

Research Report

Cytotoxicity test of 0.78% xanthone from mangosteen pericarp (*Garcinia mangostana L.*) and 0.2% chlorhexidine gluconate toward BHK-21 fibroblast cells

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ABSTRACT

Background: Chlorhexidine gluconate is one of endodontic irrigants that has excellence capability to penetrate into dentin tubules and kill the pathogenic bacteria there. On the other hand, chlorhexidine gluconate has side effects to cause allergic reactions of the tissue and discoloration of the teeth. Xanthone from mangosteen pericarp can be considered as a natural alternative irrigant that usually has a good tolerance to the body. **Purpose:** The aim of this study compared the cytotoxicity between 0.78% xanthone from mangosteen pericarp and 0.2% chlorhexidine gluconate toward BHK-21 fibroblast cells. **Methods:** This study used experimental post-test only control group design. Xanthone from mangosteen pericarp preliminary cytotoxicity tested in various concentrations. Xanthone from mangosteen pericarp classified as a non-toxic concentration at 0.78%. Cytotoxicity of 0.78% xanthone from mangosteen pericarp compare with cytotoxicity of 0.2% chlorhexidine gluconate using MTT assay method. Cytotoxicity of material can be seen from % of cell viability. Viable cell measured by the result of optical density that read by ELISA reader 620 nm. **Result:** 0.78% xanthone from mangosteen pericarp showed lower cytotoxicity than 0.2% chlorhexidine gluconate toward BHK-21 fibroblast cells. One-way ANOVA showed a significant difference between the study groups ($P < 0.05$). **Conclusion:** 0.78% xanthone from mangosteen pericarp showed lower cytotoxicity than 0.2% chlorhexidine gluconate toward BHK-21 fibroblast cells.

Keywords: Xanthone from mangosteen pericarp; chlorhexidine gluconate; cytotoxicity; BHK-21.

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INTRODUCTION

The success of root canal treatment is inseparable from the cleanliness effect of the root canals of all microorganisms and prevention of re-infection¹. Unclean root canal walls provide a breeding ground for bacteria, reduce attachment of obscuration material, increase apical gaps, and cause root canal blockage².

Mechanical root canal cleaning alone is not enough to make the root canal free of bacteria³. Irrigation materials are needed to minimize the presence of bacteria and clean the root canals from the remnants of organic tissue. Some examples of irrigation materials commonly used are sodium hypochlorite (NaOCl), ethylenediamine tetra-acetic acid (EDTA), hydrogen peroxide (H₂O₂), and chlorhexidine gluconate⁴. Chlorhexidine gluconate is a type of root canal irrigation material that has broad spectrum antibacterial activity⁵. Chlorhexidine gluconate as a root canal irrigation material has deficiencies which can cause allergic reactions in tissues and tooth discoloration³.

Various studies mentioned that chlorhexidine gluconate has a high level of toxicity. Chlorhexidine gluconate cytotoxicity test on human fibroblast cells showed toxic effects related to decreased cell protein synthesis⁶. Chlorhexidine gluconate can inhibit mitochondrial activity of periodontal ligament fibroblast cells⁷. Chlorhexidine gluconate in low concentrations can induce apoptosis and at high concentrations can cause necrosis of fibroblast cell periodontal cells⁸.

One of the herbal ingredients that can be considered as an alternative to root canal irrigation is mangosteen peel extract. Some of the advantages of using herbal ingredients as an alternative ingredient in the health field are fewer side effects, cheaper, and better tissue tolerance⁹.

Mangosteen peel extract has been shown to have antibacterial properties obtained through xanthone-derived compounds namely alpha-mangostin. One study said alpha-mangostin taken from mangosteen peel had greater effectiveness in killing the bacteria *Enterococcus faecalis* (*E. faecalis*) compared with chlorhexidine. In the same

study, alpha-mangostin cytotoxicity testing of human gingival fibroblasts showed no cytotoxic effect¹⁰.

Not only is antibacterial properties needed to obtain the ideal irrigation agent but the material must also have a biocompatible effect on tissue¹¹. Until now, there has been no further study on the cytotoxicity test of mangosteen peel xanthone extracts against fibroblast cells compared with chlorhexidine gluconate 0.2% in an effort development of alternative root canal irrigation materials.

According to the previous studies, it was found that the mangosteen peel xanthone extract was declared non-toxic at a concentration of 0.78%. Further study was conducted by comparing the results of the cytotoxicity test of mangosteen peel xanthone extract concentration of 0.78% and chlorhexidine gluconate 0.2% against BHK-21 fibroblast cells. This study aims to determine differences in the level of toxicity between 0.78% mangosteen peel xanthone and 0.2% chlorhexidine gluconate against BHK-21 fibroblast cells.

MATERIALS AND METHODS

This type of study was an experimental laboratory research design with post-test only control group design. The study was conducted at the Veterinaria Farma Center (PUSVETMA) Surabaya. The ingredients used were mangosteen peel xanthone, 0.2% chlorhexidine gluconate, BHK-21 fibroblast cell culture, culture media containing Eagle’s minimum essential medium, kanamycin, 1% extractor, fetal bovine serum (FBS) 10%, fungizone 100 units / ml , phosphate-buffered saline (PBS), and dimethylsulfoxide (DMSO), sterile aquadest (Otsuka), and MTT reagents [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] (Sigma -USA).

Xanthones were obtained from the isolation of ingredients in mangosteen peel extract with hexane alcohol acetate and chloroform solvents. Xanthones were diluted with sterile aquadest to obtain a concentration of 0.78%.

The cytotoxicity test used the MTT Assay method, consisting of 4 groups with a total sample of 6 wells planted on a 96 well microplate. Well in column group 1 was as a media control filled with culture media as much as 100 µl. Well in column group 2 as a control cell filled with cell culture dissolved in 100 µl of culture media. Well in groups

3 and 4 were filled with cell culture with the addition of 0.78% mangosteen peel skin in group 3 and chlorhexidine gluconate 0.2% in group 4. Microplate was incubated under 5% CO2 and 37°C, for 20 hours. After incubation, the test material and culture media were taken with a syringe and washed with PBS and replaced with new culture media as much as 100 µl with the addition of MTT 5g / ml in PBS 25µl. Microplate was re-incubated for 4 hours. Culture media and MTT were taken using a syringe and DMSO was added to dissolve formazan crystals, then the microplate was stirred mechanically using a shaker for 5 minutes.

The optical density (OD) value of the formazan crystals formed was read using an ELISA reader with a wavelength of 620 nm and the percentage of living cells was calculated by the formula¹²:

$$\% \text{ living cell} = \frac{\text{OD treatment} - \text{OD media}}{\text{OD cell control} - \text{OD media}} \times 100\%$$

RESULTS

The level of toxicity of a substance can be observed based on the percentage of cell life after treatment. To find out the percentage of cell life, OD data was entered into the formula for calculating the% of cell life. Graph of the average yield of the cell life of each treatment and control can be seen in Figure 1.

Data from the study were analyzed with One-way ANOVA statistical test to see the significance of the difference in mean data results between each treatment and control group. Before the test was conducted, it was necessary to test the normality of data distribution using the Kolmogorov-Smirnov test and the homogeneity test of the data variance using the Levene’s test.

Kolmogorov-Smirnov test results showed a significance greater than 0.05 which means that the data distribution was normal (p> 0.05). Homogeneity variance test results with Levene’s test obtained p = 0.650 which means that all data variance was homogeneous (p> 0.05). After finding out that all groups have a normal distribution and homogeneous variance, One-way ANOVA analysis can be performed. One-way ANOVA test results obtained a significance value of 0.000 which proves there were differences between the results of the data of each treatment and control group (p <0.000).

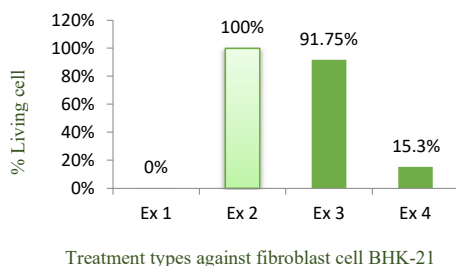


Figure 1. Graph of the percentage of cell life after treatment
Information: Ex 1 = Media control; Ex 2 = cell control; Ex 3 = Xanthone mangosteen peel 0.78%; Ex 4 = Chlorhexidine gluconate 0.2%.

Table 1. Results of statistical analysis of treatment and control group data

Group	1	2	3	4
1	-	0.000*	0.000*	0.044*
2		-	0.080	0.000*
3			-	0.000*
4				-

Information:
* = shows significant differences
Group 1 = Media control
Group 2 = Cell control
Group 3 = mangosteen skin Xanthones 0.78%
Group 4 = Chlorhexidine gluconate 0.2%

Furthermore, further analysis was conducted, such as Tukey post-hoc test to find out whether the differences obtained were significant or not. Significance of less than 0.005 ($p < 0.005$) indicates that there were significant differences between the two data groups.

Table 1 showed the average results of the 0.78% mangosteen peel xanthone treatment group had a significant difference between the chlorhexidine gluconate 0.2% treatment group and the media control but did not have a significant difference with the cell control group. The average results of the 0.2% chlorhexidine gluconate treatment group had a significant difference with the 0.78% mangosteen skin xanthone treatment group, cell control group, and media control group.

DISCUSSION

Chlorhexidine gluconate is an antiseptic agent that can be used as an irrigation agent with the advantage of being able to penetrate into the dentine tubules and kill pathogenic bacteria in it⁵. On one side chlorhexidine gluconate has side effects causing allergic reactions in tissues and tooth discoloration. *Garcinia mangostana L.* has potential as an alternative to natural irrigation which generally has good tolerance to the body. Mangosteen skin xanthenes are shown to have antibacterial activity obtained from their derivative compounds, alpha-mangostin. Alpha-mangostin has the effectiveness of killing *E. faecalis* bacteria greater than chlorhexidine¹⁰. This study was conducted to determine the level of toxicity of mangosteen skin xanthone 0.78% compared to chlorhexidine gluconate 0.2% as an effort to utilize material as an alternative to root canal irrigation.

The cytotoxicity test is a preliminary consideration in evaluating a material to be used for biomedicine¹³. A cytotoxicity test in this study was conducted by using the MTT assay method on BHK-21 fibroblast cell culture¹⁴.

An ingredient is categorized as a non-toxic concentration if the percentage of cell life that is exposed to the material is more than 90%. In the chlorhexidine gluconate 0.2% treatment group, the average cell life percentage was 15.28%. The average percentage of cell life in the mangosteen skin xanthone treatment group showed more than 90%, while in the chlorhexidine gluconate 0.2% treatment group it showed a figure of less than 90%. This gives an understanding that mangosteen skin xanthone 0.78% belongs to the category of non-toxic to cells while chlorhexidine gluconate 0.2% is toxic to cells.

The administration of mangosteen peel xanthone at a concentration of 0.78% against BHK-21 fibroblast cells resulted in a high average cell life of 91.75%. The results of the study are in accordance with the theory of the existence of a cell protection system in the xanthone content of mangosteen skin through antioxidant power. The antioxidant activity of mangosteen xanthone peel works to prevent cell damage and death through DNA protection from oxidative damage¹⁵.

Free radicals and other oxidative agents can be formed during the process of cell metabolic activity. Oxidative free radicals are unstable compounds that tend to damage the structure of proteins, lipids, and DNA of cells that can cause aging, and cell death¹⁶. Xanthone mangosteen peel has a strong antioxidant power in capturing various harmful radical compounds such as hydroxyl radicals, superoxide, and nitric oxide. The special mechanism of the antioxidant activity of mangosteen xanthone skin is to inhibit the occurrence of lipid peroxidation. Lipid peroxidation is the key to various pathological events. The presence of cell protective activity at the onset of lipid peroxidation will prevent the oxidative damage of LDL cells¹⁵. The cytoprotective power of mangosteen xanthone skin helps prevent cell death thereby allowing high cell survival rates and low toxicity results.

The administration of 0.2% chlorhexidine gluconate material to BHK-21 fibroblast cells showed a very low average percentage of cell life of 15.28%. This is supported by theories that have been proven by previous studies. Chlorhexidine gluconate has a toxic effect in inhibiting mitochondrial activity, DNA synthesis, cell proliferation, and decreased cell protein synthesis⁶⁻⁸. This results in a risk of apoptosis and cell necrosis thus the number of cells that die after administration of 0.2% chlorhexidine gluconate gives a lot of risk of cell apoptosis and necrosis high levels of material toxicity result.

Based on preliminary test results obtained non-toxic mangosteen skin xanthone concentration at a concentration of 0.78%. In the cytotoxicity test of mangosteen skin xanthone 0.78% and chlorhexidine gluconate 0.2%, it was found that xanthone mangosteen peel concentration 0.78% was less toxic than chlorhexidine gluconate 0.2%. According to this result of study, it can be concluded that 0.78% mangosteen peel xanthone has lower toxicity compared to 0.2% chlorhexidine gluconate against BHK-21 fibroblast cells.

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