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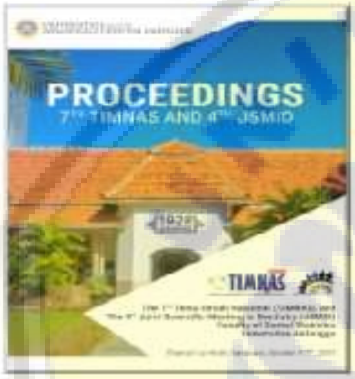

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Foreword: Come and join us in the 7thTemu Ilmiah Nasional (TIMNAS) - the 4th Joint Scientific Meeting in Dentistry (JSMID)! We extend you a warm welcome in 2017, to the 7th Temu Ilmiah Nasional (TIMNAS) - the 4th Joint Scientific Meeting in Dentistry (JSMID) held in Shangri-La Hotel 5th – 7th October 2017. The 7th TIMNAS – 4th JSMID is a global initiative of the internationally renowned in all aspect of Dentistry sharing and exchange of technical know-how among dental professionals and conducted with the main purpose to contribute in shaping the world of Dentistry. This event also has a mission to provide a stage for researchers and clinicians that are at the cutting edge of life science and a clinical innovation whereby they can share their work and discuss its future application potential in a non-conventional setting. Dental education in Indonesia has entering a new paradigm. The education is conducted in concerning with a human health. It stresses prevention over intervention. It **(More)**

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Paper

Cytotoxicity and Effectiveness of 100% Mangosteen Pericarp Extract in Cleaning the Smear Layer of Root Canal Dentine

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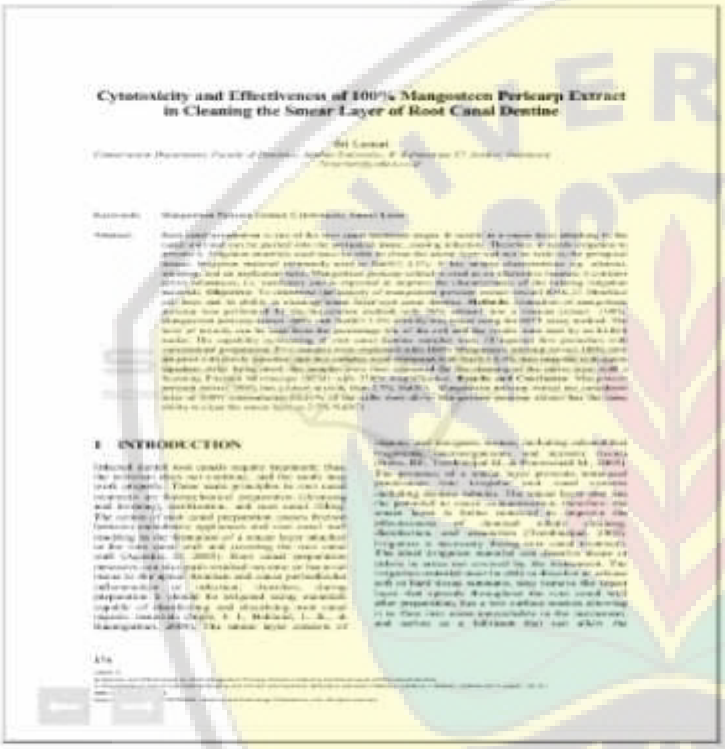
Cytotoxicity and Effectiveness of 100% Mangosteen Pericarp Extract in Cleaning the Smear Layer of Root Canal Dentine

Sri Lestari

1 INTRODUCTION

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Keyword(s): Mangosteen pericarp extract, cytotoxicity, smear layer

Abstract: Root canal preparation is one of the root canal treatment stages. It results in a smear layer attaching to the canal wall and can be pushed into the periapical tissue, causing infection. Therefore, it needs irrigation to prevent it. Irrigation materials used must be able to clean the smear layer and not be toxic to the periapical tissues. Irrigation material commonly used is NaOCl 2.5%. It has unique characteristics e.g. odorous, irritating, and an unpleasant taste. Mangosteen pericarp extract is used as an alternative because it contains active substances, i.e. xanthones and is expected to improve the characteristics of the existing irrigation materials. Objective: To determine the toxicity of mangosteen pericarp extract toward BHK-21 fibroblast cell lines and its ability in cleaning smear layer root canal dentine. Methods: Extraction of mangosteen pericarp was performed by the maceration method with 96% ethanol into a viscous extract 100%. Mangosteen pericarp extract 100% and NaOCl (More)

Cytotoxicity and Effectiveness of 100% Mangosteen Pericarp Extract in Cleaning the Smear Layer of Root Canal Dentine

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Keywords: Mangosteen Pericarp Extract, Cytotoxicity, Smear Layer.

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1 INTRODUCTION

Infected dental root canals require treatment; thus, the infection does not continue, and the tooth may work properly. Three main principles in root canal treatment are biomechanical preparation (cleansing and forming), sterilization, and root canal filling. The action of root canal preparation causes friction between endodontic appliances and root canal wall resulting in the formation of a smear layer attached to the root canal wall and covering the root canal wall (Agustin, D. 2005). Root canal preparation measures can also push residual necrotic or bacterial tissue to the apical foramen and cause periradicular inflammation or infection; therefore, during preparation it should be irrigated using materials capable of disinfecting and dissolving root canal organic materials (Ingle, J. I., Bakland, L. K., & Baumgartner, 2008). The smear layer consists of organic and inorganic tissues, including odontoblast fragments, microorganisms, and necrotic tissues (Beltz, RE, Torabinejad M, & Pouresmail M., 2003). The presence of a smear layer prevents intracanal penetration into irregular root canal systems including dentine tubules. The smear layer also has the potential to cause contamination; therefore, the smear layer is better removed to improve the effectiveness of dentinal tubule cleaning, disinfection, and obturation (Torabinejad, 2003). Irrigation is necessary during root canal treatment. The ideal irrigation material can dissolve tissue or debris in areas not covered by the instrument. The irrigation material must be able to dissolve or release soft or hard tissue remnants, may remove the smear layer that spreads throughout the root canal wall after preparation, has a low surface tension allowing it to flow into areas unreachable to the instrument, and serves as a lubricant that can allow the

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instrument to move inside the root canal (Nasution, S. S. N., 2006).

The irrigation process at the time of root canal preparation often causes irrigant contact with soft tissue under the roots of periapical tissue or contact with gingiva. This is caused by a very strong irrigant spray, and also reaches the gingiva because irrigant exceeds the cavities of teeth. It should be avoided, as it may irritate the soft tissues and cause inflammation even up to necrosis.

The root canal irrigation material commonly used is *Sodium Hypochlorite* (NaOCl) 2.5%. NaOCl is an economical (inexpensive) solution, easy to obtain, easy to use, capable of dissolving organic smear layer components and has a bactericidal effect (WaltonR. E., dan Torabinejad, et al., 2008). Sodium hypochlorite (NaOCl) is known for its antibacterial activity, which can kill bacteria very quickly even in low concentrations. The deficiency of NaOCl is very toxic to soft tissue, and can irritate the eyes and skin (Nasution, Syahnita Sari Nugraha, 2006).

The use of natural ingredients has been widely developed as an alternative to medicine, especially for dental materials that are more expensive and have side-effects. One of the natural ingredients that can be considered as an alternative for root canal irrigation is mangosteen pericarp. Mangosteen fruit (Garciniamangostana L.) is abundantly found in Indonesia. Mangosteen pericarp contains active compounds, e.g. xanthones, saponins, flavonoids, tannins, and alkaloids (Poeloengan Masniari & Praptiwi, 2010). The xanthone compounds play a role in the inflammatory process, as a chemical mediator that can decrease prostaglandin E2 (PGE2) thereby increasing the proliferation of fibroblasts by stimulating the Basic Fibroblast Growth Factor (BFGF). In normal cells, xanthones do not exhibit toxic properties of fibroblast tissue because of their function as antioxidants and anti-inflammatory (Pedraza Jose C. et al., 2008). It is also related to the statement of the existence of a cell protection system in the mangosteenxanthone, as an antioxidant. The antioxidant activity of the mangosteenxanthone serves to prevent cell damage and death through DNA protection from oxidative damage (Fu C, Loo A, Chia FP, & Huang D, 2007)

Based on the active substance content and its ability to dentin tissue and soft tissue the researcher wanted to know whether mangosteen pericarp extract can eliminate the smear layer on the root canal wall and its toxicity when in contact with soft tissues of the oral cavity that begins with the test of cytotoxicity to cell line fibroblasts. A smear layer cleanliness test and cytotoxicity of mangosteen pericarp extract 100% was compared with NaOCl irrigation materials 2.5%

2 MATERIALS AND METHODS

100% of mangosteen pericarp extract (MPE) is obtained from inner mangosteen pericarps that have been separated from their hard outer shells and extracted using ethanol. A cleanliness test of the dentine root canal smear layer used the root of the first premolar teeth of the maxilla. The dental crown was cut at cervical level and the root canal was prepared by the conventional technique until K file number 50, then irrigated using sterile aquades, irrigated using 100% mangosteen pericarp extract while prepared with file number 50, and irrigated again using sterile aquades; the irrigation solution was absorbed using a disposable syringe. The prepared root was cut at 1/3 apical, and the 2/3 cervical section was cut longitudinally in the mesio-distal direction. Subsequently, the prepared root canal wall was examined with 5000x magnification of SEM. Five other samples were irrigated with 2.5% NaOCl.

Assessment of the cleanliness of prepared surface dentine on SEM used a transparent sheet installed according to the photo size and divided into 10 boxes. The transparent sheet was affixed to the photo and then scoring was conducted on each box. Observations were made to determine the mode (frequency distribution). Cleanliness assessment was based on the Hulsmann score system (Hülsmann, M., Gressmann, G., & Schäfer, F., 2003) as follows:

1	=	all tubular dentin orifices were open, and the surfaces were free from a smear layer
2	=	some tubular dentin orifices were open and there was little smear layer
3	=	only a few tubular dentin orifices were open, and the smear layer covers some part of the surface
4	=	all tubular dentin orifices were closed, and the entire surface was covered with a smear layer
5	=	Heavy smear layer. A thick smear layer was present on all surfaces and tubular dentin orifices.

The cytotoxicity test against BHK-21 cells was examined with a MTT Assay. Each group of 8 wells was planted on a microplate 96 well. The well in column 1 contained 100 µl culture medium, group 2 column contained cell culture dissolved in 100 µl medium, group 3 contained cell culture with the addition of 100% mangosteen pericarp extract, and

group 4 contained cell culture with the addition of 2.5% NaOCL. The microplate was incubated in a CO 2.5% incubator at 37 °C for 24 hours. After incubation, the test material and culture medium were washed with PBS and replaced with a new culture medium of 100 µl, with 10 µl of Tetrazolium Salt (MTT) dissolved in PBS 5mg/ml. The microplate was incubated for ± 3 hours at 37 °C. Culture media and MTT were taken using a syringe and DMSO was added to dissolve the formazan crystals; then the microplate was stirred mechanically using a shaker tool for 5 minutes. The optical density (OD) values of formazan crystals formed were read using an ELISA reader with a wavelength of 620 nm and the percentage of living cells was calculated by the following formula:

$$\text{Living Cells} = \frac{\text{group test + media}}{\text{cells+ media}} \times 100\%$$

Cytotoxicity was observed from the amount of absorbance value (Optical Density) from reading using the ELISA reader. The absorbance value was the color change induced by the mitochondrial activity to become a blue formazan. Absorbance was used to calculate the percentage of living cells in response to MTT reagents. The thicker the color, the higher the absorbance value, and the higher the value of the OD. The higher the OD value, the higher the living cells, meaning that the cytotoxicity was lower.

3 RESULTS

The cleanliness of the smear layer on the surface of the prepared root dentin was examined using a 5000x magnification scanning electron microscopy. The results of examination with SEM are shown in Figure 1.

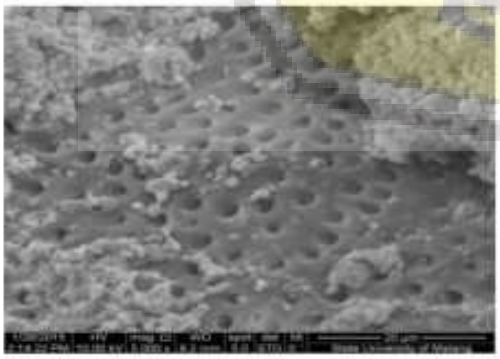

Figure 1: SEM examination of the smear layer cleanliness on the surface of prepared dentin root canal and exposed using 100% of mangosteen peel extract (Garcinia mangostana L.).

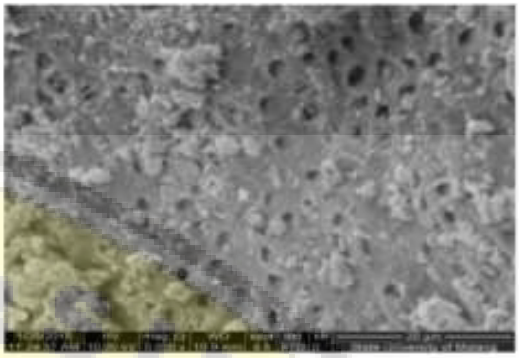

Figure 2: SEM examination of the smear layer cleanliness on the surface of prepared dentin root canal and exposed using NaOCl 2.5%.

The cleanliness of the smear layer was observed by 3 people by calculating the score of the smear layer existence on each box resulting in a frequency score (mode), shown in Table 1.

The the cleanliness of the smear layer on dentin prepared and irrigated with MPE 100% and NaOCl 2.5% had the same score of 3 (only a few tubular dentin orifices were open and the smear layer covered part of the surface).

Table 1: The cleanliness of the *smear layer* on the surface of prepared dentin root canal.

Group	N	Mangosteen Peel Extract (MPE)100 %	NaOCl 2.5%
Dentin root canal	5	3	3

Table 2: The mean of OD (Optical Density) dan living cells (%).

Groups	MPE 100%	NaOCl 2.5%
Optical Density	0.88	0.17
Living cell	92.21	23.41

Cytotoxicity can be seen from the value of OD from the ELISA Reader. The average value of OD can be seen in Table 2.

Table 1 shows that the value of OD and the average of living cells exposed to NaOCl 2.5% is less than MPE 100% meaning that NaOCl 2.5% had cytotoxicity to tissue greater than 100% MPE.

The Living Cell is classified as follows (Heravi, F, 2013). :

A. Living Cells > 90%, non-toxic
B. Living cells 60% - 90%, slightly toxic
C. Living cells 30% - 59%, quite toxic
D. Living cells<30% can be, very toxic

Based on independent T test on the living cells and OD values showed that p <0.05, meaning that there was a difference in the living cells and OD value between 100% of mangosteen pericarp extract and 2.5% of NaOCl.

4 DISCUSSION

Good irrigation materials are necessary during root canal treatment. The ideal irrigation material should be capable of dissolving tissue or debris in areas unreachable by the instrument, releasing soft or hard tissue debris, removing the smear layer that spreads throughout the root canal wall after preparation, not toxic, non-irritant, and antibacterial. Based on the results of the research it is observed that all materials used for irrigation on dentin canal root can eliminate the smear layer. The cleanliness of the smear layer on the prepared dentin has a score of 3, i.e. only a few tubular dentin orifices are open and the smear layer covers part of the dentin surface. This means that 100% mangosteen pericarp extract (Garciniamangostana L.) and 2.5% NaOCl have the same ability to clean the smear layer on the dentine root canal.

The above is likely because the Mangosteen Pericarp Extract (Garciniamangostana L.) is acidic to pH 4 because of its phenolic acid content (Viranda, Mariska. 2009). The phenolic acid compound is a weak acid compound. If the acidic materials come into contact with root canal wall it will dissolve hydroxyapatite and release $Ca2^{+}$and HPO_4^{2-} ions which are soluble in water and demineralization occurs. The more acidic a material the more hydroxyapatite dissolved (Wulandari, E. 2006).

The reaction can be explained as follows:

$$Ca_{10}(PO4)_6(OH)_2+8H^{+} \longleftrightarrow \underbrace{10Ca^{2+}+6(HPO4)^{2}}_{\text{dissolved}}+2H_2O$$

In addition, mangosteen pericarp extract (Garciniamangostana L.) contains 100% saponins. Saponin, which acts as an emulgator (detergent), can reduce surface tension. Decreased surface tension causes dentine permeability to increase so as to facilitate the penetration of adhesive materials. Saponin content consists of hydrophilic groups and hydrophobic groups where hydrophilic groups will bind to polar compounds from an organic smear layer and hydrophobic groups will bind to a non-polar compound of an inorganic smear layer. Saponin also has a particular physicochemical characteristic, i.e. foaming when rubbed with water. The chemical structure of saponins comprising glycosides (polar compounds) and triterpenes (non-polar compounds) shows that saponins belong to a soap-like surfactant group that can dissolve polar and non-polar compounds. It allows the mangosteen pericarp extract (Garciniamangostana L.) 100% to be able to clean the smear layer.

It proves that both materials can clean up the smear layer on the dentin root canal with the same level of cleanliness. The irrigation materials used frequently come into contact with both periapical and gingival tissues. It is hoped that irrigation materials that are in contact with the soft tissues are not irritating and even toxic. To know that the substance does not cause irritation or toxicity, a toxicity test on soft tissue was necessary to be performed. The soft tissues of the oral cavity contain fibroblasts. Therefore, the toxicity test performed is the exposure of BHK-21 fibroblast cell line using the MTT assay method.

The MTT assay method is based on the change of tetrazolium salt [*3-(4,5-dimetiltiazol-2-yl)-2,5difeniltetrazolium-bromide*] (MTT) becoming formazan in mitochondria active in living cells, in this case the mitochondria of fibroblast cells. Yellow MTT is absorbed into fibroblast cells and broken down by the reduction reaction by the enzyme mitochondrial succinic dehydrogenase. This enzyme converts MTT into blue formazan crystals indicating that the cell is alive (Walter LD, 1999).

Formazan is an enzyme substrate complex formed by MTT and a succinic dehydrogenase enzyme. The blue formazan concentration can be visually-spectrophotometrically determined and is proportionally related to the number of living cells because reduction only occurs when the reductase enzyme is present in the cell respiration pathway in active mitochondria. The stronger the intensity of the blue color formed, the higher the absorbance, the more MTT is absorbed into the living cells and broken down by the reduction reaction by the

reductase enzyme in the mitochondrial respiration chain, thereby the formazan formed is also abundant. The absorbance value is used to calculate the percentage of living cells. The intensity of the blue color formed is directly proportional to the number of cells that actively perform the metabolism. The greater the absorbance the greater the number of living cells (Nevi Y, 2009).

The results showed that 100% of MPE has an average of living cells of 92.21% meaning that it is not toxic; 2.5% NaOCl gave a result of 23.47% meaning that it is toxic to BHK-21 fibroblast cells. This is probably due to xanthones compound in MPE 100% known to play a role in the inflammatory process, namely as a chemical mediator that can decrease Prostaglandin E2 (PGE2). A decrease in PGE2 will increase the proliferation of fibroblasts by stimulating the Basic Fibroblast Growth Factor (BFGF). In normal cells, xanthones do not exhibit toxic properties of fibroblast tissue because of their function as antioxidants and anti-inflammatory property (Pedraza Jose C, et al., 2008). This is related to the cell protection system in the xanthone content of mangosteen pericarp. Xanthone serves as an antioxidant. The antioxidant activity of mangosteenpericarpxanthone prevents cell damage and death through DNA protection from oxidative damage. Xanthones also have strong antioxidant power, capturing various harmful radical compounds, e.g. hydroxyl radicals, superoxides, and nitric oxide. Xanthonecytoprotective power helps prevent cell death, thus allowing high survival of cell counts and low toxicity results yielded (Fu C, Loo A, Chia FP, & Huang D, 2007). Saponin content in 100% of MPE can stimulate Transforming growth factor ß (TGFß). TGF-ß serves to stimulate the proliferation of fibroblasts, cell differentiation, and other functions needed for cell life, e.g. protecting healthy tissues and stimulating collagen production (Ramadhani, Putri, 2013).

The content of flavonoids in 100% MPE stimulates the proliferation of fibroblast cells. One of the derivatives of flavonoids is luteolin. Luteolin has anti-inflammatory, neuroprotective, and anti-allergic properties. Luteolin may inhibit the secretion of excessive anti-inflammatory substances in cells, e.g. prostaglandins that can be produced by all types of cells with nuclei (almost all nucleated cells) that can stimulate the proliferation of fibroblasts (Nove H, Adiastuti E & Mintarsih D, 2013).

NaOCl is toxic and damaging to tissues. This is because the chlorine content in NaOCl is able to release free radicals that may enter the cell protoplasm and will destroy the cell. 2.5% NaOCl is known as the most toxic root canal irrigation material. Several previous studies have also stated that 5% NaOCl is able to eradicate 100% of target cells within 15 minutes.

5 CONCLUSIONS

The effectiveness of 100% mangosteen pericarp extract in cleansing the dentine root canal smear layer is equal to NaCl 2.5%. 100% mangosteen pericarp extract has a lower toxicity than that of 2.5% NaOCl. Mangosteen pericarp extract is not toxic, while NaOCl is toxic.

Further clinical research on the biocompatibility of mangosteen pericarp extract is necessary to be conducted, if it is to be commoditized as an alternative to irrigation materials.

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