



Brief Report

Anti-Graying Effects of External and Internal Treatments with Luteolin on Hair in Model Mice

Machiko Iida ^{1,2} , Takumi Kagawa ¹, Ichiro Yajima ^{1,2} , Akihito Harusato ¹, Akira Tazaki ^{1,3} ,
Delgama A. S. M. Nishadhi ¹, Nobuhiko Taguchi ^{2,4} and Masashi Kato ^{1,2,3,*}

- ¹ Department of Occupational and Environmental Health, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan; machikoi@inst-hsc.jp (M.I.); kagawa.takumi.n1@s.mail.nagoya-u.ac.jp (T.K.); harusato.akihito.n8@f.mail.nagoya-u.ac.jp (A.H.); delgama.nishadhi.j7@s.mail.nagoya-u.ac.jp (D.A.S.M.N.)
- ² Units of Environmental Health Sciences, Department of Biomedical Sciences, College of Life and Health Sciences, Chubu University, 1200 Matsumoto-cho, Kasugai-shi 487-8501, Japan
- ³ Activities of the Institute of Innovation for Future Society, Nagoya University, Nagoya 464-8601, Japan
- ⁴ General Research and Development Institute, Hoyo Co., Ltd., 1-12 Rouboku, Nagakute-shi 480-1136, Japan
- * Correspondence: kato.masashi.r6@f.mail.nagoya-u.ac.jp

Abstract: Little is known about the anti-graying effects of antioxidants on hair. The anti-graying effects of three antioxidants (luteolin, hesperetin, and diosmetin) on hair were investigated according to the sequential processes of hair graying that were previously clarified in model mice [Ednrb(+/-);RET-mice]. External treatment with luteolin, but not that with hesperetin or diosmetin, alleviated hair graying in Ednrb(+/-);RET-mice. Internal treatment with luteolin also mitigated hair graying in the mice. Although both luteolin treatments had very limited effects on hair cycles, the treatments suppressed the increase in p16^{ink4a}-positive cells in bulges [senescent keratinocyte stem cells (KSCs)]. Both of the treatments also suppressed decreases in the expression levels of endothelins in KSCs and their receptor (Ednrb) in melanocyte stem cells (MSCs) and alleviated hair graying in the mice. Luteolin is a special antioxidant with an anti-graying potency through improvement of age-related dysfunction in signaling between endothelins in KSCs and their receptor in MSCs. Luteolin for topical and oral use is commercially available to people in the form of supplements. Similar processes of hair graying in Ednrb(+/-);RET-mice and humans have been reported. These results are encouraging for the practical application of luteolin as a medicine with an anti-graying effect on hair in humans.

Keywords: antioxidant; hair graying; model mouse; luteolin; senescence



Citation: Iida, M.; Kagawa, T.; Yajima, I.; Harusato, A.; Tazaki, A.; Nishadhi, D.A.S.M.; Taguchi, N.; Kato, M. Anti-Graying Effects of External and Internal Treatments with Luteolin on Hair in Model Mice. *Antioxidants* **2024**, *13*, 1549. <https://doi.org/10.3390/antiox13121549>

Academic Editor: Alessandra Napolitano

Received: 22 October 2024

Revised: 2 December 2024

Accepted: 12 December 2024

Published: 17 December 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Hair graying has a negative impression of senescence [1–3]. Therefore, there is a worldwide demand for medicines that can prevent hair graying or reduce the progression of hair graying [4]. However, there are limited medicines that are available for anti-graying of hair with solid scientific evidence.

A period of many years is required to investigate the effects of medicines on hair graying with aging in humans. A model animal in which age-related hair graying occurs much more rapidly than that in humans would be useful for developing medicines with an anti-graying effect on hair. Model mice [Ednrb(+/-);RET-mice] for hair graying with aging [5] have recently been established by crossing RET-transgenic mice carrying a RET oncogene [6] and heterozygously endothelin receptor-B (Ednrb)-deleted mice [Ednrb(+/-)-mice] [5]. The mechanism of hair graying was investigated with a focus on follicular keratinocyte stem cells (KSCs) [cytokeratin15 (CK15)-positive cells] and follicular melanocyte stem cells (MSCs) [dopachrome tautomerase (Dct)-positive cells] in bulges, which are stem cell pools in hair follicles in Ednrb(+/-);RET-mice [5,7,8]. The sequential hair graying processes of (1) age-related accumulation of division and self-renewing for KSCs and MSCs in every

hair cycle, (2) decreased expression of endothelins (ligands) in senescent KSCs with an increased expression level of the representative senescent marker p16^{ink4a} by age-related accumulation of hair cycles, (3) decreased number of MSCs with decreased expression of endothelin receptor B (Ednrb) with aging, and (4) decreased MSCs in bulges and subsequent decreased number of descendant melanocytes in hair bulbs resulted in (5) the promotion of hair graying in the mice [5]. The major processes for hair graying, consisting of depleted survival factor of MSCs through decreased endothelins (ligands) in KSCs and decreased Ednrb (their receptor) in MSCs with aging, were shown to be comparable in model mice and humans in our previous study [5]. It might be possible to develop a medicine for alleviating hair graying if any part(s) in the entire process can be suppressed.

Luteolin (3',4',5,7-tetrahydroxyflavone), a plant-based flavonoid, not only has anti-aging and anti-oxidative effects [9–12] but also contributes to skin biology [13–17]. However, there is no direct evidence for the effects of luteolin on hair graying. In this study, the effects of luteolin and other antioxidants, including hesperetin and diosmetin, on hair graying with aging were investigated in Ednrb(+ / –);RET-mice.

2. Materials and Methods

2.1. Mice

Signalings of both RET and endothelins are biological modifiers for hair growth [18–20] and melanin production [21]. Correspondingly, Ednrb(+ / –);RET-mice [6] that were established by crossing RET-transgenic mice [22] and heterozygously endothelin receptor-B (Ednrb)-deleted mice [Ednrb(+ / –)-mice] provided by the Jackson Laboratory [23] were used as model mice for hair graying [5]. Clipping [5] or shaving [18] hairs in the back skin of Ednrb(+ / –);RET-mice was performed following previous studies.

2.2. External and Internal Treatments with Luteolin

Luteolin (Cas# 491-70-3) was purchased from LKT Laboratories (Saint Paul, MN, USA). External treatment for Ednrb(+ / –);RET-mice was performed using the previously described methods [24] with a slight modification. External treatment using 200 µL of 1.0% luteolin lysed in 70% ethanol (*w/v*) on back skin was performed daily for 16 weeks. Internal treatment using luteolin (0.5 mg/g body weight/day) for mice was performed daily for 16 weeks according to a previously described method [25].

2.3. External Treatment with Hesperetin and Diosmetin

Hesperetin (Cas# 520-33-2) and diosmetin (Cas# 520-34-3) were purchased from LKT Laboratories (Saint Paul, MN, USA) [26,27]. External treatment for Ednrb(+ / –);RET-mice was performed using the previously described methods [24] with a slight modification. External treatment using 200 µL of 1% hesperetin or diosmetin lysed in 70% ethanol (*w/v*) on the back skin was performed daily for 16 weeks.

2.4. Immunohistochemical Analysis

Paraffin sections were treated with 10 mM sodium citrate (pH 6.0) for antigen retrieval as previously reported. Anti-p16^{ink4a}/INK4A (1:3000, F-12, Santa Cruz Biotechnology, Dallas, TX, USA), anti-endothelins (1:100, Santa Cruz Biotechnology, Dallas, TX, USA), and anti-dopachrome tautomerase (Dct) (1:100, Santa Cruz Biotechnology, Dallas, TX, USA) were used as first antibodies. Fluorescence-labeled secondary antibodies, Alexa Fluor 594 anti-goat IgG, Alexa Fluor 594 anti-rabbit IgG, and Alexa Fluor 488 anti-mouse IgG (1:1000, Invitrogen, Waltham, MA, USA) were used for detection of target molecules. Fluorescence intensities of stained cells were evaluated using WinROOF (Mitani Corporation, Tokyo, Japan) using the method previously described.

2.5. Laser Capture Microdissection and Quantitative Polymerase Chain Reaction (qPCR)

Methods for laser capture microdissection [5] and qPCR [28] were previously described.

2.6. Statistical Analysis

Statistical differences between two groups were analyzed by the two-sided Mann–Whitney U test as previously described [29,30]. All statistical analyses were performed using JMP Pro (SAS Institute, Cary, NC, USA) [31].

3. Results and Discussion

In this study, the anti-graying effect of luteolin on hair was investigated in *Ednrb*(+/-);RET-mice in which hairs on back skin were clipped (Figure 1a) or shaved (Figure 1b–g). External treatment with luteolin had no effect on hair cycles in the model mice (Figure 1a). The percentage of p16^{ink4a}-positive cells in bulges in luteolin-treated mice was significantly lower than that in mice not treated with luteolin (Figure 1b). Our previous study demonstrated that the expression of other latent senescence markers, including phosphorylated p53 and p21, was undetectably low in hair follicles of wild-type mice as well as hair graying model mice at any stage or age [5]. Moreover, there were no senescent MSCs that were double positive for p16^{ink4a} and Dct in bulges, which are pools for KSCs and MSCs in follicles, in *Ednrb*(+/-);RET-mice [5]. Therefore, the p16^{ink4a}-positive cells in bulges depicted in Figure 1b can be identified as KSCs, suggesting that luteolin suppresses the senescence of follicular KSCs.

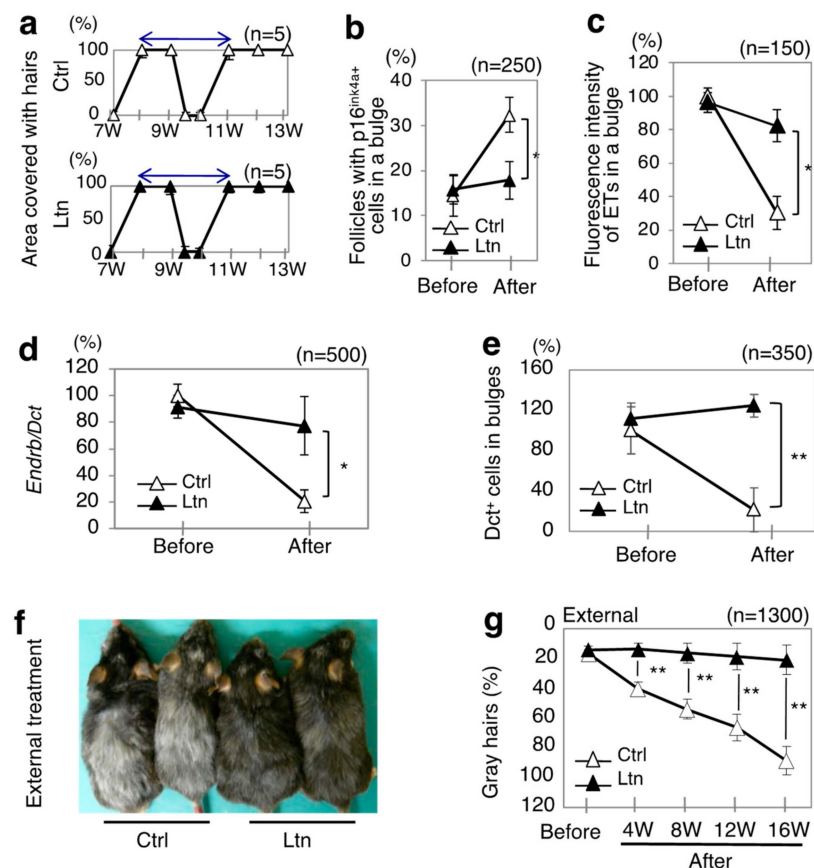


Figure 1. Anti-graying effect of external treatment with luteolin on hair in model mice. (a–g) Results of external treatment with luteolin (Ltn, closed triangle) ($n = 5$) or a solvent (Ctrl, open triangle) ($n = 5$) in model mice for hair graying [*Ednrb*(+/-);RET-mice] from 7 weeks to 13 weeks of age (a) and from 20 weeks of age (before treatment; Before) to 36 weeks of age (after treatment; After) (b–g) are presented. Ratios (means $\pm$ SD) of indicated items before and after treatment with 1% luteolin and after treatment with the solvent of luteolin relative to that before treatment with the solvent of luteolin in the model mice with hairs on their backs shaved once every 4 weeks are shown (b–g). (a) Ratios

(means $\pm$ SD) of the skin area covered with hairs after clipping at 7 weeks of age (first clipping) and at 9 weeks of age (second clipping) in luteolin-treated mice (bottom) and solvent-treated mice (top). Two-way arrows show one hair cycle. (b) Ratios of follicles with p16^{ink4a}-positive cells in telogen bulges [senescent keratinocyte stem cells (KSCs)] ($n = 250$). (c) Ratios of fluorescence intensity of endothelins (ETs) in telogen bulges ($n = 150$). (d) Ratios of Ednrb transcript expression levels to Dct transcript expression levels in telogen bulges (MSCs) isolated by laser capture microdissection ($n = 500$). (e) Ratios of Dct-positive cells in telogen bulges (MSCs) ($n = 350$). (f) Representative macroscopic appearances 16 weeks after treatment with luteolin (Ltn) or its solvent (Ctrl). (g) Ratios of gray hairs ($n = 1300$) for mice at indicated weeks after treatment with luteolin and the solvent. * and **, significantly different (* $p < 0.05$; ** $p < 0.01$) by the Mann–Whitney U test. W, weeks.

The fluorescence intensities of endothelins in telogen bulges (Figure 1c) were significantly reduced in luteolin-treated mice compared to those in untreated mice. Similarly, the expression levels of Ednrb transcripts in Dct-positive cells in bulges (MSCs) (Figure 1d) were also decreased. Moreover, the percentages of Dct-positive cells in bulges (MSCs) (Figure 1e) and the percentages of gray hairs (Figure 1f,g) were significantly lower in the luteolin-treated group. Since endothelin signaling and Ednrb signaling act as survival factors for MSCs [5,32], these findings suggest that luteolin improved the signaling dysfunction between endothelins in KSCs and their receptor in MSCs, ultimately alleviating hair graying.

Hesperetin and diosmetin, two plant-based flavonoids [33,34], have been demonstrated to suppress reactive oxygen species-mediated cellular damage induced by oxidants such as hydrogen peroxide and ultraviolet radiation [35–38]. Consequently, the effects of external treatments with hesperetin and diosmetin on hair graying were investigated in Ednrb(+/-);RET-mice. Despite the similar chemical structures of hesperetin, diosmetin, and luteolin (Figure 2a–c), hesperetin and diosmetin had no significant effect on Dct-positive cells (MSCs) in telogen bulges (Figure 2d,e) as well as the percentages of gray hairs (Figure 2f,g) in the model mice. These results suggest that other antioxidants (hesperetin and diosmetin) had no effect on hair graying in the mice. The differences in the preventive effects of the three chemicals on hair graying remain unclear. These variations may be influenced by the different adsorption properties of hair proteins toward the three chemicals [39]. On the other hand, the anti-graying effect of external treatment with luteolin in model mice is expected to be reproducible in humans due to the chemical similarity of hair proteins, such as keratin, between mice and humans [40,41]. Further studies focusing on proteins in hair are needed to elucidate these points.

Previous studies have demonstrated that internal treatment with luteolin exhibits antioxidative effects in both humans and mice [42–46]. More is known about its internal treatment than its external treatment in relation to the pharmacological effects of luteolin [47–51]. Therefore, it was next investigated whether internal treatment with luteolin can improve the age-related hair graying in the model mice in the condition of no hair shaving, which modulates hair cycles [5,18,19]. Internal treatment with luteolin had no effect on hair cycles (Figure 3a). However, internal treatment significantly suppressed the age-related hair graying process. This suppression was characterized by the following changes in luteolin-treated model mice compared to untreated model mice: an increase in the ratios of p16^{ink4a}-positive cells in bulges (senescent KSCs) (Figure 3b), a decrease in fluorescence intensities of endothelins in telogen bulges (Figure 3c), reduced levels of Ednrb transcript expression in MSCs (Figure 3d), a decrease in the ratios of Dct-positive cells in bulges (MSCs) (Figure 3e), and a reduction in the percentages of gray hairs (Figure 3f,g). These results suggest that both external and internal treatments with luteolin potentially suppress hair graying through a similar mechanism, while internal treatment with luteolin showed a weaker anti-graying effect on hair than the effect of external treatment with luteolin.

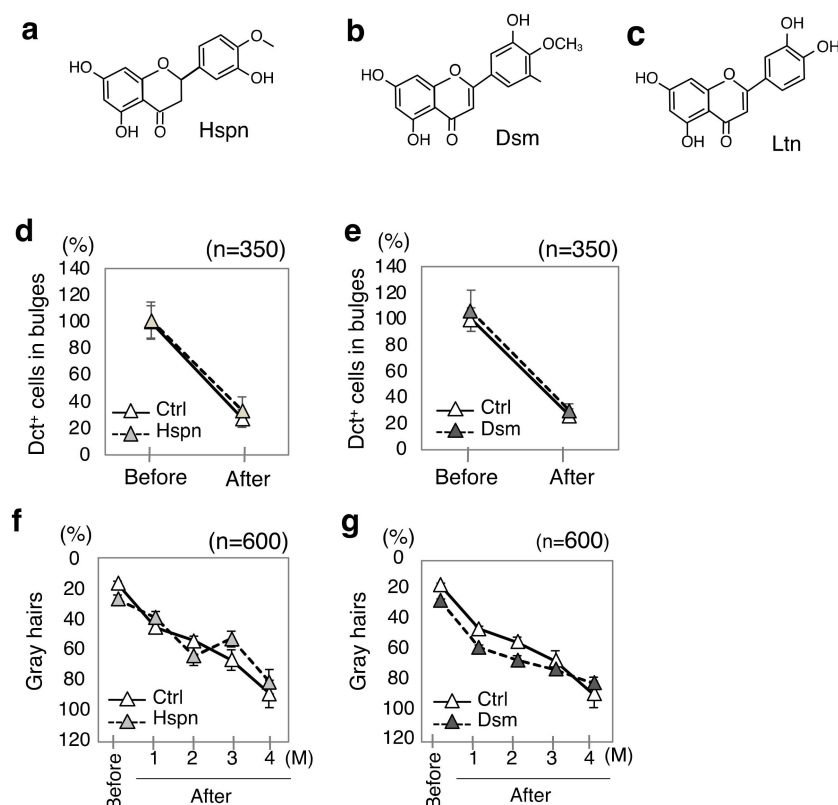


Figure 2. Effects of external treatment with hesperetin and diosmetin on hair graying. (a–c) Chemical structures of luteolin (Ltn), hesperetin (Hspn), and diosmetin (Dsm) are shown. (d–g) Results (means $\pm$ SD) of external treatment with 1% hesperetin (d,f) (Hspn, closed triangle, $n = 5$), 1% diosmetin (e,g) (Dsm, closed triangle, $n = 5$) and their solvent (d–g) (Ctrl, open triangle, $n = 5$), for model mice of hair graying [Ednrb(+/-);RET-mice] from 20 weeks (before treatment; Before) to 36 weeks (after treatment; After) of age are presented. Ratios of the indicated items before and after treatment with hesperetin (d,f) or diosmetin (e,g) and after treatment with their solvent relative to that before treatment with their solvent in the model mice with hairs on their backs shaved once every 4 weeks are shown (d–g). Ratios of Dct-positive cells (MSCs) in telogen bulges (d,e) ($n = 350$) and gray hairs (f,g) ($n = 600$) are presented. The Mann–Whitney U test was used because different control mice were used for treatment with different chemicals. No significant differences were found between control mice and treated mice. Note that the corresponding results regarding the effects of external treatment with luteolin on Dct+ cells in bulges (%) and gray hairs (%) are presented in Figure 1e,g.

Thus, this study indicated that external treatment with luteolin, but not that with hesperetin or diosmetin, could alleviate the hair graying in Ednrb(+/-);RET-mice, despite the fact that the chemical structures of the antioxidants are similar. This study also suggested that not only external treatment but also internal treatment with luteolin has a suppressive effect from the upstream process of decreased expression levels of endothelins in senescent KSCs to the downstream process of decreased number of follicular MSCs with decreased expression level of Ednrb, resulting in the alleviation of hair graying.

Our qPCR analysis further showed that luteolin decreased the transcript expression level of the senescent marker p16^{INK4A} (Supplemental Materials Figure S1a) and increased the transcript expression level of endothelin-1 (Supplemental Materials Figure S1b) in cultured human nontumorigenic skin keratinocytes (HaCaT cells) [52,53]. Luteolin had a limited effect on the expression levels of the endothelin-2 transcript in HaCaT keratinocytes, while the expression level of the endothelin-3 transcript was undetectably low. Our previous study demonstrated that endothelin-1, but not endothelin-2 or endothelin-3, was a key molecule involved in hair graying in Ednrb(+/-);RET-mice [1]. It is difficult to extrapolate the in vitro results derived from HaCaT keratinocytes to the in vivo findings observed in

KSCs from the model mice for gray hair. However, these results suggest the potential involvement of endothelin-1 signaling in keratinocytes in mediating the effects of luteolin.

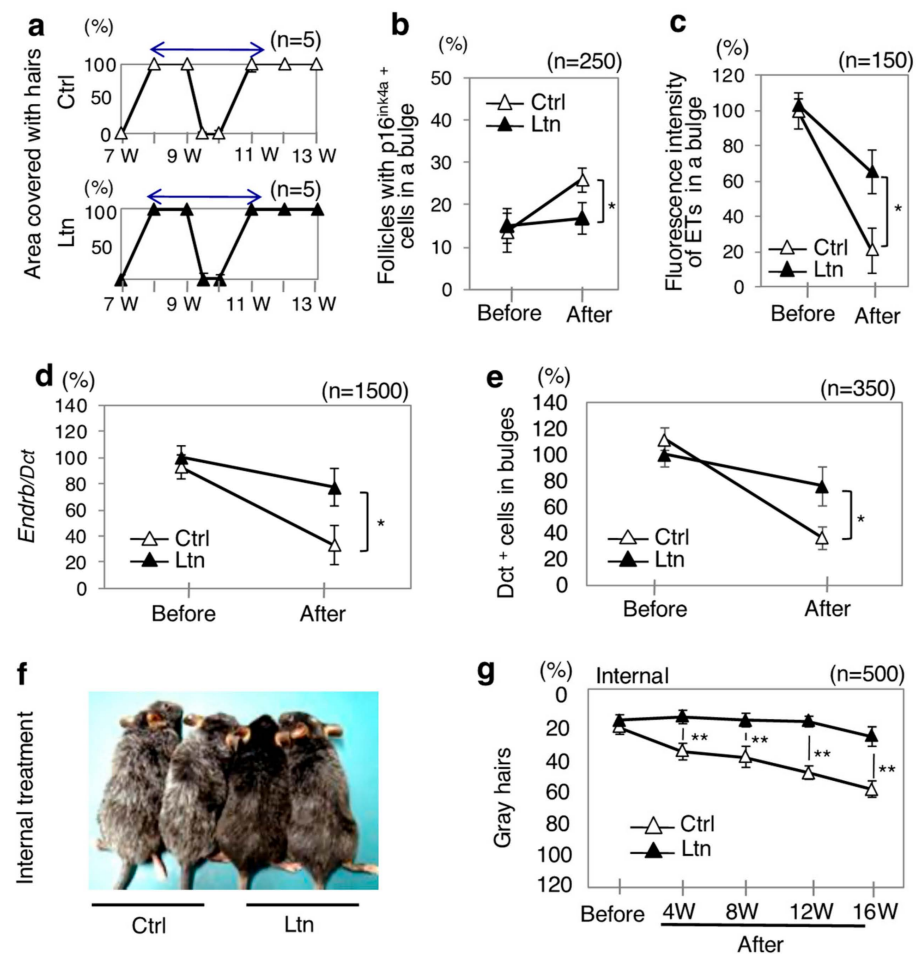


Figure 3. Anti-graying effect of internal treatment with luteolin on hair in model mice. (a–g) Results of internal treatment (0.5 mg/g body weight/day) with luteolin (Ltn; $n = 5$) or the solvent of luteolin (Ctrl; $n = 5$) for model mice of hair graying [Ednr^b(+/-);RET-mice] from 7 weeks to 13 weeks of age (a) and from 20 weeks of age (before treatment; Before) to 36 weeks of age (after treatment; After) (b–g) are presented. Ratios (mean $\pm$ SD) of the indicated items before and after treatment with luteolin and after treatment with the solvent of luteolin relative to that before treatment with the solvent of luteolin in the model mice without shaving hairs (b–g) are presented. (a) Ratios of the skin area covered with hairs after clipping at 7 weeks of age (first clipping) and at 9 weeks of age (second clipping) in luteolin-treated mice (bottom) and solvent-treated mice (top). Two-way arrows show one hair cycle. (b) Ratios of follicles with p16^{ink4a}-positive cells in telogen bulges [senescent keratinocyte stem cells (KSCs)] ($n = 250$). (c) Ratios of fluorescence intensity of endothelins (ETs) in telogen bulges ($n = 150$). (d) Ratios (means $\pm$ SD) of Ednr^b transcript expression levels to Dct transcript expression levels in telogen bulges (MSCs) isolated by laser capture microdissection ($n = 1500$). (e) Ratios of Dct-positive cells in telogen bulges (MSCs) ($n = 350$). (f) Representative macroscopic appearances 16 weeks after internal treatment with (Ltn) or without treatment (Ctrl). (g) Ratios of gray hairs ($n = 500$) for mice at indicated weeks after treatment with luteolin and the solvent. * and **, significantly different (* $p < 0.05$; ** $p < 0.01$) by the Mann–Whitney U test. W, weeks.

Our previous study demonstrated that the phosphorylation of histone H2AX (γ H2AX), a marker of DNA damage, increased in genetically engineered model mice for hair graying [5]. Since DNA damage is often induced by oxidative stress [54,55], these results suggested a latent promotive role for oxidative stress in hair graying [56–59]. To more directly

investigate this hypothesis, wild-type mice were treated with tert-butyl hydroperoxide (t-BOOH), an inducer of oxidative stress [60–62]. Consistent with our hypothesis, wild-type mice treated with t-BOOH developed hair graying (Supplemental Materials Figure S2a). Conversely, treatment with luteolin, an inhibitor of oxidative stress, significantly attenuated t-BOOH-induced hair graying (Supplemental Materials Figure S2a,b). These findings suggest not only the promotion of hair graying with an increase in oxidative stress but also reduction in hair graying by a decrease in oxidative stress in our model mice.

4. Conclusions

This study showed for the first time that both internal treatment and external treatment with luteolin prevented hair graying in Ednrb(+ / –);RET-mice. This study also provided the first evidence for an anti-aging effect of luteolin on stem cells *in vivo*. These results suggest that luteolin is a latent medicine with an anti-graying effect on hair in model mice.

Topical and oral administration of luteolin showed an anti-graying effect on hair without exhibiting any visible signs of toxicity in mice. Similar mechanisms of hair graying in Ednrb(+ / –);RET-mice and humans were shown in our previous study [5]. Therefore, this study may represent the first step toward establishing a new application of luteolin as a contributor to anti-aging and esthetic well-being in humans. To the best of our knowledge, however, no comprehensive studies have demonstrated the safety of luteolin, including its appropriate concentration, for external and internal treatments in humans. Further research is needed to investigate not only its efficacy but also its safety in human applications.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/antiox13121549/s1>, Figure S1: Luteolin increased ET-1 expression levels with decreased expression level of p16^{INK4A} in human HaCaT keratinocytes; Figure S2: Luteolin attenuates t-BOOH-induced hair graying.

Author Contributions: Conceptualization, M.K.; methodology, M.I., A.T. and N.T.; investigation, M.I., I.Y., A.T. and D.A.S.M.N.; data curation, M.I., I.Y. and A.T.; writing—original draft preparation, M.I. and M.K.; writing—review and editing, M.I., T.K., A.H. and M.K.; supervision, M.K.; resources, M.K.; project administration, M.K. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported partly by Grants-in-Aids for Scientific Research (A) (19H01147) and (B) (23H03147 and 23K27837) and Fund for the Promotion of Joint International Research (22KK0145) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) and research foundation for university investigators' participation in the research program in Chubu University. The funders had no role in the study design, data collection and analysis, decision to publish, or manuscript preparation.

Institutional Review Board Statement: This study was approved by the DNA Advisory Committee in Chubu University (approval no. 12-06-01) and Nagoya University (approval no. 20-24), Japan. The study was also approved by the Animal Care and Use Committee in Chubu University (approval no. 2810030) and Nagoya University (approval no. M240193-002).

Data Availability Statement: The data underlying this article will be shared on reasonable request to the corresponding author.

Acknowledgments: The authors wish to acknowledge Division for Medical Research Engineering, Nagoya University Graduate School of Medicine for technical support.

Conflicts of Interest: This work was partially supported by a grant from Hoya Co., Ltd. Though N.T. is a shareholder and an employed person of Hoya Co., Ltd., the situation has no influence on the authors' adherence to the policies of the journal on sharing data and materials. M.K., who is an inventor with a patent filed by Chubu University and Nagoya University for the model mice for hair graying used in this study, received the fee for university investigators' participation in the research program in Chubu University and the option fee for the patent license from Hoya Co., Ltd. The remaining authors declare no competing financial interests.

References

1. Ungvari, A.; Kiss, T.; Gulej, R.; Tarantini, S.; Csik, B.; Yabluchanskiy, A.; Mukli, P.; Csiszar, A.; Harris, M.L.; Ungvari, Z. Irradiation-Induced Hair Graying in Mice: An Experimental Model to Evaluate the Effectiveness of Interventions Targeting Oxidative Stress, DNA Damage Prevention, and Cellular Senescence. *GeroScience* **2024**, *46*, 3105–3122. [\[CrossRef\]](#) [\[PubMed\]](#)
2. Hofmeier, S.M.; Runfola, C.D.; Sala, M.; Gagne, D.A.; Brownley, K.A.; Bulik, C.M. Body Image, Aging, and Identity in Women over 50: The Gender and Body Image (GABI) Study. *J. Women Aging* **2017**, *29*, 3–14. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Rosenberg, A.M.; Rausser, S.; Ren, J.; Mosharov, E.V.; Sturm, G.; Ogden, R.T.; Patel, P.; Soni, R.K.; Lacefield, C.; Tobin, D.J.; et al. Quantitative Mapping of Human Hair Greying and Reversal in Relation to Life Stress. *eLife* **2021**, *10*, e67437. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Triwongwaranat, D.; Thuangtong, R.; Arunkajohnsak, S. A Review of the Etiologies, Clinical Characteristics, and Treatment of Canities. *Int. J. Dermatol.* **2019**, *58*, 659–666. [\[CrossRef\]](#)
5. Iida, M.; Tazaki, A.; Yajima, I.; Ohgami, N.; Taguchi, N.; Goto, Y.; Kumasaka, M.Y.; Prévost-Blondel, A.; Kono, M.; Akiyama, M.; et al. Hair Graying with Aging in Mice Carrying Oncogenic RET. *Aging Cell* **2020**, *19*, e13273. [\[CrossRef\]](#)
6. Kumasaka, M.Y.; Yajima, I.; Hossain, K.; Iida, M.; Tsuzuki, T.; Ohno, T.; Takahashi, M.; Yanagisawa, M.; Kato, M. A Novel Mouse Model for de Novo Melanoma. *Cancer Res.* **2010**, *70*, 24–29. [\[CrossRef\]](#)
7. Rabbani, P.; Takeo, M.; Chou, W.; Myung, P.; Bosenberg, M.; Chin, L.; Taketo, M.M.; Ito, M. Coordinated Activation of Wnt in Epithelial and Melanocyte Stem Cells Initiates Pigmented Hair Regeneration. *Cell* **2011**, *145*, 941–955. [\[CrossRef\]](#)
8. Nishimura, E.K.; Granter, S.R.; Fisher, D.E. Mechanisms of Hair Graying: Incomplete Melanocyte Stem Cell Maintenance in the Niche. *Science* **2005**, *307*, 720–724. [\[CrossRef\]](#)
9. Zhang, K.; Li, J.; Dong, W.; Huang, Q.; Wang, X.; Deng, K.; Ali, W.; Song, R.; Zou, H.; Ran, D.; et al. Luteolin Alleviates Cadmium-Induced Kidney Injury by Inhibiting Oxidative DNA Damage and Repairing Autophagic Flux Blockade in Chickens. *Antioxidants* **2024**, *13*, 525. [\[CrossRef\]](#)
10. Păcularu-Burada, B.; Cîrîc, A.I.; Begea, M. Anti-Aging Effects of Flavonoids from Plant Extracts. *Foods* **2024**, *13*, 2441. [\[CrossRef\]](#)
11. Zumerle, S.; Sarill, M.; Saponaro, M.; Colucci, M.; Contu, L.; Lazzarini, E.; Sartori, R.; Pezzini, C.; Rinaldi, A.; Scanu, A.; et al. Targeting Senescence Induced by Age or Chemotherapy with a Polyphenol-Rich Natural Extract Improves Longevity and Healthspan in Mice. *Nat. Aging* **2024**, *4*, 1231–1248. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Xing, X.; Peng, J.; Zhao, J.; Shi, R.; Wang, C.; Zhang, Z.; Wang, Z.; Li, Z.; Wu, Z. Luteolin Regulates the Distribution and Function of Organelles by Controlling SIRT1 Activity during Postovulatory Oocyte Aging. *Front. Nutr.* **2023**, *10*, 1192758. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Almatroodi, S.A.; Almatroudi, A.; Alharbi, H.O.A.; Khan, A.A.; Rahmani, A.H. Effects and Mechanisms of Luteolin, a Plant-Based Flavonoid, in the Prevention of Cancers via Modulation of Inflammation and Cell Signaling Molecules. *Molecules* **2024**, *29*, 1093. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Mu, J.; Ma, H.; Chen, H.; Zhang, X.; Ye, M. Luteolin Prevents UVB-Induced Skin Photoaging Damage by Modulating SIRT3/ROS/MAPK Signaling: An In Vitro and in Vivo Studies. *Front. Pharmacol.* **2021**, *12*, 728261. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Gendrisch, F.; Esser, P.R.; Schempp, C.M.; Wölflle, U. Luteolin as a Modulator of Skin Aging and Inflammation. *Biofactors* **2021**, *47*, 170–180. [\[CrossRef\]](#)
16. Ueda, H.; Yamazaki, C.; Yamazaki, M. Inhibitory Effect of Perilla Leaf Extract and Luteolin on Mouse Skin Tumor Promotion. *Biol. Pharm. Bull.* **2003**, *26*, 560–563. [\[CrossRef\]](#)
17. Zhou, W.; Hu, M.; Zang, X.; Liu, Q.; Du, J.; Hu, J.; Zhang, L.; Du, Z.; Xiang, Z. Luteolin Attenuates Imiquimod-Induced Psoriasis-like Skin Lesions in BALB/c Mice via Suppression of Inflammation Response. *Biomed. Pharmacother.* **2020**, *131*, 110696. [\[CrossRef\]](#)
18. Kato, M.; Takeda, K.; Kawamoto, Y.; Tsuzuki, T.; Dai, Y.; Nakayama, S.; Toriyama, K.; Tamada, Y.; Takahashi, M.; Nakashima, I. RET Tyrosine Kinase Enhances Hair Growth in Association with Promotion of Melanogenesis. *Oncogene* **2001**, *20*, 7536–7541. [\[CrossRef\]](#)
19. Kato, M.; Takeda, K.; Kawamoto, Y.; Tsuzuki, T.; Kato, Y.; Ohno, T.; Hossain, K.; Iftakhar-E-Khuda, I.; Ohgami, N.; Isobe, K.I.; et al. Novel Hairless RET-Transgenic Mouse Line with Melanocytic Nevi and Anagen Hair Follicles. *J. Investig. Dermatol.* **2006**, *126*, 2547–2550. [\[CrossRef\]](#)
20. Martino, P.; Sunkara, R.; Heitman, N.; Rangl, M.; Brown, A.; Saxena, N.; Grisanti, L.; Kohan, D.; Yanagisawa, M.; Rendl, M. Progenitor-Derived Endothelin Controls Dermal Sheath Contraction for Hair Follicle Regression. *Nat. Cell Biol.* **2023**, *25*, 222–234. [\[CrossRef\]](#)
21. Hayashi, H.; Sone, M.; Ito, S.; Wakamatsu, K.; Kato, M.; Nakashima, I.; Nakashima, T. A Novel RFP-RET Transgenic Mouse Model with Abundant Eumelanin in the Cochlea. *Hear. Res.* **2004**, *195*, 35–40. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Kato, M.; Takeda, K.; Kawamoto, Y.; Tsuzuki, T.; Hossain, K.; Tamakoshi, A.; Kunisada, T.; Kambayashi, Y.; Ogino, K.; Suzuki, H.; et al. C-Kit-Targeting Immunotherapy for Hereditary Melanoma in a Mouse Model. *Cancer Res.* **2004**, *64*, 801–806. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Hosoda, K.; Hammer, R.E.; Richardson, J.A.; Baynash, A.G.; Cheung, J.C.; Giaid, A.; Yanagisawa, M. Targeted and Natural (Piebald-Lethal) Mutations of Endothelin-B Receptor Gene Produce Megacolon Associated with Spotted Coat Color in Mice. *Cell* **1994**, *79*, 1267–1276. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Wölflle, U.; Esser, P.R.; Simon-Haarhaus, B.; Martin, S.F.; Lademann, J.; Schempp, C.M. UVB-Induced DNA Damage, Generation of Reactive Oxygen Species, and Inflammation Are Effectively Attenuated by the Flavonoid Luteolin In Vitro and in Vivo. *Free Radic. Biol. Med.* **2011**, *50*, 1081–1093. [\[CrossRef\]](#) [\[PubMed\]](#)

25. Jang, S.; Kelley, K.W.; Johnson, R.W. Luteolin Reduces IL-6 Production in Microglia by Inhibiting JNK Phosphorylation and Activation of AP-1. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 7534–7539. [\[CrossRef\]](#)
26. Takiyama, M.; Matsumoto, T.; Watanabe, J. LC-MS/MS Detection of Citrus Unshiu Peel-Derived Flavonoids in the Plasma and Brain after Oral Administration of Yokukansankachimpinange in Rats. *Xenobiotica* **2019**, *49*, 1494–1503. [\[CrossRef\]](#)
27. Odbayar, T.O.; Kimura, T.; Tsushida, T.; Ide, T. Isoenzyme-Specific up-Regulation of Glutathione Transferase and Aldo-Keto Reductase mRNA Expression by Dietary Quercetin in Rat Liver. *Mol. Cell. Biochem.* **2009**, *325*, 121–130. [\[CrossRef\]](#)
28. Yajima, I.; Tazaki, A.; Ohgami, N.; Kato, M. Calcitriol Inhibits Arsenic-Promoted Tumorigenesis through Regulation of Arsenic-Uptake in a Human Keratinocyte Cell Line. *Am. J. Cancer Res.* **2022**, *12*, 5019–5028.
29. Kurniasari, F.; Htike, M.T.; Tazaki, A.; Kagawa, T.; Al Hossain, M.M.A.; Akhand, A.A.; Ahsan, N.; Ohnuma, S.; Iwasaki, N.; Kato, M. Beneficial and Adverse Effects of Dam Construction in Canal Tannery Wastewater Effluent with a High Content of Chromium in Hazaribagh, Bangladesh. *Chemosphere* **2024**, *350*, 141047. [\[CrossRef\]](#)
30. Nachar, N. The Mann-Whitney U: A Test for Assessing Whether Two Independent Samples Come from the Same Distribution. *Tutor. Quant. Methods Psychol.* **2008**, *4*, 13–20. [\[CrossRef\]](#)
31. Sakata, S.; Kunimatsu, R.; Tanimoto, K. Protective Effect of Ergothioneine against Oxidative Stress-Induced Chondrocyte Death. *Antioxidants* **2024**, *13*, 800. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Takeo, M.; Lee, W.; Rabbani, P.; Sun, Q.; Hu, H.; Lim, C.H.; Manga, P.; Ito, M. Ednrb Governs Regenerative Response of Melanocyte Stem Cells by Crosstalk with Wnt Signaling. *Cell Rep.* **2016**, *15*, 1291–1302. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Lee, H.J.; Lee, S.H.; Hong, S.K.; Gil, B.I.; Lee, K.A. In Vitro Biological Activities of Hesperidin-Related Compounds with Different Solubility. *Antioxidants* **2024**, *13*, 727. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Sun, Z.; Liu, K.; Liang, C.; Wen, L.; Wu, J.; Liu, X.; Li, X. Diosmetin as a Promising Natural Therapeutic Agent: In Vivo, in Vitro Mechanisms, and Clinical Studies. *Phyther. Res.* **2024**, *38*, 3660–3694. [\[CrossRef\]](#)
35. Parhiz, H.; Roohbakhsh, A.; Soltani, F.; Rezaee, R.; Iranshahi, M. Antioxidant and Anti-Inflammatory Properties of the Citrus Flavonoids Hesperidin and Hesperetin: An Updated Review of Their Molecular Mechanisms and Experimental Models. *Phyther. Res.* **2015**, *29*, 323–331. [\[CrossRef\]](#)
36. Kim, J.Y.; Jung, K.J.; Choi, J.S.; Chung, H.Y. Hesperetin: A Potent Antioxidant against Peroxynitrite. *Free Radic. Res.* **2004**, *38*, 761–769. [\[CrossRef\]](#)
37. Wójciak, M.; Feldo, M.; Borowski, G.; Kubrak, T.; Plachno, B.J.; Sowa, I. Antioxidant Potential of Diosmin and Diosmetin against Oxidative Stress in Endothelial Cells. *Molecules* **2022**, *27*, 8232. [\[CrossRef\]](#)
38. Guo, G.; Dong, J. Diosmetin Attenuates Oxidative Stress-Induced Damage to Lens Epithelial Cells via the Mitogen-Activated Protein Kinase (MAPK) Pathway. *Bioengineered* **2022**, *13*, 11072–11081. [\[CrossRef\]](#)
39. Malinauskyte, E.; Shrestha, R.; Cornwell, P.A.; Gourion-Arsiquaud, S.; Hindley, M. Penetration of Different Molecular Weight Hydrolysed Keratins into Hair Fibres and Their Effects on the Physical Properties of Textured Hair. *Int. J. Cosmet. Sci.* **2021**, *43*, 26–37. [\[CrossRef\]](#)
40. Rogers, M.A.; Langbein, L.; Praetzel-Wunder, S.; Winter, H.; Schweizer, J. Human Hair Keratin-Associated Proteins (KAPs). *Int. Rev. Cytol.* **2006**, *251*, 209–263. [\[CrossRef\]](#)
41. Zhang, W.; Fan, Y. Structure of Keratin. *Methods Mol. Biol.* **2021**, *2347*, 41–53. [\[CrossRef\]](#) [\[PubMed\]](#)
42. Taliou, A.; Zintzaras, E.; Lykouras, L.; Francis, K. An Open-Label Pilot Study of a Formulation Containing the Anti-Inflammatory Flavonoid Luteolin and Its Effects on Behavior in Children with Autism Spectrum Disorders. *Clin. Ther.* **2013**, *35*, 592–602. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Imran, M.; Rauf, A.; Abu-Izneid, T.; Nadeem, M.; Shariati, M.A.; Khan, I.A.; Imran, A.; Orhan, I.E.; Rizwan, M.; Atif, M.; et al. Luteolin, a Flavonoid, as an Anticancer Agent: A Review. *Biomed. Pharmacother.* **2019**, *112*, 108612. [\[CrossRef\]](#) [\[PubMed\]](#)
44. Zhu, M.; Sun, Y.; Su, Y.; Guan, W.; Wang, Y.; Han, J.; Wang, S.; Yang, B.; Wang, Q.; Kuang, H. Luteolin: A Promising Multifunctional Natural Flavonoid for Human Diseases. *Phyther. Res.* **2024**, *38*, 3417–3443. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Wang, I.C.; Lin, J.H.; Lee, W.S.; Liu, C.H.; Lin, T.Y.; Yang, K.T. Baicalein and Luteolin Inhibit Ischemia/Reperfusion-Induced Ferroptosis in Rat Cardiomyocytes. *Int. J. Cardiol.* **2023**, *375*, 74–86. [\[CrossRef\]](#)
46. Nazari, Q.A.; Kume, T.; Takada-Takatori, Y.; Izumi, Y.; Akaike, A. Protective Effect of Luteolin on an Oxidative-Stress Model Induced by Microinjection of Sodium Nitroprusside in Mice. *J. Pharmacol. Sci.* **2013**, *122*, 109–117. [\[CrossRef\]](#)
47. Taheri, Y.; Sharifi-Rad, J.; Antika, G.; Yilmaz, Y.B.; Tumer, T.B.; Abuhamdah, S.; Chandra, S.; Saklani, S.; Kiliç, C.S.; Sestito, S.; et al. Paving Luteolin Therapeutic Potentialities and Agro-Food-Pharma Applications: Emphasis on in Vivo Pharmacological Effects and Bioavailability Traits. *Oxidative Med. Cell. Longev.* **2021**, *2021*, 1987588. [\[CrossRef\]](#)
48. Luo, Y.; Chen, S.; Zhou, J.; Chen, J.; Tian, L.; Gao, W.; Zhang, Y.; Ma, A.; Li, L.; Zhou, Z. Luteolin Cocrystals: Characterization, Evaluation of Solubility, Oral Bioavailability and Theoretical Calculation. *J. Drug Deliv. Sci. Technol.* **2019**, *50*, 248–254. [\[CrossRef\]](#)
49. Tan, C.; Fan, H.; Ding, J.; Han, C.; Guan, Y.; Zhu, F.; Wu, H.; Liu, Y.; Zhang, W.; Hou, X.; et al. ROS-Responsive Nanoparticles for Oral Delivery of Luteolin and Targeted Therapy of Ulcerative Colitis by Regulating Pathological Microenvironment. *Mater. Today Bio* **2022**, *14*, 100246. [\[CrossRef\]](#)
50. Song, K.; Zhou, L.; Wang, C.; Yuan, Z.; Cao, Q.; Wu, X.; Li, M. Novel Luteolin@pro-Phytomicelles: In Vitro Characterization and in Vivo Evaluation of Protection against Drug-Induced Hepatotoxicity. *Chem. Biol. Interact.* **2022**, *365*, 110095. [\[CrossRef\]](#)

51. Boeing, T.; de Souza, P.; Specia, S.; Somensi, L.B.; Mariano, L.N.B.; Cury, B.J.; Ferreira dos Anjos, M.; Quintão, N.L.M.; Dubuqoy, L.; Desreumax, P.; et al. Luteolin Prevents Irinotecan-Induced Intestinal Mucositis in Mice through Antioxidant and Anti-Inflammatory Properties. *Br. J. Pharmacol.* **2020**, *177*, 2393–2408. [[CrossRef](#)] [[PubMed](#)]
52. Boukamp, P.; Petrussevska, R.T.; Breitkreutz, D.; Hornung, J.; Markham, A.; Fusenig, N.E. Normal Keratinization in a Spontaneously Immortalized Aneuploid Human Keratinocyte Cell Line. *J. Cell Biol.* **1988**, *106*, 761–771. [[CrossRef](#)] [[PubMed](#)]
53. Deyrieux, A.F.; Wilson, V.G. In Vitro Culture Conditions to Study Keratinocyte Differentiation Using the HaCaT Cell Line. *Cytotechnology* **2007**, *54*, 77–83. [[CrossRef](#)] [[PubMed](#)]
54. Schütz, C.S.; Stope, M.B.; Bekeschus, S. H2A.X Phosphorylation in Oxidative Stress and Risk Assessment in Plasma Medicine. *Oxidative Med. Cell. Longev.* **2021**, *2021*, 2060986. [[CrossRef](#)]
55. Williamson, J.; Hughes, C.M.; Burke, G.; Davison, G.W. A Combined γ -H2AX and 53BP1 Approach to Determine the DNA Damage-Repair Response to Exercise in Hypoxia. *Free Radic. Biol. Med.* **2020**, *154*, 9–17. [[CrossRef](#)]
56. Seiberg, M. Age-Induced Hair Greying—The Multiple Effects of Oxidative Stress. *Int. J. Cosmet. Sci.* **2013**, *35*, 532–538. [[CrossRef](#)]
57. O’Sullivan, J.D.B.; Nicu, C.; Picard, M.; Chéret, J.; Bedogni, B.; Tobin, D.J.; Paus, R. The Biology of Human Hair Greying. *Biol. Rev.* **2021**, *96*, 107–128. [[CrossRef](#)]
58. Shi, Y.; Luo, L.F.; Liu, X.M.; Zhou, Q.; Xu, S.Z.; Lei, T.C. Premature Graying as a Consequence of Compromised Antioxidant Activity in Hair Bulb Melanocytes and Their Precursors. *PLoS ONE* **2014**, *9*, e93589. [[CrossRef](#)]
59. De Tollenaere, M.; Chapuis, E.; Auriol, P.; Auriol, D.; Scandolera, A.; Reynaud, R. Global Repigmentation Strategy of Grey Hair Follicles by Targeting Oxidative Stress and Stem Cells Protection. *Appl. Sci.* **2021**, *11*, 1533. [[CrossRef](#)]
60. Taguchi, N.; Uemura, N.; Goto, Y.; Sakura, M.; Hara, K.; Niwa, M.; Iida, M.; Yanagishita, T.; Watanabe, D.; Kato, M. Antioxidative Effects of Cherry Leaves Extract on Tert-Butyl Hydroperoxide-Mediated Cytotoxicity through Regulation of Thioredoxin-2 Protein Expression Levels. *J. Toxicol. Environ. Health Part A Curr. Issues* **2011**, *74*, 1240–1247. [[CrossRef](#)]
61. Martins-gomes, C.; Nunes, F.M. *Thymus* Spp. Aqueous Extracts and Their Constituent Salvianolic Acid A Induce Nrf2-Dependent Cellular Antioxidant Protection Against Oxidative Stress in Caco-2 Cells. *Antioxidants* **2024**, *13*, 1287. [[CrossRef](#)]
62. Kučera, O.; Endlicher, R.; Roušar, T.; Lotková, H.; Garnol, T.; Drahota, Z.; Červinková, Z. The Effect of Tert -Butyl Hydroperoxide-Induced Oxidative Stress on Lean and Steatotic Rat Hepatocytes in Vitro. *Oxidative Med. Cell. Longev.* **2014**, *2014*, 752506. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.