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Exploring the Anti-Seborrheic Dermatitis Effects of Apricot Kernel Oil Using the Rat–Dinitrofluorobenzene Induced Model: An Investigation Using Biochemical, Molecular. and Histopathological Methods

Exploración de los Efectos Antiseborreicos del Aceite de Semilla de Albaricoque Utilizando el Modelo Inducido por Rata-Dinitrofluorobenceno: Una Investigación através de Métodos Bioquímicos, Moleculares e Histopatológicos

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SUMMARY: Apricot Kernel Oil (AKO) is utilized for medicinal purposes to enhance strength and control hemorrhage. AKO has a long history of use due to its antibacterial, anti-inflammatory, and anti-seborrheic effects. This study aimed to explore the underlying mechanisms of AKO's anti-inflammatory and anti-seborrheic properties, particularly regarding hair restoration and seborrheic dermatitis (SD), through its sebum-suppressing effects and its role in preventing hair loss. We conducted our research on 50 Wistar rats, which were divided into five groups (n=10/group): a normal group, a group induced with 0.5 % dinitrofluorobenzene (DNFB) ointment at 2 mg/kg, a DNFB group treated with 200 and 400 mg/kg of AKO ointment, and a normal group treated with 400 mg/kg of AKO ointment. At the end of the study, we measured tissue levels of key inflammatory cytokines (IL-6, IL-1β, IL-10, and TNF-α) and various oxidative stress parameters, including the total antioxidants capacity and lipid peroxidation levels. Additionally, we assessed the expression of MMP-9, MMP-2, CCR-5, and VEGF at both gene and protein levels in skin tissue. Results showed that DNFB administration caused significant changes in tissue antioxidant levels, inflammatory markers, and the expression of genes and proteins associated with SD in the skin. AKO treatment demonstrated dose-dependent effects in alleviating these changes especially at the 400 mg/kg. Histopathological and molecular evaluations indicated structural improvements in skin tissue with AKO treatment. In conclusion, this study underscores the potential of AKO to provide anti-SD benefits in DNFB-induced conditions.

KEY WORDS: Apricot Kernel Oil; Inflammation; Apoptosis; Cerebral hemorrhage.

INTRODUCTION

Seborrheic dermatitis (SD) is a chronic inflammatory skin condition affecting areas rich in sebaceous glands, such as the scalp, face, and chest. Affecting 3-10 % of the population, it is more common in males and individuals with certain neurological disorders or compromised immune systems. SD is characterized by flaky, erythematous skin and is linked to factors like genetic predisposition, stress, hormonal changes, and the presence of Malassezia yeast. Recent studies highlight the crucial role of Malassezia species, which metabolize skin lipids, triggering an inflammatory response that is exacerbated by an imbalance in pro- and anti-inflammatory cytokines (Clark *et al*., 2015). The immune response involves T-helper cells, particularly Th1 and Th17, contributing to chronic inflammation. Additionally, SD is associated with impaired skin barrier function, leading to increased susceptibility to irritants. Emerging treatments include biologics targeting specific cytokines (e.g., IL-17, IL-23), and novel topical agents addressing inflammation and barrier dysfunction (Cohen *et al*., 2020). Probiotics and prebiotics are also being explored to restore skin microbiome balance. Future research is focusing on the

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broader role of the skin microbiome and personalized medicine approaches to better manage SD. These advances hold promise for more effective long-term treatment strategies, particularly for severe or recurrent cases of this common skin disorder (Lin *et al*., 2021).

Dinitrofluorobenzene (DNFB) is a potent hapten extensively used in immunological research to study skin sensitization, contact dermatitis, and immune responses. It binds to skin proteins, forming complexes that trigger immune recognition and subsequent inflammation, making it a common agent for inducing contact dermatitis in animal models (Tang *et al*., 2021). These models help elucidate the mechanisms of allergic contact dermatitis, particularly the roles of innate and adaptive immunity, including the activation of Langerhans cells and sensitized T cells. DNFB is also employed to explore inflammatory pathways, such as the production of pro-inflammatory cytokines like IL-1β, TNF-α, and IFN-γ, which are central to dermatitis development. Although DNFB is not directly used to model seborrheic dermatitis, its role in inflammation research provides insights applicable to various inflammatory skin conditions, including seborrheic dermatitis (Guo *et al*., 2018). Studies have compared DNFB-induced dermatitis with seborrheic dermatitis to understand differing immune mechanisms, highlighting the relevance of cytokine and chemokine profiles in both conditions (Huang *et al*., 2024). DNFB models are instrumental in testing anti-inflammatory drugs, corticosteroids, and other therapies, offering potential applications for treating seborrheic dermatitis through immune modulation. Recent research has highlighted the potential of plant extracts and polyphenols in managing SD due to their anti-inflammatory, antioxidant, and antimicrobial properties. These compounds work by modulating the immune response, reducing oxidative stress, and inhibiting the growth of Malassezia yeast, which is implicated in SD pathogenesis (Chen *et al*., 2020a). Key plant extracts studied include green tea, *Centella asiatica* L., *Kochia scoparia* L., *Triticum aestivum* L., *Ginkgo biloba* L., and effective herbal ingredients such as resveratrol and curcumin (Lee *et al*., 2018; Lee *et al*., 2020). Clinical studies have shown that topical applications of these extracts can significantly reduce symptoms such as redness, scaling, and itching, often with fewer side effects compared to conventional treatments. Some studies have even found that plant extract-based treatments can be as effective as standard therapies, like antifungal agents, and may provide synergistic benefits when combined with other natural or synthetic agents (Lin *et al*., 2020). Mechanistic studies reveal that these natural compounds influence signaling pathways involved in inflammation, such as NF-κB and MAPK, and affect gene expression related to skin barrier function and oxidative stress (Chen *et al*., 2020b).

Apricot kernel oil (AKO), extracted from *Prunus armeniaca* L. seeds, is highly regarded for its rich composition of fatty acids, vitamins A and E, and antioxidants, making it a valuable natural remedy for various skin conditions in both humans and animal models (Pawar & Nema, 2023). Traditionally, AKO has been used to treat cardiovascular disorders, diarrhea, anorexia, and skin lesions. LC-ESI-MS/MS analysis has identified a range of bioactive compounds in AKO, including isoflavonoids like daidzein and luteolin, flavonoids such as apigenin and quercetin, saponins, carotenoids, essential fatty acids, anthocyanins, and amygdalin derivatives. The high levels of oleic and linoleic acids in AKO are essential for maintaining skin barrier function and hydration (Lin *et al*., 2017). Its anti-inflammatory, moisturizing, and antioxidant properties have been shown to alleviate inflammatory skin conditions such as eczema and psoriasis, promote wound healing, and offer anti-aging benefits by protecting the skin from oxidative damage (Pawar & Nema, 2023). In animal studies, AKO has proven effective in treating dermatitis, enhancing skin regeneration, and improving skin barrier function. It is generally well-tolerated, with minimal risk of irritation, making it suitable for sensitive skin, including that of infants and individuals with compromised skin barriers (Lin *et al*., 2017). However, despite its widespread use in cosmetics and traditional medicine, clinical research specifically on AKO's effects on skin diseases is limited, with most evidence being anecdotal. Comparative studies often indicate that AKO performs as well as or better than other natural oils in moisturizing and reducing inflammation. Further research is needed to explore its potential in treating conditions like atopic dermatitis, its antimicrobial properties, and the benefits of combining it with other agents for enhanced therapeutic effects. AKO exhibits anti-inflammatory and anti-apoptotic effects by inhibiting interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), leading to downregulation of nuclear factor kappa B (NF-κB) and modulation of the apoptotic pathway via MAPK and PI3K-PKB/Akt signaling pathways (Hyun *et al*., 2019). Flavonoids in AKO, such as apigenin, quercetin, and daidzein, primarily reduce inflammation and pain related to dermatitis by inhibiting cyclooxygenase-2 and lipoxygenase, key enzymes in the inflammatory cascade (Karuppagounder *et al*., 2016; Beken *et al*., 2020). This study investigates the anti- SD effects of AKO using a DNFB-induced rat model, employing biochemical, molecular, and histopathological methods.

MATERIAL AND METHOD

Preparation of AKO. Fresh apricot kernels (8000 grams) were first dried at 30 °C in a dark environment, as verified by a botanist. The dried kernels were then ground into a powder using a soil grinder. This powder was mixed with a

solvent blend of ethanol and acetone (50:50 v/v) and incubated at 40 °C for 48 h. The mixture was filtered through a paper filter and subsequently concentrated using a rotary evaporator. The final ointment, weighing 415 g, was stored at 4 °C. An allergy test was conducted on 5 rats, with a screening period of 48 h under study conditions (Kawahara *et al*., 2017).

Experimental design. Fifty male Wistar rats, each weighing approximately 210±10 g, were randomly divided into five groups of ten rats each. Before starting the study, the rats were given a 72-hour acclimation period to adapt to the experimental conditions, including temperature, food, and water. The animals were housed in propylene cages maintained at 25±4 °C with a relative humidity of 40±3 %, and were kept on a 12-hour light/dark cycle. They had unrestricted access to standard food pellets and tap water. All procedures related to the rats' care and euthanasia were conducted under the supervision and approval of the ethics committee at Chang'an Hospital, adhering to established guidelines for laboratory animal care. SD was performed by applying 100 µl of a 0.5 % dinitrofluorobenzene (DNFB, Sigma, US) ointment, mixed with 38 % olive oil, 20 % acetone, and 5 % DMSO (v/v/v), to the inner surfaces of both ear lobes and both shaved inguinal regions on day 1. On day 20, skin-fold thickness at these sites was measured with a micrometer (Schnelltester, System Kröplin) immediately before the challenge, and again 8 and 24 h after the challenge. An increase in thickness was used to gauge inflammatory swelling. The rats were sedated with isoflurane for ease of handling. The control group received a cutaneous application of 0.5 ml PBS, while the SD group was treated with 0.5 % DNFB. For the co-treatment groups, SD rats were administered 200 or 400 mg/kg of AKO ointment topically. Additionally, the healthy+400 AKO group received 400 mg/kg of AKO ointment topically. The allergy test aimed to determine the effective, non-toxic dose of AKO, with daily administration at 9 am for 20 days based on preliminary studies and existing research on collagenase and AKO (Tian *et al*., 2016; Karaboga *et al*., 2018).

Stereological assesments of skin layers. For evaluating the anti- SD effects of the AKO ointment, the rats were anesthetized with an intraperitoneal injection of 0.5 ml diazepam (50 mg/ml). Skin tissues were then carefully excised and each specimen was fixed in 10 % formalin. After fixation, the tissues were embedded in paraffin and sectioned into 5 mm thick slices using a microtome (Model No. SM2010RV1.2, LEICA, Germany). The sections were subsequently deparaffinized and rehydrated through an ethanol gradient. Hematoxylin and eosin (H&E) staining was applied to the sections for histological examination. Mallory Trichrome staining is used to differentiate muscle from collagen fibers in skin tissue sections. First, skin tissue is fixed in Bouin's solution for at least 24 h, followed by thorough rinsing with running tap water to remove excess fixative. The tissue is then dehydrated through a graded ethanol series (70 %, 95 %, and 100 % ethanol) and cleared in xylene before being embedded in paraffin wax. Sections are cut at 4-6 µm thickness using a microtome. The sections are deparaffinized in xylene for 2-3 min (with two changes) and rehydrated through a graded ethanol series (100 %, 95 %, and 70 %) for 2-3 min each. They are then stained with Weigert's Iron Hematoxylin solution for 10 min, followed by Acid Fuchsin for 2-5 min, and rinsed in distilled water. The sections are differentiated in Phosphomolybdic acid solution for 5-10 min and stained with Aniline Blue for 5-10 min. Finally, the sections are mounted on glass slides and dried. Histological analysis is performed using a light microscope at ×400 magnification, with images captured using a BX61TRF light microscope system (Olympus, Japan) and analyzed with ImageJ software. To assess the average thickness of various skin layers and structures, including the epidermal (both stratum corneum and cellular layers), dermal, and hypodermal layers, measurements were obtained using a BX61TRF light microscope fitted with a KEcam (KEcam Technologies, Nigeria) and Top View software (Version 3.7) (Fig. 1) (Tabaei *et al*., 2020).

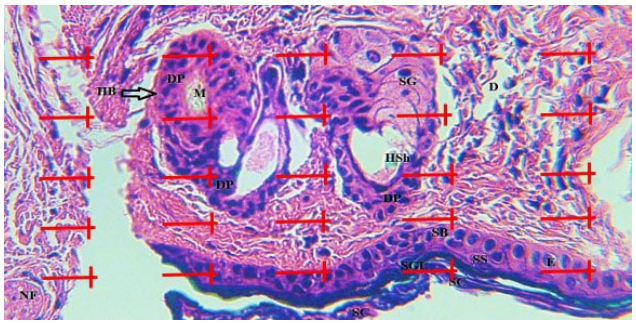

Fig. 1. Line probe (25 lines) to estimate the surface density of the skin structures (b). The number of lines located on each structure (∑p), the length of each of the lines in the probe is given by linear magnification (l/p) and the number of lines located on the internal part of structures (Sl). Finally, 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. SG: Stratum granulosum, SB: Stratum basale, E: Epidermis layer, D: Dermis layer, DP: Dermal papillae, Stratum corneum (SC), Stratum spinosum (SS), Stratum basale (SB), Dermis (Papillary) (DP), Neural fillament (NF), Hair body (HB), Hair medulla (M), Dermis (Reticularis) (DR), SG: Sebaceous gland, Dermal papillae (DP), Excretory ducts of the sweat glands (SGL).

Skin tissue total antioxidant capacity (TAC). To evaluate the total antioxidant capacity (TAC) in skin tissue, we utilized the ferric reducing antioxidant power (FRAP) assay. First, skin tissue from peri-ovarian fat was homogenized.

Following this, 100 µl of the homogenized tissue was mixed with 200 µl of cold PBS and transferred to a 2 ml polyethylene tube. Next, 10 µl of FRAP solution was added to the mixture, which was then incubated at 25 °C for 15 min. After incubation, the mixture was centrifuged at 12,000 g for 10 min, and the absorbance was measured at 593 nm (Gupta *et al*., 2016).

Skin tissue lipid peroxidation levels. To measure lipid peroxidation levels in skin tissue, we used the thiobarbituric acid reactive substances (TBARS) assay. Initially, 100 µl of homogenized skin tissue was placed in a 2 ml polyethylene tube. To this, 100 µl of TBARS solution was added. The mixture was then incubated at 37 °C for 30 min, after which the absorbance was measured at 593 nm (Badawi, 2022).

Skin tissue thiol levels. To measure thiol levels in skin tissue, a key indicator of tissue antioxidant capacity, we mixed 100 µl of homogenized skin tissue with 20 µl of 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB). This mixture was incubated at 37 °C for 15 min and then centrifuged at 12,000 *g* for 5 min. The absorbance of the resulting supernatant was assessed at 412 nm using an ELISA reader (Badawi, 2022).

IL-6, IL-1β, IL-10, TNF-α, MMP-9, MMP-2, CCR-5, and VEGF genes expression. Total RNA was extracted using TRIzol Reagent (Invitrogen) and subsequently purified with the RNeasy Mini Kit (Qiagen), following the manufacturer's protocols (Ambion, China). Five micrograms of the purified RNA were reverse transcribed into cDNA using random primers. Quantitative real-time PCR was performed with SYBR Green (Vazyme, China) on a fluorescence real-time PCR system (ABI7900, CA, USA). The PCR conditions were set as follows: an initial incubation at 50 °C for 2 min, followed by a denaturation step at 95 °C for 10 min, then 40 cycles of amplification with 95 °C for 30 s and 60 °C for 30 s. Primer sequences used in this study are detailed in Table I. Gene expression levels were normalized to GAPDH, and relative expression levels were calculated using the threshold cycle (Ct) method, with ΔΔCt and fold change formulas applied.

Table I. Primer sequences.

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

The fold formula change = $2^{-\Delta\Delta CT}$; ΔΔCt = [(Ct sample - Ct GAPDH gene) - (Ct sample – Ct control)] (Hu *et al*., 2018).

Expression MMP-9, MMP-2, CCR-5, and VEGF proteins in skin with western blotting. The samples were lysed in RIPA buffer that contained protease and phosphatase inhibitors for 30 min. The protein concentration was assessed using a BCA protein assay kit (Beyotime, China). Then, 40 µg of protein was separated on a 10 % SDS–polyacrylamide gel through electrophoresis and transferred to a PVDF membrane. The membranes were closed with a closure solution [5 % (w/v) skim milk powder] for 1 h and then incubated overnight at 4 °C with the following antibodies: anti-MMP-9 (Cat. No. ab38898; 1:400), anti-MMP-2 (Cat. No. ab97779; 1:400), anti-CCR-5 (Cat. No. ab287959; 1:500), and anti-VEGF (Cat. No. ab51745; 1:500) (Abcam, UK). After three thorough washes with PBST, the membranes were exposed to the HRP-conjugated secondary antibody (1:5000) for 1 h. The bands were visualized using ECL (Amersham Pharmacia Biotech, Piscataway, NJ), and their intensity was quantified using Image J gel analysis software. All experiments were conducted three times (Chen *et al*., 2017).

Statistical analysis. Statistical analyses were performed using GraphPad Prism 7.0 (San Diego, CA, USA). Data are presented as mean ± SD and were analyzed using one-way analysis of variance (ANOVA). A p-value of less than 0.05 was considered statistically significant.

RESULTS

Stereological assesments of skin layers. In the stereological analysis of skin structures, the surface area density of sebaceous glands, hair follicles, blood vessels, and sweat glands was significantly reduced ($p < 0.05$), while interstitial structures were significantly increased ($p < 0.05$) in the SD group compared to the healthy group. However, treatment with 200 mg/kg of AKO (in the SD + 200 AKO group) significantly increased ($p < 0.05$) the area of sebaceous glands and hair follicles compared to the SD group.

Table II. The density of sebaceous glands, sweat glands, hair follicles, blood vessels, and interstitial structures in the skin (mm^2) was measured based on surface density

Body regions	Sebaceous gland	Sweat gland	Hair follicle	Blood vessels	Interstitial structures
Healthy	0.641±0.035	0.322±0.021	0.73±0.02	0.39±0.11	0.16±0.03
SD	0.142±0.034 [a]	0.110±0.019 [a]	0.29±0.09 [a]	0.18±0.03 [a]	0.34±0.04 [a]
Healthy+400 AKO	0.502±0.016	0.342±0.022	0.61±0.07	0.41±0.02	0.29±0.03
SD + 200 AKO	0.249±0.076 [b]	0.162±0.045	0.39±0.07 [b]	0.29±0.06	0.22±0.04
SD + 400 AKO	0.432±0.065 [b]	0.311±0.027 [b]	0.63±0.16 [b]	0.34±0.06 [b]	0.19±0.02 [b]

(SV) and reference volume. Data are presented as mean ± SD.

Furthermore, treatment with 400 mg/kg of AKO (in the SD + 400 AKO group) significantly increased ($p < 0.05$) the surface area density of sebaceous glands, hair follicles, blood vessels, and sweat glands, while significantly decreasing ($p < 0.05$) the interstitial structures compared to the SD group (Table II; Figs. 2a and 2b).

In the stereological analysis of skin layer thickness, the thickness of cellular epidermis and dermis was significantly reduced ($p < 0.05$), while corneal epidermis were significantly increased ($p < 0.05$) in the SD group compared to the healthy group. However, treatment with 200 mg/kg of AKO (in the SD + 200 AKO group) significantly increased ($p < 0.05$) the thickness of cellular epidermis and dermis compared to the SD group. Furthermore, treatment with 400 mg/kg of AKO (in the SD + 400 AKO group) significantly increased ($p < 0.05$) the thickness of cellular epidermis and dermis, while significantly decreasing ($p < 0.05$) the corneal epidermis compared to the SD group (Table III; Figs. 2a and 2b).

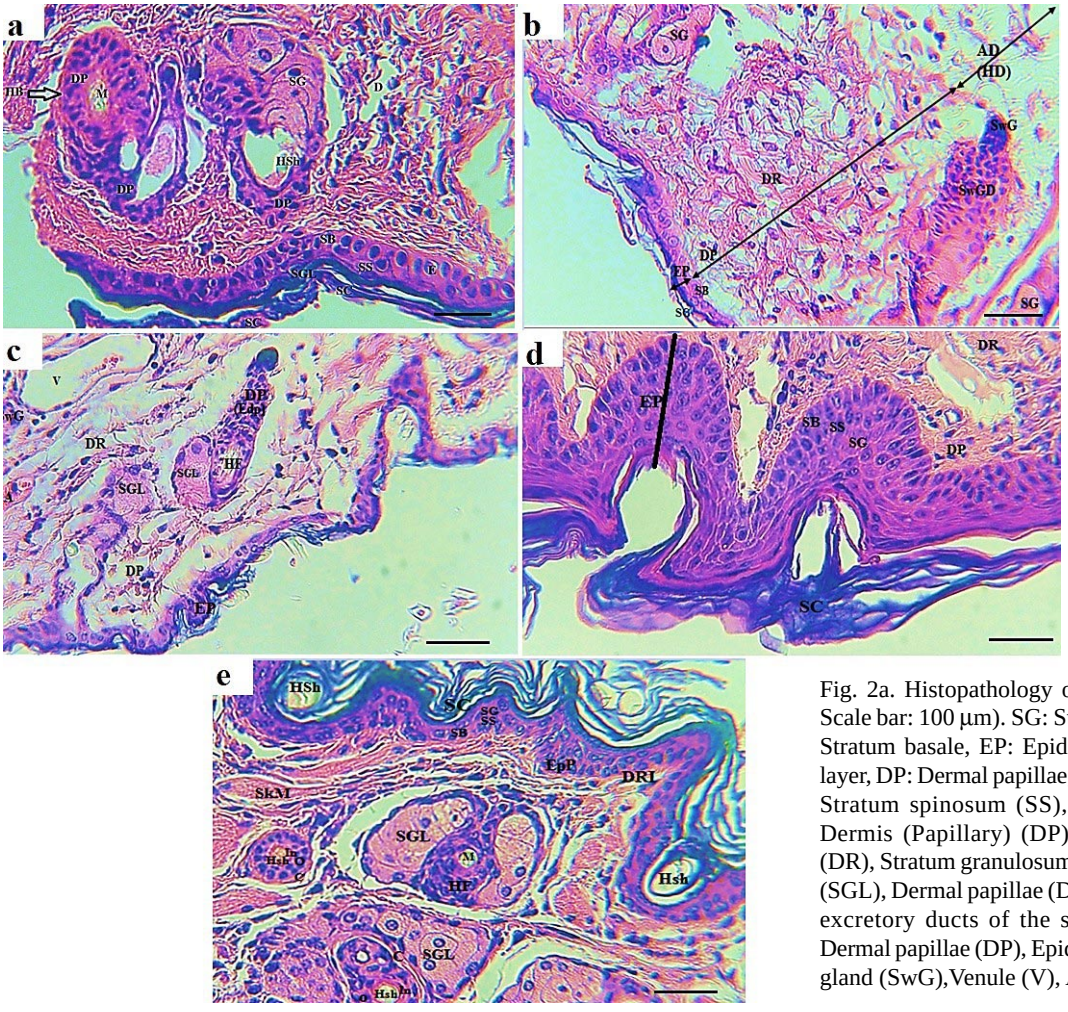

Fig. 2a. Histopathology of the skin (H&E, ¥40; Scale bar: 100 µm). SG: Stratum granulosum, SB: Stratum basale, EP: Epidermis layer, D: Dermis layer, DP: Dermal papillae, Stratum corneum (SC), Stratum spinosum (SS), Stratum basale (SB), Dermis (Papillary) (DP), Dermis (Reticularis) (DR), Stratum granulosum (SG), Sebaceous gland (SGL), Dermal papillae (DP), Sweat gland (SwG), excretory ducts of the sweat glands (SwGD), Dermal papillae (DP), Epidermal peg (Edp), Sweat gland (SwG),Venule (V), Arteriol (A).

Table III. Skin layer thicknesses (mm). Data are presented as mean ± SD.

Groups	Epidermal thickness		Dermal thickness	Total thickness
	Corneal	Cellular		
Healthy	3.4±1.9	28.2±2.9	401.1±16.6	493.4±41.6
SD	9.4±0.9 [a]	5.4±0.6 [a]	211.8±19.6 [a]	191.4±22.6 [a]
Healthy+400 AKO	3.8±1.6	29.3±5.3	421.14±79.2	429.2±44.1
SD + 200 AKO	7.6±0.6	10.2±2.1 [b]	235.4±12.3 [b]	228.9±19.7 [b]
SD + 400 AKO	4.9±1.1 [b]	24.8±5.8 [b]	393.1±23.6 [b]	461.6±23.1 [b]

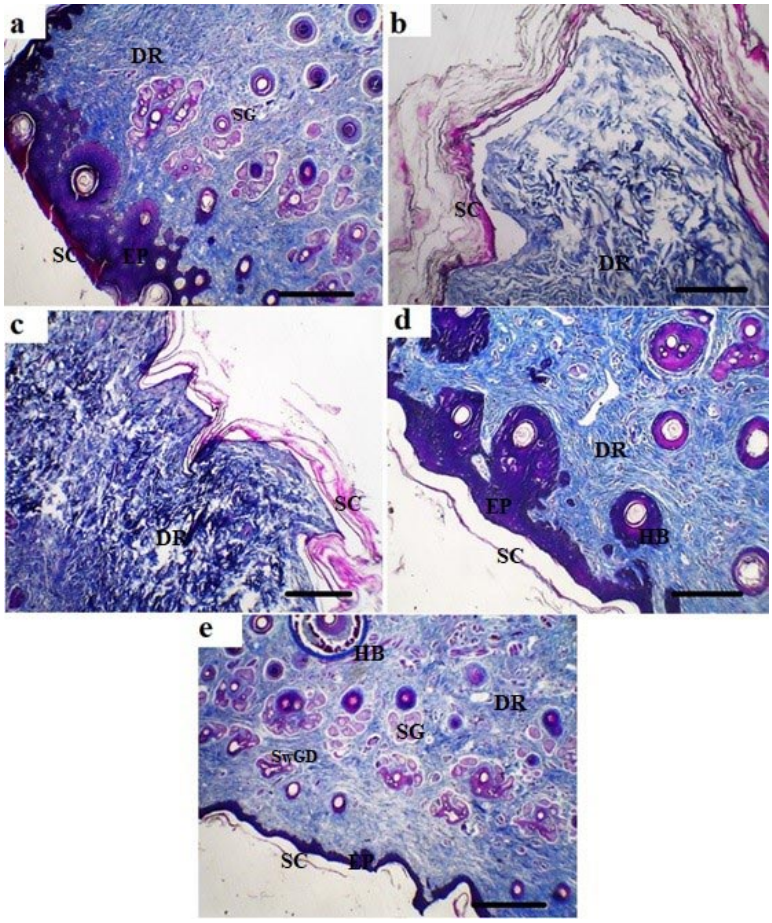

Fig. 2b. Histopathology of the skin (Mallory trichrome, ×40; Scale bar: 100 µm). EP: Epidermis layer, Stratum corneum (SC), Dermis (Reticularis) (DR), Stratum granulosum (SG), Sebaceous gland (SGL), Dermal papillae (DP), Sweat gland (SG), excretory ducts of the sweat glands (SwGD).

Effects of AKO and SD on skin thiol, FRAP, and TBARS levels. Thiol, FRAP, and TBARS levels were utilized as critical indicators of overall antioxidant capacity and lipid peroxidation in tissues. The results revealed that SD significantly ($p < 0.05$) reduced the levels of these markers compared to the healthy group. However, treatment with AKO, known for its potent antioxidant properties, resulted in a dose-dependent increase in these levels compared to the SD group. Notably, this increase was significant ($p < 0.05$) at the 400 mg/kg dosage (in the SD+400 AKO group) relative to the SD group (Fig. 3).

Effects of AKO and SD on expression of skin IL-6, IL-1b, IL-10, TNF-a, MMP-9, MMP-2, CCR-5, and VEGF genes. The analysis of gene expression associated with apoptosis and inflammatory pathways tissue showed that SD treatment led to a significant ($p < 0.05$) upregulation of TNF-α, IL-6, MMP-2, MMP-9, CCR-5, and IL-1β, alongside a significant ($p < 0.05$) downregulation of VEGF and IL-10 in skin tissue compared to the healthy group. In the SD+200 AKO group, there was a significant ($p < 0.05$) reduction in MMP-9 and CCR-5 expression compared to the SD group. The most substantial effects were observed in the SD+400 AKO group, where the expression of TNF-α, IL-6, MMP-2, MMP-9, CCR-5, and IL-1β significantly ($p < 0.05$) decreased, while VEGF and IL-10 expression significantly ($p < 0.05$) increased in comparison to the SD group (Fig. 4).

Effects of AKO and SD on expression of skin MMP-9, MMP-2, CCR-5, and VEGF proteins. The analysis of protein expression related to apoptosis and inflammatory pathways revealed that SD treatment significantly ($p < 0.05$) increased the levels of MMP-2, MMP-9, and CCR-5, while significantly ($p < 0.05$) decreasing VEGF expression in skin tissue compared to the healthy group. In the SD+200 AKO group, a significant ($p < 0.05$) decrease in CCR-5 expression and an increase in VEGF expression were observed compared to the SD group. The most pronounced effects were seen in the SD+400 AKO group, where MMP-2, MMP-9, and CCR-5 levels were significantly ($p < 0.05$) reduced, and VEGF expression was significantly ($p < 0.05$) elevated compared to the SD group (Fig. 5).

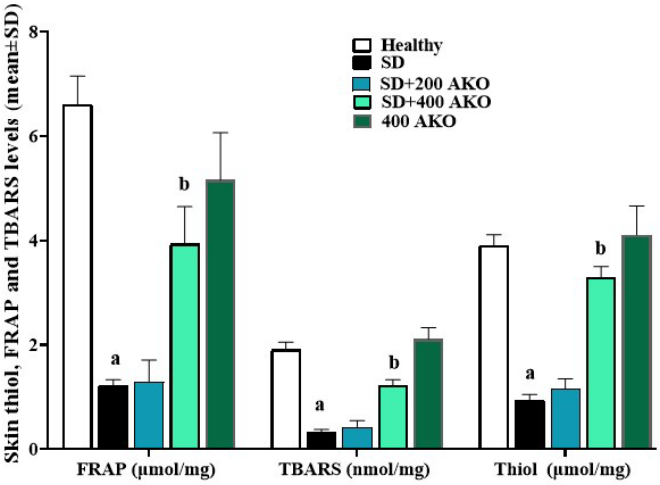

Fig. 3. Skin tissue levels of TBARS (nmol/mg) and FRAP (µmol/mg) (means ± SD; n=10/group) in experimental groups. a ($p<0.05$) SD vs. healthy groups; b ($p<0.05$) SD+200 and 400 AKO treated vs. SD groups.

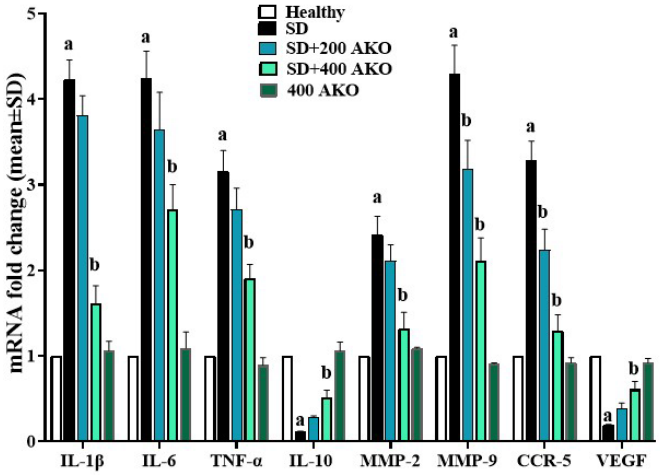

Fig. 4. IL-6, IL-1b, IL-10, TNF-a, MMP-9, MMP-2, CCR-5, and VEGF genes expression (means ± SD; n=10/group) in experimental groups. a ($p<0.05$) SD vs. healthy groups; b ($p<0.05$) SD+200 and 400 AKO treated vs. SD groups.

DISCUSSION

AKO effectively suppressed inflammatory pathways and inhibited the activity of matrix metalloproteinase, (MMPs) helping to preserve the structure of skin layers from SD damage induced by DNFB. SD development is influenced by factors such as an abnormal immune response, Malassezia fungal colonization, and oxidative stress, which generates reactive oxygen species (ROS) that overwhelm the skin's antioxidant defenses, leading to cellular damage. Studies show that oxidative stress exacerbates inflammation and skin barrier dysfunction in SD (Clark *et al*., 2015). Human and animal studies have shown that following SD, an increase in serum and tissue levels of malondialdehyde (MDA) and a decrease in antioxidant capacity occur. Also, during the inhibition of endogenous antioxidant enzymes following SD, the cells of the cellular layer of the epidermis undergo apoptosis and the thickness of the stratum corneum (acellular) increases (Cohen *et al*., 2020). Animal studies have shown that oxidative stress can trigger skin inflammation similar to SD, with inflammatory cytokines like TNF-a and IL-6 often being upregulated in response to oxidative damage, leading to increased skin irritation and immune responses. Inflammation in SD is driven by the activation of various immune cells, including macrophages, lymphocytes, and neutrophils, which release cytokines and chemokines that sustain the inflammatory response. Macrophages play a key role by secreting pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, amplifying the inflammation (Guo *et al*., 2018). Lymphocytes, particularly T-helper cells (Th1 and Th17), further exacerbate the condition by releasing cytokines like IFN-γ and IL-17. The cytokine profile in SD is typically pro-inflammatory, with elevated levels of TNF-α, IL-1β, IL-6, and IL-8 contributing to the chronic inflammation, while the suppression of anti-

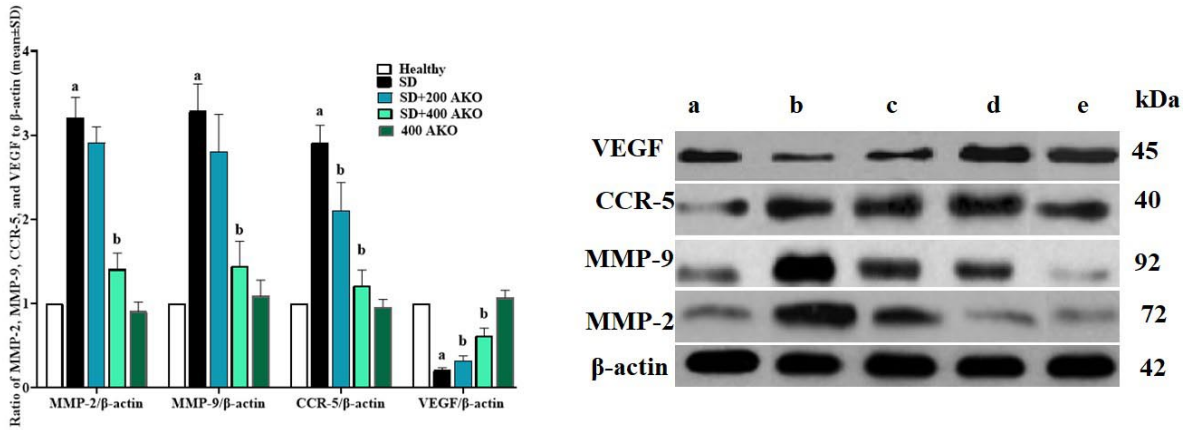

Fig. 5. MMP-9, MMP-2, CCR-5, and VEGF proteins expression (means ± SD; n=10/group) in healthy (a), SD (b), SD+200 AKO (c), SD+400 AKO (d), and 400 AKO (e). a ($p<0.05$) SD vs. healthy groups; b ($p<0.05$) SD+200 and 400 AKO treated vs. SD groups.

inflammatory cytokines like IL-10 may hinder the resolution of the inflammatory process. Increased apoptosis has been noted in keratinocytes within SD lesions, likely due to oxidative stress and cytokine activity (Huang *et al*., 2024). Animal models mirror these findings, showing that oxidative stress and inflammation can trigger skin changes resembling SD. The present study demonstrated that, following SD, there were changes in total antioxidant capacity (measured by FRAP levels), thiol levels, and lipid peroxidation (assessed by TBARS levels). These alterations indicate the onset of oxidative stress in tissues, which in turn accelerates the inflammatory process. Consequently, the levels of pro-inflammatory cytokines IL-6, IL-1β, TNF-α, and CCR-5 increased, while the level of the anti-inflammatory factor IL-10 decreased.

MMPs, including MMP-2 and MMP-9, are enzymes crucial for extracellular matrix remodeling and repair, playing roles in inflammation and wound healing. In SD, elevated levels of MMP-2 and MMP-9 contribute to extracellular matrix degradation and inflammation, exacerbating skin damage (Ertugrul *et al*., 2018). Human studies show increased MMP expression in SD lesions, correlating with heightened inflammation and tissue destruction. Animal models also reveal that MMP overexpression impairs skin integrity and promotes chronic inflammation (Szalus & Trzeciak, 2024). Additionally, CCR-5 (C-C chemokine receptor-5), a chemokine receptor involved in immune cell migration, is upregulated in SD, aiding in the recruitment and activation of inflammatory cells. This receptor's increased expression contributes to the chronic inflammatory state seen in SD. Studies in animal models suggest that targeting CCR-5 could mitigate inflammation and improve skin lesions (Zhou *et al*., 2024). Both MMPs and CCR-5 are integral to SD pathogenesis, with their elevated levels in human and animal studies highlighting their roles in inflammation and tissue damage, offering potential targets for therapeutic intervention. Research indicates that VEGF levels are suppressed in SD lesions in both human and animal models, contributing to increased vascularization and inflammation. Elevated VEGF expression is linked to enhanced inflammatory responses and epidermal hyperplasia (Samochocki *et al*., 2016). In animal studies, VEGF upregulation mimics SD's pathological features, including increased vascular permeability and inflammation. Targeting VEGF or its signaling pathways may offer potential therapeutic benefits by reducing inflammation and limiting excessive blood vessel growth in SD lesions (Kim, 2017). Understanding VEGF's role in SD could lead to new treatments aimed at improving skin health by modulating VEGF activity. In the current study, the levels of MMP-2 and MMP-9, which are involved in the pathogenesis of SD, were elevated. This increase was associated with a thickening of the stratum corneum and a reduction in the epidermal cell layer. Activation of TNF and IL-1β pathways occurs during the initiation of immediate/ early-phase reaction in SD leading to the cascade of other changes including IL-6-pathway activation (Zhao *et al*., 2017). Indeed, IL-6 expression was strongly up-regulated in our study. It has been shown that IL-6 is produced mainly by keratinocytes during the elicitation phase of SD. In addition to its role in hematopoietic progenitor cell expansion and synthesis of acute-phase response proteins, IL-6 can induce differentiation of monocytes to macrophages and terminal differentiation of B cells which can induce expression of CCR-5. Expression of VEGF was also strongly decreased in skin exposed to SD. These models help in understanding how VEGF contributes to the pathology of SD and the associated inflammation (Zhou *et al*., 2024).

AKO is a versatile oil rich in bioactive compounds, making it valuable in nutrition, cosmetics, and medicine. Its phytochemistry includes a high content of fatty acids, primarily oleic acid (60-70 %) and linoleic acid (20-30 %), which provide moisturizing and anti-inflammatory benefits. It also contains phytosterols like beta-sitosterol, which supports skin barrier function, and vitamins E and A, known for their antioxidant and anti-aging properties. AKO is also rich in polyphenols, including quercetin and catechins, which protect the skin from oxidative stress and UV damage. Additionally, it contains minor components like squalene, which enhances its moisturizing properties, and carotenoids like beta-carotene, which supports skin health (Pawar & Nema, 2023). A study on mice with induced skin wounds demonstrated that AKO accelerated wound healing and reduced inflammation. The oil promoted the re-epithelialization of wounds, likely due to its high concentration of fatty acids, vitamin E, and squalene, which together enhance cell proliferation and reduce oxidative stress (Lin *et al*., 2017). Additionally, AKO reduced the levels of inflammatory markers such as TNF-α and IL-1β in the wound area (Sharma *et al*., 2019). In this study, AKO effectively suppressed inflammatory pathways by reducing pro-inflammatory cytokines (IL-6, IL-1β, TNF-α, and CCR-5) and increasing anti-inflammatory cytokine (IL-10). In another mouse model, AKO was applied topically to skin exposed to UV radiation. The study found that AKO reduced UV-induced inflammation by decreasing the infiltration of inflammatory cells and the expression of COX-2, a key enzyme involved in the inflammatory response. This effect was attributed to the oil's antioxidant properties, particularly due to the presence of polyphenols like quercetin (Zhang *et al*., 2020). Tian *et al*. (2016), demonstrated that AKO, administered at doses of 200 and 400 mg/kg, effectively suppresses inflammatory pathways in rats treated with

cyclophosphamide. This suppression led to a decrease in interleukin (IL)-2, IL-12, and tumor necrosis factor-α (TNF-α) levels. Additionally, AKO reduced tissue levels of malondialdehyde, a marker of lipid peroxidation, and decreased the activities of endogenous antioxidant enzymes, including superoxide dismutase and glutathione peroxidase (Tian *et al*., 2016). A clinical study involving patients with mild to moderate atopic dermatitis found that AKO significantly improved skin hydration and reduced itching and redness after regular application over four weeks. The study suggested that the oil's fatty acid composition helps restore the skin barrier, while its anti-inflammatory properties reduce the symptoms of dermatitis (Ramadan *et al*., 2020). A small clinical study explored the effects of AKO on patients with psoriasis. Regular application of the oil led to a noticeable reduction in the severity of psoriasis plaques, with decreased redness, scaling, and itching. The anti-inflammatory effects were linked to the oil's ability to inhibit the expression of pro-inflammatory cytokines and improve skin barrier function (Lin *et al*., 2017). In studies focused on intestinal and colon epithelial tissues, Karaboga *et al*. (2018), and Minaiyan *et al*. (2014), found that AKO protects epithelial cells from damage by inhibiting inflammatory pathways and oxidative stress induced by ethanol and diethylnitrosamine. These studies revealed that AKO enhances angiogenesis in the submucosal area by increasing VEGF expression, which aids in the restoration of the parenchymal area and epithelial covering (Minaiyan *et al*., 2014; Karaboga *et al*., 2018). In the present study, AKO not only exhibited antioxidant and anti-inflammatory effects but also upregulated VEGF expression in the dermis, promoting the repair of the epidermal cellular layer and reducing the thickness of the stratum corneum. This effect also increased the surface density of blood vessels in the dermis. By inhibiting MMPs, AKO facilitated the differentiation of epidermal basal cells into glandular cells (sebaceous and sweat), contributing to the maintenance and formation of hair follicles. Conversely, in seborrheic dermatitis (SD), the surge of inflammatory factors and free radicals suppressed basal cell differentiation and noticeably reduced the surface density of skin glands (sebaceous and sweat) adjacent to hair follicles. The findings suggest that AKO, as a potential prodrug or complementary compound, may possess anti-SD properties, warranting further investigation in human clinical trials and other animal models of skin repair.

CONCLUSION

In conclusion, this study demonstrates the significant potential of AKO as an effective treatment for SD. Through a detailed investigation utilizing a DNFB-induced rat model, AKO exhibited potent anti-inflammatory, antioxidant, and anti-seborrheic properties. These effects were evidenced by the dose-dependent improvements in skin tissue structure, reduced inflammatory markers, and enhanced antioxidant capacity, particularly at the 400 mg/kg dosage. AKO effectively suppressed key inflammatory cytokines and modulated gene and protein expressions related to SD, such as MMP-9, MMP-2, CCR-5, and VEGF. The histopathological analysis further confirmed the restorative effects of AKO on the skin's structural integrity. Overall, these findings highlight AKO's therapeutic potential in managing SD and possibly other inflammatory skin conditions, advocating for further clinical research to explore its application in human dermatology.

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

LI, Y.; LIU, Y.; WANG, S. & LI, W. Exploración de los efectos antiseborreicos del aceite de semilla de albaricoque utilizando el modelo inducido por rata-dinitrofluorobenceno: una investigación utilizando métodos bioquímicos, moleculares e histopatológicos. *Int. J. Morphol., 43(2)*:564-573, 2025.

RESUMEN: El aceite de semilla de albaricoque (AKO) se utiliza con fines medicinales para mejorar la fuerza y controlar las hemorragias. El AKO tiene una larga historia de uso debido a sus efectos antibacterianos, antiinflamatorios y antiseborreicos. Este estudio tuvo como objetivo explorar los mecanismos subyacentes de las propiedades antiinflamatorias y antiseborreicas del AKO, en particular en relación con la restauración del cabello y la dermatitis seborreica (SD), a través de sus efectos seborrepresores y su papel en la prevención de la caída del cabello. Realizamos nuestra investigación en 50 ratas Wistar, que se dividieron en cinco grupos (n = 10/grupo): un grupo normal, un grupo inducido con ungüento de dinitrofluorobenceno (DNFB) al 0,5 % a 2 mg/kg, un grupo DNFB tratado con 200 y 400 mg/kg de ungüento AKO, y un grupo normal tratado con 400 mg/kg de ungüento AKO. Al final del estudio, medimos los niveles tisulares de citocinas inflamatorias clave (IL-6, IL-1β, IL-10 y TNF-α) y varios parámetros de estrés oxidativo, incluida la capacidad antioxidante total y los niveles de peroxidación lipídica. Además, evaluamos la expresión de MMP-9, MMP-2, CCR-5 y VEGF a niveles tanto de genes como de proteínas en el tejido cutáneo. Los resultados mostraron que la administración de DNFB provocó cambios significativos en los niveles de antioxidantes tisulares, marcadores inflamatorios y la expresión de genes y proteínas asociados con SD en la piel. El tratamiento con AKO demostró efectos dependientes de la dosis para aliviar estos cambios, especialmente con la dosis de 400 mg/kg. Las evaluaciones histopatológicas y moleculares indicaron mejoras estructurales en el tejido cutáneo con el tratamiento con AKO. En conclusión, este estudio subraya el potencial de AKO para proporcionar beneficios anti-SD en condiciones inducidas por DNFB.

PALABRAS CLAVE: Aceite de semilla de albaricoque; Inflamación; Apoptosis; Hemorragia cerebral.

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