

Topical Rice Bran Oil (*Oryza sativa L.*) Has The Same Effect With 2% Topical Minoxidil To Prevent Senile Alopecia Through Improved Vascular Endothelial Growth Factor (VEGF) Protein Expression, Hair Follicle Diameter And Length In Wistar Rats (*Rattus norvegicus*) Exposed To Ultraviolet B Light

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Abstract

Background: The hair aging process also known as senile alopecia occurs follicle miniaturization, shortening of the anagen phase and increasing proportion of hair in the telogen phase which results in hair loss and hair thinning. Rice bran oil contains γ -Oryzanol which has the effectiveness as the natural antioxidant to increase hair growth. Tocopherol, policosanol, γ -linolenic acid, linoleic acid, and oleic acid have been shown to have role in inhibiting 5 α -reductase enzymes, increasing hair growth factors such as VEGF and reduce inflammation.

Method: This study was an experimental analytic research post-test only control group design that was perform at Faculty of Medicine, Udayana University. This study used 30 Wistar male rats (*Rattus norvegicus*) and divided into three groups: Negative control group with administration of paraffin oil (P), positive control group with administration of 2% minoxidil (M), and treatment group with administration of 100% rice bran oil (R) for 21 days.

Results: there was a significant difference in VEGF levels between the M (153.49 ± 1.7 ng/L) and P (85.5 ± 1.88 ng/L) ($p < 0.001$), and R (152.25 ± 0.94 ng/L) and P ($p < 0.001$), but there was no difference between the M and R groups ($p = 0.088$). There was a significant difference in hair follicle diameter between the M (10.7 ± 4.87 μ m) and P (5.73 ± 2.18 μ m) ($p = 0.030$) and R (9.72 ± 3.19 μ m) and P ($p = 0.013$). However, there was no difference in hair follicle diameter between the M and R ($p = 0.857$). There was a significant difference in hair length between the M (12.02 ± 2.09 mm) and P (7.53 ± 1.12 mm) ($p < 0.001$) and R (13.12 ± 2.14 mm) and P ($p < 0.001$). However, there was no difference in hair length between the M and R ($p = 0.495$).

Conclusion: Rice bran oil has the same effect with 2% topical minoxidil as a gold standard to prevent senile alopecia through improved VEGF protein expression, the follicle diameter and hair length of wistar rat that were induced to ultraviolet B. The results of this study are expected to be used as a reference for further research.

Keywords: rice bran oil, 2% minoxidil, VEGF, diameter hair follicle, hair length

1. Introduction

Aging is a biological process characterized by decrease components and body function. Population with aging will experience many changes both mentally and physically, including hair organs. (Son, Park and Lee, 2019) Senile alopecia affected by combination of intrinsic factors and extrinsic factors. Intrinsic factors include age which causes cellular aging, accumulation of Deoxyribo Nucleic Acid (DNA) damage, decrease stem cell, mitochondrial dysfunction, hormonal, vascularization and genetics, whereas extrinsic factors include nutrition,

stress levels, drugs, disease, and various free radicals such as pollution, ultraviolet (UV) rays, smoking and alcohol. (Goodier and Hordinsky, 2015)

Countries with tropical climates will always be exposed to the sun throughout the year. Ultraviolet B (UVB) radiation plays an important role in the formation of Reactive oxygen species (ROS), thereby reducing the diameter and length of hair, DNA damage, reducing keratinocyte proliferation and increasing apoptosis, stimulating the production of Transforming Growth Factor- β 2 (TGF- β 2) and decreased hair follicle Insulinlike Growth Factor-1 (IGF-1) expression, triggering the early catagen phase, and inducing perifollicular mast cell degranulation. (Gherardini *et al.*, 2019)

Pathophysiologies of senile alopecia are change in the hair growth cycle, the anagen phase is shortened and there is premature regression of the hair during catagen and telogen phases. It has an impact on progressive hair miniaturization, gradual reduction in the diameter of the follicle and hair shaft, and finally the hair becomes easy to fall out. (Park, Khan and Rawnsley, 2018) Around 50–60 hairs are usually lost per day, which has no noticeable effect on appearance, but excessive loss (>100 per day) can lead to baldness. Hair is considered as the crown of the head, any problems with hair will cause various problems such as experiencing anxiety, stress to psychosocial disorders and reduce quality of life. (Williams, Pawlus and Thornton, 2020)

Nowdays, science continues to develop, including the science of Anti-Aging Medicine (AAM). With the existence of the AAM theory, it has caused a paradigm shift in the aging process that aging can be considered like a disease that can be prevented or cured and even returned to its initial conditions, humans can maintain their quality of life as they age. Similarly to the problem of hair loss in the elderly population, knowing the intrinsic and extrinsic causes of hair loss allows us as clinicians to apply the new concept of

AAM to its prevention and treatment. (Son, Park and Lee, 2019)

Minoxidil is the gold standard for hair loss treatment, however topical minoxidil therapy has several side effects such as irritation, allergic contact dermatitis, and hypertrichosis. Currently herbal plant extracts are often chosen as an alternative therapy to reduce hair loss. One of the extracts from plants that have bioactive contents to promote hair growth is rice bran oil (*Oryza sativa* L.). Rice bran is a by-product of rice milling but has a high nutritional and antioxidant content. (Yum *et al.*, 2018)

Gamma oryzanol is the main antioxidant in rice bran which can induce and prolong the anagen stage, increase the expression of hair growth factors in the form of VEGF, IGF-1, and KGF and reduce hair growth inhibiting factors TGF- β , increase the Wnt/ β -catenin pathway, reduce Inflammation-related cytokine expression thus has a positive impact on increasing hair growth and thickening of hair follicle diameter. (Choi *et al.*, 2014)

This study aims to determine the effectiveness of topical rice bran oil same effective as 2% topical minoxidil to prevent senile alopecia by improved VEGF protein expression, follicle diameter and hair length in wistar rat exposed to ultraviolet B.

2. Method

This experimental research used a randomized post-test-only control group design method. This study was performed from July 2022 to December 2022 at Faculty of Medicine Udayana University in Bali. The subjects used in this study were 30 male Wistar Rat, aged 3-4 months, weigh approximately 180-200 g, without any hair and skin disease. The sample was divided into three groups, ten rat each. The negative control group was given topical paraffin oil and UVB light exposure (P), the positive control group was given topical 2% minoxidil and UVB light exposure (M), and the treatment group was given 100% rice bran oil and UVB light exposure (R). Each group was adapted for seven days, and the treatment was given for 21 days. Exposure to UVB was given 3 times a week, on Mondays, Wednesday and Friday, dose 65 mJ/cm² for 65 seconds for each rat, with a wavelength of 311 nm with a distance of 5 cm above the wistar rat. The total radiation given is 585 mJ/cm².

The rats were euthanized using an intramuscular injection of 0.3 cc of ketamine and 0.3 cc of xylazine in the thigh of the rat, and continued with cervical dislocation. The variables observed in this study were

VEGF levels, hair follicle diameter, and hair length. VEGF as one of the growth factors that play an important role in the control of hair growth, the size of the diameter of the hair follicle to determine the fecundity of the hair, while the length of the hair can be used as a parameter of the speed of hair growth.

VEGF was measured using the Enzyme-Linked Immunosorbent Assay (ELISA) method. The sample used in the VEGF examination was a tissue biopsy of the skin in the treated area. The VEGF ELISA kit used in this study was the VEGF-A ELISA Kit from the Bioassay Technology Laboratory (BT LAB). VEGF measurement results are expressed in units of nanograms per litre (ng/L).

Hair follicle diameter examination was performed by skin biopsy on rats of 1 cm x 1 cm in the treated area. The diameter of the rat's hair follicles obtained by histological examination using Hematoxylin and Eosin (H&E) staining. Diameter examination observed using the Optilab Pro camera with 100x magnification. Calculation of the diameter of the hair follicles was seen from three fields of view, and then the largest was found in each field of view. The average diameter of the follicles in each rat was calculated and expressed in micrometre units (μm).

Ten strands of the longest hair are taken by plucking by tweezers. Hair length was measured from the base of the hair follicle to the tip of the hair shaft. Hair length was measured using a calliper with a sensitivity of up to 0.1 mm or 0.01 cm and is expressed in millimetres (mm).

Statistical analysis was performed using SPSS software for Windows version 23.0. The normality test used Shapiro-Wilk test. The homogeneity test used Levene test. The significance test used One-Way Anova (parametric test) with post hoc Least Significant Difference (LSD) on homogeneous data and Games Howell on non-homogeneous data.

3. Results

The mean of VEGF levels in the P, M and R groups were $85,5 \pm 1,88$ ng/L, $153,49 \pm 1,70$ ng/L, and $152,25 \pm 0,94$ ng/L, respectively. The results showed that there were significant differences in VEGF levels between the M and P groups (p value < 0.001) and R and P with (p value < 0.001). However, there was no significant difference in VEGF levels between the M and R groups (p value = 0.088) (Figure 1).

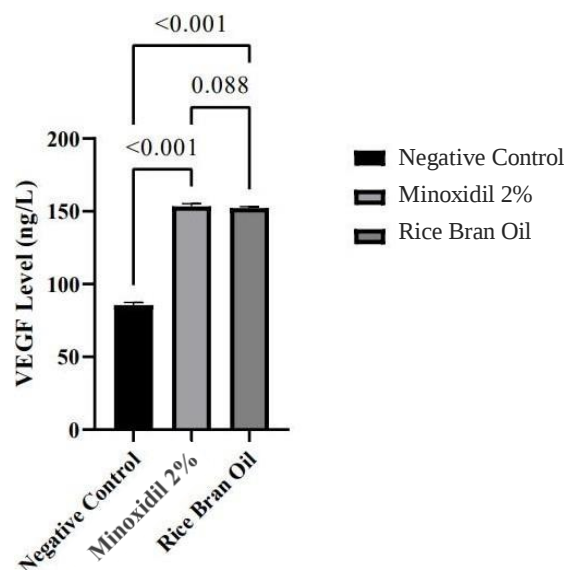


Figure 1. The comparison of VEGF Expression Between Group

The mean hair follicle diameters in the P, M and R groups were $5,73 \pm 2,18 \mu\text{m}$, $10,7 \pm 4,87 \mu\text{m}$, and $9,72 \pm 3,19 \mu\text{m}$, respectively. The results showed that there was significant difference in hair follicle diameter between the M and P groups ($p = 0.030$) and R and P ($p = 0.013$). However, there was no significant difference in hair follicle diameter between the M and R groups ($p \text{ value} = 0.857$) (Figure 2).

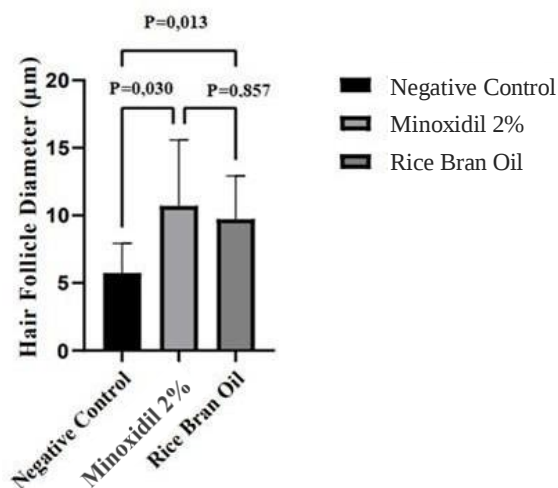


Figure 2. The comparison of Diameter Between Group

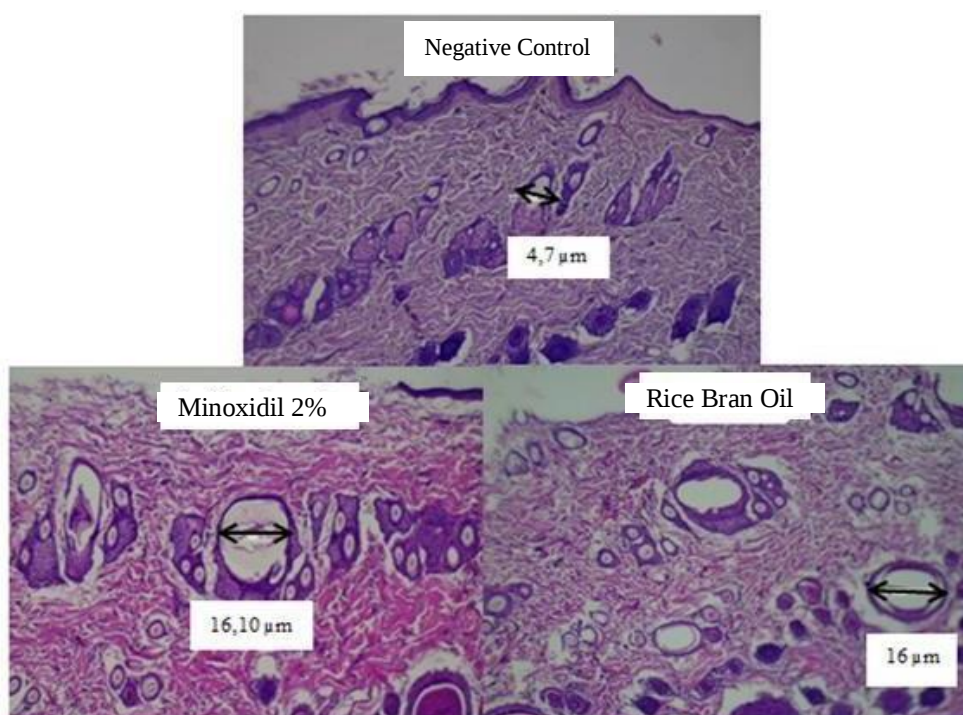


Figure 3. Histopathology of hair follicles diameter

The mean hair length in the P, M and R groups were $7,53 \pm 1,12$ mm, $12,02 \pm 2,09$ mm and $13,12 \pm 2,14$ mm, respectively. The results showed that were significant differences in hair length between the M and P groups ($p < 0.001$) and R and P ($p < 0.001$). However, there was no significant difference in hair length between the M and R groups (p value = 0.495) (Figure 4).

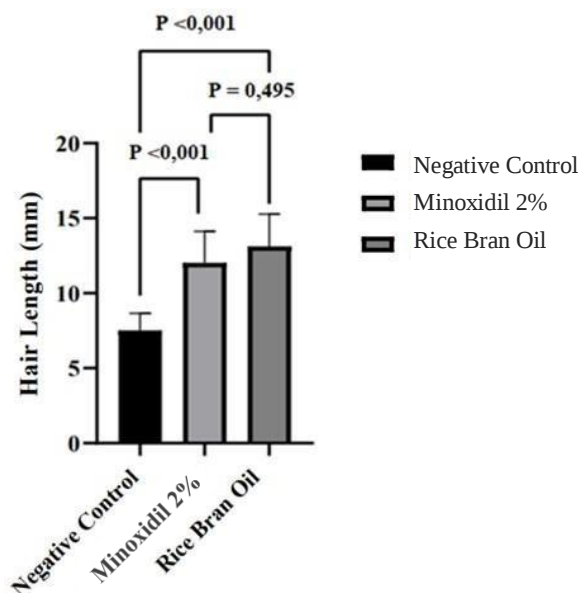


Figure 4. The comparison of Hair Length Between Group

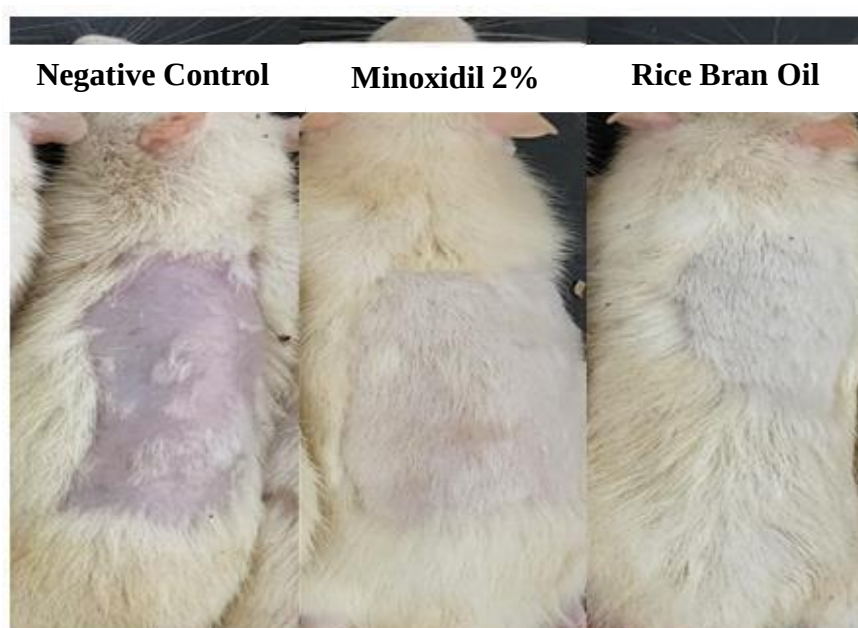


Figure 5. Wistar Rat Hair Between Group

Various cytokines and growth factors play an important role in controlling hair growth. The most important phase in hair growth is anagen phase, where it is very important to maintain the expression of growth factors such as IGF-1, FGF, KGF, and VEGF. (Choi *et al.*, 2014) VEGF is a signalling protein that plays an important role in angiogenesis and vascular permeability, it makes VEGF plays an important role in hair growth by being responsible for maintaining blood vessels around hair follicles during the anagen growth phase. (Kapoor and Shome, 2018)

In this study, topical administration of rice bran oil at a dose of 0.2 ml twice a day for 21 days on the skin of rats that had been shaved with a size of 4 x 4 cm in the R group showed a significant increase in VEGF levels compared to the P group. The results also showed that there was no difference in VEGF levels between the M group and the R group.

The mechanism that play a role in increasing VEGF protein synthesis is enhances the WNT/ β -catenin pathway, and inhibits the cytokines and enzymes responsible for inducing anagen to catagen and telogen transitions including TGF- β and 5 α -reductase. Rice bran oil contains bioactive γ -oryzanol and linoleic acid molecules that can also activate the Wnt/ β catenin pathway. (Hashemi *et al.*, 2022) Previous studies have shown that topical rice bran can increase the expression of hair growth factors in the form of VEGF, IGF-1, and KGF and decrease the inhibiting factor in hair growth TGF- β . (Choi *et al.*, 2014)

Minoxidil is the gold standard for hair growth. Minoxidil has the effect of increasing VEGF levels by inducing hypoxia-inducible factor (HIF) and inhibiting prolyl hydroxylase-2 (PHD-2) so that it can increase angiogenesis and have a positive effect on hair growth. (Yum *et al.*, 2018)

Aging of the hair causes changes in the diameter and length of the hair which will contribute to senile alopecia. Aging of the hair begins with a decrease in hair density in early adulthood, the appearance of thinning is offset by a concomitant increase in hair diameter during the third decade of life, then the hair diameter begins to decrease gradually. The main feature of senile alopecia is a decrease in hair diameter and length. (Fernandez-Flores, Saeb-Lima and Cassarino, 2019)

The results of this study were seen histologically, the diameter of the hair follicles in the R group showed a significant increase in hair diameter compared to P group. While on histopathological examination, there was no statistically significant difference in the R group with the M group.

Topical rice bran has an antiandrogenic effect on mRNA steroid 5-alpha reductase (SRD5A1, SRD5A2, and SRD5A3). SRD5A2 activity is mostly involved in the reduction of the hormone testosterone to DHT in hair follicles, therefore topical rice bran can indirectly prevent miniaturization of hair follicle diameter. (Khantham *et al.*, 2021) Topical rice bran can increase follicle diameter and inhibit hair follicle regression because rice bran is rich in α -, β -, γ -, and δ -tocopherol hence it can reduce SRD5A and 5 α reductase gene expression, rich in antioxidants and can reduce inflammation. (Khantham *et al.*, 2022)

Minoxidil can also increase the diameter size of hair follicles by accelerating the turnover of hair in the catagen and telogen phases into the anagen phase, so that it can induce the change of vellus hair into terminal hair. Increasing hair diameter can increase hair volume and improve cosmetic appearance (Suchonwanit, Thammarucha and Leerunyakul, 2019) Minoxidil also has the benefit of being a vasodilator so that there is an increase in the supply of nutrients and oxygen-rich blood to the hair follicles. Good vascularization around the hair follicle can increase the diameter of the follicle. (Rossi *et al.*, 2012) Minoxidil can also act as an antiandrogenic, significantly reducing 5 α -reductase type 2 gene expressions. (Gupta *et al.*, 2021)

Senile alopecia also changes the overall hair growth cycle, shortens the anagen phase and premature regression of hair occurs during its catagen and telogen phases. As a result of each shorter growth cycle, one of them has an impact on decreasing hair length and easy hair loss. (Park, Khan and Rawnsley, 2018) In this study, R group showed a significant increase in hair length compared to the P group. However, there was no difference in hair length between the R group and the M group.

Rice bran which contains bioactive linoleic acid and Oryzanol has the potential to accelerate the growth of hair length through increasing collagen type I, fibronectin, ALP, collagen type IV and activating Wnt/ β -

catenin signalling pathway activation. Wnt/ β -catenin-mediated signalling pathways play an important role in the regulation of hair follicle morphogenesis, hair shaft and follicle differentiation. (Kim *et al.*, 2017) Linoleic acid can increase hair length through increasing the Wnt/ β -catenin pathway and hair growth factors. The Wnt/ β -catenin pathway has a role in accelerating the hair cycle and hair regeneration, increasing gene expression to maintain the anagen phase and repairing hair damage. (Ryu *et al.*, 2021)

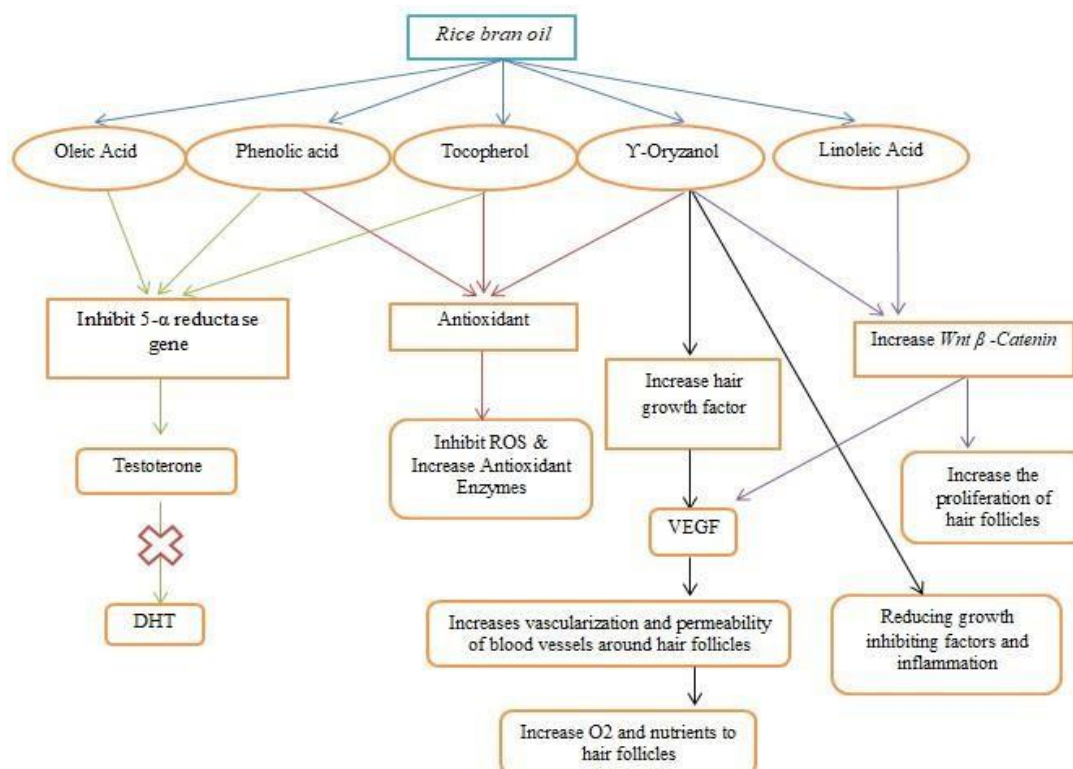


Figure 6. Mechanism of Rice Bran Oil

This study has proven that the administration of Topical rice bran oil is same effective as topical 2% minoxidil (gold standard for hair loss) in increasing VEGF protein expression, hair diameter and hair length. The advantage of rice bran oil compared to minoxidil is that rice bran has a high antioxidant content, γ-oryzanol, tocopherols and phenolic acids, so that rice bran can prevent the formation of ROS and lipid peroxidation, thereby preventing cell damage that can induce senile alopecia. (Choi *et al.*, 2014)

6. Conclusion

In conclusion, rice bran oil has the same effect with 2% topical minoxidil as a gold standard to prevent senile alopecia through improved VEGF protein expression, the follicle diameter and hair length of wistar rat that were induced to ultraviolet B. The results of this study are expected to be used as a reference for further research.

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References

- Choi, J. S. *et al.* (2014) „In vivo hair growth-promoting effect of rice bran extract prepared by supercritical carbon dioxide fluid“, *Biological and Pharmaceutical Bulletin*, 37(1), pp. 44–53. doi: 10.1248/bpb.b13-00528.
- Fernandez-Flores, A., Saeb-Lima, M. and Cassarino, D. S. (2019) „Histopathology of aging of the hair follicle“, *Journal of Cutaneous Pathology*, 46(7), pp. 508–519. doi: 10.1111/cup.13467.
- Gherardini, J. *et al.* (2019) „Transepidermal UV radiation of scalp skin ex vivo induces hair follicle damage that is alleviated by the topical treatment with caffeine“, *International Journal of Cosmetic Science*, 41(2), pp. 164–182. doi: 10.1111/ics.12521.
- Goodier, M. and Hordinsky, M. (2015) „Normal and aging hair biology and structure “Aging and Hair”“, *Current Problems in Dermatology (Switzerland)*, 47. doi: 10.1159/000369594.
- Gupta, A. K. *et al.* (2021) „Minoxidil: a comprehensive review“, *Journal of Dermatological Treatment*. doi: 10.1080/09546634.2021.1945527.
- Hashemi, K. *et al.* (2022) „A Systematic Review: Application of Rice Products for Hair Growth“, *Journal of drugs in dermatology : JDD*, 21(2), pp. 177–185. doi: 10.36849/jdd.6345.
- Kapoor, R. and Shome, D. (2018) „Intradermal injections of a hair growth factor formulation for enhancement of human hair regrowth—safety and efficacy evaluation in a first-in-man pilot clinical study“, *Journal of Cosmetic and Laser Therapy*, 20(6). doi: 10.1080/14764172.2018.1439965.
- Khantham, C. *et al.* (2021) „Effects on steroid 5-alpha reductase gene expression of thai rice bran extracts and molecular dynamics study on SRD5A2“, *Biology*, 10(4). doi: 10.3390/biology10040319.
- Khantham, C. *et al.* (2022) „Antioxidation, Anti-Inflammation, and Regulation of SRD5A Gene Expression of Oryza sativa cv. Bue Bang 3 CMU Husk and Bran Extracts as Androgenetic Alopecia Molecular Treatment Substances“, *Plants*, 11(3). doi: 10.3390/plants11030330.
- Kim, Y. M. *et al.* (2017) „Rice bran mineral extract increases the expression of anagen-related molecules in human dermal papilla through wnt/catenin pathway“, *Food and Nutrition Research*, 61. doi: 10.1080/16546628.2017.1412792.
- Park, A. M., Khan, S. and Rawnsley, J. (2018) „Hair Biology: Growth and Pigmentation“, *Facial Plastic Surgery Clinics of North America*. doi: 10.1016/j.fsc.2018.06.003.
- Rossi, A. *et al.* (2012) „Minoxidil Use in Dermatology, Side Effects and Recent Patents“, *Recent Patents on Inflammation & Allergy Drug Discovery*, 6(2). doi: 10.2174/187221312800166859.
- Ryu, H. S. *et al.* (2021) „Activation of hair cell growth factors by linoleic acid in malva verticillata seed“, *Molecules*, 26(8). doi: 10.3390/molecules26082117.
- Son, D. H., Park, W. J. and Lee, Y. J. (2019) „Recent advances in anti-aging medicine“, *Korean Journal of Family Medicine*. doi: 10.4082/kjfm.19.0087.
- Suchonwanit, P., Thammarucha, S. and Leerunyakul, K. (2019) „Minoxidil and its use in hair disorders: A review“, *Drug Design, Development and Therapy*. doi: 10.2147/DDDT.S214907.
- Williams, R., Pawlus, A. D. and Thornton, M. J. (2020) „Getting under the skin of hair aging: the impact of the hair follicle environment“, *Experimental Dermatology*, 29(7), pp. 588–597. doi: 10.1111/exd.14109.
- Yum, S. *et al.* (2018) „Minoxidil induction of VEGF is mediated by inhibition of HIF-prolyl hydroxylase“, *International Journal of Molecular Sciences*, 19(1). doi: 10.3390/ijms19010053.