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Original Research Article

Topical Virgin Coconut Oil Ameliorates Oxidative Stress and Improves Skin Structure in a Mouse Model of D-Galactose-Induced Aging

Ha TT. Nguyen[1], Trang Q. Tran[1], Phong X. Pham[2], Hanh M. Pham[3], Phuong HT. Tran[3], Chau Anh T. Phan[1], Quang V. Trinh[1], Van Anh T. Pham[1]*

[1]*Hanoi Medical University, Hanoi 10000, Vietnam*
[2]*Military Institute of Traditional Medicine, Hanoi 10000, Vietnam*
[3]*Vi Dieu Nam Medicine and Pharmacy Limited Company, Hanoi 10000, Vietnam*

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ABSTRACT

Skin aging, primarily driven by oxidative stress, results in functional and structural decline. This study investigated the protective effects of a Vietnamese virgin coconut oil, namely *Lao Nha Que* (LNQ), against D-galactose-induced skin aging in a mouse model. To induce aging, *Swiss* mice were treated with subcutaneous D-galactose (1000 mg/kg) for 42 days. Experimental groups (n = 10) received daily topical applications of saline (control), D-galactose only (disease), D-galactose + 1% vitamin E (0.2 ml/day), D-galactose + LNQ virgin coconut oil (0.2 ml/day), or D-galactose + LNQ virgin coconut oil (0.4 ml/day). Compared to the disease group, topical application of virgin coconut oil, particularly at 0.4 ml/day, significantly increased skin moisture content and hydroxyproline (HYP) levels, while decreasing malondialdehyde (MDA) levels ($p < 0.001$ for moisture and MDA, $p < 0.01$ for HYP). Virgin coconut oil (0.4 ml/day) also significantly enhanced epidermal and dermal thickness ($p < 0.01$ and $p < 0.05$, respectively). Histological improvements, including restoration of collagen and vascular networks, were observed, comparable to vitamin E treatment. Topical virgin coconut oil demonstrates significant anti-aging effects in D-galactose-treated mice, likely by mitigating oxidative stress and improving skin structural integrity. These findings support its potential as a natural agent against skin aging.

Keywords: antioxidative activities, D-galactose, mice, skin aging, virgin coconut oil.

Introduction

The global rise in life expectancy and societal emphasis on youthful appearance have driven significant demand for effective anti-aging therapies.[1] Preventing or mitigating skin aging, particularly its visible signs, is a priority in cosmetic and dermatological research.[2] It addresses both aesthetic desires and the need to maintain skin health as a barrier against environmental stressors.[3] Skin aging is an intricate, multifactorial biological process marked by progressive deterioration of skin structure and function.[4] Clinically, this manifests as wrinkles, diminished elasticity, altered pigmentation, and impaired barrier function.[5] At the molecular level, oxidative stress, driven by an accumulation of reactive oxygen species (ROS) such as free radicals and peroxides, plays a pivotal role. ROS can damage essential biomolecules, including proteins, lipids, and DNA, disrupting cellular homeostasis and accelerating senescence.[6] This oxidative damage is exacerbated by intrinsic factors (e.g., chronological aging) and extrinsic factors like ultraviolet (UV) radiation and environmental pollutants.[7,8]
To experimentally study anti-aging interventions, models mimicking natural aging are crucial. D-galactose, a reducing sugar, is widely employed to induce an accelerated aging phenotype in rodents. Chronic administration of D-galactose leads to the accumulation of advanced glycation end products and elevated oxidative stress, closely mirroring changes observed in naturally aged skin, such as reduced elasticity, thinning, and wrinkle formation.[9] The relevance of this model lies in its ability to simulate oxidative stress-driven aging, enabling the evaluation of interventions targeting ROS-mediated damage.[10] In this study, the D-galactose-induced mouse model was chosen for its established reliability in assessing skin aging parameters.
There is growing interest in natural products derived from botanical sources in the search for effective anti-aging solutions.[11,12] This interest stems from several factors, including consumer preference for safer and more sustainable ingredients than synthetic compounds.[13,14] Furthermore, natural products often contain complex mixtures of bioactive molecules that may offer synergistic effects or target multiple pathways involved in aging, potentially leading to broader benefits and fewer side effects than single synthetic agents.[13]
Plant-derived oils have garnered particular attention among these natural products. Virgin coconut oil (VCO), extracted from fresh coconut (*Cocos nucifera* L.) solid endosperm without chemical refining, is recognized for its moisturizing, anti-inflammatory, and antioxidant properties.[15] Rich in medium-chain fatty acids (like lauric acid) and phenolic compounds, VCO has shown promise in various studies in promoting skin hydration, enhancing skin barrier function, and protecting against oxidative damage.[16,17] Despite the growing body of evidence supporting VCO's dermatological benefits, research specifically characterizing Vietnamese VCO products and evaluating their efficacy against established anti-aging agents is limited. The unique geographical and processing factors in Vietnam could influence the specific phytochemical profile and biological activity of local VCO. However, empirical evidence supporting its specific efficacy in mitigating skin aging is lacking.
Therefore, the novelty of this research lies in its focus on evaluating the anti-aging efficacy of a specific Vietnamese virgin coconut oil in a D-galactose-induced mouse model, with a direct comparison to vitamin E,

*Corresponding author. E mail: phamvananh@hmu.edu.vn
Tel: +84 91 559 56 90

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a well-established antioxidant. We hypothesized that VCO would ameliorate signs of skin aging by reducing oxidative stress and improving skin structure. By assessing key parameters, including skin morphology, moisture content, collagen levels (via hydroxyproline), malondialdehyde (MDA) as an oxidative stress marker, and gross appearances with detailed histopathological changes, this research sought to provide scientific validation for the anti-aging potential of this specific Vietnamese VCO product.

Materials and Methods

Preparation of virgin coconut oil

Lao Nha Que (LNQ) virgin coconut oil (VCO) was manufactured by Vi Dieu Nam Medicine and Pharmacy Limited Company. VCO was made by cold-pressing the liquid from the solid endosperm of mature coconut (Cocos nucifera L.) using an oil expeller machine (Dongguan Sanzhong Machinery Co., Ltd, China). The oil was packaged in 120 mL plastic bottles, compliant with ISO 22000:2018 standards. Throughout the study, VCO was stored in a dry environment below 30°C and with a humidity of under 75%, protected from direct sunlight to maintain its physicochemical integrity.

Experimental animals

Healthy Swiss mice of both sexes, weighing approximately 30 ± 2 grams, were sourced from the National Institute of Hygiene and Epidemiology, Vietnam. Animals were allowed to adjust to the research environment with a standard 12-hour light/dark cycle at the Pharmacology Department – Hanoi Medical University for one week before any experiments. Throughout the study, animals were fed regular rodent food provided by the National Institute of Hygiene and Epidemiology, with unlimited water access. Daily checks were performed to monitor for signs of distress, and weekly weights were recorded until euthanasia. All protocols used in this study involving animals were approved by the Scientific Board Committee of Hanoi Medical University (ref number: IRB00003121).

Study protocol

All methods were carried out following ARRIVE guidelines and the National Institutes of Health guide for the care and use of laboratory animals. This study was designed to evaluate the effects of topical VCO on D-galactose-induced skin aging in mice, following protocols adapted from previous studies.[18,19] Before the experiment, dorsal hair was removed from all mice using an electric clipper to ensure consistent treatment application. Fifty mice were randomly assigned to five groups (n = 10 per group), as outlined in Table 1.

Table 1: Treatment intervention in *Swiss* mice with D-galactose-induced aging skin

Group (n = 10)	Treatment
Group 1 - Control	Normal saline (s.c.) + topical saline, 0.2 mL/day
Group 2 – D-galactose	D-galactose (1000 mg/kg, s.c)[10,18] + topical saline, 0.2 mL/day
Group 3 – Vitamin E	D-galactose (1000 mg/kg, s.c) + topical Vitamin E 1% (0.2 ml/day)
Group 4 – "LNQ" 0.2	D-galactose (1000 mg/kg, s.c) + topical LNQ VCO (0.2 ml/day)
Group 5 – "LNQ" 0.4	D-galactose (1000 mg/kg, s.c) + topical LNQ VCO (0.4 ml/day)

Mice in the D-galactose-treated groups (Groups 2–5) received daily subcutaneous injections of D-galactose (1000 mg/kg, dissolved in normal saline) for 42 consecutive days to induce skin aging. Control mice (Group 1) received daily subcutaneous injections of an equivalent volume of normal saline. Concurrently, following each injection, the shaved dorsal skin of mice was topically treated as follows: Group 1 (control) and Group 2 (D-galactose) received 0.2 mL/day of topical normal saline; Group 3 received 0.2 mL/day of 1% vitamin E (prepared in olive oil); Group 4 received 0.2 mL/day of LNQ VCO; and Group 5 received 0.4 mL/day of LNQ VCO. Twenty-four hours after the final treatment (Day 43), all mice were anesthetized with ketamine (100 mg/kg, i.p.). Dorsal skin samples were collected for macroscopic examination (including assessment of the subcutaneous vascular system), biochemical analysis, and histopathological evaluation.

Determination of skin moisture content

A 1 cm² section of dorsal skin was excised from each animal, and the wet weight was recorded. Samples were dried in an oven at 80°C for 12 hours to determine the dry weight. The tissues were weighed using a digital scale (Precisa, Swiss, Model 321LX Type 2200C). Skin moisture content (%) was calculated using the following formula (Equation 1):

$$\text{Moisture content (\%)} = \frac{(\text{Wet weight} - \text{Dry weight}) \times 100}{\text{Wet weight}}$$

Determination of hydroxyproline (HYP) and malondialdehyde (MDA) content

Skin samples (approximately 200 mg per animal) were excised, rinsed with ice-cold saline, and stored at −20°C until analysis. For analysis, samples were thawed, homogenized in 2 mL phosphate-buffered saline (PBS, pH 7.4), and centrifuged at 10,000 rpm for 15 minutes at 4°C. The supernatant was collected and stored at −20°C until analysis. Supernatants were analyzed for hydroxyproline (HYP) and malondialdehyde (MDA) content using commercial enzyme-linked immunosorbent assay (ELISA) kits, following the manufacturer's protocols. Specific kits used were the Mouse HYP ELISA Kit (MBS703512) and Mouse MDA ELISA Kit (MBS269473), both obtained from MyBioSource (USA).

Assessment of the skin structure

Eight out of ten animals in each group underwent histopathological analyses of skin samples, including assessments of general skin appearance, subcutaneous vascular system, microstructural arrangements, and epidermal/dermal thickness. The epidermal and dermal thicknesses were measured using ImageJ software (NIH, LOCI, USA). Each reported value is the average of three measurements at three different locations on the same sample. After dorsal skin removal, the tissues were preserved in 10% formalin for fixation. Sections were stained with hematoxylin and eosin and examined under a light microscope (Olympus BX10, Japan). The images were snapped by an attached digital camera (Olympus DP12 camera, Japan) with a magnification of 100x.

Statistical analysis

Data obtained were analyzed using SPSS Statistics 27.0 software. Researchers were unaware of the treatment allocations throughout the analysis. The final results were reported as mean ± standard deviation (SD). Differences between groups were analyzed using One-way ANOVA, followed by the TUKEY test, with statistical significance at $p \leq 0.05$.

Results and Discussion

Skin aging, a complex process driven by intrinsic and extrinsic factors, manifests as wrinkles, reduced elasticity, and impaired barrier function, significantly impacting quality of life and skin health.[20] In response to growing consumer demand for safer and sustainable anti-aging solutions, natural compounds, particularly plant-derived oils, have gained attention for their antioxidant and anti-inflammatory properties.[21] Investigating the anti-aging activity of herbs on experimental models plays a crucial role in developing a natural product for medical and cosmetic purposes.[7,22] This study aimed to evaluate the anti-aging potential of LNQ VCO in a D-galactose-induced skin aging mouse model, providing empirical evidence for its further applications.

In this study, D-galactose was used to induce skin aging, given that the role of D-galactose in accelerating skin aging in animals is widely published in the existing literature.[10,23,24] Inside the body, D-galactose is metabolized into D-galacto-hexodialdose, hydrogen peroxide, and galactitol. They build up in cells, increase osmotic pressure, and activate redox reactions, accumulating free radicals and oxidants.[10] Subsequently, these impair essential skin biological components, including proteins such as collagens, lipids, and nucleic acids, resulting in structural fragmentation and functional damage typical of aging.[25]
To enhance the originality of this study, we investigated not only the protective and restorative effects of LNQ VCO on D-galactose-induced skin aging in Swiss mice but also its efficacy relative to a well-established anti-aging agent, vitamin E. By benchmarking VCO against vitamin E, a proven antioxidant known to mitigate oxidative stress and inflammation, this study provides a robust framework for evaluating VCO's potential as a natural anti-aging therapeutic.[26] Vitamin E interferes with various forms of oxidative stress, neutralizing free radicals and preserving skin structure during aging, making it an ideal positive control for assessing VCO's anti-senescence properties.[6,27]
All mice tolerated the 42-day intervention well, with no observed signs of toxicity or mortality. Body weight, food intake, and water consumption remained consistent across all groups, indicating no adverse effects from D-galactose, vitamin E, or VCO treatments.

Macroscopic skin improvements with VCO treatment
Macroscopic examination of the dorsal skin revealed distinct differences among the groups after 42 days (Figure 1). Compared to the control group, which exhibited smooth, pink skin with dense, fine hair (Figure 1A), mice receiving only D-galactose (disease group) displayed characteristic signs of accelerated skin aging, including dryness, roughness, increased wrinkles, reduced elasticity, and sparser, coarser hair (Figure 1B). In contrast, topical application of 1% vitamin E (Figure 1C) or LNQ VCO at both 0.2 mL/day (Figure 1D) and 0.4 mL/day (Figure 1E) markedly mitigated these D-galactose-induced changes. The skin in these treatment groups appeared healthier, smoother, and more hydrated, closely resembling the control group.

Skin moisture content improvements with VCO treatment
D-galactose significantly reduced skin moisture content in the disease group compared to the control group ($p < 0.001$; Figure 2). Topical application of 1% vitamin E or LNQ VCO (0.2 or 0.4 mL/day) significantly increased moisture content compared to the disease group ($p < 0.001$ for all treatments). No significant differences were observed among the vitamin E and VCO groups ($p > 0.05$), indicating comparable efficacy in restoring skin hydration.

Effect of VCO treatment on hydroxyproline (HYP) and malondialdehyde (MDA)
Skin hydroxyproline (HYP) levels, indicative of collagen content, were significantly lower in the disease group compared to the control group ($p < 0.001$; Figure 3A). Treatment with 1% vitamin E or LNQ VCO (0.4 mL/day) significantly increased HYP levels compared to the disease group ($p < 0.01$), with no significant difference between these two treatments ($p > 0.05$). The lower VCO dose (0.2 mL/day) showed a non-significant trend toward increased HYP levels ($p > 0.05$), suggesting dose-dependent efficacy. These results indicate that VCO, particularly at the higher dose, promotes collagen synthesis and re-epithelialization, likely due to its potential to increase skin protein content, which supports extracellular matrix integrity.[28]
In our study, VCO may also protect the skin barrier through retaining skin moisture and promoting collagen synthesis, as demonstrated by increased water and HYP content compared to the diseased mice. Lauric acid, a medium-chain fatty acid, has been shown to penetrate the skin effectively, supporting fibroblast proliferation and collagen deposition during wound healing.[29] The lipid-rich composition of VCO likely reduces transepidermal water loss and enhances stratum corneum hydration.[16] Previous in vitro studies have associated VCO application with the upregulated expression of aquaglyceroporin 3, an integral channel responsible for transporting water and small molecules across cell membranes.[30] Additionally, VCO could increase filaggrin, a key structural protein that maintains stratum corneum hydration and physiological pH balance.[15] These effects are consistent with clinical studies on patients having atopic dermatitis, where reduced transepidermal water loss was associated with the topical application of VCO.[31]
Malondialdehyde (MDA) levels, a marker of oxidative stress, were significantly elevated in the disease group compared to the control group ($p < 0.01$; Figure 3B). Treatment with 1% vitamin E or LNQ VCO (0.2 or 0.4 mL/day) significantly reduced MDA levels compared to the disease group ($p < 0.01$ for vitamin E, $p < 0.001$ for VCO). Notably, both VCO doses showed a non-significant trend toward greater MDA reduction compared to vitamin E ($p > 0.05$).
Our findings further emphasize that VCO significantly improved the skin conditions of mice despite D-galactose administration, with effects equivalent to 1% vitamin E. These beneficial outcomes of VCO could result from its ability to resolve the excessive oxidative stress linked to aging. Indeed, studies have found in virgin coconut oil the presence of multiple fatty acids, such as lauric acid and linoleic acid, as well as phenolic compounds including ferulic acid.[17,32] These bioactive constituents have been reported to delay oxidation by scavenging free radicals and reducing lipid peroxidation,[17,32,33] in addition to their ability to inhibit pro-inflammatory cytokines at both genetic and protein expression levels.[15] VCO was associated with a non-significantly greater reduction of MDA than 1% vitamin E, possibly attributable to the combined effects of its diverse antioxidant components.[34]

Histopathological and structural improvements with VCO treatment
Histopathological analysis revealed significant reductions in epidermal and dermal thickness in the disease group compared to the control group

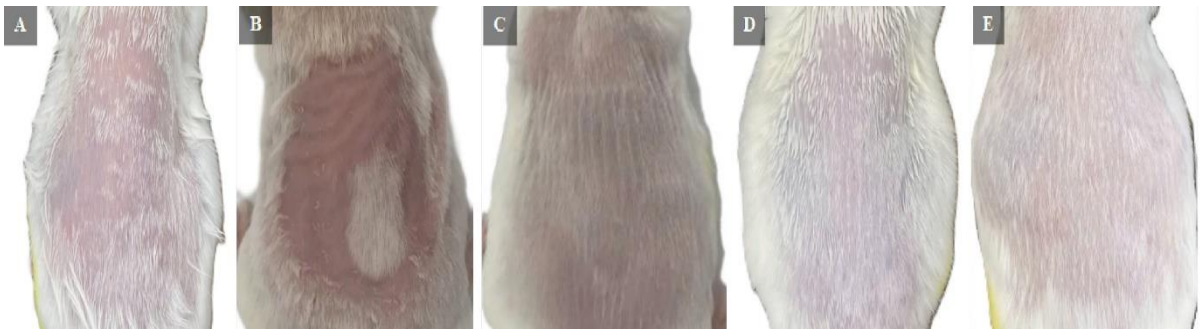

Figure 1: Dorsal skin from *Swiss* mice after 42 days of treatment. (A) Control (normal saline, s.c. + topical saline, 0.2 mL/day), showing smooth, pink skin with dense, fine hair; (B) D-galactose model (1000 mg/kg/day, s.c. + topical saline, 0.2 mL/day), exhibiting dry, rough skin with wrinkles and sparse, coarse hair; (C) D-galactose + 1% vitamin E (0.2 mL/day, topical); (D) D-galactose + LNQ VCO (0.2 mL/day, topical); (E) D-galactose + LNQ VCO (0.4 mL/day, topical). Treatment groups (C–E) show improved skin smoothness and hair density compared to the model group.

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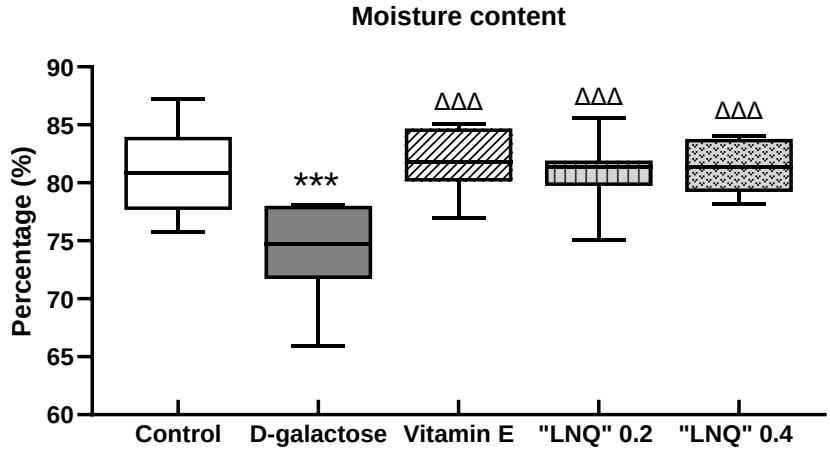

Groups	Wet weight (mg) (n = 10)	Dry weight (mg) (n = 10)
Control	66.53 ± 9.77	12.78 ± 3.71
D-galactose	45.07 ± 6.12	11.64 ± 2.78
Vitamin E	70.88 ± 9.81	12.80 ± 2.75
LNQ "0.2"	65.63 ± 12.27	12.49 ± 2.29
LNQ "0.4"	73.87 ± 9.81	13.63 ± 2.69

Figure 2: Skin moisture content in *Swiss* mice with D-Galactose-induced skin aging. Box-and-whisker plots showing skin moisture content (% wet weight) in dorsal skin samples from Swiss mice (n = 10 per group) after 42 days of treatment. Groups: Control, D-galactose (1000 mg/kg/day, s.c. + topical saline, 0.2 mL/day), D-galactose + 1% vitamin E (0.2 mL/day, topical), D-galactose + LNQ VCO (0.2 mL/day, topical), and D-galactose + LNQ VCO (0.4 mL/day, topical). Boxes represent the 25th to 75th percentiles, lines indicate the 50th percentile, and whiskers mark the 5th and 95th percentiles. ***: $p < 0.001$, compared to control group; $^{\Delta\Delta\Delta}$: $p < 0.001$, compared to D-galactose group.

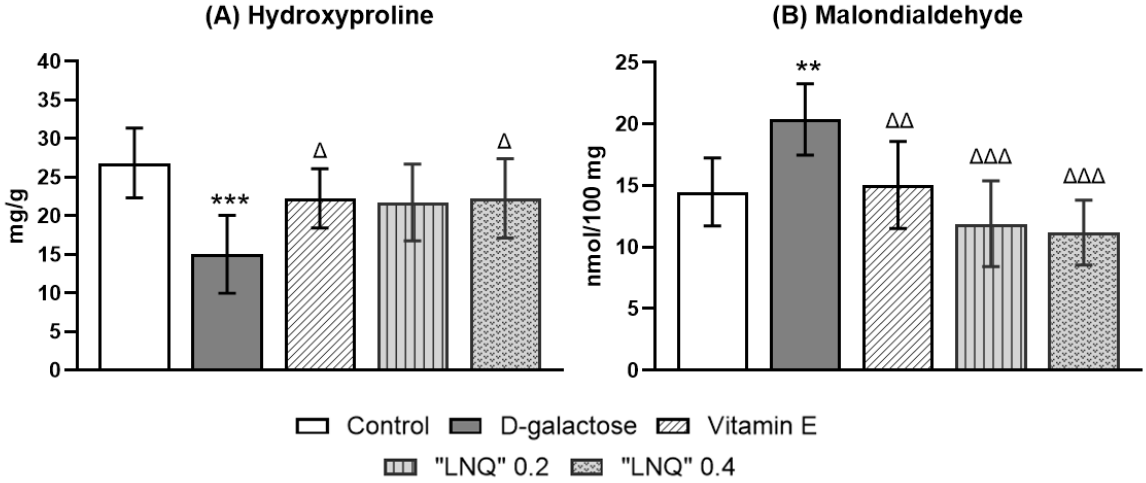

Figure 3: Hydroxyproline (HYP) and malondialdehyde (MDA) levels in *Swiss* mice skin homogenates**.** Bar charts showing (A) HYP and (B) MDA levels in dorsal skin samples from *Swiss* mice (n = 8 per group) after 42 days of treatment. Groups: Control, D-galactose (1000 mg/kg/day, s.c. + topical saline, 0.2 mL/day), D-galactose + 1% vitamin E (0.2 mL/day, topical), D-galactose + LNQ VCO (0.2 mL/day, topical), and D-galactose + LNQ VCO (0.4 mL/day, topical). **, ***: $p < 0.01$, $p < 0.001$, compared to control group; $^{\Delta}$, $^{\Delta\Delta}$, $^{\Delta\Delta\Delta}$: $p < 0.05$, $p < 0.01$, $p < 0.001$ compared to D-galactose group.

($p < 0.001$ for epidermis, $p < 0.05$ for dermis; Figure 4). Treatment with 1% vitamin E or LNQ VCO (0.4 mL/day) significantly increased both epidermal ($p < 0.01$) and dermal ($p < 0.05$) thickness compared to the disease group. The lower VCO dose (0.2 mL/day) showed a non-significant change toward improved thickness ($p > 0.05$). Examination of the subcutaneous tissue and associated vasculature (Figure 5) indicated reduced vascular density (fewer visible blood vessels) and skin thickness in the disease group (Figure 5B) compared to controls (Figure 5A), contributing to a paler appearance. Treatment with vitamin E (Figure 5C) and both doses of VCO (Figures 5D and 5E) appeared to maintain better vascular networks than the disease group. VCO treatment markedly enhanced vascular endothelial growth factor receptor 2 (VEGFR2) expression in human umbilical vein endothelial, fibroblast, and retinal ganglion cells, indicating that VCO-induced angiogenesis was associated with the activation of the VEGF signaling pathway.[35] Additionally, linoleic acid participates in the

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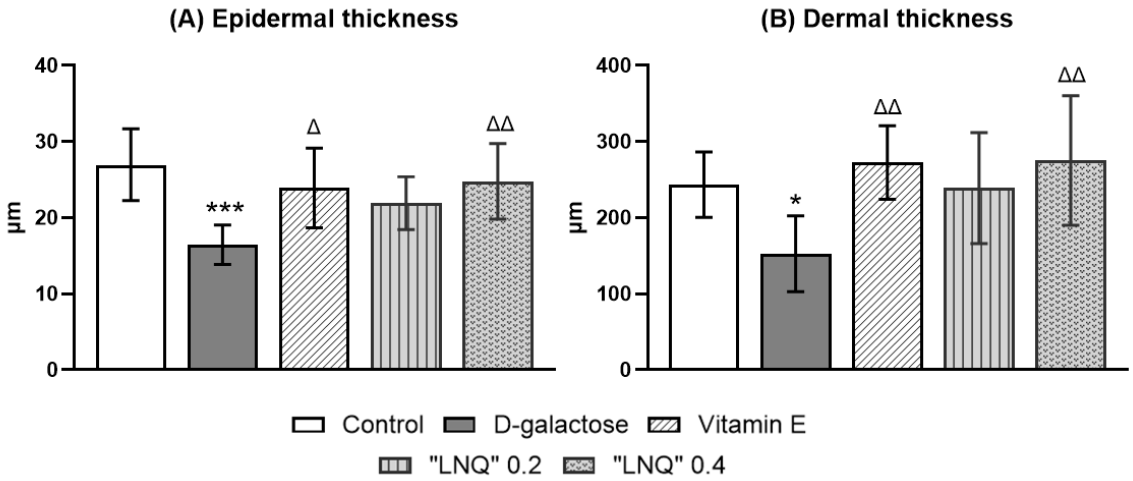

Figure 4: Epidermal and dermal thickness in *Swiss* mice skin. Bar charts showing (A) epidermal and (B) dermal thickness in dorsal skin samples from Swiss mice (n = 8 per group) after 42 days of treatment. Groups: Control, D-galactose (1000 mg/kg/day, s.c. + topical saline, 0.2 mL/day), D-galactose + 1% vitamin E (0.2 mL/day, topical), D-galactose + LNQ VCO (0.2 mL/day, topical), and D-galactose + LNQ VCO (0.4 mL/day, topical). **, ***: $p < 0.01$, $p < 0.001$, compared to control group; $^{\Delta}$, $^{\Delta\Delta}$: $p < 0.05$, $p < 0.01$, compared to D-galactose group.

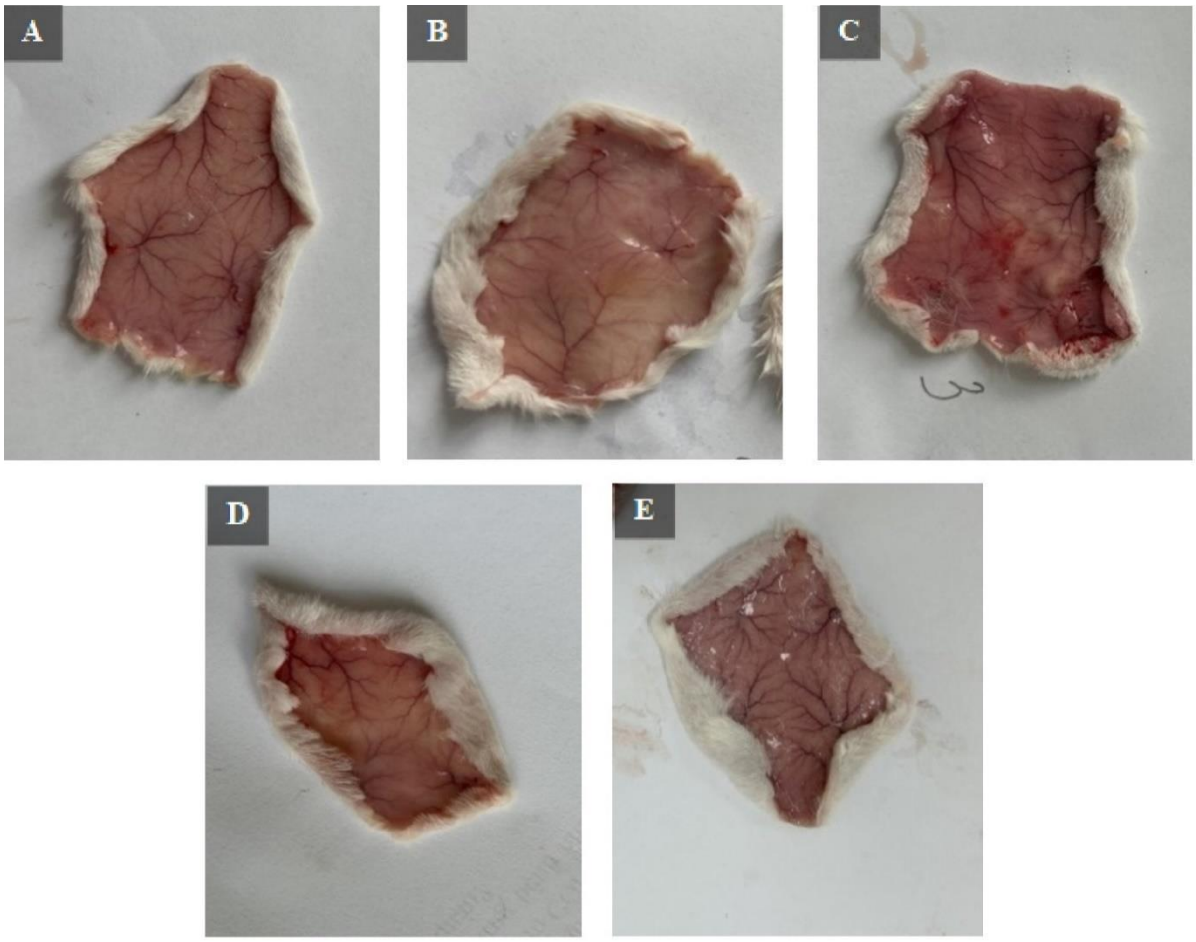

Figure 5: Gross appearance of dorsal skin and subcutaneous vasculature in *Swiss* mice after 42 days of treatment**:** (A) Control (normal saline, s.c. + topical saline, 0.2 mL/day), showing dense vascular networks; (B) D-galactose (1000 mg/kg/day, s.c. + topical saline, 0.2 mL/day), displaying reduced vascular density and paler appearance; (C) D-galactose + 1% vitamin E (0.2 mL/day, topical); (D) D-galactose + LNQ VCO (0.2 mL/day, topical); (E) D-galactose + LNQ VCO (0.4 mL/day, topical). Treatment groups (C–E) show restored vascular density compared to the model group.

neovascularization process under the skin, thereby diversifying the vascular network,[36] as demonstrated in our study. Lauric acid and linoleic acid found in VCO have been reported to promote collagen generation, likely by stimulating fibroblast activity and upregulating collagen gene expression,[37,38] while potentially downregulating the action of matrix metalloproteinases-1 (MMP-1), a collagenase responsible for degrading extracellular matrix components, particularly type I and III collagen, which are predominant in the skin.[39] This vascular restoration likely supports nutrient delivery and oxygenation, further enhancing skin repair and structural integrity. However, current literature still lacks direct evidence linking VCO to MMP-1 downregulation. Therefore, our results could pave the way for future studies to thoroughly address this relationship.

Microscopic evaluation of Hematoxylin and Eosin (H&E) stained skin sections revealed structural differences across all layers (Figure 6). The epidermis appeared relatively uniform in control mice, with no abnormality detected. It was accompanied by a highly organized collagen structure, fibrous elastic bundles, and normal capillaries in the dermis. The hypodermis comprised 10-20 rows of neatly arranged adipocytes interspersed with well-developed hair follicles (Figure 6A). In contrast, the disease group exhibited structural impairments across all skin layers: multiple sites of epidermal atrophy, sparse connective tissue and collagen network with disorganized elastic bundles, diminished vascular system in the dermis, and uneven development of hypodermal adipocytes, averaging 5-10 rows (Figure 6B). Vitamin E and both doses of VCO positively affected the skin structure in aging mice following D-galactose injections. These included relatively even epidermal structure, moderately increased collagen density and vascular network, with no significant disruption observed in the connective tissue (Figure 6C-E). The hypodermis collected was also more similar to that of the control than the disease samples, as demonstrated by 10-20 rows of organized adipocytes scattered with hair follicles. Skin samples from mice given VCO 0.4 ml/day improved the collagen network more positively than those receiving the lower dose of 0.2 ml/day (Figure 6D).

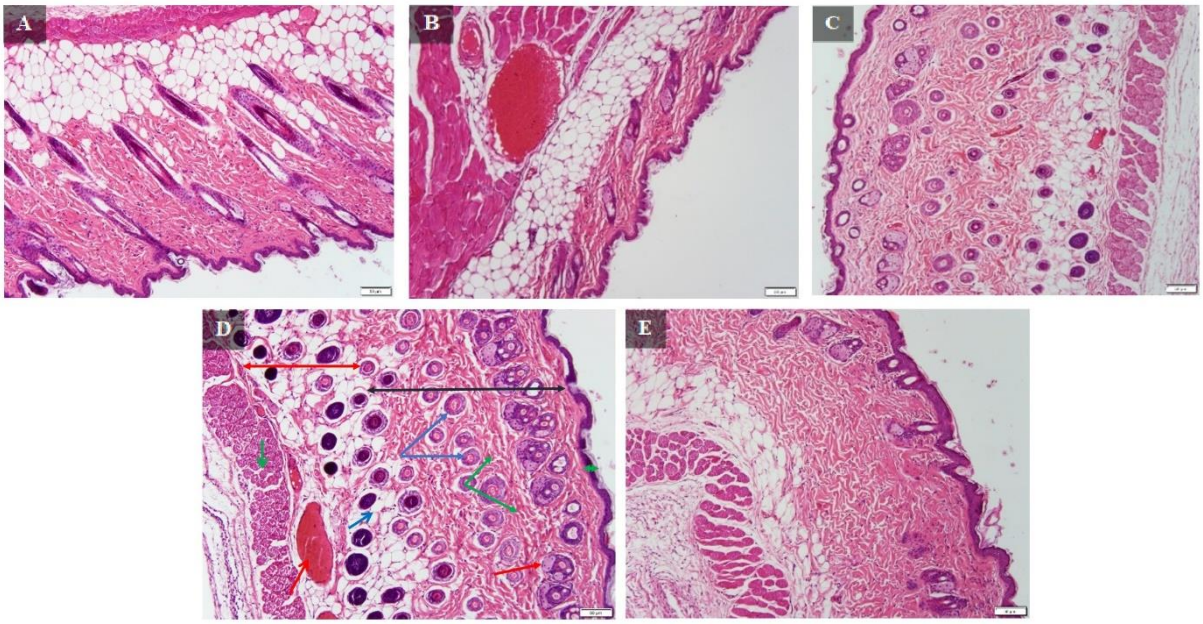

Figure 6: Hematoxylin and eosin (H&E)-stained dorsal skin sections from Swiss mice after 42 days of treatment (100× magnification). (A) Control (normal saline, s.c. + topical saline, 0.2 mL/day), showing uniform epidermis, organized collagen, and hypodermis with 10–20 adipocyte rows; (B) D-galactose (1000 mg/kg/day, s.c. + topical saline, 0.2 mL/day), exhibiting epidermal atrophy, disorganized collagen, and hypodermis with 5–10 adipocyte rows; (C) D-galactose + 1% vitamin E (0.2 mL/day, topical); (D) D-galactose + LNQ VCO (0.2 mL/day, topical); (E) D-galactose + LNQ VCO (0.4 mL/day, topical). Treatment groups (C–E) show restored epidermal structure and collagen organization. The following structures are indicated: Two-headed arrows: green for epidermis, black for dermis, red for hypodermis. Solid arrows: green for collagen fibers, blue for hair follicles, red for sebaceous glands. Open arrows: green for skeletal muscle, blue for adipose tissue, red for blood vessels.

Despite these promising results, this study has limitations. While biochemical and histological parameters were assessed, deeper mechanistic insights, such as the impact of VCO on endogenous antioxidant enzymes (e.g., superoxide dismutase), were not explored. In addition, the mouse model, though well-established, may not fully reflect human skin aging due to anatomical and physiological differences, necessitating clinical trials to validate LNQ VCO's efficacy in humans. Finally, the 42-day intervention may not capture VCO's long-term safety or effectiveness, warranting extended studies to evaluate sustained benefits and potential adverse effects.

In summary, this study employed a mouse model of D-galactose-induced skin aging to investigate the beneficial effects of VCO on improving skin conditions after 42 days of administration, with effects comparable to vitamin E, likely through antioxidant and anti-inflammatory mechanisms. Our findings show that VCO, especially at the higher dose of 0.4 ml/day, significantly improved macroscopic and microscopic skin features, such as increased moisture, HYP concentrations, and epidermal-dermal thickness, while lowering MDA levels. The comparative approach underscores the novelty of this research, as it positions LNQ VCO—a Vietnamese product potentially unique due to regional and processing factors—within the context of established dermatological agents, highlighting its comparable efficacy and potential synergistic benefits derived from its complex bioactive profile. Future research should focus on elucidating molecular pathways, confirming effects in human trials, and assessing long-term outcomes.

Conclusion

This study demonstrates that topical LNQ VCO, particularly at 0.4 mL/day, significantly mitigates D-galactose-induced skin aging in *Swiss* mice, with efficacy comparable to 1% vitamin E. VCO enhances skin moisture, collagen content, epidermal and dermal thickness, and histological structure while reducing oxidative stress. These benefits, likely mediated by VCO's antioxidant and anti-inflammatory

components, support its potential as a natural anti-aging agent for dermatological applications. Future research should elucidate underlying molecular mechanisms, validate efficacy in human clinical trials, and assess long-term safety and optimal dosing to facilitate VCO's therapeutic use.

Conflict of interest
The author declares no conflict of interest.

Authors' Declaration
The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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