

The Cytotoxicity of Green Okra (*Abelmoschus esculentus*) on Gingival Fibroblasts

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ABSTRACT

Sodium hypochlorite and EDTA, as irrigating solutions, when they come into contact with periapical tissue, are detrimental. Therapeutic material derived from the medicinal plant green okra (*Abelmoschus esculentus*) is known for its antioxidant and anti-inflammatory properties, making it a promising candidate. However, prior to clinical application, its cytotoxicity must be evaluated.

Purpose: To evaluate the cytotoxicity of green okra (GO) on human gingival fibroblasts (HGF) using the MTT assay.

Method: HGF stock cell primary culture was employed in this experimental facility. The group tests: GO (6.25%-100%), NaOCl (1.25%, 2.5%), 17% EDTA. The solutions were applied to the HGF after they were seeded in a 96-well flat-bottom microplate and cultured for 48 hours. Using the MTT assay, the cell viability was examined.

Result: GO maintained cell viability above 94% across all concentrations, indicating non-cytotoxicity. NaOCl at 1.25% was also non-cytotoxic, while 2.5% NaOCl showed slight cytotoxicity. In contrast, 17% EDTA was severely cytotoxic, with viability reduced to < 2%. Kruskal-Wallis analysis revealed significant differences among groups ($p < 0.05$).

Conclusion: The green okra is non-cytotoxic to gingival fibroblasts.

Keywords: cytotoxicity; green okra; irrigant solution

INTRODUCTION

Eliminating bacteria, debris, and necrotic tissue from the root canals is the primary objective of endodontic therapy.^[1] Irrigation is a critical component of this process, as it removes microorganisms, flushes out debris, lubricates root canal instruments, and dissolves organic tissue. An ideal irrigation solution should be non-toxic, possess broad-spectrum antibacterial activity, dissolve necrotic pulp tissue, inactivate endotoxins, and facilitate smear layer removal.^[2,3] The combined use of ethylenediaminetetraacetic acid (EDTA) and sodium hypochlorite (NaOCl) has been widely recognized as the gold standard for achieving these objectives.^[4]

Sodium hypochlorite (NaOCl), known for its strong antibacterial and tissue-dissolving properties, has long been used in root canal irrigation.^[5] However, it can cause injury when in contact with periapical tissue. Penetration of NaOCl into this tissue may lead to pain, ulceration, hemolysis, and edema, as well as swelling, allergic reactions, and an unpleasant odor and taste.^[6]

Similarly, while 17% EDTA is considered the most effective chelating agent for removing the smear layer by eliminating its inorganic components,^[1,7,8] extrusion into periapical tissues can result in cytotoxicity, inflammation, necrosis, and apoptosis.^[9,10,11]

These limitations highlight the need for safer, natural alternatives. One promising candidate is green okra (*Abelmoschus esculentus*), which contains antibacterial compounds such as alkaloids, flavonoids, saponins, and tannins, and also exhibits anti-inflammatory properties.^[12] Any natural material intended for intraoral use must be biocompatible, with cytotoxicity being a key parameter to evaluate, as it reflects the material's potential to damage cells.^[13] In light of this, the present study aims to analyze the effect of green okra extract on cell viability, addressing the need for endodontic irrigants that are less harmful to periapical tissues than current conventional agents.

MATERIALS & METHODS

A post-test-only control group design in an experimental laboratory investigation was used in this research. The research was carried out at the Biomolecular Laboratory of Medicine CDAST, University of Jember, and used a stock of primary human fibroblast cell culture. There were eight solution tests, as follows: 100% green okra extract, 50% green okra extract, 25% green okra extract, 12.5% green okra extract, 6.25% green okra extract, 1.25% NaOCl, 2.5% NaOCl (OneMed, Indonesia), 17% EDTA (PREVESDenPro, USA).

Manufacturing of green okra extract

Green okra is made in the form of an extract by slicing the green okra into tiny pieces and leaving it to aerate at room temperature for two days. It is then baked in an oven for 24 hours at 400°C. Once dry, blend and sieve with a 40-mesh sieve until it becomes a fine powder. Following that, a three-day maceration process using 96% ethanol is conducted, with stirring every twenty-four hours. To get 100% green okra extract (GOE), the solution was concentrated using a rotary evaporator set at 500°C and rotating at 90 RPM.^[14] Then, dilution is carried out using the serial dilution method by adding DMSO to get 50%, 25%, 12.5%, and 6.25% GOE.

Cytotoxicity assessment (MTT assay)

In a 96-well flat-bottom microplate, cells were seeded, and they were incubated for the entire night at 37 °C, 95% humidity, and 5% CO₂. 100%, 50%, 25%, 12.5%, 6.25% GOE, 1.25% and 2.5% NaOCl, and 17% EDTA were applied to the samples. For an additional 48 hours, the cells were incubated. Each well received 20 µL of the MTT staining solution after being twice cleaned with Phosphate Buffered Saline (PBS). The plate was then incubated at 37°C. Four hours later, each well received 100 µL of dimethyl sulfoxide (DMSO) to dissolve the formazan crystals, and the optical density (OD) was measured.^[15] The percentage of viable cells is measured by the following formula:

$$\frac{\text{Absorbance treatment} - \text{Absorbance control media}}{\text{Absorbance control cells} - \text{Absorbance control media}} \times 100$$

Statistical Analysis

Data were analyzed using the Kruskal-Wallis test followed by the Mann-Whitney test with a significance level of $p < 0.05$.

RESULT

As illustrated in Figure 1, the average viable cells are as follows: 100% GOE, 50% GOE, 25% GOE, 12.5% GOE, 6.25% GOE, 1.25% NaOCl, 2.5% NaOCl, and 17% EDTA. The results from the normality test for all groups

showed a significant value ($p > 0.05$), indicating that the data were normally distributed. Based on Levene's Test, the significance value shows ($p < 0.05$), so it can be concluded that the data is not homogeneous. There was a significant difference ($p < 0.05$) in the average viable cell between each group, according to the Kruskal-Wallis test. Table 1 displays the Mann-Whitney test result.

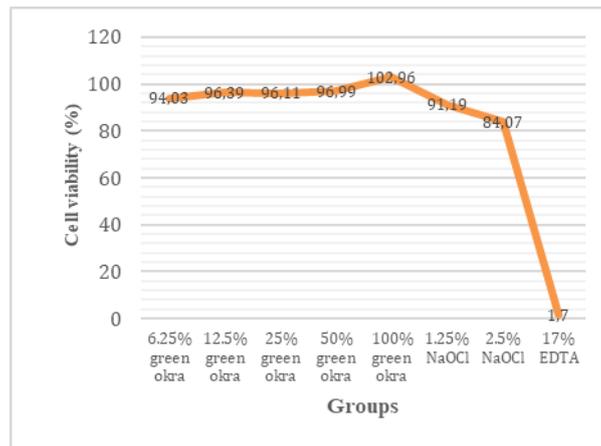


Figure 1. The average viable cell value.

Table 1. The Mann-Whitney test result.

Group	100% GO	50% GO	25% GO	12.5% GO	6.25% GO	2.5% NaOCl	1.25% NaOCl	17% EDTA
100% GO								
50% GO	0.827							
25% GO	0.827	0.827						
12.5% GO	0.827	1	0.827					
6.25% GO	0.376	0.513	0.275	0.827				
2.5% NaOCl	0.05*	0.05*	0.05*	0.05*	0.05*			
1.25% NaOCl	0.275	0.275	0.127	0.275	0.513	0.05*		
17% EDTA	0.05*	0.05*	0.05*	0.05*	0.05*	0.05*	0.05*	

*Significantly different

DISCUSSION

An ideal root canal irrigant should be safe for tissues, kill a wide range of bacteria, dissolve dead pulp tissue, neutralize harmful toxins, and remove or prevent the buildup of the smear layer.^[2,3] During endodontic treatment, irrigation fluid may leak through the apical foramen, potentially causing inflammation of the periapical tissues and delaying healing and regeneration.^[6,15] As irrigating solutions may come into contact with periradicular tissues, their biocompatibility is crucial. An irrigation solution that is not biocompatible can cause chemical injury to the periapical tissue, making low cytotoxicity a key requirement for an ideal irrigant.^[11]

The present results show that the highest rate of cell death occurred with 17% EDTA, as indicated by the lowest overall cell viability. This outcome may be explained by EDTA's strong chelating ability, which allows it to bind essential divalent metal ions such as Ca^{2+} and Mg^{2+} . The removal of these ions disrupts cell adhesion and leads to a decline

in MTT activity.^[15] EDTA interacts with inorganic ions of endogenous metals within cells, and since DNA has a high affinity for free metal ions, this chelation indirectly affects DNA stability. Although EDTA poorly penetrates cell membranes, it acts extracellularly to bind ions, altering membrane permeability and structure. This process can result in mitochondrial dysfunction.^[16] Even at low concentrations, EDTA extrusion into periapical tissues may trigger inflammatory and immune responses associated with periapical lesions.^[15] However, Botton et al.^[17] found EDTA to be the least cytotoxic among common irrigants when used alone or in combination, suggesting that cytotoxicity may be influenced by concentration, exposure time, and application method.

Sodium hypochlorite (NaOCl) remains widely recommended for root canal irrigation due to its dual ability to dissolve necrotic and vital tissue and to kill bacteria. Although higher concentrations enhance its antimicrobial and tissue-dissolving effects,

they also increase toxicity risk.^[6] Consistent with this, our study showed that cells treated with 1.25% NaOCl exhibited higher viability than those exposed to 2.5% NaOCl. The cytotoxicity of NaOCl is primarily linked to available chlorine content rather than pH or osmolarity. When NaOCl produces hypochlorous acid (HOCl) upon contact with organic tissue, chlorine release can trigger free radical formation and increase reactive oxygen species (ROS) levels.^[18] Its high pH also promotes hydroxyl ion release, which damages mitochondria and compromises the cytoplasmic membrane by forming mitochondrial permeability transition pores. This damage reduces ATP production, impairs oxidative phosphorylation, and leads to oxidative stress, resulting in lipid peroxidation, reduced protein synthesis, DNA damage, and ultimately, cell death.^[19] Cells exposed to green okra extract showed higher viability than those treated with either NaOCl or EDTA, indicating lower cytotoxicity. This protective effect is likely due to the phytochemical composition of green okra, such as polyphenols, flavonoids, tannins, alkaloids, saponins, terpenoids, and steroids. Flavonoids, for example, have strong antioxidant and anti-inflammatory properties that promote fibroblast proliferation, enhance collagen synthesis, and improve oxygen diffusion to cells.^[20,21] Saponins, another key component, possess anti-inflammatory and antibacterial activities and can modulate cell viability in a concentration-dependent manner, stimulating growth at low concentrations and inducing apoptosis at higher levels. Likewise, tannins have antioxidant and anti-inflammatory properties. These compounds also contribute to reducing oxidative DNA damage, enhancing angiogenesis, and accelerating wound healing. In okra extract, the synergistic action of flavonoids, saponins, and tannins likely promotes fibroblast proliferation and migration, supporting faster tissue repair.^[22,23,24] Previous studies support the antibacterial potential of green okra, particularly against *Staphylococcus aureus*.¹⁴ However, at

concentrations of 12.5%, 25%, 50%, and 100%, okra extract was less effective in inhibiting *Streptococcus mitis* growth compared to 2.5% NaOCl and 17% EDTA.^[25] Despite this, its superior biocompatibility profile is notable. Toxicity classification based on cell viability categorizes agents as non-toxic (>90% viability), slightly toxic (60–90%), moderately toxic (30–59%), and highly toxic (<30%).^[26] According to this scale, 17% EDTA was highly toxic, 2.5% NaOCl was slightly toxic, and 1.25% NaOCl was non-toxic. All tested concentrations of green okra extract (100%, 50%, 25%, 12.5%, and 6.25%) fell into the non-toxic category. Interestingly, higher okra concentrations supported greater cell survival, likely due to the increased abundance of active compounds. This highlights the potential of natural materials, such as green okra, as safer alternatives in endodontics.

CONCLUSION

Green okra maintained high cell viability in gingival fibroblasts across all concentrations tested, indicating non-cytotoxic effects.

Declaration by Authors

Ethical Approval: Approved

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