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Seborrheic Dermatitis Treatment Potential of Virgin Coconut Oil: Insights from a Rat-Dinitrofluorobenzene Model via Biochemical, Molecular and Histopathological Analyses

Potencial del Aceite de Coco Virgen para el Tratamiento de la Dermatitis Seborreica: Perspectivas de un Modelo de Rata-Dinitrofluorobenceno Mediante Análisis Bioquímicos, Moleculares e Histopatológicos

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SUMMARY: Virgin coconut oil (VCO) has traditionally been used for medicinal purposes, such as enhancing strength and controlling bleeding. Its long-standing use is supported by its antibacterial, anti-inflammatory, and anti-seborrheic properties. This research aimed to investigate the mechanisms underlying VCO's anti-inflammatory and anti-seborrheic effects, particularly focusing on hair regrowth and seborrheic dermatitis (SD) by examining its ability to reduce sebum production and prevent hair loss. The study involved 50 Wistar rats divided into five groups (n=10 each): a normal control, a group induced with 0.6 % dinitrofluorobenzene (DNFB) at 3 mg/kg, a DNFB group treated with 5 mg/kg VCO, a DNFB group treated with 10 mg/kg VCO, and a normal group treated with 10 mg/kg VCO. At the end of the experiment, we analyzed skin tissue for levels of key inflammatory cytokines (IL-6, IL-1β, IL-10, and TNF-α), along with oxidative stress markers such as total antioxidant capacity and lipid peroxidation. We also evaluated the expression of genes and proteins—including MMP-9, MMP-2, CCR-5, and VEGF—in the skin tissue. Our findings revealed that DNFB administration induced significant alterations in antioxidant status, inflammatory biomarkers, and the expression of SD-related genes and proteins. VCO treatment showed dose-dependent improvement, notably at 10 mg/kg, reversing these changes. Histopathological and molecular analyses demonstrated structural restoration of skin tissue following VCO intervention. In conclusion, this study highlights VCO's promising potential in mitigating seborrheic dermatitis symptoms and modulating skin inflammation, suggesting its possible use in managing DNFB-induced SD.

KEY WORDS: Virgin coconut oil; Inflammation; Apoptosis; Seborrheic dermatitis.

Abbreviations. VCO: Virgin Coconut Oil; SD: Seborrheic Dermatitis; DNFB: Dinitrofluorobenzene; IL-6: Interleukin-6; IL-1β: Interleukin-1 Beta; IL-10: Interleukin-10; TNF-α: Tumor Necrosis Factor Alpha; MMP-2: Matrix Metalloproteinase-2; MMP-9: Matrix Metalloproteinase-9; CCR-5: C-C Chemokine Receptor Type 5; VEGF: Vascular Endothelial Growth Factor; ROS: Reactive Oxygen Species; TAC: Total Antioxidant Capacity; MDA: Malondialdehyde; FRAP: Ferric Reducing Antioxidant Power.

INTRODUCTION

Seborrheic dermatitis (SD) is a longstanding chronic inflammatory skin condition predominantly affecting areas rich in sebaceous glands, such as the scalp, face, eyebrows, and chest. It impacts approximately 3-10 % of the global population, with higher prevalence observed among males and individuals with neurological disorders or compromised immune function (Dall'Oglio *et al*., 2022). Clinically, SD manifests as flaky, inflamed, and erythematous skin, often accompanied by itching. Its development is multifactorial, involving genetic predisposition, hormonal fluctuations, stress, and the presence of Malassezia yeasts—some lipophilic fungi that metabolize skin lipids and trigger inflammatory responses (Borda *et al*., 2019). Recent advances highlight the crucial role of Malassezia in disease pathogenesis; its enzymes and metabolites provoke inflammation, which is further amplified by an imbalance between pro- and anti-inflammatory cytokines, particularly involving Th1 and Th17 cell subsets. Additionally, SD is associated with compromised skin barrier integrity, rendering it more susceptible to irritants and environmental

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triggers (Adalsteinsson *et al*., 2020). Emerging therapies focus on targeting specific cytokines such as IL-17 and IL-23, along with innovative topical formulations aimed at reducing inflammation and restoring barrier function; probiotic and prebiotic approaches are also under investigation for modulating skin microbiota (Cohen *et al*., 2020). Future research aims to deepen our understanding of the skin microbiome's role and develop personalized treatments to improve management, especially for severe or recurrent cases.

The epidemiology of SD reveals a characteristic pattern, with peak incidences during infancy and adolescence—periods marked by hormonal changes and increased sebaceous activity—and continued persistence into adulthood as a relapsing condition often exacerbated by environmental and emotional factors (Cavallo *et al*., 2025). Males tend to be more affected, possibly due to androgenic stimulation of sebaceous glands and immune modulation, while associations with neurological conditions like Parkinson's disease and immune suppression such as HIV infection underscore the complex interplay between immune status and disease susceptibility (Li *et al*., 2025). The pathogenesis involves an intricate network of microbial, genetic, and immune factors; colonization by Malassezia spp. plays a central role, producing enzymes that promote inflammation and lipid metabolites that irritate the skin. Its proliferation is driven by increased sebum production—stimulated by androgens—and skin barrier dysfunction, which collectively facilitate fungal overgrowth and immune activation (Kido-Nakahara *et al*., 2025). Recent insights also emphasize the significance of oxidative stress; an imbalance favoring reactive oxygen species (ROS) results in cellular damage, lipid peroxidation (e.g., elevated malondialdehyde levels), and diminished antioxidant defenses such as superoxide dismutase, catalase, and glutathione peroxidase (Jahan *et al*., 2021). These oxidative processes activate key transcription factors like NF-κB and AP-1, fueling pro-inflammatory cytokine cascades and perpetuating inflammation. Experimental models using rats and mice have been instrumental in elucidating these pathways, revealing increased oxidative markers and cytokine expression that mirror clinical findings, and providing valuable platforms for testing potential therapeutic agents (Truong *et al*., 2024).

In SD, a range of cytokines such as IL-6, IL-1β, IL-10, and TNF-α play pivotal roles in mediating inflammatory responses; notably, IL-6 and TNF-α levels are elevated, contributing to inflammation and keratinocyte proliferation, with IL-6 promoting Th17 differentiation and correlating with disease severity, while IL-1β facilitates immune cell recruitment and skin scaling (Tosun, Yasak Güner, & Akyol, 2022). Conversely, IL-10, an anti-inflammatory cytokine, is generally decreased in affected skin, impairing the regulation of inflammation and exacerbating symptoms. Alongside these cytokines, oxidative stress markers are markedly altered in SD, with studies showing reduced total antioxidant capacity (TAC) that diminishes the skin's defenses against reactive oxygen species (ROS), and increased lipid peroxidation—evidenced by elevated malondialdehyde (MDA)—which contributes to cellular damage and amplifies inflammatory processes (Liu *et al*., 2025). At the molecular level, the upregulation of matrix metalloproteinases (MMP-2 and MMP-9) facilitates tissue remodeling and compromises skin barrier integrity, while heightened expression of chemokine receptor CCR-5 promotes immune cell infiltration, perpetuating inflammation (Akkoç, 2019). Additionally, increased levels of vascular endothelial growth factor (VEGF) contribute to angiogenesis, skin hypervascularity, and further immune cell recruitment, exacerbating lesion severity (Liu *et al*., 2024). These findings collectively underscore that the pathogenic landscape of SD involves a complex interplay of pro-inflammatory cytokines, oxidative stress, and molecular pathways driving tissue degradation and immune activation, which represent potential targets for therapeutic intervention.

Virgin Coconut Oil (VCO) is extracted from the fresh, mature kernels of the coconut palm (*Cocos nucifera* L.), a tropical plant cultivated extensively in regions such as Southeast Asia, the Pacific Islands, India, the Philippines, and the Caribbean. Belonging to the Arecaceae family, this palm thrives in coastal, sunny environments, with VCO production typically involving cold-pressing or wet-milling methods that preserve its natural bioactive compounds, ensuring high purity and nutritional value. Traditionally, coconut oil has served diverse roles in tropical cultures, including as a cooking oil to support energy and lipid metabolism, a medicinal remedy for skin infections, cuts, burns, and wounds owing to its antimicrobial properties, and as a moisturizer for skin and hair, as well as a preservative and cleansing agent (Suryani *et al*., 2020; Wiyani *et al*., 2020). It is also incorporated into religious ceremonies and healing massages. Modern scientific research has corroborated many of these traditional uses, highlighting VCO's broad pharmacological benefits such as antimicrobial and antiviral activity—mainly due to medium-chain fatty acids like lauric, caprylic, and capric acids—along with anti-inflammatory, antioxidant, cardiovascular, and neuroprotective properties that improve lipid profiles, reduce oxidative stress, and serve as alternative energy sources for brain cells (Ramesh *et al*., 2020). Nutritionally, VCO is rich in vitamins E, K, and beta-carotene precursors, as well as essential minerals like

magnesium, calcium, iron, and phosphorus. Its polyphenolic compounds—including phenolic acids, flavonoids, and tocotrienols—further enhance its antioxidant, anti-inflammatory, and antimicrobial effects (Priya *et al*., 2022). In skin health, VCO is used clinically to improve hydration, elasticity, and barrier function; to combat eczema, dermatitis, and bacterial infections such as acne through its anti-inflammatory and antimicrobial properties; and to accelerate wound healing by stimulating collagen synthesis, promoting angiogenesis via growth factors like VEGF, and reinforcing lipid barriers by enhancing ceramide production and reducing transepidermal water loss (Chew, 2019). Cellular and molecular studies support these benefits, demonstrating VCO's ability to neutralize reactive oxygen species, inhibit NF-κB-mediated inflammatory pathways—lowering cytokines like IL-1β, IL-6, and TNF-α—and disrupt microbial cell membranes through lauric acid, thereby reducing pathogen proliferation (Konar *et al*., 2020). Additionally, VCO fosters tissue regeneration by activating dermal repair mechanisms and supporting skin barrier integrity, making it an effective natural agent in managing various skin disorders (Pupala *et al*., 2019). In this study, we evaluated the antiseborrheic effects of VCO in an animal model of SD, focusing on its impact on inflammatory and antioxidant pathways, as well as its role in repairing skin damage induced by dinitrofluorobenzene (DNFB).

MATERIAL AND METHOD

Preparation of VCO. The 2200 grams of coconut juice and melt were thoroughly combined in equal parts with a 50:50 (v/v) mixture of ethanol and acetone and incubated (40 °C / 48 h). After incubation, the resulting sample was filtered and kept in the dark at room temperature for an additional 48 hours. The mixture underwent filtering through filter paper and concentrating using a rotary evaporator. Following 48 hours of fermentation, two distinct layers formed, with the upper layer containing the oil, which was collected after refrigeration. This oil was isolated via centrifugation (8000 rpm / 15 min) and then filtered to obtain a pure oily layer. The final ointment, weighing 230 g, was stored at 4 °C. An allergy test was performed on five rats, with a screening period of 48 hours under controlled conditions (Mirzaei *et al*., 2019).

Experimental design. After a one-week acclimatization period, the rats, weighing approximately 180±20 g, were randomly allocated to five groups of ten animals. The rats were maintained in propylene cages under a 12-h dark / light cycle at 26±3 °C with a humidity of 40±3 %. They could drink tap water and eat a standard pellet diet. Seborrheic dermatitis was induced by applying 50 µl of a 0.6 % (2 mg/kg) DNFB (Sigma, USA) ointment, prepared with 42 % olive oil, 15 % acetone, and 8 % DMSO (v/v/v), onto the inner surfaces of both ears and shaved inguinal regions on day 1. On day 30, skin-fold thickness at these sites was measured using a micrometer immediately before the challenge and then 6 and 12 hours afterward, with increases indicating inflammatory swelling. For ease of handling, the rats were sedated with isoflurane. The control group received 0.1 ml of PBS applied topically, while the SD group was treated with 0.6 % DNFB. In the treatment groups, SD rats received topical applications of VCO ointment at doses of either 5 or 10 mg/kg. Also, the normal+10 VCO group received 10 mg/kg of VCO ointment topically. The study aimed to determine the effective, non-toxic dose of VCO through daily topical administration at 10 a.m. for 30 days, based on preliminary data and previous research involving collagenase and VCO. All procedures involving the animals' care and euthanasia were performed following the guidelines of the ethics committee at Xi'an Central Hospital, in accordance with established guidelines for laboratory animal welfare (Adeyemi *et al*., 2020; Li *et al*., 2025).

Assessment of total antioxidant capacity (TAC) and lipid peroxidation (LPO) in skin tissue. The ferric-reducing antioxidant power (FRAP) assay assessed the TAC of skin tissue. Skin specimen collection wa done from peri-ovarian fat and homogenized thoroughly. From each homogenate, 100 µl was mixed with cold phosphate-buffered saline (PBS) (200 µl) and transferred into a polyethylene tube (2 ml). Subsequently, 10 µl of the FRAP reagent was mixed with each sample, and the mixture underwent incubation at 25°C for 15 min. Then, the samples underwent centrifugation (12,000 g / 10 min), and the supernatant's absorbance was recorded at 593 nm (Li Pomi *et al*., 2025).

For measuring LPO levels, the thiobarbituric acid reactive substances (TBARS) assay was used. An aliquot of 100 µl of the skin tissue homogenate was located into a polyethylene tube (2 ml), followed by adding 100 µl of TBARS reagent. The mixture received incubation at 37 °C for 30 min to allow the reaction to proceed. Then, the absorbance was assessed at 593 nm to quantify LPO (Li Pomi *et al*., 2025).

Assessment of thiol levels in skin tissue. To assess thiol concentrations in skin tissue, an important marker of antioxidant status, 100 µl of the skin homogenate was added to 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB; 20 µl), followed by incubation (37°C / 15 min), and centrifugation (12,000 g / 5 min). The supernatant's absorbance was evaluated at 412 nm by an ELISA plate reader (Boo, 2022).

Assessment of VEGF, CCR-5, IL-10, MMP-2, TNF-α, MMP-9, IL-6, and IL-1β genes expression in skin tissue. Total RNA was isolated by TRIzol Reagent (Invitrogen) as instructed. The isolated RNA integrity was verified by running a 1 % agarose gel, while its purity and concentration were measured with a NanoDrop spectrophotometer (Bio-TeK, USA). Subsequently, the RevertAid First Strand cDNA Synthesis Kit reverse transcribed 2000 ng of the purified RNA into cDNA (Thermo Fisher Scientific), as instructed. The produced cDNA was kept at -20 °C until further application. Quantitative real-time PCR was conducted using SYBR Green (Vazyme, China) on an ABI 7900 fluorescence PCR system (ABI, USA), and its protocol included a primary step (50 °C / 2 min), then denaturation (95 °C / 10 min), then 40 amplification cycles consisting of 30 s at 95 °C and 30 s at 60 °C. Table I lists primer sequences employed. Expression levels of the genes were normalized to β-actin, and relative quantification was performed using the Ct method, applying the ΔΔCt calculation and corresponding fold change formula.

$2^{-\Delta\Delta CT}$; ΔΔCt = [(Ct sample - Ct β-actin gene) - (Ct sample – Ct control)] (Zhou *et al*., 2023).

Table I. Primer sequences.

Gene	Sequences (5' 3')
MMP-2	F: GATACCTGGATGCCGTGTT
	R: GGCATCCAGGTTATCGGGGA
VEGF	F: TGCAGATTATGCGGATCAAACC
	R: CTCACACCGCCTTGGCTTGT
IL-1β	F: GCAACTGTTCCTGAACTCAACT
	R: ATCTTTTGGGGTCCGTCAACT
MMP-9	F: TGGACCGCTATGGTTACA CC
	R: CGGCAAACTGCGTAATCCAG
TNF- α	F: CCCACGTCGTAGCAAACCACCAA
	R: GGGATGAACCAGGGTCTGGGCC
CCR-5	F: GATGGTGGTCTTCCTGCTGT
	R: GGGTCAGCAGAGTGACCATC
IL-6	F: GCCCTTCAGGAACAGCTATG
	R: TGTCAACAACATCAGTCCCAAGA
β -actin	F: TGAAGGTCGGAGTCAACGG
	R: AGAGTTAAAAGCAGCCCTGGTG

Assessment of MMP-9, MMP-2, CCR-5, and VEGF proteins in skin tissue with western blotting. The RIPA buffer lysed specimens that included phosphatase and protease inhibitors over 30 min. The protein level was evaluated by a BCA protein assay kit from Beyotime (China). Subsequently, isolation of protein (40 μg) was done on a 10 % SDS-polyacrylamide gel by electrophoresis, folowed by transferring to a PVDF membrane. A solution of 5 % (w/v) skim milk powder blocked the membranes for 1 hour, and then an overnight incubation was done at 4 °C using the following antibodies: anti-VEGF (Cat. No. ab51745; dilution 1:500), anti-MMP-2 (Cat. No. ab97779; dilution 1:400), anti-CCR-5 (Cat. No. ab287959; dilution 1:500), and anti-MMP-9 (Cat. No. ab38898; dilution 1:400), all sourced from Abcam (UK). After washing the membranes three times with PBST, they underwent incubation with an HRP-conjugated secondary antibody (dilution 1:5000) for 1 h. Band visualization was carried out using Bio-Rad's Stain-Free Gels, and the band intensity was quantified via Image J gel analysis software (Zhang *et al*., 2022).

Statistical analyses. SPSS 16 conducted data analysis. The Kolmogorov-Smirnov test evaluated the normality of the data. Data are shown as mean ± standard deviation (SD). Data were analyzed using one-way analysis of variance (ANOVA). A P-value of < 0.05 was considered significant.

RESULTS

Role of VCO and SD on stereological assessments of skin layers. The SD group exhibited markedly reduced levels of sweat glands, blood vessels, hair follicles, sebaceous glands, and *versus* the normal group ($p<0.05$). Receiving 5 mg/kg VCO and 10 mg/kg VCO significantly improved the density of sebaceous glands, sweat glands, and hair follicles *versus* the SD group ($p<0.05$). The SD + 10 VCO group indicated enhanced densities in sebaceous glands (0.391±0.09), sweat glands (0.298±0.07), and hair follicles (0.55±0.09), all significantly different from the SD group ($p<0.05$). Additionally, there were improvements in blood vessel density and interstitial structures in the SD + 10 VCO group *versus* the SD group ($p<0.05$) (Table II).

Table II. Density measurements (in mm^2) of sebaceous glands, sweat glands, hair follicles, blood vessels, and interstitial structures in the skin were assessed based on surface density (SV) and reference volume.

Groups	Sebaceous gland	Sweat gland	Hair follicle	Blood vessels	Interstitial structures
Normal	0.446±0.11	0.241±0.11	0.51±0.06	0.42±0.09	0.16±0.04
SD	0.169±0.05 #	0.124±0.09 #	0.26±0.08 #	0.21±0.04 #	0.41±0.05 #
Normal+10 VCO	0.516±0.10	0.291±0.14	0.59±0.06	0.39±0.03	0.21±0.05
SD + 5 VCO	0.198±0.08 *	0.155±0.05	0.42±0.08 *	0.29±0.07	0.34±0.09
SD + 10 VCO	0.391±0.09 *	0.298±0.07 *	0.55±0.09 *	0.36±0.08 *	0.22±0.06 *

Data are presented as mean ± standard deviation (SD). # $p < 0.05$ indicates a significant difference between the normal and SD groups; * $p < 0.05$ indicates significant differences among the SD + 5 VCO and SD + 10 VCO groups compared to the SD groups.

Table III. Thickness measurements of skin layers (in mm).

Groups	Epidermal thickness		Dermal thickness	Total thickness
	Corneal	Cellular		
Normal	2.9±0.2	30.3±1.6	386.2±21.1	385.1±21.3
SD	11.1±0.9 #	11.2±1.2 #	189.1±11.2 #	165.2±18.4 #
Normal+10 VCO	3.3±0.2	28.6±0.9	394.2±21.1	411.6±12.9
SD + 5 VCO	6.2±0.4	19.6±1.1 *	211.4±15.2 *	199.1±11.2
SD + 10 VCO	3.4±0.9 *	25.6±2.2 *	321.2±16.1 *	329.7±16.1 *

Data are presented as mean ± standard deviation (SD). # $p < 0.05$ indicates a significant difference between the normal and SD groups; * $p < 0.05$ indicates significant differences among the SD + 5 VCO and SD + 10 VCO groups compared to the SD groups.

The SD group displayed markedly enhanced epidermal thickness (11.1±0.9 µm) and reduced dermal thickness *versus* the normal group ($p<0.05$). Conversely, the SD + 5 VCO and SD + 10 VCO groups demonstrated significant improvements in epidermal and dermal thickness *versus* the SD group ($p<0.05$). The SD + 10 VCO group demonstrated a notable reduction in epidermal thickness (3.4±0.9 µm) and an increase in total thickness (321.2±16.1 µm) *versus* the SD group ($p<0.05$). Additionally, the SD + 5 VCO group also exhibited significant enhancements in dermal thickness (19.6±1.1 µm) and total thickness (211.4±15.2 µm), indicating effective intervention *versus* the SD group ($p<0.05$) (Table III; Figs. 1 and 2).

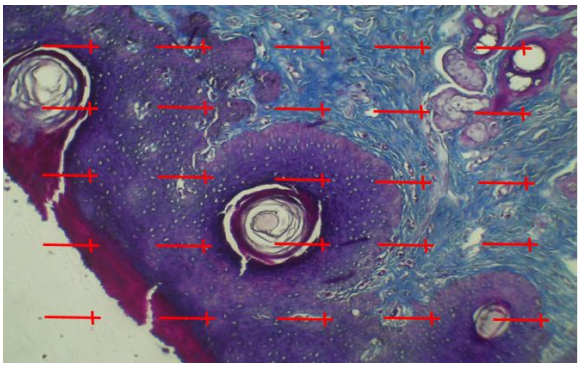

Fig. 1. Line probe (25 lines) to estimate the surface density of the skin structures. The number of lines located on each structure (∑p), the length of each of the lines in the probe is given by linear magnification (l/p) and the number of lines located on the internal part of structures (Sl). Finally, count the numbers in the following to calculate the density of the level: Sv=2× Sl/Sp × l/p (H&E staining, ×40). Light photomicrograph of the skin. EP: Epidermis layer, D: Dermis layer, DP: Dermal papillae, Corneal epidermis (CEP), Cellular epidermis (CLEP), Hair follicle (HF), SG: Sebaceous gland (SG), Sweat gland (SWG), and Blood vessels (BV).

Role of VCO and SD on skin thiol, FRAP, and TBARS levels

The SD group exhibited markedly reduced levels of thiols (0.94 mmol/mg) and FRAP (1.22 mmol/mg) *versus* the normal group ($p<0.05$). However, the administration of 10 VCO and 5 VCO significantly improved thiol and FRAP levels *versus* the SD group ($p<0.05$). The SD + 10 VCO group showed a notable increase in thiols (3.29 mmol/mg) and FRAP (3.94 mmol/mg), both significantly higher than the SD group ($p<0.05$). Additionally, TBARS concentration was notably lower in the SD + 10 VCO group (0.18 nmol/mg) than in the SD group ($p<0.05$), indicating a decrease in LPO (Fig. 3).

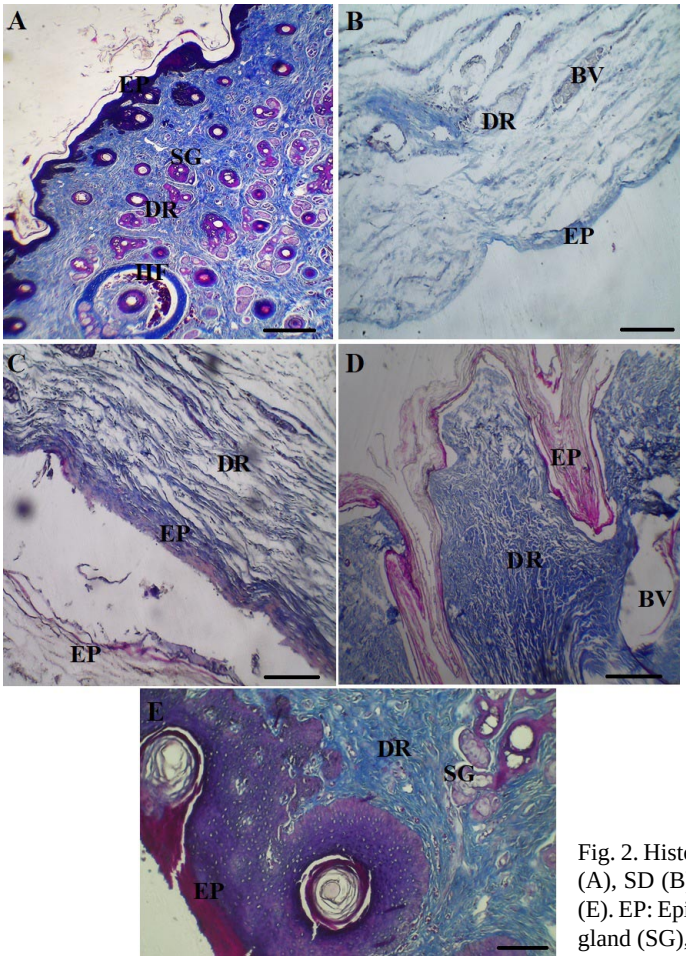

Fig. 2. Histopathology of the skin (H&E, ¥40; Scale bar: 100 µm) in normal (A), SD (B), SD + 5 VCO (C), SD + 10 VCO (D), and normal + 10 VCO (E). EP: Epidermis layer, D: Dermis layer, Hair follicle (HF), SG: Sebaceous gland (SG), and Blood vessels (BV).

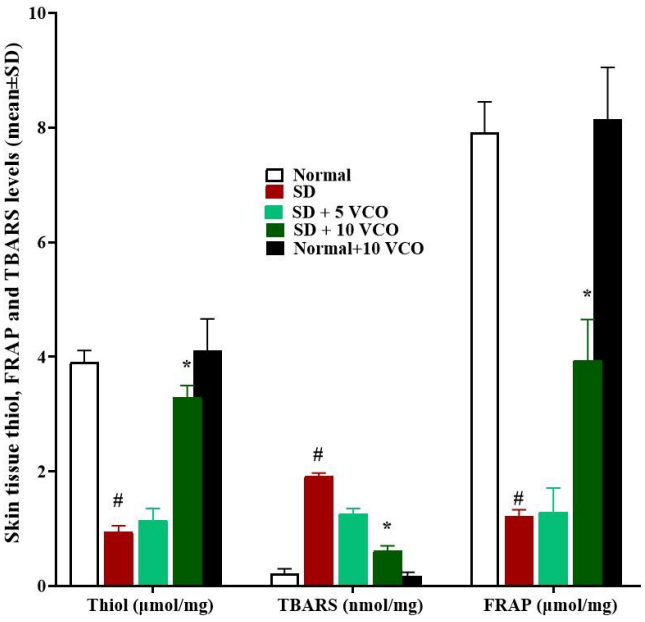

Fig. 3. Skin tissue levels of TBARS (nmol/mg) and FRAP (µmol/mg) (means ± SD; n=10/group) in experimental groups. # $p < 0.05$ indicates a significant difference between the normal and SD groups; * $p < 0.05$ indicates significant differences among the SD + 5 VCO and SD + 10 VCO groups compared to the SD groups.

Role of VCO and SD in regulating skin TNF-a, IL-1b, MMP-9, IL-6, IL-10, MMP-2, CCR-5, and VEGF genes expression

The expression levels of regulatory and pro-inflammatory cytokines, as well as matrix metalloproteinases (MMPs) and growth factors, were significantly changed across the groups. The SD group exhibited markedly elevated levels of IL-1b (4.23), CCR-5 (3.29), and MMP-9 (4.29) *versus* the controls ($p<0.05$). However, treatment with 10 VCO and 5 VCO caused significant reductions in these inflammatory markers. Notably, the SD + 10 VCO group showed a substantial reduction in MMP-9 (2.11), CCR-5 (1.29), and IL-1b (1.61), indicating a positive response to the treatment ($p<0.05$). Also, the TNF-a and MMP-2 expression levels were significantly lower in the SD + 10 VCO group (1.91 and 1.32, respectively) in comparison to the SD group ($p<0.05$). In contrast, IL-10 concentrations were significantly enhanced in the SD + 10 VCO group (1.06) *versus* the SD group (0.11), indicating the treatment's anti-inflammatory impact. Furthermore, VEGF levels increased markedly in the SD + 10 VCO group (0.92) *versus* the SD group (0.19), indicating enhanced angiogenesis (Fig. 4).

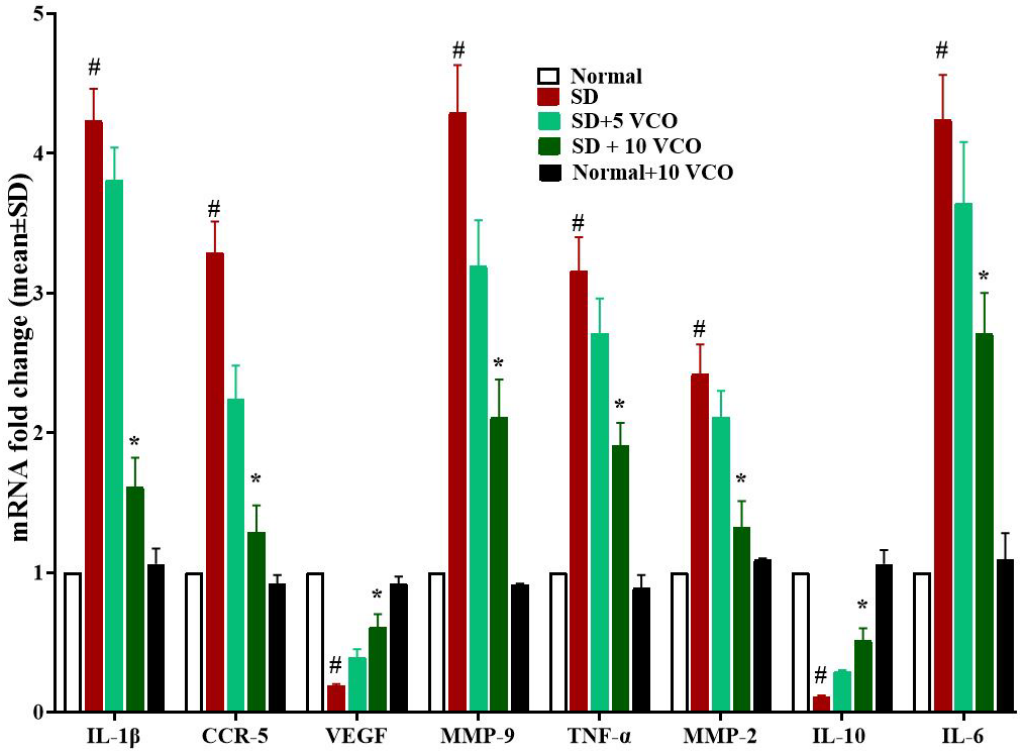

Fig. 4. IL-6, IL-1β, IL-10, TNF-α, MMP-9, MMP-2, CCR-5, and VEGF genes expression (means ± SD; n=10/group) in experimental groups. # p < 0.05 indicates a significant difference between the normal and SD groups; * $p < 0.05$ indicates significant differences among the SD + 5 VCO and SD + 10 VCO groups compared to the SD groups.

Role of VCO and SD in regulating skin CCR-5, MMP-2, MMP-9, and VEGF protein expression

The expression levels related to key proteins associated with inflammation and tissue remodeling were significantly altered across the experimental groups. The SD group exhibited elevated levels of CCR-5 (2.51), MMP-9 (4.29), and MMP-2 (4.21) *versus* the normal group ($p<0.05$). Receiving 5 VCO and 10 VCO resulted in significant reductions in their expression. The SD + 10 VCO group demonstrated a marked decrease in CCR-5 (1.21) and MMP-9 (1.45), indicating a reduction in inflammatory signaling pathways ($p<0.05$). Additionally, MMP-2 concentrations were significantly lower in the SD + 10 VCO group (0.91) in comparison to the SD group (4.21), further supporting the anti-inflammatory effects of the treatment. VEGF expression also indicated a significant increase in the SD + 10 VCO group (1.07) in comparison to the SD group (0.16), suggesting increased angiogenic activity in response to the treatment (Figs. 5a and 5b).

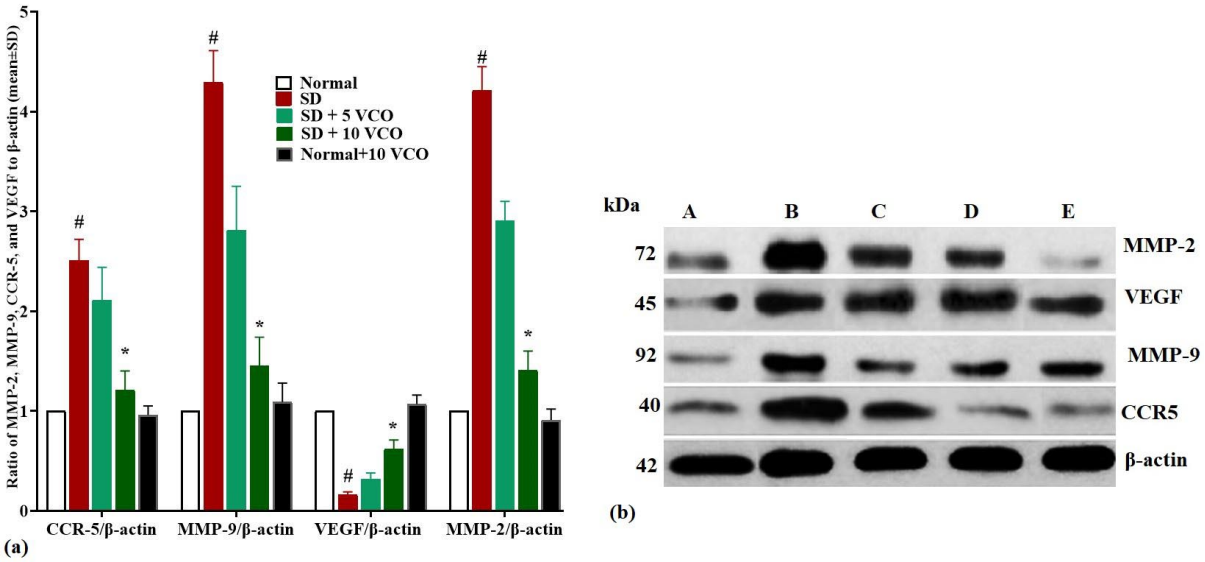

Fig. 5. MMP-9, MMP-2, CCR-5, and VEGF proteins expression (means ± SD; n=10/group) in normal (A), SD (B), SD + 5 VCO (C), SD + 10 VCO (D), and normal + 10 VCO (E). # $p < 0.05$ indicates a significant difference between the normal and SD groups; * $p < 0.05$ indicates significant differences among the SD + 5 VCO and SD + 10 VCO groups compared to the SD groups.

DISCUSSION

VCO has demonstrated significant potential in suppressing inflammatory pathways and suppressing the activity of MMPs, leading to the preservation of skin structure from damage due to SD in our study utilizing a DNFB model. The SD development is multifactorial, affected by Malassezia fungal colonization, an abnormal immune reaction, and oxidative stress (OS). These factors cause the ROS generation, which overwhelm the antioxidant defenses of the skin and cause cellular damage. OS can exacerbate inflammation and disrupt skin barrier function in SD (Papaccio *et al*., 2022). Evidence from both human and animal studies indicates that SD is associated with elevated serum and tissue MDA concentrations and a corresponding decrease in antioxidant capacity. Furthermore, the inhibition of endogenous antioxidant enzymes during SD causes apoptosis in epidermal cells and an increase in the thickness of the stratum corneum (Nakai & Tsuruta, 2021).

The inflammatory response in SD is driven by various immune cells, like macrophages, lymphocytes, and neutrophils, which release cytokines and chemokines that sustain inflammation. Macrophages are linked to this process by secreting pro-inflammatory cytokines, like IL-6, IL-1β, and TNF-α, amplifying the inflammatory reaction (Tomaszewska *et al*., 2020). Lymphocytes, particularly T-helper cells (Th1 and Th17), further exacerbate the condition by producing cytokines such as IFN-γ and IL-17. The typical cytokine profile in SD is characterized by high IL-1β, TNF-α, IL-6, and IL-8 concentrations, contributing to chronic inflammation, while inhibiting anti-inflammatory cytokines like IL-10 impedes inflammation resolution. Increased apoptosis in keratinocytes has been observed within SD lesions, likely due to OS and cytokine activity (Liu *et al*., 2023). Our findings corroborate these observations, revealing significant alterations in total

antioxidant capacity, thiol levels, and lipid peroxidation, which show the onset of OS and subsequent acceleration of the inflammatory process.

MMPs, particularly MMP-2 and MMP-9, are crucial enzymes involved in extracellular matrix remodeling and repair, playing significant roles in inflammation and wound healing. Elevated levels of these MMPs in SD contribute to extracellular matrix degradation and inflammation, exacerbating skin damage (Abotorabi *et al*., 2020). Human studies have shown increased MMP expression in SD lesions, correlating with heightened inflammation and tissue destruction. Similarly, animal models indicate that MMP overexpression compromises skin integrity and promotes chronic inflammation (Oliveira *et al*., 2019). Additionally, the upregulation of CCR-5, a chemokine receptor involved in immune cell migration, contributes to the chronic inflammatory state in SD. Targeting CCR-5 has been suggested as a potential therapeutic strategy to mitigate inflammation and improve skin lesions (Li *et al*., 2025). Both MMPs and CCR-5 are integral to the pathogenesis of SD, highlighting their roles in inflammation and tissue damage as potential therapeutic targets.

Furthermore, our study found that levels of VEGF were suppressed in SD lesions, contributing to increased vascularization and inflammation. Elevated VEGF expression is associated with enhanced inflammatory responses and epidermal hyperplasia (Xian *et al*., 2019). In animal studies, upregulation of VEGF mimics the pathological features of SD, including increased vascular permeability and inflammation. Targeting VEGF or its signaling pathways may provide therapeutic benefits by reducing inflammation and limiting excessive blood vessel growth in SD lesions (Jiang *et al*., 2020). Understanding the role of VEGF in SD could lead to novel treatments aimed at enhancing skin health through modulation of VEGF activity.

In conclusion, our findings indicate that VCO effectively mitigates the inflammatory processes associated with SD by reducing pro-inflammatory cytokines and MMP activity while enhancing antioxidant defenses. The oil's rich composition of bioactive compounds, including fatty acids, phytosterols, and polyphenols, underpins its therapeutic efficacy. These compounds not only provide moisturizing and anti-inflammatory benefits but also protect the skin from oxidative stress and promote healing. The results of this study suggest that VCO holds promise as a complementary treatment for seborrheic dermatitis, warranting further investigation in human clinical trials and other animal models to fully elucidate its therapeutic potential.

CONCLUSION

This study underscores the significant potential of VCO as a therapeutic agent in managing SD. Our findings demonstrate that VCO effectively mitigates the inflammatory processes associated with SD by significantly reducing the expression of pro-inflammatory cytokines such as IL-6, IL-1b, and TNF-a, while enhancing levels of the anti-inflammatory cytokine IL-10. Furthermore, VCO treatment resulted in a marked decrease in MMP-2 and MMP-9 and CCR-5, which are critical mediators of inflammation and tissue degradation. The histopathological and molecular analyses revealed that VCO not only restored skin structure but also improved the overall antioxidant status, as indicated by increased total antioxidant capacity and decreased lipid peroxidation. The dose-dependent response observed, particularly at 10 mg/kg, highlights the efficacy of VCO in reversing the oxidative stress and inflammatory changes induced by DNFB. Given the multifactorial nature of SD, characterized by abnormal immune responses, oxidative stress, and fungal colonization, VCO's diverse bioactive compounds—including fatty acids, phytosterols, and polyphenols—provide a multifaceted approach to treatment. These components contribute to the oil's anti-inflammatory, moisturizing, and skin barrier-enhancing properties, making VCO a promising natural remedy for SD. In conclusion, our research supports the use of VCO as a complementary treatment for seborrheic dermatitis, advocating for further clinical trials to validate its efficacy and explore its mechanisms of action in human subjects. The potential for VCO to improve skin health and alleviate the symptoms of SD presents an exciting avenue for future therapeutic strategies.

Ethical Approval. The experimental protocols of this study were approved by Xi'an Central Hospital ethics committee.

GAO, R. & LI, D. Potencial del aceite de coco virgen para el tratamiento de la dermatitis seborreica: Perspectivas de un modelo de rata-dinitrofluorobenceno mediante análisis bioquímicos, moleculares e histopatológicos. *Int. J. Morphol., 43(5)*:1692-1701, 2025.

RESUMEN: El aceite de coco virgen (ACV) se ha utilizado tradicionalmente con fines medicinales, como para aumentar la fuerza y controlar el sangrado. Su uso prolongado se basa en sus propiedades antibacterianas, antiinflamatorias y antiseborreicas. Esta investigación tuvo como objetivo investigar los mecanismos subyacentes a los efectos antiinflamatorios y antiseborreicos del ACV, centrándose especialmente en el recrecimiento capilar y la dermatitis seborreica (DS), examinando su capacidad para reducir la producción de sebo y prevenir la caída del cabello. El estudio involucró a 50 ratas Wistar divididas en cinco grupos (n=10 cada

uno): un control normal, un grupo inducido con 0,6 % de dinitrofluorobenceno (DNFB) a 3 mg/kg, un grupo DNFB tratado con 5 mg/kg de ACV, un grupo DNFB tratado con 10 mg/kg de ACV y un grupo normal tratado con 10 mg/kg de ACV. Al final del experimento, analizamos el tejido cutáneo para determinar los niveles de citocinas inflamatorias clave (IL-6, IL-1β, IL-10 y TNF-α), junto con marcadores de estrés oxidativo como la capacidad antioxidante total y la peroxidación lipídica. También evaluamos la expresión de genes y proteínas, incluyendo MMP-9, MMP-2, CCR-5 y VEGF, en el tejido cutáneo. Nuestros hallazgos revelaron que la administración de DNFB indujo alteraciones significativas en el estado antioxidante, biomarcadores inflamatorios y la expresión de genes y proteínas relacionados con SD. El tratamiento con ACV mostró una mejoría dependiente de la dosis, especialmente con 10 mg/kg, revirtiendo estos cambios. Los análisis histopatológicos y moleculares demostraron la restauración estructural del tejido cutáneo tras la intervención con ACV. En conclusión, este estudio destaca el prometedor potencial del ACV para mitigar los síntomas de la dermatitis seborreica y modular la inflamación cutánea, lo que sugiere su posible uso en el tratamiento de la dermatitis seborreica inducida por DNFB.

PALABRAS CLAVE: Aceite de coco virgen; Inflamación; Apoptosis; Antiseborreico.

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