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TOXICITY TEST OF SMALL WHITE GINGER EXTRACT ON BHK-21 FIBROBLAST CELLS IN VITRO

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ABSTRACT
Background: *Denture stomatitis is inflammation of the oral mucosa which supporting the denture that caused by Candida albicans. Candida albicans contamination can be prevented by immersing dentures into denture cleanser solution. One of the alternative ingredients that can be used as denture cleanser is small white ginger rhizome (Zingiber officinale var. Amarum).* ***Objective:*** *This study aimed to analyze whether small white ginger extract (Zingiber officinale var. Amarum) was toxic to BHK-21 fibroblast cells using the MTT assay method. Method: This study was conducted in 7 groups. Five groups consisted of extracts of 30%, 40%, 50%, 60%, 70% and 2 control groups comprised of media control and cell control. Absorbance was read using ELISA reader and cell viability was calculated.* ***Results:*** *The percentage of living cells of all groups of small white ginger extract treatment was 100%. The parametric analysis of One Way Annova showed p = 0.498 (p> 0.05)* ***Conclusion:*** *Small white ginger extract (Zingiber officinale var. Amarum) is not toxic to BHK-21 fibroblast cells using the MTT Assay method because cell viability of all concentration groups is ≥ 60%.*

Keywords: *BHK-21 fibroblast cells, small white ginger extract, toxicity test*

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INTRODUCTION

Denture stomatitis is an inflammation of the oral mucosa which supporting the denture caused by *candida albicans*. The intensity of using permanent denture will cause inflammation or *denture stomatitis* in the oral cavity.[1] *Candida albicans* contamination can be prevented by soaking the denture into a *denture cleanser* solution.[2]

One of the *denture cleansers* that is often used by the public to clean denture is *alkaline peroxide*. *Denture cleanser* material that circulates in the community is difficult to obtain because it is *imported* goods and is relatively expensive. For this reason, natural ingredients have now been developed which can be used as alternative ingredients for *denture cleanser,* one of which is a small white ginger rhizome (*Zingiber officinale var. Amarum*). According to the results of the study Saputera et al (2017) stated that the ethanol extract of small white ginger with a concentration of 70% was the same as *chlorhexidine* in inhibiting the growth of *candida albicans* which attached to the *heat cured* acrylic plate.[3] Small white ginger has antifungal activity against *Candida albicans*. The antifungal effect of small white ginger is due to the content of essential oil consisting of active compound *gingerol*, *shagaol*, and *zingeron* which are included in phenol compounds that may denaturate *Candida albicans* cell membrane protein bonds. [4]

The use of traditional medicinal plants must be accompanied by knowledge of the safety level of preparations obtained through toxicity test so as not to cause harmful effects. Small white ginger extract has not been studied regarding the level of safety so it is necessary to do a toxicity test for small white ginger extract on BHK-21 fibroblast cells. One method for assessing the toxicity of a material is enzymatic test using *3- (4,5-dimethylthiazol-2-yl) reagent 2,5-diphenlyl tetrazolium bromide (MTT)*. Based on the description above, further research is needed on small white ginger extract against BHK (*Baby Hamster Kidney*) fibroblast 21 with the MTT *assay* method to determine the safety limit of small white ginger rhizomes as *denture cleanser*.

METHOD AND MATERIAL

This research has through ethical clearence tests by the Health Research Ethics Commission of

the Faculty of Dentistry, Lambung Mangkurat University No.141 / KEPKG-FKGULM / EC / I / 2009. This study was a pure experimental study (*true experimental)* with a *posttest-only design with control group design*. The population of this study was BHK-21 fibroblast cells consisting of 5 groups of small white ginger extract treatments with concentrations of 30%, 40%, 50%, 60%, 70% and 2 control groups were media control and cell control. The number of repetitions for each treatment was 4 times using the Federer formula.

Production Of Small White Ginger Extract was used Maceration Methode

One kg of small white ginger was cleaned and dried in an oven at 45^{o}C for 48 hours. Then cut into small pieces and mashed using a *blender* until powder was formed and carried out to produce 130 grams of simplicia. The powder was then mixed with 70% ethanol, stirred evenly with a ratio of simplicia and ethanol which was 1: 5, then filtering and replaced with a new solvent every 1x24 hours. The filtrate was collected and put into a *rotary evaporator,* then evaporated with a *waterbath,* so that dark brown extract of 11.5 grams were obtained. Small white ginger extract in thick preparations was then dissolved with DMSO to make a series of concentrations of 30%, 40%, 50%, 60%, 70%.

Production of BHK-21 Fibroblast Cells

BHK-21 fibroblast cells were cultured in flask/roux bottles which were incubated using 37^{o}C incubator for 48 hours. Breeding was done until the BHK-21 fibroblast cell attached and filled the wall of the flask/roux bottle. After the cell was full, the *eagle's* media solution and FBS were removed in the flash/roux bottle. Flask/roux bottles were washed with PBS 3 times. T*ripsine versene* ½ ml then transfered to a *96-well microplate* according was added to the number of samples and control using *a multichannel micropipette*.

Toxicity Test of Small White Ginger

BHK-21 fibroblast cells were transferred to the *96 well microplate*. The treatment was given to BHK-21 fibroblast cells with small white ginger extract according to the concentration then incubated using CO_2 incubator for 24 hours. After incubation, the sample was removed and a yellow MTT reagent of 10 µ L was added for each well. The samples were Re-incubate for 2-4 hours using a CO_2 incubator. After incubation, the MTT solution was discarded and given by DMSO. *Microplate* was put in a shaker for 5 or 10 minutes so that the cessation of the reaction could occur evenly in each cell, thos it releases formazan. *Microplate 96 well was* entered into ELISA *reader* with a wavelength of 620 nm for the cell viability reading process. The percentage of cell viability was calculated and IC_{50} analyzed with SPSS (One Way Anova analysis).

$$\%\text{Viability} = \frac{(\text{Abs. treatment} - \text{Abs. media})}{(\text{Abs. cell control} - \text{Abs. media})} \times 100\%$$

Based on calculations with the formula above, the material was non-toxic when the cell percentage was ≥60%.[1]

RESULT

The Toxicity test of small white ginger extract on BHK-21 fibroblast cells on *microplate 96 well* had a color change that was dark purple. This showed that the older purple was produced, the more BHK-21 fibroblast cells (*Baby Hamster Kidney 21*) are life (Figure 1). The results of the calculation of the viability of BHK-21 fibroblast cells in all treatment groups were ≥60% (Table 1 and Figure 2). It shows that small white ginger extract is not toxic to BHK-21 fibroblast cells at concentrations of 30%, 40%, 50%, 60%, 70%.

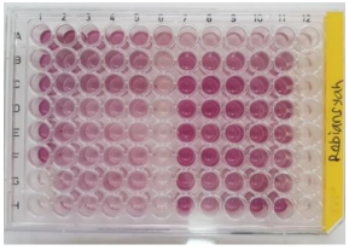


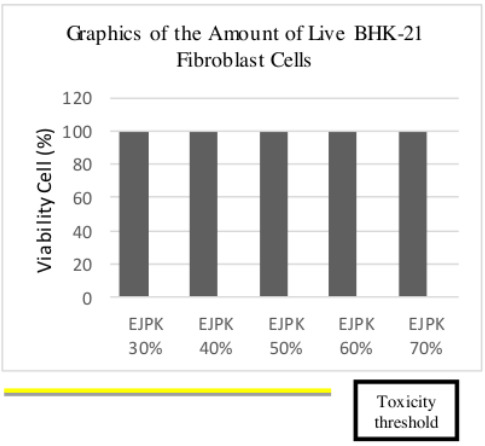
Figure 1. *Microplate 96 Well* Given the Treatment of Small Puth Ginger Extract and MTT Staining.

VIABILITY	MEAN ± SD
EJPK 30%	110.2478 ± 14.98235
EJPK 40%	108.4460 ± 9.20483
EJPK 50%	105.6307 ± 111.90916
EJPK 60%	102.0273 ± 7.73668
EJPK 70%	97.5225 ± 8.99755

Information:
A1 - F1: Cell Control
G1, H1: Media Control
A2 - A5: EJPK concentration of 30%
B2 - B5: EJPK concentration of 40%
C2 - C5: EJPK concentration of 50%
D2 - D5: EJPK concentration of 60%
E2 - E5: EJPK concentration of 70%

Table 1. Mean and SD Viability of Small White Ginger Extract Cells

Figure 2. Viability of BHK-21 Fibroblast Cells



The results of the measurement of viability tests were then carried out statistical analysis. The results of the normality and homogeneity test data showed all the Sig. in all groups is p>0.05, which means the distribution of data was normally distributed and homogeneous then continued parametric analysis using the *One Way Anova* hypothesis test with a confidence level of 95%. The results of the One Way Anova statistical test showed a value of p=0.498 (p>0.05) which means there were no significant differences from each group.

DISCUSSION

From the results of research, all groups show small white ginger extract with concentrations of 30%, 40%, 50%, 60%, and 70% was non-toxic to BHK-21 fibroblast cells. The results of the calculation of the viability formula showed that all extract groups have a percentage of living cells of more than 60% which mean that small white ginger extract was non-toxic against BHK-21 fibroblast cells. In the present study, the higher concentration, decreasing percentage rate of fibroblast cells. This is in accordance with the research conducted by Apriasari (2014) on the toxicity of mauli banana stem extract against BHK-21 fibroblast cells which proved that the higher the concentration of the extract, the lower the viability of fibroblast cells.[5]

At the concentration of 70% it had the smallest viability among other concentration groups. The resulting effect on the oak's Trak viability fibroblast cells that change cell membrane permeability. The effect of toxicity from cytotoxins results in changes in cell membrane permeabilit. This can cause cells to become non-viable and cause cell death.[6]

MTT method has a principle that is the reduction of *MTT* tetrazolium yellow salt (3- (4,5-dimethylthiazol-2-il) -2,5-diphenyltetrazolium bromide) by the reductase system. The mechanism of MTT starting from the yellow tetrazoliu salt is reduced in cells that have metabolic activity. Living cells from mitochondria that play an important role are those that have dehydrogenase. If dehydrogenase is inactive due to toxic effects, *formazan* will not be formed which is according to enzymatic activity. The purple intensity is formed based on the number of living cells.[7,8] In this study, the highest intensity of purple was shown by extract concentration of 30%. This showed that fibroblast cells that live at a concentration of 30% was more than the other groups. Cells with the ability to maintain cell permeability are most likely because there is an active chemical compound in small white ginger extract that can stimulate proliferation of fibroblast cells.[5,6] Morphology of BHK-21 fibroblast cell welding which has been treated with small white ginger extract at a concentration of 70% using an inverted microscope with 100x magnification can be seen in **Figure 3.**

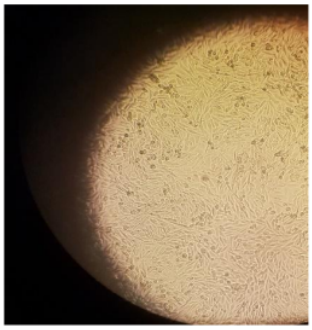


Figure 3. Fibroblast BHK-21 morphology 100x magnification on a 70% concentration.

Phenol content in small white ginger extract can activate *Nuclear factorerythroid-2 related factor* (Nrf2) which is an antioxidant defense process. NRF2 then undergoes translocation to the nucleus and binds to the *Antioxidant Respone Element* (ARE).[9] Antioxidants enzyme, such as superoxide dismutation (SOD), will convert superoxide radicals (O_2 -) to hydrogen peroxide (H_2O_2), which are still reactive. Then, H_2O_2 will be changed again to H_2O (water) and O_2 (oxygen) by catalase (CAT) and glutathione peroxidase (GPx). These enzymes can neutralize ROS, so the cell will survive.[10] Based on the results of this study it is concluded that small white ginger *(Zingiber officinale var. Amarum)* extract is non-toxic to the

cells of fibroblast BHK-21 by using MTT *assay* for cell viability in each of all concentration is ≥60%.

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