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The Effectiveness of Snail (Achatina Fulica) Slime Cream toward Inhibiting the Formation of Sunburn Cells on Mice (Mus Musculus) Balb/C Skin (Experimental Study with Induced UVB Radiation)

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ABSTRACT

Background ─ Sunburn is a radiation burn of the skin by exposure to UV light. UVB is the most dangerous UV light for humans and can cause skin cancer risk. UVB rays can damage DNA so that it can cause a response in the form of apoptosis of keratinocyte cells or also called sunburn cells.

Objective ─ Determined the effectiveness of snail (*Achatina fulica*) slime cream on inhibited the formation of sunburn cells on the skin of BALB/c mice (*Mus musculus*) induced by UVB radiation.

Methods ─ Experimental research using randomized post-test only control group design with 35 male BALB/c (*Mus musculus*) mice divided into 7 groups. There was a group without treatment, a negative control group, a control group with Parasol Face Sunscreen Cream, and 4 treatment groups with topical application of snail slime cream with varying doses. All groups except the untreated group were irradiated with UVB 200 mJ/cm^2 for 1x irradiation. The mice's back skin was taken 24 hours later to make histology slide and sunburn cell counting. Data were analyzed using One Way ANOVA comparative test, Post-hoc LSD test, and Spearman's rho correlative test with each significance value $p<0.05$.

Results ─ There are a significant difference in the mean number of sunburn cells in One Way ANOVA test $p=0.00$ ($p<0.05$) between all groups. LSD post-hoc test shows the group with the most significant mean difference (16.20) was between the negative control group and treatment group 4 (X4) with $p=0.00$ ($p<0.05$). There is a relationship between the dose of snail slime cream and the percentage of sunburn cells in the Spearman's rho test with $p=0.00$ ($p<0.05$) and the strength of the relationship between the dose of snail slime cream and the percentage of sunburn cells is very strong indicated by the Pearson Correlation (r)= -0.900.

Conclusion ─ Snail slime cream effectively inhibits the formation of sunburn cells with the most effective dose in this study is the 10% dose.

KEYWORDS: UVB, Sunburn Cells, Snail Slime Cream, Achatina fulica

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INTRODUCTION

Sunburn cells are keratinocytes that undergo apoptosis after exposure to Ultraviolet B (UVB) light, causing severe damage to DNA or other chromophores to irreversible damage.[7] UVB light is the main light that can cause Sunburn cell (SBC) resulting in sunburn. The DNA structure in skin cells, especially in the epidermis, is directly damaged by UVB rays because UVB rays have slightly greater energy than UVA rays.[1] The formation of thymine-thymine compound formation after UVB exposure makes the body initiate a response to repair DNA, including cell apoptosis and the release of inflammatory media. Symptoms of sunburn are shown in the form of reddish skin with a slight stinging pain due to excessive activation of pain receptors in the skin due to UVB exposure.[5]

The ultraviolet index value on Java Island is high, ranging from 7.8 to 13.6, with an average of 7, especially in Central Java.[9,12] High exposure to ultraviolet light received by

the skin can increase the potential for non-melanoma skin cancer, the most common skin cancer in the world with 1 million new cases found each year.[3] The primary photoprotection factor that is proven to protect the skin from the risk of SBC is the use of sunscreen. Sunscreen works by absorbing or deflecting UV rays so as to minimize the keratinocyte apoptosis process.[18]

The tendency of people to use traditional medicine is getting higher lately, so the utilization of natural ingredients tends to increase, one of which is snail slime. Snail slime has been widely used in the treatment of wounds by the community, including people in Java. This material has the potential to be an alternative sunscreen additive because it is known for its rich content of anti-inflammatory and antioxidant substances.[13,19]

Based on previous research, the application of snail slime with a concentration of 5%, 10%, and 15% using a UV-VIS spectrophotometer resulted in maximum protection and ultra protection of the skin up to SPF>15 from UV rays. Snail slime is proven to have antioxidant activity that can protect the skin from the inside.[16] According to research conducted by Soetrisno *et al* in 2021, the use of 75% aloe vera gel which is high in anti-inflammatory and antioxidant content reduces the number of sunburn cells.[14]

Research on the effectiveness of snail (*Achatina fulica*) slime cream on the formation of sunburn cells in BALB/c mice (*Mus musculus*) induced by UVB radiation has never been done. This encourages researchers to conduct research on the effectiveness of snail (*Achatina fulica*) slime cream on the formation of sunburn cells in BALB/c mice (*Mus musculus*) induced by UVB radiation.

MATERIAL AND METODS

The instruments used in this study include a mice cage measuring 26 cm x 20 cm x 19 cm, a clay jar measuring 30 cm x 40 cm, a hair clipper, a 1 cc syringe, a minor set, tweezers, scissors, an aquarium, a spatula, a beaker glass, gloves, a funnel, a measuring cup, a dropper pipette, filter paper size 10 cm x 10 cm, cotton, tissue, digital analytical scales with accuracy of 0.1 mg, water spray, cotton sticks, UVB lamp Exo-terra Solar Glo 160 W, irradiation box, micro pipettes, pipette tips, test tubes, stirrer, paraffin block holder, microtome, staining rack, and microscope. The main material needed in this study is adult A. fulica snail slime, with a shell size of 6 - 8cm. Other materials are Hematoxillin Eosin (HE) painting, 10% formalin, mice feed pellets, stearic acid, cethyl alcohol, glycerol, potassium hydroxide, propyl paraben, methyl paraben, and distilled water.

This study is an experimental study using a randomized post-test only control group design. This research has obtained permission from the Research Ethics Committee of the Faculty of Medicine, Jenderal Soedirman University based on the ethical approval letter number 006/KPEK/PE/VIII/2022.

Before slime collection, *Achatina fulica* was washed under running water and dried, then placed in a glass aquarium covered with wire mesh and allowed to walk around in it for 2 hours. The snail is then returned to the crock. The slime left in the aquarium was collected using a spatula and placed into a beaker glass. The collected slime was then centrifuged at 5000 rpm for 10 minutes twice, then filtered with filter paper.[15] The filter results were made into vanishing cream material with doses of 0%, 2%, 5%, 7%, and 10% in the Laboratory of the Faculty of Mathematics and Natural Sciences Unsoed.

The research subjects used were 35 male BALB/c mice (*Mus musculus*), aged 8-11 weeks weighing 20-25 g, and had never been used in other studies. Subject exclusion criteria such as mice have anatomical abnormalities, and have skin disorders on the back before treatment. Mice were obtained from the Pharmacology Laboratory of Muhammadiyah Purwokerto University.

Mice were divided into 7 groups. XO group without treatment, XB control group with UVB irradiation and placebo cream, XSB control group with UVB irradiation and Parasol sunblock, and X1, X2, X3, and X4 as treatment groups that were irradiated and received snail slime cream with doses of 2%, 5%, 7%, 10% respectively with an irradiation frequency of 1x for the group that received UVB irradiation.

The cream was applied as much as 8mg (2mg/cm^2) on the back skin of mice that had been shaved the day before. UVB irradiation was given 1 hour after the mice were applied with the cream at a distance of 20 cm for 3 minutes using an Exo-terra Solar Glo 160 W MED 200 mJ/cm^2 light source in an irradiation box.[4] One day after the irradiation was completed, the mice were decapitated and then 4 cm^2 of mice back skin was taken using a minor set. There were mice that died and escaped after the irradiation process, leaving 32 mice.

Skin samples that have been taken are preserved in formalin solution to be processed into tissue preparations with Hematoxylin Eosin (HE) staining. The counting of sunburn cells was carried out using the help of a microscope and recorded the average results per 100 keratinocyte cells in 5 field of view.[14]

Data analysis in this study used the help of the SPSS (Statistical Product and Service Solutions) application version 26. One Way ANOVA comparative test was conducted to determine the difference in the number of sunburn cells in the control group and the treatment group of sunburn model mice. Prior to analysis, a normality test was performed using Saphiro-Wilk with a significance level of $p>0.05$. In the comparative test, Levene's variance test was also carried out with a significance level of $p>0.05$. The analysis continued with the Least Significance Different (LSD) post hoc test because $p<0.05$ or there was a significant difference between groups. The results of the normality test

for correlative analysis in this study were p<0.05, which means that the data distribution was not normal. Therefore, the test used in this study is the Spearman's rho non-parametric correlative test with a significance value of p<0.05 and a correlation coefficient (*r*) that can show the level of variable relationship.

RESULTS

1. Univariate dan Bivariate Analysis

Table. 1 Total Amount of Sunburn Cells per Group

Group	n	Minimum Value (cell/FW in percents)	Maximum Value (cell/FW in percents)	Median (cell/FW in percents)	Mean (cell/FW in percents)	Standard Deviation (cell/FW in percents)
XO	5	2,00	4,80	2,60	2,84	1,12606
XB	4	8,80	16,40	12,20	12,40	3,21662
XSB	4	2,40	4,00	3,10	3,15	0,77244
X1	5	7,00	9,00	8,00	7,84	0,85323
X2	5	5,20	9,00	6,60	6,84	1,50599
X3	4	3,60	5,00	4,40	4,35	0,66081
X4	5	2,40	5,40	3,60	3,60	1,15758

Table 1 shows that the lowest to highest mean number of sunburns in order are in groups XO, XSB, X4, X3, X2, X1, XB.

Table 2. Results of Bivariate Analysis of the Number of Sunburn Cells

Group	n	Mean ± SD	*Saphiro Wilk* (p)	*Levene Test* (p)	*One Way ANOVA* (p)
XO	5	2,84±1,12	0,333	0,436	0,000
XB	4	12,40±3,21	0,979		
XSB	4	3,15±0,77	0,408		
X1	5	7,84±0,85	0,410		
X2	5	6,84±1,50	0,408		
X3	4	4,35±0,66	0,513		
X4	5	3,60±1,15	0,611		

The One-Way ANOVA test showed that the administration of snail slime cream gave a significant difference to the number of sunburn cells, so the bivariate test was continued with the Post Hoc LSD test to analyze the difference in the mean number of sunburn cells, in order to find out which treatment group had the most effect on reducing sunburn cells. The results of the Post Hoc LSD test are shown in Table 3.

Table 3. LSD Test Results

Post Hoc Test	Mean Difference (persentage)	*P*
XO vs X1	11,3	.000
XO vs X2	9,40	.000
XO vs X3	4,18	.037
XO vs X4	2,16	.238
XB vs X1	7,02	.001
XB vs X2	8,96	.000
XB vs X3	14,18	.000
XB vs X4	16,20	.000
XSB vs X1	10,32	.000
XSB vs X2	8,38	.000
XSB vs X3	3,16	.126
XSB vs X4	1,13	.553
X1 vs X2	1,94	.289
X1 vs X3	7,16	.001
X1 vs X4	9,18	.000
X2 vs X3	5,22	.011
X2 vs X4	7,24	.000
X3 vs X4	2,02	.296

Table 3 shows that the most significant difference in the mean number of sunburn cells is between the negative control group and treatment group 4 by 16.20%. This makes treatment group 4 the most significant in reducing the number of sunburn cells.

Table 4. Saphiro-Wilk Normality Test Results

Variable	***Saphiro-wilk p***
Sunburn cells percentage	0,444
Snail slime cream dosage	0,10
logDosage	0,002
SQRTDosage	0,007

Normality test using the Saphiro-Wilk test because the number of samples is less than 50, the results obtained p<0.05 even though the transformation experiment has been carried out which means that the data is not normally distributed as shown in Table 4. So Spearman's rho non-parametric correlative test was conducted to measure the effect of two variables in this study.

Table 5. Results of Spearman's rho Correlation

			Snail Slime Cream Dosage	**Sunburn Cells Percentage**
Spearman's rho	Snail Slime Cream Dosage	*Correlation Coefficient*	1,000	-,900**
		p (2-tailed)		,000
		N	23	23
	Sunburn Cells Percentage	*Correlation Coefficient*	-,900**	1,000
		p (2-tailed)	,000	
		N	23	23

The strength of correlation that occurs between the two variables is very strong with a negative correlation direction indicated by the value of r = -0.900 (**). The higher dosage of snail slime cream in this study, the lower sunburn cells percentage. While p = 0.000 is still smaller than the significance value of p = 0.05; meaning that there is a significant difference in the effect between the dose of snail slime cream on the percentage of sunburn cells (0.000 < 0.05).

2. Histological Effect on Mice Skin

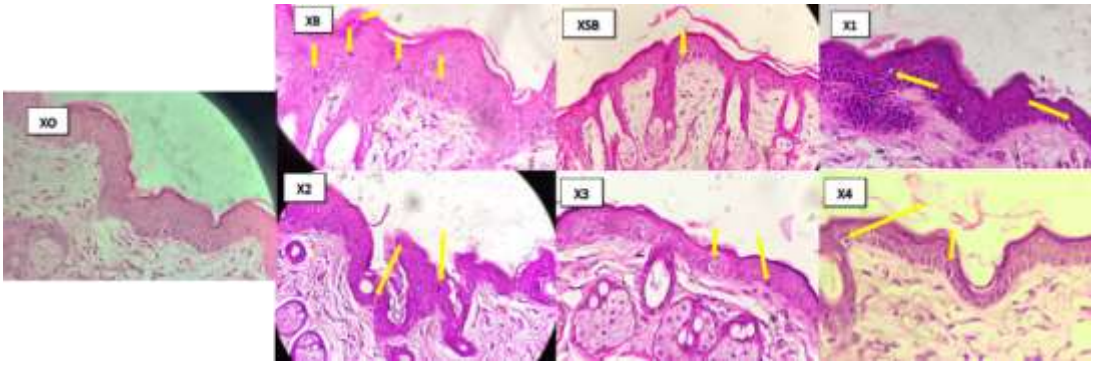

Figure 1. Histological view of mice skin.

Healthy skin will show an intact epidermal skin layer, keratinocyte cells that produce keratin, langerhans cells that produce the immune system and melanocyte cells for pigmentation.[8] In the specimen other than XO, there are keratinocyte cells that have undergone apoptosis as indicated by the yellow arrows in the image with picnotic nuclei and

eusinophilic cytoplasm. Pycnotic nucleus is the shrinkage of the nucleus as a result of cytoplasmic homogenization and eosinophilic increase which is the initial stage of apoptosis.[11]

DISCUSSION

This study examines the effectiveness of snail (*Achatina fulica*) slime cream using varying doses 2%, 5%, 7%, and 10% doses made from 100% snail slime towards the number of sunburn cells in UVB-induced mice skin. Based on Spearman's rho analysis, the correlation value is shown by *r* = -0.900 (**). The strength of the correlation that occurs between the two variables is very strong with a negative correlation direction. The higher the dose of snail slime cream in this study, the lower the percentage of sunburn cells. These results are in line with the results of previous research by Suhesti *et al*, that different concentrations of snail slime use provide different protection results against phytochemical effects and different concentrations of snail slime also provide different antioxidant activity values. The use of snail slime with a concentration of 5% obtained a strong antioxidant activity value and at a concentration of 15% snail slime obtained an antioxidant activity value with a very strong group.[16]

The difference in the results of antioxidant activity towards the number of sunburn cells is caused by variations in the dose of snail slime cream. Based on the results obtained, the best dose in inhibiting the formation of sunburn cells in this study is the highest dose used. The higher the dose of snail slime cream, the higher the polar bioactive compounds contained in the sample because more compounds are dissolved by solvents such as methanol, one of which is used in making snail slime cream because this methanol solvent is also polar so that it can dissolve almost all organic compounds contained in the sample.[6]

Based on bivariate analysis of the results of statistical tests using the One Way ANOVA test, the p value is 0.000 (p <0.05), meaning that there is a difference in effectiveness in dosing snail slime cream on the number of sunburn cells. These results are in line with previous research, where the administration of different concentrations of slime, namely 5%, 10%, and 15% concentrations, provides different effectiveness on its photoprotective effect on the skin of sunburn model rats.[16] Another study that supports the results of this study is regarding the topical administration of aloe vera in reducing the number of sunburn cells after UVB exposure, where differences in results were obtained between the administration of 75% concentration aloevera and the control group. This study also shows the potential of aloe vera as an anti-inflammatory and antioxidant against sunburn cell formation.[14] The antioxidant and anti-inflammatory content of aloevera which is also found in snail slime shows that this natural antioxidant and anti-inflammatory content is very potential in reducing the number of sunburn cells due to UVB exposure.

Theoretically, the body will naturally produce antioxidants, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and ceruloplasmin which are referred to as endogenous antioxidants. The amount of oxidative stress received by the body by prolonged UV exposure cannot be balanced by the amount of endogenous antioxidants, so exogenous antioxidants or antioxidants from outside are needed that can compensate for exposure to oxidative stress from this environment. These exogenous antioxidants can be obtained naturally from plants or animals that contain these compounds or obtained synthetically. The reduction ability of antioxidants as free electron binders of free radicals is required by the body when exposed to oxidative stress from the activation of ROS by exposure to environmental oxidative stress. In addition, tissue damage and cell death that has occurred by UV exposure needs immediate repair by the body which can be catalyzed by the provision of anti-inflammatory and cell regeneration agents from outside the body such as the content of heparan sulfate, achasin and glycosaminoglycan contained in snail slime.[2]

Superoxide dismutase (SOD) in snail slime acts as an antioxidant enzyme, anti-inflammatory agent and can also prevent precancerous cell changes. This enzyme is used in cosmetics and personal care products as an anti-aging ingredient and antioxidant due to its ability to reduce free radical damage to the skin, thereby preventing wrinkles, fine lines, and age spots, and also aids in wound healing, softening scarring, protecting against UV rays, and reducing other signs of aging.[20] *Achatina fulica* contains chitosan which acts as a source of exogenous antioxidants. Exogenous antioxidants balance the oxidant/antioxidant system due to the limited number of endogenous antioxidant systems to neutralize ROS. The SOD enzymes, catalase, glutathione peroxide (GPX), and non-enzymatic antioxidants are intracellular antioxidants with defense mechanisms against tissue damage due to free radical formation.[17]

The human body's antioxidant system that works against ROS mainly consists of antioxidant enzymes and non-enzymatic antioxidants that can prevent oxidative damage to human skin. Antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR). Catalase (CAT) in fighting ROS has a mechanism of Nrf2 and SIRT1/FOXO signaling pathways that can express antioxidant enzymes to reduce oxidative stress-induced ROS production, thereby preventing ROS-mediated oxidative damage. In addition, the SIRT1/FOXO signaling pathway protects cells through autophagy, thereby reducing skin cell aging, inflammation, and cancer.[10]

CONCLUSION

Snail (*Achatina fulica*) slime cream effectively inhibits the formation of sunburn cells with the most effective dose in this study is a dose of 10%.

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