Topical application of cocoa extract (Theobroma cacao L.) to prevent photoaging by increasing superoxide dismutase levels on wistar rats skin exposed to ultraviolet light A

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ABSTRACT

Background: The latest trend in anti-aging prevention is projected through the use of antioxidants derived from natural ingredients with minimal side effects compared to chemicals. Cocoa bean (Theobroma cacao L.) is one of the sources of natural antioxidants with high flavonoid antioxidant content. This study aims to determine the effect of topical application of cocoa extract to prevent photoaging in wistar rats exposed to ultraviolet A (UVA) light.

Methods: Experimental research with animal models using 36 wistar rats exposed to UVA and divided into 2 groups, given 10% cocoa extract cream and basic cream on the back area. The parameters assessed were superoxide dismutase (SOD) levels in skin tissue. Comparative analysis with independent T-test using SPSS version 21 to compare SOD levels of both groups.

Results: Superoxide dismutase levels in both groups were found to be normally distributed (p>0.05). The results of the comparative test with the Independent T-test found that the mean SOD levels of the treatment group were higher than those of the control group at 8.07 ± 1.99 ng/ml and 5.04 ± 1.54 ng/ml (p<0.001; 95% CI: 1.82-4.24).

Conclusion: Topical application of 10% cocoa extract cream on the skin of Wistar rats exposed to UVA light resulted in higher levels of superoxide dismutase in the rat skin tissue than the control.

Keywords: cocoa extract, photoaging, Theobroma cacao, superoxide dismutase, ultraviolet A.

INTRODUCTION

Free radicals are a major factor in the skin aging through the accumulation of reactive oxygen species (ROS). An imbalance between ROS production and the natural antioxidant system will result in oxidative stress.1 Several studies have shown that UVA waves can not only cause skin aging and wrinkles but can also cause skin cancer in animals exposed to high doses of radiation for a long time.2

To protect against ROS damage, the skin contains a series of antioxidant enzymes, including superoxide dismutase (SOD). Antioxidants are the body’s defense mechanism against oxidants and play a role in maintaining the balance between oxidants and antioxidants.3 Murine studies have shown that UVA radiation to the skin disrupts enzymatic and non-enzymatic antioxidants. Some studies have also shown that SOD activity decreases drastically after exposure to UV radiation, leading to oxidative stress and skin damage.4

Cocoa beans (Theobroma cacao L.) are one of Indonesia’s most widely developed sources of natural antioxidants and are a rich source of flavonoid antioxidants. Cocoa is rich in catechins, and based on their structure, they are classified as flavan-3-ols. Flavan-3-ols are known to have several candidate target proteins that play an important role in the formation of aging.5,6 This study aims to determine the effect of topical application of cocoa bean extract cream (Theobroma cacao L.) on superoxide dismutase levels in preventing photoaging in the skin of Wistar rats exposed to UVA light. This study is expected to contribute to utilizing natural materials, namely 10% cocoa extract cream, topically in preventing photoaging in Wistar rats exposed to ultraviolet A light.

METHODS

This research is pure experimental (true-experimental) using experimental animals with a randomized posttest-only control group design. The variables evaluated were 10% cocoa extract cream as the independent variable, superoxide dismutase levels as the dependent variable, and rat strain, age, body weight, and sex as the control variable. The parameter observed in this study is superoxide dismutase level. If there is an increase,
it is a sign of a protective effect of 10% cocoa extract cream. This research was conducted at the Animal Unit of the Pharmacology Laboratory of the Faculty of Medicine, UNUD, and the Integrated Biomedical Laboratory of the Faculty of Medicine, UNUD. While the manufacture of cocoa extract was carried out at the Laboratory of Agricultural Technology, Faculty of Agricultural Technology, UNUD, the manufacture of cocoa extract cream was at the Pharmacy Laboratory of Stikes Mahaganesha. Superoxide dismutase levels were examined by the enzyme-linked immunosorbent assay (ELISA) method.

This study was conducted for four months, from May 2021 to August 2021. The subjects used were adult, female, and healthy wistar rats aged 10-12 weeks, weighing 200 - 250 grams. Exclusion criteria if it was found that rats did not want to eat, were overreactive, and bit other rats and drop-out criteria if Wistar rats died during the study. The rats were divided into 2 groups: the treatment group was given 10% cocoa bean extract cream, and the control group was given the base material on the skin of the back. After applying the topical material, irradiation was carried out with a device producing ultraviolet A light for 8 weeks. It was applied twice daily to each control and treatment group, 20 minutes before and 4 hours after irradiation. Topical material application was still carried out on days without irradiation.

The use of animals will be carried out following the rules of research ethics regulated by the Helsinki Declaration, and ethical clearance will need to be obtained from the ethics commission and scientific coordinator of health research at FK-UNUD. At the end of the study, post-mortem treatment of animals is carried out as usual by placing them in paper or plastic bags and burning them in an incinerator. After that, the data was analyze by using SPSS. Comparative analysis with independent T-test using SPSS version 21 to compare SOD levels of both groups.

**RESULTS**

Superoxide dismutase levels in both groups were found to be normally distributed (p>0.05). The results of the comparative test with the Independent T-test (Table 1) found that the mean SOD levels of the treatment group were higher than those of the control group at 8.07 ± 1.99 ng/ml and 5.04 ± 1.54 ng/ml (p < 0.001; 95%ci: 1.82–4.24).

**DISCUSSION**

Skin aging is influenced by intrinsic (genetic) and extrinsic factors, mainly caused by exposure to sunlight or ultraviolet light. Aging caused by exposure to ultraviolet (UV) radiation on the skin is called photoaging. In contrast, changes caused by other factors in the aging process, either hormonal or metabolic, are called intrinsic or chronological aging. What Indonesia is a tropical country with year-round exposure to ultraviolet sunlight, making the Indonesian population highly susceptible to extrinsic skin aging (photoaging).

Ultraviolet (UV) radiation is one of the main causes of oxidative stress in the skin. The photon energy in UV light, especially UBV with a wavelength of 280-315 nm and UVA with a wavelength of 315-400 nm, stimulates changes that eventually accumulate and cause manifestations in skin aging and cancer. A recent trend in anti-aging research is projected through plant-derived antioxidant compounds. Several studies have shown that plant-derived polyphenols and flavonoids can protect the skin against damage caused by UV radiation and have antiaging effects due to their photoprotective effects with minimal side effects compared to treatment mainly with chemicals.

This study showed that in the treatment group, there were higher SOD levels in the skin tissue of rats compared to the control. Another study with similar results showed that SOD levels began to increase significantly at a dose of 140 mg and continued to increase up to a dose of 280 mg. It was found that the effective dose of cocoa bean extract that affects SOD levels is 140 mg. Many studies have evaluated cocoa bean extract’s or cocoa powder’s antioxidant effects. A 5% cocoa bean extract has been shown to increase antioxidant activity and attenuate inflammatory responses in vivo. It is also mentioned that the increase in antioxidants is due to the increased expression of SOD and catalase, especially mitochondrial SOD. Many studies have evaluated cocoa bean extract’s or cocoa powder’s antioxidant effects. A 5% cocoa bean extract has been shown to increase antioxidant activity and attenuate inflammatory responses in vivo. It is also mentioned that the increase in antioxidants is due to the increased expression of SOD and catalase, especially mitochondrial SOD. The antioxidant properties of cocoa powder are due to the high levels of several bioactive substances, especially flavanols. Flavanols such as monomeric epicatechin and proanthocyanin polymers have strong antioxidant properties. Flavanols from cocoa powder have been shown to stimulate nitric oxide synthetase, increase perfusion rate, maintain arterial elasticity, lower blood pressure, anticoagulation, and anti-inflammation.

Superoxide dismutase is classified as an enzymatic antioxidant and functions as a first-line antioxidant in the body, so it is also called a primary antioxidant. The presence of dismutase on superoxide anion radicals in SOD will prevent the liberation of iron ions so that the formation of ROS, such as hydroxyl radicals (OH·), does not occur. In addition, SOD can also prevent vascular activation by preventing the reaction of NO+ with O2 so that the very dangerous reactive nitrogen species (RNS) ONOO- is not formed. Superoxide dismutase also has anti-inflammatory and anti-angiogenic effects by inhibiting the expression of nuclear factor-kappa B (NF-kB), protein kinase C (PKC) and hypoxia-inducible factor-1a. The enzyme SOD has been known to function in cell rejuvenation and repair, as well as reducing the effects of free radical damage.

Due to UV exposure, fibroblasts can experience impaired growth, morphological and functional changes, and increased ROS concentrations.

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**Table 1.** Comparative test of SOD levels of treatment and control groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Groups</th>
<th>p-value</th>
<th>95% CI (min-max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD level</td>
<td>Intervention</td>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Mean±SD</td>
<td>8.07±1.99</td>
<td>5.04±1.54</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Median (min-max)</td>
<td>8.06 (5.13–13.49)</td>
<td>4.97 (2.96–8.03)</td>
<td>1.82–4.24</td>
</tr>
</tbody>
</table>

*Significant if p<0.05; *Analyze using independent T-test; SD: standard deviation; 95% CI: 95% confidence interval; SOD: superoxide dismutase.
resulting in adaptive upregulation of SOD2 at the mRNA and protein levels. Superoxide dismutase-2 (SOD2) upregulation in human fibroblasts can also be induced from paracrine mechanisms either through UV exposure or the release of keratinocyte soluble factors such as interleukin-1α and tumor necrosis factor (TNF)-α. Overexpression of exclusive SOD2 in vitro was found to increase H$_2$O$_2$ concentration by activation of specific signaling pathways and transcription factors, among which is AP-1 heterodimer, which increases transcription and activation of matrix metalloproteinases. Unbalanced overexpression of SOD2 results in increased accumulation of H$_2$O$_2$, which will lead to degradation of interstitial collagen in the skin, a hallmark of skin aging. The limitation of this study is that it did not analyze other factors associated with sod levels and evaluate the therapeutic effect and toxicity at different concentrations.

CONCLUSION

Topical application of 10% cocoa extract cream to the skin of Wistar rats exposed to UVA light resulted in higher levels of SOD in rat skin tissue compared to control.

ETHICS IN PUBLICATION

This study was approved by the Research Ethics Commission of the Faculty of Medicine, Universitas Udayana, with Ethical Clearance number 1952/UN1.4.2.7.VII.14/LT/2021.

CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the publication of this article.

FUNDING

A Research and Community Service grant from Universitas Udayana funded this research.

AUTHORS CONTRIBUTION

Author IGAAEI contributes to idea construction, literature review, proposal preparation, sample preparation and intervention, data analysis, manuscript preparation and publication. Authors NS, MW, IGNU, and IGGAAP contributed to idea construction, literature review, data analysis, and manuscript review.

REFERENCES


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