Original Article

Comparison of the Cytotoxicity of Nano Curcumin-based Paste and Zinc Oxide Eugenol: An in vitro Study

Rasoul SahebAlam

Associate Professor, Department of Pediatric dentistry, Faculty of Dentistry, Mashhad University of Medical Sciences, Mashhad, Iran PO Box 91735-346, Mashhad, Iran / Tel: 0099151103629 / E-Mail: SahebalamR@mums.ac.ir

Fereshte Naderi

Student Research Committee, Faculty of Dentistry, Mashhad University of Medical Sciences, Mashhad, Iran / PO Box 91735-346, Mashhad, Iran / Tel: 0099215144805 / E-Mail: NaderiF991@mums.ac.ir

Alireza Sarraf Shirazi

Professor, Department of Pediatric dentistry, Faculty of Dentistry, Mashhad University of Medical Sciences, Mashhad, Iran / PO Box 91735-346, Mashhad, Iran / Tel: 0099153104778 / E-Mail: SarrafA@mums.ac.ir

Maryam Valizadeh (Corresponding Author)

Assistant Professor, Dental Research Center, Mashhad University of Medical Sciences, Mashhad, Iran / PO Box 91735-346, Mashhad, Iran /Tel: 00985138832301

E-Mail: Valizadehmr@mums.ac.ir

Dr.m.valizadeh70@gmail.com

Phone number: 00985138832301 Fax number: 00985138829500

Abstract

Zinc oxide eugenol (ZOE) is a common material in dental treatments but presents issues like cytotoxicity and potential allergic reactions. In contrast, curcumin boasts anti-inflammatory and antimicrobial attributes. This study set out to compare the cytotoxicity of Nano curcumin-based pastes to that of ZOE. The research involved examining the cytotoxic effects of Nano curcumin-based pastes, ZOE, and Metapex® on L929 mouse fibroblast cells using the MTT assay. Two variations of Nano curcumin pastes, curcumin paste (CP) and modified curcumin paste (MCP), were developed and tested in five concentration dilutions (1/1, 1/2, 1/4, 1/8, and 1/16) with three replications. Results after a 24-hour exposure indicated that both Nano curcumin pastes had reduced cytotoxicity at the 1/1 and 1/2 dilutions compared to ZOE. However, after 72 hours, CP's cytotoxicity at the 1/1 dilution exceeded that of ZOE. Yet, MCP maintained its lesser cytotoxicity than ZOE for the same dilutions. The study's conclusion highlighted that MCP showcased a more favorable cytotoxic profile than ZOE, aligning more closely with the results observed for Metapex®.

Keywords: Cytotoxicity, Curcumin, Metapex, Zinc Oxide Eugenol

1. Introduction

Early loss of deciduous teeth due to deep caries in both anterior and posterior teeth can cause transient or permanent problems such as malnutrition, aesthetics and lower oral health quality of life. In cases pulp opening into the environment occurs for any reason (due to caries or traumatic accident), pulp treatments such as pulpectomy are essential for preserving the deciduous teeth. The properties of

ideal obturating materials for filling the canal of deciduous teeth include earlier or concurrent absorption of material with root absorption of deciduous teeth, lack of adverse effects on the periapical tissue and the underlying permanent tooth bud with antiseptic properties, radioopacity, easy bonding with the walls of the tooth canal, proper sealing, and easy extractability of the material if necessary (Deepak et al., 2021). Currently, there is no material possessing all the mentioned features. The materials that are mostly used in pulpectomy are zinc oxide eugenol (ZOE), mineral trioxide aggregate (MTA), KRI paste, and Metapex®. ZOE is one of the most common filling materials for deciduous teeth canals (Deepak et al., 2021) and is also applied in other dentistry fields such as periodontics, endodontics, and restoration. Previous research showed that the eugenol in the ZOE compound can cause the development of allergic and inflammatory reactions (Brar et al., 2019). It is found that ZOE remains in the alveolar bone after extracting teeth undergoing pulpectomy using this material (Fuks & Peretz, 2016; Pilownic et al., 2017). According to some studies, although Metapex® has low cytotoxicity (Brar et al., 2019; Nashaat et al., 2021), its usage is more suitable compared to ZOE. Nonetheless, it causes no significant increase in treatment success compared to ZOE (Bommareddy et al., 2022).

Curcumin is a material that is obtained from the root of turmeric and has anti-inflammatory, antimicrobial, antioxidant, anticancer, and other properties (Sharifi-Rad et al., 2020). Further, it reduces inflammatory mediators caused by microorganisms such as *Porphyromonas gingivalis*, thus it is recommended for periodontal diseases (Kocaadam & Şanlier, 2017). In spite of the antimicrobial properties of this material against different microorganisms and its alleviating and anti-inflammatory effects, no comprehensive study has so far focused on the use of this material for dental pulp therapies.

To the best of our knowledge, only one study has investigated the cytotoxicity of the curcumin paste (CP) on human pulp cells (Mandrol et al., 2016), but the cytotoxicity of this material has not been compared with that of other applied common materials in dentistry. Accordingly, the current study sought to compare the cytotoxicity of two Nano curcumin-based pastes with ZOE and Metapex® on L929 murine fibroblast cells via the MTT test.

2. Materials and methods

Nano curcumin (Sinacurcumin, Oxir Nanosina, Iran) was used to prepare curcumin-based pastes. Curcumin with two primary bases, containing Nano curcumin 50 wt% combined with ZO (CrZO) known as CP and modified curcumin paste (MCP) consisting of Nano curcumin 25 wt% plus ZO eugenol (CrZOE) were applied in the present study.

ZOE (Kemdent works, Cricklade Rd, Purton, Swindon, UK) and Metapex® (Meta BioMed Co., LTD, South Korea) were applied based on the study purpose. Dulbecco's Modified Eagles' (DMEM) culture medium, trypan blue, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma (Sigma Chemical Co., St. Louise, Missouri, USA). Moreover, penicillin-streptomycin, trypsin, EDTA, and fetal calf serum (FCS) were provided from Gibco (Gibco Chemical Co., Carlsbad, CA, USA), and finally, dimethyl sulfide (DMSO) was procured from Merck (Merck KGaA, Darmstadt, Germany).

This project has been approved by the institutional review board and received the confirmation of the Ethics Committee of Mashhad University of Medical Sciences for protection of human subjects and animals in research with registration number IR.Mums.sd.REC.1394.205

2.1. Preparation of the samples (the extract of pastes)

The addition of pastes to the culture medium containing the test cells causes physical damage to the cells. It specifically precipitates in the culture medium and changes the results of the study. Accordingly, different culture media containing the extract of pastes were prepared and added to the cell-containing plates in this study. Then, 0.1 g of each of the tested materials (Metapex®, ZOE, MCP, and CP) was measured using a digital balance with an accuracy rate of 0.001 g. Next, they were mixed with 10 cc of the culture medium in order to achieve 10 mg/ml for each paste. The prepared culture medium contained DMEM and FCS 10% plus 1% antibiotic (penicillin-streptomycin). Then, the tubes containing the culture medium and tested materials were placed inside the incubator at 37 ˚C with a humidity rate of 98% in order to homogenize the medium for all the prepared mixtures. After 24 hours, the prepared culture media with the extract of the tested materials were added to two 96-well plates containing L929 murine fibroblast cells kept inside nitrogen tanks and then placed inside the incubator.

2.2. Cell culture method

The L929 mouse fibroblasts cells in the DMEM culture medium, to which FCS 10% and penicillin-streptomycin 1% had been added, were cultured under 37 ˚C. Once the flask floor filled up to the density of 70-80%, the cells were divided into several flasks with cell passaging (Ciapetti et al., 1993). L929 cells need cell passaging every 2-9 days, and trypsin was used for this purpose (Javidi et al., 2017). For the passage of the attached cells to the surface of the flask, the old culture medium was discarded, and 2 ml trypsin 0.25% was added to the flask and placed inside the incubator for 5 min. Immediately after cell separation, 5 ml of the new culture medium was added to the flask. The percentage of live cells after cell passaging was determined via the trypan blue test (Ciapetti et al., 1993). To conduct this test, 20 µL of the mixture containing trypan blue dye was transferred to a hemocytometer, with the cells counted under a microscope with 10X magnification. The dead cells were differentiated from the live ones with blue color. The live cell count was considered approximately from 10^4 cells/ml. Once the viability of cells was ensured through the trypan blue test, the cells were transferred to the wells of two 96-well plates (10^4 cells in each well) and underwent cell culture for 24 h inside the incubator so that the cells would attach to the bottom of the well and adapt to the medium.

The culture media containing the extract of the prepared pastes were prepared as 1/1 dilution (10 mg/ml), whereby four dilutions including 1/2 (5 mg/ml), 1/4 (2.5 mg/ml), 1/8 (1.25 mg/ml), and 1/16 (0.625 mg/ml) were obtained through adding the DMEM culture medium. (Figure 1)

To prepare the culture media consisting of lower dilutions of sealer, the primary prepared sample was diluted as the serial dilution by adding the DMEM culture medium. Next, the culture medium with the mentioned dilutions was poured into 60 wells of each 96-well plate in dilution descending order, and each material was examined three times in the tested dilutions. Thereafter, the first and second plates were incubated for 24 and 72 h, respectively. Eventually, the cytotoxicity extent of the tested sealers was measured by the MTT assay method.

2.3. Examination of cell viability via the MTT method

After the completion of cell incubation, 0.25 g of the MTT powder (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was dissolved in the phosphate-buffered saline solution in order to achieve the concentration of 5 g/ml. Next, 20 ml of the MTT solution (5 mg/ml) was added to each of the wells of the plate and covered with an aluminum foil, and again placed inside the incubator for 4 h. After removing the plate from the incubator, the top culture medium was discharged, and then 100 µl of the DMSO solution was added to each well.

The MTT solution is yellow while it reduces to formazan (purple) after its addition to the medium, where the intensity of its purple color is a criterion for the number of live cells. The added DMSO solution to the wells caused the dissolution of the reduced formazan color. The intensity of the obtained color for each well was measured by the ELISA reader (Biotech Instrument, Inc., Winooski, VT, USA).

Additionally, the half-maximal inhibitory concentration (IC50) index (the maximum concentration of a drug causing inhibition of 50% of special biological and biochemical functions of cells in laboratory studies) was assessed for all control and test types of pastes.

2.4. Statistical methods and sample size

According to the protocols of cell toxicity tests and a similar study ([Javidi et al., 2017), there was no need to determine the sample size in this study. Three-factor ANOVA (investigating the effect of three factors including the type of the paste, time, and dilution) and two-factor ANOVA (examining the effect of type of the paste and dilution on each other) were used for data analysis by SPSS-23, respectively, and the significance level of the tests was considered 5%.

3. Results

In this study, the samples were investigated in terms of the number of live cells under the effect of some factors including the type of the paste, time, and dilution. Based on three-factor ANOVA, all factors affected each other ($P<0.001$). Thus, each of these factors was separately examined, data were analyzed within 24- and 72 h.

3.1. Analysis within 24 h

According to data in Table 1, the mean difference of the number of live cells across various dilutions after 24 h is significant in all pastes except for MCP (P=0.122). The comparison results of each of the dilutions with the control are shown in Table 1 and Figure 4 as follows:

In the CP paste, the mean live cells were significantly lower than control cells only at the dilution of 1/1 (P=0.001). Other dilutions (1/2, 1/4, 1/8, and 1/16) failed to affect the viability of L929 cells (P>0.05). (Figure 2)

In a paired comparison of pastes after 24 h, the cytotoxicity of CP (CrZO) was significantly lower at 1/1 and 1/2 dilutions (P<0.001, P=0.008) compared to ZOE. MCP (CrZOE) paste also showed significantly lower cytotoxicity in comparison to ZOE at 1/1 and 1/2 dilutions (P<0.001).

3.2. Analysis within 72 h

Based on the results in Table 2, the mean difference of the number of viable cells across various dilutions after 72 h is significant in all pastes. Table 2 and Figure 4 present the comparison results of each of the dilutions with the control group.

The mean viable cells were significantly lower than the control at dilutions 1/1 and in the CP paste ½ (P<0.001). (Figure 3) In addition, the mean viable cells decreased significantly at all dilutions in MCP except for 1/16 (P=0.192).

Statistical analyses indicated that after 72 h, the cytotoxicity of CP (CrZO) at 1/2 concentrations was significantly higher than that of ZOE (P=0.007), Metapex (P<0.001), and MCP (P<0.001) in the paired comparison of pastes. At 1/1 concentrations, it was significantly (P<0.001) higher than that of MCP and Metapex. On the other hand, MCP at 1/1 and 1/2 concentrations demonstrated significantly lower cytotoxicity compared to ZOE (P<0.001 and P=0.016, respectively).

The half maximal inhibitory concentration (IC50) is a measure of the potency of a substance in inhibiting a specific biological or biochemical function. Half-maximal inhibitory concentration (IC50) is the most widely used and informative measure of a drug's efficacy. IC50 is the dose of the substance which inhibits the proliferation of half of the cells (Sebaugh, 2011). As depicted in the IC50 diagram

(Figure 5), the percentage of viable cells remained below 50% in ZOE after 24 and 72 h at the initial dilution (i.e., 1/1) and CPP after 72 h at 1/1 and 1/2.

4. Discussion

This study attempted to investigate and compare the cytotoxicity of pastes containing Nano curcumin (CP and MCP) with two common pastes (ZOE and Metapex®) in pulp treatment. ZOE is one of the most extensively applied filling materials for the canal of deciduous teeth with established anti-allergic and inflammatory properties ((Brar et al., 2019; Deepak et al., 2021). Metapex® with low cytotoxicity and suitable antimicrobial properties (Brar et al., 2019; Nashaat et al., 2021) is another drug that is commonly employed in the pulp treatment of deciduous teeth. Furthermore, curcumin is a drug that is obtained from turmeric extract. Based on the evidence, this material has biological, anticancer, antioxidant, antimicrobial, palliative, and anti-inflammatory effects and is also effective in accelerating wound healing (Masloub et al., 2016). The findings of a previous study on the cytotoxicity of curcumin in medicine confirmed the very low toxicity of this material (Zhao et al., 2018). It is noteworthy that this material can be recommended in combination with other materials for treating inflammatory diseases and cancers because of its desired antimicrobial and anti-inflammatory properties (Campos et al., 2013; Chang et al., 2013; Elburki et al., 2017; Liu et al., 2016; Terby et al., 2021). Nevertheless, only one study has so far examined the cytotoxicity of curcumin on human pulp fibroblast cells (Mandrol et al., 2016).

The extracts of the study materials were prepared and exposed to cells in order to assess their cytotoxicity. This method prevents physical damage to cells and a false increase in dead cells (He et al., 2016; Nashaat et al., 2021). Different concentrations of pastes containing curcumin and ZO compounds were prepared and applied according to the study by Bin et al. (Bin et al., 2012) because, to the best of our knowledge, the present study is the first one to evaluate the cytotoxicity of these materials.

The cytotoxicity of ZOE was significantly higher than that of control within both 24 and 72 h across all dilutions. The ascending trend of cytotoxicity with concentration elevations was observed based on the results ($P<0.001$). Different studies have reported high cytotoxicity of ZOE, which is in line with the result of the present research. The most important reason for this high cytotoxicity was the presence

of free eugenol in the composition of this material. Yilmazi et al. (Yilmaz et al., 2012) compared the cytotoxicity of ZOE-based sealers with that of calcium hydroxide-based sealers and concluded that ZOE-containing sealers had greater cytotoxicity. The cytotoxicity and neurotoxicity effects of ZOE are highly prominent (Yilmaz et al., 2012). Likewise, Arun S et al. (Arun et al., 2017) compared and measured the effect of adding pachymic acid on the cytotoxicity of four dentistry sealers. Tubliseal (ZOE-based) was one of the tested sealers in their study. They attributed the high cytotoxicity of Tubliseal to free eugenol, the cytotoxic nature of zinc ion, benzoic acid, and methyl salicylic in the composition (Arun et al., 2017).

The cytotoxicity of Metapex® could be compared to the control group at three low concentrations of the study within 24 and 72 h ($P>0.05$). Brar et al, (Brar et al., 2019) compared the cytotoxicity of three applied pastes in pulpectomy, including ZOE, Metapex®, and Chitra HAP and found that Metapex® had the minimum cytotoxicity within the experimental time. Nevertheless, according to the author and other performed studies, the cytotoxicity extent of Metapex® increases over time because of the effect of the lipid in the Metapex® composition and the increased effect of calcium hydroxide in this paste (Brar et al., 2019).

Further investigation of the obtained results represented a significant reduction in cells at 1/1 dilution in CP after 24 h compared to the control group while the number of cells did not reach below 50% ($P<0.003$). Based on the results, the lower concentration of curcumin composition led to a decrease in the cytotoxicity of this compound over 24 h. Conversely, this compound had high cytotoxicity at 1/1 and 1/2 dilutions after 72 h, where a dramatic reduction was observed in the number of cells and the level of viable cells decreased to below 50% ($P>0.001$). However, the control group demonstrated no significant difference in the number of cells at 1/4, 1/8, and 1/16 dilutions.

The findings of the present study indicated that the number of viable cells in MCP within 24 h had no significant reduction in any of the dilutions compared to the control ($P=0.122$). This result did not remain stable within 72 h, and a significant decline was detected in the number of viable cells over the ascending order of dilutions. Contrarily, regarding the Metapex® paste, the number of cells did not reach below 50% in any of the dilutions. The positive effects of the MCP paste in comparison with the

CP can be attributed to a reduction in the curcumin concentration and the synergistic effects between curcumin and ZOE. Nevertheless, further studies are required in this regard.

To our knowledge, the latest study examining the cellular toxicity of curcumin on human pulp fibroblast cells revealed that curcumin resulted in an elevated number of fibroblast cells and their proliferation rather than causing cytotoxicity (Mandrol et al., 2016). Unlike our study, pure curcumin compounds with concentrations of 25, 50, and 100% were employed in the above-mentioned study. According to our observations, the number of murine fibroblast cells increased at the 1/16 dilution of the CP after 72 h compared to the control group although this rise was not statistically significant. A recent study compared the histological results of the pulpotomy of rat teeth with curcumin and MTA. The results confirmed that curcumin has wound-healing properties and the potential to change into a predictable and economical vital pulp treatment (Prabhakar et al., 2019).

Based on the above-mentioned explanations, using this paste as a drug in pulp treatment can prevent the complications resulting from other applied drugs in this type of treatment while improving inflammation in the site of interest. Nevertheless, this study had some limitations including the lack of awareness about the precise time of the setting of curcumin-based pastes for investigating the cytotoxic effects before and after its usage, as well as the lack of a long-term investigation of the cytotoxic effects of curcumin pulp pastes.

5. Conclusion

The results of this research indicated that MCP had less cytotoxicity compared to ZOE and was comparable to that of Metapex®. This compound would be presented as a novel drug if it is also suitable in terms of other required characteristics for dentistry treatments, especially pulp treatments. Evidently, determining the proper dilution of MCP for clinical uses requires further studies.

Acknowledgements

The authors appreciate Professor Jalil Tavakol-Afshari, who guided the laboratory part of this study. In addition, the authors appreciate research deputy of Mashhad University of Medical Sciences and Bo Ali Research Institute for their help and support.

6. References

Arun, S., Sampath, V., Mahalaxmi, S., & Rajkumar, K. (2017). A Comparative Evaluation of the Effect of the Addition of Pachymic Acid on the Cytotoxicity of 4 Different Root Canal Sealers-An In Vitro Study. *J Endod*, *43*(1), 96-99. https://doi.org/10.1016/j.joen.2016.09.004

Bin, C. V., Valera, M. C., Camargo, S. E., Rabelo, S. B., Silva, G. O., Balducci, I., & Camargo, C. H. R. (2012). Cytotoxicity and genotoxicity of root canal sealers based on mineral trioxide aggregate. *Journal of endodontics*, *38*(4), 495-500.

Bommareddy, C. S., Ramkumar, H., Dakshinamurthy, S., Paulindraraj, S., Jayakaran, T. G., & Shankar, K. (2022). Clinical and Radiographic Evaluation of Curcumin as an Obturation Material in Deciduous Teeth: A Randomized Controlled Trial. *International Journal of Clinical Pediatric Dentistry*, *15*(S1), S35-S39.

Brar, G. S., Bajaj, N., Bhola, M., & Brar, J. K. (2019). Clinical evaluation of root resorption and its correlation with obturation quality in pulpectomized deciduous teeth with different obturating materials: An In vivo study. *Contemporary Clinical Dentistry*, *10*(2), 243.

Campos, C. A., Gianino, J. B., Bailey, B. J., Baluyut, M. E., Wiek, C., Hanenberg, H., Shannon, H. E., Pollok, K. E., & Ashfeld, B. L. (2013). Design, synthesis, and evaluation of curcumin-derived arylheptanoids for glioblastoma and neuroblastoma cytotoxicity. *Bioorganic & medicinal chemistry letters*, *23*(24), 6874-6878.

Chang, P.-Y., Peng, S.-F., Lee, C.-Y., Lu, C.-C., Tsai, S.-C., Shieh, T.-M., Wu, T.-S., Tu, M.-G., Chen, M. Y., & Yang, J.-S. (2013). Curcumin-loaded nanoparticles induce apoptotic cell death through regulation of the function of MDR1 and reactive oxygen species in cisplatin-resistant CAR human oral cancer cells. *International journal of oncology*, *43*(4), 1141-1150.

Ciapetti, G., Cenni, E., Pratelli, L., & Pizzoferrato, A. (1993). In vitro evaluation of cell/biomaterial interaction by MTT assay. *Biomaterials*, *14*(5), 359-364.

Deepak, B. M., Prabhakar, A. R., Karuna, Y. M., Sugandhan, S., Zahoor, N., & Shagale, A. M. (2021). Evaluation of the Antibacterial Activity of Triclosan-incorporated Root Canal Filling

Materials for Primary Teeth against Enterococcus faecalis. *International Journal of Clinical Pediatric Dentistry*, *14*(3), 393.

Elburki, M., Moore, D., Terezakis, N., Zhang, Y., Lee, H. M., Johnson, F., & Golub, L. (2017). A novel chemically modified curcumin reduces inflammation-mediated connective tissue breakdown in a rat model of diabetes: periodontal and systemic effects. *Journal of periodontal research*, *52*(2), 186-200.

Fuks, A. B., & Peretz, B. (2016). Pediatric endodontics: past and present perspectives and future directions. In *Pediatric Endodontics* (pp. 1-5). Springer.

He, Y., Zhu, Q., Chen, M., Huang, Q., Wang, W., Li, Q., Huang, Y., & Di, W. (2016). The changing 50% inhibitory concentration (IC50) of cisplatin: A pilot study on the artifacts of the MTT assay and the precise measurement of density-dependent chemoresistance in ovarian cancer. *Oncotarget*, *7*(43), 70803.

Javidi, M., Dastmalchi, P., Zarei, M., Rad, M. S., & Ghorbani, A. (2017). In vitro cytotoxicity of a new nano root canal sealer on human gingival fibroblasts. *Iranian endodontic journal*, *12*(2), 220.

Kocaadam, B., & Şanlier, N. (2017). Curcumin, an active component of turmeric (Curcuma longa), and its effects on health. *Critical reviews in food science and nutrition*, *57*(13), 2889-2895.

Liu, M., Zhang, J., Li, J.-F., & Wang, X.-X. (2016). Roles of curcumin combined with paclitaxel on growth inhibition and apoptosis of oral squamous cell carcinoma cell line CAL27 in vitro. *Shanghai kou qiang yi xue= Shanghai journal of stomatology*, *25*(5), 538-541.

Mandrol, P. S., Bhat, K., & Prabhakar, A. (2016). An in vitro evaluation of cytotoxicity of curcumin against human dental pulp fibroblasts. *Journal of Indian Society of Pedodontics and Preventive Dentistry*, *34*(3), 269.

Masloub, S. M., Elmalahy, M. H., Sabry, D., Mohamed, W. S., & Ahmed, S. H. (2016). Comparative evaluation of PLGA nanoparticle delivery system for 5-fluorouracil and curcumin on squamous cell carcinoma. *Archives of oral biology*, *64*, 1-10.

Nashaat, Y., Sabry, H., & Hassan, S. A. (2021). Evaluation of the Cytotoxicity and Apoptotic Effects of Nano Triple Antibiotic Paste with Nano Anti-Inflammatory Drug as an Intracanal Medicament. *European endodontic journal*, *6*(1), 82.

Pilownic, K. J., Gomes, A. P. N., Wang, Z. J., Almeida, L. H. S., Romano, A. R., Shen, Y., Felix, A. d. O. C., Haapasalo, M., & Pappen, F. G. (2017). Physicochemical and biological evaluation of endodontic filling materials for primary teeth. *Brazilian dental journal*, *28*, 578-586.

Prabhakar, A., Mandroli, P. S., & Bhat, K. (2019). Pulpotomy with curcumin: Histological comparison with mineral trioxide aggregate in rats. *Indian Journal of Dental Research*, *30*(1), 31.

Sebaugh, J. (2011). Guidelines for accurate EC50/IC50 estimation. *Pharmaceutical statistics*, *10*(2), 128-134.

Sharifi-Rad, J., Rayess, Y. E., Rizk, A. A., Sadaka, C., Zgheib, R., Zam, W., Sestito, S., Rapposelli, S., Neffe-Skocińska, K., & Zielińska, D. (2020). Turmeric and its major compound curcumin on health: bioactive effects and safety profiles for food, pharmaceutical, biotechnological and medicinal applications. *Frontiers in pharmacology*, *11*, 1021.

Terby, S., Shereef, M., Ramanarayanan, V., & Balakrishnan, B. (2021). The effect of curcumin as an adjunct in the treatment of chronic periodontitis: A systematic review and meta-analysis. *The Saudi Dental Journal*, *33*(7), 375-385.

Yilmaz, Z., Dogan, A. L., Ozdemir, O., & Serper, A. (2012). Evaluation of the cytotoxicity of different root canal sealers on L929 cell line by MTT assay. *Dental materials journal*, *31*(6), 1028-1032.

Zhao, G., Dong, X., & Sun, Y. (2018). Self-assembled curcumin–poly (carboxybetaine methacrylate) conjugates: potent nano-inhibitors against amyloid β-protein fibrillogenesis and cytotoxicity. *Langmuir*, *35*(5), 1846-1857.

Note. IC50: Half-maximal inhibitory concentration.

Tables:

Table 1. Mean comparison of the number of viable cells in the study dilutions within 24 h for each paste

p-value	1/1	1/2	1/4	1/8	1/16	Control	Dilution / Paste
0.003*	0.69±0.05	0.85±0.10	0.91±0.09	0.93±0.06	0.87±0.04	1.00±0.02	Cp
	0.001	0.072	0.341	0.598	0.118		
<0.001*	0.05±0.00	0.58±0.03	0.70±0.08	0.83±0.11	0.78±0.04	1.00±0.04	ZOE
	<0.001	<0.001	<0.001	0.017	0.003		
0.122	0.74±0.08	0.87±0.08	0.90±0.09	0.89±0.11	0.85±0.09	1.00±0.13	Mcp
0.015*	0.67±0.04	0.83±0.00	0.96±0.02	0.91±0.06	1.09±0.16	1.00±0.07	Meta
	0.018	0.192	0.978	0.663	0.651		

Results are reported as standard deviation ± mean

*: significant

Table 2. Mean comparison of the number of viable cells in the studied dilutions after 72 h for each paste

p-value	1/1	1/2	1/4	1/8	1/16	Control	Dilution / Paste
<0.001*	0.03±0.00	0.39±0.10	0.90±0.14	0.96±0.09	1.04±0.10	1.00±0.03	Cp
	<0.001	<0.001	0.531	0.981	0.983		
<0.001*	0.04±0.00	0.61±0.03	0.77±0.03	0.82±0.03	0.88±0.05	1.00±0.03	ZOE
	<0.001	<0.001	<0.001	<0.001	0.003		
<0.001*	0.66±0.01	0.80±0.02	0.87±0.06	0.86±0.10	0.91±0.06	1.00±0.01	MCP
	<0.001	0.002	0.043	0.029	0.192		
<0.001*	0.67±0.09	0.86±0.03	0.92±0.06	0.89±0.02	0.90±0.08	1.01±0.01	Meta
	<0.001	0.038	0.246	0.108	0.127		

Results are reported as standard deviation ± mean

*: significant

Figures:

Figure 1. dilutions of the curcumin paste (CP) and medium mixture.

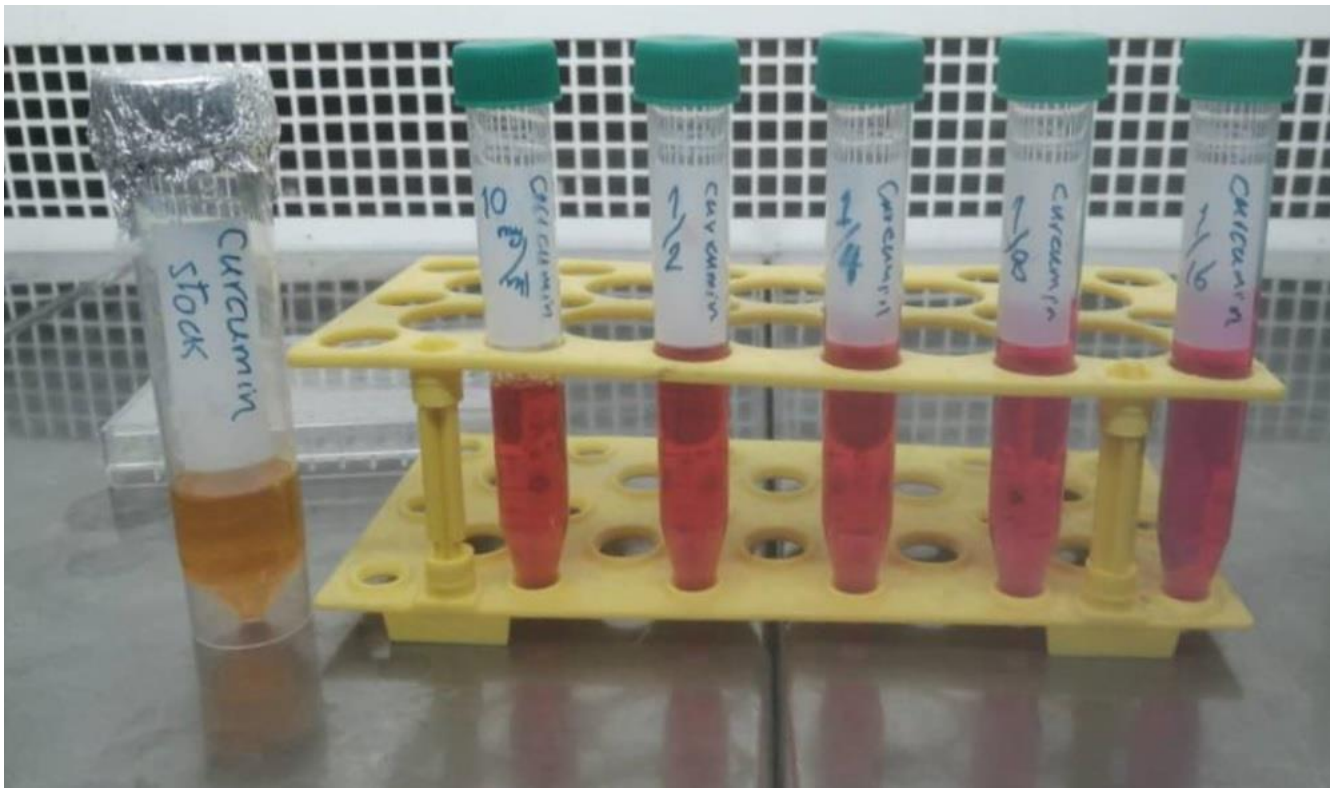

Figure 2. Optical microscopy images (scale bar 20 μm) of L929 mouse fibroblasts' response to different concentrations of curcumin paste (CP) after 24 h incubation: control (a), 1/1(b), 1/2(c), 1/4(d), 1/8(e), 1/16(f)

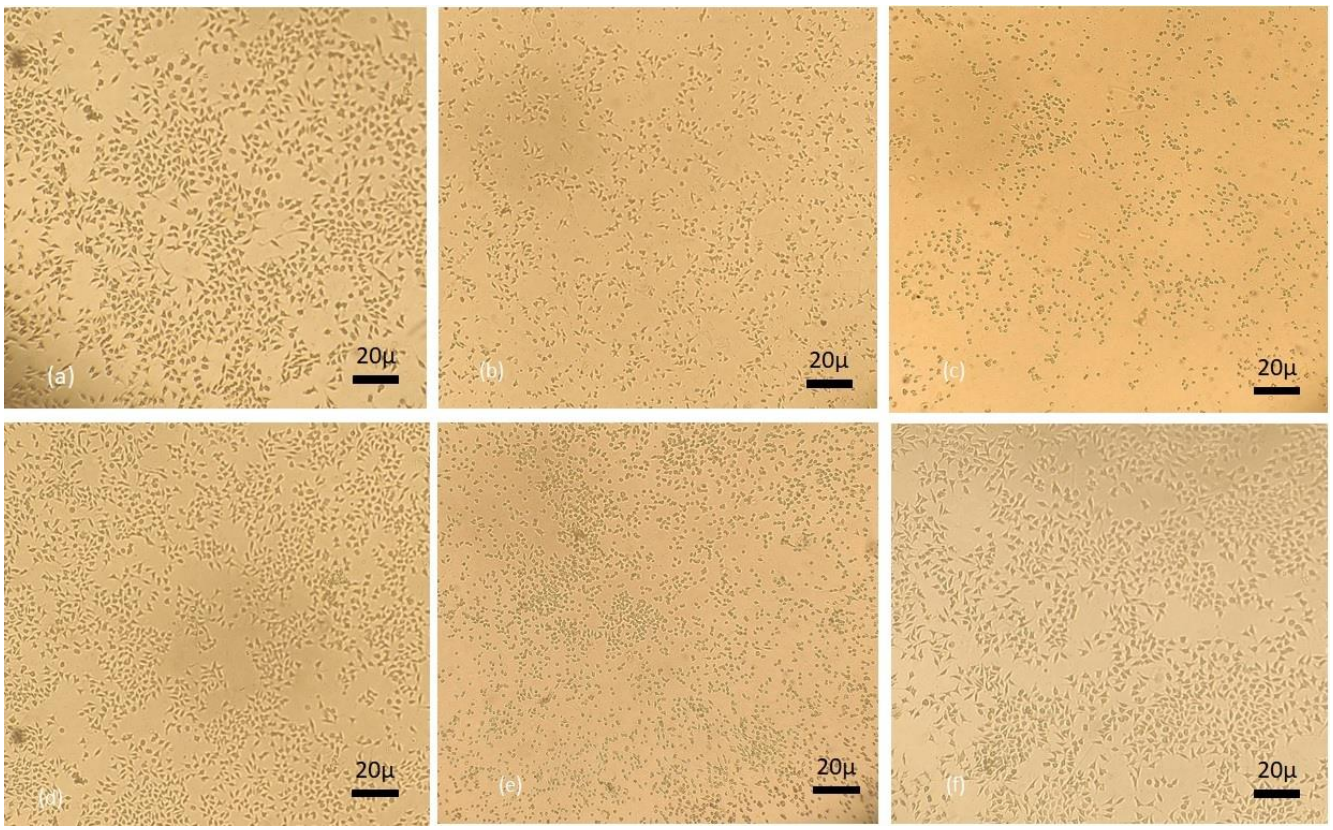

Figure 3. Optical microscopy images (scale bar 20 μm) of L929 mouse fibroblasts' response to different concentrations of curcumin paste (CP) after 72 h incubation: control (a), 1/1(b), 1/2(c), 1/4(d), 1/8(e), 1/16(f)

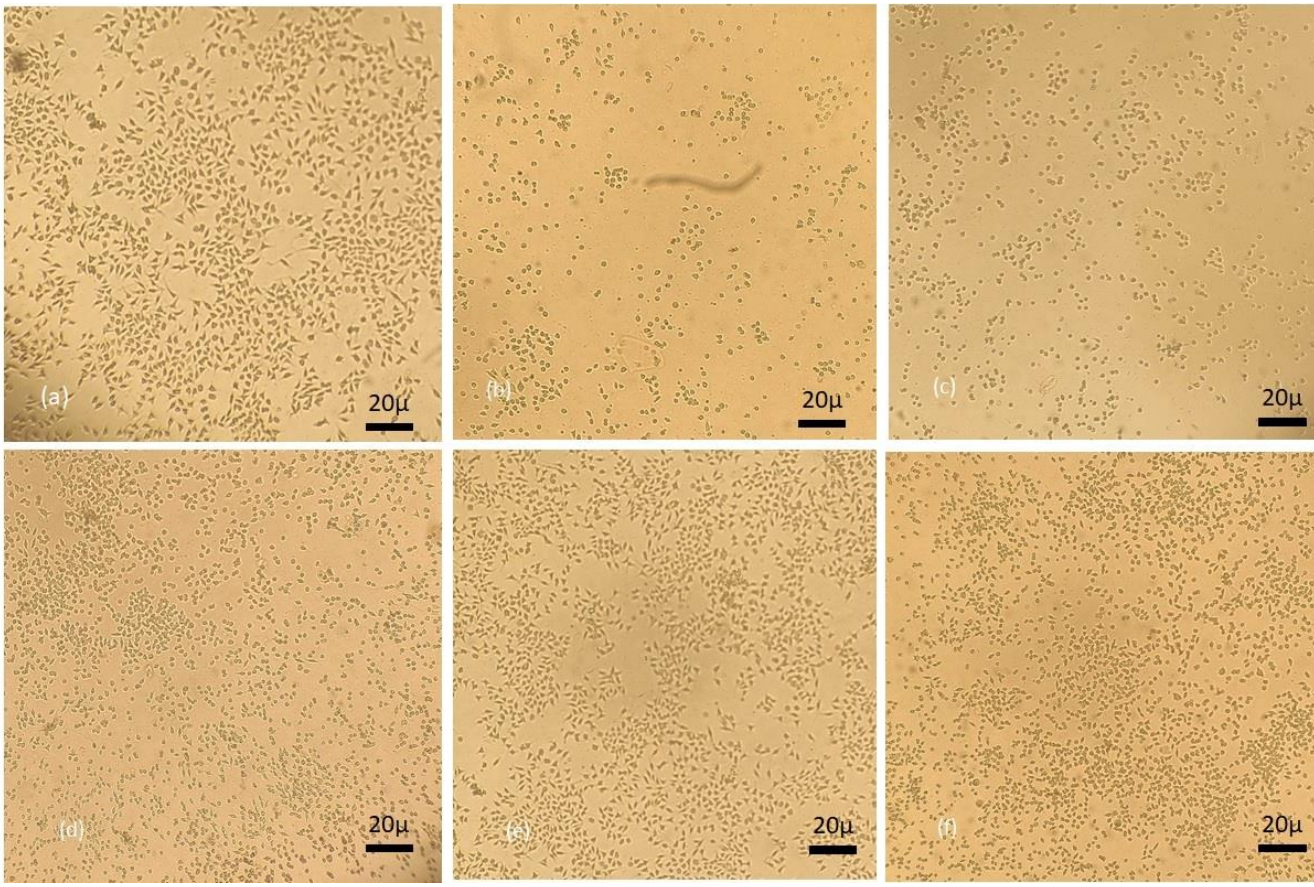

Figure 4. Mean comparison of the number of viable cells in the study dilutions within 24 h and 72 h for each paste

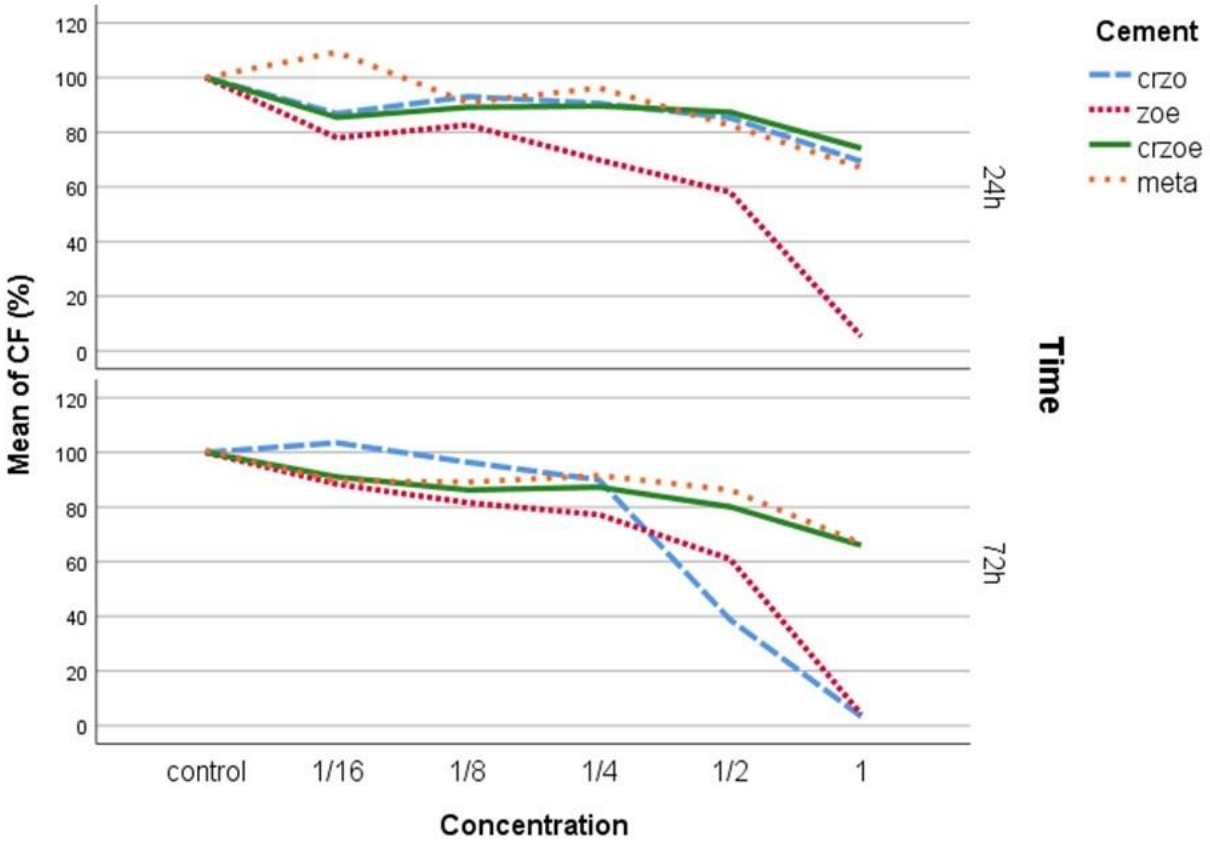

Figure 5. Investigation of the IC50 of pastes at the tested times and concentrations