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The immunomodulatory activity of dioscorea alata on spleen histology of rats induced with 50% alcohol

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ABSTRACT

Dioscorea alata is frequently consumed and has several health advantages. The immune system-protecting spleen may be protected by bioactive substances in Dioscorea alata tubers. This study examined the influence of immunomodulatory ethanol extract of Dioscorea alata (EEDA) on the diameter of the white pulp of the spleen in 50% alcohol-induced rats (Rattus norvegicus). This research is quasi-experimental using post-test only controlled group design. Twenty-five Rattus norvegicus were separated into five groups. The normal control group (C) received Carboxymextyl Cellulose (CMC), the negative control group (NC) received CMC and 50% alcohol, and three groups received EEDA at doses of 50, 250, and 500 mg/kg for 14 days and 50% alcohol. The diameter of the white pulp of the spleen data analyzed with Kruskal-Wallis and Mann-Whitney tests. The study found a significantly increase in the diameter of the white pulp of the spleen ($p<0.05$) in the negative control group, EEDA doses of 50 and 250 mg/kg. The control group and EEDA doses 500 mg/kg groups had smaller the diameter of the white pulp of the spleen. The conclusions of this research proved that EEDA has an immunomodulatory activity, EEDA was immunostimulant at doses 50 and 250 mg/kg and immunosuppressant at doses 500 mg/kg.

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INTRODUCTION

Alcohol is a prevalent harmful substance worldwide, associated with over 200 diseases and injuries, resulting in approximately 3 million deaths each year (WHO, 2022). In Indonesia, alcohol consumption declined from 0.39 liters per capita in 2020 to 0.33 liters per capita in 2022; however, its adverse health effects remain considerable (Badan Pusat Statistik, 2023). Excessive alcohol consumption adversely affects the spleen. The spleen, a component of the lymphatic system, is

crucial for immune maintenance via blood filtration, production of white blood cells (WBCs), and synthesis of antibodies (Chaudhry et al., 2023). Excessive alcohol consumption can lead to spleen damage, including necrosis and hemorrhage resulting from the destruction of red blood cells (Hidayati et al., 2018). The extent of this damage is primarily influenced by the quantity and duration of alcohol intake (Boule & Kovacs, 2017). Moreover, prolonged alcohol exposure can impair immune function, thereby elevating the risk of infections and various diseases (Barr et al., 2016). It is essential to safeguard the spleen and reduce the adverse impacts of alcohol on this organ.

Studies indicate that certain medicinal plants may offer protective effects against spleen damage. Dioscorea alata, commonly referred to as yam, is identified as a potentially protective plant. This plant contains numerous bioactive compounds and demonstrates health benefits, particularly in addressing hypertension, diabetes, hepatotoxicity, and inflammation (Khan et al., 2014). Dioscorea alata possesses various bioactive components, such as flavonoids and saponins (Obidiegwu et al., 2020). These compounds exhibit immunomodulatory properties, specifically the capacity to regulate the immune system to reduce organ damage (Meiliana, 2016; Setiawan et al., 2021). The research results of (Makiyah & Ni'mah, 2024) prove that administering ethanol extract of Dioscorea alata at doses of 450, 2,475 and 4,500 g/kg for 90 consecutive days is safe and has a good effect on the spleen, as evidenced by the diameter of the white pulp of the spleen in all treatment groups, which is not significantly different from the control group. This study examined the influence of immunomodulatory ethanol extract of Dioscorea alata (EEDA) on the diameter of the white pulp of the spleen in 50% alcohol-induced rats (Rattus norvegicus).

RESEARCH METHOD

The study was a quasi-experimental laboratory investigation with a post-test only controlled group design. This study utilized independent variables of ethanol extract of Dioscorea alata administered at doses of 50 mg/kg body weight per day, 250 mg/kg body weight per day, and 500 mg/kg body weight per day over a period of 14 consecutive days, alongside the induction of spleen damage using 50% alcohol.

This study's dependent variable is the diameter of the white pulp of the spleen of rats (Rattus norvegicus), Sprague Dawley strain. The study utilized male white rats (Rattus norvegicus), aged 2 months and weighing between 100-150 grams. These ratts were acclimatized for one week, housed in a controlled environment with consistent lighting, provided standard BR food, and given distilled water for hydration.

The ethanol extract of Dioscorea alata was obtained by macerating simplisia in 70% ethanol for a 7 days. The solution was subsequently evaporated to yield a concentrated extract. The ethanol extract of Dioscorea alata is administered orally via a sonde every morning for 14 consecutive days. Rats categorized into five groups, with each group comprising five rats. The study comprises five groups: the control group (C), administered 2cc of Carboxymethyl Cellulose (CMC) solution; the negative control group (NC), receiving 2cc of CMC solution alongside alcohol induction; treatment group 1 (T1), which is given ethanol extract of Dioscorea alata at a dose of 50mg/kg body weight (BW) with 50% alcohol induction; treatment group 2 (T2), receiving ethanol extract of Dioscorea alata at 250mg/kg BB with alcohol induction; and treatment group 3 (T3), administered ethanol extract of Dioscorea alata at 500mg/kg BB with alcohol induction.

On the fifteenth day, the test subjects were dissected, and the spleen was extracted for preparations utilizing the paraffin block method. The preparations were subsequently stained with hematoxylin and eosin. The diameter of the alba pulp in the spleen of mice was observed using a light microscope at 10x10 magnification across 10 fields of view.

The diameter of the white pulp of the spleen was determined by calculating the average of the maximum diameter and the perpendicular maximum diameter of the white pulp of the spleen. The observation yielded the average diameter of the white pulp of the spleen. The data analysis a

normality test was conducted using the Kolmogorov-Smirnov test, along with a homogeneity test employing Levene's test. Data analysis utilized the Kruskal-Wallis test, followed by the Mann-Whitney test.

The Research Ethics Committee of the Faculty of Medicine and Health Sciences at Muhammadiyah University of Yogyakarta has reviewed and approved all procedures in this study, as indicated by letter number 010/EC-HC-KEPK FKIKUMY/VII/2024.

RESULTS AND DISCUSSIONS

This study examined the influence of immunomodulatory ethanol extract of Dioscorea alata (EEDA) on the diameter of the white pulp of the spleen in 50% alcohol-induced rats (Rattus norvegicus). The histological feature of the diameter of the white pulp of the spleen of the rats can observed in the Figure 1.

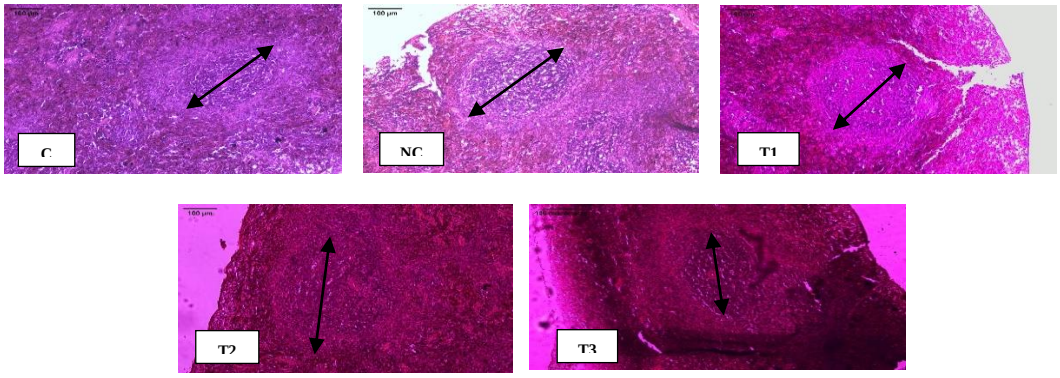

Figure 1. Histological features of the diameter of the white pulp of the spleen of rats with HE staining at 10x magnification in the control group (C), negative control group (NC), and treatment group of ethanol extract of Dioscorea alata at a dose of 50 mg/kg (T1), 250 mg/kg (T2), 500mg/kg (T3) for 14 consecutive days and 50% alcohol induction. ↔ : Diameter of splenic pulp alba

The average of the diameter of the white pulp of the spleen of rats are seen in Figure 2.

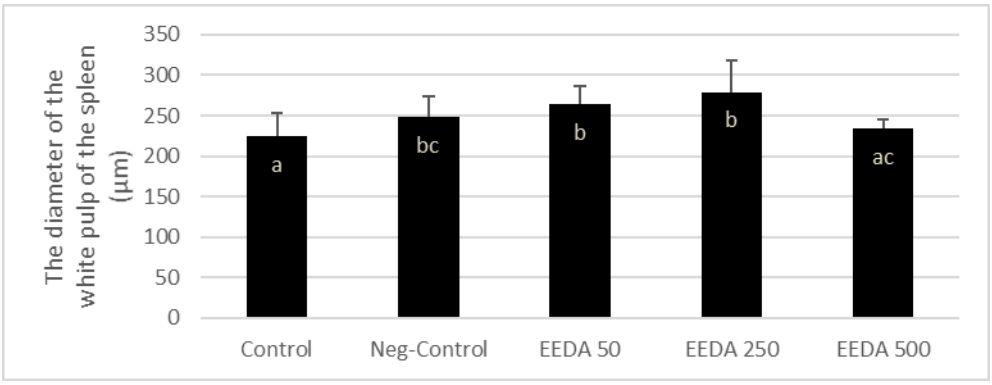

Figure 2. The diameter of the white pulp of the spleen of rats (Rattus norvegicus) which were administered ethanol extract of Dioscorea alata at doses of 50, 250, and 500 mg/kg for 14 days and induced with 50% alcohol compared with control without any treatment and negative control induced with 50%alcohol.

Group C without any treatment had the smallest of the diameter of the white pulp of the spleen (224 ± 29.68 µm). The alcohol-treated NC group showed an increase in the diameter of the

white pulp of the spleen, but it was still lower than the EEDA-treated group. The EEDA dose of 50 mg/kg (T1) and EEDA dose of 250 mg/kg (T2) groups experienced an increase in the diameter of the white pulp of the spleen, with T2 groups being the largest. In contrast, the EEDA dose of 500 mg/kg group (T3) decreased of the diameter of the white pulp of the spleen significantly. This indicates that in addition to causing an increase in immune system activity, at certain doses ethanol extract of Dioscorea alata can cause a decrease in immune system activity.

Data were then analyzed using SPSS. The Kolmogorov-Smirnov normality test showed a Sig. value below 0.05, which means the data is not normally distributed. The homogeneity test results showed a significance value of 0.044, indicating heterogeneous data. Therefore, the analysis was continued with the Kruskal-Wallis non-parametric test, which showed a significance of 0.000, indicating a difference in mean spleen diameter between treatment groups. The Mann-Whitney test was performed to determine significant differences between groups, with the results showing significant differences ($p<0.05$) between groups C, T1 and T2. However, no significant difference was found between groups C and T3 ($p>0.05$).

The results showed that the control group had the smallest splenic pulp diameter because this group did not receive a stimulus in the form of foreign antigens that could affect the activity of the immune system.

In the alcohol-induced negative control (NC) group, there was an increase in the diameter of the alba pulp, indicating the activation of the immune system due to damage caused by oxidative stress and inflammatory responses due to alcohol (Das, 2023; Ruiz-Cortes et al., 2022). Alcohol can impair the function of immune cells, including B cells, T cells and macrophages, contributing to inflammation and further organ damage (Hidayati et al., 2022). The immune response to antigens involves an increase in B cell density and immunoblast proliferation, which can be observed as an increase in the diameter of the splenic white pulp (Makiyah & Wardhani, 2017; Sulistyaningsih et al., 2024).

The group given Ethanol extract of Dioscorea alata at a dose of 50 mg/kgBB (T1) and 250 mg/kgBB (T2) experienced an increase in the diameter of the pulp alba compared to the control group (C) and negative control (NC), this indicates that flavonoids can increase the activity of the immune system (Perdana, 2021).

Other studies prove that flavonoids can work as immunostimulants by increasing IL-12 activity and lymphocyte proliferation. Flavonoids interact with immune cells such as macrophages and dendritic cells, which then stimulate the release of cytokines, increase CD4+ cell activity, and cause lymphocyte proliferation and Th1 cell activation. Activated Th1 cells release IFN-γ, which activates macrophages and enhances their phagocytosis ability (Putra et al., 2020)

In addition, the saponins found in Dioscorea alata., can also act as immunostimulants and immunosuppressants, depending on the dose given. At low doses, saponins function as immunostimulants by increasing antibody production and encouraging T cells and other immune cells to fight antigens. In addition, saponins can also trigger nonspecific immune responses, such as inflammation and lymphocyte proliferation. Saponins play a role in producing cytokines, including interleukins and interferons, which react to foreign antigens, and work with antigen presenting cells (APCs) such as macrophages and dendritic cells to enhance antigen presentation to T cells, which is very important in immune responses (Kurnianingtyas et al., 2013; Makiyah & Djati, 2018; Putra et al., 2020).

In the P3 group, which was given a dose of 500 mg/kgBB of Ethanol extract of Dioscorea alata, there was a decrease in the diameter of the splenic pulp alba that was not significantly different from the control group. This finding is in line with research by Makiyah & Wardhani (2017) which shows that flavonoids can reduce the diameter of splenic pulp in mice. Flavonoids, as natural antioxidants, can act as immunosuppressants by capturing free radicals and suppressing the production of proinflammatory cytokines. The mechanism of action includes inhibition of enzymes that regulate the inflammatory response, reduction of arachidonic acid production, and

suppression of cytokine transcription, especially IL-2, which results in decreased immune cell activity and antibody production (Kim et al., 2004; Makiyah et al., 2014; Perdana, 2021). These findings suggest that high doses of ethanol extract of Dioscorea alata can reduce immune cell activity as indicated by a decrease in the diameter of the splenic pulp alba.

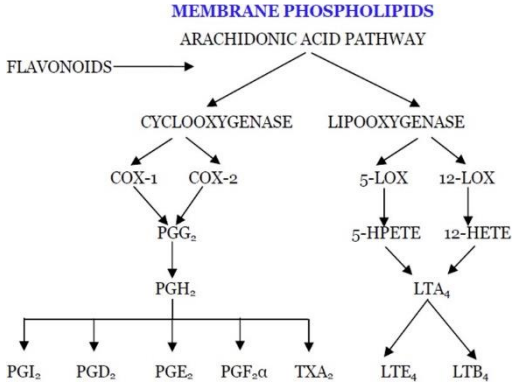

Figure 3. The mechanism by which flavonoids function as immunosuppressants (Makiyah & Wardhani, 2017)

Additionally, higher doses of saponin can function as an anti-inflammatory agent. Saponins function as immune system suppressants through interactions with diverse membrane lipids, such as phospholipids, prostaglandin precursors, and other inflammatory mediators. Saponins inhibit exudate formation and prevent increased vascular permeability, thereby contributing to the attenuation of the inflammatory response (Zaputri et al., 2023).

CONCLUSION

The research findings indicate that the administration of ethanol extract of Dioscorea alata on rats (Rattus norvegicus) induced by alcohol resulted in significant variations in the diameter of white pulp of the spleen among the different groups. The ethanol extract of Dioscorea alata. demonstrates potential as an immunomodulator, exhibiting an immunosuppressant effect at a dose of 500 mg/kgBW and an immunostimulant effect at doses of 50 mg/kgBW and 250 mg/kgBW.

The results of this study indicate that Dioscorea alata has potential as an immunomodulatory agent, but its application in humans still requires further validation through clinical trials and the optimal dose found in this study needs to be adjusted for humans by considering metabolic factors and individual tolerance to bioactive compounds in Dioscorea alata.

The next step in this research is to test the effectiveness of Dioscorea alata in other more complex animal models, such as non-human primates, to assess immune responses closer to humans. Future studies could conduct human clinical trials to evaluate the immunomodulatory effects of Dioscorea alata in a wider range of pathological conditions, such as in patients with autoimmune disorders or chronic infections.

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