Effect of single clove garlic (*Allium sativum L.*) on clinical symptoms of photoaging in Wistar rats exposed with UV-B

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ABSTRACT

**Background:** Photoaging is a premature aging that occurs on skin due to the ultraviolet light exposure that causes the clinical symptoms, one of which is wrinkle. One kind of material that can prevent photoaging is antioxidant. Single clove garlic has the highest antioxidant activity compared to the other elements.

**Aim:** This research aimed at investigating the effectivity of single clove garlic in inhibiting the clinical symptom of photoaging.

**Method:** This research was an experimental pre and post-test control group design involved 30 male Wistar rats. They were divided into 6 experimental groups and all of them were exposed to UV-B light with the amount of 840 mJ/cm². Control group (P1) was only exposed to UV-B while the other groups were treated with placebo cream (P2), sunblock (P3), 5% garlic cream (P4), 10% garlic cream (P5) and 20% garlic cream (P6) respectively. The clinical symptom in the form of wrinkle was observed by using dermascope, and the observations were categorized based on Glogau Scale. The statistical analysis utilized the Wilcoxon and Kruskal-Wallis test which was followed by the Mann-Whitney test.

**Result:** There was a significant difference in the apparent wrinkle on P1 and P3 so that P5 and P6. Meanwhile, the comparison between P1, P2 and P4 was not significant.

**Conclusion:** The single clove garlic extract can prevent photoaging and has a similar protective effect for the skin as of sunblock.

**Keywords:** Antioxidant, single clove garlic, photoaging

INTRODUCTION

Photo-ageing is a premature aging process on the skin due to the UV light exposure which causes damages on the skin’s primary structural components.¹ The damages related to photoaging are dominantly caused by UV-B.² UV-B triggers the formation of ROS, induces the activation of AP-1 and NF-κB factors. The effect caused is the increase in the production of MMP-1 which degrade the type 1 collagen and results in wrinkles.³ In addition, a translocation from active NF-κB to the nucleus may occur that will stimulate cytokine to create inflammation response, thus further oxidative stress occurs.⁴

Antioxidant is a material that may inhibit photoaging occurrence and may be found in garlic plant. Garlic contains antioxidant contents of SAC (S-Allylcysteine), uracil, and CA (caffeic acid).⁵ In addition quercetin content is also found in garlic.⁶ Based on the previous researches, this antioxidant was found to be able to reduce skin thickness and wrinkles. This antioxidant content may also maintain the elastin and collagen fibers from the effects of UV light exposure as well as increase the production of type I procollagen.⁵⁷ The antioxidant contents in garlic is almost similar to the single clove garlic, yet the single clove garlic contains higher antioxidant activity compared to the other types.⁸

METHOD

This research was an experimental study by applied the randomize pre and post-test control group design. This research was conducted at the Integrated Biomedical Laboratory of the Faculty of Medicine of Universitas Udayana, from July to August 2019. The target population was the male Wistar rats (*Rattus norvegicus*) which were obtained from the Integrated Biomedical Laboratory of the Faculty of Medicine of Universitas Udayana. The random sampling divided the real sample into 6 treatment groups. The determination of the number of samples, which was 5 rats in the respective groups, was calculated based on Federer formula so that the total samples were 30 male Wistar rats. The inclusion criteria were active male Wistar rats aged 3-4 months old with 150-200 grams in weight.

The single clove garlic was obtained from the garlic merchant at Badung Market, Denpasar which was then identified at the Determination Research
Center of Eka Karya Bedugul Botanical Garden. Such single clove garlic was then extracted until 100 grams of single clove garlic powder were obtained, which was then continued by maceration technique using 95% ethanol solvent which was conducted at the Integrated Biomedical Laboratory of the Faculty of Medicine, Universitas Udayana. The result of such maceration was then evaporated by vacuum rotary evaporator until viscous extract was obtained, which was conducted at the Post-Graduate Laboratory of the Faculty of Medicine, Universitas Udayana. The basic cream (placebo) and the single clove garlic extract cream were produced at the Pharmaceutical Academy of Saraswati University. The production of the essential cream used the ingredients of stearic acid, Adeps lanae, paraffin liquid and nipagin. The single clove garlic extract cream with the same ingredients was added with 5%, 10% and 20% single clove garlic extract. All of the sample groups were exposed to UV-B light which was produced by the UV-B source lamp made in Poland with the type of Philips UV-B-311 nm (pl-s 9 w/01).

The control group (P1) was not treated with cream and the group which was treated with placebo cream (P2), sunblock (P3), 5% single clove garlic cream (P4), 10% (P5) and 20% (P6). The male Wistar rats which had been acclimatized, shaved on the back to be treated with UV-B light exposure and to be treated with creams for respective treatment groups. The UV-B light was exposed 3 times a week for 4 weeks with a total amount of 840 mJ/cm². The P2, P3, P4, P5 and P6 groups were treated by cream with the amount of 0.05mg/cm² of the rat's skin surface area, applied 2 times a day, namely 20 minutes prior to being illuminated and 4 hours after the illumination for 4 weeks. After 4 weeks, observation on the wrinkle on the Wistar rats' back skin was conducted based on the staging criteria of Glogau Scale Measure by using a dermascope equipment. The Glogau Scale Measure criteria, consisted of 4 grades namely grade I for the skin condition which is absent of wrinkles, grade II for skin condition with wrinkles when there is any movement, grade III for the skin condition with wrinkles even during resting condition, and grade IV for the skin condition which has more severe wrinkles if compared to grade III. The criteria for photoaging degree was measured only based on the wrinkles because Wistar rats' skin is not able to be pigmented.

The research result data was entered into the ordinal data which would be analyzed by using non-parametric statistics. Wilcoxon analysis test was used for the data comparison of the pre and post-test scores of each group, meanwhile Kruskal-Wallis analysis test was used for the data comparison of the post-test scores of the six groups which was then continued by Mann Whitney test to find out the different groups. This research has been granted an ethical eligibility permit by the Research Ethics Commission of the Faculty of Medicine, Universitas Udayana. The register number was 1194/UN14.2.2.VII.14/LP/2019.

**RESULTS**

The skin conditions of each rat group on pre-test were categorized in the same grade – grade I (Figure 1). The photoaging degree score results that dominated the post test of group P1 and P2 was grade 3, as much as 60%. The dominant photoaging degree score of groups P3 and P5 was grade 1, as much as 60%. Group P4 provided photoaging degree score results of grade 2 as much as 100%, meanwhile group P6 obtained degree score of grade 1, as much as 80%.

The result of the post-test observation by using dermascope can be seen on Figure 2. The arrow on Figure 2 shows that wrinkles were present which was seen on the rat's skin after being exposed to UV-B light. The wrinkles were significantly seen in
groups P1, P2 and P4, but wrinkles were not seen on the skin of the rats in groups P3, P5 and P6. As to investigate the difference between the degree scores of the pre-test and post-test degree of each group, Wilcoxon test was conducted with the results as shown in Table 2.

The significant results (p<0.05) were shown by groups P1, P2 and P4, meanwhile the non-significant results (p>0.05) were shown by groups P3, P5 and P6. This shows that there were different gradings among the pre-test and post-test skin conditions on groups P1, P2, and P4 which were indicated by the appearance of the clinical picture of photoaging which was caused by the UV-B light exposure.

The Kruskal-Wallis test on the pre-test phase provided a non-significant result (p>0.05) due to the absent of photoaging degree difference on the pre-test and the post-test condition of the six groups. However, the Kruskal Wallis test on the post-test phase showed a significant difference (p<0.05) on the data of the photoaging degree scores of the six groups (Table 3). After being indicated that wrinkles appeared with different gradings for each treatment groups, the analysis was then continued by observing the grading difference on each treatment groups.

The analysis based on Mann Whitney test shows a significant difference (p<0.05) on the comparison of the groups between P1 and P3, P1 and P5, P1 and P6, P2 and P3, P2 and P5, P2 and P6, as well as P4 and P6. This significant difference indicated that there were differences in the clinical pictures of wrinkles which occurred between several groups.

Non-significant difference (p>0.05) could be shown on the comparison between the groups of P1 and P2, P1 and P4, P2 and P4, P3 and P4, P3 and P5, P3 and P6, P4 and P5 as well as P5 and P6. This non-significant difference indicates that there was no difference in the clinical pictures of wrinkles which occurred between several groups.

**DISCUSSION**

The comparison of photoaging pictures in pre-test and post-test on groups P1, P2 and P4 resulted differently. This shows that aging occurred in post-test with the increase of photoaging grade. The different post-test data of groups P1 and P2 had no significant results. This shows that the placebo or basic cream treatment on group P2 had no effect on the occurrence of photoaging clinical pictures. The comparison between P1 and P3 shows significant difference. This shows that sunblock cream could protect the skin from photoaging effect. The observation result on the post-test data

### Table 2. Pre and post-test clinical picture scores of photoaging on Wistar rats’ skin

<table>
<thead>
<tr>
<th>Pre and post-test treatments</th>
<th>n</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>5</td>
<td>0.038*</td>
</tr>
<tr>
<td>P2</td>
<td>5</td>
<td>0.038*</td>
</tr>
<tr>
<td>P3</td>
<td>5</td>
<td>0.157ns</td>
</tr>
<tr>
<td>P4</td>
<td>5</td>
<td>0.025*</td>
</tr>
<tr>
<td>P5</td>
<td>5</td>
<td>0.157ns</td>
</tr>
<tr>
<td>P6</td>
<td>5</td>
<td>0.317ns</td>
</tr>
</tbody>
</table>

Note: *, significance at p<0.05; ns, not significance; all based on Wilcoxon test.

### Table 3. Clinical picture scores of photoaging on the post-test

<table>
<thead>
<tr>
<th>Treatments</th>
<th>n</th>
<th>Mean Rank</th>
<th>Min</th>
<th>Max</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>P1</td>
<td>5</td>
<td>23.50</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>5</td>
<td>23.50</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td>5</td>
<td>10.30</td>
<td>1</td>
<td>2</td>
<td>0.002*</td>
</tr>
<tr>
<td>P4</td>
<td>5</td>
<td>17.50</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>P5</td>
<td>5</td>
<td>10.30</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>P6</td>
<td>5</td>
<td>7.90</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

Note: *, significance at p<0.05; ns, not significance; all based on Kruskal-Wallis test.
Single clove garlic extract is also known to be able to increase sirtuin 6. Sirtuin 6 can reduce NF-κB as well as inhibit the occurrence of cell aging and apoptosis. The inhibition of NF-κB activity trajectory will have an effect on the inhibition of expression of MMP thus reducing the collagen damage occurrence in photoaging. Furthermore, DATS can inhibit the formation of iNOS and COX-2 protein, so that the production of NO and PGE2, which can be the cause of inflammation, is also decreasing.

CONCLUSION

The administration of basic or placebo cream and 5% single clove garlic cream did not have significant effect on skin protection from photoaging effect. The inhibiting effects of photoaging could be shown with the administration of 10% and 20% single clove garlic which had the same effectiveness as the administration of sunblock cream.

REFERENCES

7. Kim HK. Garlic supplementation ameliorates UV-Induced photoaging in hairless mice by regulating antioxidative activity and MMPs expression. Molecules. 2016; 21(1).
12. Wen SY et al. Diallyl trisulfide suppresses doxorubicin-induced cardiomyocyte apoptosis by inhibiting MAPK/
