Viability Test of Water Hyacinth Leaf Extract (*Eichornia Crassipes*) on Human Gingival Fibroblast Cells

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Abstract

Periodontal disease is a chronic inflammatory disease caused by colonization of bacteria that affects soft tissues and hard tissues that support the teeth, inflammation and rejuvenation of alveolar bone are signs of periodontal disease. Fibroblast also play a role in producing and maintaining extracellular matrix, cell proliferation and cell differentiation in response to prolonged tissue injury and chronic inflammation. The components of water hyacinth are very beneficial for health such as phenols, alkaloids, flavonoids, tannins. This study aimed to determine the concentration of EcengGondok leaf extract which can maintain the viability of human gingival fibroblast cells for 24 hours. The method human gingival primary cell culture was harvested, placed on a 96-well microplate. Each well on the microplate was given water hyacinth leaf extract with a concentration of 1 mg/ml, 0.5 mg/ml, 0.25 mg/ml, 0.25 mg/ml, 0.125 mg/ml, 0.0625 mg/ml, 0.0312 mg/ml, 0.0156 mg/ml for 24 hours. MTT assay was carried out by adding MTT solution after 24 hours of incubation. Formazan optical density values are read by ELISA reader with a wavelength of 590 nm, viability is obtained by calculating the viability formula. Results are viability of human gingival fibroblast cells was good starting in the treatment group 0.125 mg/ml, 0.0625 mg/ml, 0.0312 mg/ml and 0.0156 mg/ml. Conclusion the highest viability of human gingival fibroblast cells in the treatment group of 0.0156 mg/ml was 75.98%.

Keywords: *Water Hyacinth leaf extract, human gingival fibroblast cells, viability, MTT assay.*

Introduction

Periodontal disease is a chronic inflammatory disease caused by colonization of bacteria that affects soft tissues and hard tissues that support the teeth. According to the results of the 2013 Basic Health Research (RISKESDAS), 25.9% of the population in Indonesia had problems with dental and oral health including periodontal disease, this number increased when compared to the results of Basic Health Research in 2007 which was 23.2%.[1]

In the case of periodontitis bone reasorbtion will be seen in the rontsenology examination. One of the basic therapies that can be given in cases of periodontal disease is scaling and root planing, surgical intervention therapy and also given materials that can accelerate the regenerative process of cells that have been damaged.[2]

Fibroblasts are the most common cells found in connective tissue throughout the body including the oral cavity and are the main source of extracellular matrix fibroblasts also play a role in producing and maintaining extracellular matrix. Fibroblasts play an important role in the wound healing process which is a response to injury and tissue [3]

In several studies, phytokomia of water hyacinth leaf

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extract has proven that there are components in water hyacinth which are very beneficial for health such as phenols, alkaloids, flavonoids, tannins and essential oils (squalene). These components can act as antimicrobial, antioxidant, anticancer, antitumor and for the wound healing process.[4]

The use of plant extracts as an alternative in the health sector should have very minimal side effects, are not toxic, do not cause allergies, are not cynogenic and do not cause complications in the body. Therefore, the use of plant extracts must be tested first, one of which is by viability testing in accordance with the terms and material in the field of dentistry.[5]

The viability test is a cell-based test that is often used for screening compounds to determine whether the test compound has an effect on cell proliferation or shows a direct cytotoxic effect that ultimately causes cell death.

Various tetrazolium compounds have been used to detect living cells. The commonly used compound is MTT (3- (4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide) [6]. In this study human gingival fibroblast cells were used because fibroblast cells are the most important cells in human periodontal tissue [7]

This study aimed to determine the concentration of EcengGondok leaf extract which can maintain the viability of human gingival fibroblast cells for 24 hours.

Material and Method

This type of research is a laboratory experiment with the design of The Post-Test Only control group design [8]. This research was conducted in the Stem Cell Institution of Tropical Disease (ITD) and Laboratory of the Faculty of Pharmacy, Airlangga University.

The tools and materials used are microscopy micro light, multichannel pipette 25 µl, shacker with magnetic stirrer, ELISA reader, scales, test tubes and shelves, rotary evaporator, grinder, filter paper, glass gourd, auctoclave, measuring cup, incubator, centrifuge, Buchner funnel, laminar flow, BIOH-T Proline micropipette 20-200 µl, bottle Roux, conical tube ep. TIPS 200µl, 96-well Falcon 3072 plate, water hyacinth leaf extract (Eichornia Crassipes), human gingival cell lines, culture media containing Alfa Modified Eagles Medium (αMEM), dimethyl sulfoxide (DMSO), ready-to-use MTT liquid, phosphate buffer saline (PBS), 10% SDS in 0.1 NHCL, 70% ethanol, sterile distilled water, trypsin versene.

Making Hyacinth Leaf Extract: Water hyacinth leaves are aerated for about seven days until dry [9]. After drying, then grinding is done using a grinder to get 40 mesh of water hyacinth powder granule, as much as 500 grams of water hyacinth leaves macerated with 1000 ml ethanol solvent, then stirred with a stirrer for 24 hours then filtered using whatmann paper No. 40 which is placed on a Buchner funnel and obtained by filtrate, then centrifuged at 9000 rpm at 4 oC for 15 minutes, the sediment obtained is then evaporated using a rotary evaporator at 60 oC, evaporation above the waterbath, evaporation results then evaporated again until there is no residual ethanol content.

Stage of Fibroblast Cell Management: Gingival tissue was taken from free gingiva in tooth P1 for extracted orthodontic treatment, gingival tissue was washed with physiological solution to clean from blood, then placed in a transport medium to be taken to the laboratory, gingival tissue washed 3 times with PBS containing penicillin and streptomycin antibiotics to avoid the possibility of bacterial contamination. The gingival tissue was cut to approximately 1mm3 and covered with deckglass, then collagenase was added for 30 minutes at 37oC, then the tissue was washed and centrifuge for 6 minutes, then the cells obtained were cultured with a growing and incubated medium in a 5% CO2 incubator with a temperature of 37oC for 3 days.

Stage of Fibroblast Cell Culture: Primary human gingival fibroblast cell culture in the Alpha Modified Eagle's Medium (αMEM). Culture was added with 150 µg/ml Fetal Bovine Serum (FBS) 10%, 10 µg/ml Fungizone 0.5%, 100 µg/ml 2% Citrate, confluent cells then dipasase to be propagated, cell medium removed, then washed with PBS, the cell is released with a 2 ml trypsin enzyme, then incubated safely 5 minutes at 37oC and 5% CO2, after the cell is removed then added a stopper and resuspended, centrifuged 25000 rpm for 6 minutes, pellets are planted on a 10 cm plate with αMEM medium .

Harvesting Cells: The cell growth medium is washed with PBS 10% as much as 5 mL to remove protein in the medium, PBS is removed using an aspirator with a wash movement, 25% trypsin as much as 2 mL is inserted to detach cells from the surface of the plate, put in an incubator for 5 minutes with temperature of 37oC and 5% CO2, cell conditions were observed with a light microscope to see cell distribution, human gingival fibroblast cells were taken from CO2 incubators

then cell conditions were observed (80% confluent cell cultures were used for harvesting), cells were harvested according to harvest protocols. Cells are seen using a microscope. The container is tapped so that the cell is floating.

Results

According to the results of reading OD (Optical Density), the average results of the research shown in table 1 are obtained.

Table 1: Absorbance results in the treatment group extracts of water hyacinth leaves for 24 hours.

Treatment	N	Mean
Media Control	6	0,087
Cells Control	6	0,647
Concentration 1 mg/ml	6	0,168
Concentration 0,5 mg/ml	6	0,318
Concentration 0,25 mg/ml	6	0,362
Concentration 0,125 mg/ml	6	0,428
Concentration 0,0625 mg/ml	6	0,441
Concentration 0,0312 mg/ml	6	0,463
Concentration 0,0156 mg/ml	6	0,513

Based on the results of the study it can be seen that the average value of the absorbance of the cell control group for 24 hours is 0.647. The lowest absorbance of the group of extracts of water hyacinth leaves at a concentration of 1 mg/ml of 0.168 and the average absorbance of the group of extracts of water hyacinth leaves was highest at a concentration of 0.0156 mg/ml of 0.513.

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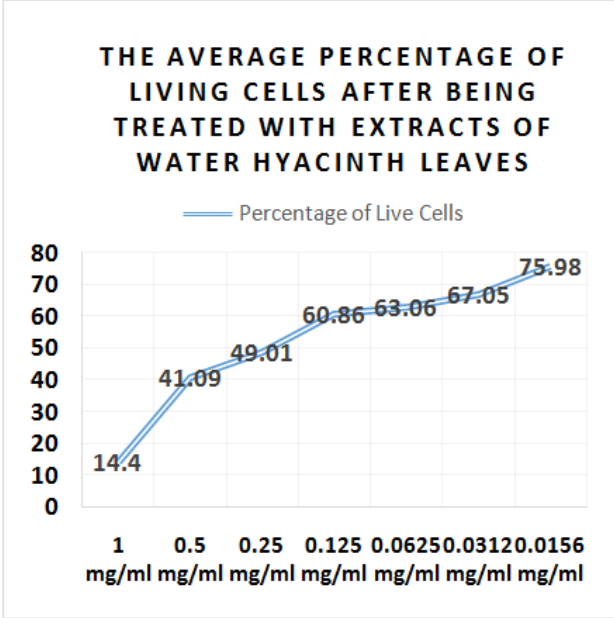
Concentration	Percentage of Live Cells (%)
1 mg/ml	14,395
0,5 mg/ml	41,094
0,25 mg/ml	49,012
0,125 mg/ml	60,858
0,0625 mg/ml	63,061
0,0312 mg/ml	67,050
0,00156 mg/ml	75,979



Graphic 1: Percentage of Live Cells

Statistical Analysis: The measurement results are tabulated according to each group sample, followed by testing the normal distribution using the Kolmogorov Smirnov Test with Sig> 0.05. It can be concluded that the data are normally distributed, then homogeneity testing using Levene's Test with Sig. <0.05 can be concluded that data is not homogeneous. After that, statistical tests were carried out using Kruskal Walis at the significance level of Sig. <0.05 and significant differences were obtained, then continued by performing multiple comparisons using Mann-Whitney which concluded that all groups had significant differences except the concentration group 0.25 mg/ml against 0.125 mg/ml; 0.0625 mg/ml; 0.0312 mg/ml, concentration of 0.125 mg/ml against 0.0625 mg/ml; 0.0312 mg/ml, concentration of 0.0625 mg/ml against 0.0312 mg/ml.

Discussion

Water hyacinth is a plant that floats on the surface of the water (weeds) that can develop roots in the mud in shallow water which eventually becomes waste because it can grow wildly on the surface of the water so that it disturbs the growing ecosystem, according to some studies water hyacinth contains several substances that can help proliferation from the cell. Then in this study using fibroblast cell cultures from human gingiva where fibroblast cells play an important role in the wound healing process. Cell culture has several advantages,

namely high cell growth speed, cell integrity is maintained and cells are able to multiply in suspension.

Research on the viability test of water hyacinth leaf extract was carried out to determine the viability of water hyacinth leaf extract on human fibroblast cells, because in the field of dentistry it requires a healing process so that each material used must meet several requirements, one of which is not having a detrimental or toxic effect on the biological environment both local and systemic. Therefore the viability test is conducted to see the level of biocompatibility of a material, one of the method is by enzymatic testing with MTT reagent (3- (4,5-dimethythiazol-2-yl) -2,5-di-phenyl-tetrazolium bromide).[10]

This tetrazolium salt dissolves in water and produces a yellow solution, living cells can reduce MTT, while dead cells cannot reduce MTT because the enzymes in the cell no longer function [11]. The basic principle is the work of mitochondrial enzymes in active cells that metabolize tetrazolium salts, so that the tetrazolium ring is broken by the dehydrogenase enzyme which causes the tetrazolium to turn into insoluble and purple formazan. Color changes are caused by tetrazolium salts being reduced by the metabolic activity of cells that form NADH or NADPH. This purple color will be measured by its absorbance, absorbance is the ratio of the intensity of light absorbed to the intensity of light that comes using a certain wavelength. The absorbance value can be said to be directly proportional to the concentration of substances contained in it, namely the more levels of substances contained in a sample, the more molecules that absorb light, the greater the absorbance value.[12]

Based on the results of the research in table 1, the absorbance results of the treatment group of water hyacinth extract for 24 hours and cell control and media control for comparison were obtained, the average value of the absorbance of the treatment group. Absorbance value is used to determine percent cell viability, if the absorbance value observed is smaller than the absorbance value of the control group, then the cell undergoes reduction or in other words the cell's ability to proliferate is low. Conversely, when the absorptive value is higher than the control, the ability of cells to proliferate is high, if the level of proliferation is too high it can cause death from cells because of the possibility of changes in cell morphology.[13]

A material cannot be said to be toxic if the percentage of living cells after exposure to the sample is more than 50% which is in accordance with CD50. Statistically, the comparison of the control group with the treatment group 1 mg/ml, 0.5 mg/ml, 0.25 mg/ml, 0.125 mg/ml, 0.0625 mg/ml, 0.0312 mg/ml, 0.0156 mg/ml had a significant difference. From the results of the percentage of live cells it can be seen that the optimum dose of the treatment group extract of water hyacinth leaves for 24 hours is 0.0156 mg/ml.[12]

Based on phytochemical tests and several scientific studies regarding extracts of water hyacinth leaves contain several substances, namely flavonoids, alkaloids and tannins. Where alkaloids play a role in increasing regulation of various types of cytokines, namely TGFβ1, CTGF, PDGF, where the three cytokines function to control cell proliferation including fibrblast cells during the wound healing process. TGFβ1 is responsible for inducing fibrosis by encouraging HSCs to differentiate into miofibroblasts and increasing TIMPs. CTGF is produced by macrophages which will produce a profibrotic signal which causes stimulation of the proliferation of collagen which will produce HSCs.[14]

Flavonoids that act as antioxidants that inhibit the increase of oxidative stress on body cells, when flavonoids are absorbed usually there will be an increase in several biological functions including protein synthesis, cell differentiation and cell proliferation and angiogenesis. Then there is tannin which neutralizes proteolytic enzymes with the help of TGFβ1 which will cause an increase in TIMPs which will inhibit degradation of the extracellular matrix and directly support the occurrence of fibrillar collagen interstitial synthesis. Which will later facilitate cell growth by preparing the environment of cells that will regenerate.[15,16]

Conclusion

Viable hyacinth leaf extract against fibroblast cells, treatment with a concentration of 0.0156% with a percentage above 75.98%.

Conflict of Interest: There is no conflict of interest.

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Ethical Clearance: This study was approved by Ethical Comission of Health Research faculty of Dentistry, University of Airlangga.

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