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REVIEW

# Effects of Caffeic Acid and Its Derivatives on Bone: A Systematic Review

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**Purpose:** Caffeic acid is a metabolite of hydroxycinnamate and phenylpropanoid, which are commonly synthesized by all plant species. It is present in various food sources that are known for their antioxidant properties. As an antioxidant, caffeic acid ameliorates reactive oxygen species, which have been reported to cause bone loss. Some studies have highlighted the effects of caffeic acid against bone resorption.

**Methods:** A systematic review of the literature was conducted to identify relevant studies on the effects of caffeic acid on bone. A comprehensive search was conducted from July to November 2020 using PubMed, Scopus, Cochrane Library and Web of Science databases. Cellular, animal and human studies reporting the effects of caffeic acid, as a single compound, on bone cells or bone were considered.

**Results:** The literature search found 226 articles on this topic, but only 24 articles met the inclusion criteria and were included in this review. The results showed that caffeic acid supplementation reduced osteoclastogenesis and bone resorption, possibly through its antioxidant potential and increased expression of osteoblast markers. However, some studies showed that caffeic acid did not affect bone resorption in ovariectomized rats and might impair bone mechanical properties in normal rats.

**Conclusion:** Caffeic acid potentially regulates the bone remodelling process by inhibiting osteoclastogenesis and bone resorption, as well as osteoblast apoptosis. Thus, it has medicinal values against bone diseases.

**Keywords:** antioxidant, bone, osteoclast, osteoblast, osteoporosis

## Introduction

Bone remodelling is a tightly coupled lifelong process, whereby old bone is removed by osteoclasts (bone resorption) and new bone is formed by osteoblasts (bone formation).[1,2] Osteocytes, which act as mechanosensors/endocrine cells, and bone lining cells[3] are also involved in bone remodelling.[4] Myriad pathophysiological factors affecting bone remodelling have been observed in skeletal diseases such as osteoporosis, arthritis and periodontal disease.[5] Oxidative stress is one of the pathophysiological factors affecting bone remodelling. Oxidative stress stimulates osteoclast differentiation, thereby enhancing bone resorption.[6,7] Reactive oxygen species (ROS) stimulate the apoptosis of osteoblasts and osteocytes, thus affecting bone formation. ROS also activate mitogen-activated protein kinases (MAPKs), such as extracellular signal-regulated kinases (ERK1/2), c-Jun-N terminal kinase (JNK) and p38, and enhance osteoclastogenesis and bone resorption.[8–11] These phenomena skew the bone remodelling process in favour of bone loss.

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Antioxidants are compounds which reduce free radicals and oxidative stress.[12] Antioxidants have been reported to promote differentiation of osteoblasts, bone formation and survival of osteocytes, as well as suppressing osteoclast differentiation and activity.[8,13–15] Some studies associate the age-related reduction in circulating antioxidants to osteoporosis in rats and women.[16–18] A decline in antioxidant levels has been reported to promote bone loss by triggering the tumour necrosis factor-alpha (TNFα)-dependent signalling pathway,[6] while administration of antioxidants, such as vitamin C, E, N-acetylcysteine and lipoic acid, have been reported to exert favourable effects in animal models of osteoporosis[19–21] and individuals with osteoporosis.[22–25]

Caffeic acid (CA) is a metabolite of hydroxycinnamate and phenylpropanoid commonly synthesized by all plant species. It is a polyphenol present in many food sources like coffee, tea, wine, blueberries, apples, cider, honey and propolis.[26] CA and its major derivatives including caffeic acid phenethyl ester (CAPE) and caffeic acid 3,4-dihydroxy-phenethyl ester (CADPE) are reported to possess potential antibacterial, antidiabetic, antioxidant, anti-inflammatory, antineoplastic and cardioprotective activities (reviewed in[27–29]). As a potent antioxidant, CA has been demonstrated to decrease lipoperoxyl radicals ($ROO^{\bullet}$) by donating a hydrogen atom to its corresponding hydroperoxide, which terminates the lipid peroxidation chain reaction. It also inhibits human low-density lipoprotein (LDL) oxidation induced by cupric ions.[30] Furthermore, it interacts with other compounds, such as α-tocopherol, chlorogenic and caftaric acids, to exert more potent antioxidant activity in a variety of different systems.[31–33] Therefore, the antioxidant activities of CA might protect against the negative effects of oxidative stress on bone cells and the skeletal system. This systematic review aims to summarise the effects of CA and its derivatives on bone cells and bone in literature.

# Materials and Methods

## Literature Review

A systematic literature search was conducted from July until November 2020 using PubMed, Scopus, Cochrane Library and Web of Science databases to identify studies on the effects of caffeic acid on bone and bone cells including osteoblasts, osteoclasts and osteocytes. The search string used was (1) caffeic acid AND (2) (bone OR osteoporosis OR osteoblasts OR osteoclasts OR osteocytes).

## Selection of Articles

Studies with the following characteristics were included: (1) original research article with the primary objective of determining the effects of caffeic acid on bone and bone cells; (2) studies using cellular or animal models, or humans; (3) studies administering caffeic acid as a single compound but not in a mixture or food. Articles were excluded if they (1) do not contain original data; (2) use food rich in caffeic acid or mixtures containing caffeic acid. The bibliography of relevant review articles was traced for potential articles missed during database search. The search results were organised using EndNote™ software (Clarivate Analytics, Philadelphia, USA). Duplicates were identified using EndNote™ and confirmed by manual checking.

## Data Extraction

Two authors (S.O.E. and K.L.P.) searched the same databases using the search string mentioned and screened the search results. All the articles that did not match the selection criteria were excluded. Next, the articles which used caffeic acid in treating models other than bone-related diseases were removed. Finally, articles which used caffeic acid in combination with other compounds were also excluded. Any disagreement on the inclusion or exclusion of articles was resolved through discussion among the two authors. The corresponding author (K.Y. C.) had the final decision on articles included if a consensus could not be reached between authors responsible for screening. This systematic review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and checklist.[34] Steps in the selection process, from identification, screening, eligibility to the inclusion of articles, are shown in Figure 1.

# Results

## Selection of Articles

From the literature search, 381 articles were identified, of which 87 were obtained from PubMed, 182 were from Scopus, 3 from Cochrane Library and 109 from Web of Science. A total of 155 duplicate articles were identified and removed. Of the 226 articles screened, 202 articles were excluded based on the selection criteria, whereby 51

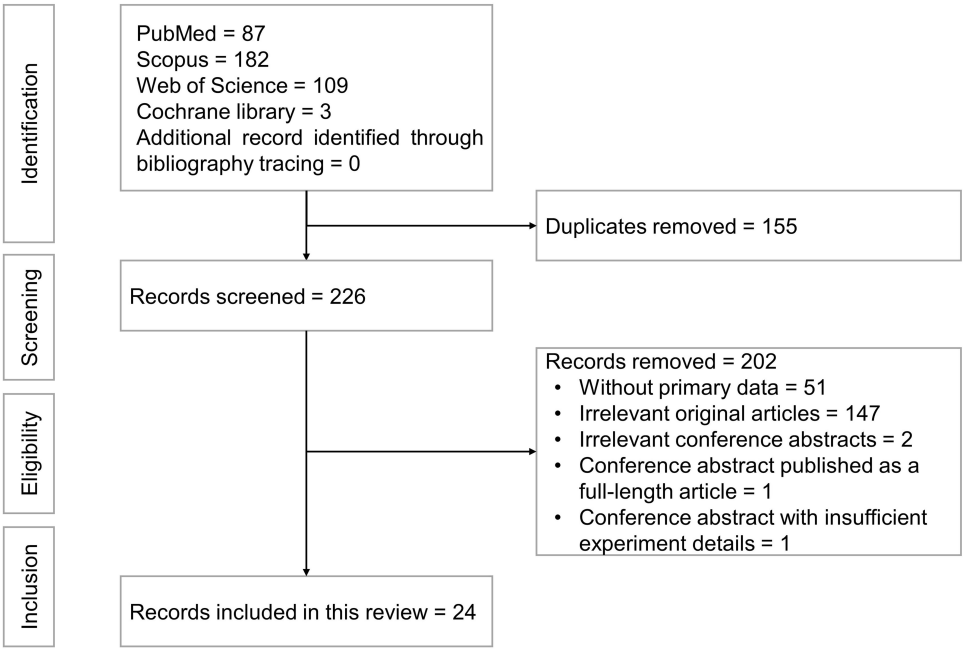


**Figure 1** Flowchart of the article selection process.

articles did not contain primary data (3 book chapters, 2 commentary and 46 review articles), 147 articles and 2 conference abstracts presented topics irrelevant to the current review, a conference abstract had been published as a full-length research article and another conference abstract did not contain sufficient experiment details (Supplementary Material). Finally, 24 articles fulfilling all criteria mentioned were included in the review.

## Study Characteristics

The included studies were published between 2006 and 2020. Seven studies were in vitro experiments using mouse bone marrow macrophages (BMMs), RAW264.7, RAW D and MG63 osteoblast cell lines[35–41] while 19 studies were in vivo studies using Sprague Dawley/Sprague Dawley albino rats, Wistar/Wistar albino rats, Balb/c mice, lipopolysaccharide (LPS)-resistant C3H/HEJ mice, C57BL/6J mice and ICR mice.[35,38,42–58] No human studies on this topic were reported.

Six in vitro studies focused on the effects of CA on osteoclast differentiation from haematopoietic cells using macrophage colony-stimulating factor (M-CSF), receptor activator of NF-κB (RANK) ligand (RANKL) or TNF-α,[35–39,41] while one in vitro study focused on the effect of CA on osteoblasts using MG63 osteoblast cell line.[40] Four in vitro studies used CA doses between 0.1–5 μM.[35,37,38,40] Ang et al.[36] used doses between 0–0.3 μM and Sandra et al.[41] and Sandra and Ketherin[39] used a dose of 10 μg/mL (55.5 μM). The treatment period was 5–7 days for the differentiation of osteoclasts.

For animal studies, Duan et al.,[55] Zawawi et al.,[58] William et al.,[51] Wu et al.,[38] Zych et al.[49] and Folwarczna et al.[48,52] used CA or its derivatives at doses between 0.5–50 mg/kg via oral or intraperitoneal (i.p.) administration. Ucan et al.,[57] Erdem et al.,[53] Cicek et al.,[54] Yigit et al.,[45] Yildiz et al.[50] and Tolba et al.[56] used doses between 10–20 μmol/kg/day (2.84–5.69 mg/kg/day) via i.p. administration. Kizildağ et al.[42–44] and Kazancioğlu et al.[46,47] used the dose of 10 mmol/kg/day (2.843 g/kg/day) for an i.p. administration, Kazancioğlu et al.[47] employed 50–100 mmol/kg/day (14.22–28.43 g/kg/day) for a localised administration, while Ha et al.[35] used a collagen sponge soaked with CAPE with the final dose of 250 μg/mouse. For oral administration, first-pass effect might affect the enteric absorption of CA or its derivatives.[59] For i.p. administration, the injection is commonly performed at the lower left or right quadrant of the abdomen. The peritoneum can absorb the compounds fast and reach systemic circulation with greater bioavailability with fewer handling errors.[60]

The bone-related disease models used included ovariectomy (OVX)- or glucocorticoids (dexamethasone)-induced osteoporosis, polyethylene particle-induced bone defect and osteolysis, electromagnetic force (EMF)-stimulated bone

loss, osteotomy- or anti-collagen antibody-induced arthritis (CAIA) and rapid maxillary expansion (RME) and LPS-induced periodontitis. The endpoints studied included bone microstructure, histomorphometry, bone remodelling and oxidative status. The effects of CA and its derivatives on bone remodelling have been summarized in Table 1.

## Evidence from Cell Culture Studies

Melguizo-Rodríguez et al. reported that 24-hour CA (1 μM) incubation increased the number of MG63 osteoblast cells compared with control.[40] Gene expression studies revealed that CA increased the expression of osteoblast-related genes such as bone morphogenetic protein-2 and -7 (*BMP-2* and *BMP-7*), transforming growth factor-beta 1 (*TGF-β1*), transforming growth factor-beta receptor 1, 2 and 3 (*TGF-βR1, TGF-βR2* and *TGF-βR3*) and osteoblastogenesis genes including Runt-related transcription (*RUNX-2*), alkaline phosphatase (*ALP*), collagen type 1 (*COL-I*), osterix (*OSX*) and osteocalcin (*OSC*).[40] Additionally, pretreatment of CA (10 μg/mL or 55.5 μM) on RAW D cells for 2 h also significantly inhibited the RANKL and TNFα-induced osteoclastogenesis with the suppression of p38 MAPK phosphorylation and tartrate-resistant acid phosphatase (TRAP)-positive osteoclast-like cells (OCLs) formation.[39] Similarly, pretreatment of CA (0.1, 1 and 10 μg/mL or 0.555, 5.55 and 55.5 μM) on RAW D cells and BMMs for 3 days significantly inhibited the RANKL and TNFα-induced osteoclastogenesis and NF-κB activity in RAW-D cells and RANKL, TNFα and M-CSF-induced osteoclastogenesis in BMMs.[41]

On the other hand, CAPE treatment (0–0.3 μM; 5–7 days) suppressed the formation of TRAP-positive OCLs on RANKL-treated RAW264.7 cells and BMMs.[36] Apoptosis occurred in CAPE-treated RAW264.7 cells with the disruption of the microtubule network in OCLs.[36] Similarly, Kwon et al. reported that CAPE treatment (0.1–5 μM) for 5 days suppressed OCLs formation from RANKL-stimulated RAW264.7 cells.[37] Another study by Ha et al. treating M-CSF and RANKL-stimulated BMMs with CAPE (0–5 μM for 5–7 days) also showed decreased OCLs formation in a concentration-dependent manner.[35] The amount of TRAP-positive OCLs was decreased upon 0.1 and 0.5 μM CAPE treatment by 30% and 95% respectively.[35] No OCL formation was observed upon 1 μM CAPE treatment.[35] The anti-osteoclastogenic activities of CAPE are mainly contributed by its anti-inflammatory and antioxidant properties. Mechanistically, CAPE reduces superoxide anion generation by downregulating the nicotinamide adenine dinucleotide phosphate oxidase 1 (Nox1) expression through the interruption of nuclear factor-kappa B (NF-κB) and c-Jun N-terminal kinase (JNK) signalling pathways.[37] CAPE suppresses RANKL-mediated activation of the NF-κB pathway by downregulating NF-κB p65 subunit expression and its nuclear translocation,[37] suppressing nuclear factor of activated T cells (NFAT) activities[36] and degradation of NF-κB inhibitor (IκBα),[36,37] as well as inducing the degradation of IκB kinase (IKK).[37] CAPE also suppresses the expression and activation of JNK and its downstream transcription factors, such as c-Fos and c-Jun, which subsequently interrupt the protein activator-1 (AP-1) complex formation.[37] Additionally, CAPE suppressed RANKL-induced activation of the Nox1 by inhibiting the Nox $p47^{PHOX}$ subunit translocation to the cell membrane and downregulation of Ras-related C3 botulinum toxin substrate 1 (Rac1) expression.[37]

On the other hand, Wu et al. reported that CADPE (0.1–5 μM for 7 days) also concentration-dependently reduced OCL formation in the M-CSF and RANKL-stimulated BMMs and RAW264.7 cells.[38] Mechanistic and characterisation examination revealed that CADPE suppressed RANKL-induced tumour necrosis factor receptor-associated factor 6 (TRAF6) activation and protein kinase B (PKB or also known as Akt) and activation of major MAPKs including ERK, JNK and p38.[38] Subsequently, CADPE suppressed downstream expression of nuclear factor of activated T-cells cytoplasmic 1 (NFATc1), nuclear translocation of c-Fos protein and expression of osteoclastic markers, such as TRAP and cathepsin K, possibly through the non-receptor tyrosine kinase c-Src signalling.[38] Interestingly, CADPE did not significantly affect the NF-kB signalling pathway and M-CSF-induced proliferation and differentiation of BMMs.

## Evidence from Animal Studies

Supplementation of CA in animal models of bone loss yielded heterogeneous findings.[48,49,52] This observation might be attributable to oral administration. Folwarczna et al. reported that CA (5 and 50 mg/kg, by stomach tube for 4 weeks) improved the bone mechanical properties by increasing the width of the trabecular metaphysis of the femur and decreasing the transverse growth in endosteal of the femur in OVX rats.[48] Folwarczna et al. then demonstrated that CA (10 mg/kg/day; oral administration for 4 weeks) could reduce the width of tibial periosteal and endosteal osteoid compared with untreated OVX rats.[52] However, CA did not promote or reduce the resorption

**Table 1** Effects of CA and Its Derivatives in Bone Remodelling

| Researcher | Study Design | Findings |
|---|---|---|
| **Cell culture studies** | | |
| Ang et al.[36] | Cell line: RAW264.7 cells, murine BMMs and human OCL cells<br>Mode of disease induction: RANKL-induced osteoclastogenesis<br>Treatment: 0–300 nM of CAPE for 5–7 days<br>Negative control: untreated cells<br>Positive control: n.a. | ↓ RANKL-induced osteoclastogenesis and TRAP-positive cells compared with negative control (RAW264.7 and BMMs)<br>↓ RANKL-mediated NF-κB activation, NF-κB p65 nuclear translocation, IκBα degradation and NFAT activation compared with negative control (RAW264.7 and BMMs)<br>↓ bone resorption and ↑ apoptosis of CAPE-treated OCL cells |
| Ha et al.[35] | Cell line: BMMs<br>Mode of disease induction: M-CSF (20 ng/mL) and RANKL (150 ng/mL)-induced osteoclastogenesis<br>Treatment: 0–5 μM of CAPE for 5–7 days<br>Negative control: untreated cells<br>Positive control: n.a. | ↓ TRAP-positive multinucleated cells compared with negative control<br>↓ RANKL-stimulated NF-κB DNA binding and transcription activity and completely abrogate NFATc1 and c-FOS induction compared with negative control<br>NS for IκB, p65, p38, JNK and ERK MAPKs phosphorylation compared with negative control |
| Wu et al.[38] | Cell line: Mouse BMMs and RAW264.7 cells<br>Mode of disease induction: M-CSF (20 ng/mL)-induced proliferation and differentiation/RANKL (30 ng/mL)-induced osteoclast differentiation<br>Treatment: 0.1, 0.5, 1, 2.5, 5 μM of CADPE for 7 days (for BMMs)/3–5 days (for RAW264.7 cells)<br>Negative control: untreated cells<br>Positive control: n.a. | Non-cytotoxic at 5μM in both BMMs and RAW264.7<br>↓ number of actin ring structure (2.5–5μM), osteoclast formation and osteoclastogenesis related markers (NFATc1, TRAP, cathepsin K, and c-Src) compared with negative control<br>↓ RANKL-induced $Ca^{2+}$ oscillation and TRAF6/c-Src interaction<br>↓ RANKL-induced phosphorylation of ERK1/2, p38, JNK and Akt<br>↓ RANKL-induced c-Fos protein nuclear translocation and AP-1 activity |
| Sandra et al.[41] | Cell line: BMMs and RAW-D cells<br>Mode of disease induction: M-CSF, RANKL and TNFα-induced osteoclastogenesis<br>Treatment: 0.1, 1 and 10 μg/mL of caffeic acid for 3 days<br>Negative control: untreated cells<br>Positive control: n.a. | ↓ RANKL, TNFα and M-CSF-induced osteoclastogenesis in BMMs.<br>↓ RANKL and TNFα-induced osteoclastogenesis in RAW-D cells.<br>↓ RANKL and TNFα-induced NF-κB activity in RAW-D cells |
| Sandra and Ketherin[39] | Cell line: RAW-D cells<br>Mode of disease induction: RANKL and TNFα-induced osteoclastogenesis<br>Treatment: 10 μg/mL of caffeic acid for 2 hours<br>Negative control: untreated cells<br>Positive control: n.a. | ↓ RANKL and TNFα-induced osteoclastogenesis and phosphorylation of p38 MAPK compared with negative control |
| Kwon et al.[37] | Cell line: RAW264.7 cells<br>Mode of disease induction: RANKL- induced osteoclastogenesis<br>Treatment: 0.1, 1 and 5 μM of CAPE for 5 days<br>Negative control: untreated cells<br>Positive control: n.a. | ↓ RANKL-induced osteoclast differentiation and bone resorption compared with negative control<br>↓ generation of superoxide anions with ↓ expression of Nox 1 and Rac1 compared with negative control<br>↓ expression and activation of the JNK and the expression of AP-1 activators such as c-Fos and c- Jun compared with negative control<br>↓ expression and nuclear translocation of NF-κB p65 and the activation of IκBα and IKKβ |

(*Continued*)

**Table 1** (Continued).

| Researcher | Study Design | Findings |
|---|---|---|
| Melguizo-Rodríguez. et al.[40] | Cell line: MG63 osteoblast cell line Mode of disease induction: No disease<br>Treatment: 1 μM of CA for 24 hours<br>Negative control: untreated cells<br>Positive control: n.a. | ↑ gene expression of osteoblast-related markers (*BMP-2*/*BMP-7*, *TGF-β1*, *TGF-βR1*, *TGF-βR2* and *TGF-βR3*) and osteoblastogenesis genes (*RUNX-2*, *ALP*, *COL-I*, *OSX* and *OSC*)<br>↑ *RANKL* expression and ↓ *OPG* expression |
| **Animal studies** | | |
| Yildiz et al.[50] | Animals: 48 Sprague Dawley rats (7 weeks old)<br>Mode of disease induction: 900-MHz and 1800-MHz EMF for 30 mins/day for 5 days/week for 28 days.<br>Treatment: 10 μmL/kg/day CAPE for 28 days (i.p administration)<br>Normal control: no treatment<br>Positive control: n.a. | ↑ spine and femur BMD compared with negative control |
| Cicek et al.[54] | Animals: 48 Sprague Dawley rats (7 weeks old)<br>Mode of disease induction: GSM 900-MHz, 2 W EMF and GSM 1800-MHz, 1.5 W EMF for 30 min/day for 5 days/week for 28 days.<br>Treatment: 0.569 mg CAPE (10 μmol/kg/day) for 28 days (i.p. administration)<br>Normal control: no treatment<br>Positive control: n.a. | ↑ breaking force, bending strength and total fracture energy compared with negative control |
| Zych et al.[49] | Animals: 40 female Wistar Cmd:(WI) WU rats (190–220g; 3 months old)<br>Mode of disease induction: Normal rats (no disease)<br>Treatment: 10 mg/kg/day of CAPE for 4 weeks (oral administration)<br>Normal control: no treatment<br>Positive control: n.a. | ↓ load of fracture at femoral neck compared with control group<br>↓ width of periosteal osteoid in tibia compared with control group<br>↓ width of epiphysis and metaphysis trabecular in femur compared with control group |
| Folwarczna et al.[52] | Animals: 48 female Wistar Cmd:(WI) WU rats (12 weeks old)<br>Mode of disease induction: OVX-induced osteoporosis<br>Treatment: 10 mg/kg/day of CAPE for 4 weeks (oral administration) after 7 days of OVX operation<br>Normal control: Sham-operated rats with no treatment<br>Positive control: 0.2 mg/kg of estradiol | ↓ bone mass and bone mineral mass compared with negative control<br>↓ bone mass/body mass ratio and bone mineral mass/body mass ratio<br>↓ width of tibial periosteal and endosteal osteoid |
| Ha et al.[35] | Animals: 15 Male ICR mice (5 weeks old)<br>Mode of disease induction: RANKL-induced osteoclastogenesis in mouse calvariae (Collagen sponge implant)<br>Treatment: 250 μg CAPE for 5 days (along with the sponge implant)<br>Normal control: Collagen sponge soaked with vehicle<br>Positive control: n.a. | ↓ TRAP-stained area under gross examination and image analysis |
| Wu et al.[38] | Animals: 18 mice (unknown strain)<br>Mode of disease induction: OVX-induced bone loss<br>Treatment: 10 mg/kg of CADPE every 2 days for 3 months (i.p. administration), starting from 7 days after OVX<br>Normal control: Sham-operated mice<br>Positive control: n.a. | ↑ BV/TV and Tb.N compared with negative control<br>↓Tb.Sp. Oc.S/BS, ES/BS, and N.Oc/B.Pm compared with negative control<br>↓ TRAP5b and CTX-1 compared with negative control |

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**Table 1** (Continued).

| Researcher | Study Design | Findings |
|---|---|---|
| Uçan et al.[57] | Animals: 63 male Sprague Dawley rats (7weeks old; 280–490g)<br>Mode of disease induction: cranial critical size bone defect<br>Treatment: 10 μmol/kg/day of CAPE for 30 days (i.p administration)<br>Normal control: critical size cranial model with no treatment<br>Positive control: n.a. | ↑ cranial bone healing level compared with negative control |
| Duan et al.[55] | Animals: 18 C57BL/6J female mice (12 weeks old)<br>Mode of disease induction: OVX-induced osteoporosis<br>Treatment: 0.5 mg/kg CAPE (in DMSO) twice a week for 4 weeks (i.p. administration) right after OVX.<br>Normal control: Sham-operated mice with PBS and DMSO (i.p. administration)<br>Positive control: n.a. | ↑ BV/TV and Tb.N compared with negative control<br>↓ N.Oc/B.Pm compared with negative control |
| Erdem et al.[53] | Animals: 39 adult male Wistar albino rats (350–400 g)<br>Mode of disease induction: Unilateral femoral lengthening (extension) osteotomy<br>Treatment: 10 μmol/kg CAPE for 22 days (i.p administration)<br>Negative control: Rats with lengthening osteotomy with 1% alcohol in physiological saline (5 mL/kg/day via i.p injection)<br>Positive control: n.a. | ↑ maximum torsional fracture momentum and degree of rigidity compared with negative control |
| Kazancioglu et al.[46] | Animals: 32 male Wistar rats (3-months; 300–330 g)<br>Mode of disease induction: Surgical-induced calvarial defects (5 mm diameter) which grafted with gelatin sponge and closed with titanium barriers<br>Treatment: Pre-mixes of 50 and 100 mmol/kg CAPE solutions with the gelatin sponges (localised administration) for 28 days or CAPE (10 mmol/kg/day) injected for 28 days (systemic i.p. administration).<br>Negative control: Equal volume of isotonic saline solution (i.p. injection) with the use of saline solution-mixed gelatin sponge<br>Positive control: n.a. | ↑ total new bone areas for systemic CAPE<br>NS between localised CAPE groups and control |
| Zawawi et al.[58] | Animals: 22 LPS-resistant female C3H/HEJ mice (6–8 weeks old)<br>Mode of disease induction: murine polyethylene (PE) particle-induced osteolysis model (2.82 x $10^9$ particles/mL)<br>Treatment: 1 mg/kg/day of CAPE at days 0, 4, 7 and 10 (subcutaneous administration)<br>Negative control: PBS with 0.04% DMSO<br>Positive control: n.a. | ↓ calvarial osteolysis, surface bone resorption and TRAP-positive cells compared with negative control<br>↑ BV compared with negative control<br>↔ CTX-1 and OSCAR levels compared with negative control |
| Folwarczna et al.[48] | Animals: 81–90 female Wistar rats (15–17 weeks old)<br>Mode of disease induction: OVX-induced osteoporosis<br>Treatment: 5 and 50 mg/kg/day of CA for 4 weeks (oral administration), started 7–8 days after OVX<br>Negative control: no treatment<br>Positive control: 0.2 mg/kg of oestradiol | ↓ transverse bone growth of endosteal in femur compared with negative control<br>↑ width of trabeculae metaphysis in femur compared with negative control |

(*Continued*)

**Table 1** (Continued).

| Researcher | Study Design | Findings |
|---|---|---|
| Kazancioglu et al.[47] | Animals: 20 3-month-old male Sprague Dawley rats (222.76 ±18.44 g)<br>Mode of disease induction: RME-induced new bone formation in rat midpalatal suture<br>Treatment: 10 mmol/kg/day of CAPE for 20 days (i.p. administration)<br>Negative control: rats with RME but no treatment<br>Positive control: n.a. | ↑ new bone formation in rat midpalatal suture after RME upon histomorphometric analysis |
| Tolba et al.[56] | Animals: 30 male Sprague Dawley rats (200–250 g)<br>Mode of disease induction: glucocorticoid (dexamethasone)-induced osteoporosis by intramuscular injection of dexamethasone disodium phosphate (7 mg/kg) once per week for 5 weeks<br>Treatment: 10 and 20 μmol/kg of CAPE for 4 weeks (i.p. administration), started a week after the dexamethasone injection<br>Negative control: no treatment<br>Positive control: n.a. | ↑ femur weight and length compared with negative control<br>↑ alkaline phosphatase and ↓ acid phosphatase and TRAP activity compared with negative control<br>↓ MDA and ↑ GSH and SOD in bone tissue compared with negative control<br>↓ caspase-3 activity compared with negative control<br>↑ Runx2 and ↓ RANKL/OPG ratio compared with negative control<br>↓ Akt activation compared with negative control |
| Williams et al.[51] | Animals: 32 Balb/c mice (6–8 weeks old)<br>Mode of disease induction: CAIA induction by intravenous injection of 150 μL of a cocktail of anti-type II collagen monoclonal antibodies on day 1, followed by 20 μL LPS on day 3)<br>Treatment: 200 μL of CAPE (1 mg/kg) in 0.4% DMSO on days 3, 7 and 10 (subcutaneous administration)<br>Normal control: PBS and PBS with 0.4% DMSO<br>Negative control: PBS and PBS in 0.4% DMSO<br>Positive control: n.a. | ↑ paw inflammation compared with normal control, marked by ↑ paw score, paw volume, tissue swelling, cartilage and bone degradation and $TRAP^+$ cells on bone surface and soft tissues compared with normal control (NS compared with negative control)<br>↓ colon toxicity score and percentage of cavitated colon goblet cells per cyst compared with negative control<br>NS for BV, and BV change to baseline, CTX-1 and C-reactive protein levels compared with negative control |
| Yiğit et al.[45] | Animals: 48 Wistar albino rats (200 ± 20 g)<br>Mode of disease induction: Ligature-induced periodontitis by placing a sterile 3–0 silk ligatures in the subgingival position around the maxillary 2nd molars for 14 days<br>Treatment: i.p. administration of 10 μmol/kg/day CAPE during the 14-day induction<br>Normal control: Rats without the subgingival ligature placement<br>Positive control: Periodontitis rats with low-dose doxycycline (10 mg/kg/day) via oral gavage | ↓ articular bone loss, histopathological scores and severity of periodontal inflammation with significantly lesser PMNLs infiltration in junctional epithelium and connective tissues compared with positive control<br>↓ periodontitis-upregulated IL-1β, IL-6, IL-10, TNFα, MDA levels and percentage of gingival apoptosis (near or lower than normal control)<br>↑ periodontitis-downregulated GSH and GPx (higher than normal control) |
| Kizildag et al.[42] | Animals: 40 male Sprague Dawley albino rats (12-week old and weight 220 −250 g)<br>Mode of disease induction: A single dose of STZ (60 mg/kg) was injected via i.p. to induce diabetes. A 10 μL of LPS (1 mg/mL) was injected into the vestibular gingival sites between the right first and second maxillary molars to induce periodontitis.<br>Treatment: Administration of 10 mM/kg/day CAPE for 15 days after the periodontitis induction (i.p. administration)<br>Negative control: Saline injection into the vestibular gingival site and daily saline i.p. injection<br>Positive control: n.a. | ↓ RANKL-positive osteoclasts, IL-1β, OSI, alveolar bone loss and histological analysis score with lesser inflammatory reactions, ulcer and hyperemia compared with negative control with diabetes and periodontitis |

(*Continued*)

**Table 1** (Continued).

| Researcher | Study Design | Findings |
|---|---|---|
| Kizildag et al.[43] | Animals: 30 male Sprague Dawley rats (3-month old and weight 220–250 g)<br>Mode of disease induction: Endotoxin-induced periodontitis by injecting 10 μL of LPS (1 mg/mL) into the vestibular gingival sites between the right first and second maxillary molars<br>Treatment: i.p. administration of 10 mmol/kg/day CAPE for 28 days after the periodontitis induction<br>Negative control: Saline injection into the vestibular gingival site and daily saline i.p. injection<br>Positive control: n.a. | ↓ LPS-upregulated RANKL-positive osteoclasts, IL-1β and oxidative stress index levels (but not CTX-1)<br>↓ periodontitis-mediated bone resorption, attachment loss and damage to the periodontal ligament<br>↑ LPS-downregulated periodontal bone support (MPBS & DPBS) |
| Kizildag et al.[44] | Animals: 40 male Sprague Dawley rats (12-week old and weight ~250 g)<br>Mode of disease induction: A 12 h-restraint procedure was applied daily from 15 days before LPS injection until 14 days after LPS injection to induce chronic stress.<br>A 10 μL of LPS (1 mg/mL) was injected into the vestibular gingival sites between the right first and second maxillary molars to induce periodontitis.<br>Treatment: Administration of 10 mmol/kg/day CAPE for 14 days after the periodontitis induction (i.p. administration)<br>Negative control: Saline injection into the vestibular gingival site and daily saline i.p. injection<br>Positive control: n.a. | ↑ periodontal bone support (MPBS and DPBS) and ↓ RANKL-positive osteoclasts, IL-1β and OSI levels, compared with negative control with chronic stress and periodontitis |

**Abbreviations**: ↑, increase or upregulate; ↓, decrease or downregulate; Akt, protein kinase B; AP-1, activator protein 1; ALP, alkaline phosphatase; BMD, bone mineral density; BMMs, bone marrow macrophages; BMP, bone morphogenetic proteins; BV, bone volume; BV/TV, bone volume fraction; CA, caffeic acid; CADPE, caffeic acid 3,4-dihydroxy-phenethyl ester; CAIA, collagen antibody-induced arthritis; CAPE, caffeic acid phenethyl ester; COL-I, collagen type 1; c-Src, cellular sarcoma tyrosine kinase; CTX-1, carboxyterminal collagen crosslinks Type-1; DMSO, dimethyl sulphoxide; DPBS, distal periodontal bone support; EMF, electromagnetic fields; ERK1/2, extracellular signal-regulated kinases 1/2; ES/BS, eroded surface/bone surface; GSH, glutathione; GSM, global system for mobile communications; GPx, glutathione peroxidase; IL, interleukin; IκBα, NF-κB inhibitor protein alpha; IKK, IκB kinase; i.p, intraperitoneal; JNK, c-Jun N-terminal kinase; LPS, lipopolysaccharide; M-CSF, macrophage colony-stimulating factor; MAPK, mitogen-activated protein kinase; MDA, malondialdehyde; MHz, megahertz; MPBS, mesial periodontal bone support; n.a., not available; NFAT, nuclear factor of activated T cells; NFATc1, nuclear factor of activated T-cells cytoplasmic 1; NF-κB, nuclear factor kappa B; N.Oc/B.Pm, osteoclast number/bone perimeter; Nox1, nicotinamide adenine dinucleotide phosphate oxidase 1; NS, not significant; OCL, osteoclast-like cells; Oc.S/BS, osteoclast surface/bone surface; OPG, osteoprotegerin; OSC, osteocalcin; OSCAR, osteoclast-associated receptor; OSI, oxidative stress index; OSX, osterix; OVX, ovariectomy; PMNLs, polymorphonuclear cells; Rac1, Ras-related C3 botulinum toxin substrate 1; RANKL, receptor activator of NF-κB ligand; RUNX2, Runt-related transcription; SOD, superoxide dismutase; STZ, streptozotocin; Tb.N, trabecular number; Tb.Sp, trabecular spacing; TGFβ1, transforming growth factor-beta 1; TGFβ-R, transforming growth factor-beta receptor; TNFα, tumour necrosis factor-alpha; TRAF6, tumour necrosis factor receptor-associated factor 6; TRAP5b, tartrate-resistant acid phosphatase 5b; W, watts.

of compact bone in the tibia of OVX-induced osteoporotic rats as evidenced by negligible changes of bone mass, bone mineral mass, bone mass/body mass ratio and bone mineral mass/body mass ratio.[52] On the other hand, Zych et al. reported that CA at a similar dose (10 mg/kg/day; by stomach tube for 4 weeks) worsened the bone mechanical properties of healthy female Wistar Cmd:(WI)WU rats by decreasing the load of fracture at the femoral neck, decreasing the width of periosteal osteoid in the tibia and decreasing the width of the epiphysis and metaphysis trabecular in the femur compared with the negative control group.[49]

CAPE is the most extensively studied caffeic acid derivative in animal studies. The beneficial effects on new bone formation and healing upon systemic administration of CAPE had been reported.[46,47,53,57] Erdem et al. reported that a low dose of CAPE (10 μmol/kg; i.p. injection for 22 days) increased new bone formation and bone strength by increasing maximum torsional fracture momentum and degree of rigidity compared with negative control in rats that underwent unilateral femoral lengthening (osteotomy).[53] Similarly, a 30-day i.p. injection of CAPE (10 μmol/kg/day) also increased bone healing level in Sprague Dawley rats with cranial critical size bone defect.[57] A higher dose of CAPE (10 mmol/kg/day, i.p. for 20 days) also further promoted the RME procedure-induced new bone formation in midpalatal suture of male Sprague Dawley rats.[47] Similarly, a longer treatment

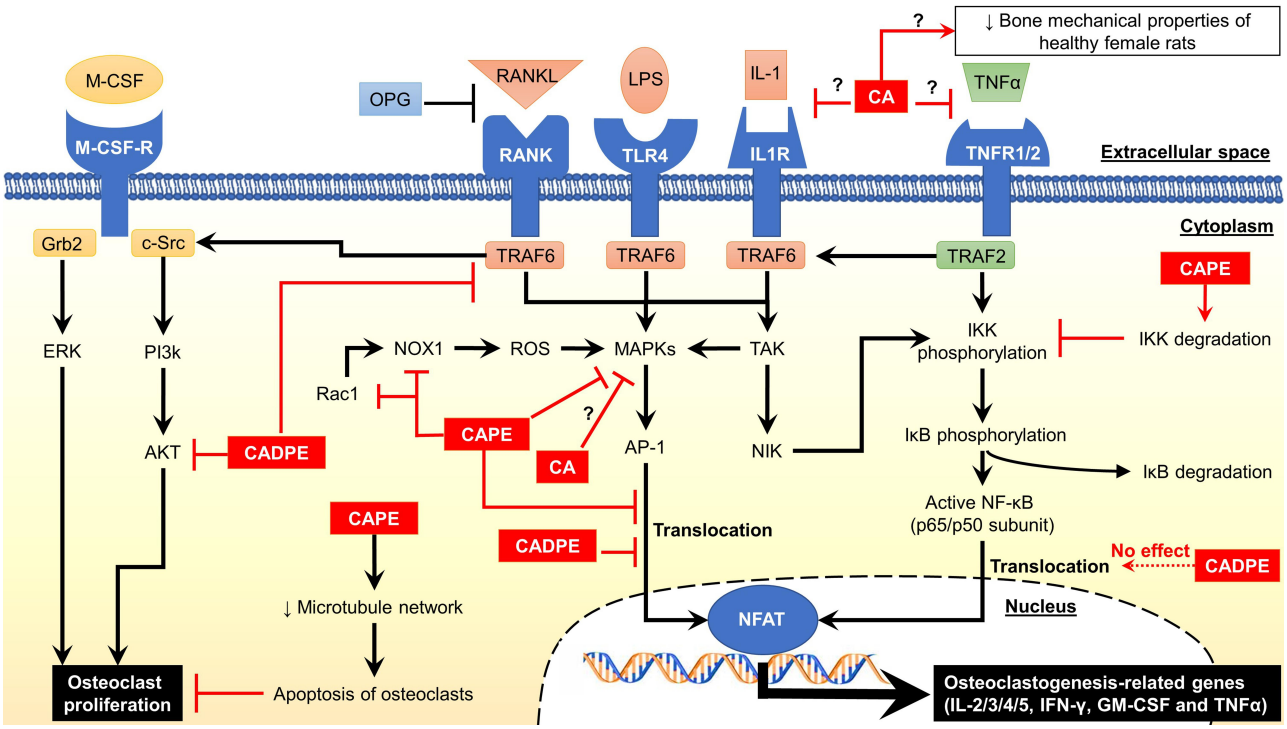


**Figure 2** Mechanism of action of caffeic acid and its derivatives.

**Abbreviations**: ↓, decrease or downregulate; ?, unknown mechanism; Akt, protein kinase B; AP-1, activator protein 1; CA, caffeic acid; CADPE; caffeic acid 3,4-dihydroxy-phenethyl ester; CAPE, caffeic acid phenethyl ester; c-Src, cellular sarcoma tyrosine kinase; ERK1/2, extracellular signal-regulated kinases 1/2; GM-CSF, granulocyte-macrophage colony-stimulating factor; Grb2, growth factor receptor-bound protein 2; IFN-γ, interferon-gamma; IL, interleukin; IL1R, interleukin-1 receptor; IκB, NF-κB inhibitor protein; IKK, IκB kinase; LPS, lipopolysaccharide; M-CSF, macrophage colony-stimulating factor; M-CSF-R, M-CSF receptor; MAPKs, mitogen-activated protein kinases; NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor kappa B; NIK, MAPK kinase kinase 14; Nox1, nicotinamide adenine dinucleotide phosphate oxidase 1; OPG, osteoprotegerin; PI3k, phosphoinositide 3-kinase; Rac1, Ras-related C3 botulinum toxin substrate 1; RANK, receptor activator of NF-κB; RANKL, receptor activator of NF-κB ligand; ROS, reactive oxygen species; TAK, MAPK kinase kinase 7; TLR4, Toll-like receptor 4; TNFα, tumour necrosis factor-alpha; TNFR1/2, TNF receptor 1/2; TRAF2, tumour necrosis factor receptor-associated factor 2; TRAF6; tumour necrosis factor receptor-associated factor 6.

period of CAPE (10 mmol/kg/day; i.p. injection for 28 days) also significantly promoted bone healing by increasing the total new bone areas in surgical-induced calvarial defects of male Wistar rats compared with the negative control.[46] However, localised administration of CAPE (28 days) on surgical-induced calvarial defects by pre-mixing 50 and 100 mmol/kg CAPE solutions with gelatin sponges did not significantly improve the new bone formation.[46]

Localised and systemic administration of CAPE was reported to be beneficial in reducing osteolysis and bone loss.[35,42–45,50,54–56,58] Ha et al. reported that collagen sponge implant impregnated with 250 µg CAPE and RANKL could reduce osteoclastogenesis with significantly lesser TRAP-stained area in mouse calvariae compared with implants with RANKL only.[35] Subcutaneous injection of CAPE (1 mg/kg/day for 10 days) reduced the polyethylene particle-induced calvarial osteolysis, surface bone resorption and TRAP-positive cells formation with an increase of bone volume (BV) on LPS-resistant C3H/HEJ female mice.[58] However, no significant changes were observed in carboxy-terminal cross-linked type 1 collagen (CTX-1) and osteoclast-associated receptor levels among untreated and CAPE-treated rats with calvarial osteolysis.[58]

Similarly, Duan et al. reported that lower dose and frequency of CAPE injection (0.5 mg/kg twice a week; i.p. injection for 4 weeks) also increased the BV and trabecular number (Tb.N) due to the decrease of bone osteoclast formation (evidenced by decreased osteoclast number/bone perimeter) in OVX mice.[55] Tolba et al. also reported that i.p. injection of CAPE (10 and 20 µmol/kg) for 3 weeks increased femur weight and length in rats with dexamethasone-induced bone loss.[56] The preservation of skeletal health in their study was associated with an improved antioxidant defence, such as higher levels of glutathione (GSH) and superoxide dismutase (SOD), and the reduction of malondialdehyde (MDA, lipid peroxidation product).[56] This event led to an increase of

osteoblastogenesis indicated by upregulation of RUNX-2 and ALP (osteoblast marker) levels[56] On the other hand, decreased RANKL/osteoprotegerin (OPG) ratio was observed with CAPE treatment, indicating the suppression of osteoclastogenesis, which was further confirmed by lower acid phosphatase level and TRAP activity.[56] In another study by Yildiz et al., CAPE (10 μmol/kg/day; i.p. injection for 22 days) also increased the spine and femur BMD in rats with EMF-induced bone loss.[50] Similarly, Cicek et al. reported a longer treatment of CAPE (10 μmol/kg/day; i.p. injection for 28 days) also significantly improved the mechanical strength of cortical bone by increasing the breaking force, bending strength and total fracture energy in rats with EMF-induced bone loss compared with negative control.[54]

Additionally, a study by Wu et al. treated mice with an OVX-induced bone loss with a moderately high dose of CADPE (10 mg/kg; i.p. injection) every 2 days for 3 months.[38] Results showed that CADPE could increase the BV fraction (BV/TV) and Tb.N, as well as decreased trabecular spacing (Tb.Sp) compared with the negative control.[38] The improvement in the bone structure was contributed by reduced osteoclast number and eroded surface on the bone.[38] Assessment of bone remodelling markers also revealed that serum TRAP5b and CTX-1 levels were reduced in CADPE-treated group compared with the negative control.[38]

On the other hand, CAPE was effective in reducing periodontitis-related bone loss and osteolysis.[42–45] CAPE (10 μmol/kg/day, i.p. for 14 days) significantly reduced the subgingival ligature placement-induced periodontitis-mediated articular bone loss, histopathological features and severity of periodontal inflammation with lesser polymorphonuclear cells (PMNLs) infiltration in the junctional epithelium and connective tissues among Wistar albino rats.[45] CAPE also suppressed the periodontitis-upregulated interleukin (IL)-1β, IL-6, IL-10, TNFα, MDA levels and the percentage of gingival apoptosis with the parallel restoration of periodontitis-downregulated GSH and glutathione peroxidase (GPx).[45] Administration of high-dose CAPE (10 mmol/kg/day; i.p. for 15 days) in streptozotocin (STZ)-induced diabetic male Sprague Dawley rats reduced RANKL-positive osteoclast number, IL-1β levels, oxidative stress index (OSI), alveolar bone loss and histological analysis score in LPS-induced periodontitis. The treated rats also suffered lesser inflammatory reactions, ulcers and hyperemia.[42] Similar changes of osteoclast number, IL-1β and OSI were observed in male Sprague Dawley rats with chronic stress and LPS-induced periodontitis treated with CAPE (10 mmol/kg/day, i.p. for 14 days).[44] In addition, CAPE also increased the mesial and distal periodontal bone supports (MPBS and DPBS) in these rats.[44] The effects of CAPE were sustained with a longer treatment period of CAPE (10 mmol/kg/day, i.p. for 28 days) on male Sprague Dawley rats with LPS-induced periodontitis.[43]

In contrast to the above findings, Williams et al. reported that subcutaneous injection of CAPE (1 mg/kg; at day 3, 7 and 10) did not reduce paw inflammation or bone loss in CAIA mice.[51] Cartilage and bone degradation, as well as TRAP-positive cells on the bone surface and soft tissues, were still apparent in the supplemented CAIA group compared with the normal control.[51]

## Discussion

This systematic review found that although CA and its derivatives is a potential anti-osteoporosis agent by suppressing the formation of osteoclasts and their bone resorption activity, it worsened bone mechanical properties in some cases. The anti-osteoclastogenesis action of CA and its derivatives was mediated by the antioxidant activities, which blocked RANKL-induced TRAF6/Akt and MAPK signalling, as well as M-CSF/c-Src signalling. In animals, CA and its derivatives (mainly CAPE) prevented bone resorption in rodent calvariae when implanted *in situ*, facilitated the healing of bone defects, preserved bone structure and improved mechanical strength in osteoporosis models induced by OVX, dexamethasone, osteotomy, LPS-mediated periodontitis and EMF. However, CA did not alter bone resorption in OVX-induced osteoporotic rats and worsened the mechanical properties in normal rats. Additionally, CAPE did not suppress bone loss in rats with CAIA-induced bone loss.

Osteoblasts are bone-forming cells derived from bone marrow mesenchymal stem cells and are responsible for the synthesis, secretion and mineralisation of bone matrix.[61] The expression of osteoblast markers was increased following CA or CAPE supplementation, an indication that CA and CAPE stimulated osteoblast proliferation, differentiation and maturation.[40,56] Osteoblasts and osteocytes regulate the formation of osteoclasts through RANKL/OPG axis. Osteoblasts and osteocytes synthesise RANKL, which binds to RANK to activate the canonical pathway for osteoclastogenesis. They also secrete OPG, which is a decoy receptor for RANKL to suppress osteoclastogenesis. The production of RANKL is

stimulated under conditions such as oestrogen deficiency[62] and oxidative stress.[63] Osteoclastogenesis can also be stimulated via a non-canonical pathway, for instance, through the binding of TNFα with TNF receptor I or II.[64] Glucocorticoids are potential modulators of RANKL/OPG axis, whereby dexamethasone is shown to downregulate OPG levels in osteoblasts.[65] Tolba et al. showed that the RANKL/OPG level reduced in rats induced with dexamethasone with CAPE treatment.[56] Other cellular studies showed that CA and its derivatives suppressed RANKL- and TNFα-induced formation of OCLs from haematopoietic cells,[35–39] indicating that CA and its derivatives suppressed both canonical and non-canonical osteoclastogenesis.

The complex formed by the binding of RANKL to RANK causes the recruitment of the adaptor molecule's tumour necrosis factor receptor-associated factors (TRAFs), including TRAF6.[66] This event leads to the activation of several downstream signalling pathways, including c-Src/Akt/phosphatidylinositol 3-kinase and MAPKs (ERK/p38/JNK). CADPE was shown to suppress RANKL-induced activation of TRAF6 activation and the subsequent signalling pathways in multiple osteoclast progenitors, such as BMMs,[38] RAW264.7[38] and RAW D cells.[39] Sandra and Ketherin suggested that the downregulation of p38 is the key step of CA-mediated osteoclastogenesis.[39] Upon activation, p38 initiates osteoclastogenesis by inducing NF-κB and NFATc1 expression.[67,68] Inhibition of p38 MAPK reduces RANKL (canonical) and TNFα-induced (non-canonical) osteoclast formation.[69]

The NF-κB pathway is another signalling pathway downstream of TRAFs critical for osteoclast differentiation and bone reabsorption activity. Upon activation, IKK (consisting of IKKα, IKKβ and IKKγ) phosphorylates and degrades IκBα, which enables translocation of NF-κB p65/p50 heterodimers into the nucleus to allow transcription of osteoclast-related genes.[70] Kwon et al. demonstrated that the anti-osteoclastogenesis effects of CAPE were mediated via the degradation of total IKKβ, thereby preventing the phosphorylation and degradation of IκBα and subsequently suppresses the nuclear translation of p65.[37] On the other hand, Wu et al. reported that CADPE did not affect phosphorylation or degradation of IκBα, as well as nuclear translocation, and DNA-binding activity of p65.[38] This observation suggests that compared with CAPE, CADPE does not influence the NF-κB signalling pathway.

ROS are one of the important secondary signals in the early stages of osteoclast differentiation.[71,72] These ROS are mainly produced as superoxide anions by Nox1.[73] Blocking of Nox1 ameliorates ROS production and the downstream MAPKs (JNK, p38 and ERK) and NF-κB activation[74] and subsequently suppresses the osteoclast formation.[71] The reduction of Nox 1 and Rac1 expression by CAPE is accompanied by RANKL-downstream signalling, denoting that anti-osteoclastogenesis effects of CAPE are dependent on suppression of Nox1-mediated superoxide anion production. Besides, dexamethasone has been reported to increase the expression of oxidative stress-related genes in human osteoblasts.[75] Tolba et al. showed that CAPE increased GSH and SOD but reduced MDA in the bone of the rats exposed to dexamethasone, indicating an improvement of redox status in the skeletal environment.[56] Additionally, CAPE also reduced the OSI and bone loss with an improvement of bone support in rats with LPS-induced periodontitis.

NFATc1 is the master regulator of osteoclast-related gene expression, and it is activated by c-Fos and NF-κB.[76] Ha et al. observed that CAPE inhibited the recruitment of NF-κB to NFATc1 promoter, and the combined effect of NF-κB inhibition on c-Fos and NFATc1 may have caused CAPE to suppress osteoclastogenesis effectively.[35] Holland et al. demonstrated a new fluorinated derivative of CAPE possesses potent anti-osteoclastogenic properties on RAW 264.7 cells by downregulating NFATc1 via suppression of c-Fos and NF-κB signalling pathways.[77] Besides, this new fluorinated CAPE also exhibits improved stability with a 2-fold higher potency than CAPE.[77] On the other hand, although CADPE did not alter NF-κB signalling, it still could suppress NFATc1 and other osteoclast-related markers, indicating other mechanisms of suppression could be involved, for instance, c-Src and MAPKs signalling pathways.[38]

Matrix metalloproteinases (MMPs), including gelatinases (MMP-2 and MMP-9) are examples of zinc-dependent extracellular matrix-degrading enzymes, which actively participate in bone resorption.[78] MMPs are expressed as inactive proenzymes or zymogens that can be activated by several mediators including AP-1, NF-κB, TNFα and TGFβ.[78] Currently, there is no study conducted to investigate the inhibitory effects of CA and CAPE on osteoclastic MMPs activity and its subsequent linkage in bone resorption; interestingly, CA and CAPE were reported to inhibit MMP-9 activity in human hepatocellular carcinoma HEP3B cells.[79,80] This observation renders

an interesting research gap in osteoclastic MMP inhibition upon CA and its derivatives treatment.

Suppression of osteoclastogenesis by CA or its derivatives have significant therapeutic potential against bone disorders induced by excessive bone resorption. Bone loss after osteotomy is a rapid process that affects both fractured and unfractured bone and may be incompletely reversible.[81] CAPE was reported to improve bone formation and mechanical strength of bone in osteotomy.[53] Exposure to EMF radiation caused by high-voltage transmission lines and transformers could affect bone health through decreased BMD, serum calcium and ALP level leading to the increase of bone resorption.[82] CAPE increased the spine and femur BMD levels[50] and increased mechanical strength of bones[54] in rats exposed to EMF radiation. Total hip arthroplasty without cement often caused osteolysis induced by polyethylene particles.[83] CAPE was shown by Zawawi et al. to prevent calvarial bone resorption in a murine polyethylene particle-induced osteolysis model.[58] Therefore, biomaterials impregnated with CA or its derivatives could be adopted to prevent osteolysis in the arthroplasty procedure. CA has been incorporated in chitosan/(3-chloropropyl) trimethoxysilane scaffold for hard-tissue engineering applications and this adopted material exhibits antibacterial and anticancer effects.[84] Ucan et al. observed that CAPE increased cranial bone healing in rats with critical size bone defect, suggesting that it could be administered systematically or locally to treat bone fracture/defect healing.[57]

Similarly, CAPE also effectively reduced the articular bone loss, inflammatory cytokines production and oxidative stress in rats with LPS-mediated periodontitis. Additionally, Wu et al.[38] and Duan et al.[55] demonstrated that CADPE prevented the ovariectomy-induced bone loss by suppressing osteoclast activity in a mouse model, while Folwarczna et al. showed increased width of trabecular metaphysis in the femur of OVX rats.[48] Similarly, Tolba et al. showed improved bone formation and skeletal health in rats with dexamethasone-induced bone loss upon receiving CAPE.[56] Additionally, CA and its derivatives may be involved in oestrogen production and signalling. Zych et al. reported that an oral administration of CA (10 mg/kg/day for 4 weeks) significantly restored the serum oestradiol levels in OVX rats.[85] Interestingly, CA at 10 and 100 μM did not cause any alteration in calcium content in the femoral-diaphyseal and metaphyseal *ex vivo* culture, suggesting its bone-protecting effect may not involve calcium metabolism and regulation.[86] Additionally, CAPE was reported as a selective human oestrogen receptor β agonist with the $EC_{50}$ value of 3.72 μM in oestrogen-responsive element transcription.[87] A recent *in silico* study by Zhao et al. suggested potential osteoimmunological effects of CAPE, which may explain its biological activities on both immune and skeletal systems.[88] However, the findings from this modelling study requires further validation through *in vitro* and *in vivo* models. As oestrogen deficiency due to menopause and glucocorticoids present the most significant cause of primary and secondary osteoporosis globally, CA and its derivatives have the potential to be used as an adjuvant therapy to existing osteoporosis management strategies. The mechanisms of action of CA and its derivatives in osteoclastogenesis have been summarized in Figure 2.

Regardless of the positive effects of CA on bone status, some studies have reported negative effects associated with supplementation of CA and its derivatives. CA supplementation did not affect the bone resorption[52] and reduced transverse growth of endosteal in femur[48] of rats with OVX-induced osteoporosis. In normal rats, CA supplementation even negatively affected their bone mechanical properties.[49] Moreover, CAPE supplementation has been reported to stimulate the synthesis of $PGE_2$,[89] which mediates osteoclastogenesis through RANKL stimulation and activation of the NF-κB pathway.[90] This event will eventually increase TRAP-positive OCLs. Similarly, Williams et al. showed that CAPE did not suppress osteoclastogenesis in rats with CAIA.[51]

In term of safety, the International Agency for Cancer Research classifies CA as Class 2B (possibly carcinogenic to humans),[91] and it was reported to induce renal tubular cell hyperplasia, forestomach hyperplasia, renal cell adenoma and forestomach cancer in rodents.[92–94] CA has been reported to be non-mutagenic and non-clastogenic.[91] Therefore, its carcinogenicity may involve epigenetic modification. Human toxicity and carcinogenicity of CA and its derivatives remain unknown. CA also showed anti-implantation activity in pregnant mice at a median effective dose of 4.26 mg/kg/day.[95] Similarly, 5 mg/kg/day and 150 mg/kg of CA in mice demonstrated anti-implantation activity in early pregnancy.[96] On the other hand, 0.15 mg/kg/day, 5 mg/kg/day and 150 mg/kg/day of CA for 21 days in mice showed no maternal toxicity, foetal teratogenesis or post-natal effects on pup development and mortality.[96] The same experiment stated that the no-observed-adverse-effect level of CA for pregnant female mice was 0.15 mg/kg/day.[96] Therefore, high-

dose CA should be cautioned in humans, especially pregnant women.

Several common limitations can be identified from the studies reviewed. Most studies did not adopt a positive control to compare against the anti-osteoclastogenesis or anti-osteoporosis effect of CA. Therefore, the therapeutic effects of CA and currently available anti-resorptive therapy cannot be compared. Although osteoblastogenesis and bone formation are also important in bone remodelling, evidence of CA on these processes is limited in the literature. The actions of CA in humans cannot be confirmed due to the lack of human clinical trials. These aspects can be improved in future studies.

The current review also has several limitations. We only considered articles indexed by PubMed, Scopus, Cochrane Library and Web of Science; therefore, non-indexed articles could be overlooked. We only selected articles studying CA or its derivatives as a single compound to understand its mechanism of action properly without other interference, but not a mixture of compounds or natural products rich in CA. CA are present in foods, and interaction with other compounds in the food matrix might alter its absorption, bioavailability and action on the target tissue. Moreover, the heterogeneous findings of CA in bone loss reduction upon oral administration further emphasise these possibilities.

## Conclusions

The current preclinical evidence agrees that CA and its derivatives exert promising skeletal protective effects by inhibiting osteoclastogenesis and bone resorption, but literature on bone formation is limited. Notwithstanding that, the skeletal effects of CA and its derivatives in models of normal bone health should be investigated because the limited studies available show undesirable effects. Human clinical trials to validate the skeletal effects of CA are lacking. Therefore, a well-planned clinical trial should be conducted to confirm the potential of CA as an antiresorptive agent. This information is critical for CA and its derivatives to be incorporated as part of the strategies to prevent bone loss.

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## Disclosure

The authors report no conflicts of interest in this work.

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