

***The Effect of Soybean Flour Extract on The Number of  
Limfosit Cell in Traumatic Ulcer  
(Study of Female Sprague Dawley Rats with Estrogen Deficiency)***

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**ABSTRACT:**

**Background:** Estrogen deficiency is a condition that often occurs in society, especially in women who experience menopause. Microscopically the gingival epithelium in postmenopausal women experiences atrophy at the gingival margins and cause the gingival tissue to easily experience desquamation. The epithelium that is thinning on the gingiva will also cause the gingiva to become susceptible to irritation and inflammation. Ulcers are lesions formed by local damage from the epithelium tissue. Ulcers that form in the oral mucosa are a picture of oral lesions that are very common in most people of various ages and genders. Phytoestrogens are a group of non-steroidal compounds that can be derived from plants that have estrogen-like behavior. There are 4 different classes of phytoestrogens, namely isoflavones, lignans, coumestenes, and stilbenes. Isoflavones contained in legumes, especially in soybeans, all processed rice, potatoes, fruits, and vegetables, while lignans are a minor element in cell walls, the fiber in seeds, fruits, vegetables, rice, and beans **Method:** This study used a laboratory experimental design in vivo using Sprague Dawley female mice as test animals. The number of samples in this study was 20 in 5 treatment groups consisting of 4 rats per group. The ELISA test was conducted to determine the concentration of estrogen in rat blood and the observation of the number of lymphocyte cells in the ulcer performed on days 1, 3, 5 and 7 with a microscope of 100x magnification with Hematoxylin-eosin (HE) staining on histological preparations. **Results:** The five groups carried out the Saphiro-Wilk normality test were obtained ( $p$ -value  $<0.05$ ) which means that the data distribution was not normal and the Kruskal-Wallis test was obtained ( $p > 0.05$ ) which means the average number of macrophage cells in all groups did not have a significant difference in the 7th day. The results of this study showed no significant difference between the number of cells of all groups on day 7. **Conclusion:** Giving soybean Tempe flour extract orally in Sprague Dawley ovariectomy rats for 30 days can increase the concentration of estrogen in the blood. Oral administration of soybean tempeh flour extract for 30 days on traumatic ulcer healing of female Sprague Dawley mice with estrogen deficiency did not affect the inflammatory phase which can be seen from the difference in lymphocyte counts, the difference was not significant.

**Keywords:** wound healing, lymphocyte, estrogen, Soybean Extract

## INTRODUCTION

Wounds are damage to the anatomical structure and function of the tissue. Damaged wounds damage the integrity of the skin epithelium or deeper, reach subcutaneous tissue and damage other tissues such as tendons, muscles, blood vessels, nerves, parenchymal organs, and even reach bone.

The wound healing process consists of 4 integrated and overlapping phases, namely hemostasis, inflammation, proliferation and tissue remodeling. These four phases must occur sequentially at the right time with optimal intensity. The inflammatory process involves many cells of the immune system, including neutrophils, basophils, mast cells, T cells, B cells, etc. Lymphocytes are a type of white blood cell that is part of the immune system. There are two main types of lymphocytes, namely, B cells and T cells. B cells produce antibodies that will attack bacteria, viruses, and toxins.<sup>1</sup>

Traumatic ulcers are oral lesions that often occur in the oral cavity, which are usually caused by damaged or sharp teeth, improper strokes. Dental instruments, bites, irritation of dentures, foreign objects, etc.

Estrogen deficiency can cause more bone absorption by cytokines produced by immune cells (monocytes and macrophages) and osteoblasts. The attachment of peridontal bacterial plaque biofilm products such as lipopolysaccharide, causes the host's immune system to produce more cytokines to activate osteoclasts which can absorb bone.<sup>2</sup>

phytoestrogens are estrogenic compounds derived from plants. Phytoestrogens can be classified as isoflavonoids and lignans. Isoflavonoids are found in legumes, especially in soybeans, all processed rice, potatoes, fruits and vegetables, while lignans are a minor component of cell walls, fibers in seeds, fruits, vegetables, rice, and beans.<sup>3</sup>

## RESEARCH METHOD

This study used a laboratory experimental design in vivo using Spargue Dawley female mice as test animals. The number of samples in this study were 20 in 5 treatment groups consisting of 4 rats per group, namely group 1 (without ovariectomy, induction of traumatic ulcer, given cognog, without soybean flour extract), group 2 (ovariectomy, induction of ulcer traumatic, given cognog and without soybean tempe flour extract), group 3 (ovariectomy, traumatic ulcer induction, non-cognate and given soybean tempe flour extract), group 4 (ovariectomy, traumatic ulcer induction, without cognog, without soybean flour extract) and group 5 (without ovariectomy, induction of traumatic ulcer, without being given cognog and without soybean tempe flour extract). The inclusion criteria in this study were female Sprague Dawley rats, age 3 months and body weight  $\pm$  170-200 grams. Exclusion criteria are mice that are pregnant.

This research was started by making soybean tempe flour extract with maceration technique. Before treatment, the test animals were adapted for seven days then ovariectomy in groups 2, 3 and 4 and then waited for seven days for the post-ovariectomy wound healing process. Seven

days after ovariectomy, in group 3 soybean flour extract was given for 30 days orally using a gastric sonde at a dose of 0.63 g / ml once a day. After giving soybean tempe flour extract for 30 days, traumatic ulcer induction was carried out in all groups. Group 1 and 2 were applied to the gingiva which had been induced by traumatic ulcers. Mandibular extraction was carried out on day 1, 3, 5 and 7 after induction of traumatic ulcers to take gingival samples which would be used as histological preparations. Histological preparations of macrophage cells were observed on days 1, 3, 5 and 7 using a 100x magnification microscope with 4 visual fields using HE staining. The normality test used is Saphiro-Wilk because the sample used in this study is less than 50. If the data distribution is normal, then the data is analyzed by one way ANOVA because the type of hypothesis of this study is comparative not paired with sample groups > 2. , if the data has a distribution that is not normal then the data analysis used is Kruskal-Wallis.

## RESULTS

The test carried out was a test of estrogen concentration by conducting an enzyme-linked immunosorbent assay (ELISA) test. Then the number of lymphocyte cells was calculated by microscopic observations in each group with haematoxylin-eosin (HE) staining

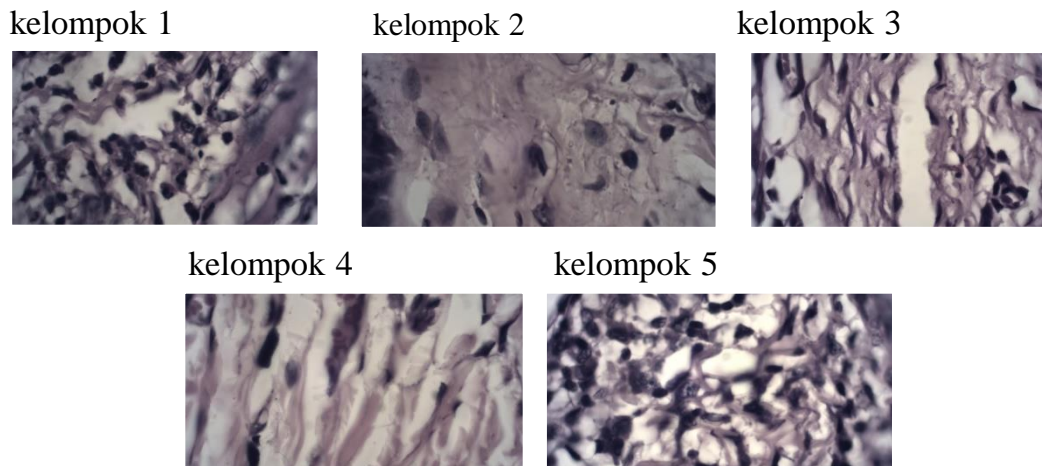
On the results of the ELISA test in the table above, it is obtained that the average estrogen group concentration 1 on the taking of the 8th day is 16.0425, on the taking of the 47th day 14.3625. Average group 2

estrogen concentration on taking the 8th day at 11.1525, on the 16th day at 10.4625, on the 47th day at 10.4125. The average estrogen concentration of group 3 on taking 8th day was 12.95, on the 16th day at 9.48, on day 47 at 13.7975. The average estrogen concentration in group 4 on taking the 8th day was 11.5625, on the 16th day at 11.1975, at day 47 of 6.42. Average group estrogen concentration 5

on taking the 8th day amounting to 15.0125, on the 47th day amounting to 13.84.

On the 30th day after the administration of soybean tempe flour extract, Group 2 decreased the concentration of estrogen to 10.4125. Group 3 experienced an increase in estrogen concentration to 13.7975 while Group 4 experienced a decrease in estrogen concentration to 6.4200. Group 1 and Group 5 decreased estrogen concentrations to 14.3625 and 13.8400.

Observation and counting the number of lymphocyte cells carried out on day 7 after traumatic ulcers. Microscopic observations with 100x magnification using Hematoxylin eosin (HE) staining with 4 visual fields for each one in group 1 (without ovariectomy, traumatic ulcer induction, given cognog, without soybean tempe flour extract), group 2 (ovariectomy, induction of traumatic ulcer, given cognog and without soybean tempe flour extract), group 3 (ovariectomy, induction of traumatic ulcer, without cognog and given soybean tempe flour extract), group 4 (ovariectomy, induction of traumatic ulcer without administration and without extract soybean flour) and group 5 (without ovariectomy, induction)



**Gambar 1.** Sel Limfosit Hari ke-7

Groups 3 4 and 5 on the 7th day were tested for Saphiro-Wilk normality, obtained (p-value <0.05) which means that the data distribution is not normal, then the Kruskal-Wallis test is obtained (p> 0.05) which means the average number of macrophage cells in all groups did not have a significant difference on the 7th day. In this study Mann Whitney test was also conducted to see whether or not there were significant differences between groups 3 and 5 and groups 3 and 4 and groups 3 and 2. obtained (p> 0.05) which means the average number of lymphocyte cells between groups does not have a significant difference without soybean flour extract, the average yield is obtained.

## **DISCUSSION**

Lymphocytes migrate to inflamed areas after the first day and can reach the maximum number on days 3 to 6, then decrease. The number of lymphocyte cells will increase in the inflammation phase to become chronic trade. inflamed tissue begins to enter the stage of wound healing that begins when the wound occurs and the loss of factors that affect the duration of the

inflammation process, which is approximately 3-7 days.<sup>4</sup>

The results of the kruskal-wallis test in groups 3 and 5 showed that there were no significant differences in the number of lymphocyte cells between groups of mice with estrogen deficiency and given tempe extract with a control group. These results indicate that soybean tempe extract can be able to replace lost estrogen after ovariectomy, this is in accordance with the theory which states that rats given tempe extract did not experience a decrease in estrogen concentration. In theory, isoflavones are a type of phytonutrients, which are rich in soybeans and soy products themselves. Isoflavones consist of four types, namely daidzein, genestein, glisitein and factor II. The structure of isoflavones is the same as the chemical structure of estrogen.

flavonoids which act as anti-oxidants that can limit the amount of free radicals. The anti-inflammatory effects obtained from flavonoids are also able to inhibit the release of degradative enzymes from neutrophils which can inhibit cross-linking of collagen<sup>5</sup>

endothelial and inhibits the proliferation phase and exudation phase of the inflammatory process. The inhibition of arachidonic acid release from inflammatory cells will cause less availability of arachidonic substrates for the cyclooxygenase and lipooxygenase pathways which will eventually suppress the amount of prostaglandin, prostacyclin, endoperoxide, hydroxatetraenoic acid, and leukotrin on the other side. Emphasis on the amount affects the inflammation process, and also leukocyte migration which will influence the suppression of the increase in the number of lymphocytes.<sup>8</sup> So, with the inhibition of the prostaglandin pathway by flavonoids and saponin it will reduce the occurrence of vasodilation of blood vessels and local blood flow so that emigration from leukocytes including lymphocytes area decreased.

Flavonoids are antioxidants that dissolve in water and cleanse free radicals which prevent oxidative cell damage and have strong anticancer activities. As an antioxidant, flavonoids provide anti-inflammatory activity<sup>10</sup>

Flavonoids can also be used as a vasculoprotector agent which is an agent to improve venous blood circulation by increasing vessel tone and reducing edema. The properties possessed by flavonoids are considered to have a role in the process of wound healing.<sup>9</sup> On the results of the kruskal wallis test between groups 3 and 4 on day 7, there was a significant difference in the number of lymphocyte cells. This is not in accordance with the theory which states that Estrogen increases humoral immunity, while androgens and

progesterone as immunosuppressors. Some physiological, pathological, and therapeutic conditions can alter serum estrogen levels, such as menstrual cycles, menopause, aging, and hormone replacement therapy, which can induce changes in immunity. Changes in immune response occur based on the phase of the menstrual cycle, which is associated with NK cell expenditure. Changes in immune response occur during the follicular phase. During the pre ovulation period there is a decrease in the cytolytic activity of NK cells, and during the luteal phase there is a change in cellular immune response to humoral. thus causing the immune system to become weak and more susceptible to microbial invasion and infection. Cytokines also play a role in the mechanism of ovarian follicle loss which will cause premature ovaries so that NK cells will decrease and T lymphocytes and B lymphocytes increase.<sup>6</sup> Group 4 incompatibility with the theory can be caused by controlled wound size or subject cleanliness after injury

In group 3 rats given soybean tempe flour extract compared to group 2 rats given cognog had no significant difference. Triamcinolone 0.1% is an ingredient in orabase, which is topical corticosteroids which generally have anti-itch and anti-inflammatory effects. The database term is showing that this drug is applied to the mouth. Database database is used for the treatment of acute and chronic lesions of the oral mucosa, this drug is recommended for use in stomatitisulserative, erosive lichen planus, denture stomatitis, desquamative gingivitis and stomatitis.<sup>7</sup>

In accordance with the table above the rats given tempeh extract did not

experience a decrease in estrogen concentration. In accordance with the theory, isoflavones are a type of phytonutrients, which are rich in soybeans and soy products themselves. Isoflavones consist of four types, namely daidzein, genestein, glisitein and factor II. The structure of isoflavones is the same as the chemical structure of estrogen.

The discussion above proves that soybean tempe flour extract can affect the

wound healing process due to various contents contained in it

## **CONCLUSION**

Based on the results of the study of calculating the number of lymphocyte cells in mice that have estrogen deficiency there is healing of traumatic ulcers, it can be concluded that: Estrogen deficiency, estrogen deficiency induced by ovariectomy shows can not affect the healing process

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