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RESEARCH

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The cytotoxic effect of cysteamine and its combinations with various endodontic intracanal medications on fibroblast cells: in vitro study

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Abstract

Background This study was established to assess cysteamine's cytotoxic effect alone and in combination with various intracanal medications on fibroblast cells, because the biocompatibility of intracanal medication is considered one of the main factors that affect the selection of specific medication for usage near vital periodontal tissues.

Methods All tested medications were prepared in a solution form. Cysteamine preparation was prepared at 200 mg/ml concentration in distilled water. The chlorhexidine–cysteamine combination was prepared by dissolving 10 mg/ml of cysteamine in chlorhexidine. Calcium hydroxide–cysteamine combination was prepared by dissolving 10 mg/mL of cysteamine in a saturated solution of calcium hydroxide (CaOH). Triple antibiotic paste (TAP)–cysteamine combination was prepared by dissolving 10 mg/mL of cysteamine in triple antibiotic paste (TAP). BHK cells were seeded in well-microtiter plates. The testing materials were filtrated using a 0.22 µm syringe filter. BHK-21 cells precultured well plates were treated with descending 12-fold serially diluted medications at 37 °C for 24 h. Residual living cells were treated with 25 µl of MTT dye. MTT was discarded, and then, dimethyl sulfoxide was added as 50 µl/well. The absorbance was conducted at 570 nm. The mean optical density and 50% cell growth inhibition (IC50) were calculated. Cell viability data showed parametric distribution, so they were analyzed using one-way ANOVA followed by Tukey's post hoc test for intergroup comparisons and repeated measures ANOVA followed by Bonferroni's post hoc test for intragroup comparisons. The significance level was set at $p \leq 0.05$.

Results Viability % and IC50 results showed that triple antibiotic paste (TAP)–cysteamine combination had the lowest cytotoxicity level compared to other intracanal combinations followed by cysteamine and the highest cytotoxicity was with chlorhexidine–cysteamine combination.

Conclusions Triple antibiotic paste (TAP)–cysteamine combination was the safest drug compared to other drug combinations with cysteamine, so it needs more research to detect its acceptance with stem cells and its effect on defense mechanisms during healing.

Keywords Cytotoxicity, Cysteamine, Intracanal medication, MTT assay, Calcium hydroxide, Triple antibiotic paste (TAP), Chlorhexidine

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Background

The usage of intracanal medications is an efficient line of treatment in the endodontic field for root canal disinfection. In the past, intracanal medication had great importance as a disinfection protocol between endodontic visits. Nowadays, its usage becomes limited after following a single visit protocol in many procedures depending on the entomb theory (Peters et al. 1995). Therefore, its usage shifts toward more specific conditions such as immature apices, trauma, and inflammatory root resorption. In addition, intracanal medications still have a great role as a treatment procedure for persistent diseases after root canal treatment, especially in retreatment cases with apical periodontitis.

Calcium hydroxide (CaOH) has been practically used by dentists for over a century. It is classified as a strong base. It releases hydroxyl ions which activate calcium hydroxide against microbes by the oxidant free radicals. The integrity of the cytoplasmic membrane is altered by the high alkalinity of calcium hydroxide (Ba-Hattab et al. 2016). Chlorhexidine is considered the gold standard of oral antiseptics and is the most widely researched medical agent in dentistry. It has been used as an irrigating substance or intracanal medicament alone or in combination with calcium hydroxide. The effectiveness of chlorhexidine (CHX) arises from its capacity to be absorbed into negatively charged surfaces in the mouth, especially dentine, and slowly released from these retention sites. So, its substantivity has been found extended from 48 h up to 12 weeks (Gomes et al. 2013). Triple antibiotic paste (TAP) is a combination of three antibiotics: ciprofloxacin, metronidazole, and minocycline. The paste's applications vary from vital pulp therapy to the recently introduced regeneration and revascularization protocol (Parhizkar et al. 2018).

The combination between calcium hydroxide and chlorhexidine had many controversies in the literature and had been compared with other combinations as triple antibiotic paste (TAP)–chlorhexidine combination and calcium hydroxide–cysteamine combination. Ballal et al. (2007), Jhamb et al. (2010) confirmed that 2% chlorhexidine was much better in antibacterial effect than its combination with calcium hydroxide (CaOH). Ghabraei et al. (2018) studied the antimicrobial effect of triple antibiotic paste and calcium hydroxide after their combination with 2% chlorhexidine. Results showed that CaOH mixed with 2% chlorhexidine was able to eradicate the EF biofilm in three days, while triple antibiotic paste (TAP) was able to eradicate the biofilm of EF in seven days.

Pandey et al. (2018) showed a non-significant difference between calcium hydroxide–cysteamine combination and the calcium hydroxide–chlorhexidine combination in bacterial eradication. Guo et al. (2016) agreed with Pandey during applying the antibacterial test in the absence of dentin powder, but there was a significant improvement in the antibacterial effect of calcium hydroxide (CaOH)–cysteamine combination in the presence of dentin powder. Therefore, cysteamine material was chosen as our target material for testing. The main objectives of the intracanal medication are restricting bacterial regrowth, supplying continued disinfection, and creating a physical barrier (Berman and Hargreaves 2020). Therefore, we need new intracanal medication biocompatible with dental tissues to achieve the most beneficial effect without harming the periodontium (Nasim and Hemmanur 2021).

Cysteamine (cys) or 2-mercaptoethylamine is an endogenously synthesized aminothiol in human body cells during the coenzyme A metabolism cycle. This material has many applications in the medical field as a treatment for cystinosis, an autosomal recessive disorder, and hyperpigmentation disorders. Cysteamine (cys) has shown to be a well-tolerated compound, due to its non-mutagenicity and non-carcinogenicity criteria (Qiu et al. 2000). Interestingly, it may inhibit the mutagenic effect of some potent mutagens. Besides, it may exert an anticancer effect as in melanoma (Tatsuta et al. 1988). Cysteamine's antibacterial effect was tested and evaluated, and it was found that it has a synergistic effect with various intracanal medications. Therefore, our objective was to detect its biocompatibility when it was mixed with other medications through cytotoxicity testing using MTT assay on fibroblast cells.

Methods

All procedures were done after ethical approval by ethics committee approval, Faculty of Dentistry, Ain Shams University, and all procedures were done with code FDASU-Rec EM01272. The manuscript of this study has been written according to Preferred Reporting Items for Laboratory studies in Endodontology (PRILE) 2021 guidelines (Nagendrababu et al. 2021a, 2021b) as in Fig. 1.

Tested medications were categorized into four groups which were applied randomly by another physician in three culture titer plates for each medication tested. The groups were as follows: group A for cysteamine preparations, group B for chlorhexidine–cysteamine combination preparation, group C for calcium hydroxide–cysteamine combination preparation, and group D for triple antibiotic paste–cysteamine combination preparation.

Medications' preparation (Pandey et al. 2018)

Cysteamine drug (Cysteamine 98%Rt, Fluka, Switzerland) and its combinations were prepared in fresh solutions

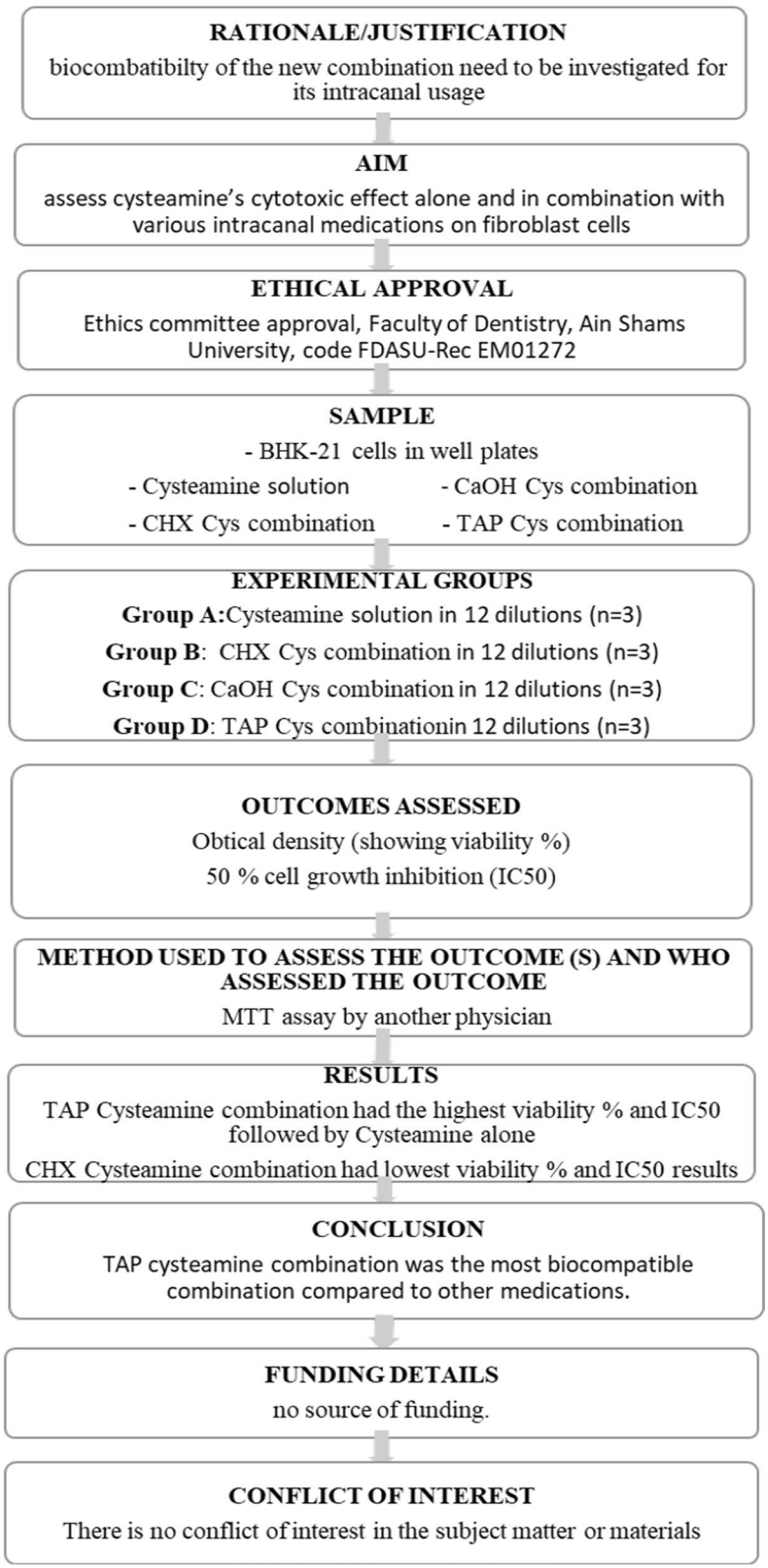

Fig. 1 PRILE 2021 flowchart of the study

to avoid oxidation during dispensing of the drug to have maximized effect (Brodrick et al. 1981; Atallah et al. 2020). The powders of the tested medications were measured and dispensed using a sensitive balance. Liquids' volumes were measured using a graduated pipette.

All tested medications were prepared in a solution form as follows: Cysteamine preparation (group A): Cysteamine was prepared by dissolving the powder in distilled water at a concentration of 200 mg/ml (Pandey et al. 2018; Guo et al. 2016). Chlorhexidine–cysteamine combination (group B): A combination of chlorhexidine 2% with cysteamine was prepared by dissolving 10 mg/ml cysteamine in chlorhexidine. Calcium hydroxide–cysteamine combination (group C): A fresh saturated solution of calcium hydroxide (CaOH) was prepared by mixing it with distilled water at a concentration of 300 mg/ml. A combination of cysteamine and calcium hydroxide (CaOH) was prepared by dissolving 10 mg/mL of cysteamine in a calcium hydroxide (CaOH) solution (Pandey et al. 2018). Triple antibiotic paste–cysteamine combination (group D): TAP powder was prepared from three antibiotics in a tablet form as follows: ciprofloxacin 250 mg, metronidazole 500 mg, and doxycycline 100 mg (Yehia et al. 2019). The ratio of drugs' concentrations is 1:1:1 (Clinical Considerations for a Regenerative Procedure 2021). This concentration ratio was mixed with 2 ml distilled water until having a paste consistency. The total amount of powder used was 4.25 g, and the total amount of water was 10 ml. A combination of cysteamine and triple antibiotic paste (TAP) was done by dissolving 10 mg/mL of cysteamine in TAP (Pandey et al. 2018). The prepared solutions were loaded in sterile syringes to be ready for usage in the MTT assay. The stock solutions were serially diluted to achieve a total of twelve concentrations (100%, 50%, 25%, 12.5%, 6.25%, 3.13%, 1.56%, 0.78%, 0.39%, 0.2%, 0.1%, and 0.05%) relative to cell volume.

Baby hamster kidney (BHK-21) fibroblast cell culture preparation (Radwan et al. 2015)

BHK cells supplied by the American Type Culture Collection (ATCC) and provided by the International Center for Training and Advanced Research (ICTAR, Egypt) were seeded in well-microtiter plates at an initial cell density of approximately 2.5×104 cells/1 ml and allowed to attach overnight. BHK cells were transferred to 12 cell culture titer plates. (Each plate contains 12 wells.)

Cytotoxicity assessment of tested groups (Radwan et al. 2015; Abdel Rahman et al. 2020)

Suspended materials were centrifuged for one hour at 12,000 rpm. The testing materials were filtrated using a 0.22 µm syringe filter. BHK-21 cells precultured well plates (Nunc, USA) were treated with descending 12-fold serially diluted medication at 37 °C for 24 h. Detached cells were washed out using phosphate buffer saline (PBS). Residual living cells were treated with 25 µl of MTT dye (0.5 mg/ml) (Sigma-Aldrich, USA) at 37 °C for four hours. MTT was discarded. Plates were PBS washed three times. Dimethyl sulfoxide (BDH, England) was added as 50 µl/well. Plates were shaken for 30 min to dissolve the produced MTT Formazan complex. The absorbance was conducted at 570 nm using the Dynatec MR5000 spectrophotometer. The absorbance values at 570 nm were relative to the number of residual viable cells. This cytotoxicity test was performed three times for each group. The mean optical density values (OD) and their standard deviations were calculated. The mean cell viability values were defined as the percentage of the OD values of the negative control (Abbaszadegan et al. 2015). Viability % was calculated by the following equation (Radwan et al. 2015):

$$\text{Viability\%} = \text{OD of treated cells/OD of untreated cells} \times 100$$

Compound concentrations that produce 50% cell growth inhibition (IC50) were calculated from curves constructed by plotting cell survival percent versus drug concentration. Cytotoxic effects were expressed as IC50 (Novohradsky et al. 2014).

Statistical analysis

Numerical data were presented as mean and standard deviation (SD) values. They were explored for normality by checking the data distribution and using the Shapiro–Wilk test. Cell viability data showed parametric distribution, so they were analyzed using one-way ANOVA followed by Tukey's post hoc test for intergroup comparisons and repeated measures ANOVA followed by Bonferroni's post hoc test for intragroup comparisons. The significance level was set at $p \leq 0.05$. Statistical analysis was performed with R statistical analysis software version 4.1.2 for Windows.

Results

Intragroup comparisons

The test was done in triplicate because the cells used are primary cells, and twelve drug concentrations were tested to have more reliable results. The cell viability % of BHK-21 fibroblasts varied according to the volume of medication added to BHK-21 cell volume. Higher volumes of the tested medication relative to BHK-21 volume were associated with less cell viability % except for the chlorhexidine–cysteamine combination that showed non-uniform changes in cell viability % in some volumes with higher cell viability % in higher volumes with a statistically non-significant difference as shown in Fig. 2.

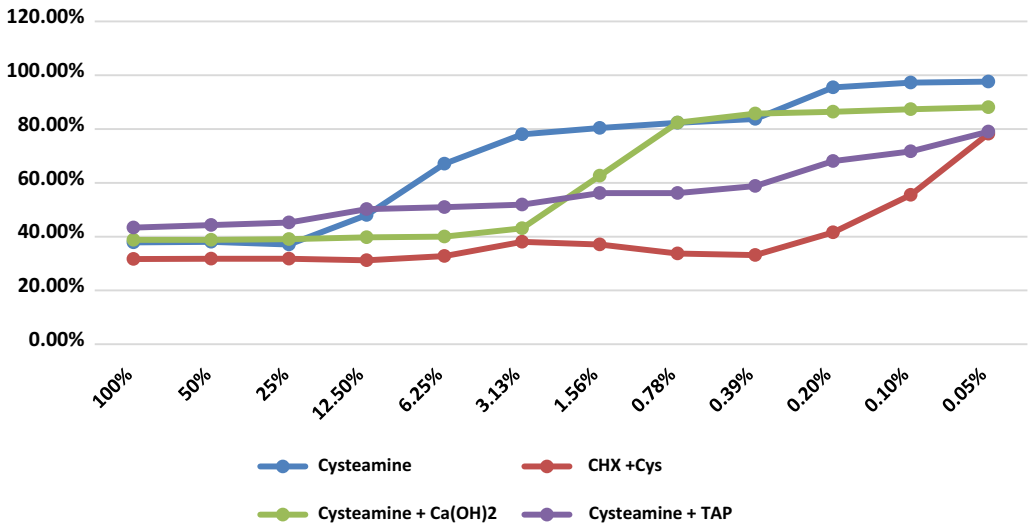

Fig. 2 Line chart showing average cell viability % for different concentrations

In the cysteamine group: 100%, 50%, and 25% concentrations of cysteamine drug incubated with BHK cells showed the lowest values of cell viability %. The lowest result was with a 25% concentration of cysteamine. There was a gradual increase in cell viability % with a decrease in the concentration of Cysteamine. The highest value was measured with 0.05% concentration. In chlorhexidine–cysteamine combination: 100%, 50%, 25%, 12.5%, and 6.25% concentrations showed a comparable highest cytotoxic effect. There was a significant increase in cell viability % and a decrease in cytotoxicity level with 0.2%, 0.1%, and 0.05% concentrations than the higher ones. In calcium hydroxide–cysteamine combination: 100% and 50% concentrations showed the most cytotoxic effect. Mean cell viability % ± SD was the same for both volumes. 1.56% showed a significant decrease in cytotoxicity level than the higher concentrations. Overall, there was a statistically significant difference between values measured at different concentrations for each combination ($p < 0.001$).

In the triple antibiotic paste–cysteamine combination: there was a statistically significant difference between the values measured at different concentrations ($p = 0.008$). The highest value was measured at (0.05%), while the lowest value was found at (100%).

Intergroup comparisons

The cell viability % of BHK-21 was statistically compared between groups. The comparison was done under the same concentration of each group as shown in Fig. 3. Some concentrations showed a non-significant difference in cytotoxic effect on BHK cells at 12.5%, 0.1%, and 0.05%. The remaining tested concentrations showed a statistically significant difference in cytotoxicity level. The lowest cell viability% was in the chlorhexidine–cysteamine combination. In 100%, 50%, and 25% concentrations of each drug, the highest cell viability % values were in the triple antibiotic paste (TAP)–cysteamine combination. In 25% concentration, there was a non-significant difference between the triple antibiotic paste (TAP)–cysteamine combination, calcium hydroxide (CaOH)–cysteamine combination, and cysteamine groups. Regarding 6.25%, 3.13%, and 1.56% concentrations of tested medications, the highest cell viability % was in the cysteamine group. In 6.25% concentration, there was a non-significant difference between cysteamine and triple antibiotic paste (TAP)–cysteamine combination. In comparing 0.78%, 0.39%, and 0.2% concentrations of each medication, the highest values were shared between the cysteamine group and calcium hydroxide (CaOH)–cysteamine combination group as the later medications showed comparable cell viability % results.

The IC50 values were determined and showed that chlorhexidine–cysteamine combination medication had the lowest value (0.12%) followed by calcium hydroxide (CaOH)–cysteamine combination (2.25%). Triple antibiotic paste (TAP)–cysteamine combination had the highest value (12.86%), followed by cysteamine with (11.19%), which indicated there was a statistically significant difference in IC50 between the triple antibiotic paste (TAP)–cysteamine combination and chlorhexidine–cysteamine combination as in Table 1 and Fig. 4.

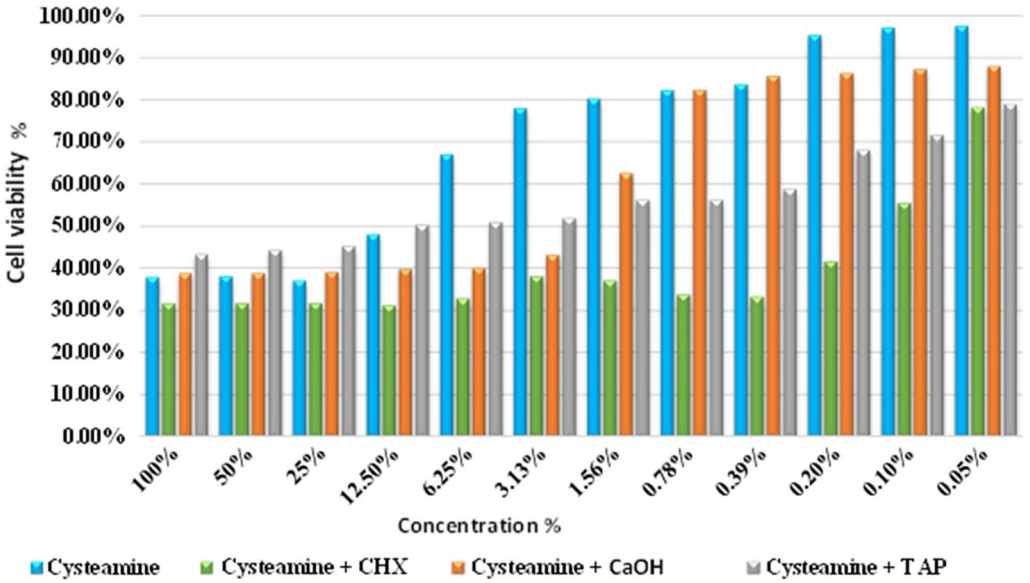

Fig. 3 Bar chart showing average cell viability % for different groups

Table 1 IC50 conc % of each testing medication

	GI Cysteamine	GII CHX + Cys	GIII CaOH + Cys	GIV TAP + Cys
IC50 conc % of drug	11.19	0.12	2.25	12.86

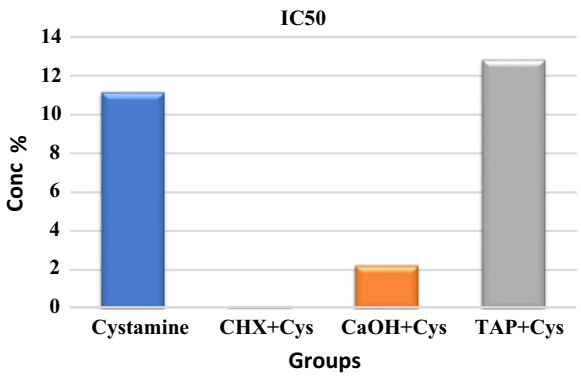

Fig. 4 Evaluation of inhibitory concentration (IC50) post-BHK cells treatment with testing medications

Discussion

Biocompatibility and antibacterial effectivity are considered essential factors for the selection of many intracanal medications. For that reason, many medicaments are less used nowadays due to marked cytotoxicity compared to their effectiveness (Ellerbruch and Murphy 1977; Kumar et al. 2019). Therefore, we regularly search for new intracanal medications which can fulfill the most requirements to be the safest and most effective disinfectants in the root canal system. Cysteamine material was selected to search for due to its approval by FDA to be used in the medical field, and it has many medical applications. It is an aminothiol endogenously synthesized by human body cells during the coenzyme A metabolism cycle. It is a well-tolerated compound demonstrating non-mutagenic and non-carcinogenic criteria. It also has an antibacterial effect (Atallah et al. 2020).

MTT assay was implemented for cytotoxicity assessment, as it is the most common in vitro method used to determine cytotoxicity. It depends on mitochondrial activity which is constant, and thereby an increase or decrease in the number of viable cells is linearly related to mitochondrial activity. The mitochondrial activity of the cells is reflected by the conversion of the tetrazolium salt into formazan crystals, which can be solubilized for homogenous measurement. Thus, any increase or decrease in viable cell number can be detected by measuring formazan concentration reflected in the optical density (Meerloo et al. 2011).

The test was proved to be more accurate and time-saving than other conventional hemocytometer counting methods (Rahayu 2018). It is suitable for the measurement of drug sensitivity in established cell lines as well as primary cells. Therefore, the most common use of it is to determine the cytotoxicity of several drugs at different concentrations as the purpose of our study.

BHK-21 cell from the fibroblast of a baby hamster's kidney was selected as it is commonly used by researchers for cytotoxicity testing of dentistry materials since it

is equivalent to a dental fibroblast, the most important cell in the components of pulp, periodontal ligament, and gingiva (Rahayu 2018). They are commonly used for endodontic research since they are available, easily cultured, and consistent in quality (Thomas et al. 2008).

Testing preparations were applied in liquid form to be easily sterilized by 0.22 μm syringe filter. Methylcellulose, which is used in gel formation of intracanal medication, is considered an inert material. Therefore, its absence will not affect the cytotoxicity results (Mozayeni et al. 2014).

The test was done in triplicate because the cells used are primary cells and twelve concentrations of each drug were tested to have more reliable results. For the dose–response curve, the stock solutions were diluted in a cell culture medium to achieve a total of twelve concentrations. The term "dose–response" simply refers to the relationship between the applied dose/concentration (the amount of substance administered to cultured cells and the effect that is observed). It is a way to determine the toxic, therapeutic, and lethal doses of drugs (Campbell and Cohall 2017; Vandenberg 2022).

In our study, the viability value of BHK cells incubated with cysteamine showed a consistent increase in cell viability % with decreasing concentration of cysteamine. This was applied also with the triple antibiotic paste (TAP) combination and calcium hydroxide combination. This consistent increase in viability agreed with Vouzara et al. (2016) who reported that antimicrobials have a direct relationship between their dose and their cytotoxic effect.

Some fluctuant changes occurred in the cytotoxicity results of the chlorhexidine–cysteamine combination, as cell viability % decreased in 1.56%, 0.78%, and 0.39% concentrations and then increased again; nevertheless, this observation did not have a significant effect. Neufeld et al. (2018) reported that some chemical compounds caused false responses across the tested concentrations in the MTT assay.

Natarajan et al. (2000) interpreted that thiol-containing antioxidant compounds, like cysteamine, reduced MTT tetrazolium salts to a blue formazan product in a dose-dependent manner, irrespective of the viability of the cells present. Accordingly, this explanation could be applied to the shift that occurred with the chlorhexidine–cysteamine combination. These changes did not occur with other combinations, although the thiol group in cysteamine existed in all groups. The reaction of cysteamine with chlorhexidine was not understood and may be different from other combinations.

The results of this study showed that cysteamine cytotoxicity level was the lowest except at 100% (2592.353 μM), 50% (1296.177 μM), and 25% (648.0883 μM) which are considered above 1250 μM (toxic concentration of Cys) as per Jeitner and Lawrence (2001) who also showed a plateau level of cytotoxicity above 160 μM. Below this value, there was a linear correlation between concentration and toxicity. They interpreted the reason for the cytotoxic effect of cysteamine by considering two causes. The first is the H_2O_2 produced, which accounts for 57% of its toxicity, as cysteamine is unstable in an aqueous solution; a rapid conversion to cysteamine occurs due to the rapid oxidation of the sulfhydryl group. The reactions of oxygen with thiols in aqueous solutions give disulfides and hydrogen peroxide (Eq. 1) or water (Eq. 2).

$$2\ RSH + O_2 \rightarrow RSSR + H_2O_2 \quad (1)$$

$$4\ RSH + O_2 \rightarrow 2RSSR + 2H_2O \quad (2)$$

The second is the inhibition of glutathione peroxidase, an enzyme having a biological role to protect the organism from oxidative damage, which accounts for the remaining 43% of its toxicity. *Enterococcus faecalis* was reported to produce high glutathione in a rich medium (Pophaly et al. 2012), and it was found that glutathione peroxidase and glutathione reductase activities are partially responsible for determining the susceptibility of cells to oxidative stress (Yang et al. 2006). Therefore, the inhibition of glutathione peroxidase may sensitize these cells, and this may be one of the causes of its antibacterial effect against *E. faecalis*.

Chlorhexidine–cysteamine combination had the highest cytotoxicity in all concentrations as agreed with Liu et al. (2018) who showed that cell survival was at a concentration of 0.002% across all tested cells including fibroblasts. Our concentration of interest (2%) is cytotoxic to all cell types.

In our study, there was a slight decrease in cytotoxicity at 0.2% with a significant reduction at 0.1% and 0.05% concentrations. These results may be approximated to Mirhadi et al. (2014) who showed less cytotoxicity detected at 0.2% concentration of chlorhexidine. He found that a combination of 2% chlorhexidine with H_2O_2 is cytotoxic, but 0.2% chlorhexidine is less cytotoxic with 1% and 3% H_2O_2 without a significant difference between both in cytotoxicity. Therefore, he recommended the usage of 0.2% chlorhexidine with 3% H_2O_2 to maximize the synergistic antibacterial effect with the least cytotoxicity. This may help to explain the synergistic effect between cysteamine and chlorhexidine as cysteamine is a source of H_2O_2.

The calcium hydroxide–cysteamine combination at lower concentrations had lesser cytotoxicity levels compared to cysteamine alone. This was in alignment with Silva et al. (2020) who evaluated the cytotoxicity of calcium hydroxide paste associated with 5% diclofenac

sodium, ibuprofen, or amoxicillin to decrease its cytotoxic effect. They found that calcium hydroxide (CaOH) pastes associated with the drugs were not cytotoxic and presented biocompatibility after implantation in rat subcutaneous tissues. Dianat et al. (2015) found that the cytotoxicity of calcium hydroxide (CaOH) in conventional and nanosized forms had a non-significant difference. Therefore, the cytotoxicity of calcium hydroxide may decrease with combination with other antimicrobials, but it is not affected by particle size.

Triple antibiotic paste (TAP)–cysteamine combination showed less cytotoxicity in higher concentrations. Similarly, Khoshkhounejad et al. (2019) compared the cytotoxicity of triple antibiotic paste (TAP) with calcium hydroxide and found that triple antibiotic paste seemed to be the safest drug for the stem cells of the apical papilla. In lower concentrations, the calcium hydroxide combination was less cytotoxic than triple antibiotic paste (TAP) as Yadlapati et al. (2014) showed that triple antibiotic paste and minocycline were the most cytotoxic materials and calcium hydroxide (CaOH) had a minimal effect on cell viability and cytokine production.

Cysteamine and triple antibiotic paste (TAP)–cysteamine combination showed a significant difference in IC50 results compared to the calcium hydroxide (CaOH)–cysteamine combination and chlorhexidine–cysteamine combination. As triple antibiotic paste (TAP)–cysteamine combination, cysteamine alone had a lower cytotoxic effect than calcium hydroxide combination and chlorhexidine combination.

Results of both cell viability %, which reflected the number of lived/metabolically active cells in a population, and IC50 which reflected the dose of the cytotoxic compound at which 50% viability on BHK fibroblast cells was achieved were in harmony as they showed that triple antibiotic paste (TAP)–cysteamine combination had the lowest cytotoxicity level compared to other intracanal combinations followed by cysteamine and the highest cytotoxicity was with chlorhexidine–cysteamine combination and usage of chlorhexidine–cysteamine combination should be in lowest concentrations as described by Mirhadi et al. (2014).

It was approved that cysteamine has an acceptable antibacterial effect and a good synergistic effect with other intracanal medications, especially with triple antibiotic paste which showed a higher antibacterial effect (Pandey et al. 2018; Elgammal et al. 2022). Our study found that the triple antibiotic paste (TAP)–cysteamine combination had the least cytotoxic effect compared to others. While observing the correlation between the antibacterial and cytotoxicity results, it was found that the chlorhexidine–cysteamine combination had the highest cytotoxicity and antibacterial effect, as the antibacterial effect may be caused by the cytotoxic mechanism of the drug. The cysteamine group also showed the second lower cytotoxicity level and lowest antibacterial effect. This correlation was not applied in the triple antibiotic paste (TAP)–cysteamine combination, as it showed the second highest antibacterial effect although it had a less cytotoxic effect. It was better for this study to be applied to human dental stem cells for saving more research time. Therefore, further research is needed to detect its acceptance for usage in in vivo studies.

Conclusions

Triple antibiotic paste (TAP)–cysteamine combination showed the best results in biocompatibility compared to other medications, so it needs to be under more research to detect its efficacy on stem cells and defense mechanisms to evaluate its acceptance in healing and regeneration.

Abbreviations

CHX	Chlorhexidine
CaOH	Calcium hydroxide
Cys	Cysteamine
TAP	Triple antibiotic paste
BHK cells	Baby hamster kidney cells
OD	Optical density

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Author contributions

The study idea was under the supervision of SE and AH. SH supervised the methodology of the test. EE applied the methodology, wrote the manuscript text, and prepared figures and tables. AH reviewed the manuscript. Finally, all authors have read and approved the manuscript.

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Availability of data and materials

All the data are available from the corresponding author upon a reasonable request.

Declarations

Ethics approval and consent to participate

The study was exempt from ethical review because it was purely laboratory research in which no patients were involved, and no experimental animals were used. All procedures were done after ethical approval by ethics committee approval, Faculty of Dentistry, Ain Shams University, and all procedures were done with code FDASU-Rec EM01272.

Consent for publication

Not applicable.

Competing interests

There are no competing interests in the subject matter or materials discussed in this study.

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