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INCIDENCE OF GINGIVAL MUCOSA KARYOLYSIS IN THE USE OF FOOD COLORING AS A DENTAL PLAQUE DETECTION MATERIAL (STUDY ON MUS MUSCULUS)

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ABSTRACT
Bacteria in dental and oral diseases can lead to systemic issues like kidney and heart disease, emphasizing the importance of maintaining oral hygiene. Disclosing solution, a plaque-revealing agent, is pivotal in assessing plaque removal efficacy. The optimal concentration of rose pink food coloring for plaque visibility is 2.5 g/20 ml water. However, the potential impact of this coloring on oral epithelium remains unstudied. Objective: This research aimed to evaluate the effect of disclosing solution food coloring on the gingival mucosal layer in mice. Pure experimental research with a control group design involved 6 mice (Mus musculus) each in treatment and control groups. Erythrocyn, derived from rose pink food coloring, was applied to mice's gingiva for 2 minutes daily over 7 days. Labio-gingival epithelial cells were then sampled and analyzed using the modified Feulgen-Roseenbeck method. 58.33% of mice showed karyolysis in gingival epithelial cells, contrasting with no karyolysis in lip mucosa. Careful management of food coloring-containing erythrosine as a plaque detection agent is crucial to prevent contact with oral mucosa or prompt cleaning if contact occurs.

Keywords: dental plaque; disclosing solution; erythrosine; food coloring; karyolysis; mus musculus

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INTRODUCTION

Gingivitis and periodontitis patients increased by 7.9 million, doubling the number from 2010, while dental caries patients increased from 5.34 to 5.84 million(Patthi, 2017). Results from the Basic Health Research in Indonesia in 2018 reported a caries teeth prevalence of 88.8% and periodontitis prevalence of 73% (Kementerian Kesehatan RI, 2018). Left untreated, dental diseases can disrupt study and work activities and may also cause systemic diseases because the bacteria in the mouth are the same as those in the kidneys and heart(Willis & Gabaldón, 2020)

Dental caries or cavities result from acid products produced by microorganisms in plaque, while the inflammatory immune response by bacteria in dental plaque can cause damage to periodontal tissue (Dommisch & Kebschull, 2015; Kilian, 2018; Sueishi et al., 2017). Therefore, to reduce gingival inflammation and tooth enamel demineralization, plaque must be removed (Gomes et al., 2015; Hayasaki et al., 2014). Plaque, being invisible, is a thin, transparent layer of biofilm that can contain more than 108 per 1mm3 weight(Chetruş & Ion, 2013). Disclosing solution, a plaque-revealing material, is used to visualize dental plaque

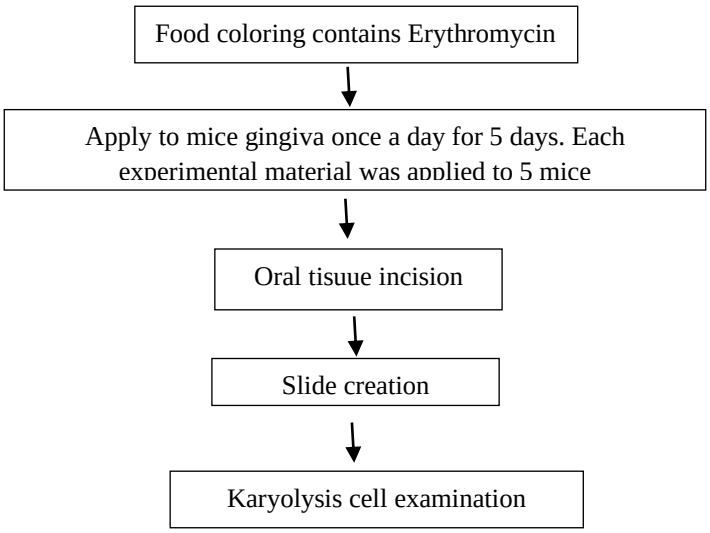
before and after brushing teeth(Datta, 2017). Disclosing solutions can also be an effective guidance in using oral hygiene tools and evaluation for oral health prevention program (Aristeidis Fasoulas et al., 2019; Kang & Min-kyung, 2018).

Finding disclosing solution materials in Jambi City is difficult as they must be purchased from dental supply stores, necessitating the search for easily obtainable and safe alternatives. Powdered food coloring in a Rose pink color that contains erythrosine, can serve as a substitute for disclosing solution and is readily available in shops and traditional markets in Indonesia. The optimal concentration of cake Sumba/rose pink food coloring to reveal plaque is 2.5 g/20 ml water(Fitria et al., 2020; Handayatun et al., 2020; Handayatun & NK, 2011).

However, the effect of Sumba on tooth surfaces and oral epithelium remains unstudied, considering that the red color of Sumba often affects the gingiva and oral mucosa and may sometimes remain on the lips. A previous study found that dental disclosing solution exhibits strong cytotoxicity against pig skin epithelium(Jung et al., 2020). Therefore, it is necessary to test the effect of the plug used as a disclosing solution on oral epithelium.

METHOD

This research adopts a quasi-experimental design with a post-test-only control group. It was conducted at the Jambi Health Polytechnic Pharmacy Laboratory for mouse maintenance and intervention, as well as slide preparation, with anatomical pathology examinations performed at Arafah Hospital in Jambi City. The research focused on mice (*Mus musculus*) as the study population. Six healthy mice weighing 200-300 grams were assigned to the control group, while another six healthy mice of similar weight comprised the treatment group. The independent variable was the "Sumba" food coloring in a Rose pink color that contained erythrosine, and the dependent variable was the occurrence of karyolysis in the gingival mucosa cells of the mice. Ethical clearance for this research was obtained from the Ethics Committee of Jambi Health Polytechnic Ministry of Health of Indonesia. The tools and materials employed included "Sumba" food coloring containing Erythrosine, mice, mouse food, syringes, paraffin blocks, equipment for slide preparation, and microscopes.
Workflow of research:



The research began by preparing food coloring containing erythrosine with a concentration of 20g/25ml. For the treatment group, a disclosing solution was applied to the gingival mucosa for 7 days. Meanwhile, in the control group, the gingival surface was only applied using cotton pellets and water. After 7 days, tissue collection of the labial gingiva of the mice using

a scalpel was done, then preserved in formalin solution to maintain its structure. The tissue is processed through dehydration, clearing, and embedding in paraffin wax for support during sectioning later. Subsequently, by using a microtome the sample was cut into thin slices/sections and then mounted onto a glass slide. The tissue sections were also stained using histological stains to highlight the cellular components. After the slides were completely dry, a careful observation under a microscope was done to find any karyolysis.

RESULTS

Among 6 samples of mice that were treated with a disclosing solution of food coloring containing Erythrosine, many gingival cells were found to have experienced karyolysis, whereas in the lip mucosa tissue, no cells were found to have undergone karyolysis. Figure 1 depicts an image of the gingival mucosa undergoing karyolysis

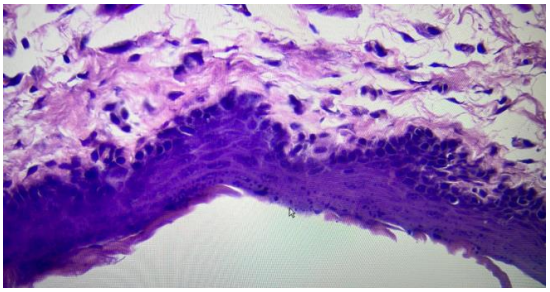


Figure 1: Microscopic examination of the gingiva of mice undergoing karyolysis

In the control group, the cells were observed to still be intact; no cells experienced karyolysis. In this instance, the mice's mucosa was applied with only water. Among the 6 samples, one sample exhibited karyolysis in the gingiva, but no karyolysis was observed in the lip mucosa. Six samples still exhibited cellular intact in both the gingival and lip mucosa.

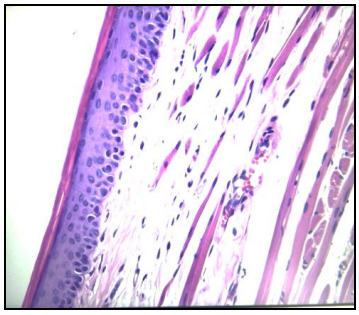


Figure 2. Microscopic examination of the gingiva of mice in control group that did not experience karyolysis.

Table 1.
Examination Results on Mice Gingiva After Application of Disclosing Solution from Food Coloring

	Karyolysis	%	Tidak kariolisis	%
Control _	1	16,7	5	83,3
Erythrosine disclosing solution	5	83,3	1	16,7

DISCUSSION

The results of the research revealed that all gingival cells in both the control group and the treatment group of mice exhibited karyolysis. This finding is consistent with previous research, where cells in the control group of Wistar mice, without any intervention, also experienced necrosis (Saputra et al., 2012). Karyolysis is one of the stages of cell necrosis,

and its frequency can serve as an indicator of tissue necrosis levels. It is characterized by a fading appearance of the cell nucleus, resembling a ghost-like image In mice treated with food coloring, a higher number of cells experienced karyolysis compared to the control group, with 83.4% of cells showing this phenomenon. This could be attributed to erythrosine, its main component. Research by Bhak et al. indicates that erythrosine can reduce the number of microbes in plaque; however, excessive consumption of erythrosine through food coloring may lead to cariogenic effects (Bhat et al., 2018)

When used as a plaque detection agent, erythrosine dissolves in water after being applied to the teeth and brushed. The previous study showed that the use of erythrosine in fast-dissolving patches can be used as a disclosing solution effectively(Tonglairoum et al., 2017). Erythrosine (E-127) is a synthetic cherry-pink food dye with a polyiodinated xanthene structure. It is used to color children's sweets and detect dental plaque. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Scientific Committee on Food (SCF) set its acceptable daily intake (ADI) at 0.1 mg/kg body weight(Piemontese et al., 2015). The FDA allows its use in food and drugs(Food and Drug Administration (FDA), 2023).

Previous studies have found that the use of disclosing solution containing erythrosine has strong cytotoxicity on oral mucosal and gingival cells. Thus, further research is needed for better and safer plaque detection(Jung et al., 2020). The use of high chronic intake can bring risk from the in vivo and in vitro test result (Merinas-Amo et al., 2019). Some studies link Erythrosine to changes in children's cognition and behavior, possibly due to dopamine receptor inhibition(Ganesan et al., 2011). Other research suggests it may cause chromosome aberrations and increase thyroid tumor risk(Chequer et al., 2012).

If toothpaste containing detergent is used during brushing, the concentration of erythrosin becomes very low. However, in this study, only 5-8 drops of water were added after applying food coloring to the mice, resulting in a high concentration of erythrosin. Consequently, during examination, karyolysis was observed in the gingival mucosa of the mice. This was consistent with a previous study, when disclosing solutions were diluted 200 and 100-fold, it showed no significant difference with the cell in the control group, but when the solution was diluted 50-fold, showed disruption in tissue healing, lost intercellular adhesion ability and were spindly and irregularly shaped(Jung et al., 2020).

Furthermore, the use of erythrosine can dye both dental plaque and surrounding soft tissue, making differentiation difficult at times. However, unlike fluorescent liquids, erythrosine does not stain dental instruments and restorations(Keerthana & Jeevanandan, 2018). But still, the use of disclosing solutions is the most effective way to improve personal self-oral care or in the use of community oral disease prevention programs(Datta, 2017; A Fasoulas et al., 2019; Kang & Min-kyung, 2018; Liu et al., 2017). Further research are needed to find the savest, easy to find, and cheap ingridients or tools to visualize dental plaque.

CONCLUSION

The disclosing solution of food coloring can induce cell karyolysis in 83.3% of the gingival mucosal epithelium of mice, while in the control group, it causes karyolysis in 16.3% of mice.

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