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| Research Report |
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Biocompatibility of 0.78% tannin of *garcinia mangostana linn* pericarp extract and 0.2% *chlorhexidine gluconate* against BHK-21 fibroblast cells culture

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ABSTRACT

Background: *Chlorhexidine gluconate* is one of endodontic irrigants potential due to its antibacterial activity. Although it is an effective antibacterial agent, *chlorhexidine gluconate* cannot dissolve organic substances and necrotic tissue present in the root canal. In addition, same as other chemicals, *chlorhexidine gluconate* also cause a cytotoxic effect. Tannin extracts of mangosteen pericarp (*Garcinia mangostana* Linn.) demonstrated various biological activities including antibacterial, antioxidant, anticancer and anti-inflammatory. Tannin extracts of mangosteen pericarp can be considered as an alternative endodontic irrigation for dental application. **Purpose:** The aim of this study was to compare the biocompatibility between 0.78% tannin extracts of mangosteen pericarp and 0.2% *chlorhexidine gluconate* to BHK-21 fibroblast cell. **Methods:** Tannins obtained from extracts of mangosteen pericarp. Preliminary test was conducted in the biocompatibility of tannin extracts of mangosteen pericarp in various concentrations of the BHK-21 fibroblast. Tannins extracts of mangosteen pericarp was at concentrations of 0.78% less toxic than the other concentrations. Then biocompatibility of 0.78% tannin extracts of mangosteen pericarp compare with 0.2% *chlorhexidine gluconate* using cytotoxicity test on BHK-21 fibroblast cells with MTT method and observed by ELISA reader. **Results:** 0.78% tannins extracts of mangosteen pericarp showed good biocompatibility with fibroblast BHK-21 than 0.2% *chlorhexidine gluconate*. There was a significant differences between the results of treatment with 0.78% tannin extracts of mangosteen pericarp and 0.2% *chlorhexidine gluconate*. **Conclusion:** 0.78% tannin extracts of mangosteen pericarp has better biocompatibility than 0.2% *chlorhexidine gluconate* as an endodontic irrigants.

Keywords: Mangosteen pericarp extract (*Garcinia mangostana* Linn.); Tannin, *Chlorhexidine gluconate* 0.2%; BHK-21; Biocompatibility; MTT assay.

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INTRODUCTION

Chlorhexidine gluconate is widely used as a root canal irrigant because it has broad-spectrum antibacterial. *Chlorhexidine* has a lower cytotoxicity, odorless and tasteless when compared with other irrigants, such as NaOCl. *Chlorhexidine gluconate* cannot dissolve organic substances and necrotic tissue present in the root canal system. *Chlorhexidine* also cannot kill all bacteria and eliminate the *smear layer*, so it is not the main irrigant. Based on several studies it is known that the exposure of *chlorhexidine* 0.125% for 120 seconds to human periodontal ligament fibroblast cells causes inhibition of almost all mitochondrial activity of these cells.^{1,2,3,4}

Based on the lack of *chlorhexidine gluconate*, an alternative material that can be developed as a root canal irrigant is needed that has better quality, biocompatible,

low prices and is easy to obtain. One of the traditional plants which is considered to have potential as a root canal irrigation ingredient includes mangosteen pericarp extract (*Garcinia mangostana* Linn.).⁵

Based on some phytochemical test results, mangosteen pericarp shows that mangosteen pericarp contains alkaloids, saponins, triterpenoids, tannins, phenolics, flavonoids, glycosides and steroids.⁶ Tannin compounds are known have several chemical activities including antibacterial, antioxidant, anticancer and anti-inflammatory.⁷

Tannin of mangosteen pericarp extract which will be developed as a root canal irrigant must be tested first with a biocompatibility test in accordance with the material requirements in the field of Dentistry. Based on the ISO-10993 standard "Biological Evaluation of Medical Devices", one source's biocompatibility is to test the toxicity of the material against the cell in vitro by MTT assay method.⁸

Chlorhexidine gluconate 0.2% as a root canal irrigant still has lack that are cytotoxic, therefore we need an alternative irrigation that comes from nature one of which is tannin of mangosteen pericarp extract. Preliminary study results showed that tannin of mangosteen pericarp extract at 0.78% concentration was biocompatible to BHK-21 fibroblast cells. Based on these results, a study is needed to determine the differences in the biocompatibility between 0.78% tannin extracts of mangosteen pericarp and 0.2% chlorhexidine gluconate to BHK-21 fibroblast cell.

MATERIALS AND METHODS

This type of study was experimental laboratory study with *post test only control group design*. The ingredients used were mangosteen pericarp extract, BHK-21 fibroblast cell culture from PUSVETMA, and culture media containing Eagle, *Fetal Bovine Serum* 10%, and MTT reagents [2- (4,5-Dimethylthiazol-2-yl) -2,5,5 -2,50 -diphenyl tetrazolium bromide], PBS (*Phosphate Buffer Saline*), DMSO (*Dimethylsulfoxide*), 90% alcohol, and *aquadest* sterile. The study was conducted at the Veterinaria Farma Center (PUSVETMA) Surabaya.

Mangosteen pericarp extract was obtained by extracting mangosteen pericarp that has been dried by maceration method using ethanol 96% solvent then evaporated with a *vacuum evaporator* at 60°C until a crude mangosteen pericarp extract is thick brown. Then tannin was isolated from mangosteen pericarp extract using acetone solvent and absolute alcohol (1: 1) and vibrated for 2 hours using a *shaker*/ shake tool and then allowed to stand until the upper fluid clear. The lower faucet on the separator was opened slowly so that the dirt that was half solid and solid comes out. Then the clear fluid was transferred to a *vacuum evaporator* until all the solvents are separated. A tannin extract in the form of a ½ solid thick liquid reddish. Then the process was conducted *freeze dry* thus the mangosteen pericarp extract tannin was obtained in the form of powder.

Biocompatibility test with cytotoxicity test using MTT assay. BHK-21 cell culture in the form of *cell-line* was planted in a *roux* bottle. After confluent culture is harvested using *trypsin* solution. Then planted again in media eagle's containing 10% bovine serum albumin incubated for 24 hours. Cells were transferred in *roux* bottles and then cultured on a *microplate* 96 well until confluent. BHK-21 cell culture was divided into 5 groups, group 1 was negative control containing culture media, group 2 was positive control containing cells in culture media, group 3 was extract control containing tannin of mangosteen pericarp extract, group 4 BHK-21 cells in culture media was exposed to tannin of mangosteen pericarp extract 0.78% and group 5 BHK-21 cells in culture media was exposed to *chlorhexidine gluconate* 0.2%. Each treatment had 6 replicas which were incubated for 24 hours in a incubator 37 ° C. After incubation, cell media were discarded, then washed with PBS, replaced with new media and MTT was added directly to the *microplate* by 10 µl. Then incubated again for

4 hours (temperature 37 ° C), next all the media in the wells and the test material were taken. After that, each addition of 50 µl DMSO was added, the *microplate* was vibrated mechanically with a *plate shaker* for 5 minutes.

Living cell is measured by *Optical Density* (OD) based on the formazan crystals that form. OD is read by spectrophotometry using *ELISA reader* (wavelength 620 nm). Live cell presentation was calculated by the formula:⁹

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

Notes:

- % of living cells = percentage of the number of living cells after testing
- treatment = formazan optical density values in each test sample
- media control = formazan optical density values in the media control
- cell control = formazan optical density value in the cell control

RESULTS

Data results of *optical density* on biocompatibility test tannin of mangosteen pericarp extract 0.78% and *chlorhexidine gluconate* 0.2% can be seen in Table 1. The study data showed that tannin of mangosteen pericarp extract at 0.78% concentration (the percentage of cell life is 67%) compared to *chlorhexidine gluconate* 0.2% had better biocompatibility with fibroblast BHK-21 cells (Figure 1).

The study data were analyzed with *post hoc* statistical test using *Tukey HSD* method to find out the differences in each group. *Post hoc* statistical test showed that 0.78% tannin of mangosteen pericarp extract treatment group had a significant difference with the entire treatment group. In the treatment group of *chlorhexidine gluconate* 0.2% had significant differences with the cell control group, tannin of mangosteen pericarp extract control group, and tannin of mangosteen pericarp extract group.

Table 1. The results of *Optical density* and percentage of cell life of tannin mangosteen pericarp extract 0.78% and *chlorhexidine gluconate* 0.2% in fibroblast BHK-21 cell.

| Treatment | Mean OD | Percentage of living cell (%) |
|-----------|---------|-------------------------------|
| A | 0.06133 | 0 |
| B | 0.6165 | 100 |
| C | 0.21583 | 0 |
| D | 0.43533 | 67 |
| E | 0.1075 | 8 |

Notes: A = The media controls; B = Cell Control; C = Control tannin of mangosteen pericarp extract; D = Tanin of mangosteen pericarp extract 0.78%; E = *Chlorhexidine gluconate* 0.2%.

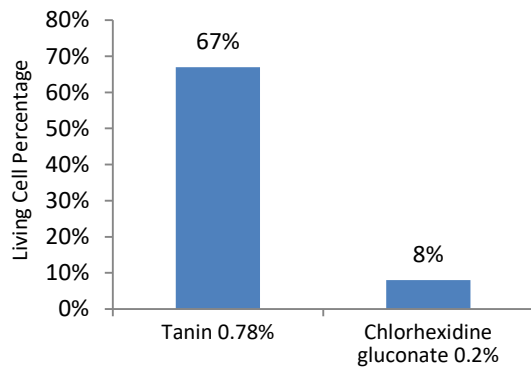


Figure 1. Graph of cell life percentage after treatment with tannin of mangosteen pericarp extract at 0.78% concentration and *Chlorhexidine gluconate* 0.2%

DISCUSSION

Root canal irrigation is one of the factors that can support the success of a root canal treatment. In the preparation of the root canal is accompanied by irrigation of the root canal because it aims to clean up small fragments of organic debris and dentine fragments from the root canal.^{10,11,12} One of the irrigant is *chlorhexidine gluconate*. *Chlorhexidine gluconate* has broad spectrum antibacterial and can be used as a cavity cleanser. As with other chemicals that are also used as cavity cleaners or *endodontic irrigants*, *chlorhexidine gluconate* also has a cytotoxic effect.¹⁴

One of the requirements for a material that can be used in the field of dentistry is biocompatible, non-toxic, non-irritating and has no adverse effects on the biological environment, both locally and systemically.¹⁵ One method of biocompatibility test is to observe the cytotoxicity of a material by enzymatic test using MTT reagents [3-(*Dimethylthiazol* 4,5--2YL); 2,5-*diphenyltetrazolium bromide*] in BHK-21 fibroblast culture cell.^{8,16} BHK-21 fibroblast cell is a cell most widely used by researchers in the material toxicity test in dentistry because they have the advantage that they are easy to grow and easy to do repetitive subcultures, have stable, sensitive characters and do not mutate.^{17,18}

In this study conducted a biocompatibility test using tannin of mangosteen pericarp extract obtained from the extraction process using maceration method with acetone solvent and absolute alcohol is intended to get more optimal tannins.¹⁹ In tannin of mangosteen pericarp extract at 0.78% concentration was an increase in the percentage of cell life of 67%. This shows that the results of the percentage of cell life more than 50%, so it can be concluded that tannin of mangosteen pericarp extract at 0.78% concentration is not toxic because the condition of a material is toxic in a way that the percentage of living cells being exposed to it is less than 50%.²⁰ Also supported by the results of cell morphological analysis on a microscope visible form normal fibroblast cells. Therefore, tannin of mangosteen pericarp extract at 0.78% concentration was chosen to be then compared with *chlorhexidine gluconate* 0.2%.

Table 2. The results of *post hoc* statistical test *Tukey HSD* method in each treatment group

| Group | A | B | C | D | E |
|-------|---|--------|--------|--------|--------|
| A | | 0.000* | 0.000* | 0.000* | 0.231 |
| B | | | 0.000* | 0.000* | 0.000* |
| C | | | | 0.000* | 0.000* |
| D | | | | | 0.000* |
| E | | | | | |

Note: * = shows a significant difference ($p < 0.05$).

In the treatment with *chlorhexidine gluconate* 0.2%, it is known that the average percentage of cell life is 7%. This means that the ability of cells to proliferate after treatment with *chlorhexidine gluconate* 0.2% is smaller than that tannin of mangosteen pericarp extract (*Garcinia mangostana* Linn.). This is because *chlorhexidine* can inhibit protein synthesis, interfere with cellular respiration by the mitochondria, inhibit DNA synthesis and cell proliferation thereby reducing the percentage of cell life.^{3,14}

Potential cytotoxicity of *chlorhexidine* can be related to how long the cell is exposed to *chlorhexidine* because the longer the cell's contact time with *chlorhexidine*, the higher the cytotoxic effect produced by the material.^{14,17} In addition, *chlorhexidine* concentration can also affect cell proliferation because the higher *chlorhexidine* concentration, the lower the cell proliferation.²¹

Differences in the ability of fibroblast cell proliferation between treatment with tannin of mangosteen pericarp extract 0.78% and *chlorhexidine gluconate* 0.2% due to tannin of mangosteen pericarp extract has antioxidant power that can inhibit *lipid peroxidation* and block the initiation of free radical formation so that *growth factors* can trigger fibroblast cell proliferation thereby increasing the percentage of cell life.^{22,23} Tannin compounds are also able to neutralize enzymes that stop the degradation of extracellular matrix by neutralizing excess MMPs needed to help create a favorable environment for fibroblast cell growth, resulting in suppression of MMP-2 and MMP-9 secretion which will cause MMP production and inhibited catalysis activity.^{24,25} In addition, tannin also acts as *chelator* a metal ion transition prevent DNA oxidation from occurring by neutralizing excess metal ions, suppressing the formation of hydroxyl radicals and stabilizing activity *prooxidative*.^{25,26,27}

Based on the results of this study showed that mangosteen pericarp extract (*Garcinia mangostana* Linn.) 0.78% compared to *chlorhexidine gluconate* 0.2% had better biocompatibility against BHK-21 fibroblast cells. To ensure that tannin of mangosteen pericarp extract is biocompatible and can be used as dental material, it is necessary to test with different methods without using tetrazolium salts such as *Luciferin ATP Assay* because this test measures the amount of ATP found in living cells produced from the catalysis process of *luciferase*²⁸ or further in vivo test can be done using experimental animals.

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