ORIGINAL ARTICLE

Toxicity test of mangosteen peel extract (*Garcinia mangostana L.)* as denture cleanser of heat-cured acrylic resin: in vitro experimental laboratory

Bertha Bening Tertya[1]
Dewi Kristiana[2]
Amiyatun Naini[2]

[1]Undergraduate study program, Faculty of Dentistry, University of Jember, Jember, Indonesia
[2]Department of Prosthodontics, Faculty of Dentistry, University of Jember, Jember, Indonesia

* Correspondence:
dewi_kristiana.fkg@unej.ac.id

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ABSTRACT

Introduction: Mangosteen peel extract can be used as a denture cleaning agent to effectively inhibit the growth of *C. albicans* because mangosteen peel contains various compounds such as mangostin, tannins, flavonoids, saponins. Denture cleaning materials that will be used in the dental field must meet biocompatibility requirements, one of which is a toxicity test. The aim of this study was to analyze the toxicity of mangosteen peel extract as a cleaner of heat cured acrylic resin denture on BHK-21 as cell culture of fibroblast cells. **Methods**: Toxicity test used the MTT assay method. Acrylic resin samples had a diameter of 3 mm and a thickness of 2 mm. The acrylic resin was soaked for 4 days, equivalent to soaking 15 minutes per day for 1 year in mangosteen peel extract with concentrations of 50, 60, 70%, and sodium hypochlorite. Analyzed data using normality and homogeneity test results showed ($p<0.05$). Kruskal Wallis test results ($0.000<0.05$) and Mann Whitney ($0.000<0.05$). **Results**: The greatest cell viability value was shown in mangosteen peel extract with a concentration of 50%, namely 97.16939%. Based on research data, it can be said that the lower the concentration of mangosteen peel extract, the lower the level of toxicity of a denture cleanser agent. Mangosteen peel extract in concentrations of 60, 70%, and sodium hypochlorite are toxic when used as a denture cleanser. **Conclusion**: Mangosteen peel extract with a concentration of 50% is not toxic to BHK-21 fibroblast cells as cell culture of and as a cleaner of heat cured acrylic resin denture on.

KEYWORDS

heat-cured acrylic resin, mangosteen peel extract, MTT assay.

INTRODUCTION

The use of complete dentures seems to be increasing on the elderly population of the world, especially at the age of over 60 years.[1] The difficulty experienced in the process of cleaning dentures on the elderly is due to a lack of motor skills.[2] Wearing clean dentures is a preventive measure to keep the oral cavity healthy.[3]
Cleaning dentures can be done by using various methods, such as brushing and soaking.[2] Until recently, acrylic resin is the basic material that is often used for removable dentures because it has many advantages such as good aesthetics, non-toxic, relatively cheap price, and easy to repair.[4,5] Acrylic resin available in powder (polymer) and liquid (monomer), and can be differentiated based on its activation method into heat cured and self cured acrylic resin. Heat cured acrylic resin is more often used due to its lower toxicity compared to self cured acrylic resin with lesser residual monomer after the polymerization process.[6]

Heat cured acrylic resin is a mixture of polymethyl methacrylate as a polymer and methyl methacrylate as a monomer, which is polymerized by heating. Heat cured acrylic resin contains ester groups so it has hydrophilic properties. This

property causes acrylic resin to easily absorb water and the remaining acrylic resin monomers can later diffuse into the water.[7] The use of denture cleaning agents, both natural and chemical, can cause toxicity to the tissue in the oral cavity due to continuous use.[8]

Denture cleaning materials derived from plants have been widely used, such as mangosteen peel extract (*Garcinia mangostana L.*). In this study, mangosteen peel extract was used because it had been proven to be effective as an acrylic resin denture cleaner against C. albicans. Mangosteen peel extract effervescent tablets were soaked for 15 minutes at a concentration of 50% that was most effective in inhibiting the growth of S. mutans.[9] Mangosteen peel extract with a concentration of 60% was effective in inhibiting C. albicans.[10] Mangosteen peel contains various compounds such as mangosteen, tannin, flavonoids, saponins, xanthones, garcione, chrysanthemin, terpenes, gartanin, vitamins B1, B2, phenol, anthocyanin.[11,12] Mangosteen peel extract as a denture cleanser is non-toxic so it does not cause irritation and allergies.[13]

The requirements of the materials to be used in the field of dentistry must be biocompatible that does not harm to the oral cavity tissue. Mouthwash is currently being reported to affect the tissue and prostheses if used for a long time.[14] A new material must pass the biocompatibility test, such as toxicity test. One kind of method used in toxicity test is the MTT assay. In the MTT assay method, the ability of living cells based on mitochondrial activity from cell cultures is measured.[9] Its mechanism is to look at the absorbance value from calculating the number of violet formazan crystals stored in the cell cytoplasm, which is produced by mitochondria activity; if the intensity of the violet color is greater, the number of living cells will increase.[15] The cell culture that will be used, namely BHK-21 type of fibroblast cells, is the most widely used by researchers to find out the toxicity of materials in the field of dentistry.[16] Fibroblasts play a major role in the repair process, cell self and alveolar bone remodeling, as well as tissue damage.[17]

Based on the description above, the aim of this study was to examine the toxicity effect of mangosteen peel extract as a denture cleanser on heat cured acrylic resin denture. In this study, immersion was carried out for 4 days, which is equivalent to immersion in a cleaning solution for 15 minutes per day for 1 year.[18] The aim of this study was to analyze the toxicity of mangosteen peel extract as a cleaner of heat cured acrylic resin denture on BHK-21 as cell culture of fibroblast cells.

METHODS

The type of research was an in vitro laboratory experiment with a research design, namely post-test with a control group design. The number of acrylic resin samples were 32 which were divided into 4 groups; each treatment group contained 8 samples. Positive control group and the treatment group. The treatment groups were using mangosteen peel extract (MPE) as a soaking material with different concentrations: 50, 60, 70%, and sodium hypochlorite, and the samples were then soaked for 4 days.

The procedure to make mangosteen peel extract was to separate the mangosteen fruit from the peel. The mangosteen peel was to be cut and mashed by pounding, then dissolved in a ratio of 100 grams of mangosteen peel. Heat cured acrylic resin samples used was the ADM (Acriylic Dental Materials, England) brand and are in disc form; with a diameter of 3 mm and 2 mm thickness. Firstly, the acrylic resin samples were soaked in mangosteen peel extract with concentrations of 50%, 60%, 70%, and then soaked in 1% sodium hypochlorite for 4 days. The soaking process was carried out by replacing the denture cleaning agent with a new one every day and at the end of the soaking, the samples were to rinsed with PBS three times.

The viability test used the MTT Assay which consisted of 4 treatment groups at concentrations of 50%, 60%, 70%, and sodium hypochlorite. First, BHK-21

fibroblast cells derived from baby hamster kidney were seeded in a 96 well microtiter and the cell culture was incubated for 24 hours. The samples were put on the well and incubated for 24 hours.[19] After 24 hours, the samples and culture media were taken and then rinsed with PBS. After that, the wells were added with 20 µl of MTT reagent and incubated for 4 hours; then added more with 100 µl of DMSO and incubated for 4 hours also. The 96-well microtiter was characterized using an Elisa reader.[20] The absorbance value formed was read using a BioTek ELISA reader with a wavelength of 550 nm and cell viability was calculated using the formula:[21]

In this study, the research field was the contact between denture cleaning agents, i.e. mangosteen peel extract (MPE) in concentrations of 50, 60, 70%, and sodium hypochlorite with acrylic resin through its soaking. To fix the toxicity of the denture cleanser, we used BHK-21 fibroblast cells on its viability using the MTT assay method. The color difference could be seen in each well, ranging from dark purple into faded. More purple, indicating that the cells had a high viability value or a high number of live cells, while for faded color, it meant a lower viability value or a low number of live cells.

The results of cell viability and toxicity will then be classified into: not cytotoxic: > 90% viable cells, mild cytotoxic: 60–90% viable cells, moderate cytotoxic: 30–59% viable cells, severe cytotoxic: < 30% viable cells.[22]

RESULTS

Table 1 shows the average uptake value after entering cell viability. The cell viability value was used to find out which solution was toxic on BHK-21 cells.

Table 1. Average absorbance value after entering the cell viability formula

Treatment	Average absorbance value	Sd (±)	Cell viability value (%)
Media control	0.054	0.002	0
Cell control	0.337	0.113	100
Acrylic resin	0.319	0.088	93.499
Sodium hypochlorite	0.057	0.006	0.752
Mangosteen peel extract 70%	0.164	0.030	38.788
Mangosteen peel extract 60%	0.176	0.033	42.857
Mangosteen peel extract 50%	0.299	0.053	86.378
Acrylic resin + sodium hypochlorite	0.093	0.053	13.622
Acrylic resin + Mangosteen peel extract 70%	0.102	0.041	16.984
Acrylic resin + Mangosteen peel extract 60%	0.108	0.025	19.018
Acrylic resin + Mangosteen peel extract 50%	0.329	0.037	97.169

Table 1 shows the results of cell viability values of acrylic resin discs that had been soaked in mangosteen peel extract at a concentration of 50%, 60%, 70%, and sodium hypochlorite and MTT assay results that were carried out on BHK-21 fibroblast cells. The highest value of cell viability was found in acrylic resin which was soaked in mangosteen peel extract with a concentration of 50% (97.169%) and it was classified as non-toxic. Meanwhile, the lowest cell viability value was for acrylic resin soaked in sodium hypochlorite (13.622%) and it was classified as severely toxic. The following is a bar diagram of the absorbance values which had been converted into the viability value formula

The results of the normality test showed that the data were not normally distributed. The results of statistical analysis using the Kruskal Wallis test showed a significance value of 0.000 < 0.05, or it could be concluded that there were differences in cell viability values between each group. Differences between groups could be determined using the Mann Whitney test. This could be proven by the significance value for each group being less than ($p<0.05$), which showed that several treatment groups had significant differences.

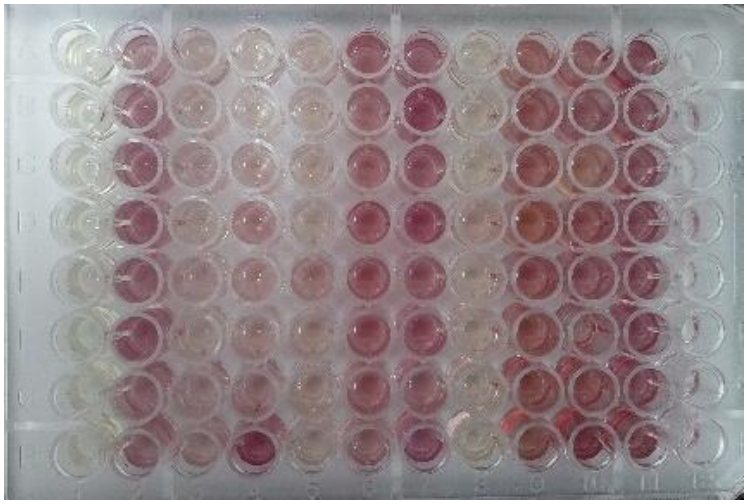
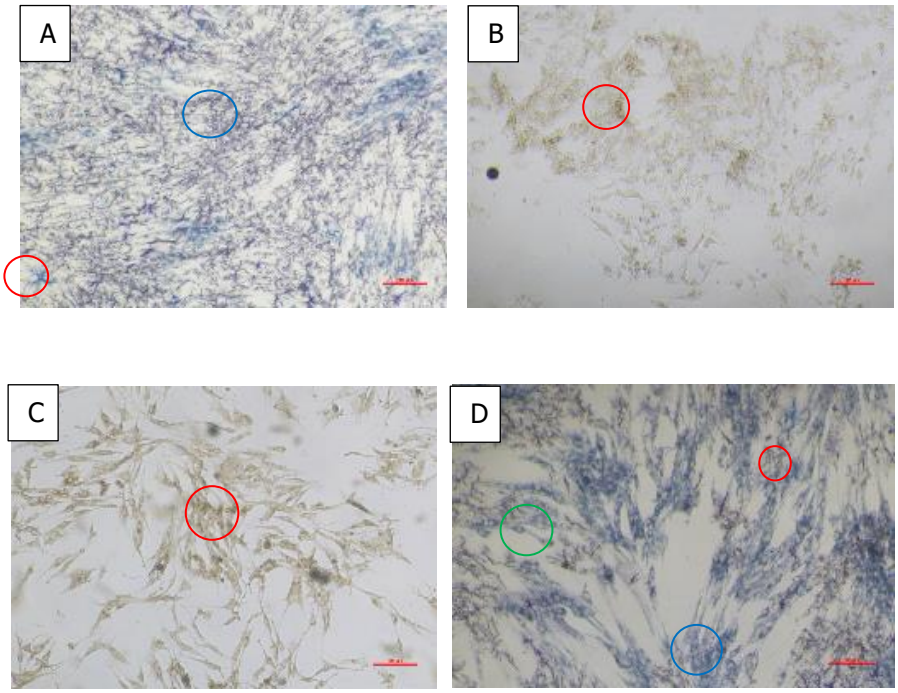


Figure 1. Staining results from toxicity tests using the MTT assay method.

The MTT test could show cell viability in reducing purple salt and dissolving it into formazan products which were then measured using a spectrophotometer with a wavelength of 490 nm. The absorbance value would be directly proportional to the number of living cells. It would decrease as the concentration of the material increased.[23] The color change on the result could be caused by tetrazolium succinate reductase which was part of the respiratory chain in the mitochondria of living cells, which was originally yellow to purple formazan. The result of the intensity of the color formed was directly proportional to the number of living cells. The more purple, many cells were living; on the contrary, the more faded, many cells had died.



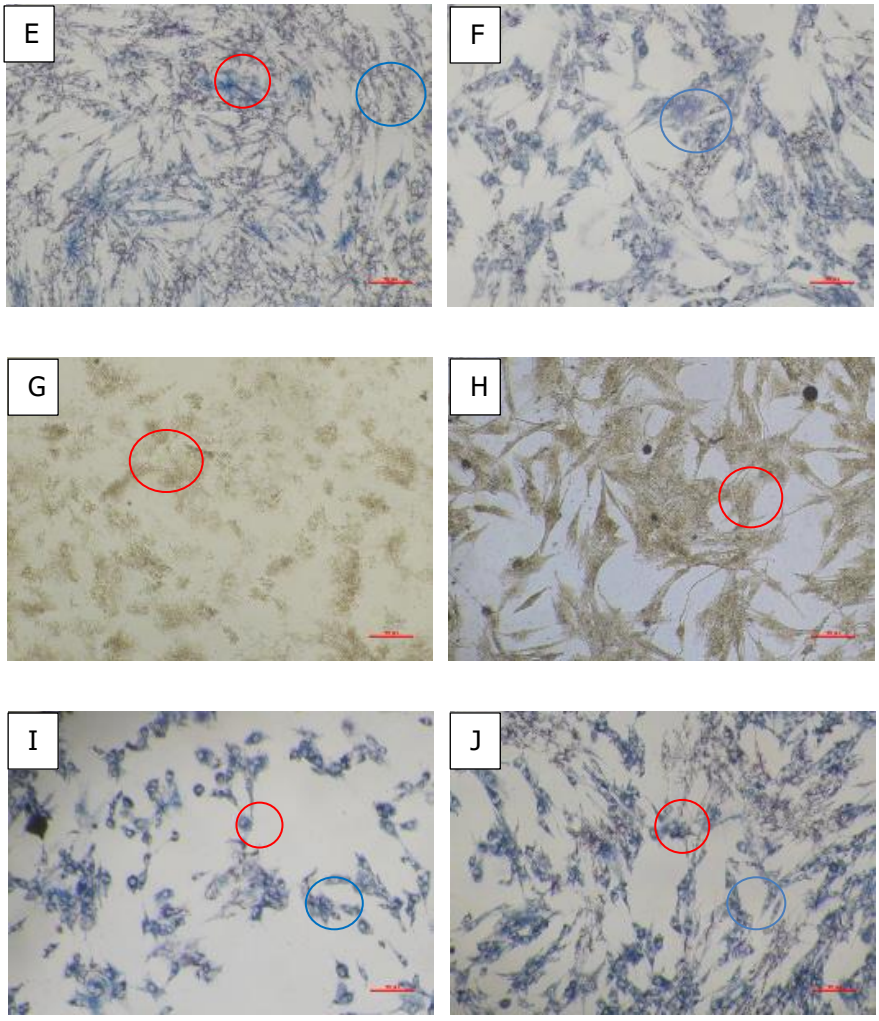


Figure 2. A. BHK-21 fibroblast cells in the control group of cells: kristal formazan), **B.** BHK-21 fibroblast cells In Acrylic resin + sodium hypochlorite (red : Fibroblast cells are dead), **C.** BHK-21 fibroblast cells In acrylic resin + 70% concentration (red : Fibroblast cells are dead), **D**. BHK-21 fibroblast cells In acrylic resin + 60% concentration (blue : fibroblasts cells are alive, red : kristal formazan, green:) the cytoplasm enlarges, **E.** BHK-21 fibroblast cells In acrylic resin + 50% concentration (blue : fibroblasts cells are alive, red : kristal formazan), **F.** BHK-21 fibroblast cells In acrylic resin of acrylic resin (blue: fibroblasts cells are alive), **G.** BHK-21 fibroblast cells In acrylic resin at sodium hypochlorite (red: fibroblasts cells are dead), **H.** BHK-21 fibroblast cells In acrylic resin at a concentration of 70% (red: fibroblasts cells are dead), **I.** BHK-21 fibroblast cells In acrylic resin at a concentration of 60% (blue: Fibroblasts cells are alive, red: the cytoplasm enlarges), **J.** BHK-21 fibroblast cells In acrylic resin at a concentration of 50%. (blue: Fibroblasts cells are alive, red: the cytoplasm enlarges)

DISCUSSION

Table 1 shows the results of cell viability values of acrylic resin discs that had been soaked in mangosteen peel extract at a concentration of 50%, 60%, 70%, and sodium hypochlorite and MTT assay results that were carried out on BHK-21 fibroblast cells. The cell viability value of 50% concentration of MPE showed 97.16939%. This value meant it was non-toxic as denture cleansing agent because the toxicity threshold value was >70%.[24] According to Widayat et al.,[25] 50% concentration of mangosteen peel extract was able to reduce the number of *Streptococcus mutans* colonies with an average number of bacterial colonies of 0.67. Mangosteen peel extract with a concentration of 50% was non-toxic on BHK-21 fibroblast cell due to its content i.e. flavonoids that could maintain cell viability. The theory stated that the flavonoids in mangosteen peel could make the cells produce ATP (Adenosine Triphosphate) that made the BHK-21 fibroblast cells to be

able to survive, because it was able to activate Ca2+ in mitochondria.[26] Meanwhile, according to Anwar et al.,[27] Moringa leaf extract has toxic effects. The flavonoid content in Moringa leaves at certain levels has the potential to cause toxicity.

Based on research conducted by Arieputri et al.,[28] it was found that the most effective concentration and time when using acrylic resin denture cleaner against Candida albicans was mangosteen peel extract effervescent tablets with a concentration of 60% which were soaked for 15 minutes. Mangosteen peel extract with a concentration of 60% was effective in inhibiting Candida albicans so it was necessary to carry out a toxicity test before use, but in this study the results showed that 60% concentration of mangosteen peel extract was toxic with a cell viability value of 19.018%. This concentration was said to be toxic because the cell viability value was less than the threshold of 70% and acrylic resin in mangosteen peel extract, a concentration of 70% with a cell viability value of 16.984%. This concentration was said to be toxic because the cell viability value was less than the threshold of 70%. When observing BHK-21 fibroblast cells after treatment with acrylic resin soaked in mangosteen peel extract at concentrations of 60% and 70%, they showed a faint yellow and purple color, which meant there was a lot of cell death or a decrease in cell proliferation. Cell death can occur because the contents of mangosteen peel extract, namely flavonoids, tannins, and saponins, at high concentrations can be toxic to cells.[11]

In this study, we also had the control group. In this group, the acrylic resin was only immersed in distilled water for 24 hours. The purpose of this treatment was to improve the toxicity value coming from the denture cleaning agent and not from the acrylic resin. Based on research by Saravi et al.,[28] it was stated that rinsing the acrylic resin is important in removing toxic chemicals, so it is recommended to soak the denture in water before used for at least one day. It also can reduce tissue hypersensitivity reactions to denture acrylic resin.

In the recent study, it was found that the viability value of acrylic resin soaked in denture cleaning material had a lower viability value when compared to the viability value of denture cleaning material. The lower viability value for group immersion in mangosteen peel extract with concentrations of 60, 70%, and sodium hypochlorite was influenced by the toxicity of mangosteen peel extract itself and from sodium hypochlorite also by the residual monomers from the acrylic resin. Based on the research conducted by Procópio et al.,[20] it was found that sodium hypochlorite at a concentration of 1% was toxic; similar with the result of this research that sodium hypochlorite was also toxic because the cell viability value was less than 30% (13.622%).

The toxicity mechanism of sodium hypochlorite is from hypochlorous acid as the oxidizing agent that has been produced. This agent will release chlorine if it is in contact with tissue. Later, chlorine will trigger the formation of free radicals that have the ability to kill cells by damaging the chemical structure of the enzyme so that the enzyme cannot function properly and the cell will be lysis. Sodium hypochlorite also has a high pH (alkaline) which will trigger the release of hydroxyl ions. These hydroxyl ions will cause changes in the integrity of the cytoplasmic membrane which will destroy the mitochondria in the cell, failing oxidative phosphorylation and reducing ATP in the cell. ATP decrease can cause cell death. Meanwhile, residual monomers from acrylic resin can cause cytotoxicity of fibroblast cells in the tissue they touch, namely mucosal tissue.[28]

Heat-cured acrylic resin is a mixture of polymethyl methacrylate and methyl methacrylate as polymer and monomers. The acrylic resin polymerization process never completed and produces residual monomer. High levels of residual monomer can cause irritation or allergies to oral tissue, so it is necessary to reduce the amount of residual monomer by immersing heat-cured acrylic resin in distilled water. The residual monomer of acrylic resin will remain even if it has been soaked in distilled water for 24 hours before treatment. Residual acrylic resin monomer can still be detected in denture bases used for up to 17 years and most of the residual monomer is released within the first 5 years.[7]

The limitation of this research is that the additional mangosteen peel extract (*Garcinia mangostana L.*) material has not been formulated into effervescent tablets or paste for use as a denture cleanser. Therefore, further research can be conducted.

CONCLUSION

50% concentration of mangosteen peel extract (MPE) that has been soaked for 4 days (equivalent to 15 minutes per day for 1 year), is the best result due to its non toxic properties as an acrylic resin denture cleanser on BHK-21 fibroblast as as cell culture of and as a cleaner of heat cured acrylic resin denture on. Other concentrations (60% and 70%), and sodium hypochlorite are toxic against BHK-21 fibroblast cells. Implication of research is that 50% concentration of mangosteen peel extract (MPE) is safe as an acrylic denture cleaner with a soaking time of 15 minutes per day.

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