

# The Effect Of Giving Black Rice Extract Ointment (*Oryza Sativa L. Indica*) On The Growth Of Wistar Rat (*Rattus Novergicus*) Hair Follicles

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## Abstract.

*Hair loss affects many people worldwide. Hair loss is common and can be treated with vitamins and minerals. Black rice (*Oryza sativa l. indica*) contains phenolic and anthocyanin-rich flavonoids. The study examined whether black rice extract ointment accelerated Wistar rat (*Rattus norvegicus*) hair follicle growth. This was an actual experiment with 30 mice. The research involved acclimating test animals in the lab, manufacturing black rice extract, and making ointment. After an Ointment Evaluation Test, Phytochemical Screening was performed. Test the FT-IR Functional Group. DPPH testing will determine antioxidant activity. Finally, four groups (control group, treatment groups 1, 2, and 3 with 10%, 20%, and 30% ointments) received treatment for 21 days. Study results were handled using SPSS. The normality test showed  $0.652 > 0.05$  significance, while the Independent Samples T-Test showed  $0.00 < 0.05$  significance. Alkaloids, flavonoids, tannins, and steroids/triterpenoids make black rice an antioxidant, according to the active compound test. For average hair growth, 10% black rice ointment dose therapy yielded 0.681 cm, 20% 0.769 cm, and 30% 0.851 cm. The study found that black rice extract contains high antioxidants (ppm concentration value  $< 50$ ) that can prevent premature skin aging and promote hair and skin health.*

**Keywords:** Hair Loss, Black Rice Extract and Alopecia.

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## I. INTRODUCTION

According to most individuals (both sexes), good hair is a sign of confidence. In terms of self-perception and social perception, hair has a role. Hair is a confidence booster: hair care, color, and style impact personal confidence and physical beauty [1]. The hair on an animal's body is one way to identify it. Human hair serves several functions, such as an adornment, a temperature regulator, and an environmental shield [2]. The building block of each hair fiber is keratin, the principal protein found in hair. Keratin comprises several proteins, lipids, minerals, water, and a significant quantity of cysteine [3]. Hair is made of the epidermis' follicle and hair shaft. Hair growth requires follicles. Over 100,000 of the body's 5 million hair follicles reside on the scalp. Hair follicle density and type may alter as they molt [4]. The hair shaft comprises the cuticle, cortex, and medulla cells [5]. The hair follicle size dictates Lanugo, vellus, and terminal hair. Lanugo hair, the initial fiber produced by the hair follicles, is long, delicate, and unpigmented. The hair follicles which make this hair are located in the embryonic stage [6]. The initial signs of growth, known as vellus or terminal hair, emerge in the ninth month and continue until the baby is born. Babies are sometimes covered in lanugo hair even before they are accepted. Velus hair that is not pigmented is short and fine. This haircut frequently conceals the prominent cheekbones and nasal bridge. It features a medulla and is long and coarse with pigmentation. It is terminal. Adolescent men's vellus hair changes into terminal hair due to hormones. This operation is visible on his beard, armpits, chest, and genitalia A [7]. In addition to being a sign of overall physical health, women may flaunt their confidence, attractiveness, and power when their hair is in good condition.

This is why women are more likely than males to experience psychological issues related to hair loss [8]. Many individuals worldwide deal with hair loss, manifesting in many body parts, including the scalp, face, and body. Genetics, hormones, dietary status, environmental exposures (radiation, environmental pollutants), and medicines are some of the many causes of hair thinning and loss [3]. Another name for hair loss is alopecia [9]. The thinning or complete absence of hair on the scalp and other usually hairy body areas is known medically as alopecia [10]. Socially and mentally, this disorder can cause a lack of self-esteem.

Androgenic alopecia, also known as widespread baldness, alopecia areata, and chemotherapy-induced alopecia, are the most frequent types of alopecia. Many factors might lead to this disorder, including stress, genetics, hormones, diet, illness, and even some drugs [11]. The hair development cycle is shortened, and hair loss can occur in two ways in all types of alopecia. A reduction in the length of the anagen phase characterizes androgenic alopecia. The result is an abnormally short telogen phase because the anagen:telogen hair ratio drops below the typical 6:1. The dermal papillae, which regulate nutrient supply and cell differentiation in hair follicles, diminish in the second. The dermal papillae get more minor as the blood arteries that provide oxygen and nutrients to the hair contract. A sudden transformation from thick, pigmented hair to deformed (thin and white) vellus hair results from these changes in the diameter and appearance of the hair [12]. Half of all men and women will reportedly lose hair at some point. Although hair loss does not affect one's ability to function, the psychological and emotional toll it takes on one's self-esteem is substantial. A person may have PTSD, anxiety, sadness, and social phobia [13]. There is a need for treatments to overcome the disruption that long-term hair loss can cause to an individual's life. Low antioxidant levels and an elevated lipid peroxidation index are expected in hair loss patients [14].

The immune system's destruction of hair follicles may be set off by oxidative stress, defined as an imbalance between the antioxidant and oxidation defense systems, and is thought to be linked to alopecia [9]. Vitamin and mineral supplements are effective treatments for hair loss [14]. Hair loss can occur if the body does not get enough vitamins and minerals for healthy cell development and function. Vitamins and minerals can be a cheap and convenient way to cure hair loss, but knowing which ones work is crucial [15]. Supplements are helpful, but those losing weight should also utilize plant extracts. In the hair care category, natural ingredients, botanical compounds, and promises of being free from hazardous components (such as sulfates, parabens, silicones, and salt) are the most sought-after attributes by customers [16]. There are a lot of all-natural, beneficial components, including black rice, which lends credence to this. The black kind of rice, *Oryza sativa L.*, is grown mainly in Asia and is characterized by its sticky texture, high nutritional content, and dark color [17]. A unique kind of black rice (*Oryza sativa l. indica*) has an endosperm covered in a dark brownish-black skin. Compared to white rice, this kind has a greater gluten level, is stickier, and needs more time in the oven. Many people love to nibble on black rice because of its distinctive color and the sweet, nutty flavor it imparts [18]. The list of nutritional and bioactive components is long and includes things like phytic acid,  $\gamma$ -oryzanol, tocopherols, tocotrienols, dietary fiber, vitamins, minerals, anthocyanins, phenolic compounds, phytosterols, and functional lipids. The distinct blackish-brown hue of the grains gives this kind of rice its name.

High concentrations of the flavonoid's anthocyanins, which act as antioxidants in the body, give this rice its characteristic black color in the kernel's pericarp (outside section) [19]. The anthocyanins and phenolic flavonoids in black rice give it potent antioxidant properties. One group of chemicals with antioxidant properties is the flavonoids, of which anthocyanins are a member. As water-soluble pigments, they often take on a deep purple hue. Depending on the pH, the hue can be anything from red to purple to blue to black. A dark hue is usually indicative of a food high in anthocyanins. Variegated varieties of black rice have a wide range of pigmentation, nutritional content, and phytochemical characteristics, contributing to the crop's rising popularity [20]. The anthocyanins in black rice have great potential as health-promoting antioxidants, helpful food additives, and dietary supplements. Another possible use is to encourage the development of new hair follicles [21]. Scientists are curious to see how applying an ointment containing black rice extract influences the growth of hair follicles in the Wistar strain of rats (*Rattus norvegicus*) in light of the given description of the phenomena.

## II. METHODS

This study employs specific quantitative research using a genuine or laboratory experimental design. True experimental research involves regulating all potential confounding factors to ensure that the experimental activities remain unaffected by outside forces [22]. This study used a pre-test-post-test methodology to examine the effects of a black rice extract ointment on the development of hair follicles in rats of the Wistar strain. The rats in the study were selected because of their ability to respond positively to

the treatment. *Rattus norvegicus* males, weighing 200-300 gr and two to three months old, were the subjects of the study. One of the most famous rodent species used in biomedical research is the rat, scientifically known as *Rattus norvegicus*. Rats share many characteristics and physiological processes with humans. To make conclusions, a certain percentage of the population must be sampled [23]. The Ferderer formula is utilized to calculate samples. Each group requires five test animals according to the sample estimate given earlier. In this study, six Wistar rats were used for each group. To ensure that all animals met the criterion, researchers included an extra tail in each group for 30 test animals in this inquiry. The animals were split into five groups at random. A variable is a measurable or observable quality that can change over time [24]. This experiment aims to determine whether or not the administration of black rice extract ointment (*Oryza sativa l. indica*) inhibits the development of hair follicles in the Wistar strain of rats (*Rattus norvegicus*).

Ointment concentrations of 10%, 20%, and 30% of black rice extract (*Oryza sativa l. indica*) are the independent variables. *Rattus norvegicus* Wistar strain rat hair follicle development is the dependent variable. Manufacturers look to a reworked study to make black rice extract (*Oryza sativa l. indica*) [25]. The effects of a 35% concentration of black rice extract on the skin and the formation of hair follicles in rats were examined in the study. We weighed the strained, macerated extract after macerating it in an acidic solution. A solution was made by macerating and filtering black rice extract at concentrations of 10%, 20%, and 30%. The filtrate was evaporated to conduct a phytochemical analysis. Then, to achieve a consistent consistency, the extract was mixed with the essential ointment. One group uses ordinary old ointment that isn't formulated with extract, whereas the other includes black rice extract. Add the melted base to the nipasol mixture and stir. Then, nipagin and a jackfruit leaf ethanol extract are mixed in. After that, the ointment is levigated with extracts of varying quantities till it becomes a homogeneous paste. Primary and secondary metabolites in plant extracts include antioxidants with potent biological activity. Researchers performed a secondary metabolite test to determine if the ointment made from rice extract contains chemicals that speed up hair development in Wistar rats and what active components are in plants generally. After adding hot water and strong hydrochloric acid to the test tubes, they were heated for 15 minutes. Findings pointed to saponins, terpenoids, alkaloids, flavonoids, and steroids. The presence of catechol tannins was indicated by a red or yellow hue, a brown or green color, and foam formation for 1-10 cm for 10 minutes. According to the findings, chemicals found in rice extract ointment can speed up the hair development process in Wistar rats.

A popular method for assessing the chemicals in black rice extract is the Fourier Transform Infrared (FT-IR) spectrophotometer. The instrument has excellent resolution, can analyze solid, liquid, and gaseous materials, and does quantitative and qualitative analyses. The functional groups of the ethanol extract of black rice were examined using the KBr pellet technique. The FT-IR characterization findings were graphed to identify the functional groups of the extract, and the spectra were studied. The DPPH technique captures free radicals and assesses antioxidant activity in vitro. Although it absorbs at 517 nm when purple, DPPH eventually becomes yellow. According to the IC<sub>50</sub> value, there is antioxidant action. For this experiment, we made DPPH and stock solutions with concentrations of 50, 100, 150, 200, and 250 ppm, in addition to a 500 ppm solution. A spectrophotometer was used to measure the absorbance at 515 nm. For the 21-day trial, 30 male Wistar rats (*Rattus norvegicus*) were shaved and given an ointment containing black rice extract. Each of the five groups of rats had six rats. We marked the rats and then applied the ointment concentration to their shaved backs. Following the protocol outlined in Sulastri's (2019) study, researchers monitored hair thickness and length after each treatment to determine the rate of hair growth. After that, the data was statistically examined. Applying the ointment at a 0.1 ml/cm<sup>2</sup> rate allowed us to examine the hair growth with a caliper. The research aimed to determine whether an ointment of black rice extract helped reverse hair loss. Data entering, cleaning, verifying, coding, and storing are all parts of data processing. Before entering data into a computer program, it is double-checked for completeness and correctness and then manually adjusted if necessary. Data cleansing is crucial in preventing mistakes and preparing data for analysis. Statistical packages such as SPSS 25.0 [26], the Kolmogorov-Smirnov test, and the independent sample T-test method were used to examine the study data for noteworthy variations.

### III. RESULTS AND DISCUSSION

#### Research Result

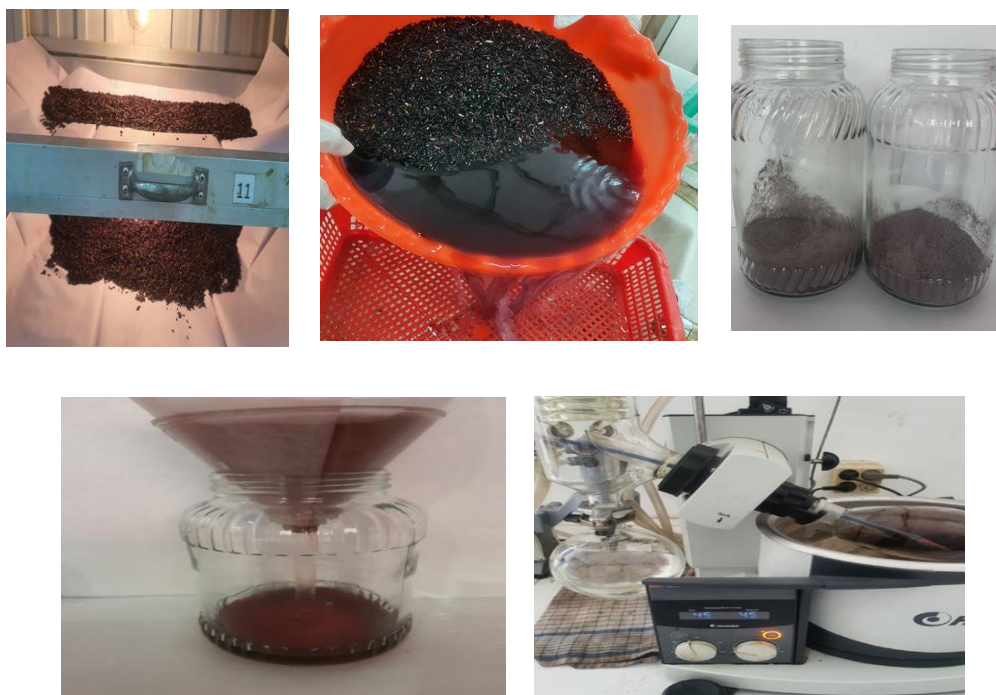
Thirty male rats will be used in this investigation, and they will be split into five groups. Researchers employed the 3R Principle, which stands for Replacement, Reduction, and Refinement, to ascertain the necessary sample size, focusing on Reduction [27], with six rats distributed across the groups. *Rattus norvegicus* male wistar rats are often utilized. When they are two to three months old, they weigh an average of 200 to 300 grams. Shaving the backs of the disinfected mice is the first step in treating them with black rice extract ointment (*Oryza sativa* L. indica). A hair shaver with a 4x4 cm area is used to shave each rat. Moreover, treat every group separately.

**Table 1.** Characteristics Of Test Animals

Component	Description
Rat Type	( <i>Rattus norvegicus</i> ) male wistar strain.
Weight	200-300 Grams
Age	2-3 Months

A kind of rice known as black rice (*Oryza sativa* L. indica) is becoming more popular as a functional food because of the positive effects it has on health. Anthocyanins, an antioxidant found in black rice, aid in preventing free radical damage to the body. In addition to promoting healthy hair and skin, antioxidants aid in the prevention of skin aging. It encourages hair development, helps restore elasticity, and keeps skin tight [19]. Combining amino acids, antioxidants, and vitamin E promotes and sustains healthy hair and skin [28]. The steps to make black rice extract are illustrated in the following image (*Oryza sativa* L. indica)

**Fig 1.** Process of Making Black Rice Extract



Researchers examined the effects of black rice extract on the skin of rats at three different concentrations: 10%, 20%, and 30%. To determine the extract yield, the macerated black rice was combined with a solvent, filtered, and then weighed. After that, the extract was blended into a uniform consistency by adding an essential ointment. Odorlessness, whiteness, light brownness, and a solid structure were all evaluated in the ointment, along with a pH level between 5.37 and 6.33. It was safe to experiment with the ointment because its spreadability was within the acceptable range of 5.0–5.9 cm in diameter. The FT-IR analysis of black rice using the KBr pellet method revealed the presence of flavonoid antioxidants. The 2 mg sample and 100 mg KBr dry powder mixture were compressed into thin pellets and characterized using an FT-IR spectrophotometer at the 4000-450 cm wavelength. The ethanol extract's functional groups were determined. Antioxidant activity may be evaluated by observing the DPPH radicals' color shift using the



DPPH technique. The reaction between the test material's hydrogen atom and the DPPH free radical produces a yellow 1,1-diphenyl-2-picrylhydrazine molecule used for this purpose. Using a spectrophotometer set at 515 nm, the procedure is rapid and precise in measuring absorbance. An IC<sub>50</sub> value of 32.38% was obtained from tests performed at a 515 nm wavelength between the 0–30-minute mark and 32.58% from the 30–60-minute mark. The results of this experiment suggest that black rice extract has strong antioxidant properties. By providing an electron or electrons to free radicals, a family of chemicals called antioxidants can neutralize them. Black rice extract (*Oryza sativa L. indica*) was administered to rats in dose-dependent phytochemical studies to identify active components that could influence hair development in rats. Using a color reagent in a testing reaction is the basis of the phytochemical screening approach. When doing phytochemical screening, choosing solvents and extraction techniques is crucial. Plants often contain secondary metabolites, including alkaloids, flavonoids, steroids, saponins, terpenoids, and tannins.

**Table 2.** Phytochemical Screening Results of Black Rice

Secondary Metabolic	Results
Alkaloid	+
Flavonoid	+
Saponin	-
Tanin	+
Steroid/Triterpenoid	+
Glikosida	-

The study aimed to determine the length of hair growth in rats treated with different concentrations of black rice extract. The control group was given distilled water twice daily for 21 days, while the treatment groups received different extract concentrations. The data was analyzed using SPSS. The rats with the highest concentration of 30% black rice extract showed the most extended hair growth, while the control group had the least. The study concluded that black rice extract can accelerate hair follicle growth in rats.

**Table 3.** Rat Hair Length

Group	Repetition	H7	H14	H21
Control -	1	0,402	0,403	0,410
	2	0,403	0,404	0,411
	3	0,402	0,403	0,410
	4	0,405	0,407	0,413
	5	0,403	0,405	0,411
	6	0,404	0,406	0,412
	Average	0,403	0,405	0,411
Control +	1	0,514	0,518	0,523
	2	0,521	0,525	0,530
	3	0,512	0,516	0,522
	4	0,521	0,525	0,530
	5	0,519	0,523	0,530
	6	0,531	0,535	0,540
	Average	0,520	0,405	0,411
Treatment 1	1	0,655	0,662	0,667
	2	0,645	0,653	0,659
	3	0,675	0,682	0,688
	4	0,673	0,681	0,687
	5	0,675	0,682	0,687
	6	0,685	0,692	0,697
	Average	0,668	0,675	0,681
Treatment 2	1	0,785	0,793	0,799
	2	0,765	0,772	0,777
	3	0,778	0,786	0,792
	4	0,795	0,803	0,809
	5	0,788	0,794	0,800
	6	0,786	0,794	0,800
	Average	0,783	0,790	0,796
Treatment 3	1	0,833	0,840	0,844

2	0,842	0,850	0,856
3	0,832	0,839	0,844
4	0,834	0,841	0,845
5	0,846	0,853	0,858
6	0,844	0,851	0,857
Average	0,839	0,846	0,851

Using the Kolmogorov-Smirnov test, the normality test determines if the data follows a normal distribution. Because it stands in for the population as a whole, normalcy is vital. Distribution is considered normal when the p-value is more than 0.05 and abnormal when it is less than 0.05 [29]. A normal distribution is shown by the 2-tailed significance result of  $0.652 > 0.05$ .

**Table 4.** One-Sample Kolmogorov-Smirnov Test

		Unstandardized Residual
N		30
Normal Parameters <sup>a,b</sup>	Mean	0E-7
	Std. Deviation	.17852798
Most Extreme Differences	Absolute	.134
	Positive	.134
	Negative	-.094
Kolmogorov-Smirnov Z		.735
Asymp. Sig. (2-tailed)		.652

a. Test distribution is Normal.

b. Calculated from data.

This test is all about the average difference between the variables being examined. This study aims to determine if there is a significant difference in hair growth after using black rice extract (*Oryza sativa L. indica*) as a topical treatment. When the Sig. (2-tailed) value is compared to the alpha value; the results may be observed. We may infer a significant difference in hair growth across groups, as seen in the table above, where the two-tailed significant value is  $0.00 < 0.05$ .

**Table 5.** Independent Samples Test Results

		F	Sig.	df	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
Result	Equal variances assumed	4.245	.066	10	-.66167	.05300	-.77977	-.54357
	Equal variances are not assumed.			7.211	-.66167	.05300	-.78626	-.53707

### Research Discussion

This research aimed to determine if white rats Wistar strain (*Rattus norvegicus*) might stimulate their hair growth by applying an ointment containing black rice extract (*Oryza sativa L. indica*). Thirty albino rats (*Rattus norvegicus* Wistar) were utilized in this research. Then, five groups with varying treatments were randomly allocated to the test animals. Treatment group-1 (K-3) administered extracts at a concentration of 10% daily to rats; negative control group (K-1) rats were merely smeared with distilled water; positive control group (K-2) rats were stained with essential ointment without black rice extract. Group 2 (K-4) received daily doses of the extract at a 20% concentration. A 30% concentration of the extract was given to Treatment Group 3 (K-5). Black rice fruit is an all-natural substance that helps keep hair healthy. It is well-known that black rice oil extract can stimulate the growth of human hair follicles. On the other hand, this study re-examined the efficacy of black rice ointment (*Oryza sativa L. indica*) in promoting hair follicle development in rats of the *Rattus norvegicus*. Black rice extract ointment has a solid structure, is odorless, and has a pH range of 5.37 to 6.33, which is still within the range suitable for human skin. Its color might be white, brown, light brown, or a combination of these shades. At the same time, the spreadability test for the ointment showed that containers weighing 0–125 g had diameters ranging from 5.0–5.9 cm. In that manner, experimental usage of the ointment won't compromise its safety. Phytochemical studies on black rice extract have identified antioxidant-active components. The reason is that it contains steroid/triterpenoids, tannins, alkaloids, and flavonoids.

Testing of black rice using Fourier Transform Infrared (FT-IR) analysis confirmed this, showing that black rice contains flavonoids. The DPPH method antioxidant test findings indicate that the antioxidant in black rice extract is highly potent, with a concentration value of less than 50 parts per million. Hence, the antioxidants in black rice are suitable for your hair and skin because they slow the aging process. Plant anthocyanins and flavonoids have many advantageous properties, including anti-aging, anti-senility, anti-liver function disorders, anti-obesity, anti-oxidation, anti-microbial, anti-inflammatory, anti-thrombogenic, anti-infertility, anticancer, antihypertensive, anti-arthritis, and anti-oxidation [30]. Maintaining healthy hair and skin is more accessible with amino acids, antioxidants, and vitamin E. [28]. Rats in Treatment Group-3 (K-5) with a 30% concentration of black rice extract had the most extended average hair growth of the other groups on the 21st observation day, measuring 0.851 cm. In contrast, rats in the control group (-) had the shortest average hair growth, measuring 0.411 cm. After collecting data over 21 days, the researchers had to ensure it was expected by running it through the One-Sample Kolmogorov Smirnov Test. Then, they moved on to the T-test by utilizing the independent t-test.

Results showing a 2-tailed significance level of  $0.652 > 0.05$  indicate that the data follows a normal distribution, as predicted by the normalcy test. Therefore, the data can pursue a normal distribution or represent the population. Repeated use of the T-test with the independent t-test ensures that customarily distributed data remains so. Since the results of this test show a significant difference in hair growth across the groups, with a two-tailed p-value of  $0.00 < 0.05$ , it may be stated that the examined factors have an average difference. White rats administered an ointment containing black rice extract had their hair development monitored in the study. This study found that hair growth rates varied throughout 10% to 30% extract concentrations. The most significant growth was noted at a 30% concentration, adding 0.851 cm of length. Treatment 3 (K5), which consisted of taking 30 percent black rice extract (*Oryza sativa l. indica*) daily for 21 days, generated the most extended hair growth outcomes compared to the other groups. So, while all concentrations of black rice extract (*Oryza sativa l. indica*) ointment helped stimulate hair growth in rats, the results showed that 30% was the quickest. Concentrations of 20% and 10% followed. This agrees with previous studies about the antioxidants found in black rice, specifically the anthocyanins, and how they aid the body in dealing with free radical damage. Premature skin aging can be averted, and overall skin and hair health can be enhanced with antioxidants. It encourages hair development, helps restore elasticity, and keeps skin tight [19].

#### IV. CONCLUSION

The study found that daily application of 10% or 20% black rice extract ointment significantly increased hair growth in rats, with the highest growth observed in the Treatment-3 group, reaching 0.851 cm on day 21. Rats' average hair follicle growth as measured by average observations reveals that treatment 3, which involved daily application of a 30% concentration of black rice extract ointment (*Oryza sativa l. indica*), had the most extended hair growth of the groups tested (0.851 cm on the 21st observation day), followed by concentrations of 20% and 10%. Black rice extract ointment (*Oryza sativa l. indica*) is characterized by its odorlessness, whiteness, brownness, homogeneity, and solid structure. Its pH ranges from 5.37 to 6.33, which is still within the range of human skin pH. Meanwhile, ointment packets weighing 0–125 g showed spreadability findings ranging from 5.0–5.9 cm in diameter. So, the ointment may be used in research without fear of side effects. According to antioxidant testing, the phytochemical findings of black rice extract (*Oryza sativa l. indica*) include active components that are effective as antioxidants. The reason is that it contains steroid/triterpenoids, tannins, alkaloids, and flavonoids. Research using Fourier Transform Infrared (FT-IR) technology on black rice has also shown that it contains flavonoids. The DPPH method antioxidant test findings indicate that the black rice extract contains highly potent antioxidants, with a concentration value of less than 50 ppm. Hence, the antioxidants in black rice are suitable for your hair and skin because they slow the aging process. It would be great if studies on humans could find out how well black rice extract stimulates hair growth (*Oryza sativa l. indica*). To bolster study findings, it is necessary to conduct a comprehensive analysis of the compounds found in black rice extract (*Oryza sativa l. indica*) and to incorporate histological observations on hair development

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