



## The effects of zinc treatment on matrix metalloproteinases: A systematic review



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### ABSTRACT

**Background:** Zinc (Zn) acts as a cofactor of matrix metalloproteinases (MMPs) and is vital for their activity and controlling their expression. Alteration of Zn in the body could affect the expression, activity, and destructive impacts of MMPs.

**Objective:** This systematic review aimed to summarize existing evidence on the effects of Zn treatment on the expression and activity of MMPs.

**Method:** International sources from Pub Med, Scopus and Google Scholar were searched for the original and English-language studies, published up to the end of May 2018.

**Results:** During the initial search, 179 records were found, and 135 articles of them remained after the exclusion of duplicate articles. 47 studies met the inclusion criteria, after multiple stages of screening and critical reviews of articles.

**Conclusion:** Approximately 62% of the included studies (29 of 47) showed an inhibitory impact of Zn on MMPs production and activities. The inhibitory or stimulatory effect of Zn on MMPs seems to depend on physiological conditions of the cells or animals used, dose of Zn used, and duration of treatment.

### 1. Introduction

Matrix metalloproteinases (MMPs) comprise a large family of multidomain Zn endopeptidases enzymes that mediate the degradation of extracellular matrix proteins and other important biological procedures [1,2]. In humans at least 24 MMPs have been identified [3] and classified in six subgroups according to their substrate specificities: membrane-type metalloproteinases (MMP-14, -15, -16, -17, -24, and -25), collagenases (MMP-1, -8, and -13), stromelysins (MMP-3, 10, 11, and -20), gelatinases (MMP-2 and -9), matrilysin (MMP-7 and -26) and other MMPs (MMPs-12, -18, -19, -20, -21, -22, -23, -27, and -28) [3,4]. All MMPs have calcium and a Zn-binding catalytic site. Therefore, these ions are involved in the activity of MMPs, and agents that chelate the ions inhibit the MMPs. After the synthesis of MMPs in cells, the cells secrete them in an inactive form (proenzymes) that could be activated via the cleavage of the propeptide sites by furin-like serine proteases or other MMPs [3,5,6]. Tissue inhibitors of metalloproteinases (TIMPs) inhibit MMPs. TIMPs (TIMP-1, TIMP-2, TIMP-3, and TIMP-4) can regulate the proteolytic activity of all MMPs [3,7–9].

MMPs play a substantial role in the pathological and physiological

procedures of tissue repair and extracellular matrix turnover. They are regulated at both the protein activation and the translational level. The dysregulation of MMPs' activity facilitates the progression of some diseases such as cancer, diabetes, multiple sclerosis, arthritis, metabolic syndrome, and periodontal disease [10–13].

It has been shown that activity and amount of MMPs increase under the conditions of oxidative stress, inflammation, hyperlipidemia, and hyperglycemia [12,14–18]. MMPs degrade many compounds of the basal membrane and extracellular matrix, such as collagen, fibronectin, elastin, matrix glycoproteins, and proteoglycan. Furthermore, they can degrade other proteins that are not part of the extracellular matrix, like growth factors, chemokines, and cell surface receptors [19,20].

Zinc (Zn) is an essential trace element that is vital for the human body with numerous functions like antioxidant activity, anti-inflammatory action, and regulation of apoptosis [21,22]. It can also regulate immune responses and oxidative stress [23,24]. Zn plays an important role in structure of more than 300 enzymes, like the antioxidant defense enzymes (metallothionein and Cu/Zn superoxide dismutase). Zn has several roles in biological functions and cellular integrity, including the growth, division, and development of cells in the

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body. The body does not have any special storage system for Zn, so human beings need to receive it daily [25,26]. The changes in intracellular Zn have a strong effect on processes such as differentiation, and proliferation [22,27]. In fact, inadequate Zn intake can be associated with various chronic diseases, like vascular diseases [28], both acute and chronic liver disease states [23,29], and diabetes [30]. Zn is also a cofactor for MMPs [21] and is vital for regulating their activity and expression. It has been evidenced that Zn deficiency increases the activity and expression of MMPs [31], and increasing the amount of Zn in the body can decrease MMPs' activity and their destructive effects [32].

Numerous studies have shown the effects of Zn on MMPs, but there is still no exhaustive article to summarize these studies. Thus, a comprehensive study is required to summarize stimulatory or inhibitory effects of Zn on MMPs and its mechanistic pathways. Therefore, this study aimed to review the effects of Zn on the MMPs and to discuss about several mechanisms offered by current researches.

## 2. Method

### 2.1. Research strategy

Databases of Scopus, Google Scholar, and PubMed were investigated for research studies from first to the end of May 2018. All original English language articles (human, *in vivo*, and *in vitro* studies) which assessed effect of Zn on MMPs' activity and expression were included.

This research was conducted using the following keywords: matrix metalloproteinases and zinc, metalloproteinases and zinc, and MMPs and zinc. These keywords from all sections of the selected articles were searched.

### 2.2. Analysis of the data

At the first stage of this study, all selected articles were transferred to the Endnote file and sorted to exclude duplicate articles. Secondly, the titles and abstracts of each article were screened to ensure that the searched articles are appropriate for the scope of the review. Following this, the complete texts of the screened articles were reviewed for eligibility. This was led to the removal of irrelevant articles and review articles. Furthermore, each article's abstract, results section, and figures and tables were reviewed for data extraction. The reference sections of the selected studies were searched again to find relevant studies that were not recognized during the database searches.

## 3. Results

At the search stage, 179 records were found, but 126 articles were only retained after excluding duplicate articles. During the screening stage, 79 articles were excluded, of which 69 had irrelevant data [articles that assessed structural  $Zn^{2+}$  ions in MMPs or the effects of various compounds on this ion in MMPs, and those assessed the effects of inter body (intracellular or intra tissue) Zn], and 10 articles were review. Finally, 47 relevant articles, which determined the relation between Zn and MMPs, remained (Fig. 1). The included studies were then categorised as animal and *in vitro* studies.

### 3.1. Animal studies

There were 16 animal studies, of which 10 indicated that the Zn treatment would decrease the MMPs level [33–42] (Table 1). For example, Mei et al. (2013) treated 70 rats with 12, 24, and 48 mg/kg of Zn (II)–curcumin complex during 10 days and demonstrated that curcumin (24 mg/kg) alone, decreased mRNA expression of MMP-9 in the gastric mucosa of rats, but the reduction was not significant. However, the mRNA expression of MMP-9 significantly in a concentration-dependent

manner decreased by the administration of Zn(II)–curcumin complex [37]. Sivalingam et al. treated 24 rats by  $ZnSO_4$  (50 mg/kg), 2 h before the administration of Indomethacin. Zn treatment was able to reduce the drug-induced increase in MMP-9 and MMP-2 activities in the small intestine of rats [38]. Yan et al. reported that Zn treatment decreased expressions of MMP-2 and MMP-9's in the aortas of the 30 rats that were treated with 3 mg/kg  $ZnSO_4$  per day for 4 weeks [42]. However, 5 studies reported increased levels of MMPs by Zn supplementation [43–47] (Table 1). For example, Shi et al. (2014) investigated possible mechanisms through which Zn supplementation inhibits the progress of liver fibrosis, that was induced by BDL (bile duct ligation), in 120 mice. They indicated that the injection of Zn (5 mg /kg  $ZnSO_4$ ) increased the MMP-1, MMP-8 and MMP-13 activities compared to the control group. Also, the MMP-13 protein level and mRNA expression in the liver significantly increased by Zn supplementation [44]. Moreover, in a study conducted by Lang et al. (2011), dietary Zn (150 mg Zn /kg of diet) had no significant effect on the mRNA expression of MMP-12 in the lung tissue of mice that were exposed to smoke [48].

### 3.2. In vitro

Thirty-one cellular studies were searched and 19 of them indicated a decreasing effect of Zn on MMPs [39,49–66] (Table 2). All studies that assessed the effect of Zn in dentin indicated a decreasing action of Zn on MMPs. For example, Osorio et al. (2011) evaluated the impact of  $ZnCl_2$  (1  $\mu M$ ) collagen degradation and MMPs activity in the demineralized dentin. They showed that  $ZnCl_2$  inhibited MMP's collagen degradation and MMPs activity [53]. Henn et al. evaluated the impact of different concentrations of zinc methacrylate (ZM) (0.5, 1, 2, 4, 8, and 16 mM) on the inhibition of MMP-2 expression in an experimental dental polymer, for 24 h. Results showed that ZM inhibited MMP-2 expression in all concentrations [64]. In another study conducted by Altinci et al. (2017) investigated the effect of  $ZnCl_2$  (0.2, 0.5, 1, 5, 10, 20, 30, or 40 mM) on matrix-bound cathepsin K and MMP activity in dentin. Zn treatment inhibited collagenolysis by decreasing the activities of MMPs and cathepsin K [50].

There are other studies indicate a decreasing effect of Zn on MMPs in different cells. For example, Szuster et al. demonstrated that Zn (30  $\mu M$   $ZnCl_2$ ) regulated ethanol and acetaldehyde-induced generation of TIMP-1 and TIMP-2, and decreased the activity of MMP-2. By the way, it did not change the activity of MMP-13 in rat hepatic stellate cells [65]. Mirza et al. evaluated the chondroprotective impact of Zn oxide nanoparticles (ZnONP) on bovine cartilage-matrix. Results showed that 1% ZnONP increased the anabolic gene expression of aggrecan and type II collagen, and decreased the mRNA expression of catabolic MMP-13 [63]. Guo et al. investigated the expression of MMP-9 in murine photoreceptor-derived cells and found that the expression of MMP-9 reduced at both mRNA and protein levels after incubation of cells with different doses (3.05, 6.10 and 12.20  $\mu M$ ) of ZnONP [62]. Takino et al. found that Zn salt of l-pyrrolidone carboxylate (Zn PCA) (10  $\mu M$ ) decreased the expression of MMP-1 and activator protein-1 (AP-1) in cultured normal human dermal fibroblasts (NHDFs). Both of them were enhanced by ultraviolet light (UV) radiation [54]. Souza et al. also examined the impacts of metal salts on the activity of the MMPs, which were obtained from gingival explants of 4 periodontitis adult patients. They showed that  $ZnSO_4$  (1540  $\mu M$ ) was a strong suppressor of MMP-9 and MMP-2 activities [60].

However, several other studies (8 studies) indicated the incremental effect of Zn on MMPs [32,43,67–72] (Table 2). For example, in a study conducted by Pan et al. (2017), it was demonstrated that  $ZnCl_2$  (100  $\mu M$ ) treatment significantly elevated the proteins levels, mRNA expression and activity of MMP-2 and MMP-9 in the human umbilical vein endothelial cells [67]. Also, Xiao et al. (2015) showed that increased mRNA expression of MMP-13 induced by  $ZnCl_2$  (100  $\mu M$ ) in the rat Nucleus Pulposus cells decreased by hypoxia and the  $Zn^{2+}$  chelator [71].

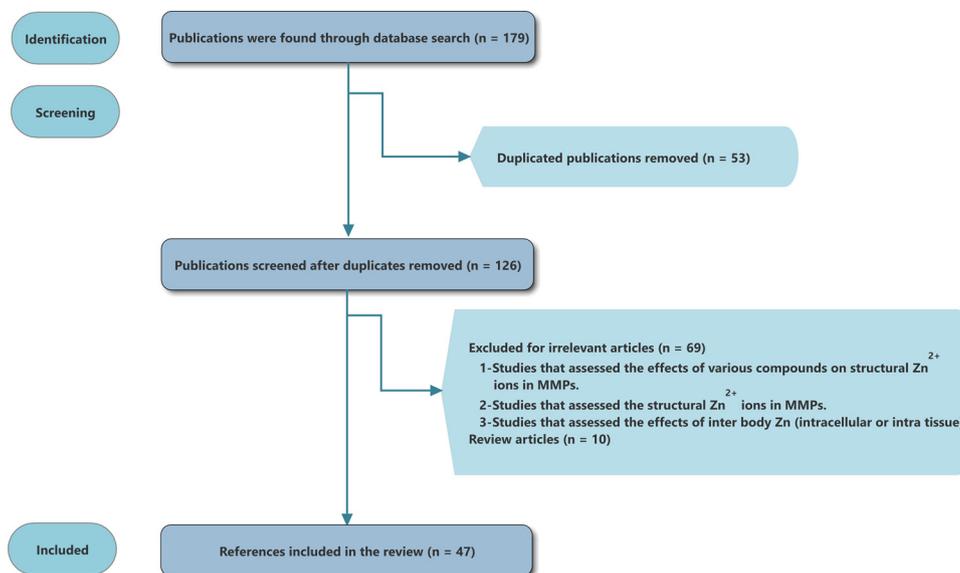


Fig. 1. Flowchart of studies included in systematic review.

Moreover, there are 4 cellular studies that indicated the null effect of Zn on MMPs [73–76] (Table 2). For instance, Xiao et al. reported that ZnSO<sub>4</sub> (0–350 μM) supplementation had no impact on MMP activity in Madin-Darby canine kidney cells [76].

#### 4. Discussion

Approximately 62% of the included studies (29 of 47) showed an inhibitory effect of Zn on MMPs production and activity, whereas the rest of the studies obtained null or opposite findings.

The inconsistency across the studies can be explained by three possibilities. The first possibility is related to the physiological condition of the cells or animals used. It appears that Zn reduces the expression and activity of MMPs in pathophysiological conditions (Osteoarthritis, sepsis, diabetes mellitus, demineralized dentin or periodontitis, etc.) [34,35,39,42,50,60], but it increases expression and activity of MMPs in physiological conditions [45,67,69,71], states like wound healing [47] or growth [43]. MMPs have important roles in normal growth, development and wound healing [43,47]. In fetal growth restriction rats, Zn treatment promotes invasion and migration of trophoblast cells, via enhancement of the activity and expression of MMP-2 and MMP-9, which contributes to the development of the placenta and improves neurodevelopmental impairment [43].

The second possibility is linked to Zn concentration used. In most cellular studies included, Zn treatment at concentrations below 50 μM reduced the activity and expression of MMPs [39,42,50,54], while at concentrations of 50 μM and above, it increased or did not influence the activity and expression of MMPs [67,69–71]. Kang et al. in a study on human hepatic stellate LX-2 cells showed that Zn treatment at a dose of 200 μM significantly increased mRNA expression and protein content of MMP-13 [32]. Uzzo et al. demonstrated that Zn treatment in the physiological levels (1.545–3.091 μM, ZnSO<sub>4</sub>) decreased the expression and secreted amount of MMP-9 in human prostate cancer cells [56]. However, in the study conducted by Boissier et al., ZnSO<sub>4</sub> treatment at a dose of 50 μM enhanced the activities of MMP-1, 2, 9, and 12 in the human breast and prostate carcinoma cells [68]. In another study, in the chicken B lymphocyte-derived DT40 cells mutants, ZnSO<sub>4</sub> treatment at a dose of 50 μM elevated the protein level and activity of MMP-9 [70].

The duration of the Zn intervention is another possible factor that may play a role in the inhibitory or stimulatory impact of Zn on MMPs. Annangi et al. demonstrated that treatment of mice embryonic

fibroblasts with sub-toxic dose of ZnO NPs (1 μg/mL) had no significant effect on the secretion activity of MMP-2 and MMP-9 during 6 weeks, but significant reduction was observed during 12 weeks [66]. Also, in another study, Zn significantly increased mRNA expression of MMP-2 in blast-injured muscle in the first 48 h, but it returned to baseline by 14-days post-blast, in the traumatic injured rats which received a diet with adequate Zn [47].

The mechanism by which Zn inhibits the activity and production of MMPs is still under investigation. Various mechanisms have been introduced for this function (Fig. 2). It has been shown that oxidative stress increases the MMP-2 and MMP-9 expression and activity [14,15]. According to the previous reports, Zn has antioxidant properties and the ability to decrease oxidative stress [23,77,78]. The stabilising properties of the sulfhydryl group and the antagonising ability of redox-active transition metals (such as iron and copper) are responsible for the acute antioxidant properties of Zn [79]. Therefore, Zn may suppress oxidative stress and subsequently MMPs expression and activity. Zn also increases the expression of metallothionein, a metalloprotein that has an antioxidant effect and reduces oxidative stress [80].

The findings of the studies included showed that Zn prohibits the MMP activity through inhibition of nuclear factor kappa-B (NF-κB) and mitogen-activated protein kinase (MAPK), which have the ability to generate reactive oxygen species. It has been shown that NF-κB can regulate transcription of MMPs and inhibition of the NF-κB can reduce the MMP-9 expression [42]. Also, in various studies, it has been shown that the MAPK pathway can regulate the expression and activity of MMPs and inhibition of MAPK pathway reduces the activity and expression of MMPs [81–84]. Zn inhibits the activation of the MAPK p38 and JNK by blocking the phosphorylation of C-Jun N-terminal kinase (JNK and p38). NF-κB activation is related to phosphorylation and proteolytic degradation of IκB (NF-κB inhibitor transcription factor). It has been shown that Zn inhibits phosphorylation of IκB (via blocking of IκB kinase (IκK) activity) [42,65].

Furthermore, it has been reported that Zn salt of l-pyrrolidone carboxylate (Zn PCA) suppresses the MMP-1 production and the AP-1 expression in NHDFs [54]. AP-1 has the ability to regulate the gene encoding of MMP-1 [85]. It has been shown that Zn deficiency enhances the binding of DNA to AP-1 and with this mechanism increases the generation of MMPs [86]. It is also known that Zn deficiency enhances AP-1 activation by increasing the oxidative stress in cells [87,88]. Zn PCA treatment leads to reduced activation of AP-1 and suppression of the oxidative stress [39].

**Table 1**  
Relation between Zn and MMPs in animal studies.

Reference	Type of subjects / Number	Physiologic status	Dosage, Zn compound, Type of administration	Duration of treatment	Method of MMPs measurement	Findings
Studies indicated a decreasing effect of Zn on MMPs						
Bortolin et al. [35]	Rat / n = 15	Type 1 diabetes mellitus	10.6 mg/day ZnCO <sub>3</sub> , supplemented dietary	12 weeks	PCR	ZnCO <sub>3</sub> decreased the mRNA expression of MMP-9 but did not change the mRNA expression of MMP-2 in the bone tissues of diabetic rats.
Hadley et al. [40]	Rat / n = 30	Long bones of growing rats	2.5, 5, 7.5, 15 or 30 mg/kg diet, (ZnCO <sub>3</sub> ), dietary	24 days	PCR	Increasing dietary Zn (15 or 30 µg/g) decreased the activity of MMP-2 and MMP-9 in the femurs of rats.
Hegedűs et al. [36]	Rat / n = 48	Neointima formation following angiotensin II	31 mg/kg body weight ZnCl <sub>2</sub> with 15 mg/kg Zn content diluted in the water	18 days	PCR	ZnCl <sub>2</sub> treatment significantly decreased the mRNA expression of MMP-9 in the carotid arteries of rats.
Huang et al. [39]	Rat / n = 60	Osteoarthritis	1.6 mg Zn/kg/day (recommended dose) or 8.0 mg Zn/kg (high dose), water-dissolved Zn (ZnSO <sub>4</sub> ·7H <sub>2</sub> O), gavage.	2 weeks	Western blotting and ELISA.	ZnSO <sub>4</sub> treatment decreased serum protein levels of MMP-1 and MMP-13.
Lin et al. [41]	Rat / n = 114	Diabetic ulcer	.01% ZnSO <sub>4</sub> (.1 mL/cm), injection	7, 14, 21 days	Immunohistochemical staining	ZnSO <sub>4</sub> decreased the expression of MMP-1 and increased the expression of TIMP-1.
Mei et al. [37]	Rat / n = 70	Gastric ulcer	12, 24, 48 mg/kg, Zn(II)-curcumin, oral administration	10 days	Reverse transcriptase PCR analysis	Zn(II)-curcumin decreased MMP-9 mRNA expressions in a dose-dependent manner in the gastric mucosa of rats.
Sivalingam et al. [38]	Rat / n = 24	Indomethacin-induced small intestinal damage	ZnSO <sub>4</sub> (50 mg/kg dissolved in water, equivalent to 11.3 mg of elemental Zn), by oral gavage	1, 12 and 24 h	Zymography and western blots.	ZnSO <sub>4</sub> reduced the activities of MMP-9 and MMP-2 and elevated significantly metalloprotein levels in the small intestine of rats.
Wessels et al. [34]	Mice / n = 30	Polymicrobial sepsis	30 (Zn-adequate) or 180 (high-Zn) mg Zn/kg diet	1 week	Customized luminex assay	High Zn diet significantly reduced the mRNA expression of MMP-9 in hepatocytes and hepatic leukocytes. Also, serum levels of MMP-9 non-significantly decreased in high Zn diet mice.
Xu et al. [33]	Rabbit / n = 24	Lipid disturbance	5 mg/day, dietary Zn	12 weeks	Western blotting	Zn inhibited MMP-2 and MMP-9's expressions in the kidney, lung, liver, thoracic aorta and left ventricle tissue from the rabbits, also, Zn decreased IL-6 and hs-CRP expressions.
Yan et al. [42]	Rat / n = 30	Abdominal aortic aneurysm	3 mg/kg, ZnSO <sub>4</sub> , daily intraperitoneal injection	4 weeks	Western blotting	ZnSO <sub>4</sub> decreased the expression of MMP-2 and MMP-9 in the aortas. Also, Zn treatment suppressed the NF-κB activation.
Studies indicated the incremental effect of Zn on MMPs						
Grommes et al. [46]	Rat / n = 66	Colon anastomosis	1.0 mg/kg body weight, C <sub>6</sub> H <sub>12</sub> N <sub>2</sub> O <sub>8</sub> Zn (zinc aspartate) / dietary	14 days	Immunohistochemistry	C <sub>6</sub> H <sub>12</sub> N <sub>2</sub> O <sub>8</sub> Zn elevated the protein expression of MMP-2 in the anastomosis at each time point (day 3; day 5; day 14) and increased protein expression MMP-8 in the anastomosis on days 3 and 5, but had no effect on the MMP-13 expression at each time point (day 3; day 5; day 14) in the colon apart from the anastomosis.
Roy et al. [45]	Mice / n = 20	Healthy	2.5 mg/ml, ZnO NPs, injection	24 h.	Western blotting	ZnO NPs increased the protein expressions of MMP-9 and caspase-1 in the macrophages.
Scrimgeour et al. [47]	Rat / n = 72	Traumatic injury	5, 30 mg/kg, Zn diet	7 weeks	Quantitative real-time PCR	Adequate Zn in diet increased significantly the mRNA expression of MMP-2 in blast-injured muscle in the first 48 h, but it returned to baseline by 14 d post-blast. Also, the mRNA expression of MMP-2, MMP-3 and MMP-9 in rats fed with low Zn diets had no change at 48 h and 14 d.
Shi et al. [44]	Mice / n = 120	Liver fibrosis	5 mg/kg, ZnSO <sub>4</sub> , injection	2 weeks	EnzCheck Gelatinase/ Collagenase Assay kit	ZnSO <sub>4</sub> increased the activity of collagenases (MMP-1, 8, and 13). Also, it increased the MMP-13 protein level and mRNA expression in the liver.
Zong et al. [43]	Rat / n = 58	Fetal growth restriction (FGR)	Zn diet (0.2 µg, kg/day)	30 days	Western blot analysis	Zn supplementation increased the expression of MMP-2 and MMP-9 in placental tissues of rats and improved memory and learning abilities of FGR rats.
Studies that indicated the null effect of Zn on MMPs						
Lang et al. [91]	Mice / n = 48	Mice exposed to smoke	150 mg Zn /kg of diet	8 weeks	Real-time-PCR	Dietary Zn had no significant effect on the mRNA expression of MMP-12 in lung tissue.

ELISA, enzyme-linked immunosorbent assay; FGR, Fetal growth restriction; Hs-CRP, high-sensitivity C-reactive protein; MMPs, matrix metalloproteinases; NF-κB, nuclear factor kappa-B; PCR, Polymerase chain reaction; TIMPs, Tissue inhibitors of metalloproteinases; ZnO NPs, zinc oxide nanoparticles; Zn, zinc.

**Table 2**  
Relation between Zn and MMPs in *in vitro* studies.

Reference	Type of subjects / Number	Physiologic status	Dosage, Zn compound, Type of administration	Duration of treatment	Method of MMPs measurement	Findings
<b>Studies indicated a decreasing effect of Zn on MMPs</b>						
<b>2.1 Dentin</b>						
Altinci et al. [50]	Dentin	Demineralized dentin matrix	0.2, 0.5, 1, 5, 10, 20, 30, or 40 mM, ZnCl <sub>2</sub>	1, 3, and 7 days	ICTP EIA kit	ZnCl <sub>2</sub> treatment inhibited collagenolysis by decreasing the activity of MMPs (from the first day of incubation, inhibition of MMPs was seen in all Zn doses).
Gerlach et al. [61]	Teeth	Demineralized dentin	0.33–10 μM of ZnSO <sub>4</sub>	40 h	Colorimetric assay, casein and gelatin zymography	ZnSO <sub>4</sub> inhibited the activity of enamel proteinases (MMPs and serine proteinases) <i>in vitro</i> . ZM inhibited the expression of MMP-2 in all doses.
Henn et al. [64]	Dental polymer	Enzymes secreted from mouse gingival tissues	0.5, 1, 2, 4, 8, and 16 mM, ZM	24 h	Gelatin zymography	ZnCl <sub>2</sub> by reducing the activity of MMP-3, inhibited cleavage by MMP-3 in the dentin.
Khaddam et al. [52]	Dentin	Demineralized dentin	3.3 mg.ml <sup>-1</sup> , ZnCl <sub>2</sub>	7 days	Western blot analysis	ZnCl <sub>2</sub> suppressed MMP-2 activity and inhibited MMP-mediated collagen degradation for all concentrations.
Oh et al. [92]	Dentin	Demineralized ground dentin by citric acid	3.33, 6.82, 13.63, 27.26 mg/ml, ZnCl <sub>2</sub>	24 h, 1 week, and 2 weeks	ELISA	Zn-bTCS decreased MMP's collagen degradation and inhibited MMPs activity.
Osorio et al. [51]	Dentin	Demineralized dentin	Zn-bTCS (Zn oxide (20 wt%))	24 h, 1 week, and 4 Weeks	ICTP	ZnCl <sub>2</sub> inhibited MMP's collagen degradation and MMPs activity.
Osorio et al. [53]	Dentin	Demineralized dentin	1 μM, ZnCl <sub>2</sub>	24 h, 1 and 3 weeks	ICTP	ZnO cement suppressed the MMP-2 and MMP-9 activity.
Santos et al. [57]	Teeth	Demineralized dentin	ZOE cements	48 h	Gelatin zymography of immunoprecipitated MMPs.	Zn inhibited collagenolysis by inhibiting of MMPs activity.
Toledano et al. [55]	Teeth	Demineralized dentin	3.33 mg/ml ZnCl <sub>2</sub>	24 h and 4 weeks	ICTP	
<b>2.2. Cell</b>						
<b>Studies indicated a decreasing effect of Zn on MMPs</b>						
Annangi et al. [66]	Mouse embryonic fibroblasts cell	Retinal cell	7.334 μM of ZnCl <sub>2</sub> , 1.22 μM of ZnO NPs	12 weeks	Gelatin zymography	Studies indicated a decreasing effect of Zn on MMPs
Guo et al. [62]	Murine photoreceptor-derived cell line 661W	Retinal cell	3.05, 6.10 and 12.20 μM, ZnONP	2 h	Real-time quantitative	ZnONP reduced both mRNA expression and protein levels MMP-9.
Huang et al. [39]	Chondrosarcoma cell line, SW1353	Osteoarthritis	incubated with 25 μM Zn (ZnSO <sub>4</sub> ·7H <sub>2</sub> O).	24 h or 48 h	PCR	ZnSO <sub>4</sub> treatment decreased the protein level of MMP-13 in the cells.
Isaksen et al. [59]	Microwell	Gelatinolytic microwell	1, 100 μM, ZnCl <sub>2</sub>	24 h	Gelatin and Casein microwell assay	Calprotectin inhibited the activity of MMPs (MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, and MMP-13), but this effect was more resulted by the addition of Zn.
Mirza et al. [63]	Bovine chondrocytes	Cartilage digested	1%, ZnONP	14 days	Quantitative polymerase chain reaction	ZnONP decreased the mRNA expression of MMP-13.
Souza et al. [60]	Human inflamed gingival cells	Periodontitis	3, 1540 μM, ZnSO <sub>4</sub>	16 h	Gelatin zymography	ZnSO <sub>4</sub> was a strong inhibitor of MMP-2 and MMP-9 activities.
Szuster-Ciesielska et al. [65]	Rat hepatic stellate cells	Liver fibrosis	30 μM, ZnCl <sub>2</sub>	24 h	ELISA	ZnCl <sub>2</sub> reduced the activity of MMP-2, by decreasing the production of reactive oxygen species, MAPK and NF-κB signalling pathways, but ZnCl <sub>2</sub> did not change the activity of MMP-13 in the rat hepatic stellate cells.
Takino et al. [54]	Cell	UVA irradiated NHDFs	10, μM Zn PCA	23 h	ELISA	Zn PCA decreased the expression and production of MMP-1 and AP-1.
Underwooda et al. [58]	Cell	THP-1 human promonocytic leukaemia cells	10 μM, Zn <sup>2+</sup> (metal ions)	16 h	Fluorescent proteolytic assay	Zn <sup>2+</sup> significantly decreased the activity of MMP-9.
Uzzo et al. [56]	Cell	Human prostate cancer cells	1.545–3.091 μM, ZnSO <sub>4</sub>	30 min	ELISA	ZnSO <sub>4</sub> decreased the expression and secreted amount of MMP-9, IL-6, IL8, and VEGF in prostate cancer cells.
Studies indicated an increasing effect of Zn on MMPs						

(continued on next page)

**Table 2** (continued)

Reference	Type of subjects / Number	Physiologic status	Dosage, Zn compound, Type of administration	Duration of treatment	Method of MMPs measurement	Findings
Bjelogrlic et al. [72]	Seven human tumour cell lines: epithelial breast cancer, osteosarcoma (U2OS), osteosarcoma cisplatin resistant, cervical carcinoma, melanoma, colon cancer, murine melanoma cells and two endothelial cell lines)	Cancer	(100, 725 µM), complex of Hfpesc with ZnCl <sub>2</sub>	24 h	Gelatin zymography	Zn <sup>2+</sup> complex elevated MMP-2 activity in tested cells.
Boissier et al. [68]	Human breast carcinoma cell	Cancer	50 µM, ZnSO <sub>4</sub>	3 h	Fluorometri-c analysis.	ZnSO <sub>4</sub> treatment reversed the inhibitory effect of bisphosphonates on the activities of MMP-1, 2, 9, and 12.
Hwang et al. [93]	Embryonic neuronal cells		10 or 300 µM, ZnCl <sub>2</sub>	15 min	Zymography	ZnCl <sub>2</sub> supplementation increased the activity of MMP-2 and MMP-9.
Kang et al. [32]	Human hepatic stellate LX-2 cells	Liver fibrosis	200 µM, Zn	24 h	Western blotting	Zn significantly elevated the mRNA expression and protein level of MMP-13.
Shuang Pan et al. [67]	Human umbilical vein endothelial cells		100 µM, ZnCl <sub>2</sub>	24 h	ELISA	ZnCl <sub>2</sub> promoted the migration and invasion of human umbilical vein endothelial cells by increasing the protein levels, mRNA expression and activity of MMP-2 and MMP-9.
Tsuji et al. [70]	Chicken B lymphocyte-derived DT140 cells mutants	Cancer	50 µM, ZnSO <sub>4</sub>	48 h	Gelatin zymography	ZnSO <sub>4</sub> treatment elevated the protein level and activity of MMP-9.
Xiao-Fan, et al. [71]	Rat nucleus pulposus cells	Hypoxic	100 µM, ZnCl <sub>2</sub>	24 h	Real-Time PCR	ZnCl <sub>2</sub> increased the mRNA expression of MMP-13.
Zong et al. [43]	trophoblast cells	Fetal growth restriction	10 µM Zn solution	48 h	Western blot analysis	Zn treatment promoted trophoblast cells invasion and migration by increasing the activity and expression of MMP-2 and MMP-9.
Studies that indicated the null effect of Zn on MMPs Guo et al. [74]	Rat vascular smooth muscle cells		0-300 µM, of Zn	24, 48 and 72 h	Gelatin zymography	Zn had no effect on the homocysteine-induced MMP-2.
Hosek et al. [75]	Human monocytic leukaemia cell line, THP-1	Leukaemia	5 µM, ZnCl <sub>2</sub>	24 h	Zymography	ZnCl <sub>2</sub> did no effect on the expression and secretion of MMP-2 but changed the pro-MMP-2/MMP-2 ratio with increasing production of matured MMP-2.
Ishii et al. [73]	Human prostate and renal cells	Cancer	0-150 µM C <sub>4</sub> H <sub>10</sub> O <sub>6</sub> Zn (Zn acetate)	24 h	Fluorometrically	C <sub>4</sub> H <sub>10</sub> O <sub>6</sub> Zn had no effect on the MMPs activity.
Xiao et al. [76]	Madin-Darby canine kidney cells		0- 350 µM, ZnSO <sub>4</sub>	12 h	Universal Fluorimetric MMP activity assay Kit	ZnSO <sub>4</sub> supplementation did not impact on MMP activity.

AP-1, activator protein; BTCS, beta-tricalcium silicate particles; ICTP, cross-linked carboxy-terminal telopeptide of type I collagen; ELISA, enzyme-linked immunosorbent assay; HS-CRP, high-sensitivity C-reactive protein; MAPK, mitogen-activated protein kinase; MMPs, matrix metalloproteinases; NF-κB, nuclear factor kappa-B; NHDFs, Normal human dermal fibroblasts; PCR, Polymerase chain reaction; Zn, zinc; ZM, zinc methacrylate; ZnONP, zinc oxide nanoparticles; ZnO NPs, zinc oxide nanoparticles; Zn PCA, zinc salt of L-pyrrolidone carboxylate; ZOE, zinc oxide-eugenol; VEGF, vascular endothelial growth factor, Zn, zinc.

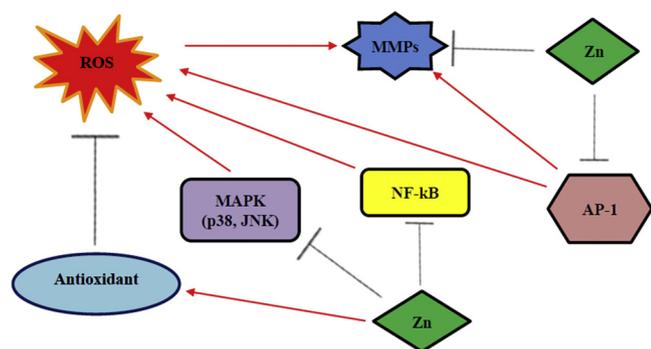


Fig. 2. mechanism of MMPs reduction by Zinc.

ROS, reactive oxygen speices; MMPs, matrix metalloproteinases; zn, zinc; AP-1, activator protein-1; NF-kB, nuclear factor kappa-B; MAPK, mitogen-activated protein kinase.

Zn can also inhibit the activity of MMPs through a direct reaction with MMPs. First, excess Zn binding changes the construction of MMP proteins or generate a Zn-hydroxide bridge which blocks the catalytic site of the MMPs. However, this action may depend on the Zn concentration [50,53,58,89]. Second, metal binding may change the consolidation of protein at the catalytic domain and inactivate the enzyme [58]. Furthermore, it has been shown that the accumulation of Zn, increases oxidative stress in the ischemic microvessels by increasing the production of the superoxide anion, which in turn leads to increased activity of MMP-9 and MMP-2 [90].

#### 4.1. Limitations and advantages of the included studies

In this review, a significant number of animal and *in vitro* studies are advantages, as these types of studies can be physiologically controlled. Another advantage is the existence of a control group, in animal studies, that decreases systematic bias and allows the possibility for comparison. In the included studies, the duration of the intervention (from 15 min to 12 weeks) and the sample sizes of animals (from 15 to 120) used, were acceptable.

However, there are several limitations for this study, and the absence of human studies was the main weakness of the research. Human studies provide definite and comprehensive data on the probability and generalisation of drug interactions in clinical treatments. The physiological and metabolic responses of humans are different from animals. Other limitations of this study are the use of different forms of Zn, different dosing, and different administration methods in the selected articles. Also, mechanistic studies regarding how Zn influences on the MMPs were limited.

#### 4.2. Conclusion

Approximately 62% of the studies included (29 of 47) showed an inhibitory effect of Zn on MMPs production and activities. The inhibitory or stimulatory impact of Zn on MMPs seems to depend on physiological conditions of the cells or animals used, dose of Zn used, and duration of treatment. However, there were few studies regarding possible mechanisms of Zn action and there is no human study in this area; hence, further research should be conducted to understand the Zn action pathway.

#### Author contributions

The authors' responsibilities were as follows—RN: designed the study; RN and SK: conducted the study; SK, RN and RG: wrote the paper; RN: has primary responsibility in the final content; and All authors have read and approved the final manuscript.

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The authors report no conflict of interest.

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