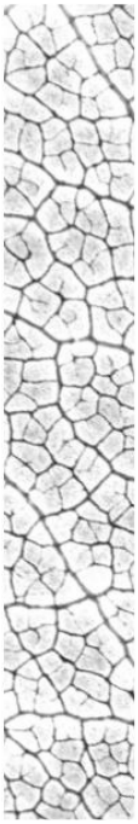
Toxicity test on Meniran extract (Phyllanthus niruri L.) as a medicated mouthwash towards fibroblast cell culture

by Michael Josef Kridanto Kamadjaja

Submission date: 19-Jun-2021 09:03AM (UTC+0800)
Submission ID: 1608826886
File name: Toxicity_test_on_Meniran_extract_Phyllanthus_niruri_L._as.pdf (246.13K)
Word count: 2521
Character count: 12911



EurAsian Journal of BioSciences
Eurasia J Biosci 14, 3791-3794 (2020)

Toxicity test on Meniran extract (*Phyllanthus niruri L.*) as a medicated mouthwash towards fibroblast cell culture

Sofi Choirum Muzamil [1], K Michael Josef [1*]

*Corresponding author: michael-j-k-k@fkg.unair.ac.id

Abstract

The main problem for denture users is denture stomatitis caused by *Candida albicans* which in a pathogenic state can release endotoxins that damage the oral mucosa. Meniran plant contains antifungal compounds that can be used as an alternative mouthwash for denture users. This study aims to determine the level of toxicity of Meniran plant extracts as mouthwash ingredients for dentures towards gingival fibroblast cell cultures. This study is an experimental research. There is a fibroblast cell control group and fibroblast cell treatment group that was added with Meniran plant extracts. All samples were incubated in the media for 24 hours. The samples were then given MTT material and incubated again for 4 hours. Cell culture on the plate was then read by ELISA reader. The results were analyzed using Kruskal-Wallis and HSD-Tukey. The results showed that there were significant differences between the cell groups and the treatment groups. The treatment results showed the percentage of living cells in the treatment group giving Meniran plants of 40%, 20%, 10%, and 5% respectively, which were 61.1%, 67.3%, 73.6%, 78.1%. Meniran plant extract is not toxic and can reduce the excess amount of *Candida albicans*.

Keywords: toxicity, Meniran plants, MTT-assay, gingival fibroblast cells

INTRODUCTION

Health Development in 2010 showed that Indonesian who received dental extraction services were 79.6%, evidenced by the high percentage of dental health service utilization for extractions which reached 79.6% (Badan Penelitian dan Pengembangan Kesehatan, 2010). Tooth loss must be replaced as a prevention towards adverse effect on oral health (Gitta Vonny 2015).

This condition causes an increase in the need for artificial teeth. The main problem for denture users is Denture stomatitis which is an inflammatory process that usually occurs in 60% of subjects who use dentures (Salerno et al. 2010). The microorganism that is commonly found in patients with denture stomatitis is *Candida albicans* (Meizarini, and dan Rianti, 2005) which in a pathogenic state can release endotoxins which damage the oral mucosa and cause it to occur. Denture stomatitis prevention can be done routinely by maintaining oral hygiene by using mouthwash. Denture stomatitis is a pathological condition of mucous tissue in the oral cavity that causes irritation trauma and is followed by ulcer and soft tissue growth at the site of the irritation (Clorinda, 2012).The use of topical medicinal ingredients is possible since they are able to penetrate and be carried by blood flow to other tissues around the oral cavity.

In Indonesia, traditional medicine is still widely used (Clorinda, 2012). Meniran plants contain antifungal compounds such as flavonoids, saponins, and tannins (Sianturi, Vonny, Suling. 2016). According to Soejono, (2006) Meniran plant (Phyllanthus niruri L.) can also be used as a urine facilitator and thrush medicine (stomatitis) (Trubus. 2000). Meniran plant extracts contain active compounds including flavonoids at 4.91%, Saponins at 3.88%, and tannins at 3.05% and Polyphenols at 4.71%. Research conducted by Melsi (2014) used Meniran extract (Phyllanthus niruri L.) with a concentration of 5%, 10%, 20%, and 40% in which each treatment can inhibit the growth of *Candida albicans* (Melsi, et al. 2013). Cell culture methods are often used to test biological effects at the initial level of a material that will be used in dentistry to determine the toxicity and effects (Anussavice Phillips, 2013). Fibroblast cells are easy to culture because they have the ability to grow and adhere to a high and fast regeneration (Freshney, 2005; Igbokwe et al, 2016).

Based on the above background, to be developed into a basic ingredient for mouthwash, toixity of the material needs to be tested against the cells in vitro by

Received: February 2020
Accepted: April 2020
Printed: September 2020

the MTT assay method. This study aims to determine the level of toxicity of Meniran plant extracts as mouthwash ingredients for dentures towards gingival fibroblast cell cultures.

MATERIAL AND METHODS

This type of research is an experimental laboratory with The Post Test Only Control Group Design. Treatment was done with Meniran plant extracts (Pyllantus niruri L.) towards gingival fibroblast cells in the laboratory of tropical disease of Universitas Airlangga.

The process of Meniran plant extracting was started by washing and cleansing the plant. It was then cut into pieces, and put in an oven at 50 ° C for ± 24 hours. After the Meniran plant was dried, it was blended to powder and sifted. Then, 400 grams of Meniran plant powder was used for the maceration process. The Meniran plant powder that has been dried was blended with ethanol 96% added as much as 400 ml until submerged (soaked solvents at least 2 times the weight or more). A solvent of about 2.5 L. was added to the closed jar and left for 24 hours. The following step was to shake the extract with a speed of 50 rpm. The liquid extract was filtered with a cloth and contained in an Erlenmeyer. The pulp was put in a jar and was added with a solvent again until it is submerged. 2 L of solvent was used and left overnight, and was put in a shaker with a speed of 50 rpm. The results of the first and second liquid extracts were put together and evaporated using a rotary evaporator. This evaporation step took 3 hours. Evaporation results were soaked in a water bath for 2 hours. At the end of this process, pure extracts were obtained.

Stages of cell isolation include gingival samples that have been taken and immediately washed using NaCl/Aquadest, then washed again using media that also has antibiotics. The washing was done for for 5 minutes. The sample was chopped and mashed using surgical scissors before being given enzymetripsin. The sample was put into an Erlenmeyer tube which already contained magnetic bar and trypsin enzyme as much as 5 ml using a pipettefiller. The tube was placed on magnetic stirrer with heater of 37 degrees Celsius for 45 minutes. After 45 minutes, 5 ml of stopper was given (with a stopper ratio: trypsin enzyme 1:1) to stop the enzimtripsin. Then, the tube was put back on the magnetic stirrer with heater for 15 minutes. The supernatant contained in the Erlenmeyer tube was poured inside a conical tube first, then a precipitate containing a lot of tissue was poured in the conical tube. The two conical tubes were inserted into the centrifuge for 5-6 minutes at 1800 rpm until the cells settle. Then the media was discarded and deposited. On each conical tube 5 ml of media was given to wash the remaining enzymes, and was then centrifuged for 5-6 minutes at 1800 rpm. Then the media was discarded and the sediment was taken. Both conical tube was given as much as 5 ml of media. The media and cell mixture were taken using a pipette filler and put in a plate. The plate was placed in an incubator with a temperature of 37 degrees Celsius until the cells grow. It was washed every 3-4 days and replanted so that the cells get adequate nutrition.

Next, the sample on the microplate which contained incubated fibroblasts cells were observed under a light microscope to see whether the fibroblasts planted in each well were enough for the treatment to be done. Each treatment has a minimum of 6 samples which were then planted in 6 wells. The treatment was carried out by giving Meniran plant extract drops to every 6 samples with concentrations of 40%, 20%, 10%, and 5%. Meanwhile, 6 samples for cell control were not dripped. The samples were then incubated for 24 hours in an incubator of 37 ℃, then transferred from the incubator.

Tetrazolium (MTT) salt was dissolved in Phosphate-Buffered Saline (PBS) of 5 mg/mL. MTT was added directly to the plate containing 10 µl of culture medium, then re-incubated for approximately 4 hours at 37° C. All media in the wells and test material were taken. Then, each well was added with DMSO (Dimethylsufoxide) as much as 50 µl. The plate was mechanically stirred with plate shaker until the formazan crystals dissolve, for 5 minutes. The living fibroblast cells would be colored with formazan and become blue, while the dead did not turn blue. Furthermore, the formazan absorbance was read spectrophotometrically by ELISA reader at a wavelength of 620 nm. The more concentrated the color, the higher the absorption value and the more the number of cells in the extract.

Analysis of the data used in this study is by using Kruskal-Wallis and HSD-Tukey to determine differences in the level of toxicity of Meniran plant extracts as a mouthwash in the use of dentures on gingival fibroblast cell cultures.

RESULTS

The analysis showed that all groups except the control media and cell control treatments had probability values of more than 0.01. This can be seen that the treatment group has a normally distributed sample. A value of 0.01 indicates that with a 99% confidence level, all groups except the control media and cell control treatments have normally distributed samples. Homogeneity test results use Levene's test shows the value of $p = 0.001$ which means the data were not homogeneous because $p < 0.01$.

Kruskal-Wallis Statistical test results show the value of $p = 0.000$ ($p < 0.01$) which means that there is a difference in the mean of the overall formazan optical density value from each Meniran plant extract (*Phyllantus niruri*) concentration and cell control groups. To find out the difference between the sample groups,

Table 1. The results of the analysis of the mean differences and standard deviations of each treatment

Treatment Group	N	Mean	% of Live Cell
40%	6	0.303	61.143%
20%	6	0.330	67.395%
10%	6	(0.368)	73,648%
5%	6	0.377	78,097%
Cell Control	6	0.478	100.00
Media Control	6	0.038	14.72%

the HSD Tukey was performed with á = 0.01.Test results obtained from the HSD- Tukey showed that there was a significant difference between the cell control group and the entire Meniran extract concentration group (*Phyllantus niruri*.). This shows that the treatment groups with concentrations of 40%, 20%, 10%, and 5% have the same effect that can reduce the number of fibroblasts after exposure to Meniran extracts that contain active ingredients (**Table 1**). All concentrations can reduce the number of fibroblast cells to a certain extent depending on the concentration of the active ingredient in the extract. From these results the LD parameter used was $_{50}$ to assess the toxicity of Meniran extract.

DISCUSSION

There was a tendency for an increase in the average density of fibroblast cells which reflected the number of living cells in the lower extract concentration group. The higher concentration of Meniran plant extracts (Phyllantusniruri), the lower the average density of fibroblast cells

Results of statistical data analysis with Kruskal-Wallis revealed a meaningful difference in the mean of all optical density values of formazan in each group of Phylalantus niruri L. plant extracts and cell control group. Based on these results it can be interpreted that the Phylalantus niruri L. extract with concentrations of 5%, 10%, 20% and 40% can reduce the percentage of living cell life to a certain extent. Based on the results of data analysis, it means that the entire Meniran plant extract group (*Phyllantus niruri* L) with concentrations of 5%, 10%, 20% and 40% have the same effect on cell control, which is to reduce the number of fibroblast cells. Statistical differences are considered to give the same output standard to determine whether the nature of the material used is toxic or not with LD 50 parameters. The LD 50 parameter was obtained through a statistical process and functions to measure the relative number of toxicities of chemicals used in dentistry.

The intended use of the LD $_{50}$parameter to detect the toxicity of a substance reveals the danger of an ingredient after administration of active compounds in Meniran plants extract and measure the biocompatibility of a substance to be tested, if the percentage of cells exposed to the material is still above 50%.

After conducting research using the MTT Assay method, *Phyllanthus niruri* plant extracts with concentrations of 5%, 10%, and 20%, and 40% of fibroblast cells reveal the percentage of living cells of 78,097%. 73,648%, 67,395% and 61,143% of the results obtained for all concentrations of plant extracts have a percentage of living cells that are still above the LD50 standard parameters. From these results it means that (*Phyllantus niruri*) extract is not toxic if the concentration is at 5% - 40%. Hence, it can be used as a mouthwash in dentistry. Based on the results of phytochemical content tests conducted at the Surabaya research and consulting industry center, Meniran plant extracts contain active compounds including flavonoids at 4.91%, Saponins at 3.88%, and tannins at 3.05% and Polyphenols at 4.71%.

Flavonoids are hydroxylated phenolic substances and are synthesized by plants in response to microbial infections (Kumar, et al. 2013). Functional compounds such as phenolic compounds can denature proteins, which are able to damage the structure of tertiary-protein and causes protein to lose its original properties. The denatured protein of the *Candida albicans* will cause fragility in the cell wall so that it is easily penetrated by other active substances that are fungistatic. If the denatured protein is an enzyme protein, the enzyme cannot work. This causes the metabolism and nutrient absorption process to be (Pringgenies et al., 2013). Protein-phenols coagulate with cellular proteins and also cause cytoplasmic membranes to undergo lysis. (Ariyanti, Ida Sang, 2012).

Saponins contribute as an antifungal by reducing the surface tension of the sterol membrane from the *Candida albicans* cell wall, so that the permeability increases. Increased permeability results in more concentrated intracellular fluid being pulled out of the cell so that nutrients, metabolic substances, enzymes, proteins in the cell exit and fungi die. Saponins are a class of compounds that can inhibit or kill microbes by interacting with the sterol membrane. The main effect of saponins on microbes is the release of proteins and enzymes from the cell. (Hardiningtyas, 2009).

Tannin is also thought to have effectiveness in inhibiting growth or killing *Candida albicans*. Tannins are shrinking and precipitating proteins from solution by forming insoluble compounds. In addition, tannins play a role in the body's defense system and have antioxidant and antiseptic activity (Sulistyawati, & Mulyati, 2009). Tannins have complex biological roles such as protein settlers. Tannins can also function as biological antioxidants (Malangngia, et al. 2012).

CONCLUSION

Meniran plant extract (*Phyllantus niruri*) with a concentration of 5% - 40% is non-toxic as a mouthwash for denture wearers, which works against gingival fibroblast cell cultures as measured using LD_{50} parameters.

Toxicity test on Meniran extract (Phyllanthus niruri L.) as a medicated mouthwash towards fibroblast cell culture

ORIGINALITY REPORT

9%	7%	2%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	repository.unair.ac.id Internet Source	5%
2	E R P Wardoyo, U Hildayati, Rachmawati, R Kurniatuhadi. "Phytochemical analysis and antifungi activity of methanol extract of Acalypha hispida Burm. F. flower against to Candida albicans (Y116)", Journal of Physics: Conference Series, 2021 Publication	1%
3	repository.unhas.ac.id Internet Source	1%
4	ijpsr.com Internet Source	1%
5	garuda.ristekbrin.go.id Internet Source	<1%
6	www.spandidos-publications.com Internet Source	<1%
7	garuda.ristekdikti.go.id Internet Source	<1%

8	Michael J. Sauder. "High Throughput Protein Production and Crystallization at NYSGXRC", Methods in Molecular Biology™, 2008 Publication	<1%

Exclude quotes	Off	Exclude matches	< 7 words
Exclude bibliography	On		

Toxicity test on Meniran extract (Phyllanthus niruri L.) as a medicated mouthwash towards fibroblast cell culture

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3