Volume 07, Issue 03 (May-June 2024), PP 44-50 **www.ijmsdr.org**
ISSN: 2581-902X

International Journal of Medical Science and Dental Research

TOXICITY TESTING OF CINNAMON BARK (*Cinnamomi burmannii Cortex*) ESSENTIAL OIL AS A MOUTHWASH ON FIBROBLAST CELL CULTURE

Vitra Nuraini Helmi[1], Ridhofar Akbar Khusnul Abdillah[1]Ira Widjiastuti[2], AnisIrmawati[3], Budiastuti[4], Mustofa Helmi Effendi[5*]

[1]*Postgraduate program, Faculty of Dentistry, Airlangga University, Surabaya*
[2]*Department of Conservative Dentistry,Faculty of Dental Medicine, Universitas Airlangga*
[3]*Department of Oral biology,Faculty of Dental Medicine, Universitas Airlangga*
[4]*Sudy Program of Pharmacy Science, Faculty of Health Science, Universitas Muhammadiyah Surabaya*
[5]*Department of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Airlangga*

ABSTRACT: *Essential oils are the active ingredient found in cinnamon which has the main ingredient of cinnamaldehyde. Previous research has revealed that cinnamon essential oils with a certain concentration have antibacterial and antifungal effects. In addition, cinnamon essential oil has been shown to have benefits as an anti-inflammatory and analgesic. With its variety of benefits, cinnamon essential oil has the potential as a mouthwash ingredient. However, to be used as a mouthwash ingredient, it needs to go through a toxicity test first. Purpose: To determine the toxicity concentration of Essential oils of Cinnamomiburmannii Cortex on fibroblast cells. Methods: This research was an experimental laboratory research using Post Test Only Control Group design. The research treatment used the administration of essential oils of Cinnamomiburmannii Cortex with 0.5%, 0.25%, 0.125%, 0.0625%, and 0.0312% concentration on fibroblast cells with 6 replications. Results: The fibroblast cell life percentage in 0.5%, 0.25%, 0.125%, 0.0625%, and 0.0312% concentrations respectively were 51%, 57%, 61% 69%, and 80%. The toxicity results were obtained using the MTT assay technique after 24 hours. The optical density absorbency values were read by an ELISA reader and represent life cell viability. Conclusion: Essential oils of Cinnamomiburmannii Cortex have no toxicity for fibroblast cells to a concentration of 0.5%.*

Keywords**:** *Cytotoxicity, Cinnamomi burmannii Cortex, fibroblast cells, Mouthwash*

I. INTRODUCTION

Teeth are one of the most important human organs which function for chewing, speaking, and supporting appearance. Every individual should ideally maintain healthy teeth throughout life. However, there are various reasons why teeth can fall out or require extraction (Syamsudin, 2007). Tooth loss can be caused by, among other things, caries, periodontal disease, or trauma (Esan, 2004). Data from Basic Health Research

(RISKESDAS) 2007 shows that the majority of people aged 55-65 years and over (23.5%) have experienced tooth loss, and 7.1% have had their lost teeth replaced (MOH RI, 2008).

Acrylic resin is still an option for making removable denture bases. Because it has several advantages, namely easy to repair, stable color, and easy to polish (Anusavice, 2003), however, acrylic resin also has disadvantages, namely it has high porosity and surface roughness, making it easier for plaque to appear due to the texture being difficult to clean which allows residues to form. food waste and microorganisms trapped in it (Daniluk, et al., 2006)

In the oral cavity, the surface of acrylic resin is the same as other surfaces of the oral cavity, namely, it is covered by saliva with a high protein content, so that a pellicle forms on the surface of the acrylic resin. Pellicles can attract microorganisms to adhere, one of which is Candida albicans. Candida Albicans is an opportunistic pathogenic flora which is the etiology of Denture stomatitis. Denture stomatitis is inflammation of the oral mucosa caused by wearing dentures without proper hygiene. It has typical signs in the form of erythema, and edema and is redder than the surrounding tissue (Lingkan, et al., 2015).

Denture stomatitis can be prevented by maintaining and cleaning dentures. One way to do this is by using mouthwash. The use of mouthwash has been proven to inhibit the formation of dental plaque quickly and easily (Inna, Atmania&Prismasari, 2010).

Indonesia is an area rich in natural ingredients. The use of plants as traditional medicine has long been carried out by Indonesian people to overcome various health problems.

One plant that can be used in traditional medicine and has antibacterial and antifungal activity is the cinnamon plant. The cinnamon plant (Cinnamomum burmannii) is a natural ingredient that has only been known as a spice in cooking, but it turns out it has medicinal properties. Based on research conducted by Fakhriyana in 2013, soaking acrylic plates with cinnamon essential oil at a concentration of 0.03% can inhibit the growth of Candida albicans. It can be concluded that the content contained in cinnamon has been proven to be an antifungal agent.

Before it can be used as a mouthwash, cinnamon bark essential oil needs to be tested for safety levels. A toxicity test is a test to detect the toxic effects of a substance on a biological system and to obtain typical dose-response data from the test preparation. Cell culture methods are often used to test the biological effects at the initial level of a material used to determine its toxicity effects (Anussavice, 2003 cit. Yuliati, 2005). Fibroblast cells are the main cells of connective tissue located in the lamina propria of the oral mucosa.

One method for assessing the cytotoxicity of a substance is to use the 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide reagent (MTT assay). MTT assay is one of the methods used in this research which has a test basis in the form of the amount of formazan crystal formation which has a positive correlation with the number of cells and their activity, and the colorimetric absorbance value indicates the number of cells that are still alive and their metabolic activity. (Li, Zhou, Xu, 2015) The parameter commonly used to determine cytotoxicity is Cytotoxic Dilution 50% (CD50). CD50 is a standard for a material to be considered toxic if the percentage of living cells after treatment is less than 50% (Telli, 1999).

Based on the background of the problem above, this research aims to determine the toxicity effect of cinnamon bark essential oil at various concentrations on human gingival fibroblast cells. Apart from that, this research was also carried out to determine the potential toxicity of cinnamon bark essential oil on human gingival fibroblast cells at concentrations of 0.5%, 0.25%, 0.125%, 0.0625%, and 0.0312%.

II. METHOD

Design, Place, and Time

This research is a type of laboratory experimental research with the research design *The Post Test Only Control Group Design.* Treatment by administering cinnamon bark essential oil to human gingival fibroblast cells. This research was conducted at the Stem Cell Research and Development Center, Airlangga University. With the research sample calculated using the Federer formula, it was found that the minimum sample size was 4 samples per test group. In the research carried out, 6 samples were used per test group so there was no need to add more samples.

The independent variables in this study were concentrations of 0.5%, 0.25%, 0.125%, 0.0625%, and 0.0312% of cinnamon bark essential oil. The dependent variable is the number of living fibroblast cells. And the controlled variables are media, tools, materials, and the way the work is done.

III. Research Stages

Preparation of Cinnamon Bark Essential Oil

A total of 5 kg of cinnamon bark was steam and water distilled with 5000 mL of water for 4 hours to isolate the essential oil. The simplicia is placed on a perforated steamer with water underneath. The boiler is assembled with steam cooling pipes and sealed to prevent leaks. The water pump is turned on and ice cubes are added to the reservoir. The distillate in the form of essential oils and water was collected in an Erlenmeyer glass covered with aluminum foil. The distillate is then put into a separator flask to separate the water phase and the oil phase. The water phase which still contains oil is added with 15 grams of NaCl per liter. The oil phase is separated and collected, then the water phase is added with hexane to draw out the dispersed essential oil. The essential oil solution in hexane was evaporated using a rotary evaporator, and the essential oil obtained was added with dried Na2SO4 to remove water droplets.

Preparation of Human Gingival Fibroblast Cells

The gingival cell isolation stage begins with washing the sample using NaCl/Aquadest and antibiotic media for 5 minutes. The samples were chopped, treated with 5 ml trypsin enzyme, and incubated on a magnetic stirrer at 37°C for 45 minutes, then stopped with a 5 ml stopper. The supernatant was separated and centrifuged. The precipitate was washed and centrifuged again before being placed on a plate and incubated at 37°C. Cell splitting is done by washing the plate using PBS and trypsin enzyme, then incubating to release the cells. Cell preparation was carried out in BSC with gingival fibroblast cell culture in Alpha MEM medium and 20% FBS, incubated at 37°C for 24 hours.

Treatment Stage

Fibroblast cells in the microplate were incubated and observed under an inverted microscope. Cells were divided into 7 treatment groups, including controls, and given cinnamon essential oil at different concentrations. Each treatment had 6 replications, then incubated again at 37°C for 24 hours.

Observation and Reading Stage of Treatment Results

Tetrazolium salt (MTT) 5 mg/ml in PBS was added 25 µl to the plate with culture medium and incubated for 4 hours at 37°C. The media and test materials were discarded, and then 200 µl DMSO was added to each well. The plate was stirred with a Plate Shaker for 5 minutes until the formazan crystals dissolved. Formazan absorbance was read with an ELISA reader at 595 nm; deeper colors indicate more living cells.

IV. RESULTS AND DISCUSSION

Research result

Based on the results of research conducted on human gingival fibroblast cells after being given drops of cinnamon essential oil with different concentrations, readings were carried out for each treatment group and control cells using an ELISA reader. The reading results using an ELISA reader in this study are in the form of optical density, which is the level of absorbance through the color change produced by mitochondrial activity to blue/purple formazan, the more intense the color produced, the higher the absorbance value.

The optical density value describes the number of living fibroblast cells so the greater the optical density value indicates the greater the number of living or proliferating fibroblast cells. alcohols, aliphatic acids and esters, amino acids, steroids, and sugars (Bankova*et al*. 1992; Koo *et al* . 1997; Kumazawa*et al*. 2004; Nagy *et al*. 1996; Park *et al*.2004).

Based on the results of observations and readings of the absorbance values of the cinnamon essential oil toxicity test on human gingival fibroblast cells via ELISA reader which was divided into treatment groups, namely 0.5%, 0.25%, 0.125%, 0.0625%, 0.0312 %, in each treatment group 6 replications were carried out, the results obtained were as shown in Figure 1.

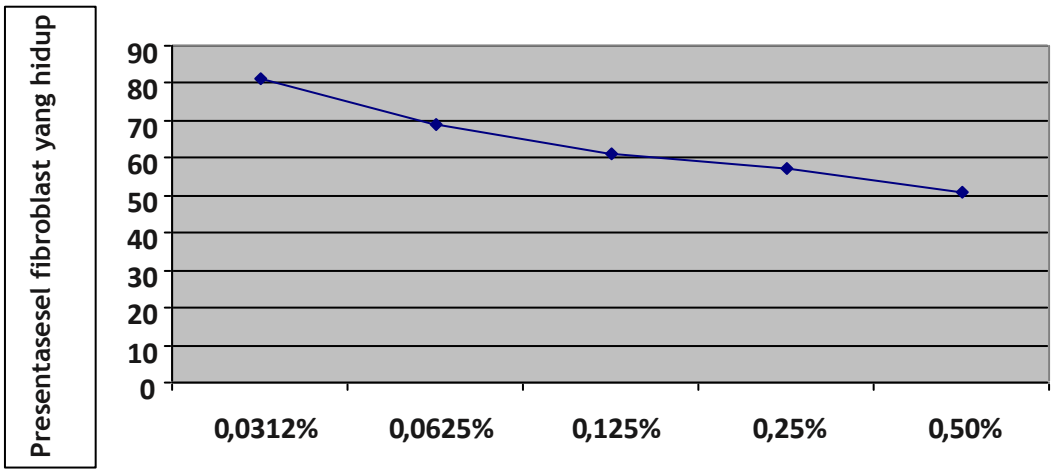

Figure 1. Essential oil concentration

Figure 1 shows the percentage of live fibroblast cells after being treated with cinnamon bark essential oil at different concentrations. The results obtained were the percentage of live fibroblast cells after being treated with cinnamon bark essential oil at a concentration of 0.5% with a live cell percentage of 51%. At a concentration of 0.25% with a live cell percentage of 57%. At a concentration of 0.125% with a live cell percentage of 61%. At a concentration of 0.0625% with a live cell percentage of 69%. At a concentration of 0.0312% with a live cell percentage of 80%.

Data analysis

The Kolmogorov-Smirnov normality test statistic was used to test the normality of the data. The data in Appendix 3 shows that the Kolmogorov-Smirnov test showed that the results of all research groups had a value greater than 0.05 (Sig > 0.05), which means the data from all research groups was normally distributed.

The Kruskal-Wallis test is used to test differences in calculated means if the sample group being tested is more than two groups that come from different populations. The p-value was obtained at 0.000, where the p-value <0.05. Based on these results, it can be said that the results of the treatment provided a significant difference because the p-value <0.05.

Data analysis continued using the Tukey multiple-comparison test to compare the differences between each concentration group and the control group. Tukey multiple-comparison test uses the null hypothesis criterion that there is no difference in the average between the two treatments and the alternative hypothesis is that there is a difference in the average between the two treatments with the criterion that H0 is rejected, if the p-value < α (= 0.05).

Based on Table 1 for optical density data, it can be seen that several treatments are not significantly different between treatments, these treatments are between concentrations of 0.25% and 0.125%. As well as 100%, 50%, 25%, 75% and 12.5% extracts, but they are significantly different from the other groups. The value written in the column is the average value, so the treatment with the highest average is cell control then cinnamon bark essential oil with a concentration of 0.0312% while the lowest is media control then cinnamon bark essential oil with a concentration of 0. 5%. From the data above, it can be concluded that the treatment that produces the highest toxicity results is cinnamon bark essential oil with a concentration of 0.5%. This also shows that all groups of cinnamon bark essential oil concentrations have the same effect, namely reducing the number of living fibroblast cells.

Table 5.2 Tukey multiple-comparison test between treatment groups

Group	N	Subset for alpha = 0.05					
		1	2	3	4	5	6
Media Control	6	.03750					
MTT 0.5%	6		.24917				
MTT 0.25%	6			.27233			
MTT 0.125%	6			.28917			
MTT 0.0625%	6				.32183		
MTT 0.0312%	6					.37083	
Cell Control	6						.44900
Sig.		1,000	1,000	.105	1,000	1,000	1,000

V. Discussion

The cinnamon plant (Cinnamomum burmannii) is an herbal plant that is only known as a spice in cooking, but it turns out it also has medicinal properties. Essential oil is the active ingredient in cinnamon which contains the main element cinnamaldehyde and several other compounds. The content of these active compounds indicates that essential oils can inhibit the growth of bacteria and fungi. Apart from that, cinnamon essential oil also has anti-inflammatory and analgesic properties.

In this research, cinnamon simplicia was obtained from the city of Padang. Then the manufacture of cinnamon bark essential oil is carried out at PT. HeptasariUnggul using the steam distillation method. Cinnamon bark essential oil was then identified in the PT laboratory. Gelora Djaja to see the contents of cinnamon bark essential oil, while the dosage of cinnamon bark essential oil was made at the Stem Cell Research Development Center, Airlangga University, Surabaya. Cinnamon essential oil has many benefits and has potential as an ingredient in the medical field, one of which is mouthwash. However, the use of drugs whether used systemically or locally must not be toxic and must not have detrimental effects on the human body (Freshney, 2000). So to develop the use of cinnamon essential oil as a mouthwash, a toxicity test must be carried out.

The toxicity test carried out in this study used the MTT Assay method. MTT assay is one of the methods used in this research which has a test basis in the form of the amount of formazan crystal formation which has a positive correlation with the number of cells and their activity, and the colorimetric absorbance value indicates the number of living cells and their metabolic activity (Li, Zhou, Xu, 2015). The parameter commonly used to determine cytotoxicity is Cytotoxic Dilution 50% (CD50). CD50 is a standard for a material to be considered toxic if the percentage of living cells after treatment is less than 50% (Telli, 1999).

The results of the toxicity test of cinnamon bark essential oil with concentrations of 0.5%, 0.25%, 0.125%, 0.0625%, and 0.0312% against fibroblast cells using the MTT Assay method respectively had a live cell percentage of 51%, 57%, 61%, 69%, 80% which can be seen in Figure 5.1. The research results obtained show that a concentration of 0.0312% produces the highest cell viability, while cinnamon bark essential oil with a

concentration of 0.5% produces the lowest cell viability. These results show conformity with the theory which states that the toxicity of a substance is directly proportional to exposure. Exposure to a material has a determining factor, namely the concentration of the material. (Rozman, Doull, Hayes, 2009).

The percentage of living fibroblast cells in all treatments with cinnamon bark essential oil showed a figure of more than 50%, which means this value is lower than the Telli benchmark regarding the toxicity parameters of a substance. Telli stated that a material can be said to be toxic if the percentage of living cells after exposure to the material is less than 50% (Telli, 1999). So, it can be concluded that cinnamon bark essential oil at this concentration is safe to use.

Previous research states that cinnamon bark essential oil at a concentration of 0.03% can kill Candida Albicans and at a concentration of 0.11% can inhibit the growth of microorganisms that stick to acrylic plates, apart from that cinnamon bark essential oil also has benefits as an anti-inflammatory and analgesic. So cinnamon bark essential oil has the potential to be used as a mouthwash for removable denture users (Fakhriyana, 2013; Damayanti, 2013).

Research shows that the main content of cinnamon bark essential oil serves as an antibacterial and antifungal cinnamaldehyde. The cinnamon bark essential oil studied in this study contained 64% cinnamaldehyde. Several previous studies stated that most of the side effects caused by using cinnamon bark essential oil were caused by cinnamaldehyde. One of the toxic mechanisms that can be caused by cinnamaldehyde is inhibiting energy metabolism in cells which will cause cells to be unable to adapt to this material (Adriani et al, 2010; Davidson, 2001). Cinnamaldehyde is the main compound in cinnamon essential oil which can cause irritation and sensitization of the skin which can cause dermatitis and can be toxic if used in large doses. Based on research conducted by Sudarmawan (2009), cinnamaldehyde does not cause toxic reactions in the oral cavity if used at concentrations below 0.25%.

Cinnamaldehyde has the strongest antifungal and antibacterial effects than other components. According to Tampieri et al. (2005), this fungistatic activity depends on the aromatic ring or the function of the aldehyde outside the aromatic ring. Cinnamaldehyde's ability to inhibit the growth of Candida albicans colonies is also caused by the free group, namely 3-phenyl, which can bind enzymes in the Candida albicans cell walls and also bind oxygen needed by Candida albicans for cell metabolism. With this bond, Cinnamaldehyde can inhibit the synthesis of enzymes in Candida albicans cell walls and inhibit the metabolic process of Candida albicans so that in the end Candida albicans will die. Apart from that, Cinnamaldehyde is also able to denature proteins and reduce surface tension so that the permeability of bacterial and fungal cells increases. Cinnamaldehyde can also inhibit glucose transport, thereby inhibiting the glycolysis process in bacterial and fungal cells.

Another compound contained in cinnamon essential oil which functions as an antibacterial agent is eugenol. Eugenol is a derivative of phenol compounds that has potential antibacterial power. The antibacterial mechanism of eugenol is related to interactions with the cell membrane, which destroys the cell membrane. However, the essential oil used in this study does not contain the eugenol component. These components are different from previous research which can be caused by several factors, namely the cinnamon used is different in the planting location, harvest season, and protocol for making the essential oil, thus affecting the content of the active compounds contained in the cinnamon bark essential oil (Cheng et al., 2009).

Apart from cinnamaldehyde, a cinnamon essential oil also contains terpenoid hydrocarbon compounds (such as α-pinene and limonene) amounting to 2.17%. These compounds can accumulate in the lipid network of bacterial cell membranes, and disrupt the structure and function of the cell membrane due to expansion (swelling) of the cell membrane and changes in the permeability of the bacterial cell membrane (Sikkema et al., 1994)

VI. CONCLUSION

Cinnamon bark essential oil has no potential toxicity to human gingival fibroblast cells at concentrations of 0.5%, 0.25%, 0.125%,0.0625%, 0.0312%

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