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Administration of 12.5% Strawberry Ethanol Extract Cream Reduced Tyrosinase Enzyme and Melanin Amount in Male Guinea Pig Skin Exposed to UVB with More Potential for Protection than Treatment

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A B S T R A C T

Skin aging occurs due to prolonged exposure to ultraviolet B (UVB) resulting in oxidative stress, which stimulates an increase in tyrosinase enzyme and melanin, causing hyperpigmentation. Chemical-based sunscreens often cause allergy and irritation, while hydroquinone (HQ) causes negative effects on the skin called ochronosis. Natural ingredients that are safe from side effects, such as strawberry (Fragaria ananassa), with active compounds of flavonoids, tannins and phenols with antioxidant and antiinflammation activity, can reduce melanogenesis. The aim of this study was to determine the effectiveness of 12,5% strawberry ethanol extract cream before and after UVB exposure in reducing levels of the tyrosinase enzyme and the amount of melanin in male guinea pigs. The research was a randomized post-test only control group design experimental study, using 30 male local strains guinea pigs, aged 3-4 months, weighing 300-350 grams, divided into three groups, consisted of 10 guinea pigs each. The control group (K0) was given UVB exposure and basic cream, treatment group 1 (K1) was given 12,5% strawberry extract cream before UVB exposure and treatment group 2 (K2) was given 12,5% strawberry extract cream after UVB exposure for 2 weeks. Skin samples were taken by biopsy and examined for tyrosinase enzyme levels using the ELISA method and melanin amount using the histopathological method of Masson-Fontana staining. The data was analyzed using One Way ANOVA and Post Hoc Tamhane. The mean levels of the tyrosinase enzyme in treatment group K1 and K2 (8,25□1,5 ng/ml and 17,47□3,09 ng/ml; p<0,05) decreased significantly compared to K0 (34,57□5,62 ng/ml). The mean amount of melanin in treatment group K1 and K2 (3,8□3,9%pixels and 5,13□1,53%pixels; p<0,05) decreased compared to K0 (21,16□10,21%pixels). There was significant difference in the decrease of tyrosinase enzyme in treatment group K1 and K2 (p<0,05), while no significant result was found in the decrease of melanin in treatment group K1 and K2 (p>0,05). In conclusion, 12.5% strawberry ethanol extract cream reduced tyrosinase enzyme level and melanin amount compared to control group applied with base cream, with better result when applied before UVB exposure (protection effect) than after UVB exposure (for treatment), with the same effectiveness in reducing the melanin amount but different effectiveness in reducing the tyrosinase enzyme.

Keywords: strawberry extract cream, ultraviolet B, tyrosinase enzyme, melanin

1. Introduction

Aging is a natural process involving psychosocial, social, physical changes and health problems that all living creatures will experience. Aging is related to genetics, hormones, air pollution and sun exposure (Pangkahila, 2019). Premature aging of the skin is characterized by loss of elasticity, blood vessel disorders, wrinkles due to dry skin and hyperpigmentation (Moyal & Fourtrainer, 2004). Hyperpigmentation is a sign of skin aging due to exposure to UVB rays, characterized by blackish brown spots on the skin, due to an increase in the amount of melanin and increased activity of the tyrosinase enzyme (Ostrowski & Fisher, 2019). Sunlight emits ultraviolet (UV) light with a wavelength of 100 nm to 400 nm. UVB radiation (280-320nm) constitutes 5% of the UV radiation that reaches the earth's surface and plays a role in carcinogenesis and sunburn (Adzhani *et al*., 2022). In contrast to UVB, UVA radiation (320-400 nm) is absorbed down to the inner dermis layer and affects keratinocyte and fibroblast cells in collagen formation and photoaging (Alcantara *et al*., 2020). UVB exposure produces tanning that is more long-lasting and has acute effects, compared to UVA which produces short-term tanning, skin damage and longer effects (Young *et al*., 2016).

Indonesia is a country with a tropical climate with high exposure to sunlight resulting in an increase in the prevalence of hyperpigmentation (Sulistiyowati *et al*., 2022). The main skin problems in Asia are acne, eczema (atopy) and hyperpigmentation (Chan *et al*., 2019). Based on dermatology research in Brazil in 2018, 3.6% of dermatology cases were and mainly in women aged 25 to 50 years with a type III-IV phenotype (Alcantara *et al*., 2020). Although hyperpigmentation does not correlate directly with death, it can cause extreme embarrassment, lack of self-confidence and even depression (Jusuf *et al*., 2019).

The use of protection in the form of sunscreen is very important to ward off solar radiation, reduce the risk of skin cancer and fight photoaging (Geoffrey *et al*., 2019). Sunscreen consists of inorganic (mineral) and organic (chemical). The most widely used organic sunscreens work by absorbing UV rays, converting them into heat waves and releasing them through the skin. Organic or synthetic sunscreens can cause side effects from dermatitis, allergies to anaphylactic shock. Therefore, sunscreen from natural ingredients is an alternative (Guan *et al*., 2021). The main topical therapy to treat hyperpigmentation is 4% HQ as the gold standard. The side effects caused are redness, skin irritation, burning sensation, hypochromia, leukoderma and ochronosis (Couteau & Coiffard, 2016). Research by Tan *et al*., found that from 2014 to 2019 there were 88 patients with exogenous ochronosis due to HQ whitening cream with 92.04% of female patients (Tansil Tan *et al*., 2020).

Based on previous in vitro research, it was proven that 0.3% strawberry (Fragaria ananassa) juice cream has an antioxidant capacity with an IC 50 (Inhibition Concentration) value of 52.59 ppm and is classified as a strong antioxidant (Ferdiansyah *et al*., 2016). In vitro research by Widyastuti and Primagara found that the ethanol extract of strawberry (*Fragaria ananassa*) consisting of flavonoids, phenolics and saponins has the ability to act as a sunscreen and tyrosinase inhibitor (Widyastuti & Primagara, 2021). Shimogaki *et al*., reported that the polyphenol compound ellagic acid found in strawberries, has the potential to inhibit the melanogenesis process both in vivo and in vitro (Shimogaki *et al*., 2000). Previous research found that 12.5% strawberry (*Fragaria ananassa*) ethanol extract cream with 20% album vaseline and 10% cera alba met the control and quality test standards, which consisted of organoleptic, pH test, spreadability test, stickiness test and protective power (Arifah *et al*., 2013). The effect of strawberry fruit extract in inhibiting the amount of melanin in vivo has been proven by Harahap *et al*., in experimental research with male guinea pigs exposed to direct sunlight (Harahap *et al*., 2022). The differences with previous research are due to differences in research methods, the source of strawberry used, comparisons with base cream and examination of tyrosinase enzyme levels.

The strawberry plant (*Fragaria ananassa*) is native to Chile (Latin America) and is often found in highland areas, especially Indonesia. Nowadays, strawberries are widely used, such as direct consumption, packaged drinks and body scrubs (Oo & Aung, 2018). In research that analyzed the antioxidant activity of 12 types of fruit, strawberries had the highest antioxidant activity compared to other fruits, related to the role of vitamin C in total antioxidant activity of around 15% (Miller *et al*., 2019). The ellagic acid content in strawberries is higher than the content in other fruits and nuts such as walnuts, green apples, red apples, pineapples, pears, bananas, kiwi, oranges (Mut Hukumaran S *et al*., 2017). The anti-inflammatory and antioxidant function of strawberries means they can ward off UV radiation. Flavonoids, tannins, phenols (ellagic acid) are related to inhibiting the inhibitory activity of the tyrosinase enzyme and binding to cooper which is related to the formation of melanin (Rapuru *et al*., 2023).

Research on strawberries as a skin lightening agent is still limited, which is why the author is interested in conducting this research. However, there has been no in vivo research that further tests and evaluates the comparative effectiveness of administering 12.5% strawberry (*Fragaria ananassa*) cream extract cream in reducing tyrosinase enzyme activity and melanin production before UVB exposure to determine the potential for protection (sunscreen) and after exposure to UVB to determine the potential for treatment (therapy) on skin with hyperpigmentation. It is hoped that this research can become the basis for further research to evaluate the effectiveness of 12.5% strawberry extract cream as an alternative to HQ gold standard therapy for hyperpigmentation, safety levels and clinical trials before widespread use in humans.

2. Material and Methods

2.1 Study Design

This research was an experimental study with a randomized post-test only control group design. Cream making and phytochemical testing of 12.5% strawberry cream ethanol extract was carried out at the Agricultural Technology in Food Analysis Laboratory, Udayana University. The research on experimental animals was conducted at the Laboratory of Animal Pharmacology Unit, Faculty of Medicine, Udayana University. Examination of tyrosinase enzyme and melanin amount was conducted at the Integrated Biomedical Laboratory and Histology Laboratory of Faculty of Medicine, Udayana University respectively. The sample needed was 30 local strain guinea pigs (*Cavia porcellus*), 300-350 gram, aged 3-4 months.

2.2 Preparation of 12.5% Strawberry Extract Cream and Base Cream

Two kilograms of strawberries were obtained from Leon Strawberry Plantation, Jl. Raya Singaraja, Pancasari Village, Bedugul, Bali. Certainty regarding the authenticity and accuracy of plants is carried out through the determination stage at the National Research and Innovation Agency (BRIN), Eka Karya Botanical Gardens, Candikuning, Baturiti, Tabanan, Bali. The result of 96% ethanol extraction from two kilograms of strawberries are 15 grams, a sour taste, dark red color, thick consistency, with strawberry aroma. The phytochemical results of 12.5% strawberry ethanol extract cream consists of active compounds containing flavonoids 4149.69mg QE/100g, phenols 681.350mg GAE/100g, tannins 1024.10mg TAE/100g, antioxidant capacity 9434.41 GAEAC mg/L and IC50 85.94 ppm. The 12.5% strawberry ethanol extract creme formula consisted of 2.5 g strawberry fruit extract, 3 g stearic acid, 2 g cera alba, 4 g vaseline album, 0.3 g triethanolamine (TEA), 1.6 g propylene glycol, nipagin 0.75 g and enough distilled water to reach 20 g (Arifah *et al*., 2013). A total of 60 grams of cream and 7.5 gram of strawberry extract were used in this study and applied to the 2 treatment groups. Basic cream was made by melting stearic acid, cera alba and vaseline album in a porcelain cup as the first mixture and TEA, propylene glycol and nipagin which were mixed evenly until homogeneous.

2.3 Experimental Animal Treatment

Experimental animals were adapted for 1 week. The animal feed given was the standard HI-GRO Medicated 552 diet, which contains 17-20% protein, 3-4% fat and 35-40% carbohydrates, amounting to 200-300 grams/head/day. Drinking water in the form of boiled water was provided ad libitum. The guinea pig was placed in a cage with iron wire measuring 100 cm x 40 cm x 40 cm, placed in a room with good circulation and ventilation, each contains 5 guinea pigs with rice husks at the bottom of the cage.

The guinea pigs were divided into 3 groups randomly, with each group consisting of 10 guinea pigs, namely:

- Control group (K0), with administration of base cream 20 minutes before and 4 hours after exposure to UVB light.
- Treatment group 1 (K1), with administration of 12.5% strawberry extract cream 20 minutes before UVB exposure, followed by base cream 4 hours after UVB exposure.
- Treatment group 2 (K2), with administration of base cream 20 minutes before UVB exposure, followed by application of 12.5% strawberry extract cream 4 hours after UVB exposure.

Exposure to UVB rays was carried out Monday, Wednesday and Friday, three times a week, at 10.00 WITA for 14 days, while cream application was carried out at 9.20 WITA and 14.00 WITA. UVB rays were applied at a dose of 65 mJ/cm2 for 65 seconds, with the total dose of UVB rays was 390 mJ/cm^2 (Ramadhani, 2021). The hair on the back of male guinea pigs was shaved on the medial dorsum in an area of 4x3 cm and repeated every two days, and 0.2 mg/cm^2 of cream was applied on the back of guinea pig.

2.4 Procedure

Guinea pig skin samples were taken after 2x24 hours of the last UVB irradiation to eliminate the influence of acute irradiation. Anesthesia was carried out with Ketamine HCl 50mg/kg and Xylazine 10 mg/kg intramuscularly. Samples were taken using the punch biopsy method with a diameter of 10 mm and a depth of 4 mm, where the hyperpigmentation area was the clearest and divided into two parts for ELISA dan histopathological examination. Euthanasia was done by dislocating the cervical spine, then burning with an incinerator. Examination of tyrosinase enzyme levels was done using the ELISA method, where the sample was placed in Phosphate Buffer Saline. Calculation of the amount of melanin by histopathological examination using Masson Fontana staining, where the sample was placed in 10% Neutral Buffer Formalin.

2.5 Data Analysis

Data were processed using SPSS 25.0. Normality test was done using Shapiro Wilk. Abnormal data was obtained so a Transformation Data Normality Test was carried out. Homogeneity test was carried out using Levenne Test. Comparability analysis was carried out using One Way Annova Test and continued with the Post Hoc Tamhane Test when $p<0.05$ to determine the differences between the three groups of data.

3. Results

Based on normality test on tyrosinase enzyme and melanin amount on the three groups using Shapiro Wilk test, the data was non-normally distributed ($p<0.05$). The data is presented in Table 1.

Table 1 - Normality Test.

Variable	Subject	n	*p*	Description
Tyrosinase (ng/mL)	K0	10	0.015	Non-normally distributed
	K1	10	0.015	Non-normally distributed
	K2	10	0.253	Normally distributed
Melanin (%pixel)	K0	10	0.415	Normally distributed
	K1	10	0.004	Non-normally distributed
	K2	10	0.827	Normally distributed

Based on the results on normality test, a data transformation test was carried out on tyrosinase enzyme and melanin amount using squareroot, and is presented in Table 2.

Table 2 – Data Transformation Normality Test.

Variable	Subject	n	*p*	Description
Tyrosinase (ng/mL)	K0	10	0.161	Normally distributed
	K1	10	0.061	Normally distributed
	K2	10	0.075	Normally distributed
Melanin (%pixel)	K0	10	0.154	Normally distributed
	K1	10	0.065	Normally distributed
	K2	10	0.922	Normally distributed

Based on the result on homogeneity test using Levene's test, the data on tyrosinase enzyme and melanin amount was non-homogeny ($p<0.05$). Data is presented in Table 3.

Table 3 – Homogenity Test

Variable	n	*p*	Description
Tyrosinase (ng/mL)	30	0.003	Non-homogeny
Melanin (%pixel)	30	0.003	Non-homogeny

Analysis on comparison between the three groups was carried out using parametric test, analyzed using One Way Annova test and comparability test between groups was done using Post Hoc Tamhane test. Data is presented in Table 4 and 5.

Table 4 – One Way Anova Test

Variable	K0 (Mean±SD)	K1 (Mean±SD)	K2 (Mean±SD)	Description
Tyrosinase (ng/mL)	34,567±5,618	8,255±1,498	17,470±3,086	0.000
Melanin (%pixel)	21,1591±10,216	3,798±3,902	5,132±1,530	0.000

Table 5 – Multiple Comparisons Test with Tamhane Test

Variable	Test	Mean Difference	*p*	Description
Tyrosinase (ng/mL)	K0-K1	26.312	0.000	Significantly Different
	K0-K2	17.097	0.000	Significantly Different
	K1-K2	-9.215	0.000	Significantly Different
Melanin (%pixel)	K0-K1	17.361	0.001	Significantly Different
	K0-K2	16.027	0.002	Significantly Different
	K1-K2	-1.334	0.705	Not Significantly Different

The results of the comparability test using Post Hoc Tamhane showed that the difference in the mean decrease in tyrosinase enzyme levels between K1 and K2 was -9.215 ($p<0.05$) and the amount of melanin was -1.334 ($p>0.05$). It can be concluded that administration of 12.5% strawberry (*Fragaria ananassa*) ethanol extract cream before UVB exposure and after UVB exposure had different effectiveness in reducing levels of tyrosinase enzyme level, but is equally effective in reducing the amount of melanin in the skin of male guinea pigs (*Cavia porcellus*).

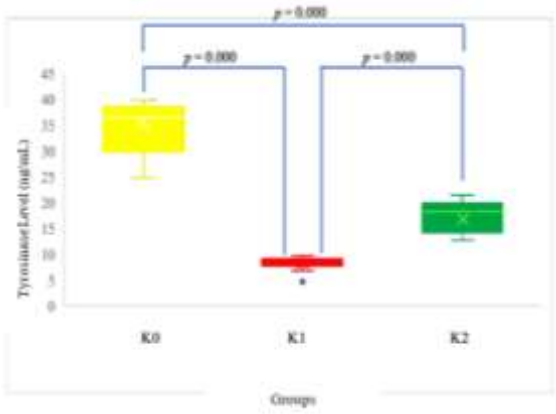

Fig. 1 – Comparison of Mean Differences in Tyrosinase Enzyme Levels (ng/mL) between Group

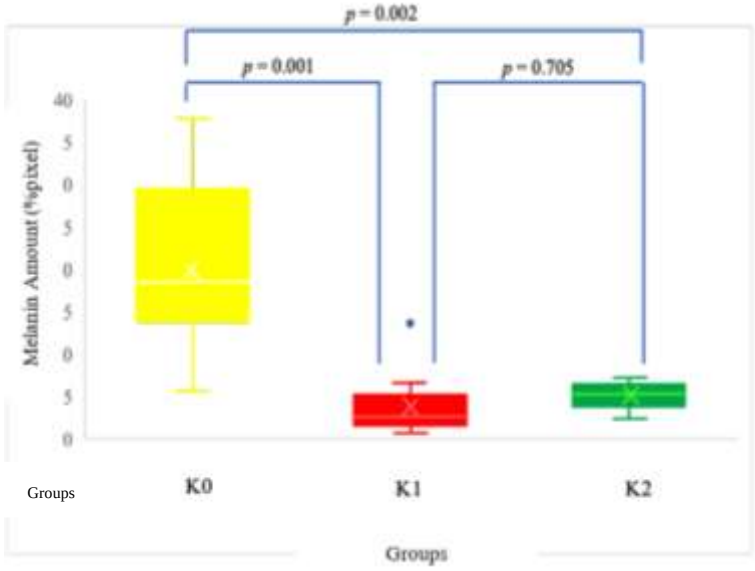

Fig. 2 – Comparison of Mean Differences in Melanin Amount (% pixel) between Groups

a. **b.** **c.**

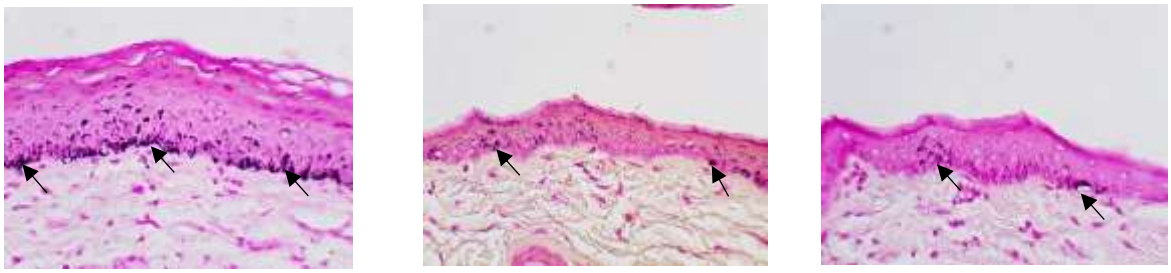

Fig. 3 – Melanin Amount in Guinea Pig's Skin using Masson Fontana Staining

Description: Histopathological tissue of the epidermis of guinea pig skin with 400x magnification. Melanin pigment is shown with black arrow. (a) The control group was given basic cream and exposed to UVB light, extensive hyperpigmented lesions were seen characterized by dense blackish brown melanin cells in the epidermal tissue. (b) Treatment group 1, given 12.5% strawberry ethanol extract cream before UVB light exposure, showed blackish brown melanin cells less frequently than the control and slightly clustered. (c) Treatment group 2, given 12.5% strawberry fruit ethanol extract cream after UVB light exposure, showed blackish brown melanin cells less frequently than the control, but appeared denser when compared to treatment group 1.

a. **b.** **c.**

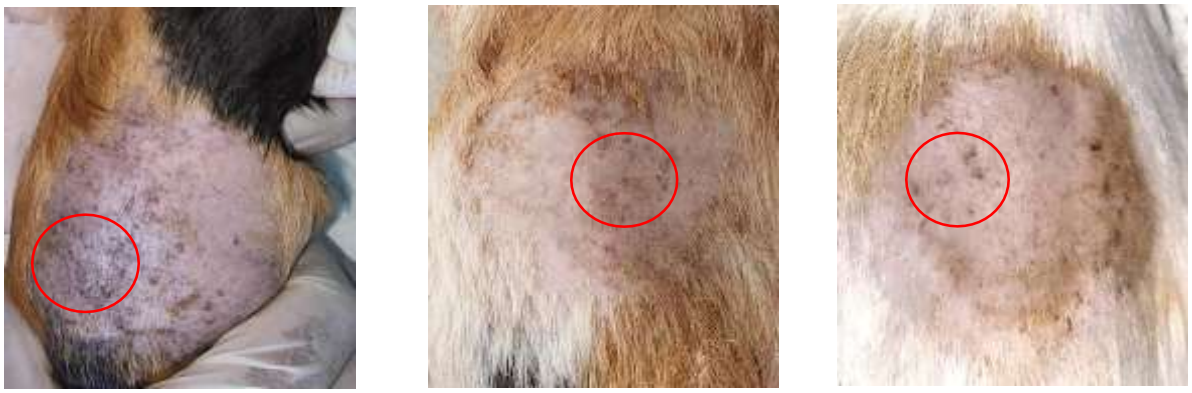

Fig. 4 – Macroscopic Appearance of Guinea Pig's Skin After Exposure to UVB

Description: (a) Control Group: the skin showed dark black spots, with hyperpigmentation visible. (b) Treatment Group 1: the skin showed minimal hyperpigmentation compared to the control group and treatment group 2. (c) Treatment Group 2: the skin showed minimal hyperpigmentation compared to the control group, however the hyperpigmentation appears darker than the treatment group 1.

4. Discussion

4.1 Effects of UVB Exposure

The results of the experiment showed that the mean levels of the tyrosinase enzyme and the amount of melanin were higher in the control group with repeated exposure to UVB light and administration of base cream. In this study, UVB exposure was chosen to provide a hyperpigmentation effect because it is more cytotoxic than UVA and the highest exposure to UVB is in equatorial areas, including Indonesia. In addition, melanin pigment is very effective at absorbing ultraviolet light to the epidermis and upper dermis layers with a wavelength of 280 to 320nm, which is the wavelength of UVB compared to absorbing ultraviolet radiation from others spectrum (Mohania *et al*., 2017). Repeated and increasing exposure to UVB light results in melanogenesis which is characterized by an increase in the number of melanocytes and transfer of melanocytes to keratinocytes from melanosomes, an increase in the tyrosinase enzyme and the spread of melanin. UVB exposure is the main cause of sunburn, resulting in longer lasting tanning and acute effects compared to UVA which produces short term tanning, damage to the skin (skin aging) and longer lasting effects (Young *et al*., 2016). This is in accordance to the macroscopic picture of the research result.

4.2 Effects of Administration of 12.5% Strawberry Ethanol Extract Cream

The results of this study showed that the mean levels of the tyrosinase enzyme and the amount of melanin decreased significantly in treatment group 1 and 2 compared to the control group ($p < 0.05$). This shows that treatment group 1 and 2 that were given 12.5% strawberry ethanol extract cream effectively reduced tyrosinase enzyme levels and the amount of melanin compared to the control group with basic cream. The mean levels of the tyrosinase enzyme in groups 1 and 2 decreased significantly, namely 8.25 □ 1,5 ng/ml and 17.47±3.09 ng/mL respectively.

In this study, it was found that the levels of the tyrosinase enzyme were reduced, which in turn also reduced the increase in the amount of melanin, where the formation of melanin pigment requires the main enzyme tyrosinase. This is shown by the mean amount of melanin in treatment groups 1 and 2 decreasing significantly, namely 3.8□3.9%pixels and 5.13□1.53%pixels, compared to the control group, namely 21.16□10.21%pixels. Based on this, it can be concluded that 12.5% strawberry ethanol extract cream has a potential effect for protection (sunscreen) when used before UVB exposure. Apart from that, it can also be concluded that 12.5% strawberry ethanol extract cream has a potential therapeutic (treatment) effect when used after UVB exposure in terms of reducing tyrosinase enzyme levels and the amount of melanin in the skin of male guinea pigs exposed to UVB.

Based on previous research by Rinandari *et al*. (2021) where clinical trial research was conducted on humans by administering 4% HQ cream for 8 weeks with MASI (Melasma Area and Severity Index) evaluation to assess the severity of melasma carried out at week 4 and week 8, the melasma lesions experienced significant changes at week 4 and week 8 (Rinandari *et al*., 2021). Other previous in vitro research found that 0.3% strawberry juice cream (*Fragaria ananassa*) is a strong antioxidant with an IC 50 antioxidant capacity of 52.59 ppm (Ferdiansyah *et al*., 2016). The use of 12.5% strawberry ethanol extract cream in this research is based on previous studies that 12.5% strawberry ethanol extract cream meets control and quality test standards, which consist of organoleptic, pH test, spreadability test, stickness test and protective capacity test (Arifah *et al*., 2013). This is reinforced by the results of previous research on ethanol extract cream from strawberries which can inhibit hyperpigmentation by reducing the amount of melanin in in-vivo experimental research on male guinea pigs with exposure to direct sunlight (Harahap *et al*., 2022).

4.3 Comparison of the Effectiveness of 12.5% Strawberry Ethanol Extract Cream Before and After UVB Exposure

Based on the Tamhane comparability test on the comparison of the mean levels of the tyrosinase enzyme, the effect of 12.5% strawberry cream extract in treatment group 1 was not as effective as in treatment group 2 ($p<0.05$). The difference in the mean decrease in tyrosinase enzyme levels was 9.21 ng/ml, with tyrosinase enzyme levels in treatment group 1 (8.26±1.5 ng/ml) being lower than in treatment group 2 (17.47±3.09 ng/ ml). This shows that 12.5% strawberry ethanol extract cream has a potential preventive (protective) effect when used before UVB exposure which is better than the therapeutic (treatment) effect when used after exposure to UVB to reduce the levels of the tyrosinase enzyme.

Based on the Tamhane comparability test on the comparison of the mean amount of melanin, the effect of 12.5% strawberry cream extract in treatment group 1 was equally effective compared to treatment group 2 with $p>0.05$ (no significant difference). The difference in the mean decrease in the amount of melanin was 1.33 ng/ml, with the amount of melanin in treatment group 1 (3.8±3.9% pixels) being lower than in treatment group 2 (5.13±1.53 % pixels). This shows that the potential preventive (protective) effect, when used before UVB exposure, and the therapeutic (treatment) effect, when used after UVB exposure, of 12.5% strawberry fruit ethanol extract cream had the same effectiveness.

Application of cream before UVB exposure has the potential to act as protection (sunscreen) which directly interferes with the absorption of radiation by limiting absorption and then converting it into heat waves, reflecting and dispersing energy. This inhibits the penetration of UVB radiation into the epidermis layer thereby preventing damage to melanocytes and keratinocytes in the deep epidermis layer (Guan *et al*., 2021). The effective reduction in tyrosinase enzyme levels and the amount of melanin in the treatment group was due to the high content of flavonoid compounds in the 12.5% ethanol extract cream of strawberries and ellagic acid as a strong competitive inhibitor of tyrosinase to inhibit the activity of the tyrosinase enzyme directly so as to reduce tyrosinase enzyme levels, as well as inhibiting tyrosine into 3,4-DOPA and DOPAquinone, which play a major role in melanogenesis (Oktaviana & Yenny, 2019).

In this study, ELISA test was limited to tyrosinase enzyme levels only, so it is possible that there is a role of TRP-1 (Tyrosinase Protein-1) and TRP-2 (Tyrosinase Protein-2) in influencing the formation of eumelanin, which was not examined in this study. In addition, the synergistic ability of antioxidant compounds in an extract can influence its capacity for preventive and therapeutic effects. However, administration of strawberry extract cream applied to the group before UVB light exposure and the group after UVB light exposure showed the same effectiveness in reducing the amount of melanin, indicating that there was an inhibition of the melanogenesis process to produce melanin, thereby inhibiting the transfer of melanocytes to keratinocytes and inhibiting the activity of the main enzyme tyrosinase.

In this study, two biomarkers were used, namely tyrosinase enzyme level and melanin amount in order to ensure that the mechanism pathway is the result of UVB exposure which stimulates the activation of the tyrosinase enzyme, then influence the formation of melanin pigment. In addition, to ensure that this mechanism is not influenced by other factors that influence melanin formation such as external factors (drugs) and internal factors (genetic, inflammation, hormonal).

4.4 Effectiveness of Administration of 12.5% Strawberry Ethanol Extract Cream Compared to Previous Researches

The results of this study are similar to previous research by Daniaswati (2022) in Udayana University, using a different extract, namely 3% snake fruit pulp extract cream, which has been proven to inhibit the increase in tyrosinase enzyme levels and the amount of melanin in guinea pig skin exposed to ultraviolet B (Daniaswati, 2022). 3% snake fruit pulp extract has the same active compound content as 12.5% strawberry ethanol extract cream, however with a lower concentration where the flavonoids are 45.21mg QE/100g, phenols 209.09 mg GAE/100g, and the antioxidant capacity is 572.79 mg GAEAC/L and IC 50 is 2,521.50 ppm.

This research has several limitations, including other ingredients that act as antioxidants and anti-hyperpigmentation that have not been analyzed, including vitamin C, and not comparing the ideal dose, duration and frequency of administration of extract cream. Other things that limited this research included the limited research duration of 14 days, the different skin colors of the guinea pigs, uneven UV light treatment on the experimental animals, and the insufficient dose of strawberry cream extract given.

Another limitation of this study is that the results show that regarding the amount of melanin, between K1 and K2, the protective effect is equivalent to the therapeutic effect. However, it is still unknown whether the protective effect is comparable with the gold standard which is HQ 4% because comparative research has not been carried out comparing strawberry ethanol extract and HQ 4%. The results of this study showed that the protective effect was better than the therapeutic effect, however this study had not evaluated the protective effect related to the SPF value and had not been compared with the protection of sunscreen base cream.

5. Conclusion

Administration of 12.5% strawberry (*Fragaria ananassa)* ethanol extract cream before exposure to UVB light and after exposure to UVB light effectively reduced levels of the tyrosinase enzyme and the amount of melanin in the skin of male guinea pigs *(Cavia porcellus)* compared to administration of basic cream. Administration of 12.5% strawberry ethanol extract cream to the skin of male guinea pigs (*Cavia porcellus*) before UVB exposure had the same effectiveness in reducing melanin amount compared to administration after UVB light exposure, but had difference effectiveness in reducing tyrosinase enzyme level. Further research is needed to compare the protective effect of strawberry extract cream with sunscreen, as well as compare its therapeutic effectiveness with the gold standard hydroquinone as a hyperpigmentation therapy. Apart from that, further research needs to be carried out with

comparison of doses or multilevel concentrations and longer duration to evaluate the protective and therapeutic effects. Furthermore, further research needs to be carried out regarding the level of effectiveness and clinical trials before it can be used on humans.

Research Ethics

The study protocol has been approved by the Ethics Committee of Faculty of Medicine, Udayana University, Bali (B/244/UN14.2.9/PT.01.04/2023).

Author Contribution

All authors contributed equally in compiling this research article

Conflict of Interest

All researches declare that there is no conflict of interest related to this article

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