal studies (92 articles), and lack of reference to the intended consequences (12 articles). Then, 16 articles were selected for systematic review, and after reviewing the full text of the articles and their reported results, 10 articles were finally analyzed.

Description of the studies: All articles selected for systematic review are published between 2002 and 2020. Of the 16 articles reviewed, 14 articles had full English text (2-15), one article had full Persian text (20) and one article had English abstract (21). In terms of study design, 15 studies were clinical trial studies (6-9, 11-21) and one study was an uncontrolled before-after study (10). Additionally, six articles were performed in Iran (7, 8, 11, 19-21), three articles in the United States (13, 16, 18), two articles in Canada (14, 15), and one article in India (6), Australia (9), Belgium (10), China (12) and Brazil (17). Of the 16 articles reviewed, 10 studies (6-8, 11, 13-15, 17, 19, 20) were meta-analyzed. The total numbers of participants in the meta-analysis studies were 603 patients and in the systematic review were 968. Dosage, dose intervals, pharmaceutical forms and duration of the intervention varied in most studies. The duration of the intervention ranged from 2 months to 12 months and the treatment interval varied from every 12 hours to every 24 hours. Details of all articles are listed in Table 2.

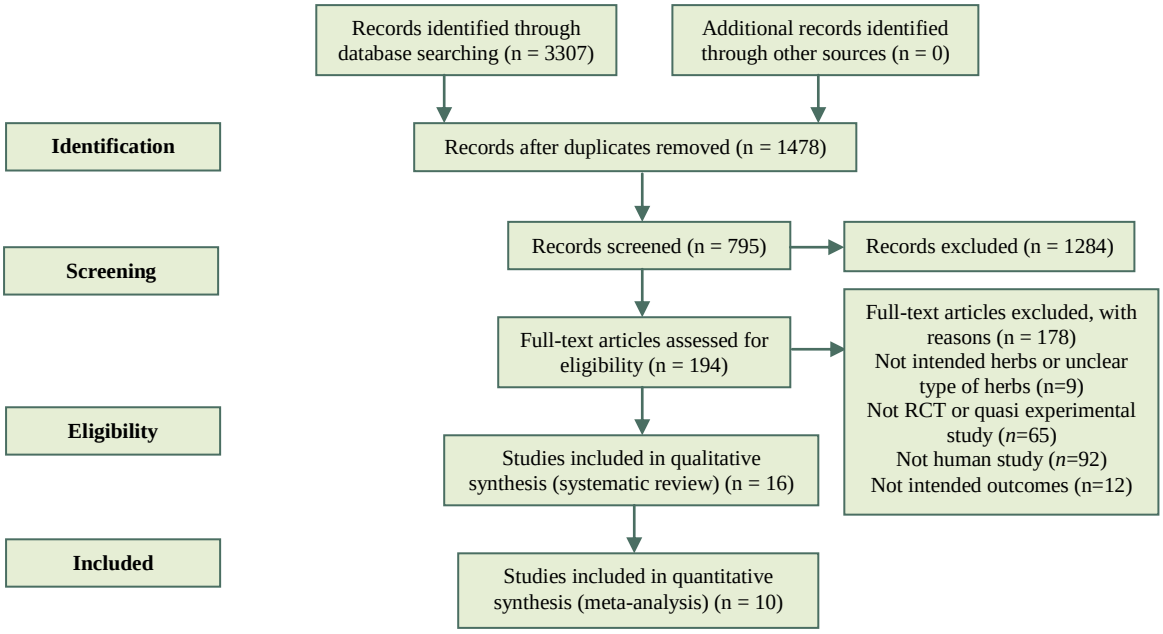


Figure 1: Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)

Risk of bias in the included studies: Of these 16 articles, one article (uncontrolled before-after study) did not meet the requirements for bias risk assessment (10), and thus 15 articles were finally reviewed. 1) Random sequence generation (checking for possible selection bias): Of the 15 articles reviewed, 5 were low-risk (6,12,14,15,19), 9 articles had unspecified risk (7-9,11,13,16-18,21) and only one article had high risk (20). 2) Allocation concealment (checking for possible selection bias): In the field of Allocation concealment bias, four articles had low risk (6, 14, 15, and 19) and 11 articles had uncertain risk (7-9, 11-13, 16-18, 20, 21). 3) Blinding of participants and personnel (checking for possible performance bias): According to text reviews, only two articles had low risk (14, 15). nine articles had unspecified risk (6-9,13,16,17,19) and four articles had high risk (11,12,18,20). 4) Blinding of outcome assessment (checking for possible detection bias): 3 articles had low risk (14,15,19), ten articles had unspecified risk (6-9,11,13,16,17,20,21) and two articles had high risk (12,18). 5) Incomplete outcome data (checking for possible attrition bias): For Incomplete outcome data, five articles had low risk (7,8,12,15,19), six articles had unspecified risk (13,14,16-18,21) and four articles had high risk (6,9,11,20), and 6) Selective reporting (checking for reporting bias): Only one article had a low risk (7) and 11 articles did not have an unspecified risk (11-21), while three articles had high risk (6,8,9) (Figures 2, 3).

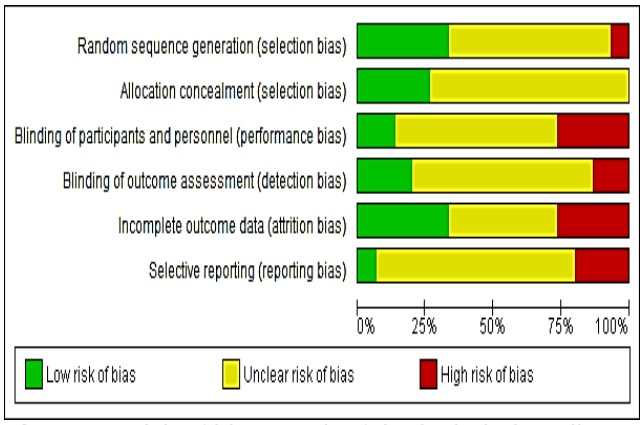


Figure 2: Risk of bias graph of the included studies

Effectiveness of Interventions

Effect of medicinal herbs on BSAP

Meta-analysis: A total of 6 RCT studies (7, 8, 11, 13, 14, and 20) with 241 participants who were analyzed and measured the effects of several different plant compounds on BSAP in different individuals were analyzed. The calculated overall effect showed that there was no significant difference between the intervention and control groups (SMD=-1.76, 95%CI: -6.85 to 3.33, p=0.50). Subgroup analysis also showed no significant difference between intervention and control groups due to *curcumin* effect (SMD=-5.03pg/L, 95%CI: -13.98 to 3.92, p=0.27), *black seed* effect (SMD=0.91 pg/L, 95%CI: There are no -3.18 to 5.00, p=0.66) and *flaxseed* effect (SMD=-0.85pg/L, 95%CI: -2.56 to 0.86, p=0.33) (Figure 4-A).

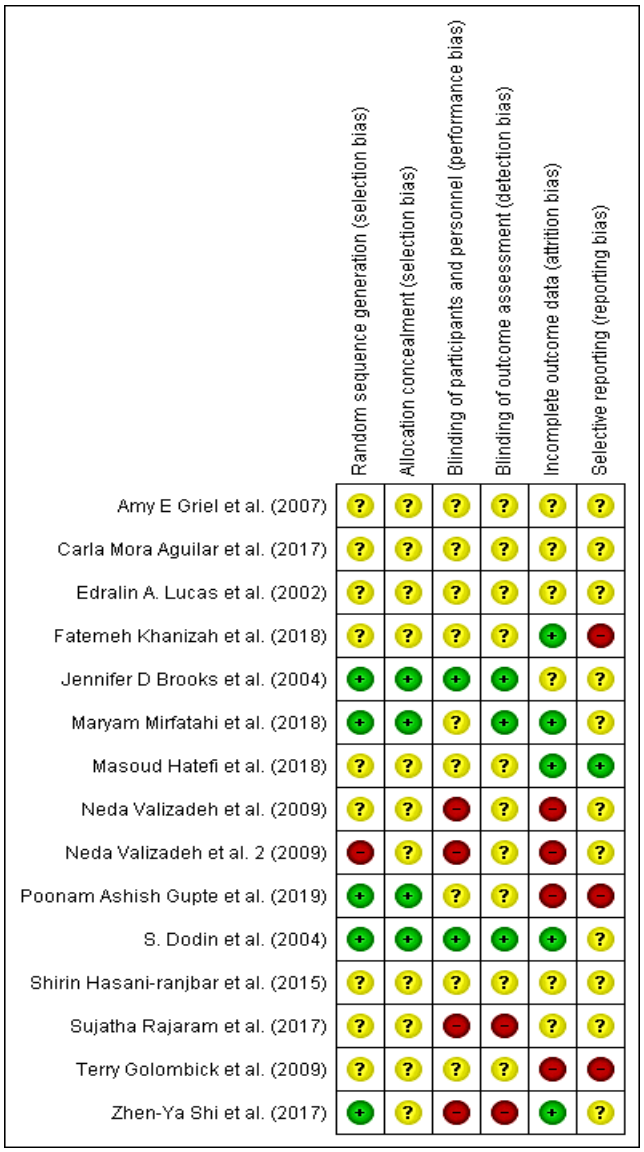


Figure 3: Risk of bias summary of the included studies

Systematic review: In the RCT study performed by Hasani-ranjbar et al (21) after six months of *black seed* consumption, there was no significant difference between the two intervention groups and placebo at BSAP levels (19.18 ± 6.61 vs 19.04 ± 6.70, p>0.05). In an RCT crossover study by Griel et al (16), α-Linolenic acid diet for 6 weeks did not significantly differ BSAP levels (p> 0.05).

Effect of medicinal herbs on CTx

Meta-analysis: In this context, 5 RCT studies (6, 7, 8, 11, and 20) with 216 participants who evaluated the effect of curcumin and *black seed* on CTx in different groups were analyzed. The calculated overall estimated effect values showed that in all studies, there was no significant difference between the intervention and control groups (SMD=-0.17ng/mL, 95%CI: -0.43 to 0.09, p=0.21). Subgroup analysis also showed that *curcumin* consumption (SMD=-0.24ng/mL, 95%CI: -0.56 to 0.08, p=0.15) and *black seed* (SMD=-0.04ng/mL, 95%CI: -0.26 to 0.18, p=0.75) also did not have a significant effect on study groups alone (Figure 4-B).

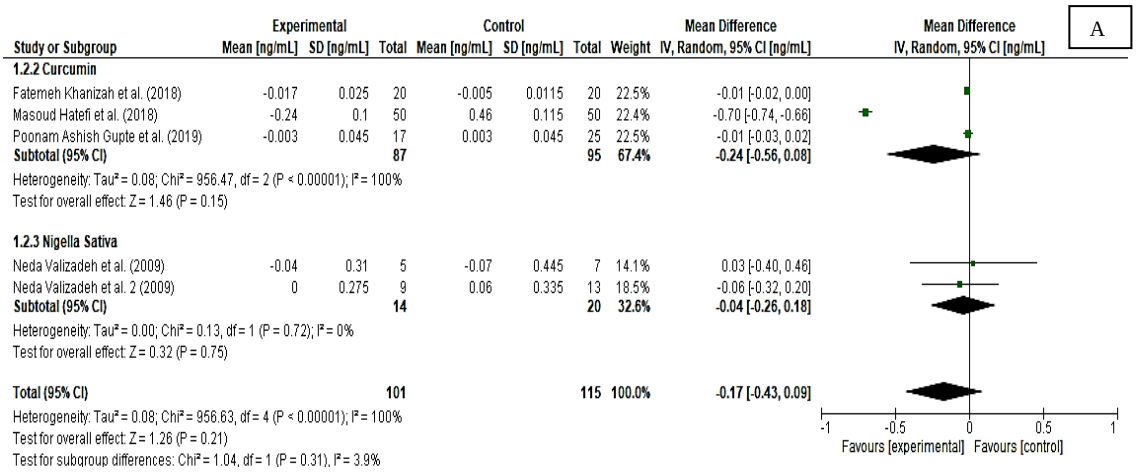
Systematic review: In the RCT by Hasani-ranjbar et al (21), six months of *black seed* consumption had no significant effect on CTx levels (0.15 ± 0.09 vs 0.19 ± 0.15, p> 0.05). In a crossover RCT conducted by Rajaram et al (18), α-Linolenic acid diet (intervention: 42–49 gr *flaxseed* oil/week plus 10 gr walnuts, three times/week), did not differ significantly CTx levels (p>0.05). Also, in the RCT by Shi et al (12), 3,6,9, and 12 months intervention by 20 gr/day QiangGuYin (containing *cinnamon)* showed significant reduction in *β*-isomerized CTX (*β*-CTX) levels (p<0.01) compared to placebo. However, Alendronate treated participants had significantly greater decreases in serum concentrations of *β*-CTX than QGY-treated participants at all-time points (*p*< 0.01). In a non-controlled trial conducted by Henrotin et al (10), after three months of taking Flexofytol capsule (bio-optimized curcumin), changes in CTx-II urinary levels were not statistically significant (p>0.05).

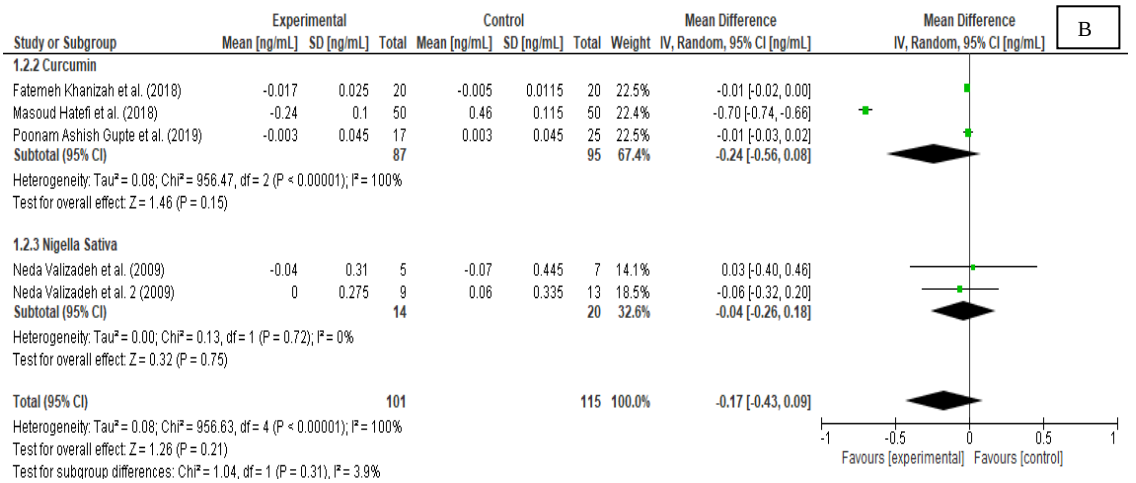
Effect of medicinal herbs on DPD

Meta-analysis: Two RCT studies (13, 14) were analyzed with 67 analyzed participants who measured the *flaxseed* effect on DPD in postmenopausal women. The calculated overall effect showed that there was no significant difference between the intervention and control groups (MD=0.82nmol/mmol, 95%CI: -0.05 to 1.68, p=0.06) (Figure 4-C).

Effect of medicinal herbs on OC

Meta-analysis: Six studies involving RCT (7, 8, 11, 17, 19, and 20) with 229 participants who evaluated the effect of curcumin, *black seed*, and *flaxseed* on OC in different groups were analyzed. The values of the overall estimated effect showed that in all studies, there was no significant difference between intervention and control groups (SMD=-2.02ng/mL, 95%CI: -4.49 to 0.45, p=0.11). Subgroup analysis showed that *curcumin* consumption did not have a significant effect on the study groups (SMD=-3.25 ng/mL, 95%CI: -7.85 to 1.34, p=0.17). Analysis of other subgroups also yielded similar results for *black seed* (SMD=-1.94 ng/mL, 95%CI: -6.77 to 2.88, p = 0.43) and *flaxseed* (SMD=-0.07ng/mL, 95%CI: -2.41 to 2.27, p=0.95) (Figure 4-D).


A
Experimental
Control
Mean Difference
Mean Difference
Study or Subgroup Mean [ng/mL] SD [ng/mL] Total Mean [ng/mL] SD [ng/mL] Total Weight IV, Random, 95% CI [ng/mL]
IV, Random, 95% CI [ng/mL]
1.2.2 Curcumin
Fatemeh Khanizah et al. (2018) -0.017 0.025 20 -0.005 0.0115 20 22.5% -0.01 [-0.02, 0.00]
Masoud Hatefi et al. (2018) -0.24 0.1 50 0.46 0.115 50 22.4% -0.70 [-0.74, -0.66]
Poonam Ashish Gupte et al. (2019) -0.003 0.045 17 0.003 0.045 25 22.5% -0.01 [-0.03, 0.02]
Subtotal (95% CI) 87 95 67.4% -0.24 [-0.56, 0.08]
Heterogeneity: Tau² = 0.08; Chi² = 956.47, df = 2 (P < 0.00001); I² = 100%
Test for overall effect: Z = 1.46 (P = 0.15)
1.2.3 Nigella Sativa
Neda Valizadeh et al. (2009) -0.04 0.31 5 -0.07 0.445 7 14.1% 0.03 [-0.40, 0.46]
Neda Valizadeh et al. 2 (2009) 0 0.275 9 0.06 0.335 13 18.5% -0.06 [-0.32, 0.20]
Subtotal (95% CI) 14 20 32.6% -0.04 [-0.26, 0.18]
Heterogeneity: Tau² = 0.00; Chi² = 0.13, df = 1 (P = 0.72); I² = 0%
Test for overall effect: Z = 0.32 (P = 0.75)
Total (95% CI) 101 115 100.0% -0.17 [-0.43, 0.09]
Heterogeneity: Tau² = 0.08; Chi² = 956.63, df = 4 (P < 0.00001); I² = 100%
Test for overall effect: Z = 1.26 (P = 0.21)
Test for subgroup differences: Chi² = 1.04, df = 1 (P = 0.31), I² = 3.9%
-1 -0.5 0 0.5 1
Favours [experimental] Favours [control]


B
Experimental
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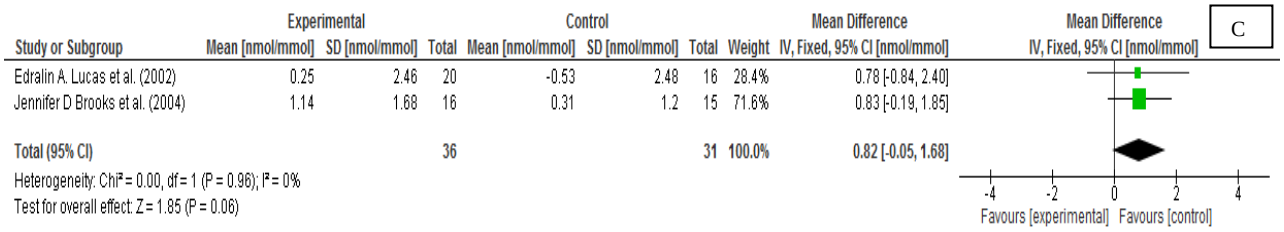
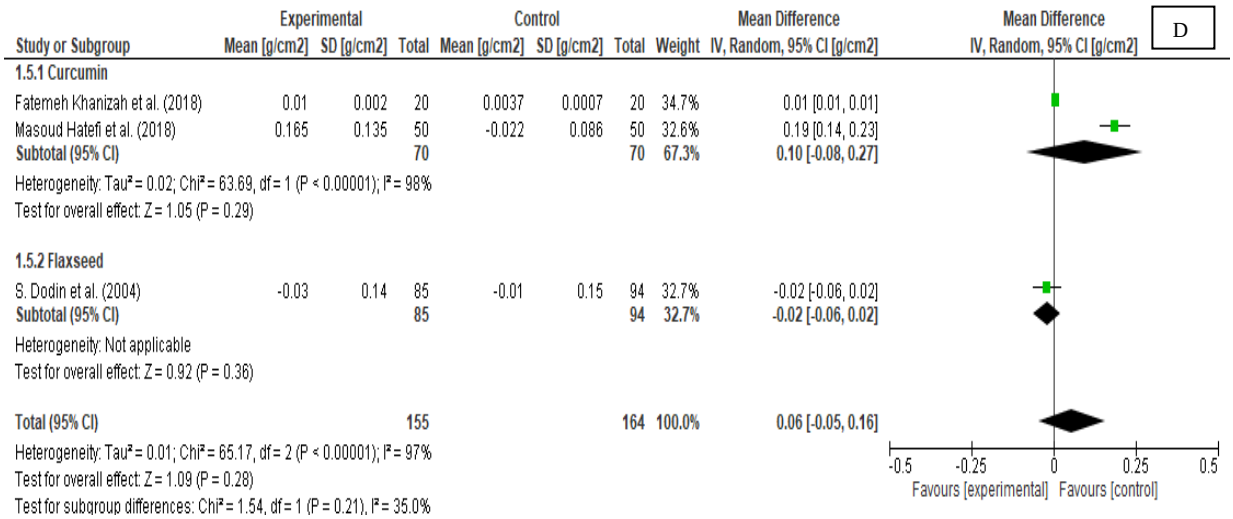
Study or Subgroup Mean [ng/mL] SD [ng/mL] Total Mean [ng/mL] SD [ng/mL] Total Weight IV, Random, 95% CI [ng/mL]
IV, Random, 95% CI [ng/mL]
1.2.2 Curcumin
Fatemeh Khanizah et al. (2018) -0.017 0.025 20 -0.005 0.0115 20 22.5% -0.01 [-0.02, 0.00]
Masoud Hatefi et al. (2018) -0.24 0.1 50 0.46 0.115 50 22.4% -0.70 [-0.74, -0.66]
Poonam Ashish Gupte et al. (2019) -0.003 0.045 17 0.003 0.045 25 22.5% -0.01 [-0.03, 0.02]
Subtotal (95% CI) 87 95 67.4% -0.24 [-0.56, 0.08]
Heterogeneity: Tau² = 0.08; Chi² = 956.47, df = 2 (P < 0.00001); I² = 100%
Test for overall effect: Z = 1.46 (P = 0.15)
1.2.3 Nigella Sativa
Neda Valizadeh et al. (2009) -0.04 0.31 5 -0.07 0.445 7 14.1% 0.03 [-0.40, 0.46]
Neda Valizadeh et al. 2 (2009) 0 0.275 9 0.06 0.335 13 18.5% -0.06 [-0.32, 0.20]
Subtotal (95% CI) 14 20 32.6% -0.04 [-0.26, 0.18]
Heterogeneity: Tau² = 0.00; Chi² = 0.13, df = 1 (P = 0.72); I² = 0%
Test for overall effect: Z = 0.32 (P = 0.75)
Total (95% CI) 101 115 100.0% -0.17 [-0.43, 0.09]
Heterogeneity: Tau² = 0.08; Chi² = 956.63, df = 4 (P < 0.00001); I² = 100%
Test for overall effect: Z = 1.26 (P = 0.21)
Test for subgroup differences: Chi² = 1.04, df = 1 (P = 0.31), I² = 3.9%
-1 -0.5 0 0.5 1
Favours [experimental] Favours [control]


C
Experimental
Control
Mean Difference
Mean Difference
Study or Subgroup Mean [nmol/mmol] SD [nmol/mmol] Total Mean [nmol/mmol] SD [nmol/mmol] Total Weight IV, Fixed, 95% CI [nmol/mmol]
IV, Fixed, 95% CI [nmol/mmol]
Edralin A. Lucas et al. (2002) 0.25 2.46 20 -0.53 2.48 16 28.4% 0.78 [-0.84, 2.40]
Jennifer D Brooks et al. (2004) 1.14 1.68 16 0.31 1.2 15 71.6% 0.83 [-0.19, 1.85]
Total (95% CI) 36 31 100.0% 0.82 [-0.05, 1.68]
Heterogeneity: Chi² = 0.00, df = 1 (P = 0.96); I² = 0%
Test for overall effect: Z = 1.85 (P = 0.06)
-4 -2 0 2 4
Favours [experimental] Favours [control]


D
Experimental
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Mean Difference
Mean Difference
Study or Subgroup Mean [g/cm2] SD [g/cm2] Total Mean [g/cm2] SD [g/cm2] Total Weight IV, Random, 95% CI [g/cm2]
IV, Random, 95% CI [g/cm2]
1.5.1 Curcumin
Fatemeh Khanizah et al. (2018) 0.01 0.002 20 0.0037 0.0007 20 34.7% 0.01 [0.01, 0.01]
Masoud Hatefi et al. (2018) 0.165 0.135 50 -0.022 0.086 50 32.6% 0.19 [0.14, 0.23]
Subtotal (95% CI) 70 70 67.3% 0.10 [-0.08, 0.27]
Heterogeneity: Tau² = 0.02; Chi² = 63.69, df = 1 (P < 0.00001); I² = 98%
Test for overall effect: Z = 1.05 (P = 0.29)
1.5.2 Flaxseed
S. Dodin et al. (2004) -0.03 0.14 85 -0.01 0.15 94 32.7% -0.02 [-0.06, 0.02]
Subtotal (95% CI) 85 94 32.7% -0.02 [-0.06, 0.02]
Heterogeneity: Not applicable
Test for overall effect: Z = 0.92 (P = 0.36)
Total (95% CI) 155 164 100.0% 0.06 [-0.05, 0.16]
Heterogeneity: Tau² = 0.01; Chi² = 65.17, df = 2 (P < 0.00001); I² = 97%
Test for overall effect: Z = 1.09 (P = 0.28)
Test for subgroup differences: Chi² = 1.54, df = 1 (P = 0.21), I² = 35.0%
-0.5 -0.25 0 0.25 0.5
Favours [experimental] Favours [control]

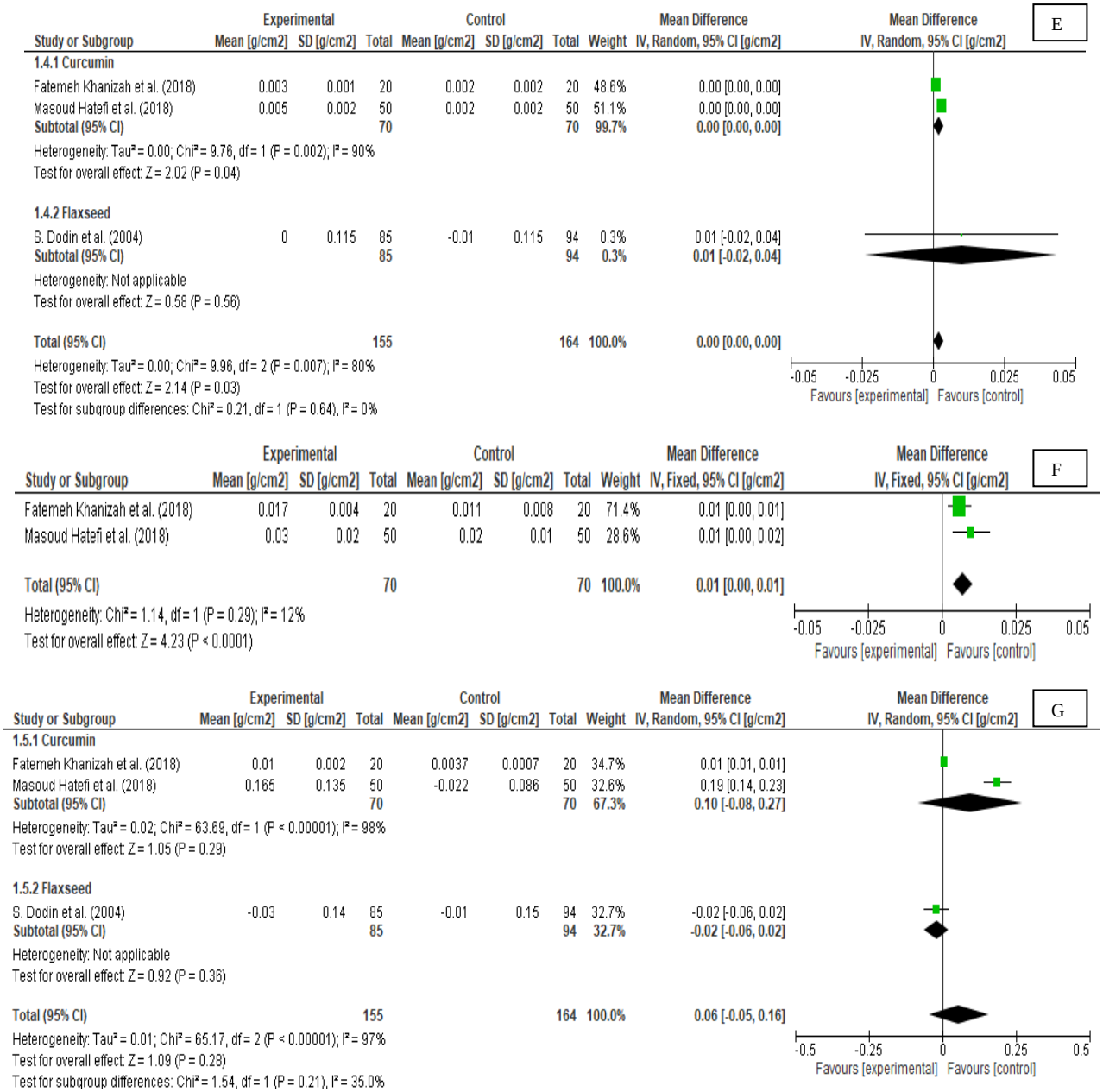

Figure 4: A. Effect of medicinal herbs on BSAP, B. Effect of medicinal herbs on CTx, C. Effect of medicinal herbs on Dpd, D. Effect of medicinal herbs on Osteocalcin, E. Effect of medicinal herbs on Femoral neck BMD, F. Effect of medicinal herbs on Total hip BMD, G. Effect of medicinal herbs on Lumbar spine BMD

Systematic review: In the RCT performed by Hasani-ranjbar et al (21), 6 months consumption of *black seed* had no significant effect on OC levels (p> 0.05). In a RCT conducted by Rajaram et al (18), OC levels did not significantly differ by α-Linolenic acid diet (p>0.05).

Effect of medicinal herbs on femoral neck BMD:

Meta-analysis: Three RCT studies (7, 8, 15) with 319 participants who measured the effect of two different herbal compounds on femoral neck BMD in different individuals entered meta-analysis. The calculated overall estimated effect showed that the difference in femoral neck BMD values in the intervention and control groups was statistically significant (SMD=0.00g/cm^2, p=0.03). Subgroup analysis showed that *curcumin* consumption significantly increased femoral neck BMD in the intervention group (SMD=0.00g/cm^2, p=0.04). But in the case of *flaxseed* consumption, there was no significant difference (SMD=0.01 g/cm^2, 95%CI:

-0.02 to 0.04, p=0.56) between the intervention and control groups (Figure 4-E).

Systematic review: In an RCT conducted by Shi et al (12), taking 20g/day QiangGuYin (containing *cinnamon*) significantly increased femoral neck BMD at months 6 and 12 (p<0.01).

Effect of medicinal herbs on total hip BMD

Meta-analysis: Two RCT studies (7, 8) with 140 participants who measured the effect of *curcumin* use on total hip BMD in patients with SCI as well as postmenopausal women with osteoporosis were meta-analyzed. The calculated overall estimated effect showed that *curcumin* consumption significantly increased BMD total hip in the intervention group (SMD=0.01g/cm^2, 95%CI: 0.00 to 0.01, p<0.001) (Figure 4-F).

Systematic review: In the RCT by Shi et al (12), total hip BMD increased significantly after consumption of 20 g per day QiangGuYin herbal compound (containing *cinnamon*) at months 6 and 12 (p<0.01).

Effect of medicinal herbs on lumbar spine BMD

Meta-analysis: Three RCT studies (7, 8, 15) with 319 participants who assessed the effect of *curcumin* and *flaxseed* on lumbar spine BMD in different groups were meta-analyzed. The values of the overall estimated effect showed that in all studies, there was no significant difference between the intervention and control groups (SMD=0.06 g/cm^2, 95%CI: -0.05 to 0.16, p=0.28). Subgroup analysis also showed that consumption of *curcumin* (SMD=0.10g/cm^2, 95%CI: -0.08 to 0.27, p=0.29) and *flaxseed* (SMD=-0.02g/cm^2, 95%CI: -0.06 to 0.02, p=0.36) alone not had a significant effect on lumbar spine BMD (Figure 4-G).

Systematic review: In the RCT conducted by Shi et al (12), receiving QiangGuYin herbal compound (containing *cinnamon*) 20 g per day increased significantly lumbar spine BMD at months 6 and 12 (p<0.01).

Effect of medicinal herbs on ALP

Systematic review: The results of an RCT study conducted by Lucas et al (13) on 58 postmenopausal women showed that supplementation with ground whole *flaxseed* compared to the control group did not have a significant effect on ALP levels (p=0.54). In a pilot study by Valizadeh et al (11) no significant effect of *black seed* extract was indicated on ALP serum levels (p=0.4). Also, in another RCT study by the mentioned author (20), supplementation with *black seed* extract did not have a significant effect on ALP (p=0.870).

Effect of medicinal herbs on P1NP

Systematic review: An RCT by Hatefi et al (7) on 100 patients with SCI showed that *curcumin* significantly increased P1NP levels (p<0.05); however, control group increased P1NP more than *curcumin*. In another RCT conducted by Shi et al (12) on 240 postmenopausal women, QiangGuYin (containing *cinnamon*) significantly increased t-P1NP (total-P1NP) levels at month 12 in comparison with placebo (p<0.01); however, reduction in t-P1NP was indicated in the QGY group by month 3 and month 6. In the RCT conducted by Rajaram et al (18) on 24 healthy adults, α-Linolenic acid diet had no significant impact on P1NP levels (p>0.05).

Effect of medicinal herbs on TRAP

Systematic review: The results of an RCT study conducted by Lucas et al (13) on 58 postmenopausal women showed that supplementation with ground whole flaxseed compared to the control group did not have a significant effect on serum TRAP levels (p = 0.75).

Effect of medicinal herbs on NTX

Systematic review: In the RCT conducted by Griel et al (16) on 23 participants, the results showed that using the α-Linolenic acid diet significantly lowered NTX levels (p<0.05). In the RCT by Aguilar et al (17), which was performed on 30 obese women in the reproductive stage, NTX-I levels in the Golden Flaxseed group increased significantly compared to the control group (p<0.05). The results of RCT performed by Mirfatahi et al (19) on 34 hemodialysis patients showed that supplementation with *flaxseed oil* significantly reduced serum NTX levels (p< 0.05).

There were no data studies on P1CP, HYP, HYL, PYD, BSP, OP, and CTSK transom biomarkers.

Adverse events: Three studies of *curcumin* consumption (6, 9, and 10) listed several side effects such as nausea, vomiting, heartburn, rash, itching, diarrhea, and abdominal cramping. Also, two studies (13, 15) raised issues such as gastrointestinal problems and difficulty with treatment intake due to *flaxseed* consumption. Other studies have shown no side effects.

Discussion

To the best of our knowledge, this is the first systematic and meta-analysis review of the five common herbal compounds on BTMs and bone mineral density in different groups. The results of the meta-analysis showed that *curcumin, black seed,* and *flaxseed* individually or in the pooled analysis did not have a significant effect on BSAP, CTx, DPD, OC,

and Lumbar Spine BMD. It was also found that curcumin significantly increased the levels of femoral neck BMD and total hip BMD, but changes in femoral neck BMD due to flaxseed consumption were not statistically significant. QiangGuYin containing cinnamon significantly increased P1NP and BMD at month 12 and decreased β-CTx at month 3, 6, 9, and 12.

Today, bone mineral density measurements and clinical risk factors are used to assess people at risk for osteoporosis. Recently, BTMs have been used as a new approach to detect osteoporosis (22, 23). They are used to provide credible information about the effectiveness of osteoporosis treatment and the state of bone metabolism and its response to treatment. High levels of BTMs may predict the risk of fractures independently of bone mineral density in postmenopausal women (22-24). Bone biomarkers are produced by the bone remodeling process which involves two stages of bone resorption and bone formation (23, 25).

BSAP is known as one of the indicators of osteoblastic activity, so the control of its levels is used to manage osteoporosis in women before and after menopause (23). OC is synthesized by mature osteoblasts, odontoblasts, and hypertrophic chondrocytes, and plays an important role in the process of bone mineralization and homeostasis. OC levels are used as a special biomarker related to osteoblastic function to assess bone formation in osteoporosis (23, 26, and 30). CTX-1 enters the serum as one of the most well-known biomarkers of bone resorption during the collagen degradation process. In fact, CTX-1 is one of the most sensitive bone biomarkers that respond rapidly to treatment with bisphosphonates in postmenopausal osteoporosis (23, 31). DPD, as one of the special biomarkers of bone resorption, is mostly found in bones and teeth. DPD is released into the bloodstream following collagen breakdown (23, 32). ALP is an enzyme that is produced in the liver, bones, intestines, and kidneys and enters the bloodstream. Studies have shown that total serum ALP levels as a bone-forming biomarker can indicate the effectiveness of drug therapy in osteoporosis (23, 33). P1NP is one of two types of type 1 procollagen that is conjugated to the bone matrix. As a bone formation biomarker, P1NP is actually a special indicator for the deposition of type 1 collagen, which enters the intercellular space and eventually the bloodstream during the formation of this type of collagen. Therefore, P1NP biomarkers are more sensitive to measuring bone formation in osteoporosis (23, 34). TRAP 5b is naturally secreted by osteoclasts during the process of bone resorption. Therefore, TRAP 5b is used as a reference for the activity of osteoclasts (23, 35-37). Urinary NTX-1 has been used as an indicator of bone resorption to assess the risk of fractures in postmenopausal women. It should be noted that urinary NTX-1, compared to serum biomarker CTX-1, is preferred for functional use because, unlike CTX-1, it is not affected by food intake and prevents the patient's blood collection (23, 38). Evaluation of bone mineral density indicators in different parts of the body, including total hip BMD, femoral neck BMD and lumbar spine BMD, is used to diagnose osteoporosis and assess the risk of fractures in bone mass (39).

In-vivo and in-vitro studies have shown that curcumin can regulate osteoclastogenesis through two major pathways including 1) Increased apoptosis and inhibition of proliferation of osteoclasts 2 and 2) Inhibition of activation of Receptor Activator of Nuclear Factor Kappa B Ligand (RANKL). *Curcumin* can also increase BMD and bone strength (7, 8). Nigella sativa or Thymoquinone (active *black seed* compound) can also prevent the formation and activation of osteoclasts through two mechanisms: 1) Inhibition of Cyclooxygenase and Lipoxygenase enzymes that make prostaglandins and leukotrienes (the main mediators of inflammation) from arachidonic acid, and 2) Neutralization of free radicals that activate Nuclear factor kappa B (NF-kB) and increase bone-resorbing cytokine levels including Interleukin-1 (IL-1) and Interleukin-6 (IL-6) (40). ALA in *flaxseed*, one of the essential omega-3 fatty acids, reduces the production and concentration of prostaglandin E2 (PGE2) in the bone. PGE2 is an eicosanoid primer that promotes osteoclast genesis (16, 18, 19). ALA also can inhibit the formation and function of osteoclasts by reducing the production of pre-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), Interleukin-1beta (IL-1β), and Interleukin-6 (16, 17, 19). Some in-vitro and animal studies have also shown that omega-3 fatty acids inhibit osteoclastogenesis by reducing RANKL expression or increasing Osteoprotegerin expression (as a decoy receptor for RANKL) (19).

In-vitro and animal studies have shown that *cinnamon* can also affect bone metabolism in two ways: 1) Increased production and activity of osteoblasts, and 2) Inhibit the production and activity of osteoclasts by reducing the expression of the

NFATc1 gene (a transcription factor) in the RANKL signaling pathway (41, 42).

Due to the lack of required data on some BTMs, including P1CP, HYP, HYL, PYD, BSP, OP, and CTSK, it was not possible to incorporate these biomarkers into this meta-analysis and systematic review. Only a small number of articles examined the side effects of supplementation, which need to be considered in future studies. In addition, the heterogeneity between the data in the studies was significant. It should be noted that the course of treatment (from 8 weeks to 12 months) and the underlying disease were not the same in all studies.

Conclusion

This meta-analysis illustrated that *curcumin*, *black seed*, and *flaxseed oil* did not have a significant effect on BSAP, CTx, DPD, OC, and Lumbar Spine BMD. It was also found that *curcumin* significantly increased the femoral neck BMD and total hip BMD. QiangGuYin containing cinnamon indicated significant effect on P1NP, β-CTx, and BMD. In the present study, most of the articles had an unclear risk of bias. Therefore, more high-quality RCTs seem necessary to evaluate the efficacy and safety of these medicinal herbs. Moreover, we find no trials investigating the effect of *cinnamon* alone as well as *ginger* alone or in combination on BMD or bone turnovers. So, further trials are suggested to evaluate the effectiveness of these herbs.

Conflict of Interests

Authors have no conflict of interests.

Acknowledgments

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