The effect of purple mangosteen (Garcinia mangostana) peel extract on collagen fiber in male Wistar rats after Ultraviolet-B (UV-B) exposure

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ABSTRACT

Background: Skin aging can be caused by long-term ultraviolet (UV) exposure that usually called as photoaging. Administered antioxidant substances can delay the photoaging process. Purple mangosteen is known to have abundant antioxidant properties such as xanthones and anthocyanins. This study aims to determine the effect of purple mangosteen peel extract on the collagen fiber in male Wistar rats after UV-B exposure.

Methods: An experimental study was conducted among 21 male Wistar rats at Histology Laboratory, Udayana University from a period of March-October 2017. The samples were divided into 3 groups as follows: control, placebo, and mangosteen extract with 7 rats in each group. Collagen fiber was assessed by using a light microscope with 400 times magnification. Statistical analysis was carried out using SPSS ver. 21 software using one way ANOVA.

Results: The study found that percentage of collagen area within control group was 61.94%, placebo group 72.90%, and mangosteen peel extract 73.63%. The mean between-group analyzed using one way ANOVA found that there was a statistically significant difference in mangosteen peel extract than control group (p=0.0000), but not statistically significant if compared with placebo group (p=0.640) on the collagen fiber in male Wistar rats.

Conclusion: there was a statistically significant difference in mangosteen peel extract than control group on the thickness of collagen fiber in male Wistar rats after UV-B exposure.

Keywords: Mask, Mangosteen Peel, Photoaging, UV-B

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INTRODUCTION

The skin is most significant organ in the integumentary system. It is a complex organ which interacts with most other organs in both physiological and pathological ways. Therefore, its function is essential for survival.1 Skin aging is a type of process which occurs because our skin is exposed to factors such as ultraviolet rays. Exposure to ultraviolet rays results in the aging of our skin. Ultraviolet rays also have the potential to cause skin cancer in the long run. The mechanism of skin aging is quite complicated. This is because in sun-protected areas the most pronounced changes occur within the epidermis and affect mostly the basal cell layer. As a result, while sun-protected aged skin appears thin, finely wrinkled, and dry, photoaged skin is characterized by deep wrinkles, laxity, and roughness.2,3

Although the fundamental mechanisms are still poorly understood, a growing body of evidence points toward the involvement of multiple pathways in the generation of aged skin. Recent data obtained by expression-profiling studies and studies of progeroid syndromes illustrate that among the most important biological processes involved in skin aging are alterations in DNA repair and stability, mitochondrial function, cell cycle and apoptosis, ubiquitin-induced proteolysis, and cellular metabolism. One of the significant factors that have been proposed to play a precise role in the initiation of aging is the physiological hormone decline occurring with age. However, hormones at age-specific levels may regulate not only age-associated mechanisms but also restrict tumor-suppressor pathways that influence carcinogenesis.4,5

Understanding the molecular mechanisms of aging may open new strategies in dealing with the various diseases accompanying aging, including cancer. One of the causes of skin aging is UV-B from sun exposure. UV exposure would lead to the activation of receptors for the epidermal growth factor, IL-1, and TNF-α in keratinocytes and fibroblasts, which then activates signaling kinases.
throughout the skin via an unknown mechanism. The nuclear transcription factor activator protein, AP-1, which controls the transcription of matrix metalloproteinases (MMP), is expressed and activated. MMP-1 is a major metalloproteinase for collagen degradation. This entire process is aided by the presence of reactive oxygen species that inhibits protein-tyrosine phosphatases via oxidation, thereby resulting in the up-regulation of the receptors mentioned above. Another transcription factor NF-κB, which is also activated by UV light, also increases the expression of MMP-9,6,7

Skin aging can be delayed or reversed with the help of a facial mask. An example of a facial mask is a gel mask. The type of gel mask which used in this research was obtained from the skin extract of the purple mangosteen. Purple mangosteen is a form of tropical evergreen tree believed to have originated in the Sunda Islands and the Moluccas of Indonesia. It grows mainly in Southeast Asia, southwest India and other tropical areas such as Puerto Rico and Florida, where the tree has been introduced. The exocarp (rind) of this fruit comprises a unique array of vitamins polyphenols, like tannin and xanthones that protect the tree against insect, plant viruses, fungi infestation and even animal predation.6 In addition, extract of mangosteen peel is also known to have antioxidant properties by reducing MDA level in rats.9 This effect may have a pivotal role in the prevention of skin aging. According to the explanation above, this study aims to determine the effect of purple mangosteen (Garcinia mangostana) peel extract on collagen fiber in male Wistar rats after Ultraviolet-B (UV-B) exposure.

METHODS
An experimental study was carried out among 21 male Wistar rats at Histology Laboratorium, Udayana University from a period of March-October 2017. The samples were divided into 3 groups, 7 rats within each group. First group was control group where the rats were only given UV-B exposure. The second group was a placebo where rats were exposed to UV-B and given a mask without purple mangosteen peel extract. And the last group was purple mangosteen extract mask group where rats were given a UV-B exposure with purple mangosteen peel extract mask. Rat’s skin was peel and analyzed histologically by using Haematoxylin-Eosin (HE) staining after 4 weeks, and the area (%) of collagen within the skin was assessed. Statistical analysis was performed using SPSS ver 21.0 software and the comparison of mean between-group was conducted using one way ANOVA test. Data on the amount of collagen in each group were tested for normality by using the Shapiro-Wilk test where P > 0.05 indicates for homogenous.

RESULT
The largest average percentage of the collagen area was found in the mangosteen skin mask group which equal to 73.63%, followed by placebo group 72.90%, and control group 61.94% based on the histological analysis (Figure 1). The significance analysis was assessed by One Way ANOVA test showed that the p-value was 0.000. This result indicated that there was a statistically significant difference in the amount of collagen within the three groups (p <0.05). In order to find out the smallest difference between different groups, the Post Hoc test using Least Significant Difference-Test (LSD) was conducted between groups. The study found that the mean area of collagen P1 has a significant difference with P2 (p=0.000). In addition, the mean area of collagen P1 also has a significant differences with P3 (p=0.000). However, there was no significant difference between P2 and P3 in the mean area of collagen (p=0.654).

DISCUSSION
The results of this study showed that there was an increase of collagen level in groups treated with mask extract of mangosteen peel in the
significant effect. The study looked only at the amount of collagen in male Wistar rats exposed to UV-B light after being given a placebo and mask of mangosteen peel extract. This study still has not distinguished the effects of mangosteen skin extract in different doses.

A similar study using 24 rats, 6-9 week old female rat weighing 20-30 grams showed significant differences in collagen thickness in rat receiving UV-B exposure alone compared with rat receiving topical mangosteen peel extract (3.67 vs. 6.68; p = 0.000) and significant differences between ethanol extract compared with mangosteen peel (3.33 vs 6.77; p = 0.000). In addition to the effect on the thickness of collagen, mangosteen peel extract also affects the density of collagen, where rat who obtain mangosteen skin extract have the mean of collagen thickness 3.89, more significant than the rat exposed only with UV-B (2.83) and given ethanol (3.06) with p = 0.000. Administration of topical mangosteen peel extracts with concentrations of 50%, and a dose of 2mg/gr of rat weight can increase the thickness and density of collagen in rats exposed to UV-B.

Another study examining the similar effect of mangosteen skin extract on skin collagen performed with human samples showed that with the administration of tablets from mangosteen peel extract at a dose of 100 mg for 4 weeks, there was inhibition of pentosidine formation. Pentosidine is an advanced glycations end products (AGE) which are produced from collagen fiber glycation processes. The presence of AGE accumulation in the human skin increases with collagen degradation resulting in skin aging. Antioxidant polyphenols contained in mangosteen peel extract (Xanthones) are thought to reduce AGE formation in in-vitro and in-vivo. In this study, it was also shown that after a month of mangosteen skin extract per-oral administration; there was an increase in skin elasticity compared to before treatment (0.663 vs. 0.715; p <0.001), where collagen levels in the skin determined skin elasticity.

A study showed that the use of mangosteen peel extract with 95% concentration could significantly change the amount of MMP-1, which MMP-1 is one of the compounds that contribute to the degradation of collagen in the skin. Mangosteen extract contains antioxidant compounds, xanthones with 95% content; it also contains anthocyanins. Antioxidant activity in mangosteen peel extract is so high that it can decrease ROS production and inhibit further collagen destruction due to UV-B exposure.

Anthocyanin which also contained in mangosteen peel extract is a powerful antioxidant because of its high reactivity as an electron donor and can stabilize unpaired electrons. Existing

Table 1. The result of Descriptive Testing on Collagen Area Percentage in the male Wistar Rats dermis after exposed to UV-B among the Group

<table>
<thead>
<tr>
<th>Collagen</th>
<th>n</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>Standard Deviation (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>45.95</td>
<td>72.84</td>
<td>61.94</td>
<td>5.89</td>
</tr>
<tr>
<td>Placebo</td>
<td>7</td>
<td>67.18</td>
<td>83.38</td>
<td>72.90</td>
<td>3.56</td>
</tr>
<tr>
<td>Mask</td>
<td>7</td>
<td>64.32</td>
<td>82.48</td>
<td>73.63</td>
<td>5.29</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>45.95</td>
<td>83.38</td>
<td>69.49</td>
<td>7.30</td>
</tr>
</tbody>
</table>

**Graphic 1.** Q-Q Plot curve in all three groups Based on Normality Test of Data Distribution with Small Sample (Saphiro-Wilk). P value for control is 0.541, placebo 0.134, and mask 0.135. All data are normally distributed (p>0.005)

**Graphic 2.** Curve Shown Mean Difference of Collagen Area Percentage in Control Group, Plasebo, and Mangosteen Skin Mask.

**Table 2.** Results of Post Hoc LSD Analysis On Male Wistar Rats Dermis Collagen Percentage That Exposed to UV-B in Each Group

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Differences</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (P1) vs Placebo (P2)</td>
<td>-10.958</td>
<td>-14.051</td>
<td>-7.863</td>
</tr>
<tr>
<td>Control (P1) vs Mask (P3)</td>
<td>-11.684</td>
<td>-14.773</td>
<td>-8.590</td>
</tr>
<tr>
<td>Placebo (P2) vs Mask (P3)</td>
<td>-0.7261</td>
<td>-3.820</td>
<td>2.376</td>
</tr>
</tbody>
</table>

Note : *= Significant different when p <0.05

dermis compared to controls, but not statistically significant when compared with the placebo group. The insignificant difference in the mean of collagen in rat given the mask of mangosteen peel extract compared to placebo may occur probably because the dosage of mangosteen skin extracts present in the mask is insufficient to cause a statistically
literature, both anthocyanin and xanthones have 3 mechanisms in protecting collagen which will be degraded by free radical by inhibiting phosphorylation of tyrosine kinase which prevents the activation of MAP kinase, JNK, and transcription of AP-1 complex, protection against TGF-β and procollagen so that new collagen synthesis is not inhibited, and inhibition of NF-KB transcription so that MMP-8 activation is inhibited. The combination of this mechanism will inhibit cAMP (cyclic Adenosine monophosphate) inhibiting protein kinase A, which is one of the MMP activators, in which MMP plays a role in degrading collagen.10,11

Based on a pre-existing literature study, studies using mangosteen peel gel masks have never been done before. Previous research done in Indonesia using topical cream of mangosteen peel extract is not gel mask. Also, that study used a sample of female rats while the sample in this study was male rats.

CONCLUSION
Based on the results of the study and discussion it can be concluded that application of mask gel from mangosteen peel extract (Garcinia Mangostana L) can prevent the decrease in the amount of dermal collagen of male Wistar (Rattus norvegicus) rat skin after exposure to UV-B rays.

REFERENCES