ABSTRACT
Introduction: UV-B exposure to the skin may produce ROS, decreasing collagen density and increasing MMP-1 expression. Longan leaf extract cream contains antioxidant and is expected to prevent decrease in collagen density and MMP-1 expression increase. Objective: The research aims at administering longan leaf extract cream to increase collagen density and decrease MMP-1 expression in BALB/c mice exposed to UV-B rays. Methods: This experimental research employed a Post Test Only Controlled Group Design. The subjects were 24 BALB/c mice, which were randomly divided into 4 groups. The Control Group (Ctr-G) was only administered with basic cream and exposed to UV-B rays, while Groups DLS-3, DLS-6, and DLS-13 were respectively administered with longan leaf extract cream at doses 3.25%, 6.5% and 13% and exposed to UV-B rays for 4 weeks at a dose of 1 MED. On day 29, a tissue excision biopsy was performed to determine the collagen density and MMP-1 expression. The collagen density preparations were made using Masson's Trichome staining and MMP-1 preparations were made using immunohistochemical staining. Results: Kruskal wallis analysis showed statistically no significant differences in collagen density and MMP-1 expression between groups (p value > 0.05). Conclusion: Administration of longan leaf extract cream at doses of 3.25%, 6.5% and 13% had proven unable to increase collagen density nor decrease the MMP-1 expression significantly after UVB exposure. Keywords: longan leaf extract, collagen density, MMP-1 expression

INTRODUCTION
Aging is certain for every living being. It is influenced by internal and external factors. One of the external factors which influence aging is UV-B ray exposure. UV-B ray exposure trigger Reactive Oxygen Species (ROS), which may then increase Activator Protein-1 (AP-1). An increase in AP-1 will be followed with an increase of Matrix Metalloproteinase-1 (MMP-1) expression and inhibition of Transforming Growth Factor-β (TGF-β) production, which is followed with a decrease in collagen density (Farage et al.,2010; Tobin, 2017). This mechanism may be prevented with administration of antioxidant containing polyphenol, which may be found in longan (Dimocarpus Longan

The Effect of Administration of Longan (Dimocarpus Longan L Steud) Leaf Extract Cream on Collagen Density and MMP-1 Expression in the Dermis of BALB/C Mice Exposed to UV-B Rays

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ABSTRAK
Latar Belakang: Kris ekstrak daun kelengkeng yang mengandung antioksidan diharapkan dapat meningkatkan densitas kolagen dan menurunkan ekspresi MMP-1 akibat paparan UVB. Tujuan: Mengevaluasi apakah pemberian krim ekstrak daun kelengkeng dapat meningkatkan densitas kolagen dan menurunkan ekspresi MMP-1 pada mencit BALB/c yang dipapar sinar UV-B. Metode: Penelitian eksperimental dengan Post Test Only Controlled Group Design, sampel berjumlah 24 ekor mencit BALB/c betina, dibagi secara acak menjadi 4 kelompok. Kelompok control (Ctr-G), hanya diberi krim dasar; kelompok DLS-3, DLS-6, dan DLS-13 masing-masing diberi krim ekstrak daun kelengkeng dengan dosis 3.25%, 6.5% dan 13%. Keempat kelompok ini kemudian dipapar sinar UV-B selama 4 minggu dengan dosis 1 MED. Hari ke 29 dilakukan ekisi kulit dan pembuatan preparat historis untuk pemeriksaan densitas kolagen dan ekspresi MMP-1. Untuk mengidentifikasi kolagen dan ekspresi MMP-1 dilakukan pengecatan masing-masing dengan Trichome's Mansonn dan Imunohistokimia. Hasil: Analisis Kuskall wallis menunjukkan bahwa densitas kolagen dan ekspresi MMP-1 tidak menunjukkan perbedaan bermakna di antara kelompok, p > 0.05. Kesimpulan: Pemberian krim ekstrak daun kelengkeng dengan dosis 3.25%, 6.5% dan 13% terbukti tidak meningkatkan densitas kolagen dan menurunkan ekspresi MMP-1 secara bermakna setelah mendapatkan paparan UVB. Kata kunci: ekstrak daun kelengkeng, densitas kolagen, ekspresi MMP-1

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The Effect of Administration of Longan (Dimocarpus Longan L. Steud) Leaf Extract Cream on Collagen Density...

L. Steud) leaf extract. Polyphenol in longan leaf extract may serve as the antagonist of the UV signaling pathway, thus hydroxyethyl (OH) discharge as the result of UVB exposure may be inhibited (Yuge Liu, 2012; Gilchrest, 2013). However, it is unknown whether longan leaf extract cream as topical antioxidant influences the increase in collagen density and the decrease in MMP-1 expression in the dermis exposed to UV-B rays.

UV rays exposure contributes to skin aging for about 80% (Listiawan, 2016). Early aging prevention has been performed generally by applying products such as whitening cream, sunblock, etc. Some products contains hazardous chemicals such as mercury or hydroquinone, which may cause a decrease in the amount of skin’s melanin. A decrease in the amount of skin’s melanin may potentially cause skin to be thinner, dry and sensitive to UV rays radiation (Pandel et al., 2013). Evidence shows that polyphenol antioxidant contained in plants may prevent free radicals from emerging and inhibit the effect of UV rays exposure (photoprotective) (Yuge Liu, 2012; Gilchrest, 2013). Polyphenol in longan leaf extract has antioxidant, anti-inflammatory, antivirus, antipyretic, antibacterial and anticancer activities (Apriyanto et al., 2016). Previous researches show that longan leaf contains bioactive contents such as polyphenol (ellagic acid, catechin, ethyl gallate, gallic acid, kaempferol and quercetin) (Salama, 2015, Qing Wu et al, 2013) phenol, steroid, saponin, flavonoid, triterpenoid (Apriyanto et al., 2016), and glycoside (Xue et al., 2015). Moreover, the polyphenol compound (ellagic acid) in longan leaf extract has antioxidant, anti-inflammatory and photoprotective effects by preventing formation of free radicals (Qing Wu et al., 2013).

Referring to the various facts, administration of longan leaf extract cream is expected to prevent MMP expression and collagen degradation, which will inhibit early aging. The purpose of this research is to evaluate if administration of longan (Dimocarpus longan (L) Steud) leaf extract cream may decrease MMP-1 expression and increase collagen density in the dermis of BALB/c exposed to UV-B rays.

METHOD

This experimental research employs a Post-test only control group design. The research subjects were 24 BALB/c mice, which were randomly divided into 4 groups, consisting of the control group (Ctr-G), which was administered only with basic cream, and groups DLS-3, DLS-6, and DLS-13, respectively administered with longan leaf extract cream at doses 3.25%, 6.5% and 13%. The four groups were then exposed to UV-B rays at a dose of 1 Minimal Erythema Dose (MED) for 8 minutes at an irradiation distance of 40 cm 5 times per week for 4 weeks. On day 29, skin excisions were performed and histological preparations were made to examine the collagen density and MMP-1 expression. Masson’s Trichome staining and Immunohistochemical staining were performed to identify the collagen density and MMP-1 expression. This research was conducted upon approval of the Commission of Ethics of the Faculty of Medicine, Unissula under No. 76/II/2018/ Komisi Bioetik at the Laboratory of the Faculty of Medicine, Unissula Semarang.

Collagen Examination

The collagen density was assessed semi-quantitatively using Masson’s Trichome staining scoring method. The preparations were observed at a 100x magnification in one field of view and photographed using an LC evolution camera into JPG format. The field of view was taken at the area with the most collagen content as marked in green. The scores would be from 0 to 4 as may be observed in table 1 below (Vita, 2016).

<table>
<thead>
<tr>
<th>Score</th>
<th>Remarks</th>
</tr>
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<tbody>
<tr>
<td>+0</td>
<td>collagen fiber is not found</td>
</tr>
<tr>
<td>+1</td>
<td>low collagen fiber density, with faint green color in &lt;1/3 of field of view</td>
</tr>
<tr>
<td>+2</td>
<td>moderate collagen fiber density, with stronger green color in &gt;1/3 and &lt;2/3 of field of view</td>
</tr>
<tr>
<td>+3</td>
<td>dense collagen fiber density, in &gt;2/3 of field of view</td>
</tr>
<tr>
<td>+4</td>
<td>highly dense collagen fiber density</td>
</tr>
</tbody>
</table>

MMP-1 Expression Measurement

MMP-1 expression stated expressed if brown stromal fibroblast cells, which expressed MMP-1 in the immunohistochemical (IHC) examination, was found. It was measured by counting the amount of stromal fibroblast cells which expressed positive brown MMP-1 using an Olympus Cx21FS1 microscope at 400x magnification in 5 (five) fields of view (Vita, 2016).

Statistical Analysis

The results of Shapiro-Wilk analysis and Levene’s test showed data in abnormal and non-homogenous distribution, thus Kruskall-Wallis analysis was then employed for the statistical analysis. The statistical test
result will be significant if $p < 0.05$ (Dahlan, 2014).

**RESULTS**

Upon administration of longan leaf extract cream to the UVB rays-exposed skin, the average collagen density and MMP-1 expression are as presented in table 2 below.

The research result shows that the highest average collagen density is with group DLS-13, followed with group DLS-6, and the lowest average is with group DLS-3 (figure 1). On the other hand, the lowest MMP-1 expression is with group DLS-13, followed with DLS-6, and the highest MMP-1 expression is with group DLS-3. A statistical analysis needs to be conducted to examine if there is significant difference between the groups. Considering that the Shapiro-Wilk analysis

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group</th>
<th>Mean (±SD)</th>
<th>N (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C-G</td>
<td>1.00 (±0.632)</td>
<td>N=6</td>
</tr>
<tr>
<td>Collagen Density</td>
<td>DLS-3</td>
<td>1.33 (±0.816)</td>
<td>N=6</td>
</tr>
<tr>
<td>(Score)</td>
<td>DLS-6</td>
<td>1.50 (±0.548)</td>
<td>N=6</td>
</tr>
<tr>
<td></td>
<td>DLS-13</td>
<td>2.00 (±0.632)</td>
<td>N=6</td>
</tr>
<tr>
<td>MMP-1 Expression</td>
<td>C-G</td>
<td>3.53 (±1.849)</td>
<td>N=6</td>
</tr>
<tr>
<td>(E)</td>
<td>DLS-3</td>
<td>2.73 (±1.638)</td>
<td>N=6</td>
</tr>
<tr>
<td></td>
<td>DLS-6</td>
<td>2.40 (±1.233)</td>
<td>N=6</td>
</tr>
<tr>
<td></td>
<td>DLS-13</td>
<td>1.33 (±0.327)</td>
<td>N=6</td>
</tr>
</tbody>
</table>

Figure 1. Histopathological image of collagen density in green color (red arrow) is stained with Masson’s Trichome obtained with a 100x microscopic magnification. Statistical analysis shows non-significant difference among the groups.

Figure 2. Histopathological image of MMP-1 expression in brown color (red arrow) is stained with Masson’s Trichome obtained with a 100x microscopic magnification. Statistical analysis shows non-significant difference among the groups.
DISCUSSION

Based on the research result, the collagen density of groups administered with longan leaf extract cream at doses (3.25%, 6.5% and 13%) exposed to UV-B rays is higher than the control group. The higher the dose, the higher the collagen density is, and the lower the MMP-1 expression. Administration of longan leaf extract cream increases collagen density and reduces MMP-1 expression although insignificantly different, based on the statistics. Some of the influencing factors are dose and bioavailability of the cream as well as inflammatory response mechanism resulting from administration of longan leaf extract cream (Yuge Liu, 2012; Muthukumarasamy et al., 2016). Therefore, in regard to dose, further research needs to be conducted on the effect of longan leaf extract cream at higher doses. In addition, cream bioavailability also influences them, since longan leaf extract cream contains cream with o/w basis, uses ethanol as solvent and contains active substances such as polyphenol (ellagic acid) and quercetin. Cream with o/w basis may be easily absorbed and vanish, thus its bioavailability is low. Dewi reports that cream containing o/w may be easily absorbed in comparison to that containing w/o even if it added propylene-glycol in a higher dose (Kurniasari, 2010). Ultraviolet (UV) rays exposure, particularly UV-B rays, is the main factor to cause skin damage (Liebel et al., 2012). UV-B rays cause depletion of skin’s extracellular matrix and an increase in matrix metalloproteinase enzymes, such as MMP-1, MMP-3, and MMP-9, which thus increase collagen degradation (Hwang et al., 2013; Jung et al., 2014). The in vitro research conducted by Pinus Desiflora states that UV-B rays exposure is capable of inducing c-jun and c-fos in mRNA expression, stimulate MMP expression and stimulate AP-1 activity, which may decrease collagen density or amount through a decrease in procollagen synthesis and an increase in collagen degradation (Jung et al., 2014).

The UV-B rays’ mechanism on skin is similar to ligand receptor’s work, which is producing ROS which will activate AP-1, increasing collagen degradation resulting from an increase in MMP-1 expression as collagen degrader. One exposure to sun’s UV radiation may disturb connective tissues by increasing MMP-1 expression and increasing dermal collagen degradation (Watson et al., 2014; Pittayapruek et al., 2016).

Longan leaf extract contains antioxidant in the form of polyphenols (ellagic acid, catechin, ethyl gallate, gallic acid, quercetin and kaempferol) which serve as free radical scavenging and antagonist of the UV signaling pathway by neutralizing hydroxyl group (OH) of which formation are triggered by UV-B rays. Consequently, elastase activity, MMP-1 expression, and collagen degradation may be inhibited and procollagen type 1 expression is otherwise increased (Yuge Liu, 2012; Sahu et al., 2013; Tilbrook et al., 2013).

This research result is in line with the research conducted by J.Bae that administration of polyphenol ellagic acid cream in berries extract is capable of preventing a decrease in collagen, decreasing skin's inflammatory response to UV-B radiation and preventing collagen degradation by blocking MMP-1 production resulting from UV-B rays (Bae et al., 2010). The research is in line with the research conducted by Afaq that polyphenols contained in plant of which fruit, leaf, etc. are taken are capable of serving as photoprotector by preventing or decreasing inflammatory process, proliferation, DNA damage, immunosuppression, preventing dysregulation of important cellular signaling pathway and preventing skin malignancy (Afaq and Katiyar, 2011). The research conducted by Y.Qing Wu states that longan leaf extract has antioxidant effect containing 8 polyphenols (gallic acid, 3,4-0-di-methyl ellagic acid, ellagic acid, ethyl gallate, quercetin, kaempferol, rhamnoside) which are capable of preventing free radicals (Panyathep et al., 2013). Moreover, the research conducted by Vicentini states that quercetin in flavonoid also exists in longan leaf extract, as proven that it is able to reduce NFκB induction for 80% and reduce inflammatory cytokines expression such as IL-1 (60%), IL-6 (80%), IL-8 (76%), and TNF-α (69%), thus quercetin is capable of blocking UV-B induction and inflammatory cytokine is not formed, AP-1 activation is inhibited and skin damage resulting from photoaging is also inhibited (Vicentini et al., 2011).

CONCLUSION

Administration of longan leaf extract cream at doses 3.25%, 6.5%, and 13% tends to increase collagen density and reduce MMP-1 expression on female BALB/c mice exposed to UV-B rays.
FUNDING
This research is conducted without any assistant or grant.

CONFLICT OF INTEREST
There is no conflict of interest in the research.

REFERENCES


Jung, H. Y. et al. (2014) ‘Pinus densiflora extract protects human skin fibroblasts against UV-induced photoaging by inhibiting the expression of MMPs and increasing type I procollagen expression’, Toxicology Reports. doi: 10.1016/j.toxrep.2014.08.010.

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