The extract of Red Beetroot (Beta vulgaris L.) Prevent Tumor Necrosis Factor-Alpha (TNF-α) Levels and Prevent Increased Sunburn in Epidermal Cell in Rats Exposed to UVB

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Abstract
Backgrounds: UVB radiation can also cause acute changes, such as sunburn and pigmentation, and chronic changes, such as photocarcinogenesis and immunosuppression. The betalain compound in beetroot extract also functions as a potent anti-inflammatory agent. **Method:** This study employed a true experimental study with a randomized post-test only control group design using 30 rats divided into three groups for treat 28 days. The negative control used placebo and without exposure to UVB (K_1). The positive control group exposed to UVB and get placebo (K_2). The treatment group get exposed to UVB light and treated red beetroot extract at a dose of 500 mg/kg BW via oral (K_3). TNF-α levels were measured using ELISA. Sunburn cells were measured by observing the epidermal cells under a microscope. Statistical using one-way ANOVA with significantly $p<0.05$. **Results:** TNF-α levels in K_1 was 33.464±1.533 ng/L, K_2 was 47.546±4.194 ng/L, and K_3 was 32.625±0.839 ng/L. The number of sunburn cells in K_1 was 0.280±0.611 cells/visual field K_2 was 0.980±0.121 cells/HPF, and K_3 was 0.300±0.054 cells/HPF. Based on the results of the Post-hoc (LSD) analysis, TNF-α levels in K_3 decreased significantly compared to K_2 ($p=0.000$), while TNF-α levels in K_3 were not significantly different from K_1 ($p=0.824$). Similarly, the number of sunburn cells in K_3 decreased significantly compared to K_2 ($p=0.000$), while the number of sunburn cells in K_3 did not differ significantly from K_1 ($p=0.665$). **Conclusion:** red beetroot extract can prevent inflammatory factor TNF-α and increased sunburn of epidermal cells in rats exposed to UVB.

Keywords: Red Beetroot Extract, TNF-α, Sunburn, UVB

1. Introduction

Aging in the skin occurs like aging in the cell in general; it is the accumulation of endogenous damage as a result of the formation of ROS during cellular oxidation metabolism (Godic et al., 2014; Kageyama and Waditee-Sirisattha, 2019; Pandel et al., 2013).The main risk factor for premature skin aging is UV radiation (Lee et al., 2019a; Lin et al., 2012; Trojahn et al., 2015). Exposure to ultraviolet radiation (UVR) is the leading cause of oxidative stress in the skin. It causes histologic differences between areas of skin exposed to UV radiation and those that are not. Moreover, UVR exposure is also categorized as a cause of age-related skin changes such as wrinkling, thinning, pigment changes, and carcinogenesis (Amaro-Ortiz et al., 2014; D'Orazio et al., 2013)

Skin photoaging is a process caused by the damage to skin cell DNA by ROS such as superoxide and hydrogen peroxide, prolonged inflammation due to the production of inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) from keratinocytes and fibroblasts, and constant degradation of structural proteins such as collagen, gelatin, and elastin by matrix metalloproteases (MMPs) (Kim et al., 2010, 2018; Tewari et al., 2014). UV radiation is an environmental factor affecting the skin's internal structure and composition. Repeated irradiation of UVB activates the transcription factors AP-1 and NF-κB. Thus, it would increase the expression and release of pro-inflammatory cytokines in human epidermal keratinocytes and dermal fibroblasts. The pro-inflammatory cytokine TNF-α is

associated with several inflammatory responses in the skin. Secreted TNF-α stimulates endothelial cells to recruit the circulating immune cells, which secrete MMPs; furthermore, it induces collagen fiber degradation, resulting in local skin damage (Lee et al., 2019a; Zheng et al., 2010)

UVB radiation can also cause acute changes, such as sunburn and pigmentation, and chronic changes, such as photocarcinogenesis and immunosuppression. UVB rays, also known as sunburn rays, have a wavelength of 290 to 320 nm and are often correlated with skin cancer than UVA rays, which have a wavelength of 320 to 400 nm (Lopes and Mcmahon, 2016; Romanhole et al., 2015; Sheehan and Young, 2002) The high risk of skin cancer due to UVB exposure which begins with sunburn on epidermal cells, has led to many studies on the use of antioxidants or antiaging to prevent and minimize skin damage caused by UVB (Romanhole et al., 2015; Tominaga et al., 2017)

The use of niacinamide (vitamin B3) has been found to have a significant repair effect on the skin. It prevents UV-induced skin damage and modulates its photoprotective mechanism against UV radiation by activating AKT and mTOR activation (Lin et al., 2012) Niacinamide is known as nicotinic acid (NA), and nicotinamide (Nam) is an active form of niacin or vitamin B3 physiologically (Makarov et al., 2018)

The existence of side effects arising from niacinamide administration triggers various other studies to identify natural antioxidants that can effectively act as antiaging, especially preventing skin tissue damage that does not cause side effects (Lin et al., 2012). One natural antioxidant that can repair tissue damage is red beetroot or beet. Red beetroot supplementation can strengthen endogenous antioxidant defenses, helping protect cellular components from oxidative damage due to increased ROS (Clifford et al., 2015)

Beetroot is a food source rich in antioxidant compounds, especially betalain pigments, which can protect cellular components from oxidative damage (Tesoriere et al., 2008). As research by Clifford et al. found that betalain compounds in red beetroot extract can inhibit linoleic damage by cytochrome C oxidase and the oxidation of lipids membrane by metmyoglobin that activated H_2O_2 and Fe. Furthermore, the study found that betalain in red beetroot extract could inhibit lipid peroxidation due to ROS very effectively (Clifford et al., 2015; Hardiany et al., 2020)

The betalain compounds in beetroot extract also function as potent anti-inflammatory agents by an interfering mechanism with pro-inflammatory signaling cascades (Clifford et al., 2015; Martinez et al., 2015; Moreno-Ley et al., 2021). Another important mechanism of the anti-inflammatory effect of red beetroot extract is to weaken the activity of the Nuclear Factor-Kappa B (NF-κB) cascade, which is the agent capable of activating and transcribing some of the target genes that regulate and amplify the inflammatory response, such as cytokines, chemokines, cell apoptosis and phagocytic which can cause chronic tissue damage (El Gamal et al., 2014)

The purpose of this study was to determine the effect of red beetroot extract in preventing the TNF-α inflammatory factor and the increasing sunburn of epidermal cells in rats exposed to UVB.

2. Methods

Research design

This research is a true experimental study with a randomized posttest-only control group design carried out from October 2021-January 2022. This study used 30 rats that were divided into three groups. Ethical permission in this study with No. B/231/UN14.2.9/PT.01.04/2021 from the Faculty of Veterinary Medicine research ethics committee, Udayana University.

Red Beetroot Extract

Red beetroot extract is an ethanolic extract obtained by extracting 1 kg of red beetroot purchased in the Bali area. The dose of red beetroot extract given was 500 mg/kg BW, dissolved in 2 mL of aquadest, and given orally once per day for 28 days.

A total of 1 kg of red beetroot was washed with flowing water, cut into small pieces, then dried in the sun. Furthermore, the red beetroot pieces were dried again in an oven at a temperature of 40-50^{o}C for 24

hours, then blended until turned into powder form. Red beetroot dry powder was dissolved in 96% ethanol in a corked Erlenmeyer and extracted by maceration for 72 hours or three days. Every 24 hours, the extraction was stirred for the effectiveness of the extraction process. After 72 hours, the solution was filtered, and the maceration result was evaporated to produce a thick extract.

Treatment of Experimental Animals

The animals needed for the experiment were 30 male rats aged 5-6 weeks, weighing 180-200 grams. The rats were adapted for seven days first and then divided into three groups randomly with ten rats, respectively. The negative control group was the group of white rats without exposure to UVB rays and treated with a placebo (2 mL of aquadest) via oral probe for 28 days. The positive control group was the group of white rats exposed to UVB rays and treated with a placebo (2 mL of aquadest) via an oral probe for 28 days. The treatment group was a group of white rats exposed to UVB rays and treated with 2 ml of red beetroot extract at a dose of 500 mg/kg BW via oral probe for 28 days.

After 28 days, skin tissue was taken with a size of 0.2-0.3 x 1.5 x 2 cm from the middle of the back of the rats. Previously the rats had been made unconscious by administering ether anesthesia. The tissue was then put into a chamber containing 10% formalin with a volume ratio of tissue: formalin was 1:20. After that, the tissue was processed and then stained the slides with hematoxylin-eosin (HE).

Measurement of TNF-α

The TNF-α level was checked using Quantikine ELISA Mouse TNF-α Immunoassay Catalog Number MTA 00B (R&D Systems, USA) with a minimum detection dose of 0.5-5.5 pg/mL and a minimum detection average of 1.6 pg/mL.

Determination of sunburn cell

The slides were observed using a microscope with a low magnification. After being clearly seen with high magnification, the sunburn number of epidermal cells was calculated; it was the cells with histological changes in the form of pyknotic nuclei and eosinophilic cytoplasm. The sunburn number of epidermal cells was determined by counting the number of sunburn cells per 100 keratinocytes using a 400x magnification by light microscope in five fields of view, where observations and calculations were carried out by shifting the slide from left to right.

Statistical Analysis

Data analysis was carried out using the 26th version of SPSS software. Data on the number of sunburn cells and TNF-α levels of each treatment group were analyzed for normality and homogeneity. Then, suppose the data were normally distributed and homogeneous. In that case, the research analysis to test the effect of the treatment can be done with One-Way ANOVA followed by Duncan's 5% real difference test to see the difference in effect among treatment groups. However, suppose the research data was not normally distributed and not homogeneous. In that case, the treatment effect test was carried out with the Kruskal-Wallis test and followed by the Mann-Whitney test to determine the difference in the effect among treatments.

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3. Results

The This study used 30 male rats aged 5-6 weeks, weighing 180-200 grams, divided into three groups, and each group consisted of 10 rats. K1 was a negative control group, a group of white rats without exposure to UVB rays and treated with a placebo (2 mL of aquadest) via an oral probe for 28 days. K2 was a positive control group, a group of white rats exposed to UVB rays and given placebo treatment (2 mL of aquadest) via an oral probe for 28 days. K3 was the treatment group, a group of white rats exposed to UVB rays and treated with 2 mL of red beetroot extract at a dose of 500 mg/kg BW via oral probe for 28 days.

After the treatment was given for 28 days, observations were made on TNF-α levels in the negative control group (K_1), positive control group (K_2), and treatment group (K_3).

The normality test to determine the distribution of the research data. In this study, the sample used was less than 50; hence, the normality test used was Shapiro-Wilk, with a significance level of 95% (p = 0.05). T

Based on the results of the normality test using Shapiro-Wilk, it is known that the data on TNF-α levels and the number of sunburn cells in each treatment group (K_1, K_2, and K_3) have a normal distribution (p>0.05).

The homogeneity test with Levene's test was carried out to determine the variance of the research data. The research data is categorized to have a homogeneous variance if the p-value >0.05. The results of the homogeneity test can be seen in Table 3 below.

Based on the homogeneity test results on the variables of TNF-α levels and the sunburn cell numbers, the p-values were 0.362 and 0.759 (p>0.05). Hence, it can be indicated that all research data has a homogeneous variance.

The results of the normality and homogeneity test indicated that the data were normally distributed, and the variance of the data was homogeneous; hence the comparability test could be performed parametrically with One Way Anova. The comparative test in this study was conducted to compare the mean value of TNF-α levels and the sunburn cell numbers in groups K_1, K_2, and K_3.

Furthermore, the One Way Anova test results indicated that the treatment groups' mean value of TNF-α levels had a significant difference (p<0.05). Similarly, on the mean value of the sunburn cell numbers among treatment groups (p<0.05). The mean numbers of sunburn cells in the red beetroot extract treatment group were significantly different compared to the negative control group and the positive control group, where the mean numbers of sunburn cells in the red beetroot extract treatment group was lower than the positive control group.

The results of the One Way ANOVA test indicated that each treatment had a significantly different mean in TNF-α level and the sunburn cell numbers (p<0.05); hence, a further test was performed with Post-Hoc (LSD). This test was conducted to determine whether a treatment group had a significant difference from other treatment groups. Based on the results of the Post-Hoc (LSD) test, there were significant differences between the mean TNF-α levels in the negative control group and the positive control group with 0.00831 ng/L (p<0.05) and between the positive control group and the red beetroot extract treatment group with 0.00870 ng/L (p<0.05). Similarly, there were significant differences in the mean number of sunburn cells in the negative control group and positive control group with 0.50690 cells/HPF (p<0.05) and in the positive control group and the treatment group with 0.46218 cells/HPF (p<0.05). There was no significant difference in the mean value of TNF-α level and the sunburn cell numbers between the negative control group and the red beetroot extract treatment group, with a mean difference of 0.00039 ng/L and 0.04472 cells/HPF (p>0 0.05) as shown in Table 1.

Table 1. Post-hoc (LSD) Test Results on TNF-α Levels and Sunburn Cell Numbers

Treatment Groups	Treatment Groups			p-value
	$K_1^{a,b}$	$K_2^{a,c}$	$K_3^{b,c}$	
TNF-α (ng/L) Levels	33.464±1.533	47.546±4.194	32.625±0.839	<0.001[a] 0.824[b] <0.001[c]
Sunburn Cell Numbers (cell/HPF)	0.280±0.611	0.980±0.121	0.300±0.054	<0.001[a] 0.665[b] <0.001[c]

Notes:

* = mean difference indicated a significant value ($p<0.05$)
p value = significance value
a = Post-hoc (LSD) test group K_1 with K_2
b = Post-hoc (LSD) test group K_1 with K_3
c = Post-hoc (LSD) test group K_2 with K_3

The comparison of the mean value of TNF-α levels among treatment groups is presented in Figure 1.

Therefore, it could be seen that the administration of red beetroot extract was effective in inhibiting the increase of TNF-α levels and the sunburn cell numbers in rats exposed to UVB rays. Red beetroot extract inhibited the increase in TNF-α levels and the sunburn cell numbers in rats exposed to UVB rays with values that were not significantly different from those without UVB exposure (K_1).

The histology of the rat epidermis supports this after treatment with red beetroot extract (Figure 3). The results of observations on epidermal histological slides taken from skin tissue biopsies of rats after 28 days of treatment then stained with HE and observed under a microscope with a magnification of 400 x in five fields showed that sunburn cells were more commonly found in the skin of rats exposed to UVB rays and only given aquadest (K_2). Meanwhile, the skin of rats exposed to UVB rays and given red beetroot extract (K_3) had the same number of sunburn cells as rats' skins without the exposure to UVB rays (K_1).

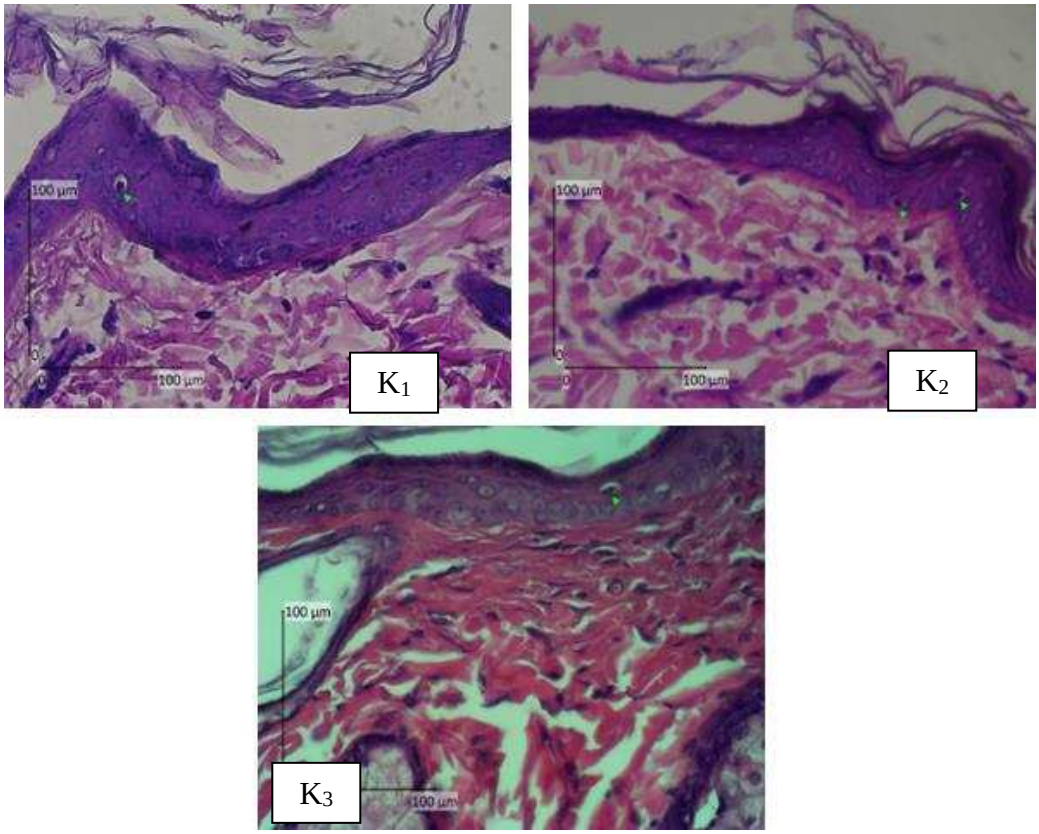

Figure 3. Sunburn cells on epidermal layer of Rat After Treatment.

4. Discussion

The Exposure to UV radiation can cause tremendous damage to the skin. UVB radiation can also cause acute changes, such as sunburn and pigmentation, and chronic changes, such as photocarcinogenesis and

immunosuppression. The energy contained in UV, especially UVA at 315–400 nm and UVB at 280–315 nm, can stimulate the accumulation of changes and trigger most of the typical manifestations of cancer and skin aging (Bosch et al., 2015; Kageyama and Waditee-Sirisattha, 2019) One of the mechanisms described in the literature is the involvement of reactive oxygen species (ROS). According to Bosch et al., ROS stimulates the peroxidation of lipid components of cell membranes, stimulates carbohydrate oxidation, and changes the function and structure of several enzymatic systems. Excessive ROS can cause oxidative stress in the skin, create histologic differences between UV radiation and unexposed skin areas, and ultimately contribute to collagen breakdown (Bosch et al., 2015).Another study found that UVA significantly increased oxidative damage to DNA, fiber damage to the collagen in the dermis, and fibroblast apoptosis leading to inflammation. Meanwhile, UVB or sunburn rays were found to increase keratinocyte proliferation and carbonylation of various proteins (Lopes and Mcmahon, 2016; Luangpraditkun et al., 2020; Sheehan and Young, 2002)

Photoaging causes various kinds of histological changes in the skin that differ from the histologic changes that occur in the aging process. Compared with young skin protected by sunlight, chronologically aging skin will experience changes in collagen fiber bundles to become short, thin, loose, and irregular (Poon et al., 2015). On the other hand, skin damaged by UV radiation is characterized by an irregular epidermis thickness, increased elastosis, collagen fragmentation under the dermal-epidermal junction, and changes in the morphology of epidermal cells. Significant changes occur in the dermis of photoaging skin (Liu et al., 2020; Nur et al., 2017). The increase in the number of glycosaminoglycans and proteoglycans can be caused by an increase in MMP with an increase in the number of hyperplastic fibroblasts. Photoaging skin may indicate an increased number of inflammatory cells such as mast cells, eosinophils, and mononuclear cells. The changes are not the same as the hypocellular nature of chronologically aged skin. In addition, it is known that UV radiation causes the release of inflammatory mediators from keratinocytes, fibroblasts, leukocytes, and the endothelial lining of blood vessels. These mediators are lipid mediators (leukotrienes, prostaglandins, platelet-activating factor), plasma mediators (plasmin, bradykinin, fibrin), inflammatory cytokines [tumor necrosis factor (TNF)-α, interleukin-1 (IL-1), IL-6] (Bosch et al., 2015)

In this study, the data indicated that red beetroot extract could prevent the inflammatory factor TNF-α and the increase in epidermal cell sunburn in rats exposed to UVB rays. This finding is essential as the preliminary evidence of the antiaging potential of red beetroot extract. Further discussion of the findings in this study will be discussed in the following sub-chapters.

The research results by El-Gamal et al. showed that red beetroot extract had a protective effect against nephrotoxic rats. The administration of red beetroot extract (250 and 500 mg/kg BW) to nephrotoxic rats for 28 days along with gentamicin was proven to increase the endogenous antioxidant status (catalase) of the kidneys as evidenced by a decrease in malondialdehyde (MDA) and nitric oxide levels; moreover, it indicated a decrease in the number of inflammation factors such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). The administration of red beetroot extract together with gentamicin at a dose of 500 mg/kg BW gave a better effect than the dose of 250 mg/kg BW (El Gamal et al., 2014)

Red beetroot also has a role in reducing sunburn due to its anti-inflammatory effect. According to Kageyama & Waditee-Sirisattha, the response induced by UV-B mainly occurs through various mediators, including nitric oxide (NO), the inducible NO synthase (iNOS), cyclooxygenase-2 (COX-2), and prostaglandin E2 (PGE2). Hence, red beetroot, which has an anti-inflammatory role, certainly contributes to reducing sunburn due to UVB exposure (Kageyama and Waditee-Sirisattha, 2019)

Red Beetroot Extract prevent Inflammatory Factor tTNF-α Rats Exposed to UVB Rays

This study showed that red beetroot extract significantly to decreased inflammatory factor TNF-α in rats exposed to UVB. Lee et al. stated that the repetition of UVB radiation activates transcription factors AP-1 and NF-κB, which can stimulate the expression and release of pro-inflammatory cytokines in epidermal keratinocytes and dermal fibroblasts. UVB radiation is also known to cause the release of inflammatory

mediators from keratinocytes, especially TNF-α. Furthermore, TNF-α signaling causes oxidative stress, which ultimately exacerbates the effects of photoaging due to UVB exposure (Lee et al., 2019b)

Tumor Necrosis Factor-α (TNF-α) has an essential role in macrovascular and microvascular circulatory disorders, both in vivo and in vitro, and is an important cytokine that can induce apoptosis and inflammation (Yang and Shao, 2016) The increase of ROS leads to the increased production of TNF-α, which in turn will induce oxidative stress. TNF-α participates in edema formation, vasodilation, and leukocyte adhesion to the epithelium through the expression of adhesion molecules. In addition, according to Zelová & Hošek, TNF-α regulates blood clotting, contributes to oxidative stress at sites of inflammation, and indirectly induces fever (Zelova et al., 2013; Zelová and Hošek, 2013)

According to Lee et al., skin photoaging can be prevented by reducing the inflammatory factor TNF-α thereby reducing NF-κB activation associated with MMP expression. Secreted TNF-α stimulates endothelial cells to recruit the circulating immune cells, stimulates MMP secretion, and further induces collagen fiber degradation resulting in local skin damage. TNF-α stimulates signal transduction pathways involved in NF-κB activation, leading to pathways that amplify NF-κB activity. TNF-α that induced NF-κB activation is required for MMP-9 expression in dermal fibroblasts and pro-MMP-2 activation via MT1-MMP induction in photoaging in human skin. In addition, overexpression of MMP-1 and MMP-13 was observed after NF-κB activation in fibroblast-like synoviocytes due to UVB exposure that induces photoaging (Lee et al., 2019b)

In this regard, red beetroot contains a group of bioactive pigments called betalains. Betalains are categorized as red-purple betacyanin pigments or yellow-orange betaxanthin pigments. Several in vivo and in vitro studies have reported the ability of betalains as anti-inflammatory and antioxidant (Vulić et al., 2014). The study results of Clifford et al. showed that two betalain metabolites, betanin and betanidin, were shown to reduce linoleic damage by cytochrome C oxidase and oxidation of membrane lipids by activated metmyoglobin H_2O_2 and Fe (AA-Fe) (Clifford et al., 2015)

So far, data on the effect of red beetroot extract with factor TNF-α. are limited El-Gamal et al. investigated the protective effect of (Beta vulgaris L.) beat root ethanolic extract (BVEE) on gentamicin-induced nephrotoxicity. The study evaluated oxidative stress (MDA, lipid peroxidation, catalase, NP-SH, and nitric oxide levels), inflammatory response (TNF-α, IL-6, NF-κB (p65), MPO, and NF-κB (p65) DNA binding), and apoptotic markers (Bax, Caspase-3, and Bcl 2). The administration of red beetroot extract (250 and 500 mg/kg BW) in nephrotoxic rats for 28 days together with gentamicin was shown to increase the endogenous antioxidant status (catalase) of the kidneys as evidenced by a decrease in the number of inflammatory factors such as TNF-α and IL-6; moreover, it decreased levels of malondialdehyde (MDA) and nitric oxide. The administration of BVEE also reduced inflammatory infiltration in the renal tubules and improved the rate of histologic injury. These findings suggest that BVEE treatment can reduce structural damage and renal dysfunction through inflammation, oxidative stress, and apoptosis in the kidney. The administration of red beetroot extract together with gentamicin at a dose of 500 mg/kg BW gave a better effect than the dose of 250 mg/kg BW (El Gamal et al., 2014)

The study of Ahmad et al. investigated the purification of betanin from red beetroot and evaluated its anti-inflammatory and antioxidant activity on LPS-activated microglial cells. Purified betanin was evaluated by the spectroscopy of Thin Layer Chromatography (TLC), High-Performance Liquid Chromatography (HPLC), Electrospray Mass (ESI-MASS), UV-visible, Fourier Transform Infra Red (FT-IR). The inhibitory effect of betanin on activated microglia was evaluated using primary microglial culture. The results indicated that betanin significantly inhibited lipopolysaccharide-induced microglia functions, including the production of ROS, TNF-α, IL-1β, IL-6, and nitric oxide free radicals. Ahmadi et al.'s further study used the in silico molecular docking analysis to investigate the interactions of betanin with TNF-α, Nitric oxide synthase (iNOS or NOS2), and IL-6. The analysis showed that betanin has a significantly negative binding energy to the active site of TNF-α, IL-6, and inducible nitric oxide synthase (iNOS) (Ahmadi et al., 2020)

Red beetroot contains betalain with potent free radicals and has antioxidant activity. Lechner & Stoner explained that the scavenging activity of betalain was comparable to that of the widely used synthetic

antioxidants such as butylated hydroxytoluene. Betalain has a role against hydroxyl, galvinoxyl, and superoxide free radicals. This compound also significantly inhibited the production of ROS in the result of culture, neutrophils and decreased DNA damage in activated cells. Betalains also have anti-inflammatory activity. Oral administration of betalains is known to inhibit the recruitment of total leukocytes, including neutrophils and mononuclear cells.[19] Furthermore, betalains significantly reduced TNF-α, IL-1β, and carrageenan-induced superoxide anions in the peritoneal fluid and increased IL-10 levels (Martinez et al., 2015)These results are also on the same page as the study of Kim et al., who isolated and tested antioxidants derived from Lactobacillus fermentation products using culture media with 1% beet powder, showed the inhibition of TNF-α, IL-1β, iNOS, nitric oxide (NO) production in RAW 264.7 cells which stimulated LPS (Kim et al., 2017)

Red Beetroot Extract Prevent the Increase in Sunburn of Epidermal Cells in Rats Exposed to UVB

The results of this study indicated that red beetroot extract significantly prevented the increase in sunburn of epidermal cells in rats exposed to UVB. The reduction in the number of epidermal cell sunburn was indicated by a decrease in the number of cells with histological changes in the form of pyknotic nuclei and eosinophilic cytoplasm. Until this study is made, no study specifically evaluates the effect of red beetroot extract on sunburn of epidermal cells. According to Kim et al., when the epidermis of the skin is exposed to UVB radiation, keratinocyte cells are dyskeratotic and cause the cytoplasm to have dark vacuoles, and nucleus enlargement occurs. This phenomenon occurs due to lysosomal damage, which reduces the ability of cells to repair DNA damage adequately. This condition will encourage keratinocyte cells to differentiate in order to form sunburn epidermal cells due to exposure to UVB (Kim et al., 2017)

UVB exposure can trigger inflammation through the induction of a cascade of cytokines, especially TNF-α, a vasoactive and neuroactive mediator in the skin, which causes an inflammatory response and form epidermal sunburn cells. UVB exposure can cause cell injury, thereby inducing damage response pathways in keratinocytes. The damage signal is in the form of p53 activation, which can change keratinocyte physiology, mediate cell cycle arrest, activate DNA repair, and induce apoptosis of keratinocyte cells. Under these conditions, TNF-α will increase and trigger cell infiltration, cause p53 accumulation, and inhibit type I collagen synthesis, thereby increasing collagen degradation by increasing metalloproteinases. This process ultimately stimulates inflammation and more severe damage, namely apoptosis of keratinocyte cells in which the nucleus undergoes pyknotic and eventually trigger the occurrence of sunburn cells (Kim et al., 2017; Lopes and Mcmahon, 2016; Ryser et al., 2014)

Lee et al. also reported that UV radiation could trigger apoptosis in keratinocytes leading to sunburn cells. However, until this study was made, no study had examined the effect of red beetroot on apoptotic keratinocytes in epidermal cells (Lee et al., 2019a). Research on the effect of red beetroot is only limited to apoptosis in the kidneys, as conducted by El Gamal et al. study. Their study evaluated the effect of red beetroot (Beta vulgaris L.) beat root ethanolic extract (BVEE) on changes in inflammatory markers and apoptosis in rat kidney tissue induced by gentamicin (El Gamal et al., 2014). Based on these studies, oxidative or nitrosative stress is known to have a significant role in mitochondrial dysfunction, which is an important initiating event in the intrinsic pathway of apoptosis. The activation of NF-κB stimulated gentamicin-induced apoptosis in rat renal tubular cells triggered by direct activation of apoptotic proteins such as caspase-3 or downregulation of antiapoptotic proteins such as Bcl-2. The administration of BVEE (250 and 500 mg/kg) significantly reduced NF-κB protein expression (p65) and DNA binding activity compared to rats given gentamicin alone. In other words, BVEE administration significantly prevented apoptosis of renal tubular necrosis (Bashir et al., 2009; El Gamal et al., 2014)

Another study stated that sunburn is an acute inflammatory skin reaction caused by high exposure to ultraviolet (UV) light (Pérez and Bashline, 2019). Related to this inflammatory process, betalain compounds in red beetroot extract also function as a potent anti-inflammatory through a mechanism that interferes with pro-inflammatory signaling cascades. This is in line with El-Gamal et al., which showed that red beetroot

extract had a protective effect against nephrotoxic rats. An essential mechanism of the anti-inflammatory effect of red beetroot extract is to weaken the activity of the Nuclear Factor-Kappa B (NF-κB). NF-κB is an agent that can activate and transcribe some of the target genes that regulate and amplify the inflammatory response, such as cytokines, cell apoptosis, chemokines, and phagocytic that cause chronic tissue damage. (El-Gamal et al., 2014) The results of this study are also supported by another study which stated that red beetroot, which contains betalain has high anti-inflammatory potential. Moreno-Ley et al. (2021)

The research of Albawali et al. aimed to determine the protective effect of beetroot extract (Beta Vulgaris L.) and silymarin against hepatotoxicity in rats induced by Cyclosporine A. The biomarkers measured in this study were mediators of inflammation, oxidative stress, DNA damage, apoptosis, and antioxidant status in liver tissue. The study of Albawali et al. (2019) proved that the administration of red beetroot extract could reduce inflammation in hepatotoxicity rats. The results showed that the administration of 500 mg/kg BW of red beetroot extract increased Glutathione S Transferase (GST) levels by 76% and reduced NF-κB levels by 10.95%. The decreased levels of NF-κB are associated with decreased inflammatory responses to prevent further damage, such as keratinocyte cell apoptosis that leads to sunburn cells (Albalawi et al., 2019)

The limitation of this study was that the dose of red beetroot extract in the treatment group was not different, which was 500 mg/kg BW, so the effect of the red beetroot extract was not observed based on the difference in dose. This study only observed the effect of red beetroot extract on TNF-α levels and sunburn of epidermal cells in rats exposed to UVB. In other words, this study did not assess the photoaging parameters due to exposure to other UVB rays, such as tyrosinase enzyme, amount of melanin, and other inflammatory mediators, namely lipid mediators (leukotrienes, prostaglandins, platelet-activating factor), plasma mediators (plasmin, bradykinin, fibrin), and inflammatory cytokines [interleukin-1 (IL-1), IL-6]. Further research is needed to determine the red beetroot extract based on different doses and research of safety index, toxicity, and clinical trials in humans; hence, the results of this study can provide benefits as they should.

5. Conclusions

Based on the results of the research that has been done, it can be concluded that the administration of red beetroot extract can prevent the TNF-α inflammatory factor and the increased sunburn of epidermal cells in rats exposed to UVB. Further studies are needed comparing red beetroot extract with the gold standard or general therapy of photoaging or UV-induced skin damage, such as niacinamide which acts as an antioxidant.

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