Systematic Review

The Interaction of Zinc as an Essential Trace Element with *Leishmania* Parasites: A Systematic Review

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Abstract

Background: The trace element of zinc (Zn) has shown great effectiveness in control of leishmaniasis infection. Hence, the present study conducted a systematic review of *in vitro* and *in vivo* studies evaluating the zinc effect in the treatment or prevention of leishmaniasis.

Materials and Methods: A systematic literature search was performed of all articles published in PubMed, SciELO, ScienceDirect, Scopus, Google Scholar, and Web of Science databases (1997–2023). The search terms were "zinc" OR "cutaneous leishmaniasis (CL)" OR "visceral leishmaniasis (VL)".

Results: Initial search yielded 89 citations, and 59 subjects were included. Data showed the zinc serum level in CL patients was lower than controls. Also, *in vitro* studies of zinc were more effective against *L. tropica* and *L. major* promastigotes compared to the amastigotes. Moreover, *in vivo* studies did not show destructive effects of zinc on the mammalian cell viability like macrophages. Furthermore, zinc depletion by specific chelators affected *L. donovani* survival and growth through promoting apoptosis and reactive oxygen species-dependent mechanisms.

Conclusion: The serum level determination of zinc could be useful for estimating the leishmaniasis pathophysiology. Environmentally or genetically determined increases in zinc levels might augment resístanse to CL. In contrast, zinc depletion using a zinc-specific chelator could be effective treatment of VL in endemic areas.

Keywords: Cutaneous leishmaniasis, systematic review, visceral leishmaniasis, zinc

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Introduction

Trace elements such as Fe, Cu, and Zn are needed for many metabolic and physiologic processes in the human body[1] such as the synthesis and structural stabilization of both protein and nucleic acid. Hence, imbalances in the optimum levels of trace elements may adversely affect biologically processes and associate with many diseases, inflammation, and infections.[2] The divalent cation of zinc is an important micro-nutrient and an essential component of more than 300 metalloenzymes and 2000 transcription factors involved in various metabolic activities, such as lipid metabolism, protein, and nucleic acid, as well as gene transcription (in zinc-finger factors).[3] Zinc also plays an important role in maintaining the cellular growth, immunity, and wound healing by regulating deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) polymerases, thymidine kinase, and ribonuclease.[3,4] With regard to the limited movement of zinc in tissues and lack of storage, continuous exterior provision of zinc for metabolic

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needs, growth, and tissue repair is important.[3] Today, about one third of the world's population, which includes most of the Southeast Asia areas of sub-Saharan Africa, and other developing countries suffer from the shortage of zinc. For example, zinc endemic deficiency in villages of Iran, Egypt, and Turkey is caused by eating whole grain bread with high fiber and phytate contents, which makes zinc almost inaccessible. The daily intake of zinc has been recommended as 0.5–1 mg/d for children, 11 mg/d for adult males, and 8 up to 12 mg/d for women during pregnancy and lactation, and it is mainly used as zinc sulfate, zinc acetate, or zinc oxide.[5] Various forms (salt) of Zn such as zinc oxide, calamine, or zinc pyrithione have been used as an adjuvant to established treatment modalities. The oral effect of zinc has been well known in humans with zinc deficiency syndromes including acrodermatitis enteropathica. Also, zinc is an essential substance for the normal function of skin, and over the years, it is used locally or systemically for a large number of skin disorders including skin inflammation (acne vulgaris, rosacea), pigmentary disorders (melasma), neoplasia (basal cell carcinoma), and infections (warts, leishmaniasis).[3]

Leishmaniasis is one of the most complex parasitic diseases that are caused by various species of *leishmania* genus, a single-cell kinotoplastic flagellated parasite transmitted by the bite of infected sandflies of Phlebotomus.[6] Leishmaniasis is common in sub-tropical and tropical regions and is native to 98 countries, and so far, about 21 human pathogenic species have been identified.[7] The disease manifests in three clinical forms: cutaneous (CL), mucocutaneous (MCL), and visceral (VL). The most severe form of the disease is VL that is caused by members of *L. donovani*. In contrast, CL is a self-healing chronic wound and has a histological form of lymphocyte and monocyte penetration to granuloma formation that is transmitted by *L. major, L. tropica,* and *L. aethiopica* complexes in the old world and *L. mexicana*, *L. guyanensis*, and *L. braziliensis* complexes in the new world. About 1.5 million new cases are reported annually in Brazil, Iran, and Afghanistan.[8,9]

There are two morphological forms of the parasite, promastigotes and amastigotes. The flagellated promastigotes are present in the insect's gut and saliva. They transfer to the mammalian host and are engulfed by macrophages, where they become non-flagellated intra-cellular amastigotes, and begin to multiply in vacuoles of the host cell, with destruction of the macrophage and infection of new macrophages.[10] The process of elimination of intra-cellular Leishmania requires a Th1 immune response. The Th1 immune response leads to the production of items such as IL-2 and INF-γ, which eliminate leishmaniasis infection, whereas a dominant of Th2 response leads to exacerbated disease.[11]

Pentavalent antimonials such as glucantime are the choiced drugs for Leishmaniasis. With regard to cardiac and renal toxicity of these chemical drugs, the discovery of novel and effective drugs is a major goal and challenge.[12,13] Trace elements like zinc have been tried with variable success, both intra-lesional and oral. It has been found effective in the management of CL, but inconsistent outcomes remain a limiting factor for its solo use.[14]

Zinc is an intra-cellular signal molecule that plays an important role in improving the function of macrophages, dendrocytes, and monocytes and cell-mediated immunity that are in turn effective in body defense against *Leishmania*.[15] Furthermore, Zn as a cofactor of gp63 (an integral part of virulence of *Leishmania* parasite) determines structural characteries of molecules involved in entrance of *L. donovani* into white blood cells (WBCs).[16] The anti-leishmanial effect of zinc sulfate is not completely clear. Therefore, the mechanism of zinc sulfate action in the leishmaniasis treatment seems to be dependent on two separate routes. The first route is the direct inhibition of Zn on the amastigote's proliferation through the impact on effective enzymes in nucleic acid metabolism. The second route is due to the immune-modulating and immune-stimulating effects of zinc against the "shift" in Th1 Th2.[17] One of the priorities set by the World Health Organization is to address the major gap in the available therapies for leishmaniasis.[18] There are also currently a dearth of reviews and meta-analyses of zinc-relevant articles on leishmaniasis in the world. Hence, in this study, we performed a systematic literature of all *in vitro* and *in vivo* studies that have evaluated the effectiveness of zinc in the prevention and treatment of leishmaniasis.

Materials and Methods

Information sources and search strategies

This systematic review was conducted of studies assessing the effect of zinc on treatment or prevention of leishmaniasis using the PubMed, SciELO, ScienceDirect, Scopus, Google Scholar, and Web of Science electronic databases.

Inclusion criteria included all original and review research studies with full text written in English without geographical restriction between 1997 and 2021 such as *in vitro/in vivo* and human-based studies (randomized control and intervention trials) reporting the role of zinc in pathogenesis or treatment of leishmaniasis. Articles without any of aforementioned criteria, including unpublished abstracts/conference proceedings, case reports, retrospective or prospective case series, letters to the editor, brief communications, and duplicates, were excluded. Additional relevant articles were obtained through citation tracking of review articles and the reference lists of included articles manually, and appropriate articles were included [Figure 1]. The full text of the selected articles (sample) was obtained directly from the aforementioned databases, when freely available, or through the Sci-hub site. The following combination of search terms in English was used: {*Leishmania* OR leishmaniasis} and {zinc OR zinc sulfate}.

Afterward, the collected references were screened carefully by two independent investigators to eliminate irrelevant articles. The titles and abstracts of each paper were read in its entirety,

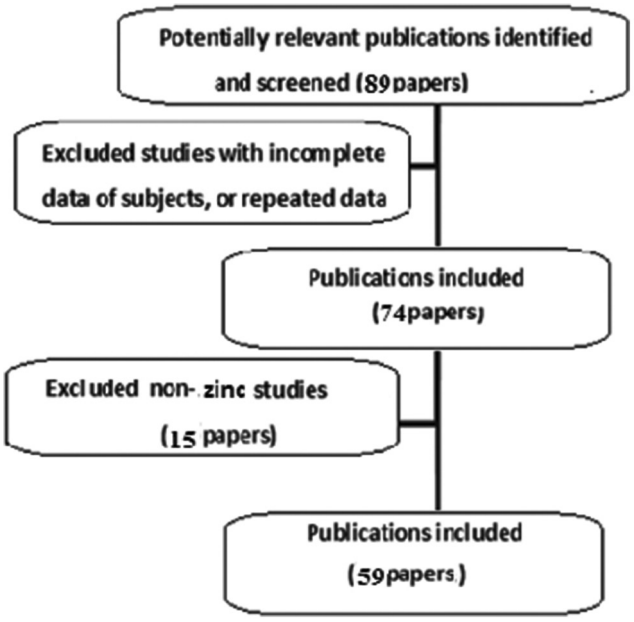

Figure 1: Flow chart of review process

and data elements were extracted by the first reviewer (Aghaei, M) and checked by another one (Aghaei, Sh) and entered by the third reviewer (koohiyan.M) into a Microsoft Excel sheet that included the first author's name, publication year, case of study, kinds of leishmaniasis, and studied trace elements. Then, the first reviewer re-checked the requisite data for accuracy before data report and interpretation. When there was any discrepancy in their report, the fourth and fifth reviewers (Hejazi.SH, Shahmoradi.ZA) were invited to resolve the issue.

Our initial literature search yielded 89 citations, and following the screening process, a total of 15 studies were excluded because they failed to answer the research question and/or offered no access to original data. Finally, 74 papers with mentioned parameters of interest were selected, and 59 articles fulfilled the inclusion criteria.

Results

A total of 74 records were found following the initial search of databases, and after removing duplicates and/or non-eligible papers, 59 papers had eligibility to be included in this systematic review.

Twenty-seven studies (% 45.7) described the relation between zinc level and pathogenicity of leshmaniasis [Table 1]. In three studies related to mice with CL, serum or plasma levels of zinc and iron were lower than copper,[19-21] and one study also showed that the ratio of serum levels of Zn/Mn is lower than the levels of other elements such as Cu/Mn and Cu/Fe.[22]

Furthermore, in three studies involving dogs with VL and non-infected dogs (control group), a significant decrease in the serum levels of zinc, selenium, and iron elements compared to copper in infected dogs was observed.[23-25] A study also showed although the serum level of zinc is higher than Cu, dogs with VL have lower levels of zinc than the control group.[26]

Seventeen studies also showed that serum or plasma levels of zinc, iron, and selenium in patients with CL, VL, and MCL (compared to control groups) are lower than copper.[2,11,27-35,37-42] Only two studies showed that the level of zinc in CL patients was higher than that of copper, but the serum zinc level was lower than that of the control group.[34,36]

In vitro studies

There were nine studies (15.2%) from India, Iraq, Iran, Notre Dame, and Brazil that investigated the inhibitory effects of zinc against various kinds of *Leishmania* species such as *L. tropica*, *L. major*, *L.donovani*, *L. amazonensi,* and *L. braziliensis in vitro* [Table 2].

Two studies showed greater sensitivity of axenic promastigotes and amastigotes of *L. major* and *L. tropica* to zinc sulfate compared to glucantime and pentostam.[43,44] In another study, eight fluorescently labeled zinc (II)-dipicolylamine (ZnDPA) probes showed selective toxicity against axenic promastigotes and intra-cellular amastigotes of *L. major* but had almost no effect on the viability of mammalian cells, including mouse peritoneal macrophages.[45] In contrast, in two studies, antimony-resistant *L. donovani* parasites were affected by N, N, N', N'-tetrakis (2-pyridinylmethyl)-1,2-ethylenediamine (TPEN) treatment, and the results showed that treating parasites with TPEN instead of $ZnSO_4$ has a significant dose- and time-dependent effect on parasite growth by promoting apoptosis-like cell death.[16,46]

Moreover, two studies showed that zinc nanoparticles (in a dose-dependent concentration) prevent infection by stimulating the production of nitric oxide and destructing promastigote and amastigote forms of *L. major* and *L. tropica*.[47,48]

A study also showed that $ZnCl_2(H3)_2$ complex inhibits the growth of promastigotes and intra-cellular amastigotes of *L. amazonensis*.[49]

Furthermore, another study showed that the application of photodynamics with zinc propyrene reduces the number of *L. brasiliensis* amastigotes (40%) in infected macrophages.[50]

In vivo studies

Considering the inclusion criteria, six articles (10.1%) against *L. tropica*, *L. major,* and *L. infantum* were selected, which were from Iraq, Notre Dame, Iran, and Italy, and examined the effect of intra-lesional or oral injection of zinc sulfate/Zn nanoparticles on the treatment or prevention of leishmaniasis *in vivo* [Table 3].

Two studies investigated the effect of oral zinc sulfate on the treatment or prevention of leishmaniasis in BALB/c mice and dogs with CL and VL, respectively. One article showed oral zinc caused dose-dependent reduction of lesions in infected BALB/c mice compared to untreated mice infected with *L. major/L. tropica* parasites.[43] Results of another study also

Table 1: Comparison of the level of zinc with other trace elements in leishmaniasis

Num	First Author/Year	Case	Sample	Leishmaniasis	Element	Result	Ref
1	Amini M/2009	Balb/c mice	Plasma	CL	Zn Cu	Zn < Cu	[19]
2	Najafzade M/2015	Balb/c mice	Serum	CL	Zn Cu	Cu > Zn > Cu/Zn	[21]
3	Sobotyk, C/2023	Balb/c mice	Serum	CL MCL	Zn Cu Fe Mg Ca Mn	Cu/Fe/Mg/Ca > Zn/Mn	[22]
4	Anstead GM/2001	Balb/c mice	Serum	VL	Zn Fe	Rats with deficient diets in iron, zinc displayed acute phase response in VL faster than iron-filled control mice	[20]
5	Pasa S/2003	Dog	Serum	VL	Zn Cu Fe	Zn , Fe < Cu	[23]
6	Heidarpour M/2012	Dog	Serum	VL	Zn Cu	Zn and Cu < control	[24]
7	Souza CC/2014	Dog	Serum	VL	Zn Cu Fe Se (selenium)	Zn , Fe, Se < Cu	[25]
8	GAZYAĞCI AN/2023	Dog	Serum	CVL	Mg Fe Zn Cu Se Cr Mn co	Mg > Fe > Zn > Cu > se > Cr > Mn > Co Se and Zn in Leishmania-positive dogs < Se and Zn in the negative ones.	[26]
9	Kocyigit A/2002	Human	Plasma	CL	Zn Fe Cu	Zn , Fe < Cu , IL-1beta	[27]
10	Van Weyenbergh J/2004	Human	Plasma	VL LCL ML	Zn Cu	1. Zn of ML/LCL/VL < control. 2. Cu and Zn in VL < LCL < ML	[11]
11	Pourfallah F/2009	Human	Plasma	CL	Zn Cu Fe	Zn , Fe < Cu	[2]
12	Lazarte C/2013	Human	Serum	CL	Zn	Reduced zinc bioavailability in a diet containing phytate	[28]
13	Faryadi M/2003	Human	Serum	CL	Zn Fe Cu	Zn , Fe < Cu	[29]
14	Mishra J/2010	Human	Serum	VL	Zn	Zn in Delhi VL patients < Zn in non-endemic controls	[30]
15	Chandra RK/1997	Human	Serum	VL	Zn Cu Mg (magnesium)	Zn < Cu, Mg	[31]
16	Farzin L/2014	Human	Serum	CL	Zn Cu Se	Zn , Se < Cu	[32]
17	Farzin L/2014	Human	Serum	VL CL	Zn Cu Se	Cu > Zn > Se in VL patients compared to CL.	[33]

Contd...

Table 1: Contd...

Num	First Author/Year	Case	Sample	Leishmaniasis	Element	Result	Ref
18	Kahvaz MS/2021	Human	Serum	CL	Se Zn Cu Fe	Cu > Fe > Zn > Se > Zn/Cu	[34]
19	Koçyiğit A/1998	Human	Serum	CL	Se Zn Cu Fe	Se > Cu > Zn > Fe	[35]
20	Kocyigit A/1998	Human	Serum	CL	Zn Cu Fe	Zn > Fe > Cu	[36]
21	Al-Hassani MKK/2020	Human	Serum	CL	Se Zn Cu Fe	Fe > Cu > Se > Zn	[37]
22	Lal CS/2013	Human	Serum	VL	Zn Cu Fe Mg Ca	Cu > Zn > Fe > Ca > Mg	[38]
23	Nahidi, Y/2021	Human	Serum	CL	Zn	Zn in both acute improved and chronic groups < Zn in control.	[39]
24	Kahvaz, M.S/2021	Human	Serum	CL	Se Zn Cu Fe	Fe > Zn > Cu > Se Zn/Cu in CL patients < controls.	[34]
25	Shakir, O.M/2023	Human	Serum	CL	Zn Mg Fe	Mg > Fe > Zn	[40]
26	Kocyigit A/1998	Human	Serum	CL	Se Zn Cu Fe	Cu > Se > Zn > Fe	[41]
27	Musa IS/2015	Human	Serum	VL	Zn	Zn in patient group < Zn in healthy control	[42]

indicated that oral zinc supplementation in dogs infected with *L. infantum* results in increased serum zinc concentration, with possible benefits in terms of faster response to treatment with allopurinol in treated animals.[51]

Moreover, three articles showed that intra-lesional injection of zinc sulfate[52,53] and/or ZnDPA[45] caused significant reduction in lesion size, less necrosis in the local host tissue, and parasitic load in spleen of BALB/c mice infected with *L. major* compared to untreated mice or mice treated with potassium antimony (III) tartrate. A study also showed that zinc nanoparticles have a synergistic effect. In combination with glucantim, they lead to the complete recovery of lesions in mice infected with *L.major*.[47]

Human studies

Seventeen studies (28.8%) described the effects of oral or injectable zinc/zinc sulfate on human CL or VL [Table 4]. Five studies showed that the use of oral zinc sulfate alone (depending on the dose) leads to healing of CL wounds or reduction of spleen regression during the treatment of VL patients.[54-58] On the other hand, only one study showed that the use of oral zinc has no effect on clinical symptoms or immune response of leishmaniasis.[59]

Furthermore, three studies showed that the intra-lesional or local efficacy of 2% zinc sulfate and oxide was more than that of glucantim in acute CL patients.[60-62] Two studies also showed that the effectiveness of their use is equal or less (33% vs. 80%).[63,64]

Two studies also used oral and injectable zinc sulfate at the same time. The results of a study showed that oral intake and intra-lesional injection of zinc sulfate have fewer side effects than glucantim.[65] In contrast, another study showed that this compound does not have sufficient therapeutic value.[66]

Moreover, three studies showed that the simultaneous use of oral zinc and intra-muscular injection of drugs such as glucantim, ketoconazole, or local injection of profile has the same effect as the mentioned drugs and leads to high wound

Table 2: The inhibitory effects of the various kinds of zinc on *leishmania* parasites *in vitro*

Num	First Author/ Year	Case	Leishmaniasis	Leishmania species	Component	Result	Ref
1	Najim RA/1998	Promastigotes Axenic amastigotes	CL	*L. major* *L. tropica*	Zinc sulfate	More sensitivities of promastigotes and axenic amastigotes of both *Leishmania* to zinc sulfate compared to glucantime and Pentostam	[43]
2	Bafghi AF/2014	Promastigotes	CL	*L. major* *L. tropica*	Zinc sulfate	Inhibitory effect of zinc sulfate on promastigotes proliferation in comparison with the glucantime	[44]
3	Rice DR/2016	Promastigotes Amastigotes	CL	*L. major*	Fluorescently labeled eight ZnDPA probe	Selective toxicity against axenic promastigotes and intracellular amastigotes of L. major, but there was almost no effect on the viability of mammalian cells, including mouse peritoneal macrophages	[45]
4	Kumari A/2017	Promastigotes	VL	*L. donovani*	Zinc-depletion Zinc-supplementation using Zinc-specific chelator TPEN Zinc Sulfate ($ZnSO_4$)	Treatment of parasites with TPEN rather than $ZnSO_4$ had a significant effect on parasite growth in a dose- and time-dependent manner	[46]
5	Saini S/2017	Promastigotes	VL	*L. donovani*	Zn-depletion by specific chelator TPEN	Reduces *Ldonovani* survival and growth by promoting cell death resembling apoptosis by a reactive oxygen species (ROS) dependent mechanism. Also antimony resistant *Ldonovani* parasites were similarly affected by TPEN treatment	[16]
6	Yadegari JG/2023	Amastigotes	CL	*L. major*	Zn nanoparticles (ZnNPs) alone and combined with glucantime	Triggering of NO production, and inhibition of infectivity rate.	[47]
7	Meaad AG/2017	Promastigotes and Amastigotes	CL	*L. tropica*	zinc oxide nanoparticles (ZnO NPs)	there is a direct destructive effect of ZnO NPs on different forms (promastigotes and amastigotes) of Leishmania tropical parasite, Also, the destruction of parasites increases with concentrations of ZnO NPs used, and the best concentration was 5 µg/ml after 72 hr.	[48]
8	Visbal G/2023	Promastigotes and intracellular Amastigotes	CL, MCL	*L. amazonensis*	$ZnCl_2(H3)_2$ complex	$ZnCl_2(H3)_2$ significantly inhibited the growth of promastigotes and intracellular amastigotes.	[49]
9	Andrade CG/2018	Promastigotes and Amastigotes	CL	*L. braziliensis*	zinc porphyrin	The number of amastigotes per macrophage was reduced by about 40% after photodynamic application. The treatment showed no considerable toxicity against mammalian cells.	[50]

healing (96%) compared to the unit effect of each drug.[67-69] In contrast, a study showed that oral zinc supplementation with glucantim injection does not have a greater clinical effect on wound healing or biochemical parameters of CL patients.[70]

Discussion

CL depending on the parasite and host factors triggers various immune responses that in turn can be affected by nutrients. In order to prevent leishmaniasis complications and other adverse effects of unbalanced diets in developing countries, the nutritional status of patients should be considered. Therefore, nutritional status studies of populations at risk for leishmaniasis are important for designing and implementing new nutrition and treatment policies.[71] Our study showed that serum concentrations of essential trace elements such as Se, Zn, Cu, and Fe change in CL patients compared to the control group. The level of Se, Zn, and Fe was lower than Cu in *in vitro* and *in vivo* studies and in CL, VL, and MCL patients compared to the control group. These changes may be a part of defense strategies of the organism and are induced by the hormone-like substances and can lead to a host's inability to clear the parasite. Zinc, as an adjuvant, has been found useful owing to its modulating actions on macrophages and neutrophil

Table 3: The effect of intralesional or oral injection of the various kinds of Zn on the treatment or prevention of leishmaniasis *in vivo*

Num	First author/Year	Case	Leishmaniasis	Leishmania species	Component	Result	Ref
1	Najim RA/1998	BALB/c mice	CL	*L. major* *L. tropica*	Oral $Zn(SO_4)$	A dose-related decrease of lesions in comparison with non-treated mice with *leishmania* parasite	[43]
2	Paola P/2017	Dog	VL	*L.infantum*	Zinc oral	To increase the serum zinc concentrations and faster response to therapy with allopurinol and the elongation of the disease-free interval time in comparison to control dogs.	[51]
3	Rice DR/2016	BALB/c mice	CL	*L. major*	ZnDPA	Lower parasitic burden nearly as well as the reference care agent, potassium antimony (III) tartrate, and with less necrosis in the local host tissue	[45]
4	Sorkhroodi FZ/2010	BALB/c mice	CL	*L. major*	Zinc sulfate	The size of the wound decreased	[52]
5	Afshari M/2016	BALB/c mice	CL	*L. major*	Zinc sulfate	There was a significant decrease in lesion sizes and parasite loads in Zn sulfate-treated group compared to the untreated group.	[53]
6	Yadegari JG/2023	BALB/c mice	CL	*L. major*	Zn Nanoparticles (ZnNPs) alone and combined with glucantime	CL lesions had completely improved in the mice received with ZnNPs in combination with MA.	[47]

functions, natural killer cell/phagocytic activity, and various inflammatory cytokines. Research showed deficiency of zinc and iron and the high level of INF-γ causes a failure of lymph node barrier function after *L. donovani* infection, which may be related to excessive production of PGE (prostaglandin E)[2] and decreased levels of IL-10 and nitric oxide.[20] Cytokines (IL-1) secreted during the response of the acute phase of the immune system, the activity of flotation peroxides, and catalase activate metallothionein (a metal-binding protein) in the liver and other tissues that alters zinc absorption and reduces zinc levels during inflammation and infection.[2,44] Therefore, the use of zinc supplements may not only result in important therapeutic assistance in both CL and VL; it can lead to prevent in people with immune suppression or residents of endemic areas.[65] Since the increased zinc levels enhance IFN-γ production, various studies have been done on the function mechanism of various forms of zinc in altering immune response in experimental animals and humans. Research has mentioned the anti-leishmanial effect of various forms of zinc on *L. major* and *L. tropica* species by inhibiting the enzymes involved in glucose metabolism and, consequently, proliferation of the parasite. Results of the present study also showed that in *in vitro* models, the *L.* major and *L. tropica's* promastigotes and amastigotes were more susceptible to the various forms of zinc such as zinc sulfate, Zn nanoparticles, ZnDPA, ZnCl2(H3)2 and so on, compared to the control group. 2% zinc sulfate inhibited growth in amastigotes and promastigotes of *leishmania* species without any effect on the viability of mammalian macrophages. Moreover, Zn nanoparticles in a dose-dependent concentration by stimulating the production of nitric oxide destructed promastigotes and amastigotes of *L. major* and *L. tropica.* Regarding the study of the effect of zinc sulfate on VL *in vitro*, the research showed that unlike CL, intra-cellular Zn depletion in the L. donovani promastigotes led to ROS-mediated caspase-independent mitochondrial dysfunction, resulting in apoptosis-like cell death.

In addition, zinc can accelerate the wound healing process through zinc metalloenzymes involved in membrane stability and collagen maturation during wound repair procedures, and it is known that wound healing is interrupted by zinc deficiency.[72] In animal studies, collected results showed that intra-lesional or oral injection of zinc sulfate/Zn nanoparticles leads to treat or prevent leishmaniasis in BALB/c mice and dogs with CL and VL, respectively. Moreover, the use of a zinc synergistic combination like ZnDPA *in vivo* has shown a promising new class of anti-leishmanial agents with potential for clinical translation. The majority of collected human studies also showed the uppermost cure rate for zinc oxide/sulfate 2% injected intra-lesional or oral (without any side effects) in individuals with VL and CL compared to drugs such as glucantim, ketoconazole, and so on. In contrast, the results of the other systematic review studies showed that zinc therapy did not demonstrate a significant clinical improvement compared to standard treatment.[14] The limitation of this study is the restriction of the search to English language so that articles in other languages that contain valuable information from Africa, the Middle East, and Asia may have been excluded. Despite this limitation, this study of systematic review represents clear and up-to-date information with respect to the effect of zinc on the treatment or prevention of leishmaniasis in studied populations of the world.

Conclusion

The results of our study showed that the serum level determination of zinc could be helpful for assessing the pathophysiology of leishmaniasis. The increased zinc levels might augment resístanse to CL. In contrast, zinc depletion

Table 4: The effects of zinc or zinc sulfate on human leishmaniasis

Num	First Author/Year	Case	Leishmaniasis	Leishmania species	Component	Result	Ref
1	Sharquie K/2001	Human	CL	*L.major*	Oral zinc sulfate	The successful dose-dependent effect of oral zinc sulfate on the treatment of CL	[54]
2	Sharquie KE/2004	Human	CL	*L.major*	Oral zinc sulfate	The successful treatment of CL	[55]
3	Sprietsma J/1997	Human	CL	*L.major*	Oral $ZnSO_4$	Oral $ZnSO_4$ was effective in changing the immune function from cellular Th1 to humoral Th2	[56]
4	Yazdanpanah MJ/2011	Human	CL	*L.major*	Oral $ZnSO_4$	The same effects of oral zinc sulfate and meglumine antigens in the treatment of CL	[57]
5	Carbone DCB/2018	Human	VL	*L.infantum*	Oral Zinc	Zinc supplementation is favoring the regression of splenomegaly in children during treatment of VL.	[58]
6	Guzman M/2014	Human	CL	*L.major*	Oral Zinc	There was not a difference in the clinical signs or the immune response at the time of healing.	[59]
7	Sharquie K/1997	Human	CL	*L.major*	Intralesional 2% $ZnSO_4$	An intralesional efficacy of ZnO 2% (94.8%) compared to antimony and sodium chloride (7%)	[60]
8	Iraji F/2004	Human	CL	*L.major*	Intralesional 2% $ZnSO_4$	The effectiveness (83.8%) of intralesional sulfate zinc 2% compared to antimony (60%)	[61]
9	Sharquie KE/2017	Human	CL	*L.major*	Topical $ZnSO_4$	The cure rate in patients treated by topical zinc sulphate was in 36 (73.4%) of 88 lesions.	[62]
10	Farajzadeh S/2016	Human	CL	*L.major*	Intralesional sulfate zinc 2%	Intralesional injection of 2% zinc sulfate has been as effective as glucantime in improving the acute dry CL	[63]
11	Maleki M/2012	Human	CL	*L.major*	Intralesional injection of zinc sulfate 2%	Intralesional injection of zinc sulfate 2% had a lower improvement than glucantime (33.3 vs 80%)	[64]
12	Firooz A/2005	Human	CL	*L.major*	Oral $ZnSO_4$ Intralesional $ZnSO_4$	Oral intake and intralesional injection of zinc sulfate have less side effects than glucantime	[65]
13	YAZDANPANAH MJ/2007	Human	CL	*L.major*	Oral $ZnSO_4$ Intralesional $ZnSO_4$	Oral and intralesional injection of 2% zinc sulfate in treatment of CL do not have sufficient therapeutic value	[66]
14	Sharquie KE/2022	Human	CL	*L.major*	Triple therapy using oral $ZnSO_4$ and oral Ketoconazole and topical Podophyllin	The cure rate was 99%. No important adverse effects were noticed	[67]
15	Farajzadeh S/2018	Human	CL	*L.major*	Niosomal topical $ZnSO_4$ with Glucantime	Combination of niosomal zinc sulfate with intralesional glucantime has equal efficacy	[68]
16	Sharquie KE/2016	Human	CL	*L.major*	Oral $ZnSO_4$ plus oral Ketoconazole	The high cure rate (96%) in the combination therapy using oral zinc sulfate and oral ketoconazole compared to the single effect of drugs	[69]
17	Guzman-Rivero M/2014	Human	CL	*L.major*	Oral zinc with Glucantime	The oral zinc supplementation along with intramuscular injection of antimony had no more clinical effect on wound healing or on biochemical parameters in CL	[70]

using a zinc-specific chelator could be effective treatment of visceral leishmaniasis in endemic areas. Unlike CL, intra-cellular Zn depletion in the L. donovani promastigotes leads to ROS-mediated caspase-independent mitochondrial dysfunction, resulting in apoptosis-like cell death. Therefore, there is a need for similar studies to complete the studies that have been done so far with a greater sample volume, changes in zinc sulfate concentration, the use of combination therapies, the identification of sub-types of *leishmania*, and their specific response to zinc sulfate for obtaining more crucial results about the therapeutic effect of zinc on CL and VL in the future.

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Conflicts of interest

There are no conflicts of interest.

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