Viability Assay Of Human Fibroblast Cells Treated by Water Hyacinth Leaf Extract After 24 Hours Incubation

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ABSTRACT

Inflammation and alveolar bone resorption are indications of periodontal disease, which is a chronic inflammatory illness caused by bacterial colonization that damages the soft and hard structures that support the teeth. In response to persistent tissue injury and chronic inflammation, fibroblasts also play a role in the synthesis and maintenance of extracellular matrix, cell proliferation, and cell differentiation. Fibroblasts play a crucial part in the healing of wounds. Phenols, alkaloids, flavonoids, and tannins are some of the health-promoting components found in water hyacinth. As a result, plant extracts must be tested first, one of which is the viability test in accordance with the requirements and materials in the field of dentistry. 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 has a direct cytotoxic effect that leads to cell death. The goal of this study is to figure out what concentration of water hyacinth leaf extract can keep human gingival fibroblast cells alive for 24 hours. Primary cell cultures from human gingiva were extracted and placed in a 96-well microplate. For 24 hours, water hyacinth leaf extract at concentrations 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 was administered to each well in the microplate. After 24 hours of incubation, the MTT assay was carried out by adding MTT solution. The optical density of formazan was measured using an ELISA reader at a wavelength of 590 nm, and viability was calculated using the viability formula. Starting at 0.125 mg/ml, 0.0625 mg/ml, 0.0312 mg/ml, and 0.0156 mg/ml, the vitality of human gingival fibroblast cells was good. In the treatment group, the greatest vitality of human gingival fibroblast cells was 0.0156 mg/ml (75.98%).

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

INTRODUCTION

Periodontal disease is a chronic inflammatory disease that affects the soft and hard tissues that support the teeth and is caused by bacterial colonization. Periodontal disorders, such as periodontitis and gingivitis, are caused by poor oral and dental health, and poor oral and dental health has the potential to induce

periodontal disease. An x-ray examination will reveal periodontitis in the case of bone resorption. Scaling and root planing, surgical intervention therapy, and materials that can expedite the regenerative process of cells that have been destroyed are some of the basic therapies that can be offered in situations of periodontal disease (Irokawa et al., 2017).

Fibroblasts are the most common cells found in connective tissues throughout the body, including the oral cavity, and are the main source of extracellular matrix. Fibroblasts also play a role in the production and maintenance of extracellular matrix, cell proliferation, and cell differentiation in response to chronic tissue injury. Fibroblasts are crucial players in the wound healing process, which is a reaction to tissue damage (Kendall & Feghali-Bostwick, 2014).

Many plants are currently being examined for their extracts in the health sector, and water hyacinth is one of them. Water hyacinth (Eichornia Crassipes) is a weed that floats on the water's surface and can grow roots in the mud in shallow water. Water hyacinth has been regarded as a waste since its existence disrupts the environment in which it grows and the animals that live there. Water hyacinth can spread over enormous regions and cover the water surface, reducing sunlight entering water bodies and causing dissolved oxygen in the water. When water levels are low, water hyacinth dies and settles at the bottom of a body of water, causing silting (Wijaya et al., 2016).

Plant extracts should have very little side effects, are non-toxic, do not induce allergies, are not casinoogenic, and should not produce issues in the body when used as a health alternative. As a result, the usage of plant extracts must first be put to the test, one of which is the viability test in line with dental standards and materials. (Permana, 2017).

To identify living cells, many tetrazolium compounds have been utilized. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is a regularly used chemical (Sakaguchi and Powers, 2012). Because fibroblast cells are the most important cells in human periodontal tissue, human gingival fibroblast cells were used in this investigation. (Yuliati, 2005)

METHOD

The research design for this type of study is an experimental laboratory with a Post-Test only control group design. Human gingival fibroblast cells were treated with water hyacinth (Eichornia Crassipes) leaf extract. Human gingival fibroblast cell culture was employed as the research sample. The Stem Cell Research and Development Center performed an MTT leaf extract experiment utilizing human gingival fibroblast cell culture. The manufacture of water hyacinth leaf extract took place at Airlangga University's Faculty of Pharmacy from October 29 to November 3, 2018.

RESULTS

After administering water hyacinth leaf extract to fibroblast cells, the following findings were obtained:

Table 1. shows the 24-hour absorbance findings in the water hyacinth leaf extract treatment group.

	Cell Control	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
Average absorbance value	0,647	0,168	0,318	0,362	0,428	0,441	0,463	0,513

The proportion of the number of living cells in each well and the average of each concentration were computed after the absorbance values from each well were obtained. The following formula is used to do calculations:

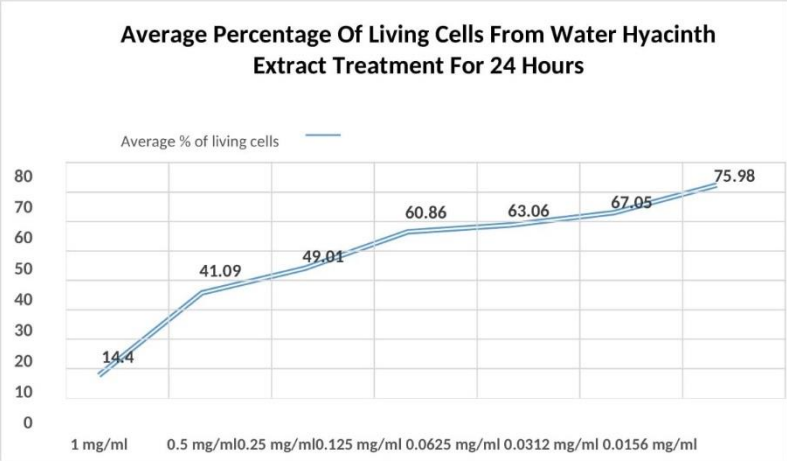
$$Live\ cell\ presentation = \frac{\text{OD Treatment} - \text{OD Media}}{\text{OD Control Cell} - \text{OD Media}}\ x\ 100\%$$

The proportion of living cells in the water hyacinth leaf extract treatment group for 24 hours averaged 14.40 percent; 41.09 percent; 49.01 percent; 60.86 percent; 63.06 percent; 67.05 percent; 75.98 percent, as shown in table 2. The concentration of 0.0156 mg/ml had the largest proportion of live cells (75.98%), while the concentration of 1 mg/ml had the lowest (14.40%). The following graph can be made from the table's results of the percentage of living cells calculation:

Table 2. Percentage of live cells in the water hyacinth extract treatment group for 24 hours

Concentration	Average % of living 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

Figure 1. Graph of the average percentage of live cells from water hyacinth leaf extract treatment for 24 hours



Research Analysis Results

The Kolmogorov-Smirnov Test normalcy test was utilized in this study, which is used for data or participants with a number greater than 30. If the value of sig.> 0.05, the data is normally distributed; if the value of sig. 0.05, the data is not normally distributed.

Table 3. The results of the normality test of the water hyacinth leaf extract treatment group for 24 hours

One-Sample Kolmogorov-Smirnov Test									
	Kontrol Media	Kontrol Sel	1 mg/ ml	0,5 mg/ml	0,25 mg/ml	0,125 mg/ml	0,625 mg/ml	0,312 mg/ml	0,156 mg/ml
Asymp. Sig. (2-tailed)	,902*	,497*	,934*	,554*	,934*	,953*	,915*	,770*	,958*

Table 3 shows that the water hyacinth leaf extract treatment group had Sig.> 0.05 for 24 hours, implying that the data from the water hyacinth leaf extract treatment group for 2 hours were typically normally distributed.

The Levene-Test was employed to determine homogeneity in this study. When Sig.>0.05, it is used to determine homogeneity.

Table 4. The results of the homogeneity test of the water hyacinth leaf extract treatment group for 24 hours

Test Homogenity of Variances			
Levene Statistic	df1	df2	Sig.
2,171	8	45	,048

Because the homogeneity test results are less than 0.05 in the table above, it may be concluded that the treatment group of water hyacinth leaf extract for 24 hours exhibited an inhomogeneous variance distribution. The Kruskal Wallis statistical test, a non-parametric test, is then applied; the test is deemed to have a significant or significant difference if the Sig value is less than 0.05. The Kruskal Wallis test results for the water hyacinth leaf extract treatment group after 24 hours are shown below.

Table 5. Kruskal Wallis . statistical test results

Treatment Group	P value	Alfa
Water Hyacinth Extract Treatment	,000	0.05

Table 5 shows that the Kruskal Wallis test results in the water hyacinth extract treatment group for 24 hours yielded Sig. 0.05, indicating that there is a significant difference between one concentration and another, as well as the control group. The alternative hypothesis is then accepted once the null hypothesis is rejected. The Kruskal Wallis test is followed by the multiple comparisons test, especially the Mann-Whitney test, when there is a significant difference.

Table 6. Mann-Whitney . test results

Mann-Whitney living cell								
Group	Control cell	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
Kontrol sel	*	*	*	*	*	*	*	*
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								*

(*) indicates that there is a significant difference

(-) indicates that there is no significant difference

According to the table above, the control group had a substantial difference from the total treatment group and control cells after being treated with water hyacinth leaf extract for 24 hours.

DISCUSSION

Water hyacinth is a weed that floats on the surface of the water and can grow roots in the mud in shallow water, eventually becoming waste because it can grow wildly on the surface of the water, disrupting the ecosystem where it grows. According to several studies, water hyacinth contains several substances that can help cell proliferation. This study used human gingiva fibroblast cell culture, as fibroblast cells play a crucial role in wound healing.

To assess the viability of water hyacinth leaf extract against human fibroblast cells, an enzymatic test using MTT reagent (3-(4,) 5-dimethythiazol-2-yl)-2,5-di-phenyl-tetrazolium bromide) was used as one of the approaches. (Meizarini and colleagues, 2005)

Because enzymes in dead cells no longer work, this tetrazolium salt is soluble in water and generates a yellow solution. Living cells can reduce MTT, but dead cells cannot. Mitochondrial enzymes function on active cells that metabolize tetrazolium salts, causing the dehydrogenase enzyme to terminate the tetrazolium ring, turning tetrazolium into insoluble formazan, which is purple in color. The reduction of the tetrazolium salt by the metabolic activity of the cells, which forms NADH or NADPH, causes the color shift. The absorbance value is directly related to the concentration of the substances contained in it, i.e., the greater the absorbance value, the more levels of chemicals contained in a sample, the more molecules that absorb light. (Neldawati et al., 2013)

Water hyacinth leaf extract contains numerous compounds, including flavonoids, alkaloids, and tannins, according to phytochemical tests and several scientific research. Where alkaloids have a part in the elevation of several cytokines, such as TGF1, CTGF, and PDGF, which influence cell proliferation, including that of fibroblast cells, during the wound healing process. TGF1 promotes the differentiation of HSCs into myofibroblasts and increases TIMPs, resulting in fibrosis. CTGF is produced by macrophages, resulting in a profibrotic signal that stimulates collagen proliferation, resulting in the production of HSCs (Adtani et al., 2018)

Flavonoids work as antioxidants, preventing oxidative stress in body cells. When flavonoids are absorbed, various biological functions, such as protein synthesis, cell differentiation, cell proliferation, and angiogenesis, are normally increased. Then there is tannin which neutralizes proteolytic enzymes with the help of TGFβ1 which will produce an increase in TIMPs which will impede the degradation of the extracellular matrix and directly support the production of interstitial fibrillar collagen. Which will boost cell

proliferation by creating the environment for cells to renew. (Gegotek et al., 2017)

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