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# THE EFFECT OF RED DRAGON FRUIT (*HYLOCEREUS POLYRHIZUS*) PEEL EXTRACT ON HISTOLOGICAL FEATURES OF THE SPLEEN IN MENOPAUSAL-MODEL RATS

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## ABSTRACT

Menopause is a physiological condition in women that is marked by the cessation of the menstrual cycle. Loss of estrogen levels as a powerful antioxidant can lead to oxidative stress. Several changes in the spleen that occur during menopause include increasing spleen weight, decreasing conventional dendritic cells, inhibition of the chemotactic index, lymphocyte proliferation, and natural killer cell activity, as well as an increase in the number of leukocytes, total lymphocytes, and monocytes. Red dragon fruit peel extract has estrogenic properties and is capable of being an immunomodulatory compound. Administering this extract could return the condition of the spleen to normal by reducing the number of lymph nodules and the diameter of the germinal centrum. However, the results of this research show an increasing trend. The P0 group had the smallest mean number of lymph nodules and germinal centrum diameter, namely 2.08 ± 0.81 and 320.95 ± 46.50 µm, followed by P1 with a value of 2.97 ± 0.99 and 346.64 ± 47.15 µm, and the largest mean gain was found in the P2 group with a value of 3.11 ± 1.06 and 432.17 ± 72.42 µm. The increase obtained was not significant based on the One-Way ANOVA parametric test with p-value = 0.124 for the number of lymph nodules and p-value = 0.368 for germinal center diameter. Therefore, the authors did not find any significant effect of administering red dragon fruit peel extract (Hylocereus polyrhizus) on the histological appearance of the spleen in menopausal model rats.

**Keywords:** ***Hylocereus polyrhizus.*****, menopause., spleen**

## INTRODUCTION

Menopause is a degenerative process that affects physiological functions in women, one of which is the body's immune system. Menopause begins to occur in women aged 45 – 55 years, which is marked by the cessation of the menstrual cycle for a period of at least 12 months. There are two sex hormones that play an important role in the normal menstrual cycle, namely the hormones estrogen and progesterone. Apart from acting as a sex hormone, the hormone estrogen also acts as a strong antioxidant by reducing levels of bad cholesterol oxidation in the body.[1] In the menopause process, there is a decrease and cessation of the formation of the hormone estrogen. This can be a threat to women's health and make women more susceptible to diseases associated with aging.

Loss of estrogen levels as a powerful antioxidant in the body can lead to a state of oxidative stress. Oxidative stress has an important role in the menopause process and results in the overproduction of free radicals, such as ROS, which can disrupt the body's antioxidant protection mechanisms. In normal metabolism, the body can produce free radicals in certain concentrations. However, excessive production of free radicals without being balanced with antioxidants can lead to oxidative stress. Antioxidants function to neutralize ROS production, which, in turn, prevents the adverse effects of oxidative stress. As we age, the body's antioxidant levels continue to decrease. Cessation of estrogen production further increases oxidative stress levels in the body.

The immune system can also be affected during menopause. In menopause, there is a decline in the immune system as a result of the loss of estrogen and the general aging process. As a result of damage to estrogen regulation in the body, there is an increase in the adaptive immune response mediated by T cells.[2] This may lead to an increase in the prevalence of autoimmune and infectious diseases.

As an active organ in the immune system, the spleen is very susceptible to damage caused by free radicals. The structure of the spleen parenchyma is composed of two parts, namely red pulp and white pulp. Red pulp functions to filter old erythrocytes.[3] Meanwhile, the white pulp is the site of initiation of the adaptive immune response.[4] Oxidative stress experienced by menopausal women can affect the structure of the spleen, resulting in an increase in the number of lymph nodules and the diameter of the germinal centrum.[5]

Antioxidants are compounds that are able to neutralize and prevent the negative effects of free radicals. Under normal circumstances, antioxidants can neutralize ROS and prevent excessive exposure to oxidative stress. However, as we age, antioxidant levels in the body decrease, causing the body to become susceptible to diseases associated with menopause.

Antioxidants have the ability to stabilize free radical molecules. The negative effects of free radicals must be prevented so that the structural integrity of cells and tissues in the immune system is maintained, so cells need adequate protection from the consumption of antioxidants. Therefore, consuming antioxidants can be beneficial in improving immunity due to menopause. Natural sources of antioxidants can be found in vegetables and fruit.

One fruit that contains antioxidants is red dragon fruit or Hylocereus polyrhizus. This fruit has become of interest in research, not only because of its unique taste, shape, and color, but also because of its bioactive compound content. Its purple color is due to its anthocyanin content, which has antioxidant, phytoestrogen, anti-inflammatory, and antimicrobial properties. Apart from the flesh, dragon fruit skin, which is often thrown away by people, also contains antioxidants.[1] Research states that the antioxidant content in red dragon fruit peel extract can increase lymphocyte proliferation and nitric oxide (NO) production, which has the potential to be an immunostimulator.[6] The potential produced by antioxidant compounds in red dragon fruit skin could be the answer to dealing with a decrease in the immune system and the histological appearance of the spleen in menopausal women.

## MATERIALS AND METHODS

This research is pure experimental research with a randomized post-test only control group design method. This research is in vivo research using rats as experimental animals and has received information on ethical feasibility by the Research Ethics Commission Unit, Faculty of Medicine, Udayana University No. 531/UN14.2.2.VII.14/LT/2023. The experimental animals were then randomly divided into three experimental groups with the following characteristics: adult female Wistar strain rats that had been ovariectomized and weighed between 180-220 grams. The subjects of this research were obtained from the Animal Laboratory Unit, Department of Pharmacology, Faculty of Medicine, Udayana University.

### Animal Preparation

Ovariectomy was performed using the modified Ingle DJ and Grith JQ methods. First, the rats were anesthetized using ketamine at a dose of 40 mg/kgBW intramuscularly, then the rats's abdominal hair was shaved and sterilized using betadine, then covered with a sterile mask. Next, a transabdominal incision is made above the uterus 1.5 – 2 cm long layer by layer until it penetrates the peritoneum. The oviducts and ovaries are cleaned from the surrounding fat and connective tissue, the distal oviducts and ovaries are then tied and removed. This process is carried out on two sides, right and left. The incision wound will then be closed with layer-by-layer sutures. Gentamicin injection was given at a dose of 60-80 mg/kgBW/day for three days, as post-operative therapy. The ovariectomized rats were then rested for 7 days while being given standard BR II food 20 grams per mouse every day and 2 ml distilled water per 200 grams of mouse body weight for drinking. These rats were then kept in cages and monitored in the Histology section of the Faculty of Medicine, Udayana University.

### Making Ethanol Extract of Red Dragon Fruit Peel (*Hylocereus polyrhizus*)

Red dragon fruit (Hylocereus polyrhizus) was obtained from a supermarket in Denpasar. The dragon fruit skin used is the skin of red dragon fruit which is ripe and red in color. Next, extract will be made from the skin by separating the dragon fruit skin from the flesh, then washing the dragon fruit skin with clean, running water. Then, chop the dragon fruit until it is thin, followed by drying by airing and then roasting to make it better. Refining the chopped pieces into coarse simplicia by blending, then sieving to produce fine simplicia. Macerate simplicia with 96% ethanol solution mixed in a ratio of 1:7 for three days with stirring twice a day. Finally, the maserate was separated using filter paper, then the results were evaporated using a rotary evaporator to obtain a thick red dragon fruit peel extract.

### Distribution of Sample Groups

This study used three treatment groups. Based on Federer's formula and considering the mortality of experimental animals, it was determined that the number of samples needed for each group was 7 animals. The treatment groups were divided as follows:

a. Treatment 0 (P0): ovariectomy group given normal saline 1 ml/day.
b. Treatment 1 (P1): ovariectomy group given ethanol extract of red dragon fruit peel at a dose of 60 mg/200 grBW/day for 30 days.
c. Treatment 2 (P2): ovariectomy group given ethanol extract of red dragon fruit peel at a dose of 90 mg/200 grBW/day for 30 days.

### Procedures for Euthanasia and Burial of Experimental Animals

The euthanasia procedure uses ketamine at a dose three times the anesthetic dose, namely 120 mg/kgBW intramuscularly. After that, the mouse spleen will be taken to make spleen histology preparations. Rats whose spleens have been removed will be wrapped in medical waste bags and buried in the ground.

### Making Histological Preparations of Rat Spleen

Histological preparations of rat spleen will be made using the paraffin method with hematoxylin-eosin staining. Making this preparation will go through several stages as follows:

1. Cutting Stage

The spleen that has been removed will be cut to the size of 1x1x1 cm. This process was carried out on all preparations to obtain 21 rat spleen incisions.

2. Fixation Stage

At this stage, the spleen will be fixed with 10% BFN solution for one hour and will be repeated twice with two different BFN solutions.

3. Dehydration Stage

The preparations will be placed in alcohol with varying concentrations (60%, 70%, 80%, 90%, absolute I and absolute II) for 2 hours in each solution.

4. Clearing Stage

Clarification is carried out to reduce the ethanol content used in the dehydration stage. Cleaning was carried out with xylol I solution (xylol and absolute alcohol in a 1:1 ratio) for 1.5 hours and continued with xylol II solution (pure xylol) for 1.5 hours.

5. Planting Stage

The spleen will be put into the cassette first, then liquid paraffin at a temperature of 60oC will be poured onto the cassette and left to harden in the refrigerator for one hour.

6. Cutting Stage

The frozen paraffin will be removed from the cassette and placed in a microtome to be cut into thin sections 5-6 µm thick. The paraffin pieces will be floated in a water bath at 56oC for 40 minutes. The glass object to be used is smeared with Mayer albumin. The cut results were placed on a glass slide and left to dry for 12 hours.

7. Coloring Stage

The resulting section will be stained using hematoxylin-eosin. The first step is to soak the preparations in xylene solutions I and II for 5 minutes in each solution. The preparation is then removed and placed in a graded alcohol solution, in order to rehydrate the preparation, starting from absolute alcohol, 95%, and 70%. The duration of soaking is 2 minutes in each solution. The preparation will then be run through running tap water for 2 minutes before being soaked in hematoxylin dye for 3 minutes. After staining using hematoxylin, the preparations were again perfused with running tap water for 2 minutes. The preparation will then be immersed in the eosin solution for 2-4 minutes and removed to be put back into the 70% alcohol solution for 5 minutes, transferred to the absolute ethanol solution for 5 seconds and repeated in 3 different absolute ethanol solutions. Cleaning will then be carried out with xylene solution (pure xylol) twice for 2 minutes each. The preparation is then dripped with Canada balsam then covered with a cover glass and allowed to dry.

**Histological Observations of Rat Spleen**

Spleen preparations that have been stained will observe the number of lymph nodules and the diameter of the germinal center. Part of the spleen will be seen in cross-section per mouse. Cross-sections were taken from the same section for all rats. Three incisions will be made in one cross-section, of which the best one will be selected and then observed in 5 fields of view. The number of lymph nodules and diameter of the germinal center will then be averaged in percentage from each field of view. The number of lymph nodules will be counted manually and assisted using OptiLab software and raster images. Meanwhile, the diameter of the germinal centrum will be measured using the image raster application which has been calibrated in micrometers (µm). Observations of the preparations will be carried out from a magnification of 40 times.

**Data Analysis**

The primary data obtained in the form of the number of lymph nodules and germinal centrum diameter will be calculated using the OptiLab application and raster images, averaged, then analyzed. A normality test will be carried out first with the Shapiro Wilk and data homogenization with the Levene test. If it is homogeneous and normally distributed, data analysis will continue with the One-Way ANOVA parametric test, because the research is comparative in nature and will be continued with a confidence interval examination with the hope that the results will be <0.05 or significant. The histology of the spleens from the three experimental groups will be presented in the form of photographs.

**RESULTS**

**Descriptive Test Results**

The descriptive test results of the two variables, namely the number of lymph nodules and germinal centrum diameter, showed the same increasing trend between the three treatment groups. Data is presented in **Table 1**. Both variables appear to have a linear increase, that is, results increase as the dose increases. The histological appearance of the spleen with the lowest results was at P0, followed by P1, and the highest results were obtained at P2. The highest number of lymph nodules was seen in group P2 with a mean value of 3.11 ± 1.06, followed by group P1 and group P0 respectively with values of 2.97 ± 0.99 and 2.08 ± 0.81. The largest number of lymph nodules was found in groups P1 and P2 with a value of 2.00.

In the germinal center diameter, the visible trends appear to be similar based on the minimum and average value parameters. The smallest average value was in group P0 with a value of 320.95 ± 46.50 µm, followed by group P1 with a value of 346.64 ± 47.15 µm, and the largest value was in group P2 with a value of 432.17 ± 72.42 µm. The smallest minimum value for germinal centrum diameter was in the P0 group with a value of 148.56 µm. However, the smallest maximum value of germinal centrum diameter was obtained by group P1 with a value of 488.73 µm, followed by P0 and P2 with values of 523.54 µm and 814.22 µm.

**Table 1.** Descriptive test results

| | Groups | Mean | N | Standard deviation | Min | Max |
|---|---|---|---|---|---|---|
| Numbers of lymphoid nodules | P0 | 2.08 | 7 | 0.81 | 1.00 | 3.20 |
| | P1 | 2.97 | 7 | 0.99 | 2.00 | 4.80 |
| | P2 | 3.11 | 7 | 1.06 | 2.00 | 4.80 |
| Diameter of germinal center (µm) | P0 | 320.95 | 7 | 46.50 | 148.56 | 523.54 |
| | P1 | 346.64 | 7 | 47.15 | 170.62 | 488.73 |
| | P2 | 432.17 | 7 | 72.42 | 179.73 | 814.22 |

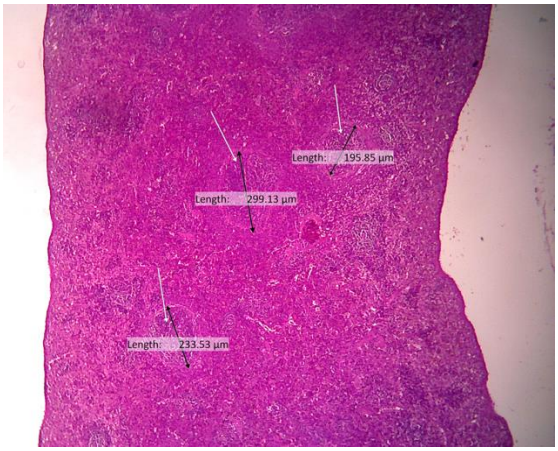


**Figure 1.** Histological image of spleen tissue with 4x objective magnification in the negative control group (P0)

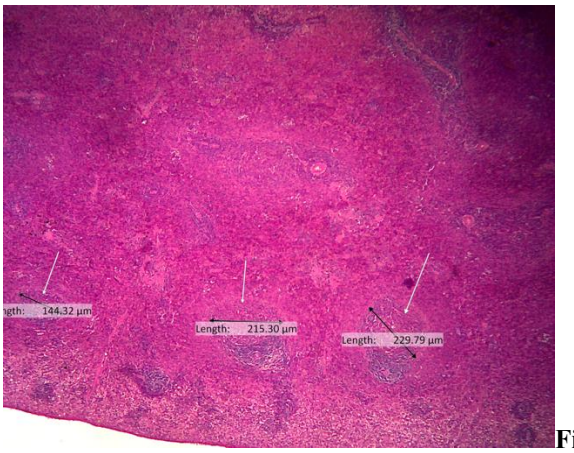


**Figure 2.** Histological image of spleen tissue with 4x objective magnification in treatment group 1 (P1)

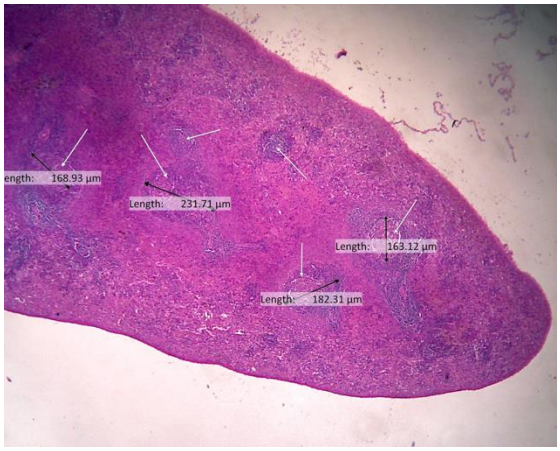


**Figure 3.** Histological image of spleen tissue with 4x objective magnification in treatment group 2 (P2). Primary lymph nodes were found without germinal center formation.

**Normality Test Results**

The results of the normality test use Shapiro-Wilk because the sample size is less than 50. This is also supported by the results of the df value < 50 based on **Table 2**, so the Shapiro-Wilk test is appropriate to use to carry out normality tests. The significance value for the two variables in each group shows a p value > 0.05, which means that the data is normally distributed. Therefore, data processing can be continued using the One-Way ANOVA parametric test.

**Table 2.** Normality test results using Shapiro-wilk showing a normally distributed data

| | Groups | Statistics | df | Sig. |
|---|---|---|---|---|
| Numbers of lymphoid nodules | P0 | 0.931 | 7 | 0.563 |
| | P1 | 0.896 | 7 | 0.309 |
| | P2 | 0.854 | 7 | 0.132 |
| Diameter of germinal center (µm) | P0 | 0.989 | 7 | 0.991 |
| | P1 | 0.925 | 7 | 0.506 |
| | P2 | 0.850 | 7 | 0.123 |

**3.1 Homogeneity Test Results**

The homogeneity test was carried out using the Levene test and gave results in accordance with **Table 3.** In both groups of dependent variables, the result was a p value > 0.05, which means that the data is homogeneous, with details of the p value for the number of lymph nodules being 0.793 and 0.914 for the germinal centrum diameter. This shows that the data group comes from a population with the same variance.

**Table 3.** Homogeneity test results using Levene test

| | Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|---|
| Numbers of lymphoid nodules | 0.234 | 2 | 18 | 0.793 |
| Diameter of germinal center (μm) | 0.091 | 2 | 18 | 0.914 |

### 3.2 Inter-Group Comparability Test Results

Because the data is normally distributed and homogeneous, a comparability test was carried out using One-Way ANOVA and gave results in accordance with **Table 4.** From the results of the parametric test, it is known that the significance values for the two variables give similar results. In the variable number of lymph nodules, it is known that the p value = 0.124 ($p > 0.05$). Meanwhile, for the germinal centrum diameter variable, the value obtained was $p = 0.368$ ($p > 0.05$). This leads to the conclusion that there is no significant difference in the average number of lymph nodules and diameter of the germinal center in the three experimental groups.

**Table 4.** Inter-group comparability test results using One-Way ANOVA showing no significant differences

| | Sum of Squares | df | Mean square | F | Sig. |
|---|---|---|---|---|---|
| Numbers of lymphoid nodules | 4.347 | 2 | 2.173 | 2.349 | 0.124 |
| Diameter of germinal center (μm) | 474.65 | 2 | 237.32 | 1.056 | 0.368 |

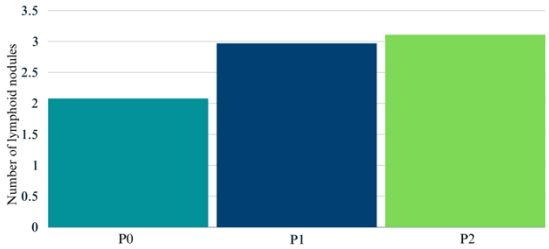


**Figure 4.** Graph of the mean regarding the number of lymph nodules. No significant differences were found between the three experimental groups.

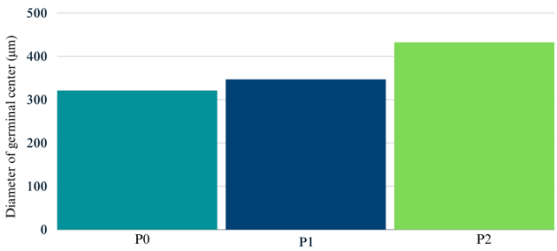


**Figure 5.** Graph of the mean regarding the diameter of germinal center. No significant differences were found between the three experimental groups.

## 1. DISCUSSION

The hormone estrogen has an important role in various aspects of body homeostasis and anabolism. This includes the role of estrogen in oxidative stress, immune function, as well as several other metabolic aspects such as the development of bone cells and fat tissue. In general, the estrogen hormone signals through two different receptors, namely ERα and ERβ which are spread in various tissues, one of which is in the immune system. Deficiency or decreased levels of the hormone estrogen in menopause can be a predisposing factor for autoimmune diseases and increase proinflammatory activity. In this study, a bilateral ovariectomy procedure was carried out in such a way as to induce menopause in experimental animals. One of the limitations of this study is that the researchers did not have a group of healthy rats without ovariectomy, so comparisons of changes in the histological appearance of the spleen could not be made. In addition, researchers found limited literature discussing the histological features of the spleen in mouse models of menopause, so researchers could only compare non-specific immune responses to menopause or ovariectomy, such as levels of B and T lymphocyte cells in the blood.

The spleen as the largest secondary lymphoid organ is responsible for the body's defense mechanism against antigens. Most of the anatomical structure of the spleen is composed of red pulp, while a small part is composed of white spleen. Red pulp is a place for red blood cells to be disposed of, while white pulp is a center for activation of T lymphocyte and B lymphocyte cells which respond to antigens and produce antibodies. In this study, the author only focused on looking at the white pulp of the spleen, namely the lymph nodules and germinal centrum more specifically. Lymph nodules have another name in the form of white pulp follicles (Malpighian corpuscles) which is a continuation of PALS. Lymph nodules are divided into two types: primary and secondary. Primary lymphatic nodules are composed of aggregations of small lymphocyte cells (naive and mature B lymphocytes), while secondary lymphatic nodules are composed of aggregations of large lymphocyte cells (active B cells) which form the germinal center. The germinal center is composed of two parts, namely the dark zone which consists of proliferating B cells, and the light zone where high-affinity B cells are selected to survive and then differentiate. The germinal centrum forms within 4 to 7 days after initiation of the B cell response by T cells. Not all conditions in the body cause the formation of the germinal centrum. According to Crane et al. (2021), the formation of the germinal center is usually the body's immune response to certain pathogens or stimuli, and can also be an indication of autoimmune and infectious diseases.[3] So theoretically, the process of menopause induction through ovariectomy in rats causes increased formation of the germinal center in the white pulp. Initially, the formation of the germinal center is initiated by the migration of Tfh cells towards the follicle. This process continues until the B cells stimulated by the antigen are activated and migrate to the follicle to form the germinal center. B cells proliferate and form a dark zone within 6 to 12 hours, so that

within 5 days 5000 progenitor cells can be created. After several stages of proliferation, the dark zone of B cells stops dividing. B cells that have divided will migrate to the light zone and are selected to recognize antigens by FDCs. Then, high-affinity B cells will exit the germinal center as plasmablasts towards the bone marrow, while B cells that fail to recognize antigens will undergo apoptosis in the light zone.[7]

There are several studies that explain changes in immunity that occur as a result of the ovariectomy procedure. One of them, research by Cunningham et al. (2016) on female mice that were ovariectomized at the age of 4 (prepuberty) and 8 weeks (postpuberty). In this study, results were obtained in the form of an increase in the spleen weight of ovariectomized mice, although this increase was not statistically significant. In addition to examining spleen weight, Cunningham et al. (2016) also examined spleen dendritic cell populations using flow cytometry. Results were obtained in the form of a significant decrease in the number of conventional dendritic cells (CD11c+ CD11b+) between mice that were not ovariectomized and mice that were ovariectomized before puberty (4 weeks of age). Meanwhile, a trend was found to increase the number of tolerogenic dendritic cells (CD11c+ CD8a+) in ovariectomized mice. However, administration of estradiol in this study was known to be able to restore the number of conventional and tolerogenic dendritic cells to normal.[8] Dendritic cells are cells that are important for maintaining tolerance and innate immune responses. Dendritic cell development needs to be considered because it has various influences on cell function. For example, conventional dendritic cells are capable of priming CD8+ T cells against extracellular antigens. Meanwhile, tolerogenic dendritic cells function to accommodate dendritic cells which play a role in immunosuppression to return the immune condition to a tolerant state. Dendritic cells, especially FDCs, are also very important for germinal center reactions. It is known that disruption of the FDC network causes premature termination of the germinal center.[9]

Not only does it cause changes in the number of dendritic cells and spleen weight, the ovariectomy procedure also causes the body to be in a state of oxidative stress. The increase in oxidative stress during ovariectomy procedures is also supported by research by Abdelrazek et al. (2019), who noted increases in nitric oxide (NO) and lipid peroxidation (malondialdehyde) as biomarkers of oxidative stress.[10] A cross-sectional study on 69 healthy women aged 45 – 60 years by Abildgaard et al. (2020) stated that there was an increase in the number of leukocytes, total lymphocytes and monocytes in postmenopausal women compared to perimenopause. This is predicted because there is an increase in visceral fat mass in menopausal women. This increase in the number of leukocytes was also accompanied by an increase in pro-inflammatory cytokines such as TNF-α and IL-6, which was possibly caused by high FSH levels. Systemic inflammation in postmenopausal women also contributes to a significant increase in T cells which also causes high levels of senescent-T cells.[11]

Based on research by Rahman et al. (2016), red dragon fruit (*Hylocereus lemairei*) skin extract can act as an immunostimulator, namely that the extract is able to increase the body's immune system specifically and non-specifically. They conducted research on male white mice by comparing the differences between two immune responses, namely specific and nonspecific immune responses. The specific immune response was assessed by observing the relative weight of the spleen to the body weight of the mice. Meanwhile, the nonspecific immune response is carried out by calculating leukocyte levels using peripheral blood smears and total leukocyte counts. Administration of dragon fruit extract at doses of 10 mg/kgBW, 50 mg/kgBW, and 100 mg/kgBW showed an increase in leukocyte cells, such as eosinophils, rod neutrophils, segment neutrophils, lymphocytes, and monocytes. The same trend also occurred in the spleen weight parameter, namely there was an increase in the relative weight of the spleen to the body weight of the spleen as the dose of dragon fruit peel extract administered increased. In this study, significant differences were found between the control group and the 100 mg/kgBB dose group, so it can be said that 100 mg/kgBB is an effective dose for increasing the immunomodulatory activity of red dragon fruit peel extract. An increase in the number of leukocyte cells and spleen weight indicates that phagocytic cells in the process of producing antibodies have also increased. B lymphocyte cells that have been activated will produce antibodies consisting of natural killer cells, T lymphocyte cells, B lymphocyte cells, macrophages, dendritic cells, and erythrocytes which will capture antigens from the blood.[12]

If we look at the content contained in red dragon fruit peel extract, there is an active compound in the form of lupeol from the terpenoid and polyphenol groups. Based on research by Maigoda et al. (2016), it was found that there were 171.79 mg/100 g flavonoids, 47.7 mg/100 g anthocyanins, 157.34 mg/100 g phenolic acids, 0.25 mg/100 g carotene, 35.92 mg/100 g alkaloids, and 88.17 mg/100 g vitamin C as antioxidants in red dragon fruit peel extract.[13] Flavonoids have activities similar to CDKs in the cell cycle. In addition, flavonoids also inhibit MAPK by providing protective effects against autoimmune, neurodegenerative, cardiovascular and allergic diseases.[14] This is also supported by a systematic review by Sakbania et al. (2021) who summarized that red dragon fruit peel extract has immunomodulatory activity by reducing levels of pro-inflammatory cytokines. In the inflammatory process, IL-1β is produced by macrophages thereby activating the MAPK and NF-κB pathways. By administering red dragon fruit peel extract, apart from inhibiting NF-κB activation, there was also a decrease in other pro-inflammatory cytokine cascade activators, leading to a decrease in the concentrations of TNF-α and IL-1β.[15] Testing the phagocytic activity of macrophages after administering red dragon fruit peel extract was also carried out by Hafid & Syachriyani, 2022 by making syrup preparations into 3 formulas: FI (5% red dragon fruit peel extract), FII (10% red dragon fruit peel extract), and FIII (red dragon fruit peel extract 15%) in mice infected with Escherichia coli bacteria. The results of this study showed that the group of mice given FIII syrup had the highest effect on the number of macrophages, although there was no significant difference in impact compared to the positive control group. Macrophages act as agents for destroying antigens circulating in mouse blood. In carrying out their role, macrophages are able to secrete IL-12 which helps differentiate CD4+ cells into Th1. These Th1 cells will then secrete macrophages.[16]

There is an experimental study in humans that supports the effect of providing antioxidants on improving the body's immune

system in menopausal women. In menopause there is also an increase in the risk of cancer due to a decrease in the production of the hormones estrogen and progesterone, resulting in a decrease in cytotoxic cell activity. Resveratrol, a natural polyphenolic compound, is often used as a nutraceutical because of the several effects it can offer, such as estrogenic, anti-inflammatory and antioxidant effects. Research by Di Credico et al. (2021) on 13 menopausal women concluded that giving RSV was able to increase the body's immunity through the cytotoxic activity of NK cells against malignant cells. In this study, an increase in the activity of CD8+ CTL and CD56+/16+ NK cells were found, although it was not significant (p value = 0.975 and 0.865). Administration of RSV therapy also resulted in a significant increase in total antioxidant capacity in cell lysates and supernatant in lymphocytes activated by IL-2. Although the mechanism of RSV administration on immune function is not yet known with certainty, the results of this study also revealed that the highest number of immune synapses were detected when RSV was administered during the activation and killing phase by cytotoxic cells. This means that administration of estrogen-like substances such as RSV can increase immune function through the formation of synapses between cytotoxic cells and target cells.[17]

In this study, although a trend was found in the form of an increase in the number of lymph nodules and the diameter of the germinal center, the administration of red dragon fruit peel extract did not have a significant effect. Discussions regarding the development and termination of the germinal center are still being debated today. The germinal centrum is a response that can occur and recover due to various specific mechanisms. The termination process is also very dependent on the antigen that affects it. Generally, the germinal center response lasts for approximately 3 weeks when induced by certain antigens, but the germinal center response due to viral infection can last longer. For example, after being infected with the VSV virus, the germinal centrum is still detectable up to 100 days after infection. This is because chronic infection due to viruses is associated with more efficient maturation and production of plasma cells and memory B cells. However, it needs to be emphasized that healing the infection does not necessarily result in termination/reducing the diameter of the germinal center. This may be due to residual antigens that are still present after the healing phase.[9]

There is research that more specifically discusses the number of lymph nodules and germinal center diameter by Trinaya et al. (2019) by giving a combination of Euphorbia milii flower tea and propolis to mice infected with Mycobacterium tuberculosis. The findings of this research show that the compounds in combination tea contain caffeic acid phenethyl esther (CAPE) which functions as an immunomodulator by increasing phagocytic activity by macrophages thereby activating IL-12. This causes the proliferation of B and T lymphocyte cells in the spleen to increase as a response to resistance against pathogens. Initially, the formation of lymph nodules occurs by T lymphocyte cells which ultimately results in the proliferation of B lymphocyte cells. The proliferation of B lymphocyte cells leads to the formation of antibodies and plasma cells, causing an increase in the diameter of the germinal center. In general, these two things occur due to spleen hyperplasia to increase phagocytic cells in the mechanism of resistance against pathogens.[18] The conclusion of this study is that in the context of infection by Mycobacterium tuberculosis, administration of combination tea can improve the condition of the body as indicated by an increase in the number of lymph nodules and diameter of the germinal center.

Other research such as administering ethanol extract of gotu kola leaves (*Centella asiatica*) to the spleen of rats induced by sodium nitrite by Afiqoh et al. (2017) gave insignificant results when administering a dose of 600 mg/kgBW/day for 42 days.[19] Insignificant results were also obtained by a similar study, namely the administration of isoflavones in soybeans to ovariectomized female rats.[10] This study showed a non-significant increasing trend in the difference in spleen weight of mice after administration of a high isoflavone diet (26.41% soy containing 1500 µg/g genistein and 800 µg/g daidzein) for 7 weeks. The consideration for giving high isoflavone dietary doses is based on soy consumption in Asian populations, namely 20 - 50 grams of soy a day. Apart from measuring the weight of the mice's spleens, the study also measured the total lymphocyte count (TLC) and lymphocytes transformation test (LTT). There was a significant increase in lymphocytes in mice that consumed soy compared to mice that consumed casein. This is thought to be because the isoflavone compounds in soybeans have estrogenic and anti-inflammatory properties by suppressing the regulation of chemokines and adhesion molecules that recruit leukocytes in the inflammatory process. In addition, the increase in lymphocytes also compensates for isoflavone resistance to oxidative stress that occurs after removal of gonad cells. In contrast to LTT parameters, administration of soy isoflavone significantly reduced lymphocyte transformation compared to the group that consumed casein and the group that was not ovariectomized. This LTT measurement aims to measure the sensitivity of the body's immune system to antigens. These results are supported by research by Elsayed et al., (2020) regarding the impact of administering isoflavone on implantation failure in mice. The results showed that administration of isoflavones had an effect on implantation failure in mice. Both of these may occur due to overregulation of isoflavone binding to estrogen receptor alpha, thereby potentially inhibiting T lymphocyte proliferation.[10] However, it is also stated that implantation failure may occur due to down-regulation of the progesterone receptor which fails to reduce overregulation of estrogen receptor alpha activation.[20]

Understanding the lymph nodules and germinal centrum is still a challenge. Several mechanisms may play a role in the development and termination of these two structures. The germinal center response does not always show observable changes. For example, the condition of the germinal center in CD80-deficient mice did not change along with decreased production of plasma cells, memory cells, and apoptosis. Another example, mice with Bam32 deficiency show defects in the germinal center response due to injection of sheep red blood cells at low doses, but not at high doses.[9] Meanwhile, in the context of menopause, so far, no research has been found that describes the condition of the germinal center during menopause or ovariectomy. The increasing trend in this study may occur due to the chronic condition of menopause which causes pro-inflammatory cytokines, which act as antigens, to continue to be produced by the body. Giving red dragon fruit peel extract for 30 days may not be enough to neutralize the body's condition back to its original state. A more potent compound is needed to maintain

the balance of free radical levels, considering that estrogen as a strong antioxidant has decreased/stopped production in the body.

**SUMMARY AND RECOMMENDATIONS**

This study found a non-significant increasing trend towards the number of lymphatic nodules and germinal center diameter due to administration of red dragon fruit (*Hylocereus polyrhizus*) peel extract to menopausal model rats. A similar study needs to be carried out by adding a group of rats that were not ovariectomized. Furthermore, analysis test of the rat white blood cells, analysis of the content fractions in the extract of red dragon fruit peel, and a longer study period needs to be conducted to support research data.

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