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Effect of Astaxanthin Cream on MMP-1 and SOD Expression in Wistar Rats Exposed to ultraviolet B

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Abstract: Ultraviolet B (UVB) exposure is the main cause of skin damage leading to photoaging, increasing Reactive Oxygen Species (ROS) and triggering the production of Matrix metalloproteinase-1 (MMP-1) enzymes that play an important role in photoaging, describing clinical signs including wrinkles, thickening, dryness and pigmentation. Astaxanthin can inhibit ageing and reduce wrinkles, has strong antioxidant potential that can neutralize ROS, and suppress MMP-1 activity. The purpose of this study was to determine the effect of astaxanthin cream on the levels of MMP-1 and Superoxide Dismutase (SOD) enzyme production in mouse skin tissue exposed to short-term UVB light experimental research with a post-test-only control group design. The experimental animals used were 28 Wistar rats divided into 4 groups, namely group K1 (healthy), group KN exposed to short-term UVB and given base cream, group P1 exposed to short-term UVB rays and given 0.05% astaxanthin cream, and group P2 exposed to short-term UVB rays and given 0.1% astaxanthin cream. The treatment was carried out for 5 days, and on the 6th day, the levels of MMP-1 enzyme production in skin tissue and SOD were examined using the immunohistochemical method. Based on One-way ANOVA, there was a significant difference in the average levels of MMP-1 enzyme production in the skin tissue of all groups ($p = 0.000$). The highest MMP-1 production was found in the KN group, while in the P2 group, the levels were lower and not significantly different from the healthy group (K1). Significant differences in SOD expression were also observed among all groups ($p = 0.000$) using the Kruskal-Wallis test. The highest SOD expression was found in group P2 and was significantly different from groups P1 and KN. Topical astaxanthin cream administration affected MMP-1 enzyme production in skin tissue and SOD expression in Wistar rats exposed to short-term UVB radiation. Astaxanthin-based cream formulation has the potential to be developed as a topical agent to prevent skin damage caused by sun exposure, and these findings require further validation through studies on human skin models or early clinical trials.
Keywords: Astaxanthin cream; superoxide dismutase expression; matrix metalloproteinase-1; ultraviolet B.

INTRODUCTION

Excessive exposure to UVB rays affects the epidermal layer, resulting in skin inflammation (Lee et al., 2021), increases Reactive Oxygen Species (ROS) and triggers the expression of Matrix metalloproteinase-1 (MMP-1), which plays an important role in photoaging (Pittayapruek et al., 2016). Photoaging describes clinical signs, including wrinkles, thickening, dryness and pigmentation (Chiu et al., 2017). Conventional topical administration can change the skin tone to darker, causing the

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effects of allergic dermatitis/irritants and ochronosis (Nautiyal & Wairkar, 2021). Subcutaneous morphological changes that affect the shape of the face impact a person's appearance according to their age (Pittayapruek et al., 2016). Skin appearance supports activities (Hamer et al., 2017). The development of alternative therapies using antioxidants has been tested to prevent and improve skin health (Yoon et al., 2014). The use of creams facilitates penetration into the skin (Hagavane et al., 2022). Astaxanthin has high UV light absorption and bioactive benefits for health, such as anti-inflammatory, so it can inhibit ageing and reduce wrinkles. Astaxanthin has a strong antioxidant potential that can neutralize ROS and suppress MMP-1 activity. (Zakaria et al, 2021). The effectiveness of astaxanthin in suppressing UVB-induced skin damage through the MMP-1 and SOD pathways requires further research.

The World Health Organization estimates that approximately 11 million cases of sunburn occur each year (Gaddameedhi et al., 2015). Sunburn, due to UV exposure and DNA damage, increases the risk of skin cancer, both melanoma and nonmelanoma(Gromkowska et al., 2021). In the United States, 34% of 31,162 respondents reported experiencing sunburn, especially in 18–29 year olds with Fitzpatrick skin types I–III (Guerra & Crane, 2022). Studies show that the frequency of sunburn and total sun exposure is directly proportional to the risk of skin damage and cancer (Holman et al., 2018),(Hung et al., 2022). In Indonesia, high outdoor activities increase the risk of skin cancer. According to the Indonesia Cancer Care Community, in 2018, there were 6,170 cases of nonmelanoma skin cancer and 1,392 cases of melanoma (Jordaniel et al., 2021).

Exposure to UVB rays is the main cause of skin damage that leads to photoaging (Chiu et al., 2017). The inability of cells to replicate due to the continuous cessation of the cell cycle due to many stressors, including oxidative stress, DNA damage, and telomere shortening that causes cells to age (Markiewicz et al., 2022). The degradation of collagen and elastic fibres occurs due to an increase in the matrix of metalloproteinases (MMPs) secreted by dermal fibroblasts and epidermal keratinocytes in response to UVB radiation. In addition, MMP secretion and activation are stimulated by various inflammatory cytokines secreted by keratinocytes by ROS from radiation-exposed cells. Hence, the suppression of inflammatory cytokines is essential for inhibiting skin damage (Tominaga et al, 2017).

Antioxidants can neutralize ROS and stabilize free radicals so that they can donate electrons (Wang et al., 2018). The formation of ROS can be inhibited by superoxide dismutase (SOD), which is widely expressed in the cytoplasm and plays an important role in protecting the skin's antioxidant defence mechanisms from oxidative stress (Andarina & Djauhari, 2017). Optimal SOD activity can reduce the negative impact of these free radicals, thereby inhibiting the photoaging process (Papaioannou et al., 2011). Using astaxanthin can increase antioxidants, elasticity and integrity of ageing facial skin as anti-ageing and inhibiting photoaging (Tominaga et al., 2017). Astaxanthin can be produced sustainably and environmentally friendly and is a natural alternative to cosmetic ingredients compared to other sources of astaxanthin (Papaioannou et al., 2011).

Several previous studies have shown that astaxanthin is effective in suppressing MMP-1 expression and increasing SOD. A study by Tominaga et al. (2017) showed the protective effect of astaxanthin on skin ageing in humans through increased skin elasticity. Studi oleh Yoon dkk. (2014) juga menunjukkan bahwa astaxanthin dapat melawan efek oksidatif dengan merangsang sistem antioksidan endogen. However, most of these studies were conducted at the cellular level or clinical studies without a specific acute UVB exposure model or have not discussed

the topical effectiveness of astaxanthin in suppressing MMP-1 and increasing SOD simultaneously in skin tissue in vivo. Therefore, this study aims to examine the effect of topical astaxanthin cream on the level of MMP-1 production and SOD expression in the skin of Wistar rats exposed to UVB rays acutely. This study is expected to provide a scientific basis for developing astaxanthin as an effective and safe topical antiphotoaging agent. Based on the description above, a study will be conducted to see the effect of astaxanthin cream on the expression of MMP-1 and SOD in the skin tissue of Wistar strain rats exposed to acute UVB rays.

MATERIALS AND METHODS

This type of research uses a Post Test Only Control Group Design research design conducted at the Integrated Biomedical Laboratory (IBL) of Sultan Agung Islamic University Semarang and at the anatomical pathology laboratory of Sultan Agung Islamic University Semarang from October to December 2024. The subjects of this study were male Wistar rats (Rattus norvegicus) aged 2-3 months, weighing 200-250 g, healthy rats with no macroscopic abnormalities, and actively eating and drinking. The research sample was 28 rats divided into 4 groups, each group consisting of 6 rats. The minimum sample size in this study was calculated based on the Federer formula, which was 6 mice per group. To avoid loss of following, 1 experimental animal was added to each group, so there were 7 experimental animals in 1 group. This sample size is representative and meets the minimum requirements for parametric statistical tests, such as one-way ANOVA, and non-parametric tests, such as Kruskal-Wallis, which are both used in this study. A sufficient number of subjects per group also helps ensure the validity of the results and strengthens the detection power of differences between groups. The healthy group (KS) is a group of mice that are given standard feed and unlimited distilled water, mice in healthy conditions without treatment for 5 days. The negative control group (KN) is a group of mice that are given standard feed and unlimited water, then exposed to short-term UVB light and applied with base cream for 5 days. Treatment group 1 (P1) is a group of mice that are given standard feed and unlimited water, then exposed to short-term UVB light and applied with astaxanthin cream at a dose of 0.05% for 5 days. Treatment group 2 (P2) is a group of mice that are given standard feed and unlimited water, then exposed to short-term UVB light and given astaxanthin cream at a dose of 0.1% for 5 days.

Ethical Clearance

This research was conducted after receiving ethical clearance approval from the Faculty of Medicine, Sultan Agung Islamic University, Semarang, with No 213/VI/2024/Bioethics Commission.

Research Materials and Tools

The materials needed in this study are: Formalin 10% neutral buffered, Paraffin, Xylol (Xylene), Graded alcohol (ethanol 70%, 80%, 95%, 100%), Distilled water / aquadest, Buffer (PBS / TBS - Phosphate / Tris Buffered Saline, pH 7.4), Antigen Retrieval Solution, Blocking agent, Primary antibody (anti-MMP-1 or anti-SOD), Secondary antibody conjugated, Reporter enzyme, HRP (Horseradish Peroxidase), AP (Alkaline Phosphatase), Substrate-Chromogen DAB (3,3'-diaminobenzidine), AEC (Aminoethylcarbazole), Counterstain, Hematoxylin, Ammonia water/solution, Mounting medium and Cover glass and glass slide (glass object).

The research equipment needed in this study is a Tissue Processor (Merk Thermo Scientific Excelsior ES), Cutter / Knife, Cutting Board, Iron Ruler, Tape, Micron

Blade, large brush, Small Brush, Tweezers, Measuring Cup, Beaker, Funnel, Tissue, Gloves, Mask, Apron, Lab Coat, Google Glass, Filter Paper, and Gauge.

Astaxanthin Cream Preparation

The preparation of astaxanthin cream with a cream formulation will be as follows: Prepare the necessary tools and ingredients; all ingredients are weighed according to their respective weights. The oil phase (Sponge 80, liquid paraffin, propyl, and paraben) is put into a porcelain cup and then melted over the bath as a mixture 1 then the water phase (sponge 80, propylene glycol, methyl parabens, and aquaades) is put in a beaker glass. Heat over a water bath, stir until all the ingredients are dissolved as a mixture of 2 phase oils (tween 80, propylparaben, liquid paraffin, and VCO) and mixture 1 is poured into mixture 2 little by little and stirred using a homogenizer until homogeneous until a creamy mass is formed (Federer, 1977).

Acute UVB exposure procedure in mice

The procedure for giving UVB exposure to rats is as follows: After adaptation, the dorsal (back) hair is shaved using a shaver to open access to the skin. The shaved area has a diameter of about 1 cm. This shaving aims to reduce the resistance of the coat layer so that UVB exposure can directly hit the skin of the rats. The back of the mice was exposed to UVB light at a distance of 30 cm, and the total dose was 160 mJ/cm2 for 15 minutes/day for 5 days. The rats in treatment groups 1 and 2 were given topical astaxanthin cream at 0.05% and 0.1% once a day for 5 days and one hour after acute UVB irradiation.

Skin tissue sampling in mice

Skin tissue sampling was carried out after 5 days of treatment; tissue sampling was carried out on the sixth day with the following stages: Mice were terminated by euthanasia using diethyl ether gas or vapour so that the mice lost consciousness and eventually died. Skin tissue sampling on the skin exposed to UVB light using the biopsy method. To prevent tissue degradation, tissue sections must be taken carefully to maintain their structure and morphology fixation with 10% buffered formalin solution. To remove water from the tissue, perform cascade ethanol sequences (70%, 80%, 90%, 95%, and 100%). The embedding process involves using liquid paraffin to cut the tissue, placing it on a mould with the appropriate orientation, and allowing the hardening of the paraffin at room temperature. Cut the paraffin-embedded tissue into thin slices (3–5 μm) using a Thermo Scientific HM325 microtome. Place the tissue slices on a glass slide with adhesive (e.g., poly-L-lysine) to prevent the tissue from coming off during staining. Dry the slides at 37–40 °C for 12–24 hours. Incubate the slides in xylene 2–3 times (5–10 minutes per step) to remove paraffin. Rehydrate with graded ethanol (100%, 95%, 90%, 80%, and 70%) and rinse with distilled water.

MMP-1 Expression Analysis Procedure Using Immunohistochemical Methods

The procedure for analyzing MMP-1 expression by immunohistochemical method is as follows: Incubation of slides in Citrate Buffer pH 6.0 antigen retrieval solution with a water bath for 10–20 minutes; slides are allowed to cool to room temperature, then rinse with PBS or FFB. Incubate the slide with serum blocking or 1–5% BSA for 15–30 minutes at room temperature to prevent non-binding of antibodies. Add a specific primary antibody against MMP-1, Incubate the slide at 4°C or 1–2 hours at room temperature, then rinse with PBS or FFB to remove the unbound antibodies. Add the secondary antibody conjugated with the enzyme and incubate for 30–60 minutes at room temperature. Rinse again with PBS or FFB, adding an enzyme substrate, such as DAB (3,3'-diaminobenzidine), which produces a brown colour at the antigen site. Incubate until colours appear (1–10 minutes), then stop the reaction by rinsing the slides with distilled water. Use a core dye such as hematoxylin to provide

contrast. Rinse the slide with running water to remove any remaining staining. Add mounting media, such as entellan or glycerol, to the slide, then cover with a coverslip. A light microscope is used to observe the expression of MMP-1; the brown colour indicates the location of the expression of MMP-1 (Ardiyan, 2020).

SOD Expression Analysis Procedure Using Immunohistochemical Methods

The procedure for analyzing SOD expression by immunohistochemical method is as follows: Incubation of slides in Citrate Buffer pH 6.0 antigen retrieval buffer solution with a water bath for 10–20 minutes; slides are allowed to cool to room temperature, then rinse with PBS or FFB. Incubate the slide with serum blocking or 1–5% BSA for 15–30 minutes at room temperature to prevent non-binding of antibodies. Add specific primary antibodies against SOD. Incubate the slide at 4°C or 1–2 hours at room temperature, then rinse with PBS or FFB to remove unbound antibodies. Add the secondary antibody conjugated with the enzyme and incubate for 30–60 minutes at room temperature. Rinse again with PBS or FFB, adding an enzyme substrate, such as DAB (3,3'-diaminobenzidine), which produces a brown colour at the antigen site. Incubate until colours appear (1–10 minutes), then stop the reaction by rinsing the slides with distilled water. Use a core dye such as hematoxylin to provide contrast. Rinse the slide with running water to remove any remaining staining. Add mounting media, such as entellan or glycerol, to the slide, then cover with a coverslip. Use a light microscope to observe the expression of SOD; the brown colour indicates the location of the expression of SOD (Ardiyan, 2020).

Data Analysis

The research data was obtained using a data normality test with the Shapiro-Wilk test and a homogeneity test with the Levene test. The results of MMP-1 expression analysis were normal and homogeneous (P>0.05), so a parametric test was carried out with the One Way Anova test to see the differences between all groups and continued with the Post Hoc LSD test to find out the differences between the two treatment groups. While the average expression of SOD was obtained from abnormal data and homogeneous data variants, the Kruskal Wallis non-parametric test was carried out to see the differences between all groups and continued with the Mann-Whitney test to find out the differences between the two groups. The decision to accept or reject the research hypothesis is based on the 5% α and data analysis processing in this research using the SPSS application.

RESULTS AND DISCUSSION

On day 6 after treatment, skin tissue samples of Wistar rats were taken to measure MMP-1 and SOD expression by immunohistochemical method.

Mean and Results of MMP-1 Expression Analysis in Rat Skin Tissue Exposed to Acute UVB Light

The average and results of MMP-1 expression analysis in rat skin tissue exposed to acute UVB light can be seen in Table 1 and Figure 1. Based on Table1 and Figure1, the average expression of MMP-1 in the healthy group (KS) was 18.16%, the negative group (KN) was 48.10%, the treatment group 1 (P1) was 28.60%, and the treatment group 2 (P2) was 23.00%. The highest expression of MMP-1 was found in the KN group, while the lowest expression of MMP-1 was KS. The results of the Shapiro-Wilk test on the expression of MMP-1 in all groups were normally distributed with a value of (p>0.05) and had a homogeneous data variant with the Leuvene Test result of 0.649 (p>0.05). The analysis results with the One-way ANOVA test obtained a value of p=0.000 (p<0.05), which means that there was a significant difference in the

mean expression of MMP-1 in all groups. The results of the analysis with the Post hoc LSD test to determine the differences between groups can be seen in Table 2.

Table 1. Results of the Average Description of MMP-1 Expression and the One-Way Anova Test

Group	KS	KN	P1	P2	*P value*
Expression MMP-1 (%)					
Mean	18.16	48.10	28.60	23.00	
SD	0.39	0.27	0.22	0.63	
Shapiro-Wilk	*0.452	*0.554	*0.078	*0.101	
Leuvene Test					*0.649
One way Anova					*0.000

*Shapiro-Wilk = Normal (p>0,05)
*Leuvene Test = Homogeneous (p>0,05)
*One Way anova = significant (p<0,05)

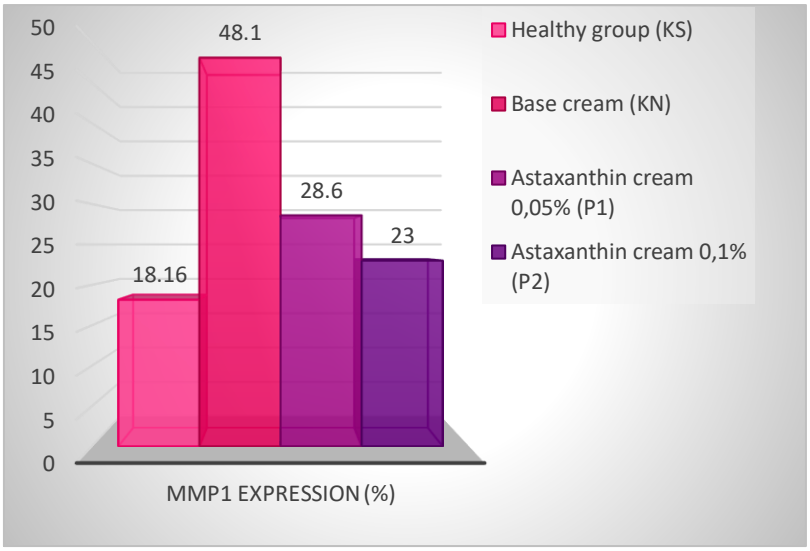

Figure 1 Mean MMP-1 Expression of Each Treatment Group

Table 2 Post Hoc LSD Test of MMP-1 Expression in Each Group

Group	**KS**	**KN**	**P1**	**P2**
KS	-	*0,000	*0,000	*0,000
KN	-	-	*0,000	*0,000
P1	-	-	-	*0,000

*Means p<0,05

Table 2 presents the results of the Post Hoc LSD test to distinguish the mean expression of MMP-1 between groups. These results show a significant difference between the KS and KN groups: KS with P1, KS with P2, KN with P1, KN with P2, and P1 with P2.

Microscopic immunohistochemical (IHC) results on MMP-1 expression were performed by 3,3'-*Diaminobenzidine* (DAB) staining; DAB staining is a technique to detect the presence of specific antigens or proteins in tissues. DAB is a chromogen that produces the brown colour that is visible under a microscope after reacting with a peroxidase enzyme, such as *horseradish peroxidase* (HRP), which is associated with secondary antibodies, as shown in Figure 2 below:

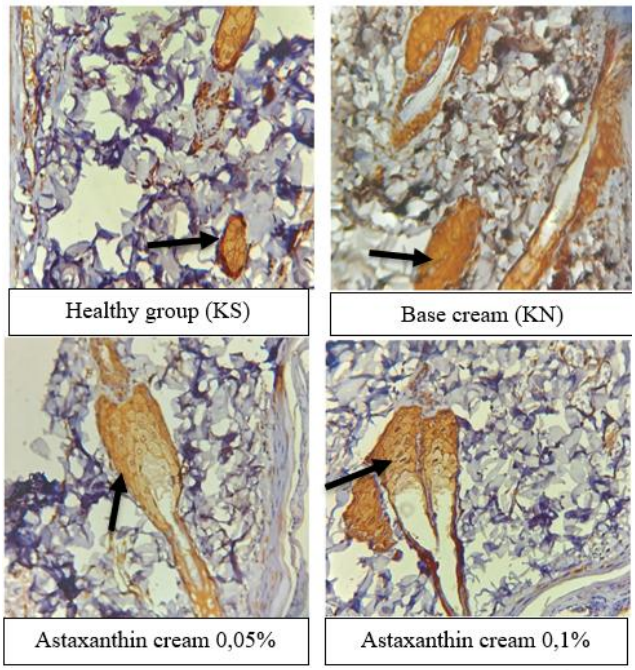

Figure 2 Immunohistopathology of MMP-1 Expression İn Each Group

Based on Figure 2. MMP-1 expression was analyzed microscopically using immunohistochemistry (IHC) by calculating the percentage of brown-positive cells from the total cells in five fields of view, analyzed using ImageJ software by an anatomical pathologist. The observation results of the lowest average MMP-1 expression were in the healthy group (KS) at 18.16%, reflecting normal skin conditions without oxidative stress or significant tissue damage. The highest average MMP-1 expression was in the negative group (KN) at 48.10%, indicating a strong induction of MMP-1 activity in response to stress exposure, such as UV light or inflammatory conditions, which triggers collagen degradation in the extracellular matrix. The average MMP-1 expression in the group (P1) was 28.60% lower when compared to KN (48.10%), indicating a protective or inhibitory effect of the treatment intervention in reducing collagen degradation. MMP-1 expression in the treatment group was still higher than in the healthy group, indicating that the treatment had not completely normalized MMP-1 activity. However, the average in P1 was still higher when compared to treatment group 2 (P2) (23%). These results show that the administration of astaxanthin cream affects MMP-1 expression in male white rats of the Wistar strain exposed to acute UVB light, where a dose of 0.1% is better than a dose of 0.05%.

Results of SOD expression analysis in rat skin tissue exposed to acute UVB rays

The average and results of SOD expression analysis in rat skin tissue exposed to acute UVB light can be seen in Table 3 and Figure 2. Based on Table 3 and Figure 3, the average expression of SOD in the healthy group (KS) was 0.11%, the negative group (KN) was 5.83%, the treatment group 1 (P1) was 9.90%, and the treatment group 2 (P2) was 16.40%. The highest MMP-1 expression was found in treatment group 2 (P2), while MMP-1 expression was lowest in the healthy group (KS). The results of the Shapiro-Wilk test on SOD expression were known to be not normally distributed with a value of ($p>0.05$) and had a homogeneous data variant with the Leuvene Test result of 0.189 ($p>0.05$). The results of the analysis with the Kruskal Wallis test obtained a value of $p=0.000$ which means that there is a significant difference in the average expression of SOD in all groups. The results of the analysis

with the Man-Whitney test to find out the differences between groups can be seen in Table 4.

Table 3 SOD Expression Mean and Analysis Results with Wallis Kruskal Test

Group	KS	KN	P1	P2	*P value*
Expression SOD (%)					
Mean	0.11	5.83	9.90	16.40	
SD	0.12	0.23	0.17	0.36	
Shapiro-Wilk	0.027	*0.421	0.006	*0.607	
Leuvene Test					**0.189
Kruskal Wallis					***0.000

*Shapiro-Wilk= Normal (p>0,05)
**Leuvene Test = Homogeneous (p>0,05)
***Kruskal Wallis = Significant (p<0,05)

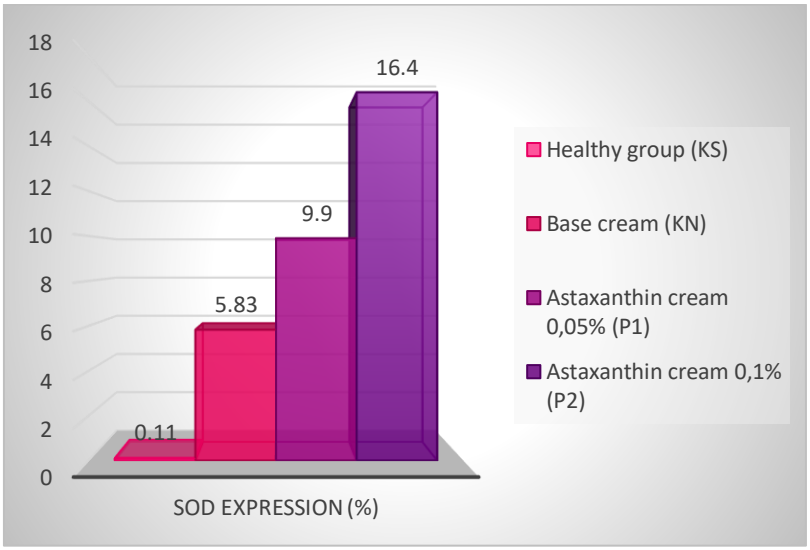

Figure 3. Average SOD Expression for Each Treatment

Table 4. Mann-Whitney Test Results for SOD Expression Between Groups

Group	**KS**	**KN**	**P1**	**P2**
KS	-	*0,004	*0,003	*0,004
KN	-	-	*0,003	*0,004
P1	-	-	-	*0,003

* significant p<0.05

Table 5.4 presents the results of the Mann-Whitney test to distinguish the mean expression of SOD between groups. These results show a significant difference between the KS and KN groups: KS with P1, KS with P2, KN with P1, KN with P2, and P1 with P2.

Microscopic immunohistochemical (IHC) results of SOD expression were performed by DAB staining, which produced a brown colour visible under the microscope, as shown in Figure 4.

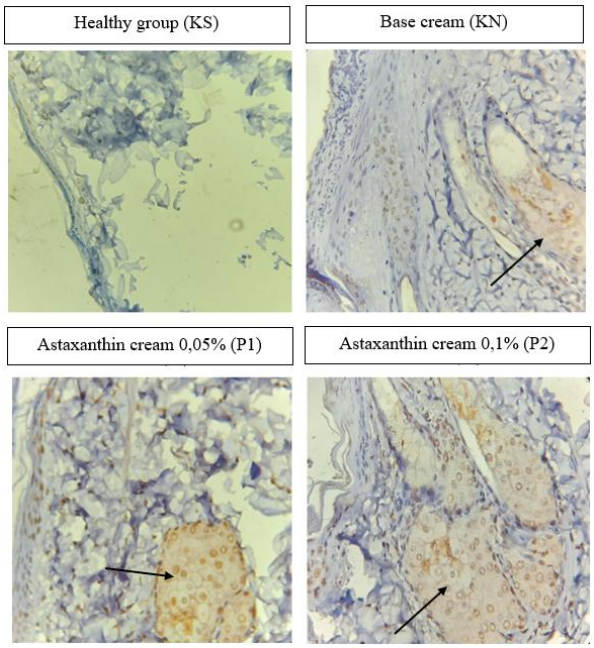

Figure 4 Immunohistopathology of SOD Expression in Each Group

Based on figure 4, IHC results showed variations in SOD expression in each treatment group. In the healthy group (KS), the average SOD expression was very low (0.11), reflecting normal skin conditions with minimal levels of oxidative stress. Healthy skin generally has a balance between free radical production and antioxidant system capabilities, so it does not require higher SOD expression. Conversely, in the negative group (KP), SOD expression was higher, indicating a higher cellular response to oxidative stress, possibly due to exposure to triggers such as UV rays or inflammation that produces higher free radicals. Higher SOD expression, this level may still not be enough to fully overcome existing oxidative stress, reflected by the high tissue damage or inflammation in this group. In treatment group 1 (P1) and treatment group 2 (P2), SOD expression was higher than the negative group. Higher SOD expression in both groups indicates a protective effect from the treatment intervention given (e.g., application of certain active ingredients or therapies), which causes higher antioxidant enzyme activity. In group P2, higher SOD expression indicates an optimal effect of the treatment in inducing the body's antioxidant defence system.

These results indicate that the treatment given to groups P1 and K4 caused significantly higher SOD expression compared to the negative group (KP) but also provided a protective effect against oxidative stress. Higher SOD expression in treatment group 2 (P2) reflects the ability of this treatment to inhibit the effects of free radicals, maintain skin tissue integrity, and reduce the risk of excessive cellular damage.

UVB exposure produces free radicals or ROS that cause oxidative stress in the skin. ROS activates the MAPK (*Mitogen-Activated Protein Kinases*) signalling pathways, especially p38 and JNK, which induce the transcription factor AP-1 (Activator Protein-1). AP-1 directly increases the expression of MMP-1. MMP-1 is a major enzyme that degrades type I and III collagen, a major component of the skin's dermis. Increased MMP-1 activity due to UVB accelerates the breakdown of dermis tissue and triggers skin ageing (Murlistyarini & Dani, 2022; Serafini et al., 2014).
UVB triggers the accumulation of ROS, especially superoxide (O_2^-). SOD is the main enzyme that converts superoxide to hydrogen peroxide (H_2O_2), which is then further

broken down by catalase or glutaposition peroxidase. Prolonged exposure can lead to a decrease in SOD activity due to oxidative stress that exceeds the skin's antioxidant capacity. With decreased SOD activity, ROS cannot be metabolized efficiently, causing damage to DNA, lipids, and proteins and worsening inflammation (Borgstahl et al., 2018; Rabe et al., 2014). When SOD activity decreases, ROS increases and activates more MMP-1 via the MAPK/AP-1 pathway, amplifying skin damage. The imbalance between antioxidants (SOD) and pro-oxidants (ROS) plays an important role in regulating MMP-1 levels and accelerating the process of skin damage due to UVB (Petruk et al., 2018).

The study's results showed that the administration of astaxanthin cream reduced MMP-1 expression in the skin tissue of Wistar rats exposed to acute UVB rays for 5 days. The administration of astaxanthin cream from EJBT at a dose of 0.05% decreased compared to the base cream (KN) group. The most significant decrease occurred in the astaxanthin cream group with an EJBT dose of 0.1% (Table 1). In line with research by Tominaga et al., 2017. Treatment with astaxanthin suppresses the UVB-related inflammatory response in keratinocytes. Also, it decreases the secretion of the metalloproteinase-1 matrix (MMP-1) by fibroblasts grown in UVB-induced keratinocytes (Tominaga et al., 2017). Sitanggang et al., 2019 also reported that an astaxanthin cream of 0.02% could prevent an increase in the amount of melanin in guinea pig skin exposed to UVB rays with the same effectiveness as hydroquinone cream 4% (Sitanggang et al., 2019).

Astaxanthin is an antioxidant that can reduce or neutralize ROS, suppress inflammatory pathways (such as NF-κB), and reduce the expression of MMP-1, helping to maintain collagen and improve skin structure and function. Topical use of astaxanthin has shown promising results in reducing MMP-1 levels and improving skin resistance by targeting oxidative stress and inflammation. Its efficacy lies in inhibiting pathways that cause collagen degradation and inflammation (Hwang et al., 2012).

The results of the study with the administration of astaxanthin EJBT cream increased SOD expression in the skin tissue of Wistar rats exposed to acute UVB rays for 5 days. The administration of astaxanthin cream from EJBT dose 0.1% experienced the most significant increase compared to other groups. Topical administration of astaxanthin protects keratinocytes and dermal fibroblasts from UVB-induced damage. This protection includes stabilizing mitochondrial function, which can trigger SOD activity to fight oxidative stress (Davinelli et al., 2018). Astaxanthin enhances SOD expression through the activation of endogenous antioxidant pathways, specifically by modulating the Nrf2 pathway, which is a key transcription factor that regulates the expression of antioxidant response element-dependent genes (AREs), including genes encoding SOD. When astaxanthin is applied, it interferes with the interaction between Nrf2 and its inhibitor, Keap1, which allows Nrf2 to translocate to the nucleus and bind to ARE, which promotes the production of antioxidant enzymes such as SOD and heme oxygenase-1 (HO-1). This reduces oxidative stress and prevents cell damage caused by ROS (Setyanto et al., 2022). Studies have shown this double-pathway modulation by astaxanthin in various models of oxidative stress and inflammation, such as ochratoxin-induced damage in mice, where significant increases in SOD levels and reductions in oxidative markers and inflammation are observed when astaxanthin is used (Hwang et al., 2012).

This mechanism makes astaxanthin a valuable component in skin care formulations that target premature ageing and skin damage due to UV rays. Astaxanthin inhibits cytokines and inflammatory pathways, such as NF-κB, which causes oxidative damage caused by UVB activities. By reducing inflammation, overall

oxidative stress on the skin is reduced, which indirectly affects the activity of SOD and other antioxidant enzymes (Xu et al., 2019; Binatti et al., 2021).

The creamy dose of astaxanthin provided an optimal effect on Wistar rats. However, this study did not compare the topical application with other methods for *astaxanthin* in protecting the skin from photoaging—study limitations, such as duration of UVB exposure or other biological parameters that have not been researched. Further research, such as testing in human models or the combination of astaxanthin with other active ingredients such as retinol, ceramide, and vitamin C, is needed to develop astaxanthin-based cream products. A review of the implications of research results for the development of astaxanthin-based cream products as a photoaging inhibitor or post-UVB therapy in humans also needs to be conducted, as well as potential commercial applications, including the stability of astaxanthin cream formulations, skin penetration, and potential side effects.

CONCLUSION

There was an effect of MMP-1 and SOD expression by administration of astaxanthin cream on the skin tissue of Wistar strain rats exposed to acute UVB rays. There was a difference in the average expression of MMP-1 and SOD in the skin tissue of Wistar strain mice that were not exposed to acute UVB light. There was a difference in the average expression of MMP-1 and SOD in the skin tissue of Wistar strain mice exposed to acute UVB light and given base cream. There was a difference in the average expression of MMP-1 and SOD in the skin tissue of Wistar strain mice exposed to acute UVB light and given astaxanthin cream 0.05%. There was a difference in the average expression of MMP-1 and SOD in the skin tissue of Wistar strain mice exposed to acute UVB light and given 0.1% astaxanthin cream. There was a difference in the mean expression of MMP-1 and SOD between the control group and the treatment group. Astaxanthin cream has potential as a preventive topical agent against UVB-induced skin damage through suppression of MMP-1 and enhancement of SOD. Further studies are recommended in human skin models or clinical trials to ensure efficacy and safety and to develop stable and optimally absorbed formulations.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest in the publication of this article.

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