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Research Article

IMMUNOMODULATORY EFFECT OF A MIXTURE OF WATER EXTRACTS OF BETEL (*PIPER BETLE* L.) LEAF AND (*UNCARIA GAMBIR ROXB*.) GAMBIER ON PHAGOCYTIC CELLS AND MODULATION ON PHOSPHATASE ENZYME OF MICE

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ABSTRACT

Objective: This study was to determine the immunomodulatory effect of a mixture of water extracts of betel (*Piper betle* L.) leaf and (*Uncaria gambir* Roxb.) gambier on phagocytic cells and modulation on phosphatase enzyme of mice.

Methods: Mixture of extracts of betel leaf and gambier (ratio, 429:71) was given to seven groups of mice for 14 days with doses of 100, 200, and 400 mg/kg body weight (BW). Two kinds of immunomodulatory drug in syrup form commonly used in the treatment of infectious were used as positive controls. The peritoneal fluid of mice containing macrophage cells was isolated by performing surgery. Immunomodulatory effect was done by calculating the number of phagocytosis activity and capacity of macrophage cells of mice, and measurement of phosphatase enzyme was done using a spectrophotometer ultraviolet visible on λ=405 nm, after 1 h intraperitoneal injection *Staphylococcus epidermidis* on each group of mice. Immunomodulatory effects of each group of a mixture of extract were compared with negative control, normal control, and positive controls.

Results: The result showed that doses administered 200 mg/kg BW of mixture extract most efficacy for both phagocytosis activity and phagocytosis capacity as well as for the results of testing for phosphatase enzyme. Based on statistical tests, it was significantly different ($p \leq 0.05$), if compared with negative controls and normal controls but not significantly different, if compared to positive controls ($p \geq 0.05$).

Conclusion: Based on the results of this study, it has been obtained that a mixture of water extracts of betel (*P. betle* L.) leaf and (*U. gambir* Roxb.) gambier with a dose of 200 mg/kg BW is very potential to be used as an immunomodulatory.

Keywords: Betel leaf, Immunomodulatory, Phagocytic macrophage, Gambier, Phosphatase enzyme.

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INTRODUCTION

Betel chewing is the number two biggest addiction in the world after smoking. This is mostly done by people living in the South Asian region, Southeast Asia, China, Pacific Islands, and several other countries in the world. The people who betel chewing will cause addiction because betel chewing can cause feelings of comfort (Euphoria) and eliminate headaches [1-3].

Betel chewing has advantages and disadvantages. The advantages of this are to prevent infectious diseases, prevent worm disease, increase immune system (immunomodulatory), eliminate bad breath, prevent toothache, cleanse the mouth and teeth, strengthen teeth, improve appetite, improve digestion, eliminate headaches, treat joint pain, prevent diabetes, eliminate constipation, cure inflammation, wound healing, as an aphrodisiac, and others [2-5]. The disadvantage of betel chewing is that it can cause oral cancer, this happens when people who betel chewing add young areca fruit. Because young areca fruit contains alkaloids; arecoline, guvacoline, guvacine, and arecaidine, where these alkaloids in the body can form derivatives of nitrosamine compounds that are carcinogenic [1,3,6,7].

On the other hand, based on research of Ramya and Anuradha (2015), on 25 betel chewers, male patients (aging ≥45 years) compared to non-chewers when chewing betel added with tobacco, obtained an increase in the level of plasma glucose, serum enzymes such as alanine aminotransferase, aminotransferase, alkaline phosphatase, aspartate, creatinine cholesterol, high-density lipoprotein, serum cholesterol, and triglycerides urea and the protein level were decreased [7] so that the disadvantage of betel chewing can be avoided, in this study, we did not use young areca fruit and tobacco. Therefore, this research was to find solutions to people who betel chewing, where they get the advantage to their health and avoid disadvantage to their health.

The number of people who betel chewing the biggest in the world is in India. It was estimated that around 170–440 million Indian residents and in the world around 550–700 million are betel chewing; therefore, there are several terms for betel chewing in India, namely, pan masala (young areca fruit, slaked lime, gambier, and other mixtures), gutka (pan masala plus tobacco), mainpuri (tobacco, young areca fruit, slaked lime, camphor, and cloves), mawa (young areca fruit, tobacco, and slaked lime), and a mixture of tobacco with slaked lime called khaini [3,10].

Research in India conducted at Tata Memorial Hospital showed that 28–30% had been diagnosed with oral submucous fibrosis due to betel chewing more than 12 times a day [7].

As we know, infectious diseases are still a major problem in the health sector in the world, with the increasing number of resistant antibiotics from time to time, so for the next few years, the morbidity and mortality rate of the world population due to infectious diseases will increase sharply, to prevent the increase of infectious diseases, it is necessary to prevent this problem. Therefore, the role of immunomodulatory drugs will be increasingly important [10].

Actually, the body has a special system to eradicate various infectious and toxic substances. Immune system cells work together with an organized division of labor to deal with various threats to the body. This system consists of blood leukocytes and tissue cells derived from

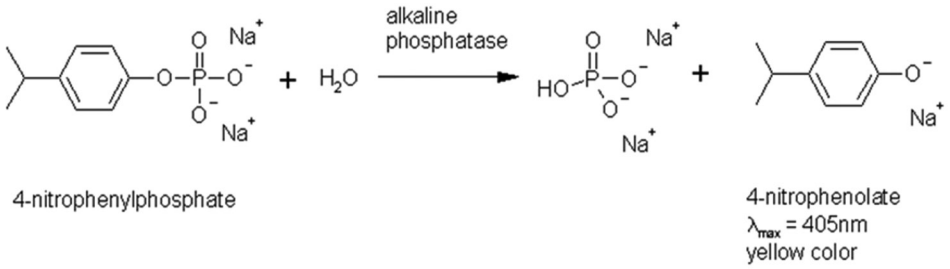

Fig. 1: The reaction process of 4-nitrophenyl phosphate (colorless) and H_2O in the presence of alkaline phosphatase forms Na phosphate and 4-nitrophenolate (yellow color) and absorbs strongly at 405 nm

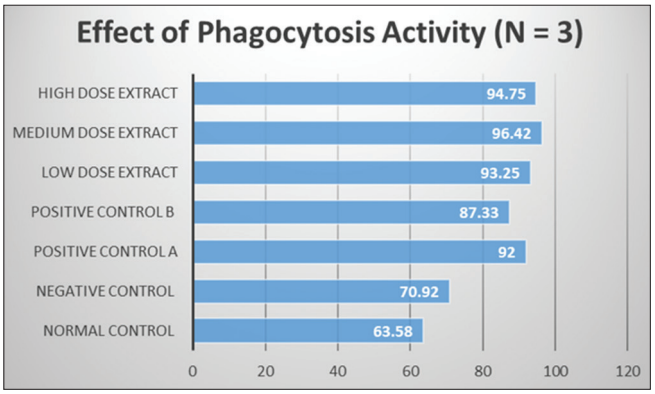

Fig. 2: Effect phagocytosis activity of mixture of betel and gambier to macrophage cell of mice

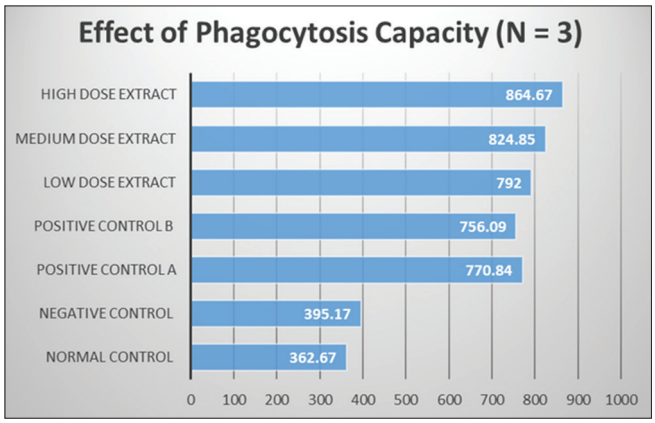

Fig. 3: Effect phagocytosis capacity of a mixture of betel and gambier to macrophage cell of mice

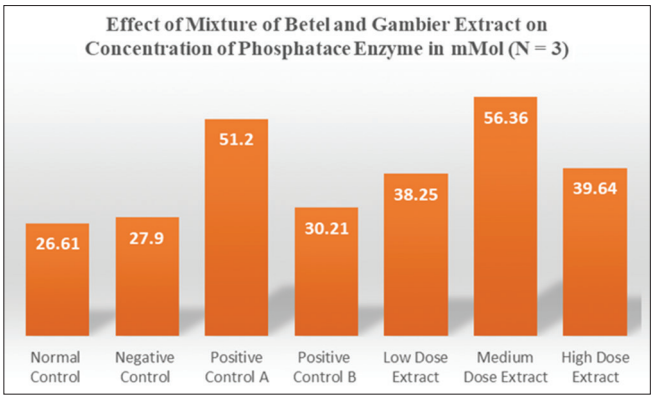

Fig. 4: The effect of a mixture of betel and gambier extracts on the concentration of the phosphatase enzyme

Measurement of the acid phosphatase enzyme

On the measurement of the acid phosphatase enzyme, as a standard solution was used a 4-nitrophenolate solution, measurement of the acid phosphatase enzyme level was done using a spectrophotometer UV visible on λ=405 nm [20-22]. The reaction process that occurs is shown in Fig. 1 [22-23].

RESULTS AND DISCUSSION

The results of the authentication for plants taxonomy that was done by Herbarium Bogoriense, Biological Research Center, Indonesian Institute of Sciences, indicating that the plant used for this research were *Piper betle* Linn. and *Uncaria gambir* Roxb.

The yield of betel chewing extract that was made with ratio betel and gambier (421:71) was obtained 9.92% dry extract. Determination of this ratio was based on the habits of people who eat chewing betel.

As shown in Fig. 2, for phagocytosis activity, the results of statistical tests showed a significant difference ($p<0.05$) between the increase in phagocytic activity by a mixture of betel and gambier extracts as well as positive control A and positive control B compared to normal control and negative control, whereas for low-dose and high-dose test preparations, there was no significant difference ($p>0.05$) in positive control A and positive control B. However, the medium dose test preparation was significantly different from positive control A ($p<0.05$).

As shown in Fig. 3, for phagocytosis capacity, the results of statistical tests showed a significant difference ($p<0.05$) between the increase in phagocytic activity by a mixture of betel and gambier extracts as well as positive control A and positive control B compared to normal control and negative control, whereas for low-dose and medium-dose test preparations, there was no significant difference ($p>0.05$) in positive control A and positive control B. However, the high-dose test preparation was significantly different from positive control 1 and positive control 2 ($p<0.05$).

As shown in Fig. 4, for the effect of the test preparation on the concentration of the phosphatase enzyme, the results of statistical tests showed a significant difference ($p<0.05$) between mixtures of betel and gambier extracts as well as positive control A compared to normal control and negative control and positive control B was no significant difference ($p>0.05$) compared to normal control and negative control, whereas for low-dose and medium-dose test preparations, there was no significant difference ($p>0.05$) in positive control A. However, the high-dose test preparation was significantly different from positive control A and positive control B ($p<0.05$).

According to Domingues *et al.* (2011), chemical compounds of gambier work to trigger an immunomodulation toward a Th2 cytokine profile, in this study, also were occurred at doses of 125 mg/kg body weight (BW) work as stimulant and at a dose of 500 mg work as immunosuppression [24].

On the other hand, according to Labro (2000) in this condition, large doses will cause interference with the immune system or certain damage to macrophage cells because there is a working relationship between