

The effect of soy tempe flour extracts on the number of macrophage cells of traumatic ulcer (the study of female *sprague dawley* rats with estrogen deficiency)

Mustiko Ajeng Widya P.¹, Dyah Triswari²

¹Mahasiswa Program Studi Kedokteran Gigi FKIK UMY

²Dosen Program Studi Kedokteran Gigi FKIK UMY

E-mail: ajengwidyap1@gmail.com

ABSTRACT

Background: Estrogen deficiency that occurs in women usually causes a variety of disturbing symptoms. Estrogen deficiency can also increase the risk of complaints in the oral cavity, one of them is traumatic ulcer. Traumatic ulcer is a lesions of the oral mucosa caused by various forms of trauma. Examples are trauma due to dentures, being bitten, traumatized by the use of toothbrushes that are too hard, sharp teeth and trauma due to dental procedures. The location of the occurrence of traumatic ulcers is usually in the lateral border area of the tongue, gingiva, mucosa of the lips and cheek mucosa. The occurrence of traumatic ulcers causes the presence of macrophage cell inflammation. Macrophage cells will immigrate to the inflamed area and phagocytes bacteria, viruses, necrotic tissue and harmful foreign particles. Phytoestrogens are a group of plants that have a structure and function similar to the hormone estrogen so that they can bind to estrogen receptors. Tempe made from soybeans is one source of phytoestrogens containing isoflavonoids which are included in the active substance phytoestrogens which are expected to increase estrogen in estrogen deficiency conditions so that it can reduce complaints in the oral cavity. **Method:** This study used a laboratory experimental design in vivo using Spargue Dawley female rats as test animals with a sample of 20 individuals in 5 treatment groups; traumatic ulcer and kenalog, ovariectomy, traumatic ulcer and kenalog, ovariectomy, traumatic ulcers and flour extract soybean tempe, ovariectomy and traumatic ulcer, only traumatic ulcer. The ELISA test was carried out to determine the concentration of estrogen in rat blood and observation of the number of macrophage 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 on the 7th day tested Saphiro-Wilk normality obtained groups 1, 2 and 3 with normal data distribution and group 4 and abnormal data distribution. Kruskal-Wallis test groups 3, 4 and 5 were obtained ($p > 0.05$) which means that the average number of macrophage cells did not have a significant difference on the 7th day. The Mann Whitney test was carried out in groups 3 and 4 of the 7th day which obtained 0.343 or ($p > 0.05$) which meant that there were no significant differences. Mann Whitney test in group 3 and 7 days 7 was obtained 0.317 or ($p > 0.05$) there was no significant difference. The Independent Sample T-test was carried out in groups 2 and 3 where 1,000 results were obtained or ($p > 0.05$), meaning that there were no significant differences in the average number of macrophage cells. The 7th day after the induction of traumatic ulcers was found to increase the pattern of the number of

macrophage cells in all groups. **Conclusion:** There are no effect of soybean flour extract on the number of macrophage cells in traumatic ulcers of female Sprague Dawley rats that have estrogen deficiency.

Keywords: wound healing, macrophage cells, estrogens, soybean tempe flour extract

INTRODUCTION

The estrogen hormone is the main sex hormone in women produced by the ovary. The imbalance of hormones in the body is influenced by menopause, old age, cancer treatment and illness that can cause estrogen hormone deficiency in women (Manurung et al., 2017). Hormone deficiency that occurs in menopausal women often causes symptoms, such as burning and redness in the skin, fatigue, anxiety, anxiety and decreased strength and calisification of bones throughout the body (Guyton & Hall, 2006). Menopause can also increase the risk of complaints in the oral cavity such as xerostomia, traumatic ulcers, gingivitis, periodontitis, and burning mouth syndorm (Farronato et al, 2012).

Estrogen affects cellular proliferation, differentiation and keratinization in the gingival epithelium (Carranza et al., 2012). The hormone estrogen also affects the effectiveness of the epithelial barrier against bacterial attacks and has an effect on the maintenance and repair of collagen. This can increase the susceptibility of traumatic ulcers (Markou et al., 2009). The occurrence of estrogen deficiency can slow or interfere with the wound healing process by inhibiting the activation of coagulation, thus disrupting the

hemostatic process that initiates the process of wound healing (Horng et al., 2017).

Traumatic ulcers that occur can be caused by various factors, such as trauma due to dentures, being bitten, the use of toothbrushes that are too hard, sharp teeth and trauma due to dental procedures. Clinical features of traumatic ulcers usually appear concave, oval and clearly demarcated which are covered by yellowish pseudomembranes, peripheral erythematous ulcers and are found to have infiltration of inflammatory cells, one of which is macrophages (Ongole and Praveen, 2013). Macrophage cells will immigrate to inflamed areas and phagocytes of bacteria, viruses, necrotic tissue and harmful foreign particles found in the area of the lesion (Guyton & Hall, 2006).

Niharika M. and Jyothi A. (2013) say that to overcome the symptoms that appear in women who experience estrogen deficiency at menopause, it can be done by consuming high concentrations of natural phytoestrogens. Isoflavones have two hydroxyl (OH) groups spaced 11.0 - 11.5 Å^o which are essentially, exactly the same as estrogen. It is this distance of 11 Å^o and OH group that becomes the main structure of a substrate in order to have estrogenic effects, so that it is able to bind to estrogen receptors which can increase estrogen in the body

(Achadiat, 2003). Anggrahini and Handayani (2014) conducted a study on the effect of consuming soy milk on complaints of menopause. The results of these studies indicate that there is an effect of consuming soy milk on a decrease in menopausal complaints.

MATERIALS AND METHODS

This study used a laboratory experimental design *in vivo* using Sprague 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 this type of hypothesis is comparative not paired with sample groups > 2, if the data has distribution is not normal then the data analysis used is Kruskall-Wallis.

RESULTS

This study aims to determine the effect of giving soybean flour extract to the number of macrophage cells on traumatic ulcers of female sprague dawley rats who have estrogen deficiency. Serum estrogen levels in rat blood were obtained by conducting an enzyme-linked immunosorbent assay (ELISA) test taken in rat blood samples before ovariectomy, on the 7th day after ovariectomy and on the 47th day after giving soybean tempe flour extract.

Table 1. Average estrogen concentration of rats blood.

Observations and calculations of the number of macrophage cells were carried out

Group	Average estrogen concentration of blood (ng/L)		
	Day 8 (before ovx)	Day 16 (7 days post ovx)	Day 47 (30 days post consuming extract)
Group 1	16.04	-	14.36
Group 2	11.15	10.64	10.41
Group 3	12.95	9.48	13.80
Group 4	11.56	11.20	6.42
Group 5	15.01	-	13.84

In the table above, the group induced traumatic ulcers and given cognog (group 1) on day 8 had estrogen concentrations totaling 16,042 then decreased to 14,3626 on day 47 (30 days after administration of group 3 extract). The group that was ovariectomized, induced traumatic ulcers and was given a cognog (group 2) on the 8th day before ovariectomy. having estrogen concentrations amounting to 11,1525 then 7 days after ovariectomy estrogen concentration decreased to 10.6425 and on day 47 after ovariectomy it decreased to 10.4125. The ovariectomy group, induced by traumatic ulcers and given soybean flour extract (group 3) on the 8th day before ovariectomy the estrogen concentration was 12,9500 and 7 days after ovariectomy decreased to 9,4800 and again increased to 13.7975 after 30 days of extraction. . The group that was ovariectomized and induced by traumatic ulcers, on the 7th day before ovariectomy the estrogen concentration totaled 11.5625 and decreased to 11.1975 7 days after ovariectomy then the 47th day after ovariectomy decreased to 6.4200. The group that was only induced by traumatic ulcers, the estrogen concentration on day 7 was 15.0125 and decreased to 13.8400 on the 47th day.

on days 1, 3, 5 and 7 after traumatic ulcers microscopically with 100x magnification using Hematoxylin eosin (HE) staining with 4 visual fields in all groups. Calculation of the number of macrophage cells in the 4 field of view carried out was obtained on average.

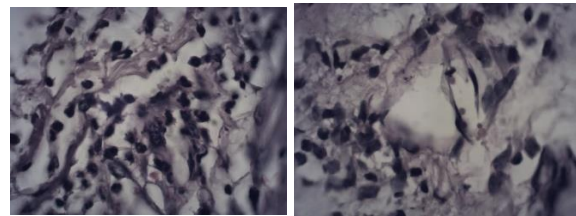


Figure 1. The microscopic picture of pacrophage cells on the 7th day in group 1 (left) and group 2 (right).

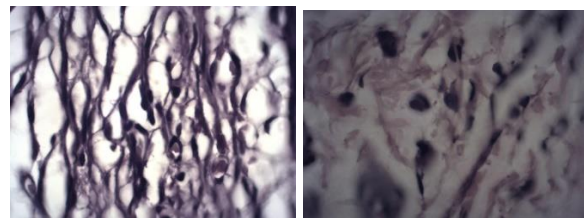


Figure 2. The microscopic picture of macrophage cells on the 7th day in group 3 (left) and group 2 (right)

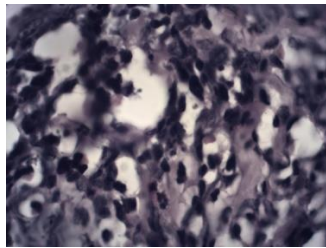


Figure 3. Microscopic picture of macrophage cells on the 7th day in group 5

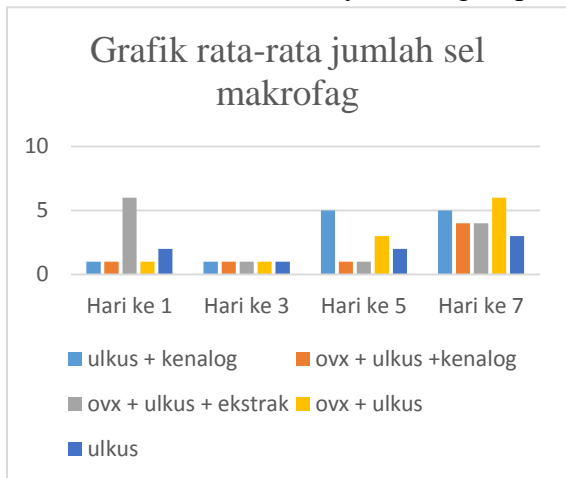


Figure 4. Graph of average number of macrophage cells in all groups on days 1, 3, 5 and 7.

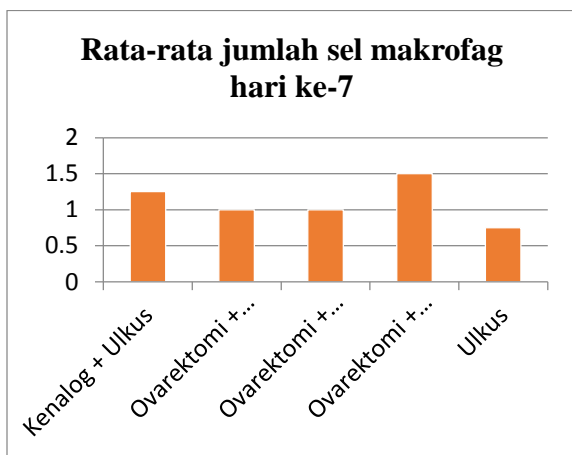


Figure 5. Graph of the average number of macrophage cells in all groups on day 7

The results of the study based on the graph in Figure 4 show that in group 1 which was induced by traumatic ulcers and given

cognog there was an increase in the number of macrophage cells on the 5th and 7th day. Group 2 which was ovariectomy, induced traumatic ulcers and given cognog increased the number of macrophage cells on the 7th day, while group 3 were ovariectomized, induced traumatic ulcers and were given soybean tempe flour extract increased on day 1 and then decreased on days. 3 and 5 then again increased on the 7th day. Group 4 which was ovariectomized and induced by traumatic ulcers increased the number of macrophage cells on the 5th and 7th day. Group 5 which was only induced by traumatic ulcers increased on day 1 and then declined on day 3 and returned to increase on the 5th and 7th days.

Based on the results of the study, showed that on the 7th day all groups experienced an increase in the number of macrophage cells. The increase in the number of macrophage cells that occurred on day 7 based on the graph in Figure 9 shows a difference in the average number of macrophage cells, but based on the results of data analysis using SPSS there were no significant differences. Increasing the number of macrophage cells that occur in all groups on the 7th day can be caused because the monocyte takes 48-72 hours to differentiate into macrophages in the injured area, so that an increase occurs on the 7th day (Hargreaves and Goodis, 2002).

Table 2. The results of the Saphiro-Wilk Normality Test for all groups on the 7th day

group	df	Sig.	
Number_of_cells	group 1	4	,406
	group 2	4	,683
	group 3	4	,683
	group 4	4	,024

group 5	4	,001
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Group 1: No ovariectomy, induction of traumatic ulcer, given cognog, without soybean flour extract.

Group 2: Ovariectomy, induction of traumatic ulcer, given cognog and without soybean flour extract.

Group 3: Ovariectomy, induction of traumatic ulcer, not given cognog and given soybean flour extract.

Group 4: Ovariectomy, induction of traumatic ulcers, without cognog, without soybean flour extract.

Group 5: Only induced traumatic ulcers.

Based on the data in Table 2, it shows that the results of the Shapiro-Wilk normality test obtained significance values for the number of macrophages in the group induced by traumatic ulcers and given cognog (group 1), groups that were ovariectomy, induced traumatic ulcers and given groups (group 2) and groups which was ovariectomy, induced traumatic ulcer and given tempel kedelao flour extract (group 3) of (p-value > 0.05) which means that the number of macrophage cells in groups 1, 2 and 3 had normal data distribution. The results of the normality test in groups 4 and 5 amounted to (p-value < 0.05). This shows that data on the number of 7th day macrophage cells in the group induced by traumatic ulcers (group 5) and the group ovariectomy and induced traumatic ulcers (group 4) had abnormal data distribution. Data testing was continued using the Kruskal-Wallis Test because the data distribution was not normal.

Table 3. Kruskal-Wallis Test results for groups 3, 4 and 5 on day 7

	Number_of_macrophage
Chi-Square	2.652
Df	2
Asymp. Sig.	.266

Based on the data in Table 3, it shows that the significance value is 0.266 or (p > 0.05). This value showed that there was no significant difference in the number of macrophage cells in group 3 ovariectomy, induction of traumatic ulcers and given soybean tempe flour extract, group 4 which was ovariectomy and induction of traumatic ulcers, and group 5 which was only induced by traumatic ulcers on the day -7.

Table 4. Mann Whitney test results between group 3 and group 5 on day 7

	number_of_macro phage
Mann-Whitney U	6.000
Asymp. Sig. (2-tailed)	.317

Based on the data in Table 4, it shows that the significance value is 0.317 or (p > 0.05) which means that there is no significant difference in the number of macrophage cells that are significant between groups 5 which are only induced by traumatic ulcer and group 3 which is ovariectomy, induced ulcer traumatic and given soybean flour extract on the 7th day.

Table 5. Mann Whitney test results between group 3 and group 4 on day 7

	number_of_macro phage
Mann-Whitney U	5.000

Asymp. Sig. (2-tailed) .343

Based on the data in Table 5, it shows that the significance value is 0.343 or ($p > 0.05$) which means that there is no significant difference in the number of significant macrophage cells between the ovariectomized groups, induced traumatic ulcers and given soybean tempe flour extract (group 3) with groups only ovariectomy and induced traumatic ulcers (group 4) on day 7.

Table 6. Independent Sample T-Test Test Results in groups 2 and 3 on day 7

Independent Samples Test			
Levene's Test for Equality of Variances		t-test for Equality of Means	
		Sig. (2-tailed)	Mean Difference
Number of macrophages	Equal variance assumed	1.000	.0000

Based on the data in Table 6, it shows that the significance value is 1,000 or ($p > 0.05$) which means there is no significant difference between the ovariectomized group, induced traumatic ulcer and given cognog (group 2) with the group ovariectomy, induced traumatic ulcer and given soybean tempe flour extract (group 3) on the 7th day.

DISCUSSION

Estrogen deficiency that occurs in menopausal women can increase the risk of complaints in the oral cavity, one of which is traumatic ulcers (Farronato et al, 2012). Decreasing the amount of estrogen in the menopause phase is one of the factors causing inflammation in the gingiva. Estrogen affects cellular proliferation, differentiation and keratinization in the Carranza et al., 2012 gingival epithelium. The effectiveness of the epithelial barrier against bacterial attack and its effect on maintenance and repair of collagen can also be influenced by the hormone estrogen (Markou et al., 2009).

Table 1 shows that the ovariectomized groups of groups 2, 3 and 4 experienced a decrease in estrogen concentration on the 7th day after ovariectomy, but group 3 again experienced an increase in estrogen hormone concentration after 30 days given soybean tempe flour extract. Groups 2 and 4 which had been ovariectomized and not given soybean tempe flour extract continued to experience a decrease in estrogen concentration on the 47th day. The results of the study based on the graph in Figure 4 show that in group 1 which was induced by traumatic ulcers and given cognog there was an increase in the number of macrophage cells on the 5th and 7th day. Group 2 which was ovariectomy, induced traumatic ulcers and given cognog increased the number of macrophage cells on the 7th day, while group 3 were ovariectomized, induced traumatic ulcers and were given soybean tempe flour extract increased on day 1 and then decreased on days. 3 and 5 then again increased on the 7th day. Group 4 which was ovariectomy and induced traumatic ulcers experienced increase in the number of macrophage cells

on the 5th and 7th day. Group 5 which was only induced by traumatic ulcers increased on day 1 and then declined on day 3 and returned to increase on the 5th and 7th days.

Based on the results of the study, showed that on the 7th day all groups experienced an increase in the number of macrophage cells. The increase in the number of macrophage cells that occurred on day 7 based on the graph in Figure 5 shows a difference in the average number of macrophage cells, but based on the results of data analysis using SPSS there were no significant differences. Increasing the number of macrophage cells that occur in all groups on the 7th day can be caused because the monocyte takes 48-72 hours to differentiate into macrophages in the injured area, so that an increase occurs on the 7th day (Hargreaves and Goodis, 2002).

Based on the Mann Whitney test showed that there was no significant difference in the group given only the treatment of traumatic ulcer (group 5), compared with the group given ovariectomy treatment, induced traumatic ulcer and given soybean flour extract for 30 days (group 3) at 7th day. The graph in Figure 5 also shows that there is no difference in the pattern of the average number of macrophage cells that are far between groups 5 which are only induced by traumatic ulcers with group 3 which are ovariectomy, induced traumatic ulcers and given estrak soybean flour. This means that the wound healing process is occurred in group 5 which had natural estrogen in the body not much different from group 3 which was ovariectomy and had estrogen derived from soybean tempe flour extract so that soybean flour flour extract could increase estrogen concentration in the blood of rats ovariectomized.

The increase in estrogen concentration was supported by the results of the Spargue Dawley ELISA test in Table 1 which showed that the estrogen concentration in group 3 which was ovariectomized increased after being given soybean tempe flour extract for 30 days. These results are in accordance with the theory by Gilani and Anderson (2002) that soybean tempeh contains isoflavones which have a structure and function similar to estrogen, which can interact with estrogen receptors so that it can increase estrogen concentration. The increase in estrogen concentration that occurred in this study was also supported by the study of Utami et al (2017) that the administration of soybean tempe flour extract for 20 days most affected the increase in weight of the uterus, endometrial thickness and thick myometrium in menopausal mice.

The average number of macrophage cells on the 7th day based on the graph in Figure 5 shows that although there was no significant difference between groups 5 which were only induced by traumatic ulcers with group 3 ovariectomy, traumatic ulcers were induced and soybean flour extract was given, but the average number of macrophage cells in group 3 was higher compared to group 5. The higher number of macrophage cells in group 3 could be due to factors consuming soybean tempe flour extract. Tempe contains Zn, Cu and Fe which can increase the activity of antioxidant enzymes, so that there is an increase in ability to inhibit oxidation reactions and body cell performance, one of which is an increase in macrophages (Ramprasath et al., 2005 and Rimbach et al., 2008).

Isoflavonoids, daidzein, phytosterol, genistein, saponins, phytic acid and protease inhibitors are active substances that are

thought to affect the ability of phagocytosis of macrophages (Koswara, 2006). This is supported by Nurrahman's (2015) study which showed that when given stimulants, phagocytic activity of macrophage cells increased in rats that consumed black soybean tempe for 30 days compared to mice that did not consume black soybean tempeh.

The Mann Whitney test results showed that there was no significant difference in the number of macrophage cells on day 7 between group 3 which was ovariectomized, induced traumatic ulcer and given soybean tempe flour extract with group 4 which was only ovariectomy and induced traumatic ulcers, but on average Descriptive number of macrophage cells in group 4 is higher than group 3. The results of the Independent T-Test based on Table 6 showed that there were no significant differences between the groups that were ovariectomized, induced traumatic ulcers and given cognog (group 2) with the group ovariectomy, induced traumatic ulcers and given soybean flour extract (group 3). Group 2 in this study uses the in-database method. Kenalog in orabase is a topical corticosteroid containing triamcinolone acetonide 0.1% which functions as an anti-inflammatory (Thantawi et al., 2014).

The absence of significant differences in the Mann Whitney test and the Independent T-Test can be caused by stress factors experienced by mice. When giving soybean tempe flour extract using gastric sonde, there were several rats who spewed soybean tempe flour extract and tended to rebel when they wanted to do the extract with the gastric sonde. Stress experienced can trigger activation of the HPA axis (hypothalamus and pituitary adrenocortical) which causes an increase in the secretion of the cortisol

hormone produced by the adrenal cortex, thereby suppressing the body's immune system which results in long healing of wounds (Boonen et al., 2013). When applying gel, the test animals also tended to rebel which caused the application of the intestinal ulcers which functioned as anti-inflammatory works less optimally, resulting in an increase in the number of macrophage cells in group 2 (ovariectomy, induced traumatic ulcers and given cognog) on the 7th day.

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