

The effectivity of edamame extract (*Glycine max* L. Merrill) as a heat-cured acrylic resin denture cleanser using in vitro toxicity studies

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Abstract

Denture cleanser that are used over a long period will produce residue that can be absorbed by the acrylic resin. The hydrophilic acrylic resin can cause denture-cleaning molecules to enter through microporosity which can be toxic to the oral tissue. Plant-based denture cleanser is commonly used one of which is edamame (*Glycine Max* L. Merrill) extract. Denture cleanser made from edamame extract needs to be tested for toxicity to determine the biocompatibility of the material in the oral cavity, specifically on fibroblast cells. This study aims to determine the toxicity of edamame extract as a denture cleanser for heat-cured acrylic resin, which is soaked for 4 days against BHK-21 fibroblast cells. This 4-day submersion equals 15 minutes of submersion per day for 1 year. The toxicity tests were carried out using the MTT assay method. The results of these MTT tests can be seen from the absorbance values and calculating the percentages of cell viability. The more cells that are still alive, the more intense the purple color produced. The research results showed that the acrylic resin group which was soaked in a 50% concentration of edamame extract was not toxic to fibroblast cells, because the percentage of cell viability had a value of >70%, which was 83.638%. This research concludes that 50% concentration of edamame extract as a denture cleanser for heat-cured acrylic resin is not toxic to BHK-21 fibroblast cells compared to 75% and 100% concentrations of edamame extract.

Keywords: Denture cleanser; Heat-cured acrylic resin; Edamame extract; BHK-21 fibroblast cells; MTT assay test

1. Introduction

Tooth loss that occurs in a person can cause problems with chewing function, reduce self-confidence, and discomfort when eating and talking, so it is best to immediately make a replacement using dentures [14]. Dentures have one part that is in direct contact with the oral mucosa, namely the denture base, which must have good biocompatibility properties, that is, it does not cause allergic reactions in the underlying tissue and is not toxic to the supporting cells [16]. The most frequently used denture base is heat-cured acrylic resin because of its advantages, namely affordable price, easy manipulation process, good aesthetics, and easy repair and polishing [11]. Acrylic resin also has the weakness of producing residual monomer which can cause irritation and inflammation of the oral mucosa, however, residual monomer can be minimized by soaking the acrylic resin in water for 1 hour before use [1]. Another weakness of acrylic resin is that it has microporosity which affects the physical, aesthetic and cleanliness of the denture base [15]. Acrylic resin denture cleansers can ideally remove and prevent bacterial plaque, remove food debris, and are not toxic [5]. However, denture cleansers containing sodium hypochlorite in the long term have the potential to be toxic to oral tissue because this material can be absorbed into the hydrophilic acrylic resin through microporosity [12]. Denture cleaning agents made from natural ingredients, namely plants, have been widely used, one of which is edamame extract which contains isoflavones, flavonoids and saponins. These ingredients are by the requirements for denture cleaning, namely anti-inflammatory and antibacterial [6]. Denture cleansers made from edamame extract need to be tested for toxicity to determine the biocompatibility of the material in the oral cavity, namely on fibroblast cells.

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2. Material and methods

The disc-shaped heat-cured acrylic resin was made with a diameter of 3 mm and a thickness of 2 mm. Edamame extract as an acrylic resin cleanser was made using the maceration method with 70% ethanol solvent for 3 days [6]. Acrylic resin is soaked in sterile distilled water first for 24 hours to reduce residual toxic monomers [1]. Acrylic resin is soaked in edamame extract in concentrations of 50%, 75%, 100%, and 0.5% sodium hypochlorite as a heat-cured acrylic resin denture cleanser for 4 days. Acrylic resin is soaked for 4 days because it is equivalent to 1 year of use with 15 minutes of soaking every day. During this soaking, the acrylic resin is also rinsed or washed with water and replaced with new edamame extract and sodium hypochlorite once every day. After that, a toxicity test was carried out using the MTT assay method, the acrylic resin was placed on a 96-well microplate containing 2×10^4 fibroblast cells/well and 100 μ L MEM-Neaa media [9]. Edamame extract and sodium hypochlorite were also placed in 20 μ L 96-well microplates and then incubated for 24 hours at 37°C with 5% CO₂ [3]. After that, 10 μ L of PBS was washed, then 10 μ L of MTT was given, incubated at 37°C for 4 hours, then 100 μ L of DMSO was given. The microplate is placed into the ELISA reader with a wavelength of 550 nm to read the absorbance value. Then the percentage of cell viability or the cell's ability to survive is calculated. A cell viability percentage that shows >70% can be categorized as indicating that the material is not toxic to cells [9].

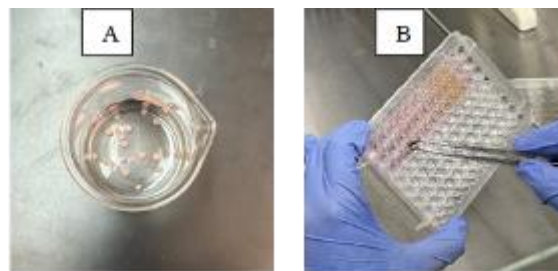


Figure 1 (A) Acrylic resin is soaked in sterile distilled water (B) acrylic resin was placed on a 96-well microplate

3. Results

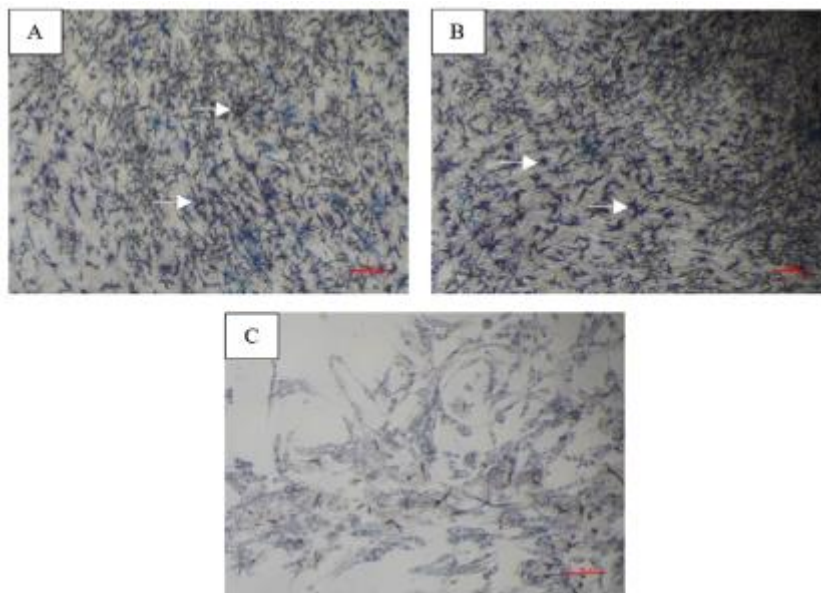


Figure 2 (A) Cell control group (B) Acrylic resin treatment group soaked in 50% edamame extract (C) Acrylic resin treatment group soaked in 0.5% sodium hypochlorite (arrows in images A and B indicate the formation of formazan crystals because the cells are capable of survive so that it can convert MTT into formazan).

This research was conducted to test the effectiveness of edamame extract in concentrations of 50%, 75%, 100%, and 0.5% sodium hypochlorite as a heat-cured acrylic resin denture cleanser which will cause toxic effects or not after soaking for 4 days on BHK fibroblast cells. -21. The results of the toxicity test can be seen from the color changes shown.

This color change occurs because water-soluble yellow salt (MTT) becomes purple formazan crystals which are insoluble in water so cell viability is maintained. The more formazan crystals produced, the higher the cell viability.

Absorbance readings were carried out using an ELISA reader with a wavelength of 550 nm. Cell viability was calculated for each group using the cell viability percentage formula. If the cell viability percentage shows more than 70%, it can be said that the material being tested is not toxic.

Table 1 Results of calculating the percentage of viability of BHK-21 fibroblast cells

| Group | Average Absorbance Value ± Standard Deviation | Cell Viability Percentage |
|---|---|---------------------------|
| KM (Media Control) | .053 ± .003 | 0% |
| KS (Cell Control) | .582 ± .031 | 100% |
| R+E50 (Acrylic Resin + Edamame Extract 50%) | .496 ± .019 | 83.638% |
| R+E75 (Acrylic Resin + Edamame Extract 75%) | .417 ± .025 | 68.722% |
| R+E100 (Acrylic Resin + Edamame Extract 100%) | .382 ± .025 | 62.115% |
| R+SH (Acrylic Resin + Sodium Hypochlorite) | .356 ± .017 | 57.583% |
| R (Acrylic Resin) | .454 ± .018 | 75.708% |
| E50 (Edamame Extract 50%) | .432 ± .059 | 71.68% |
| E75 (Edamame Extract 75%) | .306 ± .029 | 47.892% |
| E100 (Edamame Extract 100%) | .213 ± .014 | 30.208% |
| SH (Sodium Hypochlorite) | .12 ± .017 | 12.775% |

Percentage of BHK-21 Fibroblast Cell Viability

The percentage of cell viability tends to be higher at lower concentrations of edamame extract. Based on the percentage of cell viability, a bar diagram of the effect of edamame extract was obtained which shows that there is a tendency for cell viability to increase as the concentration decreases.

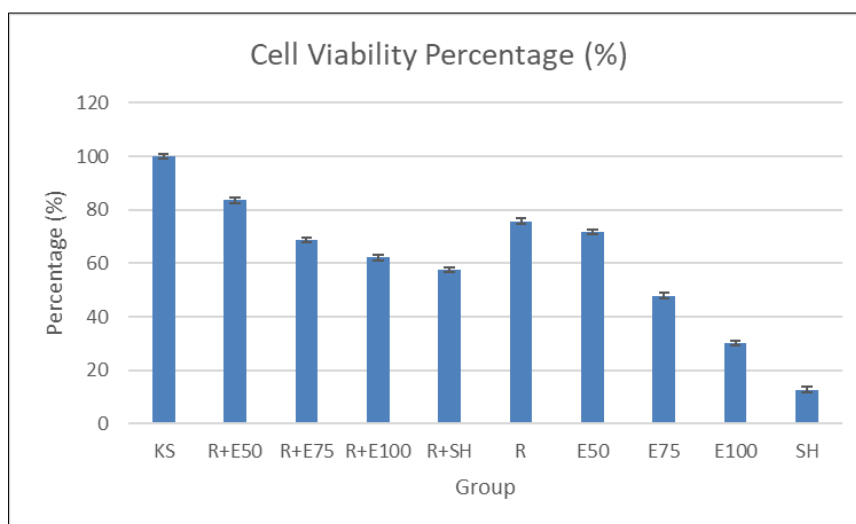


Figure 2 Bar Chart of Cell Viability Percentage

The results of the toxicity test that can be seen on the microplate are that there are different colors in each well, namely purple and yellow. The yellow MTT solution changes to purple formazan because there are cells that are still alive and actively metabolizing, whereas if there are dead cells the MTT solution will remain yellow. After all, the cells are unable to convert MTT into formazan.

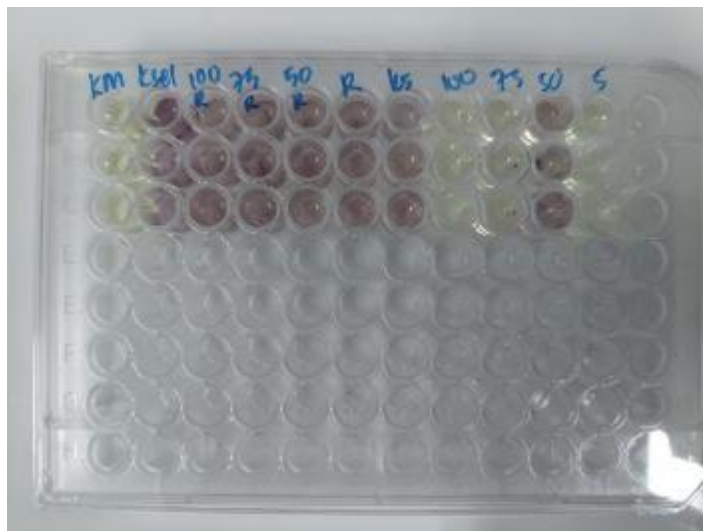


Figure 3 Results of Color Changes in BHK-21 Fibroblast Cells

4. Discussion

Heat-cured acrylic resin has residual monomers which can be toxic to the oral cavity. This can be prevented by reducing the amount of residual monomer of acrylic resin before use in the oral cavity. Reducing the amount of residual monomer of acrylic resin is done by soaking the acrylic resin in water. Soaking acrylic resin in water can help release residual monomers that diffuse into the water. The recommended soaking time for acrylic resin is 24 hours [1]. The acrylic resin used in this research was also tested for toxicity after being soaked in water for 24 hours and not treated with edamame extract and sodium hypochlorite. The results shown in table 1 show that acrylic resin is non-toxic, namely with a cell viability percentage of 75.708% so if there are toxic groups then the toxicity does not come from the acrylic resin.

Acrylic resin is soaked in edamame extract which acts as a denture cleanser. This soaking causes the edamame extract to seep through the microporosity of the acrylic resin. Heat-cured acrylic resin which consists of polymethyl methacrylate will form ester groups so that the acrylic resin has hydrophilic properties. The ester of polymethyl methacrylate will react with the phenol from edamame which is in contact with the acrylic resin. This causes the polymer chain bonds of the acrylic resin to be disrupted and weakens the physical properties of the material so that these compounds can enter the surface of the acrylic resin [2].

Based on table 1, shows that there are significant differences in the average cell viability values in several groups. The average cell viability value is used to find out how many cells are still alive. Cells that are still alive can be seen from the change from yellow tetrazolium salt to purple formazan. The tetrazolium salt will be reduced by the dehydrogenase enzyme in the mitochondria of actively metabolizing cells to become formazan. The more intense the purple color produced, the higher the number of living cells [8].

The acrylic resin group that had been soaked in 50% concentration of edamame extract showed a cell viability percentage above 70%, namely 83.638% so that 50% concentration of edamame extract was effective for use as a denture cleanser because it is not toxic to the oral cavity. Edamame extract in 50% concentration as a denture cleanser does not cause toxic effects because it contains isoflavone and flavonoid activity as antioxidants which function to capture free radicals that have the potential to cause damage to cells so that fibroblast cells remain alive [3]. Flavonoids can also activate Ca^{2+} in mitochondria so that cells can produce ATP and survive [13]. The phytosterols and vitamins in edamame also function as antioxidants which can donate electrons to free radicals, thereby preventing oxidative processes in cells and reducing cell damage [7].

The acrylic resin group that had been soaked in 75% and 100% concentrations of edamame extract as a denture cleanser was proven to be toxic because the percentage of cell viability was less than 70% with cell viability of 68.722% and 62.115% respectively. Flavonoids in edamame extract at high concentrations will act as prooxidants which can trigger the formation of free radicals and reactive oxygen species (ROS) resulting in toxic effects on cells [3]. Flavonoids, which are polyphenolic compounds, have acidic hydroxyl groups. Flavonoid concentrations that are too high will cause the environment to become increasingly acidic, which can interfere with the ability of antioxidants to neutralize free radicals [4]. Another ingredient contained in edamame extract is saponin which has amphipathic molecules (having

hydrophilic and hydrophobic parts) that can disrupt the permeability of cell membranes, causing fibroblast cells to lyse or rupture and die [17].

The acrylic resin group that has been soaked in 0.5% sodium hypochlorite is not effective for use as a denture cleanser and is toxic because it has a cell viability percentage of 57.583%, which means it is below the toxicity limit value of 70%. Sodium hypochlorite is toxic because it has a high pH of 12-13 and is caustic, causing functional disorders in cells. Sodium hypochlorite contains hypochlorous acid which, when in contact with tissue, releases chlorine. Chlorine will interfere with cell metabolism by inhibiting the work of enzymes and damaging DNA synthesis so that cells become lysed or die [10].

5. Conclusion

A 50% concentration of edamame extract is effective as a heat-cured acrylic resin denture cleanser without causing toxic effects on BHK-21 fibroblast cells within 1 year of use. Edamame extract concentrations of 75%, 100%, and 0.5% sodium hypochlorite are not effective as heat-cured acrylic resin denture cleansers because they cause toxic effects on BHK-21 fibroblast cells within 1 year of use.

Compliance with ethical standards

Acknowledgements

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Disclosure of Conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

The animal study protocol was approved by the Ethics Committee of Faculty of Dentistry, University of Jember, No.2446/UN25.8/KEPK/DL/2024.

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