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Original Article

In vivo study on the effect of astaxanthin cream on preventing inflammation in skin tissue exposed to acute UVB by reducing MDA and IL-6 levels

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A B S T R A C T

Background: Continuous UVB exposure induces lipid peroxidation and inflammation, with Malondialdehyde (MDA) as an indicator, leading to interleukin-6 (IL-6) secretion. Astaxanthin, a powerful antioxidant, is 50-100 times stronger than vitamin C, but its effects on MDA and IL-6 in UVB exposed skin have not been studied.

Objective: This study aims To determine the effect of astaxanthin cream on MDA and IL-6 levels in individuals exposed to acute UVBrays.

Methods: An experimental post-test-only control group design with four groups: healthy (K1), negative control (K2), and two treatment groups (K3, K4) exposed to UVBand given 0.05% and 0.1% astaxanthin cream, respectively. UVBirradiation was at 302 nm for 15 minutes, 5 days. MDA levels were measured using the TBARS method, and IL-6 levels were measured using ELISA. Data were analyzed using the Kruskal-Wallis and ANOVA tests.

Results: Average MDA levels were K1 (0.688 nmol/mL), K2 (0.766 nmol/mL), K3 (0.486 nmol/mL), and K4 (0.731 nmol/mL), with significant differences found (p=0.001). Average IL-6 levels were K1 (8.78 ng/L), K2 (6.23 ng/L), K3 (6.49 ng/L), and K4 (7.59 ng/L), with significant differences (p=0.003).

Conclusion: Astaxanthin cream reduces MDA and IL-6 levels following acute UVB exposure.

INTRODUCTION

Human skin contains melanin as a defence against ultraviolet (UV) radiation, a physical barrier and absorber.[1] Photoaging is a significant problem, especially in tropical areas with constant sun exposure.[2] Data shows that 83% of 1,400 participants in Australia experienced moderate to severe photoaging, while in Indonesia, 57% of individuals aged 25 years and over who were aware of signs of ageing showed visible skin ageing. In Indonesia, prolonged UV exposure also increases the risk of skin cancer, with a prevalence of 6,170 cases of non-melanoma skin cancer and 1,392 cases of melanoma skin cancer in 2018.[3-5]

Excessive UV exposure damages human skin by increasing reactive oxygen species (ROS), accelerating ageing, stimulating melanocyte proliferation, and causing dry skin and elastin fragmentation.[6] UVB exposure also triggers inflammation, leading to interleukin-6 (IL-6) secretion, which contributes to the acute inflammatory response. Malondialdehyde (MDA) levels, a marker of lipid peroxidation, reflect ROS-induced premature ageing, highlighting the need for natural photoprotectors.[8] Astaxanthin, a potent antioxidant carotenoid, protects against ROS and is 50–100 times more potent than vitamin C. It has a variety of pharmacological benefits, including anti-inflammatory and anticancer properties.[9–12] Topical antioxidants offer superior protection against free radicals compared to sunscreen alone, which does not entirely prevent free radical formation. Previous studies have shown that 0.01% to 5% astaxanthin concentrations effectively reduce UVB-induced skin damage. [13-15]

Although previous studies have examined the in vitro effects of astaxanthin and its role in wound healing and melanin reduction, no studies have explored its impact on MDA and IL-6 levels in UVB-exposed mice. The present study evaluated whether astaxanthin can reduce lipid peroxidation and inflammation in UVB-exposed skin, as indicated by lower MDA and IL-6 levels.

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METHOD

Study Design

This study uses an experimental method with a post-test only control group design.[16]

Study Site

The research was conducted in October 2024 at Integrated Biomedical Laboratories (IBL), Faculty of Medicine, Unissula.

Materials

Preparing astaxanthin cream requires sterile storage containers, sterile glass spoons, juicers, and vacuum dryers. For handling mice, necessary tools include cages, mouse restrainers, scales, small syringes, razors, and gloves. Equipment used in specimen preparation consists of scalpels, tweezers, cutting boards, sieves, tissue cassettes, automatic tissue processors, vacuum infiltration machines, embedding machines, freezers, microtomes, glass slides, cover slips, and water baths maintained at 46°C.

Experimental Procedure

This study used 28 male Wistar rats (230–250 g, aged 2–3 months) with no dropouts. Subjects were exposed to 302 nm UVBlight at 160 mJ/cm² for 15 minutes, followed by topical application of 0.05% or 0.1% astaxanthin cream for five consecutive days. Rats were divided into four groups: a healthy group (K1) receiving no treatment, a negative control group (K2) exposed to UVB and given base cream, and two treatment groups K3 received 0.05% astaxanthin cream, and K4 received 0.1%, both after UVB exposure. All creams were applied evenly to UVB exposed skin areas.

Preparation Before Treatment

UVB exposure was performed after preparing all equipment and materials. After adaptation, rats had a 2×3 cm area on their backs shaved for irradiation. They were exposed to UVBlight (302 nm) at a distance of 20 cm with a total dose of 160 mJ/cm² for 15 minutes per day over 5 days. Fifteen minutes after each exposure, treatment groups 1 and 2 received 0.5g of astaxanthin cream topically at concentrations of 0.05% and 0.1%, respectively, once daily for 5 days.[17]

Astaxanthin Cream Making

All tools and materials were prepared, and each ingredient was weighed accordingly. The oil phase (Span 80, liquid paraffin, propylparaben, and paraben) was melted in a porcelain cup over a water bath (mixture 1). The water phase (Tween 80, propylene glycol, methylparaben, and distilled water) was heated and stirred in a beaker until fully dissolved (mixture 2). Mixture 1 was then gradually added to Mixture 2 and homogenized until a uniform cream was formed.

In Vivo Procedure

Animal Preparation

To take skin samples in rats, a biopsy was performed, euthanasia was carried out first with xylazine 20 mg/kgBB IM and ketamine 60 mg/kgBB IM. After the mice are confirmed dead, biopsy sampling can be done.[18]

Experimental Prosedure

Skin tissue sampling was performed on day 6, after 5 days of treatment. Once the rat was confirmed dead, the area with active skin lesions was marked, cleaned with antiseptic, and covered with a sterile drape. A 6 mm punch biopsy collected tissue from the UVB exposed area. Samples were placed in PBS (pH 7.4), homogenized at 4°C, and centrifuged at 2000–3000 rpm for 20 minutes to obtain the supernatant. If not analyzed immediately, samples were stored at –20°C. MDA levels were measured using the TBARS method with a spectrophotometer at 532 nm, while IL-6 levels were analyzed using an IL-6 ELISA Kit and read at 450 nm.

Data Analysis

One-way ANOVA assessed differences in MDA levels between groups, while IL-6 levels were analyzed using the Kruskal–Wallis test.

Ethical Considerations

Ethical clearance for this research was obtained from the Bioethics Commission of the Faculty of Medicine, Sultan Agung Islamic University, Semarang, under No. 217/VI/2024.

RESULTS

MDA Level Results

The effect of astaxanthin cream on MDA and IL-6 levels in UVB exposed mice is presented in Table 1 and Figure 1. The highest average MDA level was observed in the negative control group (K2: 0.766 ± 0.022 nmol/mL), while the lowest was in the K3 group (0.486 ± 0.063 nmol/mL), which received 0.05% astaxanthin cream. The K4 group, which received a higher dose (0.1%), showed higher MDA levels (0.731 ± 0.035 nmol/mL) than K3, suggesting a better effect at the lower dose. Statistical analysis using the Kruskal-Wallis test revealed a significant difference between groups ($p = 0.001$), indicating that astaxanthin cream significantly reduced MDA levels in UV-B–exposed mice.

Table 2 presents the results of the Mann Whitney test to compare the average difference in MDA levels between the two groups. These results showed that there were significant differences between K1 and K2 groups (p=0.016), K1 and K3 (p=0.001), K2 and K3 (p=0.004), K2 and K4 (p=0.002), and K3 and K4 (p=0.004), but there was no significant difference between K1 and K4 groups (0.150).

Table 1. Results of descriptive analysis of MDA and IL-6 levels between treatment groups

Variable	K1	K2	K3	K4	p-value
MDA	0.688 (±0.562)	0.766 (±0.022)	0.486 (±0.063)	0.731 (±0.035)	
Shapiro Wilk	0.729	0.752	0.474	0.522	0.002
Levene					0.098
Kruskal wallis					0.001
IL-6	8.78 (±0.83)	6.23 (±0.91)	6.49 (±1.02)	7.59 (±1.57)	
Shapiro Wilk	0.713	0.675	0.002	0.086	0.293
Levene					0.781
Anova					0.003

Exp: K1 is a group of healthy mice without treatment; K2 is a group of mice exposed to acute UVBand applied with base cream for 5 days; K3 is a group of mice exposed to acute UVBand applied with astaxanthin cream at a dose of 0.05% for 5 days; K4 is a group of mice exposed to acute UVBand given astaxanthin cream at a dose of 0.1% for 5 days.

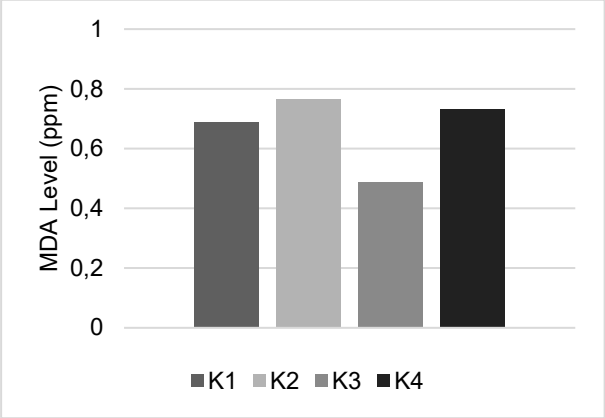

Figure 1. Graph of Average MDA Levels Between Groups

Table 2. The average difference in MDA levels between the 2 groups with the Mann Whitney test

Group	K1	K2	K3	K4
K1		0,016	0,001	0,150
K2			0,004	0,002
K3				0,004
K4				

Exp: K1= Healthy rat group; K2= Rat group exposed to UVBrays and given base cream; K3= Rat group exposed to UVBrays and given astaxanthin cream at a dose of 0.05%; K4= Rat group exposed to UVBrays and given astaxanthin cream at a dose of 0.1%.

IL-6 Level Results

Based on table 1 and figure 2, it shows that the average level of Il-6 is highest in K1 (8.78±0.83 ng/L) and the lowest level is in K2 (6.23±0.91 ng/L). The average IL-6 level in K4 was higher when compared to K3 (7.59±1.57 ng/L >< 6.49±1.02 ng/L). The average IL-6 level in K4 (group given astaxanthin cream at a dose of 0.1%) was better when compared to K3 (group given astaxanthin cream at a dose of 0.05%). The results of the Anova test showed a significance value of p of 0.003, indicating that there was a significant difference in IL-6 levels between the treatment groups.

The results of the Anova test showed a significance value of p of 0.003, indicating that there was a significant difference in IL-6 levels between the treatment groups. Table 3 shows the results of the post-hoc LSD test used to compare the mean difference in IL-6 levels between the two groups. The test results showed significant differences between K1 and K2 (p=0.001) and between K1 and K3 (p=0.002), but there were no significant differences between K1 and K4 (p=0.80), K2 and K3 (p=0.692), K2 and K4 (p=0.49).

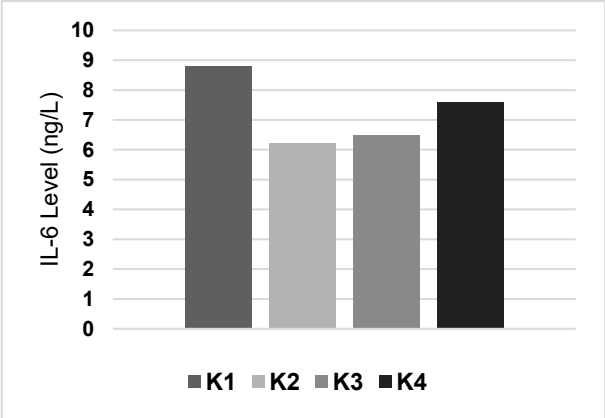

Figure 2. Graph of Average IL-6 Levels Between Groups

Table 3. Difference in average IL-6 levels between 2 groups with post-hoc LSD test

Group	K1	K2	K3	K4
K1		0,001	0,002	0,80
K2			0,692	0,49
K3				0,105
K4				

DISCUSSION

The findings of this study indicate that topical application of astaxanthin cream can reduce MDA and IL-6 levels in skin tissue exposed to acute UVB radiation. The effectiveness of astaxanthin is greatly influenced by the dose and formulation used, as both play an essential role in minimizing reactive oxygen species (ROS). Acute UVB exposure increases MDA levels due to lipid peroxidation caused by excess ROS, which damages cell membranes and other cellular components.[19]

Unlike MDA, IL-6 levels showed a different response, indicating that although astaxanthin has antioxidant and anti-inflammatory properties, its ability to suppress IL-6 may be limited under certain UVB exposure conditions. IL-6 is released in a variety of physiological and pathological conditions, such as acute infection, chronic inflammation, obesity, and stress, and is associated with diseases such as atherosclerosis, cardiovascular disorders, and stroke. Genetic variability in IL-6 gene expression may further

complicate accurate assessment, creating limitations in interpreting IL-6 levels.[20]

The potent antioxidant and anti-inflammatory activities of astaxanthin have been reported to be more powerful than other carotenoids, such as lutein and zeaxanthin, which are central to its therapeutic potential. However, in this study, the cream concentration tested was insufficient to significantly reduce IL-6 levels in UVB-exposed skin, highlighting the need for optimization in dose and formulation.[12,24] Several factors, including the health condition of the subjects, formulation and application method, duration of UVB exposure, overall study duration, and dose, influence the efficacy of astaxanthin. While previous studies support the anti-inflammatory effects of astaxanthin in animal models, optimal results require precise concentration and administration. [25]

The limitations of this study include the inability to determine the ideal dose and formulation to reduce IL-6 levels significantly. Further studies are needed to evaluate the appropriate duration of treatment, cream application method, and subject selection. MDA and IL-6 levels are influenced by age, body weight, stress, and underlying health conditions.[26,27]

CONCLUSIONS AND RECOMMENDATION

Topical administration of astaxanthin cream significantly reduced MDA and IL-6 levels, helping to prevent inflammation in skin tissue exposed to acute UVB radiation. These findings highlight astaxanthin's antioxidant and anti-inflammatory potential. However, further research with extended observation periods and combination formulations is needed to optimize skin protection against UVB induced oxidative stress.

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