THE EFFECTIVENESS OF HAEMATOCOCCUS PLUVIALIS EXTRACT ON SUPEROXIDE DISMUATSE LEVEL OF RATTUS NORVEGICUS POST ULTRAVIOLET B EXPOSURE

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ABSTRACT

Background : Exposure of ultraviolet B from the sun can trigger the formation of free radicals, and causing oxidative damage on skin. One of the treatments for skin damage caused by UV B exposure is using 2% glutathione cream. and using green algae Haematococcus pluvialis extract cream.

Objective : To analize the differences in the effectiveness of giving Haematococcus pluvialis extract cream and glutathione cream on white rats post ultraviolet B exposure.

Method : The type of research used in this study is experimental with the post test only control group design approach. The number of samples was calculated using the Federer formula, with a total sample of 30 white rats that are devided into 6 groups. The parameter is superoxide dismutase enzim that is measured using spectrophotometer.

Result : As a normal control group, 5 white rats were not exposed to UV B and were not given any cream while 25 white rats were exposed to UV B light for 2 hours in 14 days, then 5 rats as negative control group were not given any cream, 5 rats as positive control group were given 2% glutathione cream, 5 rats were given 10% Haematococcus pluvialis cream, 5 rats were given 20% Haematococcus pluvialis cream, and 5 rats were given 30% Haematococcus pluvialis cream. The results of this research showed that rats that were given 20% and 30% Haematococcus pluvialis cream has higher superoxide dismutase levels than the other groups.

Conclusion : Haematococcus pluvialis extract cream is effective in treating oxidating damage on white rats post ultraviolet B exposure.

Keyword : Haematoccus plucialis estract cream, glutathione cream, superoxide dismutase, ultraviolet B.

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INTRODUCTION

Sunlight contains ultraviolet light which has many benefits that can be felt by humans, which is the formation of vitamin D3, bone formation, and maintaining immunity. Ultraviolet rays are divided into ultraviolet A, ultraviolet B, and ultraviolet C. Ultraviolet A and ultraviolet B can penetrate the ozone layer so they can penetrate the skin. Ultraviolet A can penetrate to the dermis layer while ultraviolet B rays are mostly absorbed by the outermost layer of skin and only a small portion penetrates of the dermis of the skin. Ultraviolet rays from the sun can have a negative impact on humans if they are exposed to excess. Ultraviolet A rays is able to penetrate the skin deeper and can damage skin DNA directly which can cause skin aging while ultraviolet B rays can cause sunburn and cancer formation as a result of oxidative stress of the skin. When the skin is exposed to ultraviolet it will stimulate the production of melanin which functions to fend off ultraviolet rays, but the longer the skin is exposed to ultraviolet light, the skin is no longer able to produce melanin, which is resulting sunburn and hyperpigmentation on the skin. Continuous sunlight exposed can also induce the growth of skin cancer. [1]

Compounds that can counteract free radicals are antioxidants, which are active ingredients that can protect the skin from oxidative damage. In this study, the antioxidants used were Glutathione cream and *Haematoccous pluvialis* extract cream which contain astaxanthin as an antioxidant that can help repair oxidative damage caused by free radicals from UV B exposed.[2]

Glutathione as an endogenous antioxidant, plays a major role in neutralizing free radicals. Glutathione has the function of protecting and repairing the brain, heart, kidneys, eyes, liver, and skin from oxidative damage. Glutathione has an anti-melanogenic effect that can lighten the skin. Glutathione works directly to neutralize free radicals by binding to free radical molecules, resulting in the detoxification of free radicals, heavy metals, and other harmful chemical reactions so that they can be removed from the body.[3,4]

The use of Haematococcus pluvialis green algae extract as an antioxidant needs to be reviewed from the astaxanthin content to protect and repair the skin from exposure to ultraviolet rays that can cause oxidative damage. According to research, astaxanthin has antioxidant activity 10 times stronger than the beta-carotene, canthaxanthin, lutein, and zeaxanthin groups. According to other studies, it was also found that astaxanthin was 100-500 times more effective than vitamin E in preventing fat peroxidation in vivo. Astaxanthin contained in Haematococcus pluvialis works by binding to singlet oxygen, which is a type of ROS that is formed due to exposure to UV light. Singlet oxygen is bound by astaxanthin through a physical mechanism, the excess energy from the singlet oxygen is transferred to the carotenoid structure which is rich of electrons and converts it into heat energy so that singlet oxygen is no longer formed and reacts with other ROS to prevent and stop the chain reaction.[5,6]

The parameter of this research is the superoxide dismutase (SOD) enzyme, which is a group of enzymes that function to catalyze the removal of free radicals which is superoxide anions into hydrogen peroxide and oxygen. The increase of oxidative stress due to chronic hypoxia happens because of excessive production of free radicals without any compensation for antioxidant enzyme activity. Several studies have shown that during hypoxia, the production of free radicals will increase, thereby suppressing the activity of the SOD enzyme. The provision of antioxidants therapy is expected to help the work of the SOD enzyme so that it can counteract free radicals in the body.[2]

The purpose of this research is to study the effectiveness of green algae *Haematococcus pluvialis* on Superoxide

Dismutase level of Wistar rats post ultraviolet B light exposed.

METHOD

This research was conducted at the Biomolecular Laboratory of the Faculty of Veterinary Medicine, Airlangga University, Surabaya using a pure experimental study with a post test only control group design using male Wistar rats (*Rattus norvegicus*). The adaptation of the rats was held for 7 days and then the hair on the back area was shaved with 5cm lenght and 4cm width. In total of 6 groups of male Wistar rats are sorted into control and intervension groups. K1 is a normal control group, the rats that were not exposed to UV B rays and not receive *Haematococcus pluvialis* extract and glutathione cream. K2 is a negative control group, the rats that exposed to UV B rays and not receive *Haematococcus pluvialis* extract and glutathione cream. K3 is a positive control group, the rats that exposed to UV B rays and smeared with 2% glutathione cream. P1 is a group of rats that exposed to UV B light and smeared with *Haematococcus pluvialis* extract cream with a concentration of 10%. P2 is a group of rats that exposed to UV B light and smeared with *Haematococcus pluvialis* extract cream with a concentration of 20%. P3 is a group of rats that exposed to UV B light and smeared with *Haematococcus pluvialis* extract cream with a concentration of 30%. The group that was exposed to UV B rays was done once a day for 2 hours in14 days with a wavelength of 294 nm 18 watts. The glutathione cream and *Haematococcus pluvialis* extract cream was given twice a day for 7 days.

At the end of the study, the experimental animals were anesthetized using Ketamine injection and then blood was taken intracardiac. The plasma was used for measuring the levels of superoxide dismutase enzyme using ELISA and microplate reader using spectrophotometer.

After the data collected, the data were processed and analyzed for normality test, homogeneity test, and hypothesis testing using SPSS system. The normality test use the Saphiro Wilk test to determine the normal distribution of the data. The homogeneity test use the Levene test to determine the similarity of the population variance. If the data were normally distributed and homogeneous, the hypothesis test will use One Way ANOVA test to determine significant differences of the treatment groups. If the result variable is not normally distributed and homogeneous, then the Kruskal-Wallis test analysis is carried out to find out whether there is a significant difference between groups and then the Post-Hoc test is carried out using the Mann-Whitney.

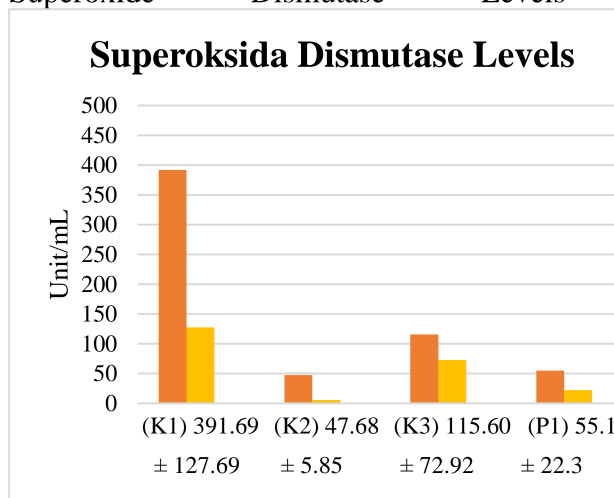
RESULT

Tabel 1. Average of Superoxide Dismutase Level

Group	**Mean ± SD (Unit / mL)**
Normal control (K1)	391.69 ± 127.59
Negative control (K2)	47.68 ± 5.85
Positive control (Glutation 2% cream) (K3)	115.60 ± 72.92
10% *Haematoccus pluvialis* extract cream (P1)	55.16 ± 22.30
20% *Haematoccus pluvialis* extract cream (P2)	449.32 ± 101.21
30% *Haematoccus pluvialis* extract cream (P3)	372.73 ± 225.51

Based on the data of superoxide dismutase enzyme levels between groups, it was found that the K2 group, which is the negative control group, had the lowest average superoxide dismutase enzyme level compared to the other groups (47.68 Unit/mL). The P2 group, which is the 20% Haematococcus pluvialis extract cream group, had the highest average superoxide dismutase enzyme level (449.32 Unit/mL).

Figure 1. Graph of Comparative Analysis of Superoxide Dismutase Levels



Tabel 2. Normality Test

Group	Significance(P)	Result
K1	0,485	Normal
K2	0,981	Normal
K3	0,191	Normal
P1	0,525	Normal
P2	0,405	Normal
P3	0,063	Normal

The table above shows that the data were using the Saphiro Wilk test and the results obtained P value> 0.05 in all groups so that it can be concluded that the data are normally distributed.

Table 3. Result of Homogeneity Test

Levene Statistic	df1	df2	Sig.
12,556	5	22	,000

Table 3 above shows the results of the homogeneity test using the Levene test, it was found that the six groups were not homogenous because the P value <0.05. Because the data is normally distributed but the variance is not homogeneous, then a non-parametric test which is Kruskal-Wallis test was carried out and the Post Hoc test will using Mann-Whitney test.

Tale 4. Result of *Kruskal-Wallis* Test

Ranks

	Group	N	Mean Rank
Result	K1	5	20,00
	K2	4	4,38
	K3	5	11,00
	P1	4	5,38
	P2	5	22,00
	P3	5	20,40
	Total	28	

Test Statistics

Chi-Square	20,857
df	5
Asymp. Sig.	,001

From the results of the Kruskal-Wallis test, the mean rank of each group is the average rank value. The highest value was obtained from the P2 group, which is the group that smeared 20% *Haematococcus pluvialis* extract cream and then in the second order was the P3 group, which is the group that smeared with 30% Haematococcus pluvialis extract cream, and followed by the K1 group, the normal control group. Furthermore, the K3 group, which is the group that smeared with 2% Glutathione cream, had a higher average rating than the P1 group, which is the group that smeared with 10% Haematococcus pluvialis extract cream. The lowest average rating value was obtained from the K2 group, which is the negative control group.

In this study, a P value of 0.001 was obtained, which indicated that the treatment had a significant effect on the levels of the Superoxide Dismutase enzyme on experimental animals post UV B exposed.

Tabel 5. Result of *Mann-Whitney* test

Group	Group	P (P<0,005)	Result
K1	K2	0,000	Significant
	K3	0,002	Significant
	P1	0,000	Significant
	P2	0,466	Not Significant
	P3	0,810	Not Significant
K2	K1	0,000	Significant
	K3	0,419	Not Significant
	P1	0,932	Not Significant
	P2	0,000	Significant
	P3	0,001	Significant
K3	K1	0,002	Significant
	K2	0,419	Not Significant
	P1	0,471	Not Significant
	P2	0,000	Significant
	P3	0,003	Significant
P1	K1	0,000	Significant
	K2	0,932	Not Significant
	K3	0,471	Not Significant
	P2	0,000	Significant
	P3	0,001	Significant
P2	K1	0,466	Not Significant
	K2	0,000	Significant
	K3	0,000	Significant
	P1	0,000	Significant
	P3	0,335	Not Significant
P3	K1	0,810	Not Significant
	K2	0,001	Significant
	K3	0,003	Significant
	P1	0,001	Significant
	P2	0,335	Not Significant

After the Kruskal-Wallis test was performed, a Post Hoc test was performed using the Mann-Whitney method to determine the difference in levels of Superoxide Dismutase between the two different groups. The basis for decision making in this method is determined by looking at the significance value or P value. If the P value> 0.05 then there is no significant difference, but if the P value <0.05 then there is a significant difference between the two groups.

Based on the analysis, the table above shows that the K1 group had a significant difference in SOD levels with the K2, K3, and P1 groups but K1 did not have a significant difference when compared to the P2 and P3 groups. The K2 group had a significant difference with the

P2 and P3 groups but K2 did not have a significant difference with the K3 and P1 groups. The K3 group had a significant difference with the P2 and P3 groups but there was no significant difference when compared to the P1 group. In the P1 group, the SOD levels were significantly different compared to the P2 and P3 groups, while in the P2 group there was no significant difference when compared to the P3 group.

DISCUSSION

From the results of the study and data analysis on superoxide dismutase levels in the 6 groups the average SOD levels in group K1 (the group that was not exposed to UV B rays and did not smeared with *Haematococcus pluvialis* and glutathione cream) was 391.69 ± 127.59; group K2 (the group that was exposed to UV B rays and did not smeared with *Haematococcus pluvialis* and glutathione cream) was 47.68 ± 5.85; the K3 group (the group that was exposed to UV B rays and smeared with 2% glutathione cream) was 115.60 ± 72.92; group P1 (the group that was exposed to UV B rays and smeared with *Haematococcus pluvialis* extract cream with a concentration of 10%) was 55.16 ± 22.30; group P2 (the group exposed to UV B rays and smeared with *Haematococcus pluvialis* extract cream with a concentration of 20%) 449.32 ± 101.21, and group P3 (the group exposed to UV B rays and smeared with *Haematococcus pluvialis* extract cream with a concentration of 30%) was 372.73 ± 225.51.

20% and 30% *Haematococcus pluvialis* extract cream showed high SOD levels, due to the effect of the astaxanthin content in *Haematococcus pluvialis* which plays a role in treating oxidative damage caused by UV B rays. This is in accordance with previous research conducted by Erick (2020) who explained that *Haematococcus pluvialis* has an antioxidant effect and *Haematococcus pluvialis* has a high astaxanthin content equivalent to vitamin C at certain doses. In addition, a previous study conducted by Tetty (2018) explained that astaxanthin has the same level of effectiveness as hydroquinone in preventing an increase in the amount of melanin. Astaxanthin contained in *Haematococcus pluvialis* works by binding to singlet oxygen which is formed from free radicals due to exposure to UV B rays. The excess energy from singlet oxygen is transferred to the carotenoid structure which is rich in electrons and converts it into heat energy so that singlet oxygen is no longer formed and reacts with other ROS to prevent and stop chain reactions.[7,8]

In this study, the results of the Mann-Whitney test which compared the 20% *Haematococcus pluvialis* extract cream and the 30% *Haematococcus pluvialis* extract cream obtained $p>0.05$, which is equal to $p=0.335$, which means that there is no significant difference between the two. The average SOD levels of the group that smeared with 20% *Haematococcus pluvialis* extract cream also showed a higher value than the group that smeared with 30% *Haematococcus pluvialis* extract cream. This is not in accordance with previous research and studies that have proven that astaxanthin is dose-dependent. The cause of these results is thought to be caused by several factors, which is the lack of a large sample used in the study so that the resulting results are less significant. Another factor that can cause these results is due to cofounding variables that cannot be 100% controlled by researchers, for example, physical activity that can affects the plasma SOD levels of the experimental animals.

In this study, 2% Glutathione cream was used as a positive control and the results were 2% Glutathione cream had the best effectiveness in treating oxidative damage after 20% and 30% *Haematococcus pluvialis* extract cream , this is in accordance with previous studies which showed that the skin burns of white mice was healed more significantly in glutathione-treated mice compared to

glutathione-untreated mice. Glutathione works by inhibiting the tyrosinase enzyme so that there is no increase in melanin production that is triggered by excessive exposure to UV B rays. In this study, 10% *Haematococcus pluvialis* extract cream had less healing effectiveness than 2% Glutathione cream, this is presumably because the low concentration makes the effectiveness of the active ingredients in it becomes low.[3,4]

In this study, the lowest oxidative damage healing was the negative control group, which is the group that was exposed to UV B rays but was not smeared with the extract or Glutathione cream. Stressors from UV B rays and the treatment given, by not giving the extract or Glutathione cream were thought to make the healing of oxidative damage in that group run slower because there was no exogenous antioxidant assistance from outside that helped the process of treating oxidative damage from UV B exposed.

CONCLUSION

Based on the results of the research that has been carried out, it can be concluded that the treatment of *Haematococcus pluvialis* extract cream has an effect on the levels of Superoxide Dismutase on Rattus norvegicus post Ultraviolet B exposed, there is a significant difference between the groups that smeared with 20% and 30% *Haematococcus pluvialis* extract cream compared to the groups that smeared with 2% Glutathione cream, and the effectiveness of astaxanthin in *Haematococcus pluvialis* extract cream is dose-dependent.

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CONFLIFT OF INTEREST

All authors declared that there is no conflict of interest regarding this publication.

AUTHOR CONTRIBUTION

All authors contributed equally in the writing of this article.

ETHIC APPROVAL

This study had been ethically approved by ethical commission of Faculty of Medicine Widya Mandala Catholic University with approval letter number 174/WM12/KEPK/MHSW/T/2021.

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