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Effect of Soybean Extract Cream on Matrix Metalloproteinase-1 (MMP-1) and Interleukin-6 (IL-6) Levels (In Vivo Experimental Study on UVB-Induced Balb/c Mice)

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ABSTRACT: UV radiation causes skin disorders that start from the formation of reactive oxygen species (ROS) and the synthesis of MMP-1 by dermal fibroblasts which plays a role in skin aging, resulting in an increase in IL-6 levels which correlates with tissue damage and inflammation that occurs. The compounds contained in soybean extract are known to act as anti-inflammatory and antioxidants which can reduce inflammation and reduce the negative impact of UV rays on the skin. This study aims to determine the effect of giving soybean extract cream on MMP-1 levels and IL-6 levels in the skin tissue of bablb/c mice induced by UVB light. This research design was a posttest only control group with a completely randomized design method. The samples studied were 36 female balb/c mice with UVB light induction with a wavelength of 302 nm and an energy of 1 MED 5 times a week for 2 weeks. This research was carried out in five groups, namely the healthy group (K1), negative control group (K2), positive control group (K3), with treatment group 1 (K4) with 10% soybean extract cream and treatment group 2 (K5) with extract cream. soybeans 20%. MMP-1 levels and IL-6 levels were analyzed using ELISA. ELISA analysis showed that there was a significant decrease in the mean MMP-1 concentration between K2 (6488 ± 805) compared to K5 (5396 ± 522) with a p value <0.05. Analysis of IL-6 concentration also showed that there was a significant decrease in the mean IL-6 concentration in K4 (270±50) and k5 (242±54) compared to K2 (661±90) with a p value <0.05. Giving soybean extract cream can reduce MMP-1 and IL-6 levels in female Balb/c mice induced by UVB light.

KEYWORDS: UVB light, soybean extract, MMP-1, IL-6

I. INTRODUCTION

Ultraviolet (UV) radiation from sunlight is one of the environmental factors involved in aging and skin disorders.[1] UV radiation increases the risk of long-term skin damage such as aging, immunosuppression, carcinogenesis, damaging skin proteins collagen and elastin, thus playing a role in various skin disorders such as photodermatosis, hyperpigmentation, skin aging, and precancerous lesions.[2] This is one of the health problems in Indonesia with a tropical climate, where there is currently a heat wave or heatwave and a high population with livelihoods in the outside environment. Skin disorders caused by UV radiation begin with the formation of reactive oxygen species (ROS) and the synthesis of MMP-1 by dermis fibroblasts that play a role in skin aging. UV light induces ROS radicals to act as secondary intermediaries to activate the MAPK family which leads to persistent genetic damage, characterized by increased expression of AP-1 and MMP. UV radiation is also able to reduce the expression of MMP inhibitors in collagen degradation, namely tissue inhibitors *of matrix metalloproteinase* (TIMP) and increased levels of IL-6 correlate with tissue damage and inflammation that occurs. In general, IL-6 is related to IL-1 and TNF-α, which means that these three cytokines can coordinate their removal from active monocytes, especially in inflammatory areas so they are often called proinflammatory cytokines (proinflammatory-cytokines).[3]

One of the natural ingredients that are widely found and contain anti-inflammatory and antioxidants is soybeans which can help relieve inflammation and reduce the negative impact of UV rays on the skin.[4] Previous research with oral administration of soybean extract doses of 10 mg and 15 mg was proven to be able to act as an antioxidant that can capture free radicals and prevent lipid peroxization so that they become more stable free radicals.[5] However, until now the dose of soybeans given topically still gets less than optimal results, so this study was conducted with increasing doses to assess MMP-1 and IL-6.

Effect of Soybean Extract Cream on Matrix Metalloproteinase-1 (MMP-1) and Interleukin-6 (IL-6) Levels (In Vivo Experimental Study on UVB-Induced Balb/c Mice)

The incidence of skin disorders due to UV rays increases every year along with the depletion of the ozone layer. The incidence of hyperpigmentation in 2020 increased by ~100,350 new cases.[6] An Australian study found that the rate of skin disorders associated with UV rays was 72% in men and 47% in women younger than 30 years.[7] This percentage increases with age, and is associated with solar keratosis and skin cancer. Epidemiological studies in North American populations with Fitzpatrick I-III skin types show a higher incidence of skin damage, which is 80-90%.[8]

Previous research said that soybeans (Soyben) is one source of protein that contains other bioactive components, one of which is isoflavonoids which are members of flavonoids that have antioxidant and antiinflammatory properties. In addition, flavonoids are also able to protect plants from UV rays and eliminate ROS produced by UV radiation and are able to inhibit gelatinase (MMP-2 and MMP-9) and neutrophil elastase (MMP-12). Meanwhile, in other studies other flavonoids such as genistein, baicalein, quercetin, and nobiletin have been reported to reduce MMP-1 expression in skin exposed to UV light.[9] Previous studies have identified the effects of soybeans on UVB skin damage. The isoflavonoid koumestrol in soybeans can prevent the formation of wrinkles by targeting FLT3 kinase. Biocanine isoflavonoid A inhibits cyclooxygenase-2 (COX-2) expression from UVB light.[10] Soybeans also have anti-inflammatory properties by decreasing the secretion of interleukin (IL)-6, IL-1, nitric oxide (NO), and prostaglandin E2.[11] The combination of soybean extract with other substances can enhance the positive effects of soybeans themselves, as in other experimental studies that used a mixture of soybean extract with *Haematococcus* extract and that used a mixture of soybean extract with collagen peptides. Both studies showed that the combination of substances with soy extract significantly increased MMP-1 inhibition.[12-13]

Several studies have proven that one of the important components contained in soybeans and acts as an antioxidant and antiinflammatory is isoflavones so that by giving soybean extract can reduce Oxidative Stress Reaction (ROS) and has the potential to reduce the negative effects of the aging process and damage due to UVB exposure to the skin. Therefore, based on this background, this study aims to determine the effect of giving soybean extract cream (soybean) at doses of 10% and 20% on MMP-1 and IL-6 levels in mice BALB/c induced by UVB light.

II. MATERIAL AND METHOD

Study Design and Experimental Animals

This research is an *in vivo* experimental research with *Post Test Only Control Group Design*. The study subjects used 30 female BALB / C mice aged 6-8 weeks weighing between 18-35 grams that met the inclusion and inclusion criteria, adapted for 1 week. This study used 5 treatment groups, a control group of healthy rats (K1), a negative control group of mice only exposed to UV light (K2), a positive control group in which subjects were only smeared with a cream base and exposed to UV-B (K3) light, treatment group 1 was the subjects were smeared with 10% soybean extract cream and exposed to UV-B (K4) light, and treatment group 2 was the subjects were smeared with 20% soybean extract cream and exposed to UV-B (K5) light, On the 15th day, female mice BALB/C were taken skin tissue samples to check MMP1 levels and IL-6 levels.

Research Materials

The research materials consist of basic cream, Soybean extract cream with a dose of 10%; 20%, UV-B lamp type *Narrowband* TL-F72-100W/12, Radiation dose meter (Dosimetry), *Standard solution, Assay diluent* A and B, Wash buffer concentrate, Substrate *solution A and B, Stop solution*, *and Plate sealer.*

Research Equipment

This study used several equipment, including tools used to make soybean extract, namely sterile storage bins, sterile glass spoons, *vacuum dryers*, rotator evaporators, blenders, and erlemeyer flasks, tools used for the maintenance of experimental mice, namely cages with complete food and drink places, 26 needles, 1 cc syringes, shavers, gloves, fixation points, and analytical scales, tools used for making preparations, namely glass *objects,* cover glass, scalpel knives, tweezers, cutting boards, sieves*, tissue*, freezers *(-20^{0}C),* microtome *machines, 46^{0}C* waterbaths*, automatic* processor machines, *vacuum* machines and blocking machines, and tools used for ELISA, namely *assay plates,* single micropipettes, multiple micropipettes. incubator, eppendorf tube, vortex.

Cream Making

Ingredients in the oil phase ; stearic acid, triethanolamine, gkycerin, potassium hydroxide and aquadest, weighed each ingredient then put soybean extract into a mortar and added tewwn to taste while stirring until homogeneous, then add basic cream M/A (vanishing cream), mix well then put the pot.

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UVB Irradiation Procedure

UV-B irradiation induces photoaging marked early by skin *erymatouse* in the exposed area and deeper wrinkles appear. Here are the steps: (1) Mice babl / c that have been adapted for 7 days. (2) Mice are anesthetized with a mixture of ketamine (60mg/kgbb) and xylasine (20mg/kgbb) intramuscularly as much as 0.5 ml. (3) The hair on the back is shaved with a size of 2 x 3 cm. (4) The back of mice is irradiated UV-B with a distance of 20 cm with a minimum erythema dose (1 MED 150mJ/cm2) for 8 minutes every 5x a week for 14 days. (5) Soybean extract is given at the same time every 10 am. (6) Mice babl/c ; The positive control group was then given topical treatment using a cream base, treatment groups 1 and 2 were given topically using soybean extract doses of 10% and 20% once a day for 14 days after UV-B irradiation.

Research Procedure

(1) Prepare 36 female mice of the BALB/C strain aged 6-8 weeks weighing 18-35 grams that have been adapted for 7 days **(2)** Shave the back of mice covering an area of 2x3 cm. **(3)** Shine the back of mice with UV-B light with a minimum dose of 1 MED with a distance of 20 cm for 8 minutes. **(4)** Give treatment to mice according to their group (each group of 6 mice), negative control: no treatment is given; Positive control: smeared cream base on shaved area, treatment 1: smeared 10% soybean extract cream, treatment 2: smeared 20% soybean extract cream, and **(5)** Repeat steps 5 times a week. **(6)** After the 14th day. Rest the mice. **(7)** Day 15, a biopsy is performed for further examination. **(8)** Check IL-6 and MMP-1 levels using ELISA. **(9)** Prepare reagents and solutions for ELISA. (**10)** Dilute the 120 *µL standard solution into the 120 µL* standard diluent. **(11)** Dilute *15 mL* wash buffer concentrate into 300 mL distilled water **(12)** Add 50 µL *standard* to each *plate*. **(13)** Add 40 µL of sample to *the plate.* **(14)** Close *the well* using a *sealer*. **(15)** Incubation for 60 minutes at 37°C. **(16)** Open the *sealer,* wash *the well* 3 times with *a wash buffer*. **(17)** Add 50 µL substrate *solution A* and 50 µL *substrate solution B* to each *well*. **(18)** Incubation for 10 minutes at 37°C. **(19)** Add 50 µL *stop solution* to each *well.* **(20)** Read absorbance results using ELISA *Reader* at 450 nm wavelength within 10 minutes after adding *stop solution.*

III. RESULT

Validasi MSCs

This study tested the effect of soybean extract cream on IL-6 and MMP-1 levels in 30 female Balb / c mice. Mice were first exposed to UVB light with a minimum dose of 1 MED with a distance of 20 cm for 8 minutes 5 x a week. Macroscopic observations were made to see *wrinkles due to UVB* exposure and based on observations it was seen that wrinkles were seen in mice exposed to UVB compared to those not exposed as shown in Figure 1. In addition, to strengthen validation, anatomical analysis was carried out using Masson Trichome staining to see the density of elastin due to UVB exposure. Based on previous research, elastin can be seen as a red image on the recording.[50] Based on the results of anatomical observations, it was found that there was a decrease in post-exposure elastin expression as shown in Figure 1.

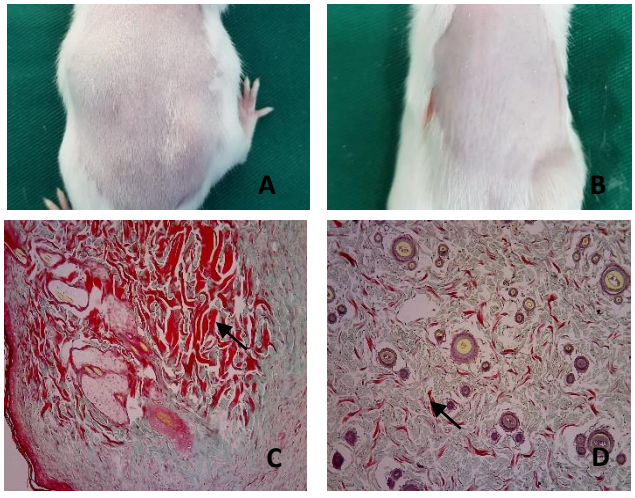

Figure 1. There are wrinkles in mice exposed to UVB (B) compared to those who are not exposed (A). Elastin shown in red (black arrow) was more prevalent in the group without UVB exposure (C), compared to the group with UV exposure (D)

The results of the IL-6 concentration analysis are shown in table 3. Based on the results of the analysis, it was found that the Healthy group (K1) had the lowest IL-6 concentration (135±37 ng/mL) which was then followed by K5 (242±54 ng/mL) and K4 (270±50 ng/mL), while the K2 group had the highest IL-6 concentration (661±90 ng/mL). Decreased IL-6 concentrations were found

in both doses of soybean extract cream compared to the K2 group. The Shapiro Wilk test was used to evaluate the distribution of normality data in each group of IL-6 concentrations within each group. The results of the Shapiro Wilk test showed that the distribution of IL-6 concentration data in the five groups was normal (P>0.05). For all five groups, the homogeneity test of data variance with the Levene test showed that the P value was greater than 0.05.

Table 1. IL-6 Analysis Data

VARIABLE	Group					P
	K1 (ng/mL) Average±SD	K2 (ng/mL) Average±SD	K3 (ng/mL) Average±SD	K4 (ng/mL) Average±SD	K5 (ng/mL) Average±SD	
IL-6	135±37	661±90	615±121	270±50	242±54	
Shapiro wilk						>0.05
Lavene test						0,227
One Way Anova						0,000

Based on normal and homogeneous data, the One Way Anova parametric test was used to determine the difference in the average concentration of IL-6 among the five groups. Based on the results of the One Way Anova test, the resulting data differed significantly (P<0.05), which showed that there was a marked difference in IL-6 concentration data in at least two groups. Then to test the relationship between groups, a Post Hoc LSD test was carried out, and data were obtained as shown in table 2.

Table 2. Differences in mean IL-6 concentrations between the two groups with Post hoc LSD Test

	K1	K2	K3	K4	K5
K1	-	0,000	0,000	0,006	0,024
K2	0,000	-	0,319	0,000	0,000
K3	0,000	0,319	-	0,000	0,000
K4	0,006	0,000	0,000	-	0,535
K5	0,024	0,000	0,000	0,535	-

The results of the post hoc LSD test of IL-6 concentration obtained p values < 0.05 for all groups compared to the K1 group. The results of the analysis also found that soybean extracts of groups K4 and K5 were significantly different compared to K2 (P<0.05). In addition, K4 and K5 also differed significantly compared to the K3 group (P<0.05). The data also showed that there was no difference between the K4 and K5 groups (P>0.05). The pattern of decline shown is dose dependent manner where the highest dose results in a significant decrease in IL-6 concentration as shown by Figure 2.

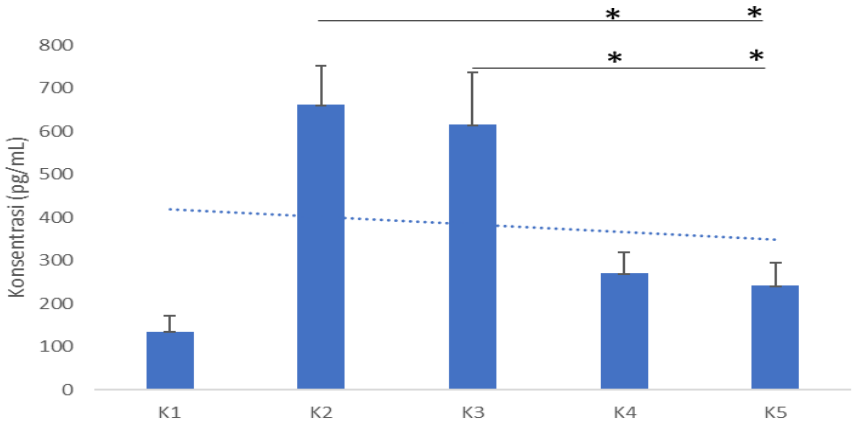

Figure 2. Graph of IL-6 concentration after soybean extract cream on mice exposed to UVB

Matrix Metalloproteinnase-1

The results of the MMP-1 concentration analysis are shown in table 3. The K2 group had the highest MMP-1 concentration (6488 ± 895), while the group with the lowest MMP-1 concentration was the Healthy group (K1) (3518±552 pg/mL) which was then

followed by the K5 group (5398±436 pg/mL). Both applications of soybean extract cream showed a decreased concentration of MMP-1. Since the MMP-1 concentration in each group is ratio data and the number of samples is not more than 50, the Shapiro wilk test is also used to analyze the distribution of normality data. The results of the Shapiro Wilk test showed that the p value in the five groups was greater than 0.05, so the distribution of MMP-1 concentration data in the five groups was normal. The results of the homogeneity test of data variants between the five groups with the Levene test resulted in a p value of 0.497 (p> 0.05) indicating that the variance of MMP-1 concentration data between groups was homogeneous. indicates that the variance of MMP-1 concentration data between groups is homogeneous.

Table 3. MMP1 Content Analysis Data

VARIABLE	Group					P
	K1 Average±SD	K2 Average±SD	K3 Average±SD	K4 Average±SD	K5 Average±SD	
MMP-1	3518±552	6488±895	6484±746	6093±440	5398±436	
Shapiro wilk						>0.05
Lavene test						0,518
One Way Anova						0,000

Based on normal and homogeneous data, the One Way Annova parametric test was used to determine the difference in the average MMP-1 concentration among the five groups. Based on the results of the One Way Annova test which yielded a P of 0.000, it showed that there was a significant difference in MMP-1 concentrations in at least two groups. The following Post hoc LSD test results showed significant differences in MMP-1 concentrations between the two groups. Post hoc LSD tests were performed to show differences in MMP-1 concentrations between the two groups and the results are shown in Table 4.

Table 4. Differences in mean MMP-1 concentrations between the two groups with Post hoc LSD Test

	K1	K2	K3	K4	K5
K1	-	0,000	0,000	0,000	0,000
K2	0,000	-	0,990	0,295	0,007
K3	0,000	0,990	-	0,301	0,007
K4	0,000	0,295	0,301	-	0,072
K5	0,000	0,007	0,007	0,072	-

The results of the post hoc LSD test of MMP-1 concentration obtained a p value of < 0.05 for all groups compared to the Healthy group. The results of the analysis also found that only the K5 group was significantly different compared to K2 (P<0.05). The pattern of decline shown is dose dependent manner where the highest dose results in a significant decrease in MMP-1 concentration as seen in Figure 3.

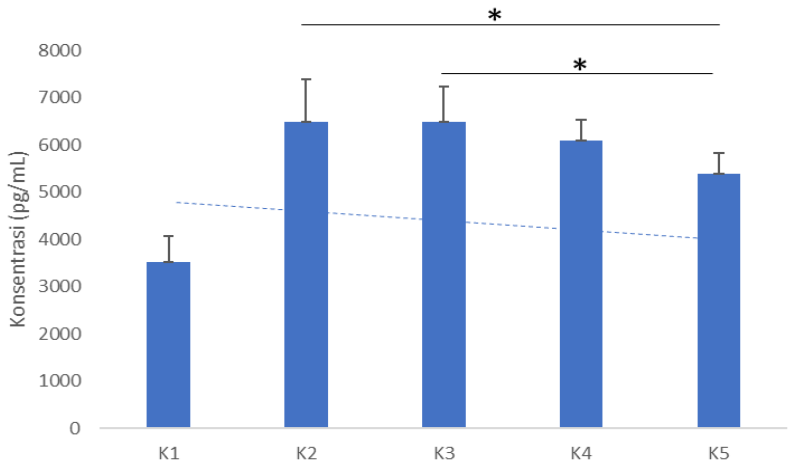

Figure 3. Graph of MMP-1 concentration after administration of soy cream extract in mice exposed to UVB

IV. DISCUSSION

The photoaging process involves a series of complex changes in the skin in response to UV exposure.[53.54] UV exposure to skin areas can cause damage to DNA that increases the formation of *reactive oxygen species* (ROS) compounds thereby increasing oxidative stress.[55] The formation of ROS leads to the activation of NF-kB local immune cells that cause inflammation characterized by an increase in pro-inflammatory cytokines such as IL-6 in the skin area.[56.57]

The inflammation that triggers production by IL-6 can cause it to attract other immune cells such as neutrophils and also monocyte cells to come to the UVB-exposed area.[57.58] These immune cells produce various lytic molecules, including MMP-1 in skin tissue and become the main driving factor in the degradation process of the extracellular matrix (MES) thus triggering the development of *photoaging*.[57.59]

The active compound contained in soybeans, namely genistein, can inhibit the inflammatory response pathway through regulating ROS balance.[60-62] Genistein has been shown to reduce ROS and inhibit the production of inflammatory cytokines, including IL-6.[63,64] In addition, one of the main mechanisms of genistein in reducing inflammation is through inhibition of the NF-κB pathway, which is a transcription factor to regulate the expression of genes involved in the inflammatory response. Genistein can inhibit NF-κB activation and block the transfer of this factor to the nucleus, thereby reducing the production of pro-inflammatory cytokines, including IL-6. [65-67]

According to previous research, one of the other active compounds contained in soybeans, namely isoflavones, has an important role in regulating the activity of Mitogen-Activated Protein Kinase (MAPK), which is a series of signaling pathways consisting of ERK1/2 (Extracellular Signal-Regulated Kinase 1/2), JNK (c-Jun N-terminal Kinase), and p38.[68] Each of these MAPK pathways is involved in a variety of cellular functions, including proliferation, differentiation, and response to stress or inflammation.

Isoflavones have been shown to inhibit MAPK activation, including ERK1/2, JNK, and p38 by inhibiting phosphorylation or ERK1/2 activation, which is a key step in the signaling pathway that regulates MMP-1 gene expression.[68] By inhibiting the MAPK pathway, isoflavones can indirectly reduce the expression of the MMP-1 gene so as to reduce damage to the extracellular matrix that leads to inhibition of photoaging.

Based on the results of research that has been done, it can be concluded that soybean extract cream can play a role in reducing IL-6 and MMP-1 concentrations through inhibition of MAPK pathways, free radicals, and inflammatory pathways. However, this study only looked at the effect of soybean peel extract on IL-6 and MMP-1 without examining pathways that regulate both proteins such as MAPK or NF-B. This has led to the inability to uncover the mechanisms behind IL-6 and MMP-1 inhibition. In addition, this study used whole extracts that cause unknown active molecules such as daidzein, and genistein which play a role in the inhibition of IL-6 and MMP-1. These things become limitations of this study which should be the basis for future research.

V. CONCLUSION

1. There is an effect of soybean extract cream (Glycine Max) on reducing the concentration of IL-6 and MMP-1 in bentina mice BALB/c exposed to UV-B.
2. There was a difference in IL-6 concentrations in the K4 and K5 groups given soybean extract cream at doses of 10% and 20% compared to the control group.
3. There was a difference in the amount of expression of Matrix Metalloproteinase-1 in the K5 group given soybean extract cream at a dose of 20% compared to the control group.

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