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Oral pre-malignant cells inhibition through expression of superoxide dismutase and caspase-3 by potential antioxidants of banana stem (*Musaceae*)

Koko Muzari[1], Ridhofar Akbar Khusnul Abdillah[1], Salsabila Nurmalia[1], Shahnaz Dwi Permata Putri[2], Gading Yudha Pratama[3], Hendrik Setia Budi[4]

[1]*Postgraduate program, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia*
[2]*Postgraduate program, Faculty of Dental Medicine, University of Indonesia,, Jakarta, Indonesia*
[3]*Genaral Practitioner of Griya Husada Hospital, Madiun, Indonesai*
[4]*Department of Oral Biology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia*

Abstract: *Introduction: Premalignant lesions are potential malignant lesions in the oral cavity. The exact etiology of premalignant lesions is not yet known. The antioxidant enzyme superoxide dismutase (SOD) plays a protective role against reactive oxygen species (ROS) and oxidative damage. Caspase-3 is involved in nuclear apoptosis and is considered an effector caspase. Decreased expression of caspase-3 can lead to the transformation of pre-cancerous lesions into malignant ones. Banana stem (Musaceae) contains various active compounds which contribute to apoptosis mechanisms and enhance the ability to combat free radicals that cause pre-cancerous lesions. Objective: To determine the antioxidant potential of banana stem by examining the expression of SOD and caspase-3 in pre-cancerous lesions in Wistar rats. Material and methods: This research uses narrative review focuses on full-text articles from scientific databases like PubMed, ScienceDirect, and Google Scholar from year 2013–2023. Discussion: The compounds found in banana, such as lectin, palmitic acid, and quercetin, have different mechanisms in increasing the expression of Caspase-3. Activated caspases perform their tasks by inducing apoptosis in damaged cells, allowing the proliferation of healthy cells in pre-cancerous lesion conditions. The constituents of banana, such as flavonoids, quercetin, and saponin, can enhance SOD expression, thereby suppressing the formation of free radicals by converting superoxide radicals (O2*) into hydrogen peroxide (H2O2). Conclusion: Banana stem (Musaceae) has antioxidant potential due to the presence of active compounds such as flavonoids (quercetin), lectin, palmitic acid, and saponin, which can enhance the expression of SOD and caspase.*

Keywords – *Antioxidants, Banana stems, Caspase-3, Oral premalignant, Superoxide dismutase*

I. INTRODUCTION

Oral premalignant lesions are lesions on the oral mucosa that can transform into malignancy [1]. The prevalence of pre-malignant lesions occurs between 1.5% and 4.5% of the world population and is more

common in males than females. The highest populations are found in Asia, South America and the Caribbean because there are geographic variations in different levels of tobacco and alcohol consumption [2]. Lifestyles such as smoking, drinking alcohol, chewing tobacco/areca nuts are risk of causing premalignant lesions [3]. Exposure to carcinogenic substances from cigarettes will produce DMBA which can induce premalignant lesions [4].

Reactive oxygen species (ROS) and free radicals play a role in neoplastic transformation. ROS can damage proteins, lipids, carbohydrates and nucleotides. Free radicals can also damage cell membranes through the production of lipid peroxide. An imbalance in ROS production and the capacity of oxidant cells can create oxidative stress that triggers carcinogenesis in cells through mutagenesis, cytotoxicity, and changes in gene expression. Antioxidant enzymes such as superoxide dismutase (SOD) can protect cells against ROS and oxidative damage. Excessive ROS production or lack of antioxidant systems can also lead to malignant transformation [5,6].

The difference between cell proliferation and apoptosis can influence the development of premalignant lesions. This dynamic balance is very important to maintain homeostasis and stability of the human body at the cellular level [7]. Apoptosis is a program of cell death that functions to eliminate useless or harmful cells in a multicellular organism. Caspase-3 is important in nuclear apoptotic changes and effector caspase. Decreased expression of caspase-3 makes pre-malignant lesions transform into malignant ones [8].

Currently, there are quite a lot of medical treatment developments that utilize plants or plants around as a source of medicine. Banana stem (Musaceae) is a tropical plant that is often used as herbal medicine. Banana stem consists of several parts that have different contents and properties. The sap extracted from banana is known to contain lectins, palmitic acid, leucocyanidin, quercetin, 3-O-galactoside, 3-O-glucoside, and 3-O-rhamnosyl glucoside, and have analgesic, anti-inflammatory, antibacterial, and antioxidants [9]. Banana stem also contain secondary metabolites such as flavonoids, tannins and saponins [10].

In addition to increasing the mechanism of apoptosis through caspase, the content of banana stem is also known to have an effect on the expression of exogenous antioxidants, namely superoxide dismutase (SOD). Evidenced by the study of Bagheri et al. (2021), who investigated SOD expression in rats receiving quercetin. The results showed that quercetin as a common polygenol with anti-inflammatory and antioxidant properties, has the effect of increasing superoxide dismutase (SOD) [13]. Secondary metabolites such as flavonoids, tannins and saponins also have an effect on increasing SOD. Flavonoids, tannins and saponins can increase the expression of SOD through activating nuclear factor erytgoid 2 related factor 2 (Nrd2) thereby increasing SOD [14]. Based on the study of Elekofehinti et al. (2012) who examined the effect of saponins on SOD and MDA activity, showed an increase in SOD activity. Saponins as antioxidants have the ability to donate hydrogen and chelate metal ions thereby increasing SOD activity. SOD converts superoxide radicals into hydrogen peroxide, which catalase converts into harmless water and oxygen [15].

Management of pre-malignant lesions and understanding of their potential to develop into malignancies will minimize morbidity and mortality so that this study will focus on finding the antioxidant effects of banana stem through nanoemulsion gel preparation on the expression of SOD and Caspase 3 in pre-malignant lesions of Wistar rats induced DMBA.

II. OBJECTIVE

This literature review aims to analyze the antioxidant effects of banana stem (*Musaceae*) in pre-malignant lesions of Wistar rats using the secondary data.

III. MATERIALS AND METHODS

The method in this research uses narrative review on the antioxidant properties of banana stem (*Musaceae*) and their potential role in treating pre-cancerous growths (pre-malignant lesions) in Wistar rats using the secondary data from PubMed, Sciencedirect, and Google Scholar databases. The literature studied is a scientific journal in full-text form which was published in the last 10 years, namely 2013-2023.

IV. DISCUSSION

Oral pre-malignant lesions are lesions in the oral cavity that have the potential to be malignant. Oral pre-malignant lesions include leukoplakia, erythroplakia, erythroleukoplakia, lichen planus, oral submucous fibrosis (OSMF), and oral dysplasia.16 Pre-malignant lesions occur in 2.5% of the population so that it becomes an urgency for the prevention of malignant lesions. The etiology of pre-malignant lesions is unknown but there are several risk factors such as chewing tobacco, smoking, and alcohol which have potential in the development of pre-malignant lesions [1].

Physiologically, cells can regulate the balance between the formation of reactive oxygen species (ROS) and their elimination, but several risk factors such as cigarette smoke can increase ROS levels and trigger loss of homeostasis. Excessive increase in ROS will cause oxidative stress conditions. Oxidative stress can induce precancerous and neoplastic initiation phases. Furthermore, carcinogens cause resistance to apoptosis where apoptosis is programmed cell death to destroy cells that are not needed. Apoptosis requires proteases namely caspase whose activation can be achieved by extrinsic and intrinsic signaling pathways [17,18].

An increase in free radicals in premalignant conditions will cause oxidative stress and cell damage. Oxidative stress causes damage to lipids and proteins, thereby damaging cell membranes and damaging DNA. Antioxidants are molecules or substances that can stabilize or deactivate free radicals before turn cells. Antioxidants are obtained exogenously or endogenously. Superoxide dismutase (SOD) is an exogenous antioxidant that forms the first line of defense against superoxide radicals and protects cells against cell damage. The SOD enzyme converts superoxide into hydrogen peroxide and oxygen and prevents the accumulation of ROS and changes the proteins in the mitochondrial membrane so that oxidative damage and cancer can be avoided [19]. Based on the results of the study by Kuthoor et al., (2022), a decrease in SOD levels in subjects indicated that patients were more susceptible to tobacco-induced oxidative stress due to increased free radicals. Low ROS levels will encourage cell proliferation and inhibit apoptosis thereby inducing tumor growth [20].

Activated Caspase-3 will affect the mechanism of apoptosis and produce several cytoplasmic and nuclear proteins. In some cases OSMF and OSCC showed a low percentage of caspase-3 positive cells. Caspase-3 specifically activates the caspase-activated DNAse (CAD) endonuclease. In cell proliferation, CAD will turn into CAD inhibitor (ICAD). In apoptotic cells, activated caspase-3 cleaves ICAD to release CAD which further degrades chromosomal DNA in the cell nucleus causing chromatin condensation. In addition, caspase-3 also induces cytoskeletal reorganization and cell disintegration to form apoptotic bodies. The actin-binding protein, gelsolin, is one of the active substrates of caspase-3 [21,22].

The reduction of SOD and Caspase activity in premalignant lesions needs to be increased through the use of antioxidants and other compounds to increase SOD and Caspase activity. Banana stem (*Musaceae*) is known to contain several compounds such as flavonoids (quercetin), tannins, saponins, lectins and palmitic acid which can affect the expression of SOD and Caspase. Flavonoids as antioxidants can directly provide hydrogen ions so that neutralizing toxic effects and indirectly by increasing gene expression through activation of erythroid nuclear factor 2 associated factor 2 (Nrf2) resulting in an increase in superoxide dismutase (SOD) [14].

Quercetin as one of the most widely found flavonoids increases the antioxidant defence system. The mechanism of quercetin in increasing antioxidant capacity is by regulating GSH levels. After free radicals are formed in the body, superoxide dismutase (SOD) quickly captures O2 and converts it into H2O2, then converts H2O2 into non-toxic H2O and this reaction requires GSH as a hydrogen donor [23]. Saponins are secondary metabolites of the polyphenol group and are found in many plants. Saponins also participate in activating Nrf2. In normal conditions Nrf-2 is inhibited. When oxidative stress occurs, Nrf-2 is activated to bind to the antioxidant response element (ARE) in the nucleus, and transcription of antioxidant genes is initiated [24].

Caspase activation is induced by the lectin content. Lectins induce caspase activation which is triggered by depolarization of the mitochondrial membrane which releases c chromium and calcium ions which act as important secondary messengers. According to the study of Singh et al. (2016) who measured the activation of caspase-8, 9 and 3 through the administration of lectins to pancreatic cancer cells, resulting in an increase in the expression of caspase-9 and caspase-3. Caspases are activated by pro-apoptotic signaling molecules such as cytochrome c and Ca2+ which are released to trigger cell death. The effect of lectins in stimulating caspase

expression is through increasing Ca2+ in cancer cells. For caspases to be activated, pro-apoptotic upstream signaling molecules such as cytochrome c and Ca2+ must be released to trigger the cell death cascade. Accordingly, mitochondrial membrane potential and calcium ion release were also evaluated. After 12 h of incubation of lectin-treated PANC-1 cells, a significant increase in the number of Ca2+ releasing cells was observed with highly disruptive mitochondrial membrane potential. resulting in the release of cytochrome c which forms the apoptotic complex and activates caspase-9. This further leads to caspase-3 activation leading to final cell death.

Besides lectins, quercetin and palmitic acid are known to also affect the apoptotic process through caspase expression. Quercetin can activate caspase-3 and caspase-9 by releasing cytochrome c and PARP (poly-ADP-ribosepolymerase). The release of cytochrome C by mitochondria is due to inhibition of Bcl-2 expression which then activates caspase-3 and triggers caspase-3-dependent pathways inducing apoptosis [11,25]. In addition, quercetin also prevents inhibition of caspase expression by caspase inhibitors [26]. Palmitic acid also affects caspase expression. Based on the results of Budi et al's research (2020) it was found that there was an increase in the concentration of caspase 3 after being given banana stem extract with palmitic acid as one of the ingredients. The mechanism of increasing caspase expression by palmitic acid is through the regulation of Bax and Bcl-2 [27].

V. CONCLUSION

Banana stems (*Musaceae*) has antioxidant potential since it contains active compounds such as the flavonoid quercetin and saponins which can increase SOD expression. Expanding the expression of SOD as an exogenous antioxidant will increase SOD activity in regulating ROS levels in premalignant lesions. Lectins, quercetin and palmitic acid were also found in banana stem which affected Caspase expression. Activated caspases can increase the body's ability to cell apoptosis.

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