SYSTEMATIC REVIEW

Influence of doxycycline on wound healing: a systematic review, meta-analysis and GRADE assessment of animal experimental trials

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Abstract

Objective To evaluate the influence of doxycycline on wound healing activity compared to control interventions, such as saline, chitosan, or other pharmacological agents.

Materials and methods The following databases Medline (PubMed), Embase, Scopus, and Web of Science were searched for the relevant studies. A total of 94 records were screened after the removal of duplicates and 16 trials were included. Considering the similarity in outcome measures, data was pooled from nine trials using Comprehensive Meta Analysis (CMA) 3.0 software. The level of significance was $p<0.05$ and the certainty of the evidence was evaluated using GRADE pro GDT.

Results A pooled analysis of nine trials assessing the wound healing in percentage (SMD 2.952, 95% CI:[0.597 to 5.307], $p<0.014$) significantly favoured doxycycline over the control agents. In the SYRCLE tool, four out of nine criteria displayed a low risk of bias in more than 50% of the included studies (more than 8 studies). This, along with low/unclear risk of bias, minimised imprecision and indirectness in the included trials, leading to high certainty of evidence.

Conclusion Doxycycline shows notable advantages in wound healing compared to placebos or other agents, but replicability in clinical settings is necessary. Definitive pooled evidence from human trials is crucial to determine its precise clinical implications.

Keywords animal trial, antibiotic, doxycycline, MMP, wound healing

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Introduction

Wound healing is a complex and dynamic process involving various cellular and molecular events to restore tissue integrity. Several factors influence the rate and efficiency of wound healing, which include inflammation, angiogenesis, collagen deposition, and tissue remodelling. Infection stands as a pivotal factor profoundly influencing wound healing dynamics. Doxycycline is a member of the broad-spectrum tetracycline antibiotic group. It is widely used for its diverse therapeutic applications and it plays a multifaceted role in wound healing. Its primary mechanism of action involves inhibiting bacterial protein synthesis by binding to the 30S ribosomal sub-unit, thereby halting bacterial growth and replication. Beyond its primary function in combating infection, doxycycline exerts additional therapeutic effects by mitigating inflammation. By inhibiting pivotal enzymes, such as matrix metalloproteinases (MMPs), responsible for extracellular matrix degradation during inflammation,

doxycycline aids in preserving tissue integrity and fostering an environment conducive to healing. Studies have suggested doxycycline plays a significant role in influencing those factors and enhances the wound healing process.[1] Doxycycline is a compelling option for clinical consideration in wound healing protocols for several reasons. Firstly, its extended half-life facilitates convenient once-daily dosing, enhancing patient compliance and simplifying treatment regimens. Furthermore, its long-standing presence in the pharmaceutical landscape, coupled with its off-patent status, renders it a cost-effective choice for healthcare providers and patients alike. Compared to its counterparts like tetracycline, doxycycline demonstrates a markedly lower incidence of side effects and adverse events, ensuring a favorable safety profile for widespread use. And, when the pharmacokinetic profile is being considered, in comparison with the first-generation tetracycline, orally-administered doxycycline is rapid and completely absorbed with exceptional tissue penetration and a longer half-life of action.[2,3] These attributes collectively strengthen the rationale for incorporating doxycycline into wound management strategies, offering a pragmatic and efficacious solution for promoting optimal healing outcomes in clinical settings.

One of the key mechanisms through which doxycycline influences wound healing is its potential anti-inflammatory properties, by inhibiting the activity of enzymes like MMPs, which are responsible for the degradation of extracellular matrix during inflammation.[4] The increased MMP load in the wound site can be attributed to a load of bacteria (infection), non-viable tissues and repetitive mechanical distress to the wound site. Obstinate elevation of these enzymes leads to uncontrolled degradation of existing and newly depositing matrix components like collagen, glycosaminoglycans and proteoglycans, and results in degradation of various growth factor proteins which are required to coordinate the process of wound healing. Moreover, doxycycline suppresses pro-inflammatory cytokines like interleukin-1 (IL-1) and tumour necrosis factor-alpha (TNF-α) which plays a pivotal role in the initiation and perpetuation of the inflammatory phase of wound healing.[5,6] By modulating their expression in the wound site, doxycycline reduces inflammation and promotes a more favourable environment for healing. Also, doxycycline has been demonstrated to upregulate the expression of vascular endothelial growth factor (VEGF), thereby enhancing the process of angiogenesis.[7,8] This action accelerates the delivery of oxygen and nutrients to the wound site expediting the healing process.[9] Collagen being the primary structural protein in the extracellular matrix plays a crucial role in the healing of any wounds, doxycycline has been shown to stimulate the fibroblast cells which are responsible for producing collagen.[10] These potential activities against MMP and other pathophysiological interactions result in an effective approach towards wound healing.

Doxycycline, in addition to its standard antibiotic properties, has demonstrated potential benefits for wound healing in cases where healing is compromised. It can serve as an effective anti-infective agent while also promoting better wound repair. Although they are emerging, there are only a few human trials in the literature exploring potential drugs for accelerating the process of wound healing. Animal experimental trials, however, are more abundant and so this review looked to them for evidence relevant to the hypothesis about doxycycline's influence on wound healing.

Methods

Review question

This systematic review was conducted following the "Preferred Reporting Items for Systematic Reviews and Meta-Analyses" (PRISMA) guidelines, to answer the following research question: "In an animal experimental trial, does doxycycline improve wound healing compared to other control agents in subjects with wounds or ulcers?"

Inclusion and exclusion criteria

The eligibility criteria were based on the PICOS checklist:

- **(P) Population:** Wound/Ulcer in subject
- **(I) Intervention:** Doxycycline used as a treatment of these wounds.
- **(C) Comparison:** Other drugs or placebo
- **(O) Outcomes:** Wound healing
- **(S) Study design:** Animal experimental trial

Original studies that addressed the "Subjects Intervention Control Outcome" parameters as per PICOS were eligible for inclusion. Ex-vivo cell studies, case reports/series, and observational studies were all excluded.

Type of the study: This systematic review included animal experimental trials, comparing the effect of doxycycline with any other placebo or positive control on wound healing parameters.

Search strategy for article identification

Two independent researchers (SS and GS) conducted an electronic search for animal trials via four databases, namely: Medline (via PubMed), Web of Science, Scopus, and EBSCO up to 4 March 2023. The search strategy for each database is summarised in Table 1. The search was limited be language to articles published in English, by time to between 2010 and 2023.

Screening of articles

Upon pooling the articles from the databases, considering the similarities in title, author, and the year of publication the duplicates were removed. The titles and abstract level of screening were performed independently by two authors (SS and GS). Studies that did not meet the specified PICO criteria were excluded following the authors' agreement. Disagreements related to the selection of articles were

resolved via discussion and consultation with a third author (RM). Then, the full text of the eligible articles was screened individually by two authors (SS and GS) and the disagreement was resolved via discussion.

Data extraction

For all studies that fulfilled the eligibility criteria, data extraction was performed by two authors (SS and GS) comprised of the following parameters: (a) author and year, (b) subjects, (c) number of sites observed in the subject, (d) location of wound, (e) test group, (f) control group, (g) assessment measure with duration of observation, (h) outcome (percentage of wound healing between the test and control group), (i) inference. This data is shown in Tables 2 and 3.

Risk of bias

The studies were independently assessed for Risk of Bias (ROB) using the SYstematic Review Centre for Laboratory Animal Experimentation (SYRCLE) tool[11] by the two review authors and disagreement among them was resolved by the third reviewer (LP). This tool is based on the Cochrane Collaboration that aims to assess methodological quality and it was adapted to appraise aspects of bias that play a role in animal experiments.[12] The ROB for individual studies and a summary graph was plotted using the software Review Manager (RevMan), version 5.0.

Effect measurement and statistical analysis

Data extracted data from the studies was entered into Microsoft Excel 2016. Then Comprehensive Meta-Analysis software Version 3.0, Biostat Inc. USA[13] was used for meta-analysis of selective studies which has outcomes in a comparable manner from systematically reviewed articles. For the outcome of 'percentage in wound healing', the standardised mean difference (MD) with 95% confidence intervals (CI) were used to summarise the data for each group. The random effect model was selected because of the expected heterogeneity between the included studies. We analysed the publication bias using Egger's test and depicted it as a Funnel plot. Statistical heterogeneity was estimated using I^2 analyses, interpreting an I^2 value greater than 50% as a significant level of heterogeneity.[14] In cases where higher heterogeneity was present, sensitivity analysis was performed considering the variance and outliers in the effect estimate of each study. Statistical significance was determined as $p<0.05$ for all analyses. Funnel plots were constructed using the Comprehensive Meta-analysis software Version 3, to assess the publication bias and identify the heterogeneity and a further grade of evidence table was generated using the Grading of Recommendations, Assessment, Development and Evaluations (GRADE) approach and the software GRADEpro GDT (https://gradepro.org/).

Results

Study selection

The studies were screened by two authors (SS and GS) based on the PICO criteria at the title and abstract level and at the full-text level including 16 articles finally for the review and based on the comparable similar outcomes in those studies, nine were utilised for generating quantitative evidence synthesis in the form of forest plot graph (Figure 1).

General characteristics

After screening at the level of title and abstract and the full text, based on the inclusion and exclusion criteria, finally, 16 articles were processed for data extraction. Although most of the studies had several test groups, only our subject of interest (doxycycline group-related data) was extracted along with its the positive or negative control and tabulated (Table 2). Among these, the wound healing parameter was the major outcome of the evaluation, in which the percentage of wound healing was extracted from the studies at varied durations ranging from day 5 to 6 weeks. The location of the wound was predominantly the skin[15–24] or cornea,[7,25–28] and in one study the wound healing parament was bone-implant osseointegration.[29] The animals used in the studies included rabbits, rats, dogs and mice. Rather than considering the number of animals per group for the sample size, the number of sites was accounted for as in some studies the counterpart side was used as a control for the same study group.

Main outcome of the study

Table 3 shows a summary of the outcome measures from the studies included in the review, in which studies evaluating the percentage of healed wounds between the groups infer that doxycycline enhances the healing of wounds compared to its control, with its activity towards reducing the proinflammatory cytokines, IL-1β, IL-6, MMP-8, MMP-9 and other molecular pathways. In one study, the healing of wounds was assessed in days to attain complete healing where it was evident that, the doxycycline group had faster wound healing by 1.73

Table 1. Search strategy of the study

Database	No.	Search strategy
Medline (PubMed)	5	(wound healing[MeSH Terms]) OR (wound healings[MeSH Terms])) AND (doxycycline[MeSH Terms])
Web of Science	80	(TS=(wound healing)) AND TS=(doxycycline)
SCOPUS	45	(TITLE-ABS-KEY ("wound healing") AND TITLE-ABS-KEY ("doxycycline"))
EMBASE	7	('Wound healing':ti,ab) AND ('Doxycycline':ti,ab)

days compared to any of the control drugs or placebo.[7] All the studies which included induced corneal ulcers for evaluation,[7,25–28] significantly favour doxycycline for wound healing.

Risk of bias

In summary, the following sections were considered: selection bias (sequence generation, baseline characteristics and allocation concealment), performance bias (random housing and blinding), detection bias (blinding of outcome assessors), attrition bias (incomplete outcome data), reporting bias, and other biases (calibration). Studies were classified as having "high risk of bias", "low risk of bias", or "unclear", for each of these sections (Figure 2). Overall, the studies were assessed for risk of bias based on these nine criteria. Among the 16 studies evaluated for ROB, four studies[7,19,25,26] were scored with more than 50% of the criteria in the low-risk category (Figure 3). Thus, overall, the risk of bias is moderate with all the included studies.

Quantitative synthesis

Meta-analysis was performed only with nine of the included sixteen studies. No further comparison was possible due to the lack of similar outcome measures.[7,17,24] Although in some studies the evaluation was in the percentage of wound closure, they were not considered because of lack of clarity in data presentation about confidence intervals.[19,25-27]Quantitative results showed a statistically significant increase in the efficiency of wound healing with doxycycline in any of its forms in comparison with placebo/ drugs (SDM=2.952; 95% CI(0.597 to 5.307); p=0.014) (Figure 4). The highest (12.11) relative weight towards the pooled estimate value was contributed by a study that favoured doxycycline.[21] Although from the graph it is evident that out of nine studies included for analysis, two studies presented results favouring the comparator, the pooled estimate along with its confidence limits favours the doxycycline for wound healing without its interruption on the line of no effect.

Publication bias

Funnel plots were constructed using the Comprehensive Meta-Analysis software Version 3, to assess publication bias and identify heterogeneity. The obtained funnel plot presented no proof of obvious publication bias for the included studies. The Egger's test was insignificant with a p-value of 0.351. Five studies out of the nine studies fell completely outside the funnel plot, suggesting that these were the main source of heterogeneity (Figure 5).

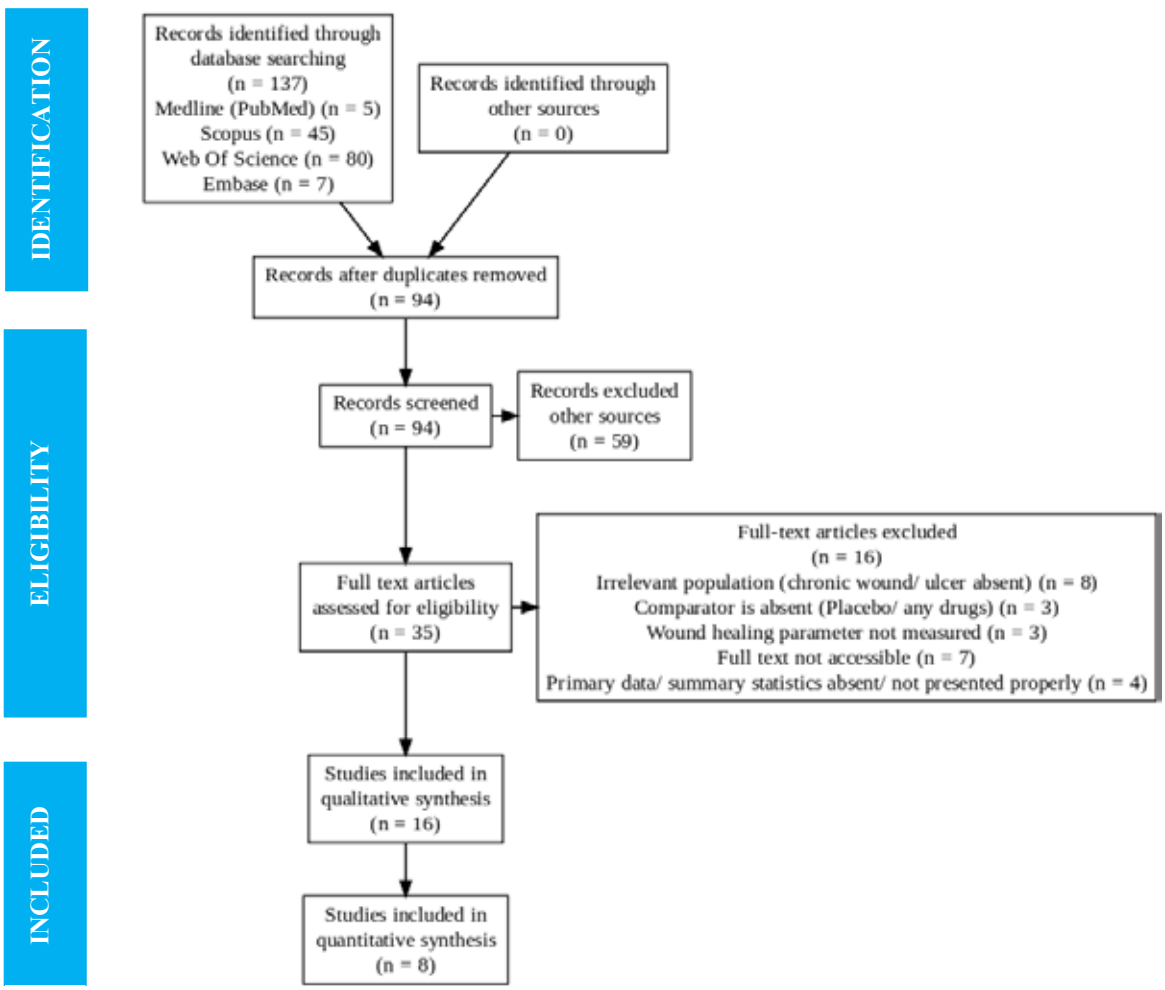

Figure 1: PRISMA Flow diagram of literature search process

Table 2. General characteristics of studies included

S.no	Author, year	Subjects	No of sites	Wound location	Test group	Control group
1	Perry HD et al 1993[27]	Dutch belted rabbits	24	Corneal epithelium	1.5mg/kg oral doxycycline 5mg/kg oral doxycycline	No intervention
2	Hebda PA et al 2003[24]	FVB white mice	14	Skin	Doxycycline (2mg/ml) treated water	Untreated water
3	Kopman JA et al 2005[29]	Sprague Dawley rats	32	Right femur	Diabetes test groups treated with Doxycycline	Diabetic control
4	Adhirajan N et al 2009[23]	Female Wistar rats	60	Skin	Doxycycline treated group	Sterile saline group
5	Chandler HL et al 2010[25]	Adult dogs	89	Corneal ulcers	Doxycycline with triple antibiotic ointment	Cephalexin with triple antibiotic ointment
6	Su W et al 2011[7]	Female Sprague-Dawley rats	120	Corneal burns	DTSH (doxycycline temperature-sensitive hydrogel)	Saline treated
7	Bian F et al 2016[26]	Female C57BL/6 mice	168	Corneal burns along with dry eyes	Doxycycline treated	Balanced salt solution treated
8	Yi Q et al 2019[28]	Sprague-Dawley rats	84	Corneal burns	Doxycycline group	Phosphate-buffered saline group
9	El-Ela FI et al 2019[22]	Albino rats	42	Skin	Doxycycline ointment	Standard Fucidin ointment
10	Hedayatyanfard K et al 2020[21]	Wistar male rats	108	Skin	• n-C/P/D- nanofiber Chitosan/Polyvinyl alcohol/ Doxycycline • f-C/P/D- film Chitosan/ Polyvinyl alcohol/ Doxycycline	• n-C/P- nanofiber Chitosan/ Polyvinyl alcohol • f-C/P- film Chitosan/ Polyvinyl alcohol
11	Tort S et al 2020[20]	Wistar Albino rats	18	Skin	Doxycycline wound dressing	Commercial wound dressing
12	Varshosaz, J et al 2020[19]	Male Wistar albino	24	Skin	Doxycycline loaded RGD (tripeptide Arg-Gly-Asp)-free membranes	Blank RGD free membranes
13	Altoé LS et al 2021[18]	Male Wistar rats	15	Skin	Doxycycline 10 mg/kg/day Doxycycline 30 mg/kg/day	Distilled water
14	Khosravian P et al 2022[17]	Female Wistar rats	84	Skin	ChMesND (Chitosan skin patch loaded with doxycycline)	ChMesN (Chitosan skin patch containing drug-free mesoporous silica nanoparticles)
15	Rozan HE et al 2022[16]	Sprague Dawley rats	20	Skin	Doxycycline	Hydroxybutyl chitosan
16	Tallapaneni V et al 2023[15]	Wistar albino rats	24	Skin	Chitosan hydrogel with doxycycline	Chitosan hydrogel

Sensitivity analysis

As there exists a higher heterogeneity value of >75% in the analysis which included 9 studies, the results from the funnel plot infer that five studies might be responsible for the existing heterogeneity. After removing those studies with higher variance (more than one)[15,16,23,28], sensitivity analysis was performed. The I^2 value remains relatively stable with higher values after removing outliers, suggesting that the observed heterogeneity in the meta-analysis is likely driven by legitimate differences between the included studies rather than extreme values from those five studies. This indicates the robustness of the meta-analysis findings regarding the degree of variation across studies (Figure 6).

Certainty of evidence

Grades of evidence describe the strength and the value of the evidence relative to how rigorous the study design was. Considering the certainty assessment parameters like inconsistency, indirectness, imprecision, risk of bias and other factors, the summary of evidence in the nine experimental trials infers the conclusion of high certainty towards the generated evidence (Table 4).

Discussion

The present review considers the drug doxycycline for its non-antibiotic anti-inflammatory activity predominantly against MMPs and other inflammatory mediators. In a similar way to doxycycline against this MMP activity, corticosteroids such as dexamethasone act against MMP-9 activity via transcriptional inhibition of MMP-9 gene expression.[30] However, Corticosteroid use was typically associated with many cutaneous side effects including delay in wound healing.[30] To this time, there is not much evidence for the effects of any MMP inhibitors other than the tetracycline antibiotic groups on wound healing.

In our review, the wound healing outcome was evaluated either through a quantitative percentage of wound closure or duration of healing in days. Out of the 16 trials included in the review, data from seven studies could not be pooled for quantitative analysis due to variability in the reporting of the outcome variable.[7,17,19,24–27] The pooled effect estimates of nine studies[15,16,18,20–23,28,29] which reported the outcome in the percentage of wound healed showed a statistically significant effect of doxycycline in comparison to control agents like saline, standard wound ointment and chitosan.

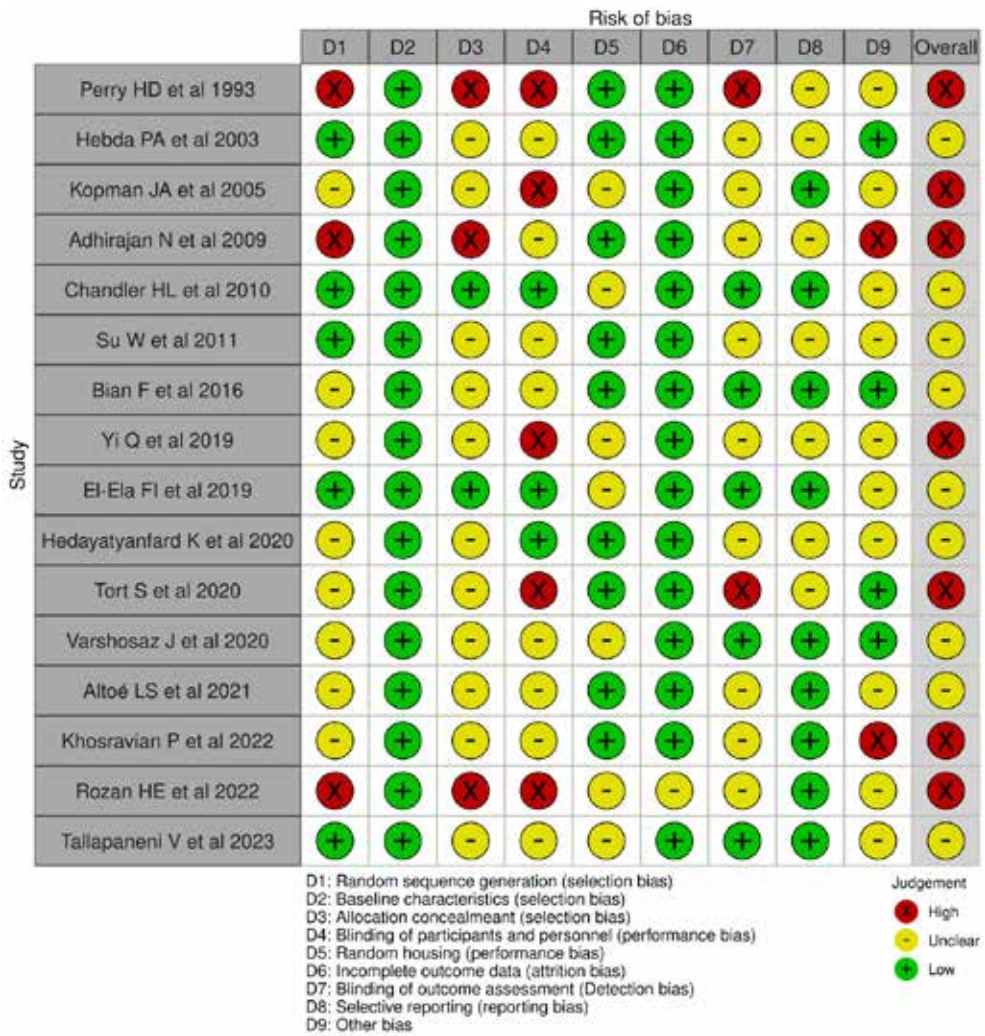

Figure 2. Risk of bias evaluation of individual studies

In contrast to all control agents, hydroxybutyl chitosan, a form of chitosan evaluated in a study by Rozan et al,[16] against doxycycline showed higher efficiency in wound healing which can be attributed to its action through MMP-23 in the dynamic process of cutaneous proliferation and wound healing. Also, it has been shown that hydroxybutyl chitosan combined with doxycycline showed a more synergistic effect towards wound healing than any other comparator. Understanding of this activity of hydroxybutyl chitosan through MMP-23 was further strengthened through a study by Haoyang Xia and colleagues in 2023.[31]

The pooled result of this review on animal trials was in line with the results from a few previous human trials with doxycycline. In one such study from 2008 by Huvenne, W et al[32] doxycycline (DC)-releasing stents were used for chronic rhinosinusitis and wound healing was evaluated against conventional stents, and showed significantly positive results towards doxycycline. Delay in healing post-sinus surgery was predominantly associated with concentrations of MMP-9. Considering the action mechanism of doxycycline investigated through these animal trials, it is evident that doxycycline is efficient in reducing these MMPs, pro-inflammatory cytokines, IL-1β etc. Thus, the human trial with rhinosinusitis wound healing is in line with the results of our review of its action.[18,20,26]

Further supporting the review of its action against MMP, a study by Serra et al[33] on venous ulcer patients inferred that low-dosage oral administration of doxycycline (20mg twice a day) for three months increased the healing efficiency with the decreased expression of MMP-9. With regards to activity in hard tissue, one of the studies we looked at investigated osseointegration, and inferred that the doxycycline group established 11.7% higher bone marrow-to-implant contact than the control group.[29] This result aligns with a study by Bedi et al[34] which demonstrated that doxycycline modulated MMP-13 activity and augmented tendon-bone healing in conditions with rotator cuff tears. Both of these human studies[33,34] support the notion that doxycycline speeds up the wound healing process, as we observed through our review of animal trials.[15,18,20,21,23,25,26,28,29]

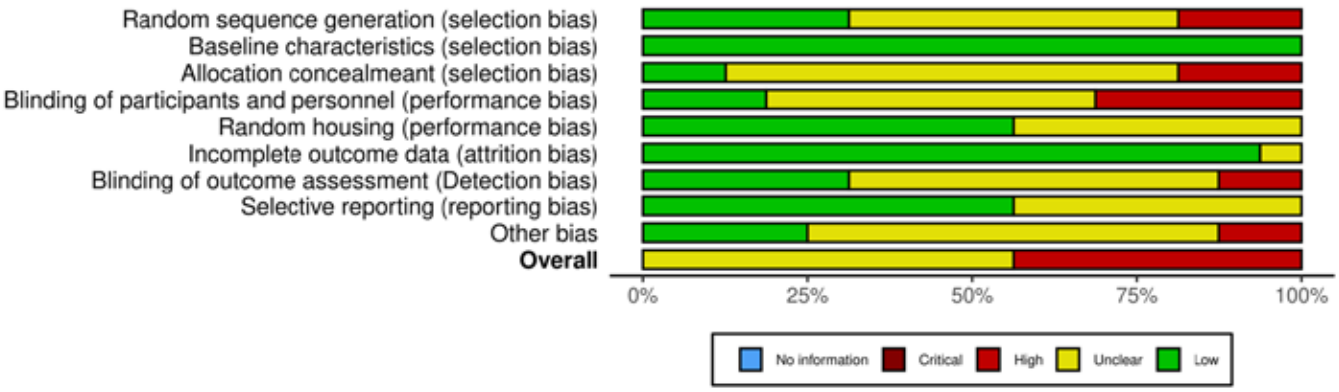

Figure 3. Summary of risk of bias using the SYstematic Review Centre for Laboratory animal Experimentation (SYRCLE) tool

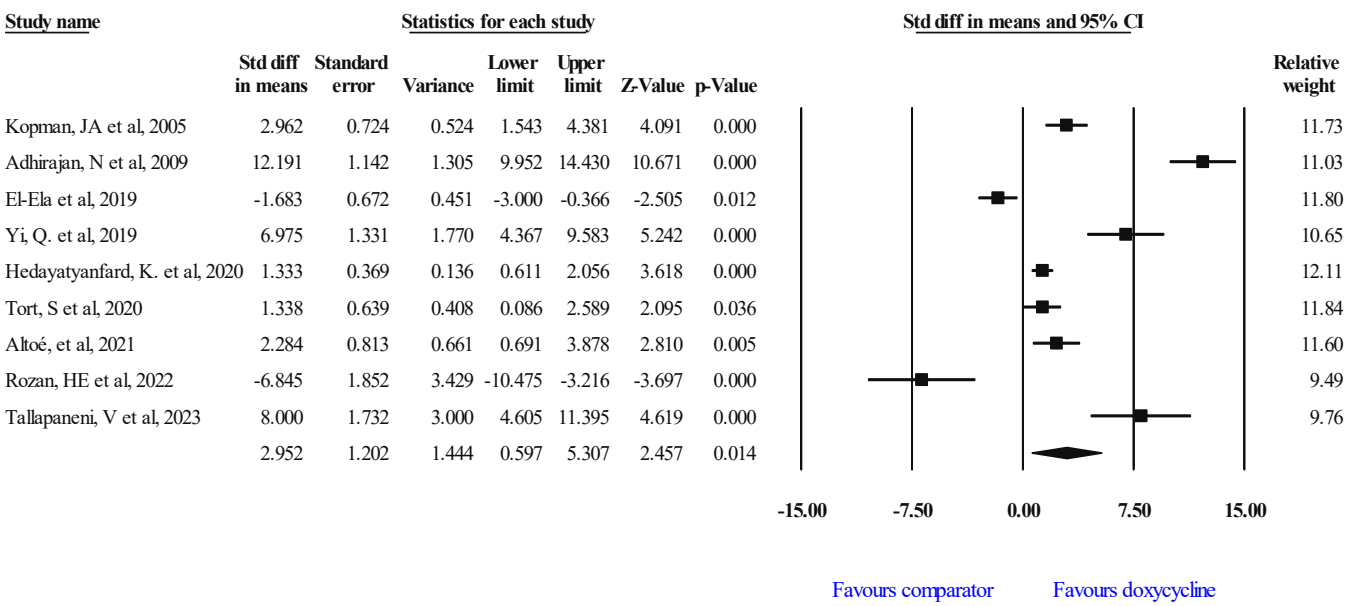

Figure 4. Meta-analysis of doxycycline and control (comparator) interventions on wound healing

Table 3. Main outcomes and inferences from the included studies

S.no	Author, year	Assessment measure (days)	Outcome	Inference
1	Perry HD et al 1993[27]	Percentage of epithelial defect healed (14th day)	1.5mg/kg oral Doxycycline 49.3% 5mg/kg oral Doxycycline 93.9% No intervention 50.0%	Doxycycline treatment resulted in **21.6% higher healing** compared to the control group, regardless of the dosage. However, for systemic administration, a higher dose of 5mg/kg is recommended for more efficient promotion of corneal re-epithelialization.
2	Hebda PA et al 2003[24]	Microscopic assessment of re-epithelialization (8th day)	Untreated water 1.9 Doxycycline treated 2.1	Accelerated collagen organization and tensile strength development was appeared in doxycycline group. Enhanced efficiency of **0.2 higher** value in wound healing score for doxycycline against the control group.
3	Kopman JA et al 2005[29]	Percentage of Bone marrow to implant contact (28th day)	Diabetes Doxycycline group 33.9% Diabetic control 22.2%	Systemic administration of doxycycline slightly enhances osseointegration compared to control. **11.7% higher bone marrow to implant contact** established with doxycycline group than control.
4	Adhirajan N et al 2009[23]	Percentage of wound healed (15th day)	Doxycycline treated 98% Control group 53%	Dressing that releases doxycycline decreased both the infection and metalloproteinase levels, therefore it has a potential application in wound healing. Doxycycline treated group showed **45% higher efficiency** than control group.
5	Chandler HL et al 2010[25]	Percentage of corneal wound ulcers healed (6th week)	Doxycycline 95% Cephalexin 40%	Corneal ulcers in the doxycycline treated group healed rapidly than the ulcers in dogs belonging to control group. **55% higher** wound healing was attained in doxycycline treated subjects.
6	Su W et al 2011[7]	Epithelial healing duration (days)	Doxycycline TSH 3.47 Saline treated 5.20	With DTSH treatment, it influences on the expression of MMPs, vascular endothelial growth factors, iNOS and IL-1β in the corneal burns thereby, **reduces the healing time by 1.73 days** when compared to control.
7	Bian F et al 2016[26]	Percentage of corneal wound healed (5th day)	Doxycycline treated 100% Balanced salt solution (BSS) treated 25%	Doxycycline treatment significantly reduces IL-1β, IL-6, MMP-8, and MMP-9, in comparison with the vehicle. Doxycycline treated subjects had **75% higher** wound closure than BSS treated group.
8	Yi Q et al 2019[28]	Percentage of corneal wound healed (14th day)	Doxycycline 97% Phosphate-buffered saline (PBS) 82%	Doxycycline treatment reduced corneal opacity increases wound healing in rats by reducing TGF-β1, MMP-9, NF-κB, and α-SMA expression. **Difference of 15%** was observed in doxycycline group compared to PBS.
9	El-Ela FI et al 2019[22]	Percentage of wound healed (16th day)	Doxycycline 85.34% Standard Fucidin 89.34%	The percentage of wound healing was **not significant** with plain doxycycline when compared to positive control like standard ointment (-4%).

S.no	Author, year	Assessment measure (days)	Outcome	Inference
10	Hedayatyanfard K et al 2020[21]	Percentage of wound healed (14th day)	n-C/P 89% n-CP/D 93% f-C/P 92% f-C/P/D 93%	The doxycycline group improves wound healing by decreasing the proinflammatory cytokines and matrix metalloproteinase and as well by increasing the TIMP levels. **4% increased wound healing** efficiency in doxycycline group was observed when compared to control group.
11	Tort S et al 2020[20]	Percentage of wound healed (14th day)	Doxycycline wound dressing 98% Commercial wound dressing 94.1%	Nanofiber wound dressing with doxycycline was not statistically significant in comparison to the commercial product in wound healing. In immunohistochemical studies, it was observed that the doxycycline dressing decreased the MMP levels and increased TIMP levels. The difference in wound closure percentage observed with doxycycline wound dressing was **3.9%.**
12	Varshosaz J et al 2020[19]	Percentage of wound healed (16th day)	Blank RGD free membranes 88.8% Doxycycline loaded without RGD 95.4%	The doxycycline loaded membrane reduced the activity of MMPs without showing any cytotoxic effects. It had improved the wound closure further proved with the histopathological evaluation (re-epithelialization, collagen deposition, and angiogenesis) in comparison to the control groups. **6.6% higher wound closure** was attained with doxycycline loaded RGD.
13	Altoé LS et al 2021[18]	Percentage of wound healed (21st day)	Doxycycline 88% Distilled water 67%	Doxycycline has antioxidant potential by increasing antioxidant enzymes. Additionally, it aids in wound closure by targeting MMP-9. The percentage of **enhanced wound closure with doxycycline was 21%.**
14	Khosravian P et al 2022[17]	Remaining wound area, square mm (21st day)	ChMesN 5.99 ChMesND 1.09	Chitosan, known for its anti-inflammatory properties, becomes even more effective when combined with doxycycline. Notably, the remaining wound area in the doxycycline added chitosan group was **4 mm²** smaller than that in the control (chitosan) group.
15	Rozan HE et al 2022[16]	Percentage of wound healed (6th day)	Doxycycline 74.7% Hydroxybutyl chitosan 86%	The percentage of wound closure attained with the doxycycline was comparatively lesser (11.3%) than that attained with hydroxy butyl chitosan. Yet varied combination with doxycycline and chitosan has proved beneficial effects in wound healing efficiency.
16	Tallapaneni V et al 2023[15]	Percentage of wound healed (21st day)	Chitosan hydrogel with doxycycline 98% Chitosan hydrogel 90%	Percentage of wound contraction attained with doxycycline was **8% higher** against the chitosan hydrogel control.

 # Bold were the differences observed in the doxycycline group compared to the control group

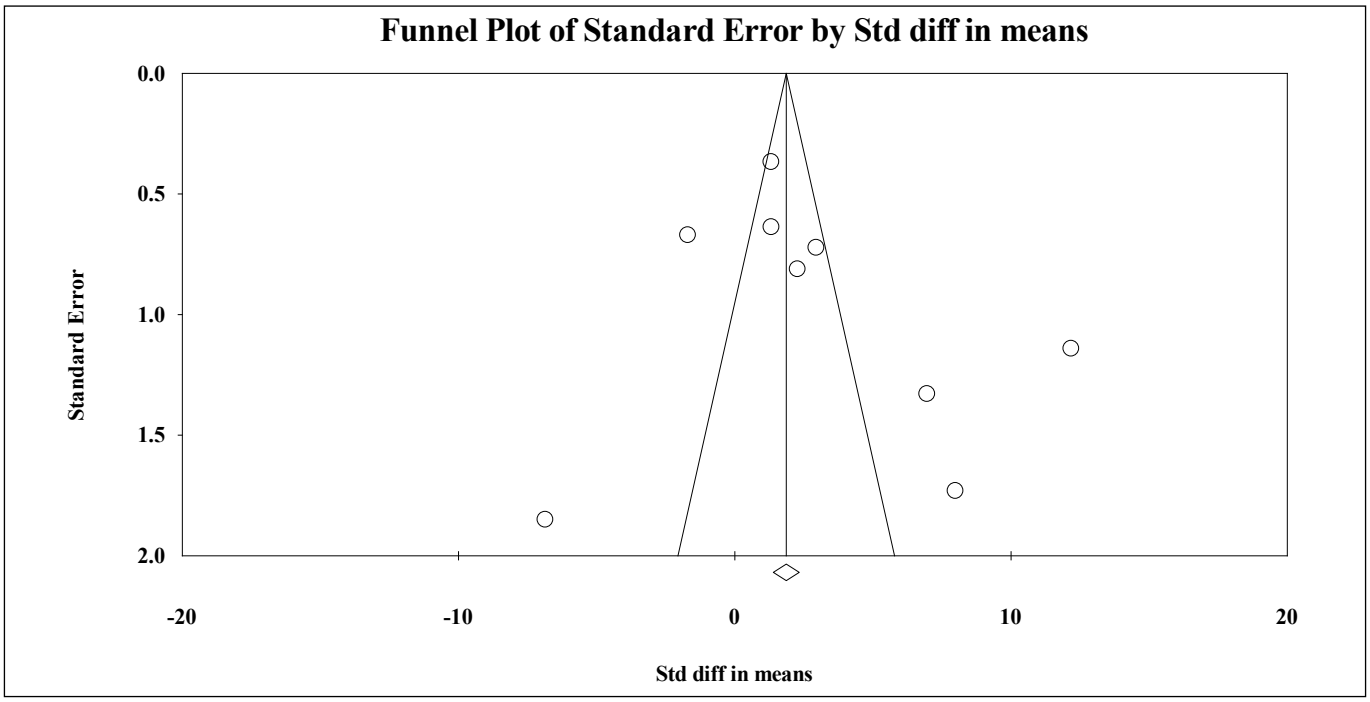

Figure 5. Funnel plots of the meta-analysis for doxycycline and control interventions on wound healing.

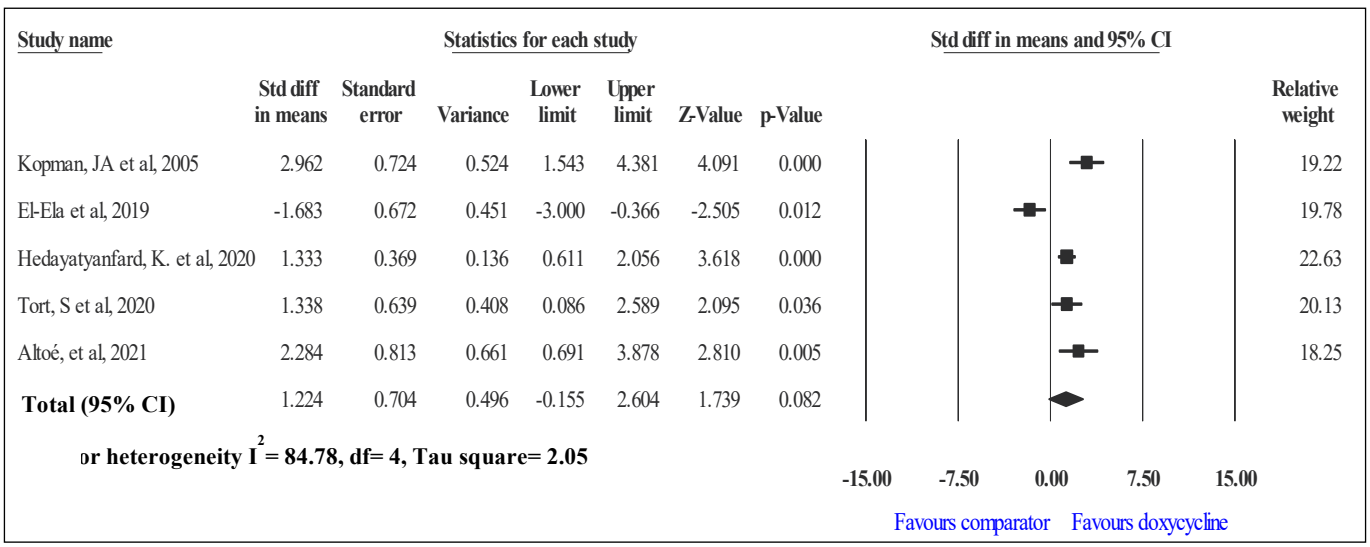

Figure 6: Sensitivity analysis for doxycycline and control (comparator) interventions on wound healing.

In the present review, two predominant sites of the wound were evaluated, skin and cornea. Studies involving the corneal ulcers as the wound site more highly favoured doxycycline compared with any other controls than studies involving the skin.[7,25–28] This was supported by a 2023 study by Juwita.[35] Her review of experimental mice trials involving alkali-induced corneal ulcers treated with doxycycline infers enhanced corneal wound healing and opacity scores with doxycycline usage, in comparison with the control agents.

In the post-wound scenario, those sites would be usually occupied by pro-oxidants and present with decreased synthesis of the antioxidant enzymes, such as superoxide, glutathione, and catalase, which impair the healing environment towards wound closure.[36] In a study by Altoe, et al[18] the amount of antioxidants present was evaluated. It was observed that there was an increase in the antioxidant enzymes (catalase and superoxide dismutase) after doxycycline exposure, which demonstrates its antioxidant potential. Furthermore, doxycycline accelerates the closure of skin wounds by a series of activities like increasing type 1 collagen and elastic fibres, and by reducing the levels of MMP. The percentage of enhanced wound closure with doxycycline was 21% higher than its comparable counterpart.

Table 4. GRADE Summary of findings for doxycycline versus placebo/ any drugs for its influence on wound healing

Certainty assessment							No. of patients		Effect		Certainty	Importance
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Doxycycline	Placebo/ any drugs	Relative (95% CI)	Absolute (95% CI)		
Wound healing (assessed with: Percentage of healing)												
9	Animal experimental trials	Serious[a]	Serious[b]	Not serious	Not serious	Strong association, dose response gradient is present	91	91	-	SMD 2.952 higher (CI= 0.597 to 5.307)	⊕⊕⊕⊕ High	Important

Explanations
a. There exists a moderate risk of bias in the included studies assessed using SYRCLE risk of bias tool.
b. Test for heterogeneity I^2 value= 95.13 which depicts there exists considerable heterogeneity between the studies.

Inference:
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

CI: Confidence interval; **SMD:** Standardised mean difference

Regarding the risk of antimicrobial resistance with the usage of doxycycline for its wound healing potential, the conventional antimicrobial doses of doxycycline can result in bacterial resistance through selection pressure and alteration of the normal commensal microflora.[37,38] This "adverse effect" was not reported, even in prolonged administration of low doses of doxycycline (20–40 mg/day). Thus, it appears that it can be safely used at a non-antibiotic anti-inflammatory dose without altering the bacterial susceptibility to these antibiotics.[39,40] The risks associated with the systemic administration of doxycycline, as described in the literature, are rare compared with other second-generation tetracyclines.[41] The main adverse events reported in studies of doxycycline so far are photosensitivity and esophageal erosion. A 2005 study, based on prescriptions recorded by the FDA between 1998 and 2003, estimated adverse events were associated with 13 per million prescriptions in the US.[42]

Limitations and recommendations

Very few studies could be pooled together considering the similarity in outcome assessed. In this study, to minimise the impact of heterogeneity among the included trials, a random effect model was used. However, such higher heterogeneity (I^2= 95.13) might still project some uncertainties in the results. The quality of evidence generated through this review is assessed to be high, considering the bias and heterogeneity in the certainty of the effect estimates. We excluded human trials from our review due to the need for consistent risk of bias assessment with the SYRCLE tool and comparable evaluation measures for quantitative evidence synthesis. However, human clinical trials with large sample sizes should be conducted to evaluate the efficacy of doxycycline in advancing wound healing, in cases where normal healing is compromised.

Conclusion

Our systematic review and meta-analysis demonstrate that doxycycline improves wound healing by significantly reducing MMPs and pro-inflammatory cytokines, thereby enhancing the wound healing environment. Doxycycline accelerates wound closure by increasing type 1 collagen and elastic fibers and boosting levels of antioxidant enzymes, such as catalase and superoxide dismutase. In animal studies it has been particularly beneficial in treating corneal ulcers and enhancing bone-to-implant contact, without the risk of developing antimicrobial resistance at low anti-inflammatory doses. These findings underscore doxycycline's potential as a valuable agent in wound management. Despite the limitations of the present review, the evidence from multiple studies with high certainty suggests that doxycycline, with minimal adverse effects at low doses, can be an efficient drug of choice for enhancing the wound healing process. This is particularly advantageous in clinical settings for patients with systemic conditions that may compromise or delay wound healing.

Author contribution

Sasidharan Sivakumar: Conceptualization of the study, designing the research methodology, and performing meta-analysis and GRADE assessment. Involved in the statistical analysis, interpretation of results, and writing of the manuscript.

Lakshmi Prasanna P: Conducted the literature search, data extraction, and analysis. Played a key role in risk of bias evaluation and in drafting the manuscript and interpreting the findings.

Elamvaluthi M: Performed data extraction and risk of bias evaluation.

Benjamin Rajasekar: Assisted with the literature search, data extraction, and provided critical insights during the data analysis phase. Contributed to the manuscript's review and revision.

Gowardhan Sivakumar: Performed literature search and screening as well as overseeing the systematic review process. Contributed significantly to the writing and critical revision of the manuscript for important intellectual content.

Protocol registration

The study protocol can be accessed through the International Prospective Register of Systematic Reviews, the PROSPERO database with the following register number: CDR42023416533.

Patient and public involvement

Patients and/or the public were not involved in the design, conduct, reporting, or dissemination plans of this research.

Ethical approval

Not applicable.

Patient consent for publication

Not applicable.

Data availability statement

Data are available on reasonable request.

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Competing interest

None declared

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