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A Histological Evaluation of Pumpkin Oil Seeds Extract on Skin Wounds That Induced by TCA in Albino Rats

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ABSTRACT

Pumpkin (Cucurbita spp.) is a nutritionally rich plant whose seeds contain high levels of bioactive compounds such as unsaturated fatty acids, tocopherols, sterols, and polyphenols. These constituents contribute to its anti-inflammatory, antioxidant, and wound-healing potential, making pumpkin seed oil (PSO) a promising candidate for dermatological applications. This study aimed to investigate the histopathological effects of pumpkin seed oil on trichloroacetic acid induced skin injuries in male Albino rats. A total of 49 rats were randomly assigned into seven groups, including a negative control, a positive control, a corn oil vehicle control, and three PSO treatment groups receiving 25%, 50%, 100% concentrations of the oil and Mebo Group treated topically with Mebo ointment. Dermatitis was chemically induced using 40% trichloroacetic acid to simulate the clinical and histological features of atopic dermatitis. Topical applications were administered daily, and tissue samples were examined histologically using hematoxylin and eosin (H&E) and Masson's trichrome staining to assess epidermal integrity, inflammatory infiltration, necrosis, and adnexal structure preservation for a period of 30 days. demonstrated a dose-dependent therapeutic effect of PSO, with the 100% PSO group showing near-complete restoration of normal skin architecture, including intact epidermis, reduced inflammation, and preserved sweat and sebaceous glands. In contrast, the positive control and corn oil groups exhibited severe tissue damage and inflammatory infiltration. The 25% and 50% PSO groups showed partial recovery with variable histological improvements. These findings highlight the regenerative and anti-inflammatory potential of PSO, particularly at higher concentrations, and support its use as a natural topical agent for managing inflammatory skin conditions.

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INTRODUCTION

Pumpkin (Cucurbita spp.) is a widely cultivated plant valued for both its nutritional and medicinal properties. Traditionally used in folk medicine, its seeds are a rich source of oil and bioactive compounds that contribute to health-promoting effects. Pumpkin seeds are particularly rich in bioactive compounds, including unsaturated fatty acids (oleic, linoleic, linolenic), tocopherols (vitamin E),

phytosterols, and phenolic acids [1,2]. These constituents have been associated with reduced inflammatory responses, decreased oxidative stress, and stabilization of cell membranes processes vital for wound healing [3,4]. Among potential natural remedies, pumpkin seed oil (PSO) has gained attention for its promising combination of anti-inflammatory, antioxidant, and immunomodulatory properties [3,5].

The Skin, as the largest organ in the human body, functions as a vital barrier protecting internal tissues from pathogens, toxins, and physical damage. Wound healing is a sophisticated physiological process that aims to restore injured tissue's structural and functional integrity through tightly regulated phases of hemostasis, inflammation, proliferation, and remodeling [6]. Apart from its anti-inflammatory profile, pumpkin seed oil has demonstrated the ability to promote angiogenesis, enhance fibroblast proliferation, and support granulation tissue formation mechanisms that are fundamental for efficient tissue repair [7,8]. Furthermore, its antioxidant components such as tocopherols and carotenoids have been shown to reduce oxidative stress, a major contributor to chronic wounds and delayed healing in diabetic and elderly populations [1,9].

MATERIAL AND METHODS:

All procedures used in this study were reviewed and approved by the Scientific Committee of the Faculty of Veterinary Medicine, University of Kufa, Kufa, Iraq, in compliance with the **ethical principles** of animal welfare (Code: UK.VET.2025.2109).

Pumpkin Seed Oil Extraction

Pumpkin seeds (Cucurbita pepo) were ground into powder, and 100 g was mixed with 1,000 mL of 99.9% hexane. The mixture was stirred for 24 hours at 45°C, then filtered through gauze and Whatman No.1 paper. The filtrate was concentrated using a rotary evaporator at 40°C and stored in airtight refrigerated containers.

Animals

A total of 49 male albino rats, 12 weeks of age, weighing between 150 to200 grams were used for the study. The animals were placed in plastic cages, with seven rats per cage, and sawdust was used as bedding. The rats were kept and maintained at the University of kufa Animal House, Faculty of Science, at a temperature range of 23 ° C to 25° C and adequate ventilation under controlled environmental conditions. All rats were fed a standard commercial diet provided by the Green World Company, with available water ad libitum. Hair removal was done using a Braun® electric shaver, followed by a smooth, hair-free surface of a Venus® shave grinder. All procedural functions were performed in a sterile environment. The rats were assigned to random experimental groups and allowed a week before the experiment began. Appropriate biosecurity measures were followed during all laboratory procedures, including laboratory coats, disposable gloves and face masks. For anesthesia, each rotten intramuscular injection of a ketamine (0.05 mg/kg) and xylazine (0.1 mg/kg) mixture was mixture using an insulin syringe. After about five minutes, when anesthesia was effective, the back area of each rat was marked using a permanent marker to indicate the shaving area.

Experimental Design

A total of forty-tow (42) adult male Albino rats were used for induction of dermatitis by trichloroacetic acid (TCA). The animals were randomly divided into seven experimental groups, with seven rats (n = 7) in each group. One group served as the negative control and did not receive any treatment or undergo skin injury induction, thereby

representing the baseline physiological state. The remaining forty-two rats were subjected to chemically induced atopic dermatitis using trichloroacetic acid (TCA) to model inflammatory skin injury. After successful induction of dermatitis, the animals were allocated into five distinct treatment groups as follows:

- **Group 1:** Negative Control (C−): Rats with no TCA-induced skin injury and no treatment; serves as the baseline physiological reference.
- **Group 2:** Positive Control (C^+): Rats with TCA-induced dermatitis receiving no treatment; represents the model of untreated inflammation
- **Group 3:** Corn Oil Group: Rats with TCA-induced dermatitis treated topically with corn oil only used as a vehicle control.
- **Group 4:** Drug-Treated Group (Mebo): Rats with TCA-induced dermatitis treated with a standard topical wound healing agent (Mebo cream), The main ingredients of Mebo (Moist Exposed Burn Ointment), a herbal ointment, are beta sitosterol, sesame oil, and beeswax. In addition, it has carbohydrates, vitamins, fatty acids, and amino acids. The ingredients in the ointment are intended to produce an environment that prevents the growth of microorganisms, so preventing infection and accelerating the healing process in the afflicted region.
- **Group 5:** Pumpkin Seed Oil 25% Group: Rats with TCA-induced dermatitis treated with 25% extract of pumpkin seed oil.
- **Group 6:** Pumpkin Seed Oil 50% Group: Rats with TCA-induced dermatitis treated with 50% extract of pumpkin seed oil.
- **Group 7:** Pumpkin Seed Oil 100% Group: Rats with TCA-induced dermatitis treated with pure (100%) pumpkin seed oil extract.

All topical treatments were applied once daily under anesthesia and aseptic conditions for a predetermined period. Then, wounds are measured every three days to evaluate the wound-healing and anti-inflammatory efficacy of the pumpkin seed oil extract in a concentration-dependent manner, and to compare its therapeutic performance with vehicle and untreated controls.

Induction of Atopic Dermatitis

The application involved the topical administration of 3.5 cc of 40% TCA to the shaved dorsal skin of rats, maintained for five minutes per day over a five-day period. This approach reliably generated visible signs of dermatitis, including erythema, edema, vesiculation, crusting, and localized hair loss clinical hallmarks of AD. Behavioral indicators such as repeated scratching, grooming, and self-inflicted trauma were also monitored as functional indicators of discomfort and inflammation severity. In instances where the inflammatory response diminished prematurely, additional low-concentration applications were considered to maintain consistent barrier disruption throughout the experimental phase. This model provided a robust platform for evaluating the therapeutic effects of pumpkin seed oil and other treatment modalities on AD-like skin lesions.

Sample Collection

At the end of the induction phase, rats were anesthetized, and full-thickness skin biopsies were collected from the treated areas. Tissues were fixed in 10% buffered formalin for 12 hours, followed by formalin replacement for sample preservation prior to histological analysis.

Histopathological Study

Following euthanasia, skin tissue samples were collected and immediately fixed in 10% neutral-buffered formalin for 48 hours, Fixed tissues were trimmed into slices approximately 0.5 cm thick and placed in plastic cassettes for automated processing using a Histo-Line ATP700 tissue processor (Italy). Samples underwent dehydration, clearing, and infiltration steps, and were subsequently embedded in paraffin blocks using a HESTION TEC2800-C tissue embedding system (China). Histopathological analysis was conducted using hematoxylin and eosin (H&E) staining to assess epidermal integrity, inflammatory responses, glandular structures, and overall tissue architecture in the skin of treated and control rats.

RESULTS

The histological section of the skin from the negative control group shows normal architecture of all skin layers. The epidermis appears intact, stratified, and well-organized. The dermis is densely packed with collagen fibers and is devoid of any pathological alterations. The hypodermis shows normal adipose tissue distribution. Additionally, the sweat glands and sebaceous glands are preserved and structurally normal. These observations confirm a baseline healthy skin state Fig. 1.

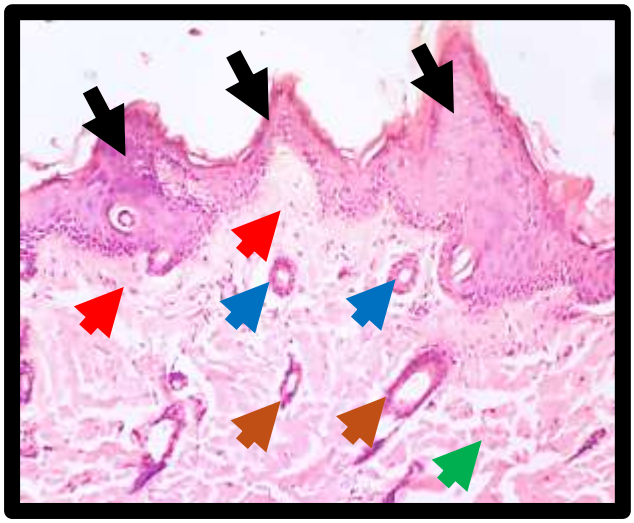

Fig. 1. The histological section of rat skin in the control negative group. The tissue is stained by H&E stain and the section is captured using digital camera and light microscope at 10 X magnifier scale. Black arrows: skin layer; Red arrows: derms; Green arrow: hypodermis; Blue arrows: sweat gland; Brown arrow: Sebaceous glands.

The positive control group, exposed to trichloroacetic acid-induced damage, demonstrated marked pathological changes. The epidermis shows severe degeneration and sloughing, indicating acute epithelial injury. The dermis exhibits dense infiltration of inflammatory cells, suggestive of a robust immune response. Necrosis is evident in the hypodermal tissue, pointing to tissue destruction extending deep into the skin layers. This image confirms the successful induction of skin lesions mimicking atopic dermatitis or chemical burns Fig. 2.

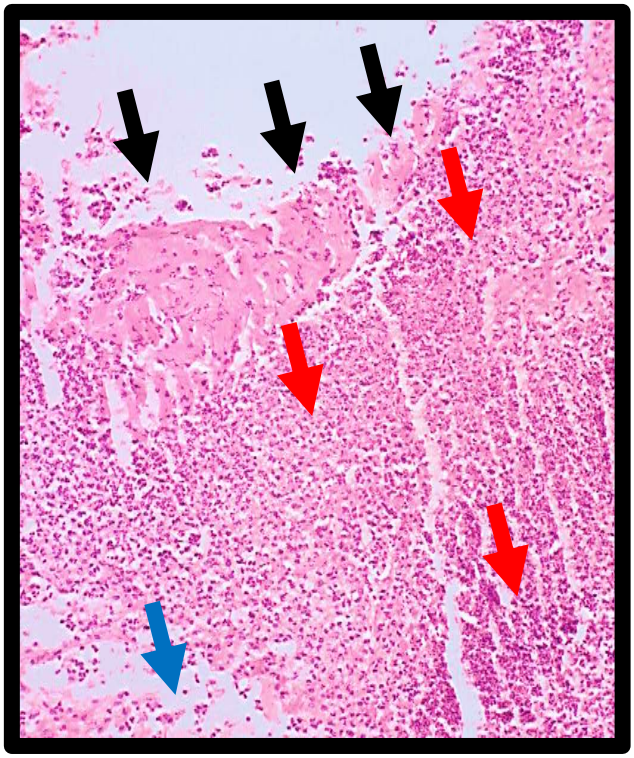

Fig. 2. The histopathological section of rat skin in the control positive group (Rats with TCA-induced dermatitis receiving no treatment). The tissue is stained by H&E stain and the section is captured using digital camera and light microscope at 10 X magnifier scale. Black arrows: skin layer; Red arrows: derms; Green arrow: hypodermis; Blue arrows: sweat gland; Brown arrow: Sebaceous glands

Similar to the positive control, the corn oil group exhibited severe epidermal loss and sloughing. Dense infiltration of inflammatory cells is observed in the dermis, and the hypodermis displays regions of necrosis. This suggests that corn oil did not exert any protective or therapeutic effect against the trichloroacetic-induced injury Fig. 3.

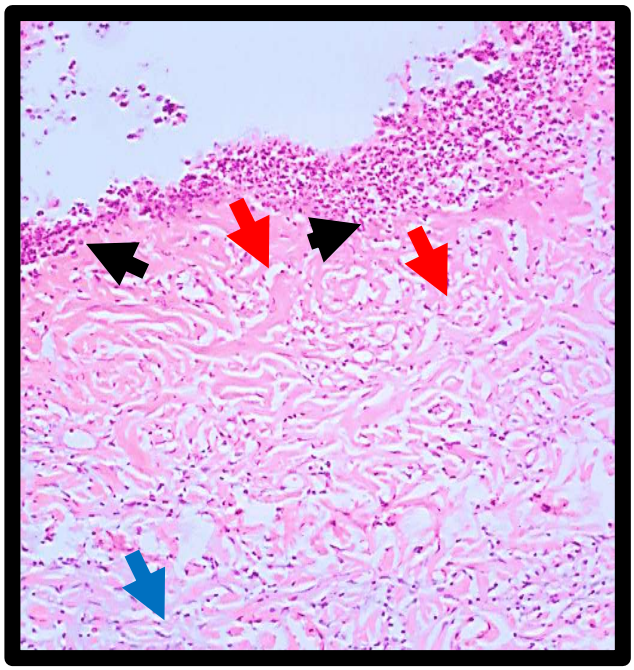

Fig. 3. The histopathological section of rat skin in the corn oil group (Rats with TCA-induced dermatitis treated topically with corn oil only). The tissue is stained by H&E stain and the section is captured using digital camera and light microscope at 10 X magnifier scale. Black arrows: epidermal skin layer; Red arrows: derms; Green arrow: hypodermis; Blue arrows: sweat gland; Brown arrow: Sebaceous glands.

This histopathological in fig. 4. represents a section of rat skin from the Mebo treatment group, which was administered to rats with trichloroacetic acid (TCA)-induced dermatitis. The histological architecture shown in this section strongly supports the efficacy of Mebo cream in promoting wound healing following chemically induced skin damage. Restoration of epidermal, dermal, and glandular structures suggests that Mebo® facilitates both anti-inflammatory and regenerative mechanisms, aligning with its known therapeutic profile in dermatological applications.

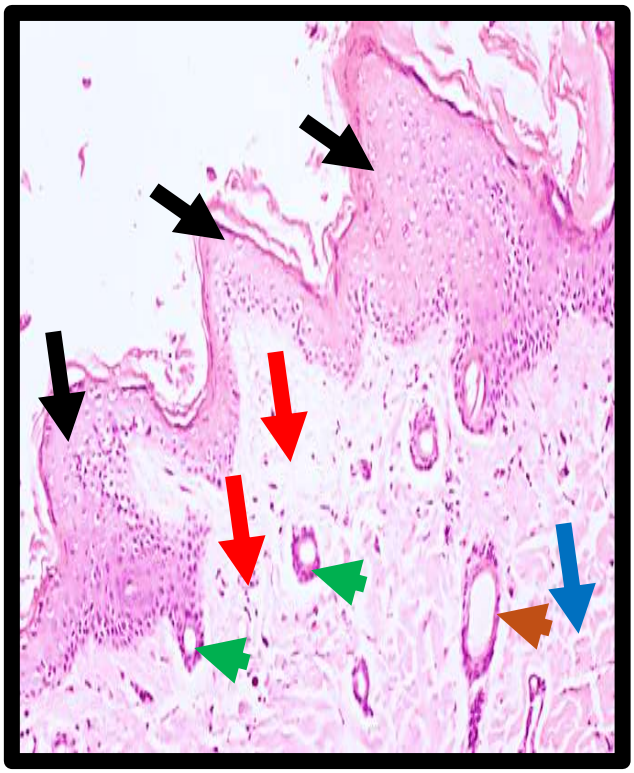

Fig. 4. The histopathological section of skin in rat in mebo treatment group (Rats with TCA-induced dermatitis treated with a standard topical wound-healing agent (Mebo® cream). The tissue is stained by H&E stain and the section is captured using digital camera and light microscope at 10 X magnifier scale. Black arrows: epidermal skin layer; Red arrows: derms; Green arrow: hypodermis; Blue arrows: sweat gland; Brown arrow: Sebaceous glands.

In the 25% treatment group, the epidermis is present but shows mild thickening, possibly due to reparative hyperplasia. The dermis contains areas of mild necrosis, while the hypodermis exhibits hyperemia, indicated by engorged blood vessels. Notably, inflammatory cell infiltration is present and sweat glands show mild-to-moderate hyperplasia. This suggests partial tissue recovery with mild inflammatory activity, representing a moderate therapeutic effect Fig. 5.

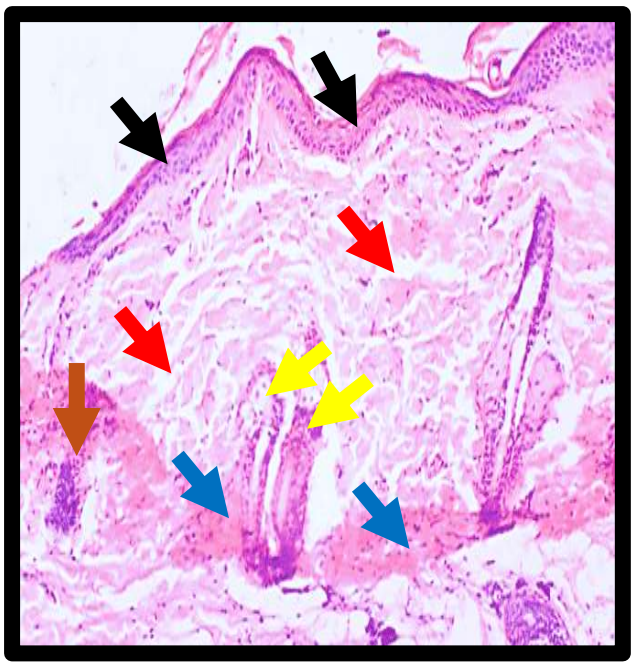

Fig. 5. The histopathological section of skin in rat in the treatment group (Rats with TCA-induced dermatitis treated with 25% ethanolic extract of pumpkin seed oil.). The tissue is stained by H&E stain and the section is captured using digital camera and light microscope at 10 X magnifier scale. Black arrows: epidermal skin layer; Red arrows: derms; Green arrow: hypodermis; Blue arrows: sweat gland; Brown arrow: Sebaceous glands.

The epidermis appears intact with mild thickening. The dermis shows limited necrosis while the hypodermis is preserved. Hypertrophy of sweat glands and presence of sebaceous glands suggest active regeneration. Overall, this group shows improved structural restoration compared to the 25% group, indicating enhanced therapeutic efficacy at this dose Fig. 6.

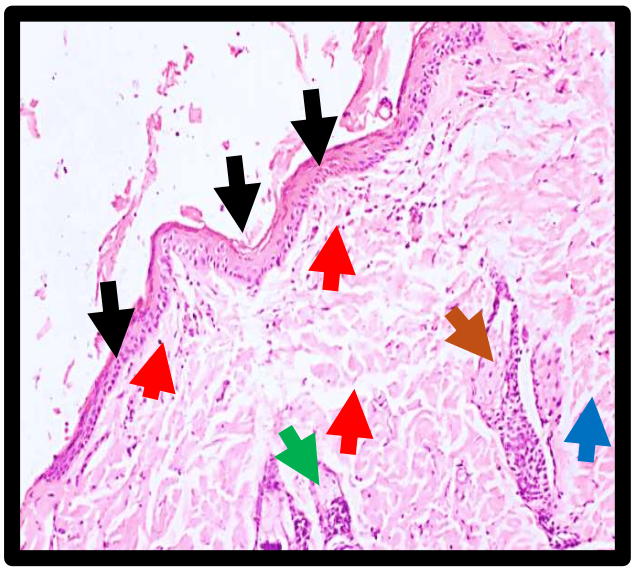

Fig. 6. The histopathological section of skin in rat in the treatment group (Rats with TCA-induced dermatitis treated with 50% ethanolic extract of pumpkin seed oil). The tissue is stained by H&E stain and the section is captured using digital camera and light microscope at 10 X magnifier scale. Black arrows: epidermal skin layer; Red arrows: derms; Green arrow: hypodermis; Blue arrows: sweat gland; Brown arrow: Sebaceous glands.

This group displayed the best histological outcomes. The skin layers — epidermis dermis, and hypodermis retained normal structure. Sweat glands are clearly identifiable and morphologically intact. There are no signs of inflammation, necrosis, or glandular atrophy, supporting a strong protective and regenerative effect of the 100% pumpkin seed oil treatment Fig. 7.

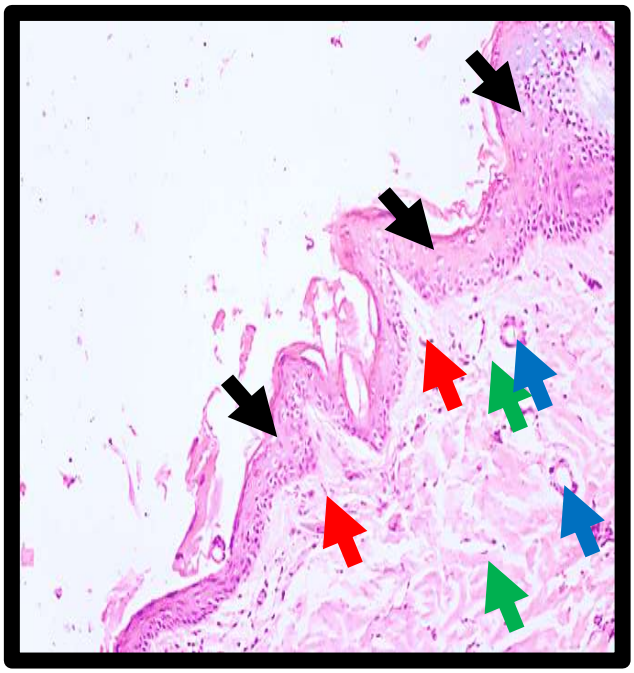

Fig. 7. The histopathological section of skin in rat in the treatment group (100% of extraction, mention what is happening in this group). The tissue is stained by H&E stain and the section is captured using digital camera and light microscope at 10 X magnifier scale. Black arrows: epidermal skin layer; Red arrows: derms; Green arrow: hypodermis; Blue arrows: sweat gland; Brown arrow: Sebaceous glands.

DISCUSSION

The histopathological investigation presented here offers critical insights into the extent of epidermal integrity, inflammatory response, and dermal-appendageal preservation under the influence of pumpkin

seed oil at varying concentrations, compared against both untreated and standard treatment controls. In the negative control group, histological features confirmed the baseline structure of healthy skin. The epidermis was intact and well-stratified, the dermis exhibited no inflammatory infiltration, and the hypodermis showed evenly distributed adipose tissue. Both sweat and sebaceous glands were preserved, establishing a reference point for evaluating pathological alterations in other groups.

Upon exposure to TCA, the positive control group exhibited hallmark features of chemically induced dermatitis, including complete epidermal sloughing, dense dermal infiltration by inflammatory cells, and hypodermal necrosis. This pattern mirrors the pathophysiology of acute eczema and severe irritant contact dermatitis, where barrier dysfunction triggers cytokine cascades, leukocyte recruitment, and oxidative tissue injury according the report by Ashcroft et al and Dubin et al [10,11]. Similar histopathological disruptions were observed in the corn oil group (Fig. 3), indicating that the vehicle alone lacked any reparative or anti-inflammatory effect, consistent with previous findings that lipid-rich carriers may fail to medisate therapeutic outcomes without bioactive constituents by Dong et al [12].

The 25% pumpkin seed oil group yielded mixed histological outcomes. In one section (Fig. 4), mild epidermal hyperplasia and sweat gland hyperplasia suggested partial regeneration. However, inflammatory cell infiltration and dermal necrosis persisted. In contrast, another section from the same group (Fig. 5) demonstrated complete epidermal erosion and glandular atrophy, suggesting that this concentration was insufficient for consistent healing. This inconsistency may stem from sub-therapeutic bioavailability of key phytochemicals such as tocopherols and sterols, which have been shown to inhibit TNF-κ signaling and reduce pro-inflammatory cytokine expression this results similar for study by Altaf et al and F. Caili et al [7,14].

The 50% of extract concentration group (Fig. 6) showed a more stable improvement in skin architecture. The epidermis appeared regenerated, sweat and sebaceous glands were preserved, and inflammatory signs were minimal. These results support the notion of a threshold concentration necessary to trigger adequate immunomodulation and tissue repair, aligning with the findings in study of Bardaa et al [15], who noted concentration-dependent wound healing benefits of Cucurbita pepo oil in burn models.

Finally, the 100% pumpkin seed oil group achieved near-complete histological normalization. All skin layers retained structural integrity, and no signs of inflammation, necrosis, or glandular degeneration were observed. These outcomes suggest that the full-spectrum phytoconstituents in unrefined pumpkin seed oil particularly polyphenols, omega-6 fatty acids, and zinc may synergistically contribute to restoring epidermal barrier function, resolving inflammation, and promoting adnexal regeneration according study by Kaur et al and Kleih et al [16,17].

The current study aimed to assess the therapeutic efficacy of pumpkin seed oil extract in managing trichloroacetic acid (TCA)-induced atopic dermatitis in male albino rats, through an integrated evaluation of gross morphological healing, inflammatory cytokine modulation, and histopathological tissue recovery. The results collectively provide robust evidence supporting the concentration-dependent healing potential of pumpkin seed oil. Gross visual assessment showed that topical application of 100% pumpkin seed oil resulted in significant morphological improvement over the 30-day treatment period. The lesions that initially

exhibited erythema, edema, crusting, and ulceration progressed toward organized granulation tissue formation as showed in previous study [18–21], in additionally to contraction of wound margins[22- 4], and complete re-epithelialization by day 30. This was accompanied by restoration of fur and cessation of scratching behaviour, indicating reduced inflammation and discomfort. These outcomes were not observed in lower concentration treatment groups or the corn oil-only group, suggesting that the therapeutic benefits are specific to the bioactive compounds within pumpkin seed oil and require a sufficiently high concentration to exert meaningful effects.

CONCLUSION

The histopathological evaluation clearly demonstrated that pumpkin seed oil extract promotes a concentration-dependent regenerative effect on TCA-induced skin injuries in male Albino rats. Notably, the 100% concentration group exhibited near-complete restoration of skin architecture, while lower concentrations (25% and 50%) led to partial histological improvement. These findings underscore the superior healing capacity of the high-concentration extract, validating its potential as a natural topical agent for enhancing dermal repair and mitigating inflammatory damage.

Overall, the results indicate that the use of pumpkin seed oil extract significantly accelerates wound healing caused by trichloroacetic acid, making it a promising candidate for future therapeutic applications in skin regeneration.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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