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RESEARCH ARTICLE

In-vitro cytotoxicity activity of potato (*Solanum tuberosum.* L*)* peel extracts against human gingival fibroblasts

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ABSTRACT

Potato peel is often regarded as waste although it contains phenolic compounds, glycoalkaloids, and flavonoid. This study aimed to evaluate the effect of different concentrations of potato peel extracts on the viability of Human Gingival Fibroblasts (HGF). Potato peel extracts were prepared by a maceration technique. The 96-well tissue culture micro titre plates were seeded with HGF at a density of 2×10^4 cells/100 µL and incubated for 24 hours. Next, 100 µL of potato peel extracts at a concentration of 62.5 µg/mL, 125 µg/mL, 250 µg/mL, 500 µg/mL, and 1000 µg/mL and a medium (control) were dispensed into the well of the cell culture. Each concentration was evaluated for its viability with 3 replicate samples. The results of the MTT test were analyzed statistically using one-way ANOVA and LSD test. The mean and standard deviation of the viable HGF after incubated with the potato peel extract at the concentration of 62.5 µg/mL, 125 µg/mL, 250 µg/mL, 500 µg/mL, and 1000 µg/mL were 98.67% ± 3.56, 88.34% ± 0.79, 55.42% ± 3.96, 28.33% ± 0.60, and 26.26% ± 0.53, respectively. The percentage of non-viable HGF increased with an increase in the concentration of the potato peel extract. The ANOVA test result showed a significant influence of various concentrations of the potato peel extract on the viability of HGF ($p<0.05$). The result of the LSD-test showed a significant difference among all the treatment groups ($p<0.05$). A higher concentration of potato peel extracts increased the viability of HGF cell line and the concentrations of 62.5 µg/mL and 125 µg/mL were considered non-cytotoxic.

Keywords: cytotoxicity; HGF cells; potato (*Solanum tuberosum* L.) peel extracts; viability

INTRODUCTION

Dental plaque is a chronic endogenous infection caused by normal oral commensal flora that destroys tooth tissue locally by bacterial fermentation of dietary carbohydrates.[1] Adhesion of the pioneer species of oral biofilm such as *Streptococcus oralis*, *Streptococcus mitis* and *Streptococcus sanguis* marks the start of the plaque and caries formation.[2] Dental plaque can be prevented in many ways. In addition to the usual mechanical removal of the biofilm by toothbrush and dental floss, plaque formation can be prevented and controlled by using antiseptic mouthwashes containing active ingredients such as chlorhexidine (CHX) and essential oil.[3]

Mouthwashes are aqueous solutions containing one or more active compounds. Basically, mouthwashes can be divided into two types, namely therapeutic and cosmetic ones. Therapeutic mouthwashes are formulated to reduce plaque, gingivitis, dental caries, and stomatitis, while cosmetic mouthwashes are formulated to aid in breath freshness using antimicrobial and flavouring agents. Mouthwashes generally contain alcohol ranging from 10% to 20%.[4] Although alcohol enhances the flavour and functions as a solubilizing agent for some flavouring agents, it elicits some unfavourable side effects, as it can cause a burning mouth syndrome when in contact with the mucus membrane of the mouth.[5] Some mouthwashes also contain CHX. CHX is one of the highly effective antiplaque agents to date. However, CHX has side effects such as staining of the teeth, restorations, and tongue, and sometimes even mouth dryness and oral ulcer.[6] Thus, the usage of natural products such as herb and plant extracts have been investigated to overcome the side effects.

Potato (*Solanum tuberosum* L.) is a member of the *Solanaceae* family, which has been ranked as the world's fourth most important staple food

crops, after maize, wheat, and rice.[7] Potato peel contains several components such as phenolic compounds, glycoalkaloids, and flavonoid which may be used as anti-inflammatory and analgesic. Potatoes are usually peeled during processing, so utilization of these by-products reduces the amount of waste and leads to sustainable production.[8]

Flavonoids are a group of plant metabolites that are thought to provide health benefits through cell signalling pathways and antioxidant effects.[9] Flavonoids are found in a variety of fruits and vegetables, along with tea and wine. The biological properties of flavonoids include antioxidant, metal chelation, anti-inflammatory, antitumor, antiviral, antibacterial, as well as cardiovascular disease prevention, and anticancer activities.[10] Phenolic compounds in potato peel have a high antioxidant activity and serve as an antibacterial material. One of the commonly found phenolic compounds in potato peel is chlorogenic acid which has a strong antioxidant activity.[11] Research conducted by Daglia et al on chlorogenic acid and a few chemical compounds have shown anti adhesive properties against *Streptococcus mutans*.[12]

All new dental materials proposed to the market need to be evaluated for their potential toxicity.[13] To detect the biological activity of potato peel, *in vitro* cytotoxicity tests can be carried out on fibroblasts and HeLa. The cell culture is later tested with MTT assay for cell proliferation and cell viability.[14] Fibroblasts are the most common cells in the connective tissue which originate locally from undifferentiated mesenchymal cells. They are responsible for the synthesis of collagen and extracellular matrix components.[15] Primary human cells from gingival and periodontal ligament fibroblasts have been used to facilitate *in vitro* cytotoxicity and genotoxicity studies of dental materials. However, due to the limited lifespan and difficulty to work with, human gingival fibroblasts (HGF) cell line is a more suitable cell to be used.[16]

MATERIALS AND METHODS

This study was approved by the Ethics and Advocacy Unit of the Faculty of Dentistry, Universitas Gadjah Mada (No. 00247/KKEP/FKG-UGM/EC/2015). The potatoes originated in Dieng, Wonosobo, Central Java and were determined and analysed at the Department of Biology Pharmacy of the Faculty of Pharmacy, Universitas Gadjah Mada. The *Solanum tuberosum* L. peel was obtained by first peeling the potatoes and cutting the peel into small pieces. The peel was then dried with a cabinet dryer at 40 °C for 24 hours and later ground into powder. One hundred gram of the fine powder was obtained from 10 kg of potatoes. After that, the sample was macerated in 70% ethanol with a ratio of one to five at a room temperature for a week. Lastly, the crude extract was filtered and evaporated in a vacuum rotary evaporator at 45 °C before being stored at -20 °C for further analysis.[17]

Sterile distilled water was first measured 5% less than the desired total volume of the media and then DMEM powdered media were added to the sterile distilled water at a room temperature with gentle stirring. To prepare a 1-liter medium, 3.7 g of sodium bicarbonate ($NaCO_3$) was added and stirred until dissolved. The pH of the media was adjusted to 0.2-0.3 units below the desired pH using NaOH and HCl as pH tends to rise during filtration. After that, sterile distilled water was added to the desired volume and the medium was sterilized immediately by using a Millipore vacuum 0.22 μm filter top. Lastly, the medium was stored at 2-8 °C in the dark until usage.[18]

The HGF cell lines were obtained from stocks stored in liquid nitrogen at the Integrated Research and Testing Laboratory – Universitas Gadjah Mada (LPPT- UGM). These cells had been already harvested from the patient biopsies under the ethics committee approval. The cells were cultured in a DMEM medium and supplemented with 10% Foetal Bovine Serum. The cell cultures were maintained at 37 °C, humidified, 5% CO_2/ air incubator. The cell cultures were removed from the culture flasks by enzymatic digestion (trypsin/ EDTA) and incubated for 10 minutes at 37 °C before being centrifuged for 10 minutes. After that, the cells were then resuspended in the culture medium.

The 96-well tissue culture microtiter plates were seeded with HGF cells at a density of 2×10^4 cells/100 µL. The cells were incubated for 24 hours. Next, 100 µL of potato peel extracts (at a concentration of 62.5 µg/mL, 125 µg/mL, 250 µg/mL, 500 µg/mL, and 1000 µg/mL, respectively) or medium (control) were dispensed and incubated for 24 hours. There were three replicates in each concentration.

The cellular viability evaluation using MTT was based on ISO 10993-5 Part 5 (2009).[19] After the cells were seeded into the 96-well plates and maintained in culture for 24 hours, a semi-confluent monolayer was formed. One hundred microliters of MTT solution were added into the well before the cells were incubated for another 4 hours. After that, 100 µL of SDS-HCL solution was added into the well and incubated overnight. The optical density (OD) of the cell viability which is directly proportional to the amount of formazan crystal generated was then determined by using an ELISA plate reader at 550 nm. Cell viability is expressed as followed[19]:

$$\text{Viab.\%} = \frac{\text{OD}_{550\ \text{treated}}}{\text{OD}_{550\ \text{control}}} \text{x } 100$$

where

$OD_{550\ treated}$: The mean of the measured optical density of the treated cells

$OD_{550\ control}$: The mean of the measured optical density of the control cells

The results of the experiment were statistically analyzed by Shapiro-Wilk normality test, Levene's test, one-way analysis of variance (ANOVA) with a level of confidence of 95% and followed by Post Hoc Least Significant Different (LSD) test using SPSS 16.0.

RESULTS

The viability of human gingival fibroblasts was evaluated using MTT assay. The cell viability was determined by comparing the optical density (OD) of each sample with the optical density (OD) of the control.

The results of the MTT test were analysed statistically using one-way ANOVA and LSD test. Figure 1 shows the mean and standard deviation of viable fibroblasts in percentage (%) after treated with potato peel extracts at different concentrations. Each concentration was evaluated for its viability with 3 replicate samples.

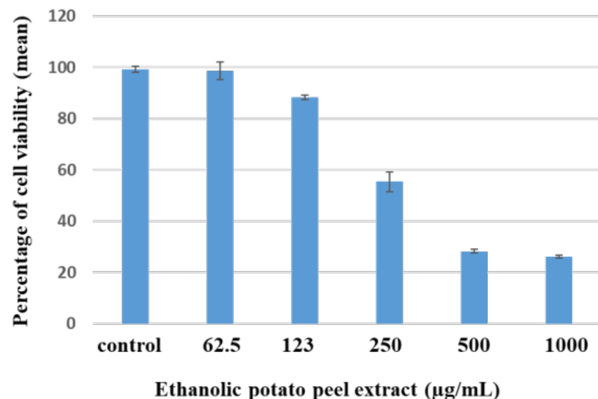

Figure 1. The effect of different concentrations of potato peel extracts on the viability of gingival fibroblasts

As shown in Figure 1, the percentage of viable cells remained more than 70% even after the cells were treated with 100 µL of 62.5 µg/mL and 125 µg/mL potato peel extracts for 24 hours. However, as the concentration of potato peel extracts increased, the percentages of the viable cells decreased and finally at the concentration of 1000 µg/mL potato peel extracts, only 26.26% of the cells were viable. These results indicated that potato peel extracts showed a significant effect on the viability of HGF cell line.

Figure 2 shows the microscopic images of HGF cells after 24-hour incubation only in DMEM (control), in 62.5 µg/mL, 125 µg/mL, 250 µg/mL, 500 µg/mL, and 1000 µg/mL of potato peel extracts, respectively. The cells were observed under a microscope with a ×100 magnification. The cells that appeared cylindrical or elongated in shape and attached to the substrate are viable cells, while the cells that appeared round and big are non-viable cells.[20]

The one-way ANOVA result (data not shown) revealed a significant difference of the cell viability percentage among the groups with different concentrations ($p<0.05$) and the F ratio was 779.142. The Fisher's LSD test was later carried out to distinguish the significance of the mean between each concentration group. The

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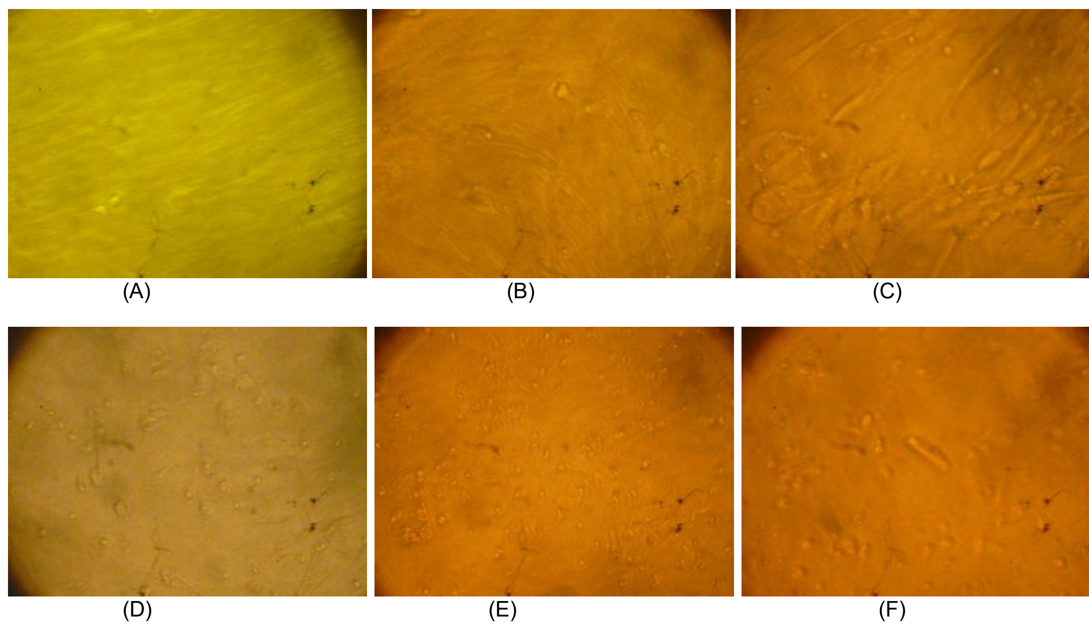

Figure 2. Microscopic images of HGF cells after 24 hours of incubation; (A) cell without any treatment (control), with (B) 62.5 µg/mL, (C)125 µg/mL, (D) 250µg/mL, (E) 500 µg/mL, and (F) 1000 µg/mL of potato peel extracts. Viable cells (red arrow), and non-viable cells (black arrow)

result from the post hoc LSD test (data not shown) showed that there were significant differences in all the concentration groups ($p<0.05$). Based on the result of the ANOVA test in Table I, different ethanolic potato peel extract concentrations can affect the viability of HGF cell line significantly.

DISCUSSION

Cell death is caused by the toxicity of a material that is influenced by the content of the active substance in the material. Round and cylindrical shape of cells can be seen in Figure 2. HGF cells attached to a substrate are bipolar or multipolar and have elongated shapes. The results indicated that potato peel extracts had a significant effect on the viability of HGF cells because as the concentration of potato peel extracts decreased, the percentages of viable cells increased. Cell death is triggered by the toxicity of a chemical and is influenced by the active substance concentration of the substance. As stated by Paracelsus (1493-1541) cited by Williams et al,[21] "All substances are poisons. There is none, which is not a poison. The right dose differentiates a poison and a remedy." Thus, the result observed that the potato peel extracts contain substances that might increase the death of HGF cells.

Cell death can be classified into two types, namely necrosis and apoptosis, which differ in their morphology, mechanism, and physiology. Necrosis is cell death that occurs when a cell loses its ability to maintain homeostasis while apoptosis or programmed cell death occurs under normal physiological conditions.[22] Cellular necrosis or extrinsic factor-induced cell death is normally caused by factors like hypoxia-ischemia, thermal insults, exposure to toxic agents, and traumatic insults.

Sherwood[23] stated that cell is sensitive and responsive to changes in its surrounding environment. Membrane applied in the well of the cell culture will alter the surrounding environment. The cells will experience a progressive loss of membrane integrity which allows the influx of ions and water, resulting in cytoplasmic swelling, nuclear pyknosis followed by a release of lysosomal and granular contents into the surrounding extracellular space, with subsequent inflammation, eventually leading to irreversible cell injury that causes cell death.[24] The study showed the potato peel extract had the potential to cause acute toxicity. As stated

by Schieber and Shaldana (2009),[8,25] potato peel contains phenolic compounds, glycoalkaloids, and flavonoids antioxidant. One or more components in potato peel extracts are believed to change the environment in the well, causing a disturbance to the enzymatic activity of the exposed HGF cells, especially the mitochondrial enzyme dehydrogenase, thus eventually leading to cell death by necrosis. Non-viable cells in Figure 2 appear round and big, which fit the theory stated by Mitchell et al. (2012),[22] that a cell might undergo necrosis after it fails to the adaptive responses such as hyperplasia, hypertrophy, and atrophy.

Glycoalkaloids are one of the components of potato peel extracts that might contribute to cell death. Potatoes produce several types of glycoalkaloids and one of which is solanine. Solanine is a cholinesterase inhibitor which has the ability to disrupt cell membranes.[26] Research by Gao et al[27] found out that solanine is cytotoxic to cell and it opens the potassium channels of mitochondria, prevents the breakdown of acetylcholine, thus decreasing membrane potential. This in turn causes Ca^{2+} to be transported into the cytoplasm, resulting in influx of Ca^{2+} that eventually triggers cell damage and apoptosis.

The cytotoxic of a substance indicates that there is a probability of a substance to be toxic when applied clinically.[28] Sjögrenet al. (2000)[29] classified the cytotoxicity of a substance based on the viable cell percentage as follows: a) Non-cytotoxic: >90% viable cell; b) Mildly cytotoxic: 60-90% viable cell; c) Moderately cytotoxic: 30-59% viable cell; d) Severely cytotoxic: ≤30% cell viability. According to ISO 10993-5 standard, a substance is considered acceptable for clinical usage when the cell viability is more than 70%, therefore, it can be concluded that potato peel extracts with the concentration of 62.5 µg/mL and 125 µg/mL with viability value more than 70% (98.67% and 88.34% respectively) may consider safe to be applied clinically in mouthwash as the concentration is mildly cytotoxic and within the tolerable limit.

CONCLUSIONS

The concentration of ethanolic potato peel extracts appears to affect HGF cell viability. The study concluded that potato peel extracts at 62.5 µg/mL and 125 µg/mL were non-cytotoxic.

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CONFLICT OF INTERESTS

The authors declared no competing interests.

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