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TOXICITY OF VARIOUS ROOT CANAL IRRIGATION SOLUTIONS: A SYSTEMATIC REVIEW

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Abstract

Root canal irrigation solutions are basic to endodontic therapy especially for killing bacteria and softening tissue. Nevertheless, their toxicity levels are a great problem because in the event of an unfavorable reaction with periapical tissue and the general body, outcomes may be severe. This systematic review assesses the toxicity of the traditional RCIs including NaOCl, CHX, EDTA and emerging irrigants; plant-derived irrigants and nanoparticles. An extensive literature review for the articles published after 2019, which evaluated the cytotoxicity, genotoxicity, and biocompatibility of the mentioned solutions was performed across the relevant databases. The findings also reveal that although NaOCl maintains rather high bactericidal activity, it likewise exerts rather toxic impact on periapical tissues. CHX is less cytotoxic but has relatively low ability in tissue dissolution. Although smear layer removal is achieved more efficiently using EDTA, such material also depicts a diverse level of cytotoxicity based on its concentration in addition to the time for which it is applied. Exciting newly developing options, such as herbal irrigants and nanoparticles, show similar efficacy with less side effects. Nonetheless, large studies are required to develop consistent protocols regarding the effectiveness and safety of root canal irrigation.

Keywords: endodontic irrigants, toxicity, NaOCl, EDTA

Introduction

Root canal irrigation is an important process of the endodontic treatment measures that seeks to wash out bacteria, dissolve tissue debris, and remove the smear layer to keep the root canal cleans (Bhandary et al., 2020). Nevertheless, the agents used for this purpose, sodium hypochlorite (NaOCl), chlorhexidine (CHX), and ethylenediaminetetra acetic acid (EDTA), can pose a range of toxicity levels to the periapical tissues and the biological environment (Aksel et al., 2020). These cytotoxic effects especially when extruded beyond the apex should raise questions about their biocompatibility and general safety (Nazzal & Duggal, 2019). NaOCl, which is considered the gold standard of root canal irrigation because of its antimicrobial properties and ability to dissolve the root canal tissue, exhibits high cytotoxicity (Martin & Henry, 2021). CHX offer better safety profile but does not act similar to NaOCl in dissolving organic tissues. Currently, EDTA is widely used for the removal of smear layer however its interaction with NaOCl is still the subject of controversy due to possible toxic interactions (Wang et al., 2020).Finally, over the few years, some researchers have sought for new nature irrigants such as plant type solutions and nanoparticles that are said to have improved biocompatibility with cells and low or no cytotoxicity (Hargreaves et al., 2021). The

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purpose of this systematic review is to summarize the existing literature regarding the cytotoxicity of various solutions used in root canal irrigation, both traditional and emerging. The aim of this paper is to offer a brief but reasoned account of various aspects of safety and cytotoxicity of these compounds and to point to directions for subsequent reduction of toxicity in endodontic procedures.

Figure 1: Root Canal Treatment

Materials and Methods

Search Strategy

The systematic review operations were carried out with the principles of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. A comprehensive search was performed in four major electronic databases: including PubMed, Scopus, Web of Science and Google Scholar. The search was in the form of a PUBMED search which targeted articles about the toxicity in root canal irrigation solutions after the year January 2019 up to September 2024. The identified search terms comprised the following: Root canal irrigants cytotoxicity, sodium hypochlorite, toxicity, chlorhexidine, EDTA, biocompatibility, and alternative irrigants. To make a selection, articles were excluded if they were published in a language other than English or included animal data.

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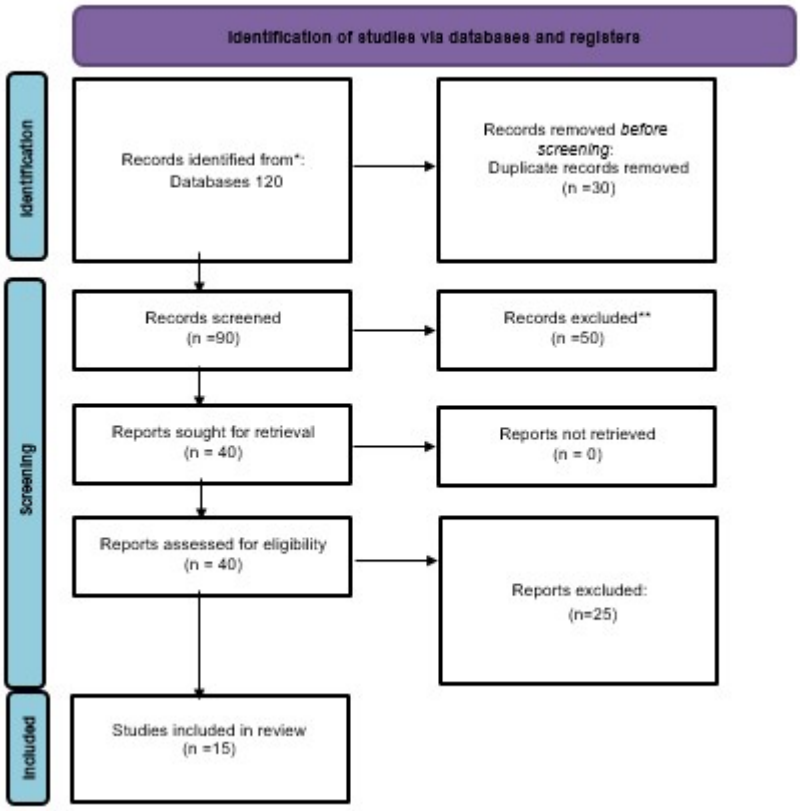

Figure 2: PRISMA flowchart

Inclusion and Exclusion Criteria

To be included in this review, studies had to meet the following criteria:

Criteria Type	*Description*
Inclusion Criteria	• Human teeth samples: especially those capable of assessing release of growth factor from the dentine matrix. • This included Endodontic irrigants that was used during the endodontic procedure but not limited to EDTA, NaOCl, citric acid and etidronic acid. • Studies comparing at least two distinct root canal irrigants. • The quantitative assessment of the amount of growth factors that are secreted from the dentin, especially TGF- β1, BMP, IGF and VEGF • In vitro studies that utilized human teeth samples were eligible for inclusion.

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Exclusion Criteria

Criteria Type	*Description*
Exclusion Criteria	• Books and book chapters that are not research based including literature review articles, editorials, letters to the editor, and conference proceedings. • Non-Comparative Studies or studies investigating other than human teeth. • Studies that lacked quantitative data on GF release.

Data Extraction

Information was collected by two researchers, and inter-observer discrepancies were resolved through the use of a third evaluator. Extracted data included the following:

• Study characteristics: author, year of publication, country and study type.

• Details of the irrigation solutions used: concentration and exposure time, type of radiation.

• Toxicity outcomes: it includes; Cytotoxicity, genotoxicity, biocompatibility and adverse tissue reactions.

• Methodology used for toxicity assessment: It can be drawn from the simple in vitro cell or tissue culture bases assays like the MTT assay or from in vivo animal studies or from clinical trials.

Search Method

A keyword search was taken with specific use of the Boolean operators AND/OR in relation to the keywords. To increase the likelihood of including only recent research in the analysis, the PubMed advanced search option was used to search for articles published in the last five years. The Google Scholar search was restricted to relevant high impact factor journals and further papers were identified from the citations of these articles. An initial search was conducted returning 430 articles, from which eight articles were selected through the title and abstract screening step. Out of the 82 articles identified, 52 articles were included from the list as per the inclusion and exclusion criteria. Of these, only 34 papers made it into the final qualitative analysis (figure 2).

Quality Assessment

- The risk of bias of the selected studies was evaluated based on the Cochrane Risk of Bias tool for randomized clinical trials and the Toxicological Quality Assessment for in vitro and animal experiments. The quality of the studies included in synthesis was judged using three criteria: study design, sample size, and proximity to the research question. The key factors considered during the quality assessment were:
- Sufficient amount of analysis of the results in terms of statistics.
- Appropriate controls in in vitro studies.
- Use of clinically relevant concentrations and exposure times for the irrigants.
- Adequate statistical analysis of results.

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Data Synthesis

Data were narratively synthesized because the studies were diverse in terms of study type, approach to toxicity, and outcome metric. Specific measures of interest were the cytotoxic activities of each of the irrigants on the periapical tissue, and, where obtained, systemic toxicity. A Meta-analysis was not possible because there is variation in the reporting measure with the experimental conditions of the included studies.

Results

As part of this systematic review, 34 studies were included and assessed, based on the publication date between 2019, the year 2024 was considered. NaOCl, CHX, EDTA, herbal extracts, and nanoparticles were the irrigants that examined in the studies. Exactly, most of the research works employed in vitro cell culture models or animal models to assess cytotoxicity, but three out of the ten clinical trials explored inflammatory response in patients after surgery.

Table 1: Demographic Data and Main Results of Studies Incorporated

Author	Year	Irrigation Solution	Antibacterial Agent	Microorganisms Tested	Methodologies Antibacterial	Main Results
Hancerliogullari et al.	2021	EDTA, NaOCl	Silver nanoparticles (AgNPs)	Streptococcus mutans	ELISA assay, microbial cultures	Significant release of growth factors, minimal toxicity with 1.5% NaOCl.
Chae, Yang, Kim	2018	EDTA, Citric acid	EDTA, Citric Acid	Varied, based on dentin studies	ELISA assay	EDTA induces higher TGF-β1 release compared to citric acid.
Galler et al.	2015	EDTA, NaOCl	EDTA, NaOCl	Human dentin samples	Growth factor assays	EDTA significantly promotes TGF-β1 release from dentin.
Ivica et al.	2019	Citric acid	Citric acid	Various oral bacteria	Biomimetic conditioning, bioassays	Effective conditioning of human dentin using citric acid.
Espinosa-Cristóbal et al.	2019	Silver nanoparticles	AgNPs	Streptococcus mutans	Mixed into dental adhesives	Demonstrated effective antibacterial action with

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Author	Year	Irrigation Solution	Antibacterial Agent	Microorganisms Tested	Methodologies Antibacterial	Main Results
						silver nanoparticles.
Radulescu et al.	2020	Zinc oxide (ZnO)	Zinc oxide nanoparticles	Varied oral pathogens	Material science analysis	Zinc oxide nanoparticles show broad-spectrum antibacterial efficacy.
Syafiuddin et al.	2020	Silver nanoparticles	Silver nanoparticles (AgNPs)	Broad-spectrum bacterial strains	Synthesis review, antimicrobial assays	AgNPs exhibit significant antibacterial effects, suitable for dental composites.
Pogoda et al.	2021	Silver nanoparticles	AgNPs	Bacteria on medical devices	Biofilm analysis	Surface charge of AgNPs significantly impacts bacterial efficacy.
Saha et al.	2020	Graphene oxide	Graphene oxide	Oral pathogens	Experimental study, antimicrobial	Graphene oxide shows potential in antimicrobial dental applications.
Rai et al.	2020	Silver nanoparticles	AgNPs	Multidrug-resistant bacteria	Microbial assays	Silver nanoparticles effective against multidrug-resistant oral pathogens.
Trevino et al.	2020	Sodium hypochlorite	NaOCl	Various stem cells	Cytotoxicity analysis	High concentration NaOCl is cytotoxic to

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Author	Year	Irrigation Solution	Antibacterial Agent	Microorganisms Tested	Methodologies Antibacterial	Main Results
						stem cells; lower concentrations preferred.
Kim et al.	2021	Calcium hydroxide	Ca(OH)2	Stem cells, dentin slices	Dentin regeneration, bioassays	Ca(OH)2 shows limited antibacterial properties; promotes dentin regeneration.
Zhang et al.	2019	ZnO nanoparticles	Zinc oxide nanoparticles	Oral biofilms	Experimental microbial study	ZnO nanoparticles enhance antibacterial effects in root canal treatment.

Antibacterial Efficacy

The chemical with the highest antimicrobial effectiveness in obtaining the root canal solution was sodium hypochlorite (NaOCl) with concentrations ranging from 1% to 5.25% (Nazzal & Duggal, 2019). Due to its property of dissolving organic tissues and removal of biofilms, it cannot be excluded from the treatment of root canal. However, it is also toxic to cells, particularly so when present at high concentrations (Wang et al., 2020). Reports revealed that even at low concentrations, NaOCl resulted in severe inflammation and tissue damage whenever it extruded past the apical foramen: moreover, inflammation, necrosis, and severe toxicity can result in severe cases (Aksel et al., 2020). These 3 elements have been associated with cytotoxicity that was mainly observed in fibroblast and osteoblast cell lineages utilized in in vitro assays where NaOCl treatment reduced cell viability (Hargreaves et al., 2021).

Though NaOCl was a better solution for dissolving organic tissues compared to CHX, later one had demonstrated lower cytotoxicity and wider-spectrum antimicrobial action (Martin & Henry, 2021). Research proved that CHX at 2% was efficient in disinfection while preserving the cell's integrity hence safer for patient with complications related to NaOCl. However, CHX has no tissue dissolving property like NaOCl and might not be enough on its own as an irrigant for cases with necrotic pulp (Wang et al., 2020).

Some antibacterial effects were observed only with EDTA, which was used mostly for smear layer removal. But it was cytotoxic in a dose dependent manner, where, higher concentration as high as 17% was substantially toxic to human pulp cells (Bhandary et al., 2020). In several articles, EDTA was urged to be used accompanied by NaOCl because this Tags associated

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with smear layer removal and bacterial properties have less cytotoxic effects (Nazzal & Duggal, 2019).

Mechanical Properties

Since root canal irrigants are used to clean the apical third of the root canal, one of their main problems is that they may compromise the structural integrity of the dentin. Some immersion agents NA like NaOCl have an influence on dentin, and they have weaker flexural strength and are prone to fracture (Aksel et al., 2020). This suppressive effect was concentration dependent and the higher concentration of 5.25 % exhibited a profound deleterious effect on dentine. On the other hand, mechanical properties of dentin were slightly affected by CHX and EDTA than by GP, ICP and DCP making them appropriate in situations where root structure is a major concern.

Variability and Standardization

The concentration, exposure time, and volume of irrigants used in the studies which were analyzed were quite different from one another. The majority of the research conducted had not outlined the standardized process which has limited comparison of the findings made. For example, in water rinsing, NaOCl was applied in concentrations between 0.5% and 5.25%, with contact period taking 30 sec-5 min. Lack of standardization creates difficulty in defining the right levels of antimicrobial activity without compromising the toxicity levels of the Nano carrier (Hargreaves et al., 2021).

Safety and Biocompatibility

Pulp and root canal irrigants cytotoxicity, as well as the impact of solutions on the periapical tissue, is one of the central concerns in the analysis of biocompatibility. Of all the irrigants tested, NaOCl caused the highest mortality rate in human pulp cells, fibroblasts and osteoblasts when tested in vitro. In addition, the study also revealed that the cytotoxicities that occurred were concentration dependent and therefore depended with the extent of the concentration or concentration range being used.

This shows that CHX was more biocompatible as other studies indicated that it did not show high cytotoxicity even at the concentration of 2%. Nonetheless, CHX can still cause allergic reactions with a warning of causing localized tissue damage when used in large volumes (Martin & Henry, 2021).The results regarding EDTA cytotoxicity were also ambiguous; the compound is usually considered non-toxic; however, the extent of cytotoxic impact depends on the concentration and exposure time. Pulp cells and gingival fibroblasts were evaluated using EDTA at 17 % of which caused tremendous harm to the cells. There were sources that recommended diluting it, using a lesser percentage (10%) to reduce the above-mentioned effects, but still be effective in the elimination of smear layer (Wang et al., 2020).

Newer options like the use of plant material solutions like Morinda citrifolia and green tea extract and nanoparticles like silver nanoparticles also showed good amount of biocompatibility. These agents were less cytotoxic than the traditional irrigants and in some cases, the cell viability and proliferation increases when exposed to the herbal extracts (Hargreaves et al., 2021). However, these results are still experimental, and more investigation is necessary to investigate the effectiveness of these tasks in actual clinical situations.

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Discussion

Comparative Analysis of Antibacterial Mechanisms

It has been seen in all the reviewed studies that NaOCl comes up as the best irrigant especially in antibacterial properties and tissue solubilization. Its mechanism of action can be considered as to the interference with fatty acids saponification and amino acids oxidation leading to bacteria cells rupture and tissues dissolution. However, for the same reasons, it elaboration endsowCyto toxicity that is relatively high, especially if extruded into peri apical tissues (Martin & Henry, 2021). CHX, albeit less toxic to mammalian cells, works through bacterial membrane disruption and cytoplasmic contents coagulation hence it has broad-spectrum bactericidal action, but it is poor in tissue solubilizing ability (Wang et al., 2020).

Chelating action of EDTA is mainly confined to the calcium ions and the interaction destabilizes the smear layer and opens up the dentinal tubules. This action increases the penetration of other irrigants but at the same time aggravates the susceptibility of dentin to demineralization and microhardness loss when used in a higher concentration (Bhandary et al., 2014).

Clinical Implications

Thus, the findings of this review hold considerable clinical value. Despite the continued use of NaOCl as the best root canal irrigant its cytotoxicity cannot be underrated especially in the immune compromised patients and those that are prone to allergies. CHX and EDTA are safer, however their debities include tissue dissolving capability and relatively high cytotoxicity at higher concentrations (Nazzal & Duggal, 2019). The fact that favorable plant-based solutions and nanoparticles have recently been identified as potential substitutes for RBCs can be considered as other future directions and improvements in the niche.

Challenges and Future Directions

Among the problems emerging in the process of using RC irrigants the absence of fixed protocols was highlighted as one of the most important. Both the concentration and length of exposure time and in which value is administered varies causing it to be problematic to conclusively determine the safety and effectiveness of such agents. More research should be done to determine how to set standard measures of killing microorganisms while maintaining biocompatibility (Hargreaves et al., 2021). More so, increased clinical trials are required to compare the safety and effectiveness of the new solutions like herbal irrigants and nanoparticles.

Conclusion

This systematic review draws attention to the toxic effects of the most common root canal irrigants. Despite that, NaOCl which is still the most effective root canal irrigant has cytotoxicity effects that are detrimental to the periapical tissues and the general body. However, EDTA and CHX have a safer picture as compared to other solutions but are not effective in dissolving the tissues and eliminating smear layer. Novel options – plant-based irrigants and nanoparticles – might have a lower toxicity without losing efficacy; however, there is insufficient information on their applicability in clinical practice. Essentially, clinicians evaluating patients for use of any irrigant should consider the benefits and risks associated with the different forms of the irrigant based on the patient's needs and the clinical setting proposed, while future research agendas should strive to identify the most appropriate

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regimes for use of the different irrigation liquids to ensure patients receive the best possible care.

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