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Research Report

Cytotoxicity of 5% *Tamarindus indica* extract and 3% hydrogen peroxide as root canal irrigation

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ABSTRACT

Background: *Preparation of root canal is an important stage in endodontic treatment. During conducting preparation, it is always be followed with root canal irrigation that has aim to clean root canal from necrotic tissue remains, grind down dentin powder, micro organism, wet the root canal to make preparation process of root canal easier, and solute root canal content at area that can not be reached by equipment. Flesh of Tamarindus indica (pulpa tamarindorum) is used as traditional medicine and it contains vitamin C (antioxidant), protein, fat, glucose, etc. Previous research shows that 5% tamarindus indica extract can clean smear layer but it is more cytotoxicity to cell line BHK–21 than sterilized aquabides.* ***Purpose****: This research is to compare cytotoxicity between 5% Tamarindus indica extract with 3% H_2O_2 as root canal irrigation material.* ***Method****: Four teen culture cell line BHK 21 divides into 2 groups. Group 1 is treated with 3% H_2O_2 and Group 2 is treated with 5% Tamarindus indica extract, for about 2.5 minutes in every group. Then, living and death cell percentage is measured. Data is analyzed with independent t test with significant level of 0.05%.* ***Result****: The research showed that death cell in group 1 was 29.3% and in group 2 was 21.1%. There was a significant different ($p < 0.05$) between group 1 and group 2.* ***Conclusion****: Cytotoxicity of 5% Tamarindus indica extract to the cell line BHK–21 is lower than 3% H_2O_2.*

Key words*: cytotoxicity, Tamarindus indica extract, hydrogen peroxide, root canal irrigation*

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INTRODUCTION

The main purpose of root canal treatment is to prevent more serious teeth damage spreading by losing microorganism and necrotic tissue inside root canal. One of important treatment step of root canal treatment is preparation that is followed by root canal irrigation. The purpose of root canal irrigation is to clean root canal from necrotic tissue remain, grind down dentin powder, microorganism, wet the root canal to make root canal preparation process easier, and solute root canal content in area that can not be reached by equipment.[1,2]

One of root canal irrigation requirement is low periapical tissue cytotoxicity.[3] This is considering that root canal irrigation solution can permeate to periapical especially at mandibular teeth with necrotic pulp in case with open foramen apical, if it contacts with periapical tissue and causing periapical complication.[3] Koulaozidou *et al*.,[4] stated that if irrigation material comes out from teeth apex it will cause periapex tissue irritation and damaging.

Root canal irrigation material used is 3% hydrogen peroxide (H_2O_2). If it contacts with organic material, it will produce nascent oxygen, that is white foam formed from oxidation result of the material and has removed out debris from the root canal mechanically. Nascent oxygen is free radical that can damage membrane and organelle from cell. Concerning about it, toxic effect from material used in endodontic treatment is very important to be paid attention. Exact root canal irrigation material must be chosen carefully because treatment failure is sometimes caused by improper material choosing.

Many kinds of plant growing in Indonesia can be used for medication purposes. One of them is *Tamarindus indica*. The flesh (pulpa tamarindorum) can be used as scurvy medication. Chemical component of the flesh is vitamin C that is determined as antioxidant, citric acid, tartrat acid, protein, carbohydrate, fat, water, etc.[5,6] Dharmayanti[7] stated that 20%, 15%, 10% and 5% of *Tamarindus indica* concentration had antibacteria of *Streptococcus viridans*. Previous research showed that 5% *Tamarindus indica* extract as root canal irrigation could clean smear layer of root canal wall.

Material that is used in oral cavity must be non toxic. To measure toxicity from a material to cell can be done by cytotoxicity test with culture cell method by using Baby Hamster Kidney-21 (BHK-21). It is used because the cell has the same characteristic with fibroblast of periapical tissue.[8] Previous research showed that cytotoxicity of 5% *Tamarindus indica* extract to cell line BHK–21 is higher than sterilized aquabides, but it has not known yet the cytotoxicity difference between 5% *Tamarindus indica* extract with 3% H_2O_2. Based on the background that has been mention above, the research is done to compare cytotoxicity between 5% *Tamarindus indica* extract with 3% H_2O_2 as root canal irrigation to cell line BHK–21. From this research hopefully can be used as therapy development base in endodontic field, whereas it can be found alternative material of root canal irrigation in cleaning root canal.

MATERIAL AND METHOD

Research materials are 3% H_2O_2, 5% *Tamarindus indica* extract, sterilized aquabides, culture cell line BHK–21 (stock), 10% bovine serum, phosphate buffer saline (PBS), 0,25% versene tripsine solution, tryphan blue, Eagle media, and neutralized salt solution. Research equipment are roux bottle, incubator, weighing balance (Triple Beam Balance, OHAUSE), tweezers, mortal and pastel to make the flesh smooth, magnetic stirring to mix the flesh with sterilized aquabides, centrifugal (Kokusan), 0.45 µm Millipore filter (Sartorius) to filter 5% *Tamarindus indica* extract, pipettes, microplate 24-well, laminar flow, flight microscope (Olympus CH 40), hemositometer to measure living and death cell.

Parental cell BHK–21 (stock) is being revival by putting it into the Roux bottle and is given Eagle media, 10%bovine serum, 0.02 ml streptomycin, 0.06% fungison. Then it is kept in 37° C incubator until confluent. Eagle media is removed and washed with 15 ml PBS twice to remove serum remain inside the bottle. After that, trypsinase is conducted with 0.25% versene trypsine as much as 1 ml. If the cell has already been separated, it must be put into microplate 24-well, each of them is 120 cell/ml. Then, new Eagle media and 10% bovine serum will be added in every well, and then it is put into 37° C incubator until confluent. The next step is preparing test material (5% *Tamarindus indica* extract).

Material test preparation is 5 gram flesh of *Tamarindus indica*, then it is added with sterilized aquabides until the volume is 100 cc and it is stirred by using magnetic stirring so that the flesh will easily soluble, it is centrifuged (2500 rpm) for 15 minutes to separate the deposit from its water. *Tamarindus indica* extract is taken over and filtered by using Millipore 0.45 µm filter so that 5% *Tamarindus indica* extract can be obtained.

Toxicity test procedure is fibroblast cell BHK–21 in microplate 24 well were divided into 2 groups. At the first group, cell inside the microplate is washed with PBS, and it is given 0.5 cc of 3% H_2O_2, and let it for about 2.5 minutes. After that, 3% H_2O_2 is removed, washed with PBS and it is given 0.1 cc versene trypsine. Then, wait until the cell is shed. After that, it was given Eagle media + 0.9 cc serum and stirred with inhale procedure and sprayed repeatedly by using micropipette until the cell is separated. Cell inside microplate is taken at about 0.025 cc and then added with 0.025 tryphan blue then it is dropped into hemositometer. This is done to conduct cell counting under the microscope. At the second group, the same procedure is done, but material that is used is 5% *Tamarindus indica* extract. All procedure is conducted inside laminary flow with the purpose to stabilize research condition in sterilized condition.

Counting procedure of cell that is living (bright) and death (dark) cell in the box from hemositometer view field is counting then put into Bird and Forrester (1981) formula so that it can be got cell death percentage. Formula:

$$\text{Percentage of death cell} = \frac{\text{Death cell}}{\text{Living cell + Death cell}} \times 100\%$$

The higher value of death cell percentage so the higher cytotoxicity of material test.

RESULT

This data shows the death cell percentage counting from cytotoxicity test with die exclusion test method by using tryphan blue (Table 1).

Table 1. Death cell percentage of from 3% H_2O_2 and 5% *Tamarindus indica extract* group

Group	Sample large	Average (%)	Deviation standard	Significant level
3% H_2O_2	7	29.3	2.17	
5% EAAJ	7	21.1	1.83	0.00

Normality test is done using Kolmogorov Smirnov Test. After normal distribution data is gotten, the independent t test with parametric statistic analysis was done. Based on homogeneity test (Levene's test), homogenous data is

got and independent t test shows that there is significantly difference between group 1 and 2 ($p < 0.05$).

DISCUSSION

Death cell percentage of cell that treated with 3% H_2O_2 is higher than that were inhibited with 5% *Tamarindus indica* extract. Hydrogen peroxide is determined as free radical that can damage cell membrane. The cell membrane contains doubled unsaturated fatty acid that is responsive to free radical. If it is broken, it will cause membrane structure and function change and the death of the cell.[9] According to Kumar *et al*.,[10] there is chain autokalisis reaction if free radical attack doubled bounding at doubled unsaturated fatty acid from membrane fat, it will create unstable peroxide and reactive that cause membrane, organelle and cellular damage. The damage of membrane will cause osmotic balance lost of protein, enzyme, co enzyme and ribonucleic acid, it will cause the death of cell.

Free radical is also formed as side product of oxidation or cell burning that is lasted at cell metabolism. Its function is to prevent from damaging caused by virus, bacteria, and also other materials. Free radical over production will attack the cell in the same way as attacking other strange thing and it will cause destructive. If imbalance condition between free radical formation and antioxidant happened, it will cause oxidative stress so that it will cause cell damaging. Imbalance will happened because lack of antioxidant and over production of free radical.[10,11] This is estimated has caused group cell damaging with the given of 3% H_2O_2 that is higher than 5% *Tamarindus indica* extract. It is because 3% H_2O_2 is included on free radical and it does not contain antioxidant.

Group with 5% *Tamarindus indica* extract has lower death percentage than group with 3% H_2O_2. This is because *Tamarindus indica* extract containt vitamin C, an antioxidant to free radical. Antioxidant has function to change free radical to be less effect molecule, catch free radical compound and also prevent chain reaction of free radical formation. This can be done by accepting or give electron to bind with free radical electron that is not paired.[9,10,12] Vitamin C is also used as co enzyme in cell metabolism process and has important role in cell regeneration.[13] *Tamarindus indica* contains glucose, fat, and protein that is used for glutathione peroxides (GSH) activity that is determined as endogen antioxidant. If GSH does not working properly, the balancing process of free radical will be uncontrolled.[10]

Cell death can be caused by low pH, so that it will cause injury to the cell. Contact time as much as 2.5 minutes is determined have big role in the death of cell in group 1 and 2. Cellular respond to injury stimuli is depend on injury type, injury period and serious condition. In certain boundary, cell can repair the damage that is caused by injury, and if stimuli are stopped, so the cell will be back to normal. Persistent injury or more will because cell go across threshold boundary to injury irreversible and will cause cell death.[10]

It concluded that cytotoxicity of 5% *Tamarindus indica* extract as root irrigation material to fibroblast cell line BHK-21 is lower than 3% H_2O_2.

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