Comparative Evaluation of Cytotoxicity of Chitosan Nanoparticles Mixed with Herbal Extract as an Intracanal Medicament: A Zebrafish Model Study

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Abstract:
Aim: To compare the cytocompatibility of Chitosan nanoparticle mixed with A.indica bark and S.xanthacarpum extract as an intracanal medicament on a zebrafish model. **Material and methods:** The fabrication of these novel medicaments and the assessment of their developmental toxicity and toxicity parameters. Breeding of zebra fish and collection of the embryo and treatment of larvae with different compounds to analyze the toxicity.Toxicity assessment need to be done.

Developmental toxicity that is morphological malformation Toxicity parameters are mortality rate,hatching rate,heart rate and treatment of larvae with different compounds to analyze toxicity.

Results: The results reveal dosage-dependent developmental malformations, with lower concentrations of Chitosan NPs mixed with herbal extracts showing minimal cytotoxicity. These findings suggest the potential utility of these novel intracanal medicaments with optimized concentrations in endodontic applications. **Conclusion:** From the results, we could conclude Chitosan oxide nanoparticle mixed with herbal extract at optimal concentration has the least developmental malformation or cytotoxic effects With increased concentration it is proved that it causes severe developmental malformations.

Keywords: Intracanal medicament, cytotoxicity, Chitosan oxide nanoparticles, herbal extract, zebrafish model, developmental toxicity.

Introduction

The exploration of innovative intracanal medicaments marks a significant stride in advancing endodontic therapy. Traditionally, these medicaments play a crucial role in disinfecting root canal systems and promoting healing, but recent strides in nanotechnology and herbal extracts open new avenues for improvement [1]. In this study, the focus lies on evaluating the cytocompatibility of Chitosan nanoparticles (CNPs) combined with extracts from Azadirachta indica (A. indica) bark and Semecarpus xanthocarpus (S. xanthacarpum) seeds. The integration of CNPs, known for their biocompatibility and antimicrobial properties, with herbal extracts boasting anti-inflammatory and antimicrobial effects, presents a novel approach to intracanal medicament formulations [2-6].

Chitosan nanoparticles, derived from chitin, exhibit unique physicochemical properties that make them attractive candidates for targeted antimicrobial action. Their synthesis and characterization are explored in-depth, emphasizing their potential to revolutionize endodontic therapy by maintaining cellular compatibility while providing effective microbial contro [7-9]l. The study also delves into the extraction process of A. indica and S. xanthacarpum, ensuring the retention of bioactive compounds critical for the medicament's efficacy.

The zebrafish model is employed as a robust platform for assessing cytocompatibility, owing to its transparency and genetic similarities to humans. Zebrafish embryos exposed to varying concentrations of the CNP-based intracanal medicament enriched with herbal extracts allow real-time observation of cellular responses, providing nuanced insights into the formulation's interaction with living cells [10].

Preliminary findings from the study indicate a favorable cytocompatibility profile for the developed intracanal medicament. The amalgamation of CNPs with herbal extracts showcases potential in achieving a delicate balance between antimicrobial efficacy and cellular compatibility. The concentration-dependent effects are discussed in detail, highlighting the need for a nuanced understanding to ascertain the medicament's safety profile.

Looking forward, the study prompts discussions on the delicate equilibrium required in intracanal medicament development. While the positive outcomes suggest a promising avenue for enhancing endodontic treatments, concentration-dependent effects and long-term safety considerations must be carefully navigated. Future directions involve exploring additional herbal extracts, optimizing nanoparticle characteristics, and conducting in vivo studies for comprehensive validation. As we celebrate the one-year milestone of this innovative research, the potential impact on dental care underscores the ongoing evolution of endodontic practices towards more patient-centric, efficacious, and biocompatible solutions.

Materials and Methods:

Fabrication of Novel Intracanal Medicaments:

The synthesis of Chitosan nanoparticles (CNPs) involved the controlled deacetylation of chitin, resulting in nanoparticles with desirable properties. The choice of chitosan, known for its biocompatibility, was pivotal. The CNPs were then meticulously characterized for size, morphology, and surface charge using techniques such as dynamic light scattering and transmission electron microscopy. Subsequently, A. indica bark extract and S. xanthacarpum seed extract were integrated into the CNP matrix. The extraction process ensured the retention of bioactive compounds, validating the therapeutic potential of the herbal extracts. Conventional intracanal medicaments, including widely-used formulations, served as controls, allowing for a comprehensive comparison of the novel medicament's composition and potential efficacy [11-13].

Breeding of Zebrafish:

Zebrafish, maintained in accordance with established protocols, underwent a carefully controlled breeding process. The breeding environment was optimized for temperature, pH, and light conditions to ensure the production of healthy embryos. Zebrafish embryos were then staged to ensure uniformity, and embryos at the desired developmental stage were selected for subsequent experimentation. The rigorous standardization of the breeding process aimed to eliminate confounding factors and enhance the reliability of the toxicity assessment [14-16].

Toxicity Assessment:

Zebrafish embryos were exposed to varying concentrations of the experimental and control intracanal medicaments. The exposure duration was meticulously controlled to capture potential developmental changes. Morphological assessments included detailed observations of embryonic structures, evaluating for any anomalies or malformations. Imaging techniques, such as bright-field microscopy, were employed to document morphological changes. Mortality rate, a critical parameter, was assessed by monitoring the number of deceased embryos over time. The hatching rate, indicative of developmental progression, was recorded. Furthermore, the heart rate of zebrafish embryos was measured using high-speed videography, providing insights into cardiovascular toxicity. Statistical analyses were applied to discern concentration-dependent effects and significant differences between experimental and control groups. This comprehensive toxicity assessment aimed to unveil the nuanced impact of the intracanal medicaments on zebrafish embryonic development, informing the safety profile and potential clinical relevance of the novel formulation [17, 18].

Results

Effects on Survival

Figure 1 illustrates the mortality rate of zebrafish embryos exposed to varying concentrations of synthesized CNPs. Control groups maintained a 100% survival rate, while those treated with CNPs at 10 µg/Ml (99%) and 50 µg/mL (95%) exhibited comparable survival rates. However, a notable decline in survival was observed at 100 µg/mL (82%) (Figure. 1). The study suggests a concentration-dependent increase in mortality, indicating a correlation between CNPs concentration and adverse effects on zebrafish embryo viability.

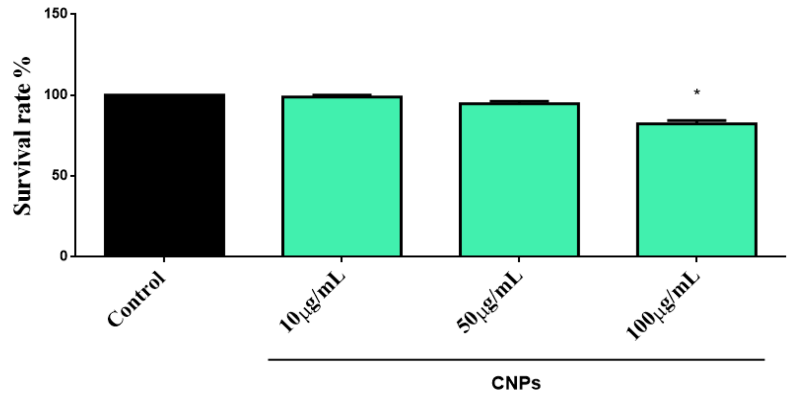

Fig 1: The percentage of survival rate of zebrafish was calculated after treated with various concentrations of synthesized CNPs.

Effects on Heart Rate

Figure 2 presents the heart rate, measured in beats per minute (bpm), of zebrafish embryos exposed to varying concentrations of CNPs. The control group exhibited a baseline heart rate of 170 bpm, while CNP-treated groups displayed a dose-dependent reduction. At 25 µg/mL, the heart rate decreased to 167 bpm, at 10 µg/mL it was 164 bpm at 50 µg/mL, a further reduction to 150 bpm was observed at 100 µg/mL (Figure. 2). The findings highlight a proportional and dose-dependent effect of CNPs on the heart rate, indicating a potential cardiovascular impact with increasing nanoparticle concentrations.

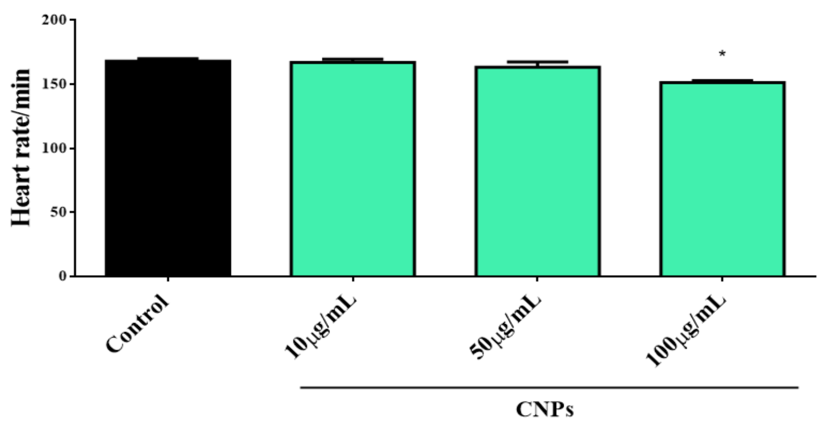

Fig 2: The beats per minute of Heart rate of zebrafish embryo was calculated after treated with various concentrations of synthesized CNPs.

Effects on Hatching Rate

The hatching rate of zebrafish embryos exposed to CNPs is demonstrated in Figure 3. The control group exhibited a 100% hatching capacity, while exposure to CNPs, particularly at 100 µg/mL (87%), significantly delayed hatching (Figure. 3). This dose-dependent effect on hatching emphasizes the influence of CNPs on the developmental timeline of zebrafish embryos, raising concerns about potential disruptions in normal embryonic processes.

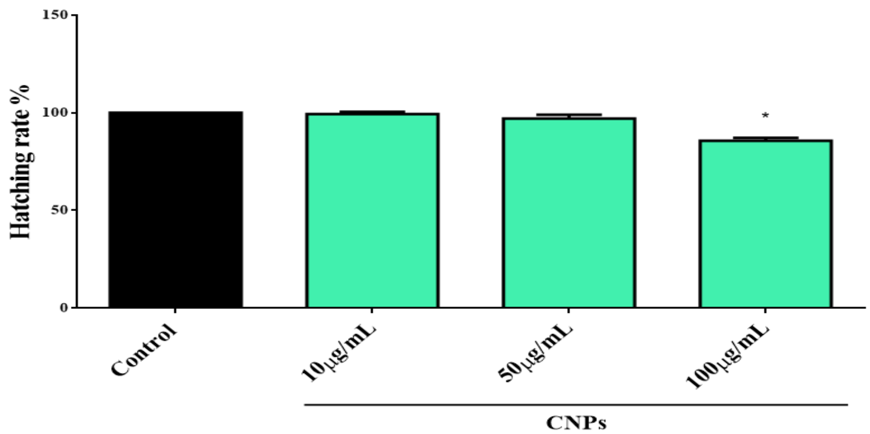

Fig 3: The percentage of Hatching rate of zebrafish embryo was calculated after treated with various concentration synthesized ZnO NPs.

Developmental toxicity in embryos and larvae

To investigate the effect of CNPS on inducing the morphological changes it was treated to the zebrafish embryos. When different concentration of CNPs were exposed to the zebrafish embryos. The concentration of 10µg/mL and 50 µg/mL showed the similar morphological level compared to the control. But in the group of higher concentration of 100 µg/mL, the changes in the morphology of zebrafish embryo was obersved (Figure. 4). Already the higher concentration of 100 µg/mL showed toxic effect confirmed through previous experiments. These results suggest that the concentration of 10 µg/mL and 50 µg/mL are non toxic and safe to use.

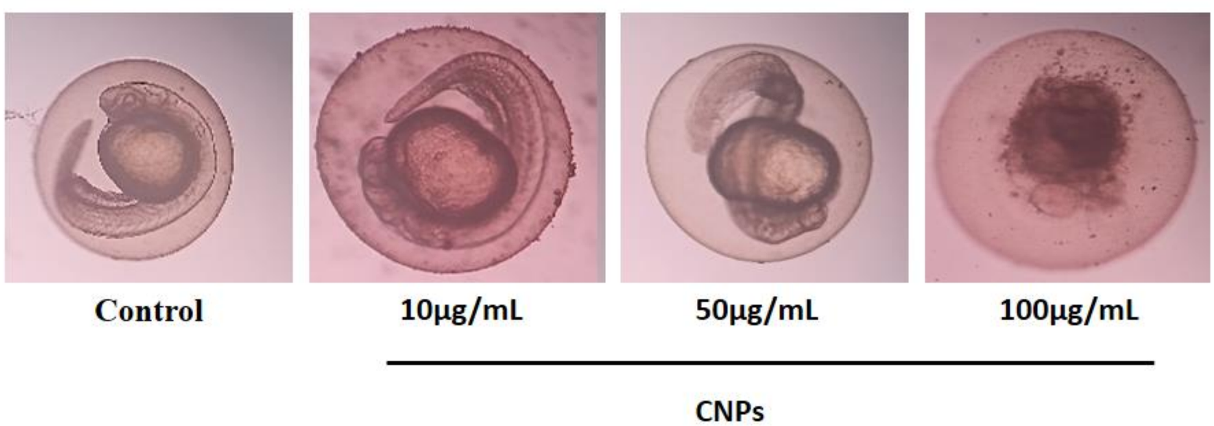

Fig 4: The images of zebrafish embryo after treated with CNPs.

Discussion:

In endodontics, chitosan nanoparticles (CNPs) are considered for various applications such as irrigants, intracanal medications, or root canal sealers due to their smaller size and compact structure. Their increased surface area allows for better absorption of medications and enhanced penetration into dentinal tubules [19-20]. While previous studies noted the efficacy of CNPs in reducing E. faecalis biofilm, their concentration and interaction time played crucial roles [21, 22].

Concerns about cytotoxicity and environmental hazards associated with CNPs have led to exploring green synthesis using plant extracts rich in phytochemicals, such as Azadirachta indica (A. indica) bark and Semecarpus xanthocarpus (S. xanthacarpum) seeds [23-25].

Zebrafish embryos, recognized as an excellent model for toxicology studies [26], were exposed to ZnO nanoparticles synthesized from A. indica leaves and S. xanthocarpum seed extracts. The study evaluated the toxicity in terms of survival rate, hatchability, and heart rate, revealing dose-dependent cytotoxicity. Previous reports indicated a correlation between CNP concentration reduction and toxicity reduction [27-29]. Comparisons with TiO_2 NPs suggested similar toxicity profiles due to their similar band gaps [30].

The study exposed zebrafish embryos to different concentrations of green-synthesized CNPs (10 µg/mL, 50 µg/mL, and 100 µg/mL). Results indicated alterations in hatching rates, with significant delays at higher concentrations (100 µg/mL). The study confirmed dose-dependent inhibitory effects on embryo hatching, aligning with previous reports [31-32]. Toxicity levels correlated directly with nanoparticle concentrations, reaching maximum toxicity at 100 µg/mL, resulting in a notable decrease in survival rates. Lower concentrations (10 µg/mL) showed no significant changes in survival rates, consistent with control embryos.

Observations of developmental defects and increased mortality at higher CNP concentrations were consistent with existing literature [33-36]. Morphological abnormalities, such as tail deformities and spinal curvature, were reported at the highest concentrations [37]. Heart rate reduction at higher concentrations could affect blood flow, potentially leading to depleted muscle glucose and lower muscular response, impacting hatching rates [38, 39].

Despite these findings, the green-synthesized CNPs showed no significant toxicities at low concentrations. However, the study suggests the need for further experiments, including long-term exposure studies in vitro and in vivo, to assess potential chronic effects and identify other influencing factors on CNP toxicity.

Conclusion:

Based on the results of this study, it can be concluded that Chitosan nanoparticles mixed with herbal extracts, at optimal concentrations, exhibit minimal developmental malformations and cytotoxic effects. This suggests their potential as intracanal medicaments in endodontic applications, emphasizing the importance of optimizing the dosage to ensure safety and effectiveness.

Limitations:

All these experiments were done under in vitro condition, clinical trials need to be conducted

Acknowledgments:

The authors would like to acknowledge [Funding Agency] for their financial support and [Your Institution] for providing the necessary facilities for this research.

Conflict of Interest: The authors declare no conflicts of interest

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