J Prima Med Sains (2024) 6(1):72-78
doi.org/10.34012/jpms.v6i1.5454

ORIGINAL ARTICLE

The effect of betel nut extract on melanin pigment levels in rats' skin exposed to Ultraviolet B radiation

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Abstract

This study aims to analyze the effect of areca nut extract on the amount of melanin pigment in the skin of Wistar rats exposed to ultraviolet-B light. The research method used was post-test with control group design. The total sample of white rats used was 20 rats, which were divided into 4 groups with each group consisting of 5 rats. The cream preparation was made using cold cream base. The rats were then exposed to UVB with a certain intensity for 5 days, characterized by erythema as a result of UVB absorption into the epidermis. Each group of treated rats received base cream, 10% areca nut extract cream, and 15% areca nut extract cream topically for 14 days before termination on day 15. The collected data were then tabulated and analyzed. To determine significant differences or influences between the test groups, analysis was carried out with t-test or Independent Samples T-Test approach at 95% confidence level ($p < 0.05$). The results of the analysis showed that the administration of areca nut extract was effective in reducing the amount of melanin pigment in the skin of Wistar rats exposed to Ultraviolet-B light. From histopathological observations, the administration of areca nut extract with a concentration of 15% is effective in removing or fading melanin pigments in the skin exposed to Ultraviolet-B light and the results of damaged skin melanin pigments appear in the epidermis of rat skin barely visible grain.

Keywords: areca nut extract, histopathology, skin melanin pigment, UVB

Introduction

With the advancement of time, cosmetics have seemingly become a primary need for some women. This has created opportunities for the cosmetics industry in Indonesia. Cosmetic manufacturers are legally required to adhere to Good Manufacturing Practices (CPKB) principles and guidelines to ensure that their products consistently meet specific efficacy standards. These standards and production methods are regulated by the Ministry of Health and the National Agency of Drug and Food Control (BPOM RI).[1,2] Currently, the cosmetics industry is focusing on developing products that utilize natural ingredients due to the positive response from the public. Natural ingredients are considered safer to use and have fewer negative impacts compared to chemical ingredients.[3] Supported by Indonesia's natural wealth, the domestic cosmetics industry can utilize phytoconstituents from various plants as active ingredients in cosmetic preparations..[4]

One plant known for its benefits for skin health is the betel nut. The plant, scientifically known as *Areca catechu L.*, has various benefits, including consumption with betel leaves (*menginang*), use as traditional medicine, textile dye, and cosmetic ingredients. The parts of the plant used as traditional medicine are the young fruit and the core.[5] Regarding the use of betel nut as medicine, Lestaridewi adds that the roots of the betel nut plant can also be used as medicine, particularly for internal wounds and appetite stimulants. The preparation involves boiling or pounding. The composition includes 5 young betel

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nuts, 9 pieces of betel nut roots, 9 young black wood leaves, and 7 glasses of water. As an ingredient, the use of betel nut in combination with betel leaves has become a hereditary practice in certain regions of Indonesia. This is also seen in the community of Ternate City, where the majority use betel nut solely for consumption. The limited knowledge of the community about the benefits of betel nut has hindered its productivity, although proper utilization of this plant could significantly impact the community's economy.[6]

Ultraviolet (UV) rays are emitted by the sun and reach the earth's surface alongside visible light and infrared rays. UV rays range in wavelength from 200-400 nm.[7] The energy from UV radiation reaching the earth's surface can cause signs and symptoms of skin burns, including redness (erythema), pain, blistering, and peeling. UVB, with a wavelength of 290-320 nm, is more effective in causing skin damage compared to UVA, which has a longer wavelength of 320-400 nm. The energy from ultraviolet radiation reaching the earth's surface can cause signs and symptoms of skin burns, including redness (erythema), pain, blistering, and peeling. UVB, with a wavelength of 290-320 nm, is more effective in causing skin damage compared to UVA, which has a longer wavelength of 320-400 nm.[8]

The use of active substances with antioxidant properties can prevent various diseases caused by UV radiation. Several groups of active antioxidant compounds, such as cinnamates, flavonoids, tannins, quinones, and others, have been studied for their ability to protect the skin from UV rays.[9] Biological and clinical changes in the skin due to UV exposure range from acute side effects like sunburn, tanning, and hyperpigmentation to chronic side effects like photoaging and skin cancer.[10] Pathologically, hyperpigmentation can be caused by an increased amount of melanin in the epidermis, as seen in lentigo, or an increased amount of melanin in the epidermis and upper dermis, as seen in melasma. Lentigo and melasma are the most commonly complained about aging-related disorders. Increased melanin synthesis can also be caused by reactive oxygen species (ROS). ROS formation due to UVB exposure can occur through direct or indirect interactions. Direct UVB interaction involves cross-linking adjacent pyrimidine bases, causing direct DNA damage and binding to aromatic amino acids, resulting in free radical formation.[11]

Indirect UVB interaction leads to ROS formation through photosensitization, which changes electrons in chromophores to singlet electrons, resulting in free radical production. Photosensitization also produces superoxide anions, followed by dismutation into hydrogen peroxide. Hydrogen peroxide, with the help of metal bonds (Fe and Cu), generates excessive hydroxyl groups on the skin, causing harmful effects such as erythema, pigmentation, and premature aging. Of all the solar radiation, only 0.2% causes erythema (redness) on the skin, which is within the UV-B spectrum (290–320 nm), while the UV-A spectrum, which causes skin darkening (pigmentation), is radical in nature.[10]

Previous research found that betel nut seed flour in facial mask preparations using rice flour as a base in traditional cosmetics still contained proanthocyanidins, which belong to the flavonoid group and are beneficial for facial skin. Betel nut seeds contain proanthocyanidins, a type of condensed tannin within the flavonoid group. This content can act as an anti-acne preparation because betel nut seeds have antibacterial properties, classifying them as bactericidal (bacteria-killing)[12]. This study aims to analyze the effect of betel nut extract on melanin pigment levels in the skin of Wistar rats (*Rattus norvegicus*) exposed to ultraviolet-B radiation.

Method

Design

This research falls into the category of experimental laboratory research or true experiment. The research method used is the post-test with control group design to evaluate the impact of betel nut extract on melanin pigment levels in the skin of Wistar rats exposed to ultraviolet-B radiation.

Sampling

In this study, the sample consisted of adult Wistar strain white rats weighing 150-300 grams and aged 2-3 months, which were in healthy condition, characterized by active movement and the absence of physical defects. The selection of Wistar strain white rats as the sample is based on their characteristics, which are similar to humans, their larger size compared to mice, and their ability to adapt to the laboratory environment. A total of 20 white rats were used, divided into 4 groups with each group consisting of 5 rats. The determination of the sample size follows the principle of "reduction" to minimize the use of animals in

research without compromising the validity of the results, in accordance with the 3R principle (Replacement, Reduction, and Refinement) that must be adhered to in in vivo research. The four sample groups are: 1) Negative control group (K-) consisting of 5 male rats without treatment; 2) Positive control group (K+) consisting of 5 male rats exposed to ultraviolet-B radiation without extract; 3) Group P1 consisting of 5 male rats exposed to ultraviolet-B radiation and given 10% betel nut extract cream topically; and 4) Group P2 consisting of 5 male rats exposed to ultraviolet-B radiation and given 15% betel nut extract cream topically.

The male Wistar rats were acclimated in the Animal House of the Faculty of Mathematics and Natural Sciences, University of North Sumatra for one week before testing. The betel nuts used were obtained from Tembung Pasar 7 Medan, North Sumatra. The extraction process was carried out using a Soxhlet apparatus with 70% ethanol as the solvent. The result was an ethanol extract of betel nut. The extract underwent an evaporation process. Finally, TLC testing, cream formulation, and result analysis were conducted for research purposes. Formula 1 contained betel nut extract, stearic acid, glycerin, propylene glycol, glycerin monostearate, TEA, methyl paraben, propyl paraben, and distilled water. Each component was present in the following percentages: 10%, 12%, 5%, 3%, 4%, 1%, 0.18%, 0.02%, up to a volume of 100 ml, functioning as active ingredients, emulsifying agents, humectants, and preservatives. Formula 2 had a similar composition with the concentrations being: 15%, 12%, 5%, 3%, 4%, 1%, 0.18%, 0.02%, up to a volume of 100 ml, serving as active ingredients, emulsifying agents, and preservatives. The main difference between the two formulas lies in the concentration of the ingredients and the function of each component in the cream formula.

Cream preparation

The cream formulation was made using a cold cream base with the following steps: spermaceti, white wax, and liquid paraffin (as part A) were heated to 70°C. Borax dissolved in hot water was added to part A. Nipagin was included in the water phase, and nipasol in the oil phase. These were then slowly mixed into part A while stirring until homogeneous. The mixture was stirred until it thickened. The base cream was then combined with the extract at the specified concentrations. Each formulation was homogenized and placed in containers to observe physical and microbiological stability during storage.

Physical stability measurement

In the cream test, the process began with a homogeneity test where a cream sample was evenly spread on a glass plate to check for uniformity. Next, the viscosity test was conducted using a Rhion viscometer to measure the cream's viscosity. The spreadability test involved measuring the cream's spread diameter by gradually adding weights to observe the impact on the spread diameter. Finally, the adhesion test involved placing the cream on an object glass and pressing it with a weight to measure its adhesion.

Microbial count test

Sterilization was carried out using an autoclave at 121°C for 15-20 minutes for sterilizing equipment and materials. The microbial count process involved aseptic sampling, dilution, bacterial colony growth on plate count agar media, and finally counting the number of bacterial colonies after incubation.

Treatment administration

The research was conducted using 20 male rats divided into 4 groups based on the Federer formula. The rats were acclimated for 1 week at the Herbarium Medanese, Faculty of Mathematics and Natural Sciences, University of North Sumatra, and were regularly fed. Anesthesia was administered with topical lidocaine on the rats' backs, which were then placed prone on a surgical table. The rats' backs were disinfected with 10% povidone iodine before shaving the disinfected area. The rats were then exposed to UVB at a specific intensity for 5 days, resulting in erythema due to UVB absorption into the epidermis. Each treatment group received the base cream, 10% betel nut extract cream, and 15% betel nut extract cream topically for 14 days before termination on the 15th day. The collected data was tabulated and analyzed for further research.

Histopathological observation

The effectiveness of the betel nut extract cream on the test animals' skin was measured by observing the increase in melanin pigment. The melanin pigment count was analyzed using digital analysis with an LC Evolution camera and an Olympus Bx51 microscope at 40x magnification. Each slide was photographed three times and saved in JPEG format. The images were analyzed for melanin pigment count using Image J software. The melanin pigment network, appearing bright red, was selected, and the histogram results from the image segmentation of the melanin pigment, in terms of pixel area, were recorded. Other tissues with different colors were selected and their histogram pixels were noted. The melanin pigment amount was calculated as the percentage of melanin pigment pixel area compared to the total tissue pixel area (sum of melanin pigment pixel area and other tissue pixel area). After obtaining the melanin pigment analysis results for each treatment group, scoring of the melanin pigment amount on UVB-exposed rat skin was conducted based on histopathological observations. A higher score indicated a better percentage in one field of view regarding melanin pigment amount on the skin.

Results

Based on the homogeneity test results, the obtained cream formulation showed that the active ingredients and other additives were evenly mixed. This indicates that the cream has good quality because the medicinal substances are uniformly dispersed in the base material. The pH test results showed that the cream formulation meets the required pH standard (5) for a topical preparation. This indicates that the betel nut seed extract cream does not cause irritation when applied to the skin.The spreadability test showed that the cream formulation meets the spreadability parameters with a diameter of 5. This indicates that the betel nut extract cream is safe to use on the skin. The viscosity test showed that the cream formulation has a viscosity speed of 30 with a value of 9700, which is 97% in percentage, meeting the testing criteria.

Table 1. Average results of skin melanin count

Repetition	K-	K+	P1	P2
1	82	60	44	25
2	81	59	42	22
3	79	56	38	19
4	86	64	43	24
5	88	66	44	26
Shoes*	+4	+3	+2	+1
Mean	83.2	61	42.2	23.2
SD	3.7	4	2.4	2.7

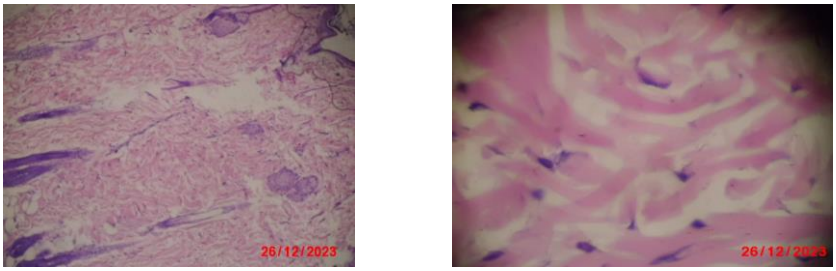
*Information:
0= No melanin pigment was found on the skin; +1= The amount of melanin pigment in the skin is small (less than 10% per field of view); +2= The amount of melanin pigment in medium skin (less than 10-50% per field of view); +3= The amount of melanin pigment in the skin is high (less than 50-90% per field of view); +4 = The amount of melanin pigment in the skin is very high (less than 50-90% per field of view)

Secondary metabolite testing was conducted to identify the organic compounds present in betel nut (*Areca catechu L.*). Several tests were performed to identify secondary metabolites, including phytochemical tests. Screening results showed that betel nut extract (Areca catechu L.) contains active compounds such as alkaloids, terpenoids, and steroids. Terpenoids are beneficial as antiseptics, expectorants, spasmolytics, anesthetics, and sedatives for the skin. Alkaloids in betel nuts stimulate the central nervous system. The tannins in betel nuts give the fruit its bitter and astringent taste but also function as antioxidants. Phenolic and steroid compounds, which are the largest group of phytochemicals in plants, have antioxidant activity and are useful in skincare. Thus, betel nut extract has many antioxidants that can benefit the skin, such as in melanin formation and skin aging.

Anesthesia was administered using a lidocaine solution topically on the back of the rats, and their backs were disinfected with 10% povidone-iodine. The rats' backs were exposed to UVB light from a distance of 20 cm with a minimal erythema dose (MED) of 160 mJ/cm², for about 15 minutes per day, for 5 days. This was marked by erythema on the rats' backs due to the cumulative effect of UVB absorption into the epidermis. The first group, the negative control group (K-), consisted of 5 male rats that did not receive any treatment. The second group, the positive control group (K+), consisted of 5 male rats exposed to UVB and given a base cream (0% extract). The third group (P1) consisted of 5 male rats exposed to UVB and given 10% betel nut extract cream topically. The fourth group (P2) consisted of 5 male rats exposed to UVB and given 15% betel nut extract cream topically. Termination was performed on the 15th day. After collecting the data, it was tabulated and analyzed.

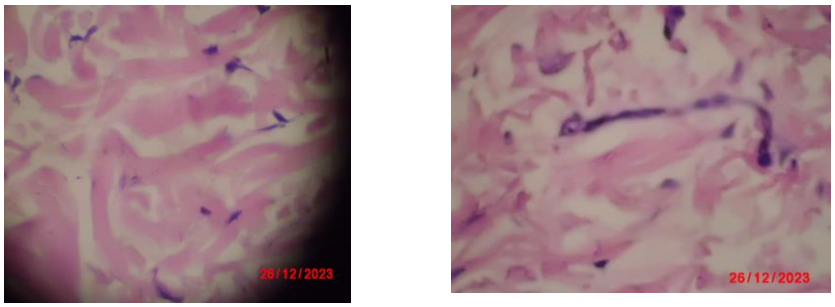
From the data in the table above, it can be concluded that the negative control group (K-), which was only exposed to UVB radiation for 14 days without treatment, had an average skin melanin pigment percentage of 83.2±3.7. Meanwhile, the positive control group (K+), which was exposed to UVB and applied

with base cream without betel nut extract, had an average skin melanin pigment percentage of 61±4. The treatment group 1 (P1), where rats were exposed to UVB and applied with 10% betel nut extract cream topically, had an average skin melanin pigment percentage of 42.2±2.4. The treatment group 2 (P2), where rats were exposed to UVB and applied with 15% betel nut extract cream topically, had an average skin melanin pigment percentage of 23.2±2.7. The analysis results showed that the P2 group, which received 15% betel nut extract cream, showed significant improvement in the amount of skin melanin pigment. Conversely, the negative control group showed the worst results without using betel nut extract cream. Further evaluation of skin melanin pigment will be conducted through histopathological images by dividing the interpretation of the amount of melanin pigment into three categories: low (<40 melanin pigment), medium (40-80 melanin pigment), and high (>80 melanin pigment).



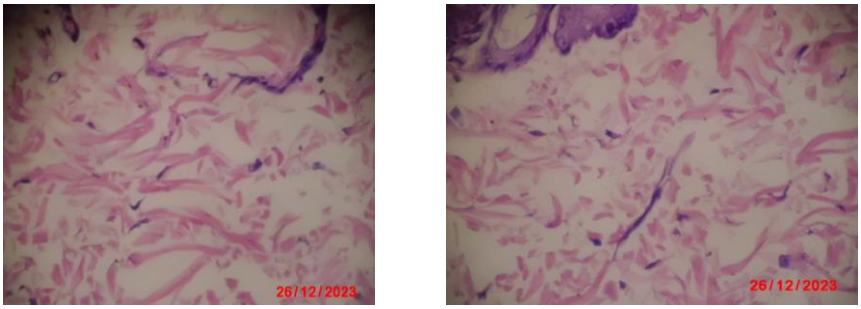
K-

Information:
For the results of damaged skin melanin pigment, it appears in the epidermis of rat skin in large numbers. This shows that the intensity of exposure obtained also affects the production of melanin pigment that is not given any treatment in the negative control group (K-)



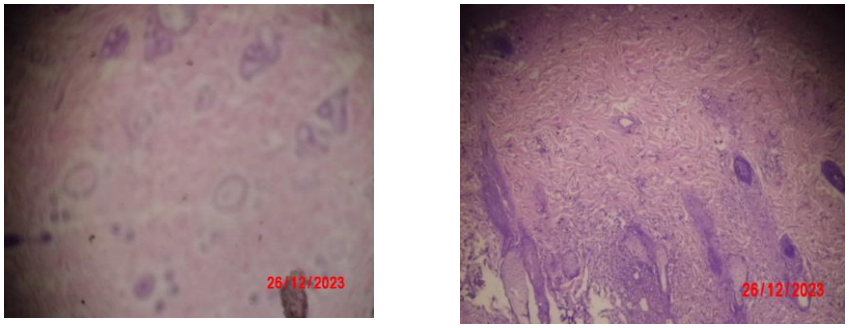
K+

For the results of the damaged skin melanin pigment seen in the epidermis of the rat skin began to decrease and began to be seen, it can be seen from the skin melanin granules that were slightly reduced from before. This shows that the intensity of exposure obtained also affects the production of melanin pigment applied with basic ointment in this positive control group (K+).



P1

For the results of the damaged skin melanin pigment, it appears that the epidermis of the rat skin has fewer grains and is seen to begin to fade. This showed that the intensity of exposure obtained also affected the skin melanin pigment exposed to *ultra violet-B* rays and was given a cream of areca nut extract with a level of 10% topically in treatment group 1 (P1).



P2

For the results of the melanin pigment of the damaged skin, it appears that the epidermis of the rat skin is barely visible. This showed that the intensity of exposure obtained also affected the skin melanin pigment exposed to *ultra violet-B* rays and received a cream of areca nut extract with a level of 15% topically in treatment group 2 (P2).

Figure 1. Histopathological description of rat skin melanin

The administration of 15% betel nut extract effectively eliminated melanin pigment in UVB-exposed skin based on histopathological observations. The damaged skin melanin pigment granules in the epidermis of rats were almost invisible. This contrasts with the negative control group (K-) that did not receive treatment during the experiment, where many damaged skin melanin pigments were visible in the rats' epidermis. The intensity of exposure appears to affect the amount of melanin pigment still present in the untreated group. Thus, betel nut extract also positively impacts the elimination or fading of melanin pigment in UVB-exposed skin.

Table 4. Normality test results

Group	df	sig
Control	5	.200
P1	5	.200
P2	5	.200
P3	5	.200

Table 5. t Test results

t	df	Sig 2 tailed
29.002	8	.000

The normality test results showed that all data were normally distributed ($p > 0.05$). Subsequently, a comparative test using the t-test was conducted. This test is generally used to compare the average values of two unrelated groups. In this study, the researcher compared the negative control group and treatment group 3. The significance value (2-tailed) was $0.000 < 0.05$, indicating a significant difference in skin melanin formation.

Discussion

The skin functions to protect the body from external influences. Damage to the skin can disrupt both human health and appearance, making it essential to maintain and protect skin health. One factor that can cause skin damage is free radicals, including ultraviolet (UV) rays. UV rays are only a small part of the sunlight spectrum, but they are the most dangerous for the skin due to the harmful reactions they induce. These adverse effects arise from oxidative stress, which occurs after UV exposure. Oxidative stress results from an imbalance between pro-oxidants (reactive oxygen species) and antioxidants (Agarwal, 2017).

This study aimed to investigate and test the effects of betel nut extract on the amount of melanin pigment in the skin of Wistar rats (Rattus norvegicus) exposed to UV-B radiation. The use of active compounds with antioxidant properties can prevent various diseases caused by UV radiation. Some groups of active antioxidant compounds, such as cinnamates, flavonoids, tannins, quinones, and others, have been studied for their ability to protect the skin from UV rays (Hogade, 2010). Regarding the use of betel nut as a medicinal ingredient, Lestaridewi added that the roots of the betel nut plant can also be used as medicine, particularly for internal wounds and as an appetite stimulant.

Phytochemical tests have shown that betel nut extract (*Areca catechu L.*) contains active compounds such as alkaloids, terpenoids, and steroids. Terpenoids are beneficial as antiseptics, expectorants, spasmolytics, anesthetics, and sedatives for the skin. Alkaloids in betel nuts stimulate the central nervous system. Tannins in betel nuts, which give the fruit a bitter and astringent taste, also function as antioxidants. Phenolic compounds and steroids, which are the largest group of phytochemicals in plants, have antioxidant activity. Phenolic derivatives are useful in skin care. Thus, betel nut extract contains many antioxidants that can benefit the skin, such as in melanin formation and skin aging.

Based on the average data obtained, the group with the most improved melanin pigment was P2, the treatment group given 15% betel nut extract cream, with an average and standard deviation of 23.2±2.7. The group with the worst average melanin pigment was the negative control group (K-), which was exposed to UV-B radiation but not given betel nut extract cream, with an average and standard deviation of 83.2±3.7.

Histopathological observations in the images above show that administering 15% betel nut extract effectively eliminates and fades melanin pigment in UV-B exposed skin, with damaged melanin pigments in the epidermis of rats almost invisible. In the normality test, all groups (K-, K+, P1, P2) showed a significant value of 0.2. Since the significance value (p) in the Kolmogorov-Smirnov test exceeds the standard margin ($p>0.05$), all data are normally distributed. The independent t-test above shows a sig. (2-tailed) value of $0.00 < 0.05$, indicating a significant difference in skin melanin formation.

Conclusion

The administration of areca nut extract was effective in reducing the amount of melanin pigment on the skin of wistar rats exposed to Ultraviolet-B light. From histopathological observations, the administration of areca nut extract with a concentration of 15% was beneficial effectively in removing or

fading melanin pigment on the skin exposed *to Ultraviolet-B* rays and the results of damaged skin melanin pigment appeared on the epidermis of the skin of mice with almost no visible grains.

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