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Topical Argan Oil Inhibited Prostaglandin E2 and Prevented Collagen Reduction in Male Wistar Rats' Skin Exposed to UVB

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Abstract: ***Argan oil contained high tocopherol, phenol, oleic acid, and linoleic acid has been used to treat skin problems especially aging skin. However, the effectiveness and argan oil mechanism to protect skin elasticity has not been studied. This study aimed to prove the effectiveness of topical argan oil (Argania spinosa) in inhibit the increase of Prostaglandin E2 (PGE2) and prevent the decrease of collagen amount in the skin of males Wistar (Rattus norvegicus) rats exposed to UVB. The experimental research using a randomized post - test only control group was carried out on 36 male Wistar rats aged 2 - 3 months and weighed 180 - 200 grams. Wistar rats were divided into two groups: the control group, smeared with paraffin oil, and the treatment group, smeared with argan oil. These two groups were exposed to UVB with a total dose of 840 mJ/cm2 for four weeks. The skin tissue was taken to examine the PGE2 levels by ELISA and collagen using Sirius Red staining. The results showed that the mean level of PGE2 in the treatment group (1.58±0.33) was lower than the control group (2.79±1.16) with p<0.05. The mean amount of collagen in the treatment group was higher (79.07±3.31) than the control group (67.6±5.23) with p<0.05. The administration of argan oil was shown to inhibit the increase in PGE2 levels and prevented a decrease in collagen in the skin of male Wistar rats exposed to UVB.***

Keywords: Argan oil, UVB, Collagen, Prostaglandin

1. Introduction

Aging caused by extrinsic factors, especially UV exposure, may cause various reactions on the skin through various mechanisms. UV light triggers ROS formation, which can activate Mitogen - Activated Protein Kinase (MAPK) and subsequently triggers the formation of Activator Protein 1 (AP - 1). AP - 1 will inhibit the work of TGF - β and cause a decrease in collagen production, thus trigger Matrix Metalloproteinase 1 (MMP1) formation, which will increase collagen degradation. [1]

The presence of antioxidants, including enzymatic and non - enzymatic antioxidants found in the body, can help the body defend against increased ROS, both produced from within the body and the environment. [2] The balance between the formation of physiological antioxidants and the formation of ROS will prevent oxidative stress. [3]

Natural ingredients are an excellent solution to meet the antioxidant needs of skin exposed to UVB rays because of their relatively low toxicity with good antioxidant capacity. Oils derived from plants have been proven to be able to overcome various skin problems. The content contained in this plant oil reduces the inflammatory process, improves the skin barrier, helps wound healing, and has antimicrobial and antibacterial effects. [4]

Argan oil is one of the plant oils often used as a mixture in cosmetic products or for single use. Argan oil is a vegetable oil produced from the Argan tree seeds (*Argania spinosa*), widely found in Morocco. [5] Argan oil has two forms, virgin edible argan oil, and virgin beauty argan oil. Virgin edible argan oil is a form of argan oil that can be consumed directly or as a salad dressing and studied for various uses, including lowering lipid profiles, preventing cancer, and others. Meanwhile, virgin beauty argan oil itself has been shown to increase skin elasticity in postmenopausal women. Argan oil is rich in oleic acid, linoleic acid, phenolics, especially ferulic acid and tocopherols. [6]

Based on the content of these active ingredients, argan oil can be used to prevent aging. [7] A study conducted on 60 postmenopausal women found that argan oil used topically and orally could reduce Trans - epidermal Water Loss (TEWL) and increase skin elasticity better than olive oil. [8] In addition, argan oil inhibits tyrosinase and dopachrome tautomerase expression in mice due to the high content of antioxidants, such as tocopherols and ferulic acid. [5]8/23/2021 4:22:00 PM

Tocopherol has the primary function of preventing lipid peroxidation. Tocopherol also functions as an anti - inflammatory by inhibiting the activity of Cyclo - Oxygenase 2 (COX - 2), which will reduce the production of prostaglandin E2 (PGE2) so that it can inhibit the decline in collagen production. [9] Tocopherol is stabilized by ferulic acid so it does not oxidized easily. [21] Interaction between ocopherol and oleic acid increase the absorption of argan oil into the skin. [20] The anti - inflammatory effect of argan oil has been studied to accelerate the healing of second - degree burns in rats. [10] However, no research has been conducted on the effects of argan oil as an anti - inflammatory that can inhibit collagen decline. [7]This study aimed to analyze the

effectiveness of argan oil to prevent the inflammatory process caused by exposure to UVB rays and its effectiveness in inhibiting collagen decline. Although the argan tree itself is not found in Indonesia, there are currently many health or beauty products such as facial moisturizers, body lotions, shampoos, and so on that use argan oil as one of their components. Argan oil alone has also been widely sold in the market.

2. Methodology

The research was conducted at the Integrated Biomedical Laboratory, Faculty of Medicine, Udayana University, Denpasar, Bali, Indonesia. The research procedure was approved by the Animal Research Ethics Commission, Faculty of Veterinary Medicine, Udayana University. (Number: B/29/UN14.2.9/PT.01.04/2021).

The Argan Oil used is Elbaamri brand argan oil, which contains 100% pure argan oil imported from Morocco by PT. Smarvan Ryasa Indonesia.

A post - test - only control group study design was performed on a population of male Wistar rats aged 2 - 3 months. According to the minimum sample calculation, 36 rats were required in the study and divided into two equal groups. The experimental animals were adapted for seven days before the intervention began. The control group was smeared with paraffin oil before and after UVB were radiated, while the treatment group was smeared with argan oil.

Radiation was given three times a week. The backs of rats were smeared with paraffin and argan oil twenty minutes before and four hours after UVB exposure. This irradiation is carried out routinely for four weeks. After the treatment was completed, an incision was made after 24 hours to exclude the acute effects of UVB exposure. [11] An incision was made on the back of the rat with a 1 x 1 cm size, a depth of 0.2 mm. During these four weeks, the total dose of irradiation was 840 mJ/cm^2, which was carried out gradually starting from the smallest irradiation dose of 50 mJ/cm^2 for the first week. The dose for the second week was 70 mJ/cm^2.
Moreover, in the third and fourth weeks, the dose of UVB rays used in experimental animals was 80 mJ/cm^2. The irradiation distance is 3 cm, and the irradiation time is 45 minutes. The measurement of the exposure dose was carried out using a UV meter.

PGE2 levels were assessed using the PGE2 Examination Kit using the MyBioSource ELISA method. The amount of dermal collagen is the percentage of pixels of collagen tissue in the form of bright red tissue with Sirius red staining compared to the pixels of all tissues that appear on the histological preparation photos and expressed in percent (%). The assessment was carried out on photos of preparations in JPEG format taken with an LC Evolution camera and an Olympus Bx41 microscope with an objective magnification of 400 times, at three times the photo field of view (microphotography) to obtain than the average percentage of dermal collagen amount from the three photo fields of view.

Data analysis was performed using the SPSS 25 device (IBM, 2019). The comparative test was conducted to compare the results in the control group and the study group. An independent t - test was performed on data with normal distribution, while the Mann Whitney test was performed on data with skewed distribution.

3. Results

3.1 Histopathology

Dermal collagen appears red on picro Sirius Red stain. The image of collagen in the dermis tissue of the rat skin observed using a microscope at a magnification of 400x is shown in the following image (Figure 1.)

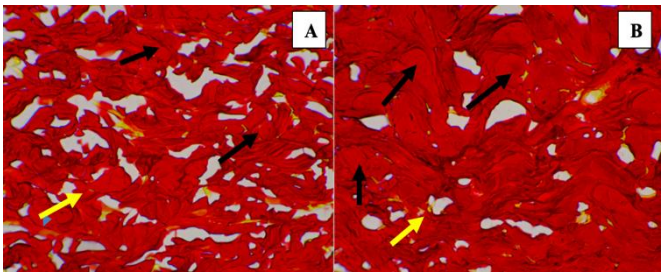

Figure 1: Histopathological Image of Dermal Collagen. (A) Control group; (B) Treatment group. The damage of collagen structure and thin arrangement of collagen fibers are more prominent in the control group. Black arrows indicate intact collagen fibers. Yellow arrows indicate incomplete collagen fibers.

3.2 Data Analysis Results

Comparative analysis on the variable levels of PGE and the amount of collagen using the Independent T - test showed a p - value <0.05 (Table 1.).

Table 1: Comparative Analysis Between Groups

Variable	Group	Mean±St. Dev	P - value
PGE2 levels (ng/mL)	Control	2.79±1.16	0.000
	Treatment	1.58±0.33	0.000
Collagen Amount (%)	Control	67.6±5.23	0.000
	Treatment	79.07±3.31	0.000

The result indicates that the topical administration of argan oil compared to the control has been shown to inhibit the increase in PGE2 levels and prevent a decrease in collagen in male Wistar rats exposed to UVB.

4. Discussion

Continuous UVB rays exposure on the skin can cause damage through several mechanisms. UV light will stimulate ROS production and activate MAPK, transcription factors AP1 and NF - κB, triggering skin inflammation and apoptotic processes in cells and cause photoaging. [12] UV light also stimulates the production of COX2, exacerbates inflammation in the skin by catalyzing the synthesis of PGE2 and iNOS (inducible Nitric Oxide Synthase). [13] COX2 and iNOS increased along with NFκB stimulation and stimulated proinflammatory cytokines. [14] Continuous exposure to UVB rays can trigger skin aging or photoaging.

The total dose of UVB irradiation used in this study was840 mL/cm^2, carried out over four weeks. From the exposure, data showed high levels of PGE2 and collagen decreased. Supported by a previous study, the exposure to UVB rays for seven consecutive days would increase COX2/PGE2 levels and decrease collagen. However, the dose used in the study was much larger, 150 mJ/cm^2. May be more similar to a study by Wiraguna, that a decrease in the collagen amount in Wistar rat's skin happened after exposure to UVB rays for four weeks with a total dose of 840mJ/cm^2. [15]

In this study, the treatment group had a lower mean of PGE2 level than the control group. In addition, in the control group, the average collagen intake was lower than in the treatment group.

This indicates that argan oil effectively inhibits the increase of PGE2 and inhibits the decrease of collagen amount due to UVB exposure in the Wistar rat's skin ($p<0.05$).

The results of the phytochemical analysis carried out at the Agricultural Technology Laboratory, Udayana University, and at Gajah Mada University show that argan oil contains 3118.12 mg/L tocopherols, 5.57% oleic acid, 47.78% linoleic acid, andphenol.544.49 µg/ml. The high tocopherol content in argan oil can function as an antioxidant. Tocopherolis a type of fat – soluble antioxidant that can protect the skin from UVB radiation. The results of this study are in line with previous studies which proved that vitamin E has antitumor and photo protective effects. [16] Besides that, tocopherols also have anti – inflammatory and anti – proliferative effects. [17] Tocopherols, especiallyγ - Tocopherol, function to inhibit Cyclo - Oxygenase 2 (COX - 2), reducing prostaglandin E2 (PGE2) acts to inhibit collagen production and trigger MMP1 expression by fibroblasts. [9, 18]

The main phenolic content in argan oil is ferulic acid. Ferulic acid acts as an antioxidant that can stabilize free radicals. Ferulic acid also plays a role in UV absorbers and has anti – inflammatory effect by inhibiting inflammatory mediators, such as IL - 8, COX, i - NOS, and NF - κB. The fatty acids contained in arganoil are mainly oleic acid and linoleic acid—the fatty acids contained in argan oil function to modify the skin barrier. Oleic acid has a high level of stability against oxidation. Combining oleic acid and antioxidants such as tocopherols has a better protective function than single tocopherols. [19] Oleic acid is one type of chemical enhancer. It's use alone or in combination can increase drug penetration in to the skin. [20] As the main PUFA component in the epidermis, Linoleic acid will be metabolized through the 15 - lipoxygenase pathway to 13 - hydroxyoctadecadienoic acid, which will act as anti - proliferativeand anti - inflammatory. This is in line with a previous research, which states a high linoleic acid content in argan oil, which reduces inflammation in the skin. [5]

It can be concluded that the tocopherol and phenolic content in argan oil acts as an antioxidant to counteract ROS due to UVB and has an anti - inflammatory effect by inhibiting COX - 2 activity and reduce PGE2 production. Meanwhile, the linoleic acid in argan oil has an anti - inflammatory role.

5. Conclusion

The skin is the outer most part of the body thus may experience aging faster due to intrinsic and extrinsic factors such as UVB. In this study, Argan oil has been shown to reduce PGE2 levels to inhibit the decrease in collagen in the skin of Wistar rats due to exposure to UVB and shows potential benefits for anti - aging. However, further studies on the effects of argan oil on humans are still needed to prove these benefits.

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