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ORIGINAL

A Meta-analysis and Systematic Review of the Antimicrobial Activities of Calcium Hydroxide Paste Mixed with Different Metallic Salts, Polymers, and Metallic Nanoparticles

Un metaanálisis y revisión sistemática de las actividades antimicrobianas de la pasta de hidróxido de calcio mezclada con diferentes sales metálicas, polímeros y nanopartículas metálicas

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Abstract

Objective: the present study were conducted to evaluate the antimicrobial activity of calcium hydroxide paste mixed with different metallic salts, polymers, and metallic nanoparticles.

Material and methods: A search of all international databases was conducted between January 2013 and May 2023 using keywords related to the study's objectives, including PubMed, Scopus, Science Direct, ISI, Web of Knowledge, and Embase. For the current study, related articles were found by using the PRISMA 2020 checklist and Google Scholar search engine. Inverse-variance method and the fixed effect model were used to calculate the 95% confidence interval for mean differences. The meta-analysis was conducted using Stata/MP v.17 software.

Results: A total of 183 abstracts were reviewed in the initial review, which removed duplicate studies. A total of 41 studies were reviewed by two authors, and ten studies were chosen for further study. The mean differences of antibacterial effect on E. faecalis between CHX + CH and CH were 0.75 (MD, 0.75 95% CI 0.67, 0.83; p=0.00). Mean differences of antibacterial effect on E. faecalis between CHT+ CH and CH were 2 (MD, 2 95% CI -5.46, 9.46; p=0.60).

Conclusion: Based on the findings of the present meta-analysis, a mixture of CH and CHX, silver nanoparticles, copper nanoparticles, and zinc nanoparticles can lead to eradicating E. faecalis as an intracanal medicine. The use of CHT as an antimicrobial adjuvant for CH-based drugs was not supported.

Key words: Nanoparticles, biofilms, root canal obturation, metal nanoparticles, chlorhexidine.

Resumen

Objetivos: el presente estudio se realizó para evaluar la actividad antimicrobiana de la pasta de hidróxido de calcio mezclada con diferentes sales metálicas, polímeros y nanopartículas metálicas.

Métodos: se realizó una búsqueda en todas las bases de datos internacionales entre enero de 2013 y mayo de 2023 utilizando palabras clave relacionadas con los objetivos del estudio, incluidas PubMed, Scopus, Science Direct, ISI, Web of Knowledge y Embase. Para el estudio actual, se encontraron artículos relacionados utilizando la lista de verificación PRISMA 2020 y el motor de búsqueda Google Scholar. Se utilizó el método de varianza inversa y el modelo de efectos fijos para calcular el intervalo de confianza del 95% para las diferencias de medias. El metanálisis se realizó utilizando el software Stata/MP v.17.

Resultados: Se revisó un total de 183 resúmenes en la revisión inicial, que eliminó los estudios duplicados. Dos autores revisaron un total de 41 estudios y se eligieron diez estudios para realizar estudios adicionales. Las diferencias medias del efecto antibacteriano sobre E. faecalis entre CHX + CH y CH fueron 0,75 (DM, 0,75; IC del 95 %: 0,67 a 0,83; p = 0,00). Las diferencias medias del efecto antibacteriano sobre E. faecalis entre CHT+ CH y CH fueron 2 (DM, 2 IC del 95%: -5,46; 9,46; p=0,60).

Conclusión: Según los hallazgos del presente metanálisis, una mezcla de CH y CHX, nanopartículas de plata, nanopartículas de cobre y nanopartículas de zinc puede conducir a la erradicación de E. faecalis como medicamento intracanal. No se apoyó el uso de CHT como adyuvante antimicrobiano para medicamentos a base de CH.

Palabras clave: Nanopartículas, biopelículas, obturación del canal radicular, nanopartículas metálicas, clorhexidina.

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Introduction

As a result of persistent microbial infection in the root canal system and surrounding radicular tissues, apical periodontitis develops, which is considered an inflammatory disorder[1]. The basis of root canal treatment is to destroy microorganisms and prevent re-infection[2]. However, for some reasons, including the anatomical complications of the root canal system, apical periodontitis may remain[3,4]. Therefore, it is recommended to use the supplement of antimicrobial drugs[5-7]. One widely used drug is Calcium hydroxide (CH) paste, which studies have reported its antimicrobial effect. CH paste creates a very alkaline environment that usually microorganisms cannot survive[8]. However, studies have reported that the effectiveness of CH paste can be limited, or some microbial species have become resistant to it[4]. It has been reported that Enterococcus faecalis (E. faecalis) and Candida albicans (C. albicans) are among the species resistant to CH paste treatment and cause root canal treatment to fail. Apical periodontitis remains[9,10]. These bacteria can tolerate high pH and have the ability to penetrate dentin[11]. Studies have tried to increase its antimicrobial activity by adding auxiliary antimicrobial substances to CH paste[12]. One of these substances is Chlorhexidine (CHX), Gram-positive and Gram-negative bacteria have been shown to be susceptible to this antimicrobial agent. This material is biocompatible and can be absorbed into dental tissues, increasing the treatment level[13]. Many studies have investigated the advantage of mixing CH with CHX. According to the studies, CHX is much more effective at killing bacteria than CH alone. However, other studies also observe contradictory findings[14-16]. Some studies have shown that using metallic nanoparticles (MNPs) can effectively control oral infections[17-19]. Studies have suggested that MNPs can be promising alternatives to traditional antimicrobial agents[20,21].

Silver, copper and zinc nanowires are among the MNPs whose antimicrobial properties have been reported by many studies[22]. Polymeric nanoparticles (PNPs) have recently been introduced as complex carriers for drug delivery[23,24]. The advantages of PNPs include biocompatibility, improved stability, biodegradability, drug release profiles, and ease of use[25]. Metallic nanoparticles have also been successfully synthesized in aqueous solutions using chitosan (CHT) as a reducing and stabilizing agent[26]. One of the advantages of CHT is that they are biocompatible and biodegradable. Studies have indicated that CHT is hemostatic, antimicrobial and analgesic[23]. A study has reported promising results in using CHT/CDP/metallic nanoparticles[27]. An investigation of the antimicrobial activity of CH paste mixed with different metallic salts and metallic nanoparticles, PMNPs, has been conducted in the present study. Therefore, the present study evaluated the antimicrobial activity of calcium hydroxide paste mixed with different metallic salts, polymers, and metallic nanoparticles.

Methods

Search strategy

A search was conducted in all international databases, PubMed, Scopus, Science Direct, ISI, Web of Knowledge and Embase, based on keywords related to the study's objectives, between January 2013 and May 2023. A PRISMA 2020 checklist[28] was used to guide the current study, and related articles were also found by using Google Scholar. Keywords and the MeSH terms:

((((((((“Periapical Periodontitis”[Mesh]) AND (“Anti-Infective Agents”[Mesh] OR “Antimicrobial Stewardship”[Mesh] OR “Biofilms”[Mesh])) AND “Candida albicans”[Mesh]) AND (“Root Canal Obturation”[Mesh] OR “Dental Pulp Diseases”[Mesh])) AND “Metal Nanoparticles”[Mesh]) OR “Silver”[Mesh]) OR “Copper”[Mesh]) OR “Zinc Oxide”[Mesh]) AND “Chlorhexidine”[Mesh]) AND (“Microbiology”[Mesh] OR “microbiology” [Subheading] OR “Microbiological Techniques”[Mesh]).

Process of data collection, data selection, and data items

A checklist that included seven items was used to extract sample specifications from the selected studies: author's name, publication year, sample size, study design, Nanoparticle type, number of control groups, assessment of the antimicrobial properties and Bacterial strain. Additionally, the findings of the studies were used to extract the data required for the meta-analysis, including the antimicrobial performance. Each record was reviewed independently by two reviewers, and each report was retrieved. According to inclusion and exclusion criteria, all studies were selected.

Eligibility criteria

Inclusion criteria: Articles published in English, in-vitro studies and studies assessing the antibacterial effect.
Exclusion criteria: Review papers, case studies, and case reports. Studies without full-text access.

Critical appraisal

A modified version of the CONSORT Criteria (Guidelines for reporting pre-clinical in vitro studies on dental materials) was used to evaluate the quality of the study[29]. The parameters were answered yes or no for each study in a review with 14 items. These items were:

A structured description of the methods, results, and conclusion of the trial; an explanation of the scientific context; specific objectives or hypotheses; and sufficient details about the intervention, including when and how it was administered, for replication. An outcome measure that is fully defined, predetermined, and includes how and when it is evaluated, how the sample size is calculated, how the random allocation sequence is generated, how it is implemented, who is responsible for creating the random allocation sequence, and who becomes blind after the intervention is administered. Statistics used to compare the groups, results from each group, estimation of effect size and precision, trial limitations, identifying potential biases and imprecisions, and where the full trial protocol can be found, including multiple analyses, funding sources and other support sources. This tool was adapted and modified from the Cochrane risk of bias tool. This tool assigned scores of 2, 1, or 0 to each item. A low bias risk score is 0 to 3, a moderate bias risk score is 4 to 7, and a high bias risk score is 8 to 10. The tool produced the lowest score of 0 and the highest score of 10[30].

Data analysis

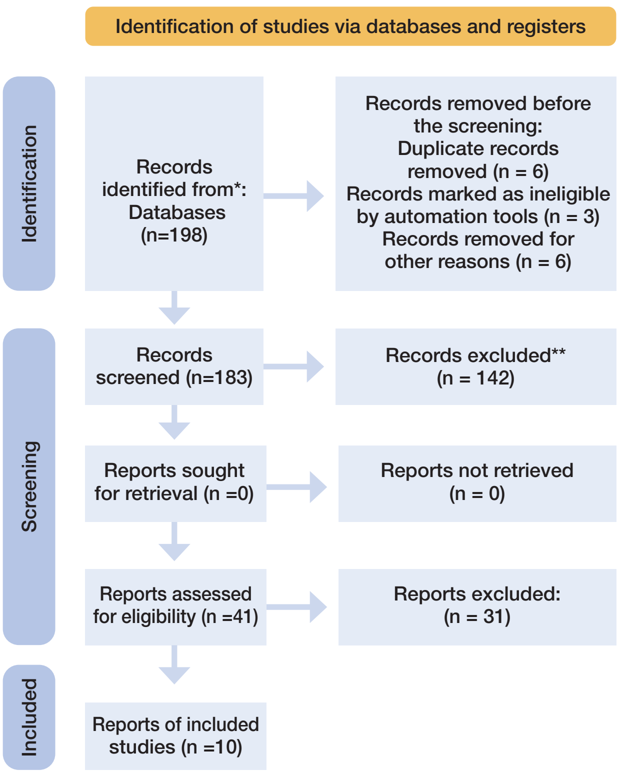
According to the I^2 coefficient, low heterogeneity can be determined with values less than 50%, moderate heterogeneity can be determined with values between 50% and 75%, and high heterogeneity can be determined with values over 75%. The fixed effects model and inverse variance method were used to calculate 95% confidence intervals for mean differences. STATA/MP. V17 software was used to conduct the meta-analysis.

Result

Study selection

All references were entered into EndNote X8 software after the initial search for articles using keywords revealed 198 articles. Six articles were duplicates, three articles were due to records marked as ineligible by automation tools, and six articles were removed for other reasons. After reviewing 183 abstracts, 142 articles that did not meet inclusion criteria were removed. The full texts of 41 articles were reviewed by two blinded observers. Ten articles were selected (**Figure 1**) after excluding 31 incomplete articles without data inconsistency with the objectives of the study.

Figure 1: PRISMA 2020 Checklist.



Study characteristics

In the present study, 536 human teeth were examined in vitro; two studies did not report the sample size. Other extracted data are reported in **table I**.

Bias assessment

Four studies received a score of 3, which indicates a low risk of bias; Three studies received a score of 4, which indicated a moderate risk of bias; and three studies received a score of 8, which indicated a high risk of bias. Two studies were low quality in the sample size section, and two were low quality in the Assessment methods section. In three methodological studies, the studies were very poorly written (**Tables II** and **III**).

Antibacterial activity

AgNPs+ CH

Mean differences of antibacterial effect on E. faecalis between AgNPs+ CH and CH were -0.39 (MD, -8.39 95% CI -8.56, -8.21; p=0.00) with high heterogeneity (I^2=99.93%; p =0.00). The results significantly reduced the E. faecalis with AgNPs+ CH than the control group (**Figure 2**).

CuNPs+ CH

Mean differences of antibacterial effect on E. faecalis between CuNPs+ CH and CH were -1.49 (MD, -1.49 95% CI -2.36, -0.62; p=0.00) with high heterogeneity (I^2=98.59%; p =0.00). The results significantly reduced the E. faecalis with CuNPs+ CH than the control group (**Figure 3**).

ZnNPs+ CH

Mean differences of antibacterial effect on E. faecalis between ZnNPs+ CH and CH were -2.54 (MD, -2.54 95% CI -3.39, -1.68; p=0.00) with high heterogeneity (I^2=96.52%; p =0.00). The results significantly reduced the E. faecalis with ZnNPs+ CH than the control group (**Figure 4**).

CHX + CH

Mean differences of antibacterial effect on E. faecalis between CHX + CH and CH were 0.75 (MD, 0.75 95% CI 0.67, 0.83; p=0.00) with high heterogeneity (I^2=99.50%; p =0.00). The results significantly reduced the E. faecalis with CHX + CH than the control group (**Figure 5**).

CHT+ CH

Mean differences of antibacterial effect on E. faecalis between CHT+ CH and CH were 2 (MD, 2 95% CI -5.46, 9.46; p=0.60). The results showed no statistically significant differences in reducing the E. faecalis between groups (**Figure 6**). The mixture of CH and AgNPs was the most effective medicament against E. faecalis bacteria, and CHX displayed the best efficacy at the lowest concentrations against E. faecalis.

Table I: Summary of study data.

N	Study. Years	Study design	Sample size	Teeth	Intervention group (n)	Number of control group	Bacterial strain
1	Rôças et al., 2023[31]	In-vitro	90	necrotic root canals of teeth	2% CHX + CH	CH	bacterial
2	Afkhami et al., 2022[32]	In-vitro	60	single-rooted, sound human teeth with mature apices	2% CHX (10) CH paste (10) CH/CHX: Mixture of CH and 2% CHX gel in 1:1 ratio (10) Group TAP: Metronidazole, ciprofloxacin and minocycline; 0.5 mg of each antibiotic was mixed with 1 mL of saline (10) Group DAP: Metronidazole and ciprofloxacin; 0.5 mg of each antibiotic was mixed with 1 mL of saline (10) CH and AgNPs suspension were mixed in a 1:1 ratio (10) 1: No drug therapy (5) 2: Saline (5)		*E. faecalis* (ATCC 29212)
3	Raza et al., 2022[33]	In-vitro	60	human premolar teeth have a single root	AgNPs + CH (30)	Unmodified CH (30)	*E. faecalis*
4	Tülü et al., 2021[34]	In-vitro	29	Human single-rooted mandibular premola's	AgNPs + CH CHX + CH	saline solution, CH	*Enterococcus faecalis, Streptococcus mutans, Lactobacillus acidophilus*
5	Sy et al., 2021[35]	In-vitro	NR	NR	AgNPs+ CH, CuNPs+ CH, ZnNPs + CH, CHX + CH, CHT	CH alone	*E. faecalis* and *C. albicans*
6	Hegazi et al., 2019[36]	In-vitro	45	human single-rooted teeth	CH+ AgNPs	CH	*E. faecalis*
7	Samiei et al., 2018[22]	In-vitro	132	single root teeth	CH+ ZnNPs, CHX + CH, CH+ AgNPs	normal saline	*E. faecalis*
8	Yousefshahi et al., 2018[20]	In-vitro	NR	NR	CH+ AgNPs, CH +CuNPs, CH +ZnNPs	CH alone	*E. faecalis*
9	Afkhami et al., 2015[17]	In-vitro	54	single-root teeth	CH+ AgNPs, CHX + CH	CH alone (6)	*E. faecalis*
10	Javidi et al., 2014[37]	In-vitro	66	human single-rooted teeth	CH+ AgNPs	CH alone	E. faecalis

AgNPs: silver nanoparticle; CHX: Chlorhexidine; CH: Calcium hydroxide paste; CuNPs: Copper Nanoparticle; ZnNPs: zinc nanoparticles; CHT: chitosan.

Table II: Quality of the included studies.

Study. Years	Item grade													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Rôças et al., 2023[31]	√	√	×	×	×	×	×	×	×	√	×	×	×	×
Afkhami et al., 2022[32]	√	√	√	√	√	√	√	×	×	√	√	√	×	×
Raza et al., 2022[33]	√	√	√	√	×	√	×	×	×	√	√	√	×	×
Tülü et al., 2021[34]	√	√	√	√	√	√	√	×	×	√	√	√	×	×
Sy et al., 2021[35]	×	×	×	×	×	×	×	×	×	√	√	√	×	×
Hegazi et al., 2019[36]	√	√	×	×	×	×	×	×	×	√	×	√	×	×
Samiei et al., 2018[22]	√	√	√	√	√	√	√	×	×	√	√	√	×	×
Yousefshahi et al., 2018[20]	×	×	×	×	×	√	×	×	×	√	√	√	×	×
Afkhami et al., 2015[17]	√	√	√	√	√	√	√	×	×	√	√	√	×	×
Javidi et al., 2014[37]	√	√	×	√	×	×	×	×	×	√	√	√	×	×

Yes: √; No: ×

Table III: Risk assessment.

Study. Years	Allocation concealment	Sample size	Blinding	Assessment methods	Selective outcome reporting	Score	Risk of bias
Rôças et al., 2023[31]	1	0	2	0	0	3	Low
Afkhami et al., 2022[32]	1	0	2	0	0	3	Low
Raza et al., 2022[33]	1	1	2	0	0	4	Moderate
Tülü et al., 2021[34]	1	0	2	0	0	3	Low
Sy et al., 2021[35]	2	2	2	2	0	8	High
Hegazi et al., 2019[36]	1	1	2	2	1	8	High
Samiei et al., 2018[22]	1	1	2	0	0	4	Moderate
Yousefshahi et al., 2018[20]	2	2	2	1	1	8	High
Afkhami et al., 2015[17]	1	1	2	0	0	4	Moderate
Javidi et al., 2014[37]	1	0	2	0	0	3	Low

Figure 2: The forest plot showed a reduction of bacterial load after AgNPs+ CH and CH.

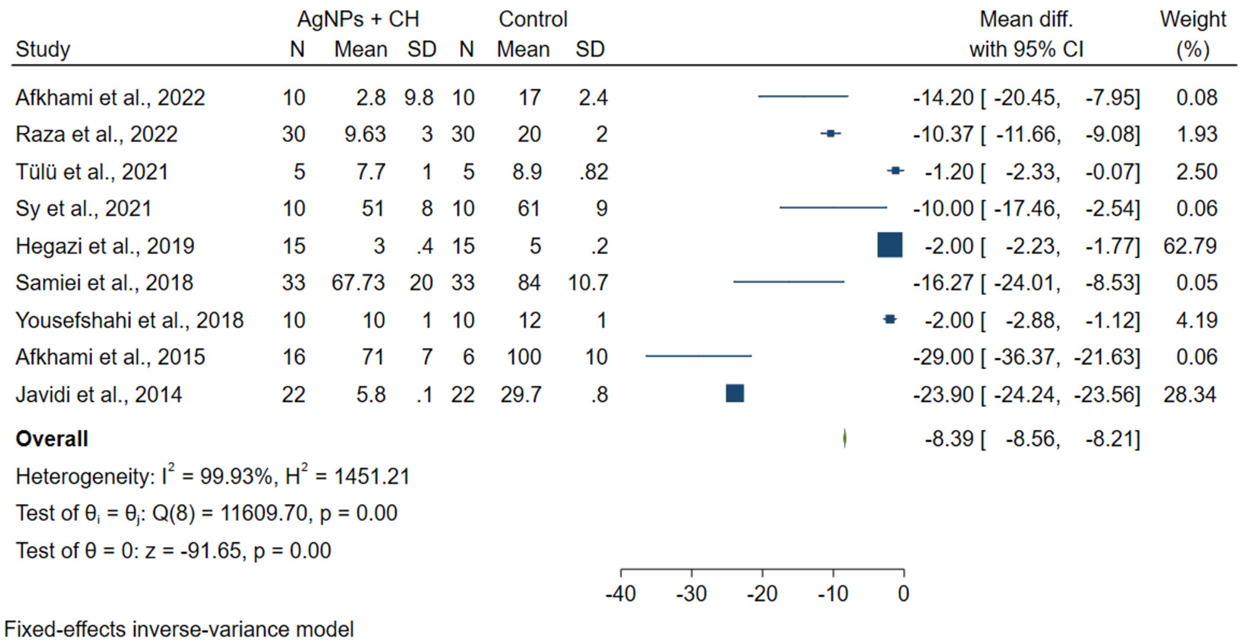


Figure 3: The forest plot showed the Mean colony count in the groups after CuNPs+ CH and CH.

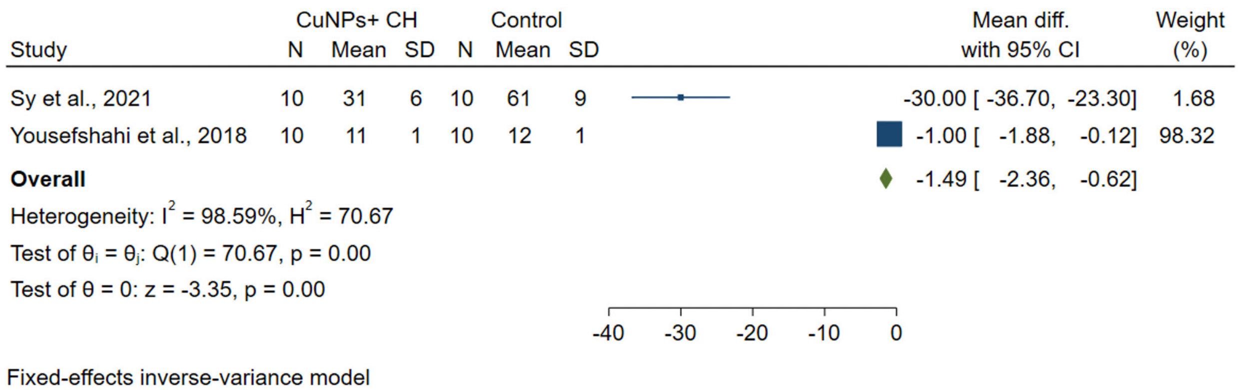


Figure 4: The forest plot showed the Mean colony count in the groups after ZnNPs+ CH + CH and CH.

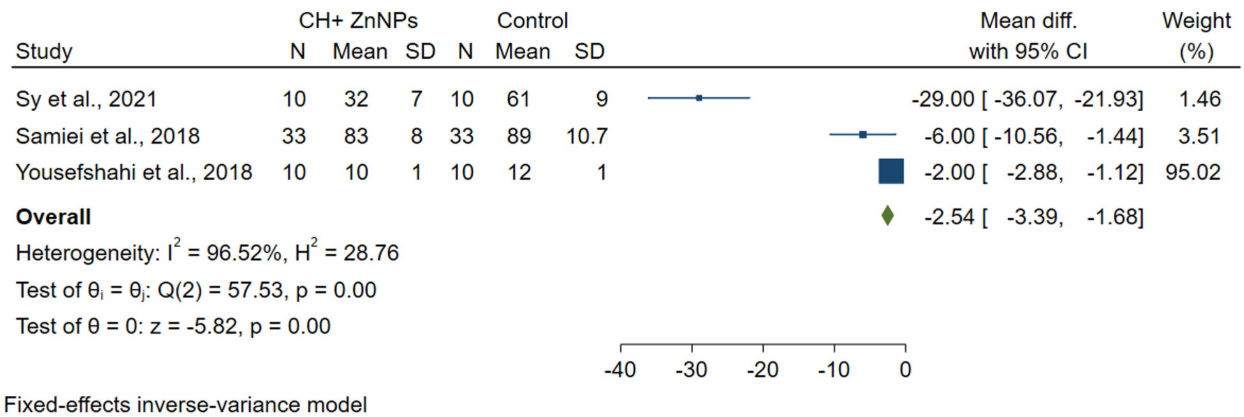


Figure 5: The forest plot showed the Mean colony count in the groups after ZnNPs+ CH + CH and CH.

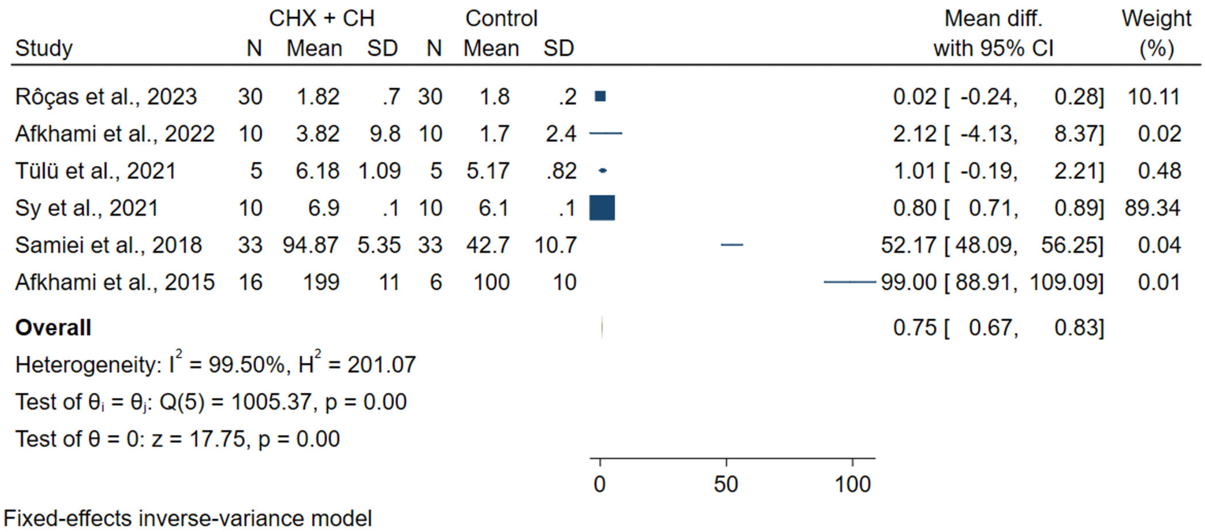
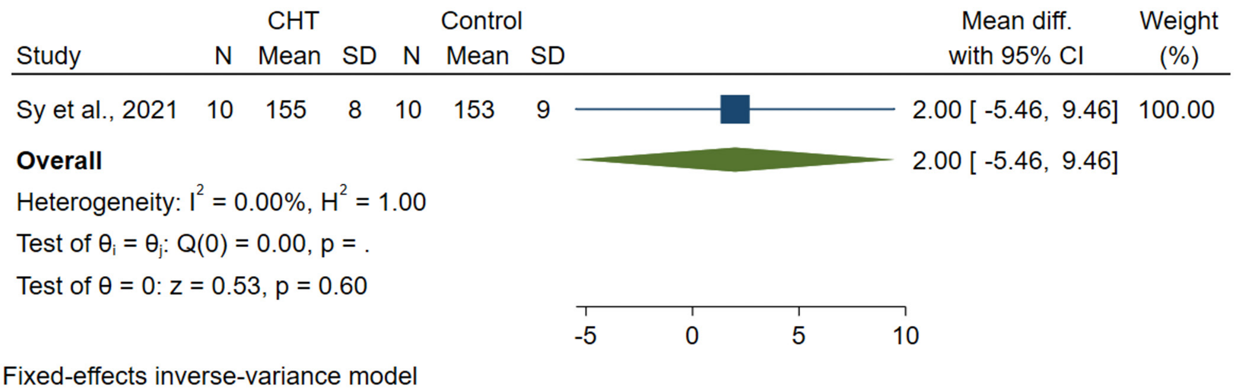


Figure 6: Forest plots showed a reduction of bacterial load after CHT+ CH and CH.



Discussion

The present study investigated the antimicrobial activity of CH mixed with metallic nanoparticles, CHT and CHX compared to CH alone. In the present study, an attempt was made to investigate studies that had performed antibacterial activity in the eradication of E.faecalis in a laboratory manner Because studies have shown that E. faecalis, which is associated with root canal infections, is resistant to CH[4,38]. One of the goals of the present study was to investigate CH paste mixed with different metallic salts (Ag2SO4, CuSO4, ZnCl2). However, due to the existence of very few studies in this field and contradictory results, it was not possible to conduct a meta-analysis. A study showed that zinc oxide has no antimicrobial effect on E. faecalis[39]. Contradictory findings have also been reported with similar metals for other bacteria found in the mouth[40,41]. Based on the findings of the present study, the combination of silver nanoparticles with CH showed an antibacterial effect compared to CH alone. The findings of most studies are in line with the study of the esophagus, while some studies could not show this advantage[17]. A high heterogeneity was found between studies, which can be attributed to differences in methodology or characteristics of nanoparticles and concentrations used. Studies have shown that the size of AgNPs less than 10 nm can increase antimicrobial activity; however, higher toxicity has also been reported[42-45]. According to the available evidence, the antibacterial activity of AgNP may also depend on the shape[46]. According to the present meta-analysis, CH + CHX is more effective at reducing the number of E. faecalis bacteria than CH paste alone. A systematic review and meta-analysis of nine studies found many findings that matched those of the present study[47], A significant difference was not observed between CH + CHX and CH alone in their effects on E. faecalis. The difference in findings can be attributed to the selection of newer studies.

Newer studies published in the last ten years can provide better results; however, high heterogeneity was observed, which could be related to the cognitive methodology of the studies. Based on the present meta-analysis, it was observed that the combination of CuNPs + CH ZnNPs + CH is effective in reducing the number of E. faecalis bacteria compared to CH paste alone. However, according to the present meta-analysis, the combination of CHT + CH was not different compared to CH paste alone. Therefore, the present study does not support using CHT metal nanoparticles as an antimicrobial adjuvant for CH-based drugs. The current study had limitations: the sample size of the studies was small, which requires more studies with a larger sample size. Also, high heterogeneity between studies was observed, which can be related to the cognitive methodology of the studies. Consequently, interpreting the results of this study should be done with caution. Also, all studies were in vitro, making it possible to approximate only a number of parameters involved in vivo. Only in most studies, the antimicrobial activity against E. faecalis was investigated. A multimicrobial biofilm consists of root pathogens, each of which is sensitive to different antimicrobial agents in vivo. These results need to be confirmed and correlated with clinical outcomes in future studies.

Conclusion

Based on the findings of the present meta-analysis, it was observed that a mixture of CH and CHX can lead to the eradication of E. faecalis as an intracanal medicine. Also, silver, copper, and zinc nanoparticles showed antimicrobial activity. The use of CHT as an antimicrobial adjuvant for CH-based drugs was not supported. Further studies are needed to confirm the results of the present study, and further studies are needed to optimize the antimicrobial activity of CHT/CDP metal nanoparticles before using them as an adjunct to endodontic therapy.

Conflict of Interest

The authors declare that there is no conflict of interest.

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