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Histological Evaluation of Cutaneous Wound Healing Treated by Local Application of Cucurbita pepo L. Seed Oil

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Abstract

Objective: The rising demand for natural medicines in pharmaceuticals has prompted scientists to investigate medicinal plants. Pumpkin seed (Cucurbita pepo L.) is a noteworthy candidate due to its remarkable pharmacological qualities for possible wound healing therapies.

Methods: A total of twelve mature male New Zealand rabbits weighing between 1.5 and 2 kg were subjected to uniform wounds on their dorsum. These rabbits were then split into two groups for healing durations of 3 and 7 days, with 6 rabbits in each group. The induced wound in the control group was allowed to heal naturally, whereas the wounds in the experimental group were treated with a daily local application of pumpkin oil (10µl). The animals were sacrificed after healing times of three and seven days. All examined groups underwent clinical assessment of wound contraction. Specimens were obtained for histological and histomorphometric analysis to evaluate inflammatory cell and blood vessel counts, as well as epithelial thickness.

Results: The average percentage of wound contraction escalated over time. A notable change was found and a statistically significant disparity in the mean values of inflammatory cells was recorded. The mean values for blood vessel count rose with a notable disparity was noted between the control and experimental groups. The evaluation of epithelial thickness indicated that mean values increased over time, with a significant difference in both experimental groups.

Conclusion: As a potential therapeutic agent for wound healing, pumpkin oil may be also beneficial for nutritional and therapeutic applications.

Keywords: Cucurbita pepo L. oil; Wound healing; Natural product.

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Introduction

The incidence of chronic wounds and their associated morbidity have rendered wound care progressively significant [1]. It is predicted that one to two percent of individuals in underdeveloped nations would experience chronic wounds at some stage in their lives [2]. The capacity for wound healing in the human body depends on various aspects, including the individual's overall health, the degree of tissue damage, and the proliferative capacity of cells [3]. Wounds compromise the integrity of the skin, resulting in changes to the adjacent anatomical structures and their functions. The complex and dynamic process of wound healing is affected by various elements, including the host's general health, the immune system, and the local environment surrounding the wound [2]. The categorization of wound healing mostly relies on the therapeutic approach and the distinct attributes of the lesion. Wound healing denotes the physiological process by which the skin repairs and restores itself after injury or damage. The stages of wound healing are commonly categorized as primary, secondary, and tertiary phases [3]. The effective regeneration of healthy, functional skin is a considerable challenge due to its intricate multilayered architecture and the organized organization of many cell types within the extracellular matrix [4]. In light of recent improvements in wound care treatments, there is a sustained interest in investigating alternative medicines sourced from natural origins, such as plant extracts, honey, and larvae [5].

The increasing interest in health-promoting foods is emphasizing specific things that have been used locally for millennia but have only recently attained global awareness [6]. Pumpkin seed oil is utilized in traditional medical and culinary applications, but recent research indicates its utility in the pharmaceutical and cosmetic industries as well [7]. Furthermore, multiple resources designate it as a possibly useful food, mostly due to its composition of pumpkin seeds, which encompass a range of advantageous components [8]. The pumpkin, scientifically known as Cucurbita pepo L., is a climbing plant that is planted annually and blooms from July to September. The maturation of the seeds takes place between August and October [6]. Pumpkin oil can be utilized as a cooking oil or a potential nutraceutical due to its abundant supply of several bioactive compounds with functional qualities [7]. The medical benefits of pumpkin seed oil, a viscous oil with a highly dichromatic appearance, have recently been the focus of research [8]. The majority of the attention that researchers have paid has been focused on the content and quantity of sterols, tocopherols, and fatty acids in pumpkin seed oil due to its beneficial health effects [9]. Furthermore, pumpkin has garnered recognition as an outstanding safeguard against various ailments, including hypertension and cancer, due to its health benefits, including its antimicrobial, antioxidant, and anti-inflammatory properties [10]. Evaluating the biochemical and oxidative stability characteristics of pumpkin seed oil would augment its value, particularly in the pharmaceutical, cosmetic, and culinary sectors [11].

Material and Methods

The experimental methods complied with the ethical standards for animal research established by the College of Dentistry at the University of Baghdad (Project number 834723). This cross-sectional study employed a sample of twelve adult male New Zealand rabbits, with an average weight between 1.5 and 2 kg. Each rabbit's dorsum was incised with two circular full-thickness wounds, utilizing a sterile biopsy punch with an 8 mm diameter; one wound served as a control and was permitted to heal spontaneously, while the other received daily applications of 10 µl of Cucurbita pepo L. oil.

The animals were individually positioned on a surgical bench and received general anesthesia by intramuscular injection. The anesthetic formulation comprised 80% ketamine (administered at a dosage of 40 mg/kg) and 20% xylazine (administered at a dosage of 5 mg/kg) [12].

The hair on the dorsal area was first clipped with a hair clipper, then a hair removal lotion was applied to eradicate any remaining hair. A 90% ethyl alcohol solution was utilized for skin disinfection. Two circular excisional incisions were made on the dorsal skin of each rabbit, separated by a distance of 2 cm. A biopsy stapler punch with an 8mm diameter was utilized to accomplish this. The localized application of oil was executed with a micropipette. A volume of 10µL of Cucurbita pepo L. oil was administered to the left wound of rabbits, while the right wound remained untreated to facilitate spontaneous healing. Scarification was conducted on animals at specific intervals (3 and 7 days) to promote the healing process. The animals had daily examinations to assess the induced wound's dimensions utilizing a vernier caliper for assessing wound contraction. Samples were subsequently acquired and submerged in a 10% freshly made formalin solution for 24 hours. Furthermore, histological and histomorphometric analyses were performed to evaluate the

presence of inflammatory cells and quantify the thickness of the epithelial layer and the count of blood vessels. The statistical analysis was conducted utilizing SPSS (Statistical Package for the Social Sciences) software version 26, employing descriptive statistics and inferential statistics through an independent paired T-test to compare the two groups.

Results

Histological findings

Three days

Histological analysis of the skin portion from the control group at the wound site reveals a scab on the surface, new epithelial tissue, hair follicles, and the presence of inflammatory cells, as illustrated in Figure 1. The microphotographic examination of the experimental group reveals a newly formed epithelium covering the wound surface, a limited presence of inflammatory cells, and the emergence of new hair follicles within the dermis, as illustrated in Figure 2.

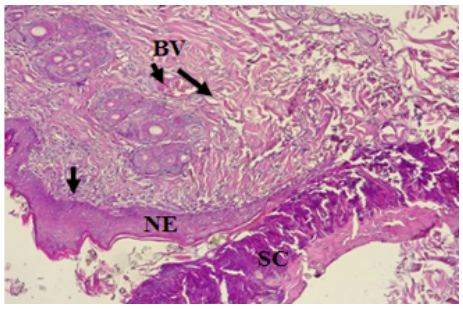

Figure 1. View of wound site showed scab (SC), new epithelium (NE), inflammatory cells (arrow), blood vessels (BV). H&EX10.

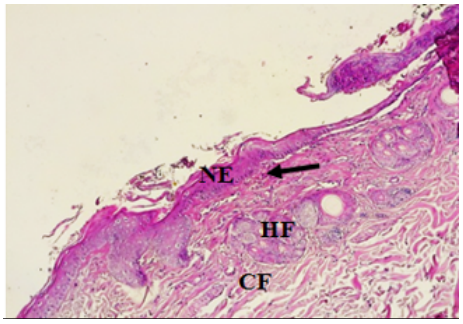

Figure 2. View of experimental group showed new epithelium (NE), inflammatory cells (arrows), hair follicles (HF), collagen fibers (CF). H&Ex10.

Seven days

A microphotograph of the wound site in the control group exhibits complete epithelialization at the surface, remodeled collagen fibers, hair follicles, and blood vessels, as depicted in Figure 3. The histological examination of the experimental group reveals a thickened, freshly created epithelium at the wound surface, whereas the dermis exhibits remodeled collagen fibers and blood vessels, as illustrated in Figure 4.

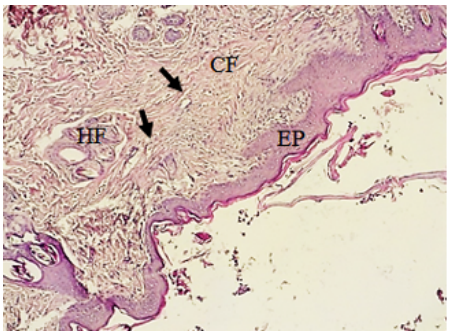

Figure 3. View of control group showed, new epithelium (NE), collagen fibers (CF), blood vessels (arrows). H&EX10.

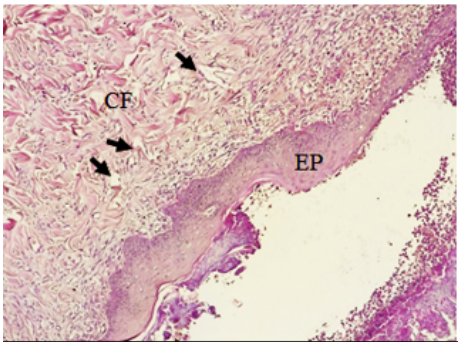

Figure 4. View of experimental group after 7 days showed new epithelium (EP), collagen fibers (CF), blood vessels (arrows). H&Ex10.

Statistical analysis

Estimation of wound contraction

The formula for determining the percentage of wound closure was as follows: ((L1- L2) / L1) x 100, where L1 represents the length of the wound on day 0 and L2 represents the length of the wound on the day of observation [13].

The assessment of wound contraction was performed in millimeters (mm). The findings of the present study demonstrate that the measured rate of wound

contraction showed a continuous increase over the duration of the research, as illustrated in Table 1. The investigation entailed observing the extent of wound contraction during the healing process for each group examined. The control groups demonstrated a minimal mean value for wound contraction, while the treated group achieved its maximum mean value on day 7.

Inflammatory cell parameter

The average inflammatory cell counts were recorded during the healing periods for all studied groups by enumerating the inflammatory cells in five fields. Cells were enumerated using a light microscope equipped with a square grid in one eyepiece, under a magnification of 40x, and the average cell count was documented for all healing intervals [14]. Table 2 displays the descriptive statistics for the inflammatory cell count data gathered at different healing intervals. The results demonstrate that the mean values were significantly lower in the experimental groups compared to the control groups on days 3 and 7.

Epithelial thickness parameter

To determine the epithelial thickness, the distance between the outermost keratin layer and the innermost basal layer of the epidermis at the wound borders was measured, averaging two readings using Image J software. The results revealed that the mean epithelial thickness exhibited a gradual increase over time across all examined groups, as shown in Table 3. Moreover, the measured thickness was consistently greater in the experimental groups with significant outcomes than in the control group.

Blood vessel count

The procedure was executed utilizing Image J software. The numerical density of blood vessels was assessed using a light microscope at 40x magnification in a 45 µm² area across three fields. The average number of blood vessels was documented. The results are shown in Table 4. The experimental group exhibited the highest mean blood vessel values over the 7-day healing period, whereas the control group had the lowest mean value during the 3-day healing period.

Table 1. Descriptive statistics of wound contraction in each healing duration.

Duration	Group	N	Mean± SD	Min	Max	T Test	P-Value
3 Day	Control	6	12.1% ± 1.5	9.8%	14.1%	-2.3	0.03*
	Experimental	6	14.9% ± 2.4	11.1%	18.5%		
7 Day	Control	6	42.8% ± 6.1	36.4%	51.2	-3.09	0.01*
	Experimental	6	55.7% ± 8.2	43.9%	65.4%		

Table 2. Descriptive statistics of inflammatory cell count in each healing.

Duration	Group	N	Mean± SD	Min	Max	T Test	P-Value
3 Day	Control	6	52.6 ± 6.5	40.5	58.1	2.28	0.04*
	Experimental	6	43.1 ± 7.7	33.5	56.4		
7 Day	Control	6	65.9 ± 7.9	54.3	72.8	7.08	0.000*
	Experimental	6	39.1 ± 4.8	32.4	45.2		

Table 3. Descriptive statistics of Epithelial thickness in each healing duration.

Duration	Group	N	Mean± SD	Min	Max	T Test	P-Value
3 Day	Control	6	5.2 ± 1.2	3.8	7.3	-3.1	0.01*
	Experimental	6	7.3 ± 0.9	5.8	8.4		
7 Day	Control	6	10.2 ± 1.4	7.8	11.6	-5.009	0.001*
	Experimental	6	15.6 ± 2.2	12.5	18.3		

Table 4. Descriptive data of blood vessel count over various healing.

Duration	Group	N	Mean± SD	Min	Max	T Test	P-Value
3 Day	Control	6	4.06 ± 0.9	2.6	5.4	-2.4	0.03*
	Experimental	6	5.2 ± 1.2	3.9	7.1		
7 Day	Control	6	8.4 ± 1.09	7.1	9.3	-2.6	0.02*
	Experimental	6	11.1 ± 2.13	8.2	12.5		

Discussion

Wound healing is a systematic sequence of actions designed primarily to restore the skin's barrier and protective function to avert more blood loss and infection [15]. The wound healing process encompasses inflammation, re-epithelialization, granulation, and angiogenesis, ultimately resulting in wound contraction [16]. Accelerating wound healing while minimizing side effects is a primary objective of contemporary medicine [17]. Natural herbal therapies have become essential in the management of dermatological conditions and infections, attributed to the adverse effects of contemporary medication and the cost-effectiveness of herbal medicines [18].

wound healing owing to their diverse array of components and phytochemicals, including alkaloids, flavonoids, fatty acids, terpenoids, saponins, and phenolic chemicals that can augment the healing process [19]. Phytochemicals can affect multiple phases of the wound-healing process by diverse methods, including the overexpression of TGF-β, vascular endothelial growth factor (VEGF), monocyte chemoattractant protein-1 (MCP-1), and interleukin-1 (IL-1), alongside the downregulation of nitric oxide (NO) and reactive oxygen species (ROS) [20]. The oil extracted from Cucurbita pepo seeds is extensively utilized as a traditional remedy due to its antibacterial and antioxidant qualities, providing a novel, safe, and cost-effective alternative to commercial wound healing treatments [21]. In contrast to the control groups, which had distal wound borders, the oil-treated groups demonstrated a more pronounced epithelial layer. There exists A significant reduction in healing time was observed in the treated groups relative to the untreated group, in accordance with the study conducted by Kamil and Al-Ghaban in 2019 [22]. Between the 1st and 7th day of wound induction, Cutaneous wound healing demonstrated that both treatment groups had enhanced wound healing, as reflected by the increased wound closure percentages compared to the control group on days 3 and 7. This outcome aligns with Rezk et al. (2023), who utilized a Pumpkin Seed Oil-Infused Chitosan/Polyvinyl Alcohol Electrospun Nanofiber Scaffold utilized as a cutaneous and oral wound dressing [23]. The findings suggest that pumpkin-infused nanofibers possess promise for dermal and oral wound healing. They offer an innovative, secure, and cost-effective alternative to traditional wound healing methods. The findings emphasize the integration of natural botanicals and advancing nanoscience for biological purposes, especially in wound healing [23]. An exaggerated inflammatory rim was more prominent around the untreated wound compared to the treated wounds, and the inflammatory cell count was reduced in the treated group relative to the untreated group. This finding can be attributed to the anti-

inflammatory and antibacterial properties of pumpkin oil [24]. The count of blood vessels and the thickness of the epithelium in the treated group are significantly greater than in the control group. Increasing evidence indicates that a humid environment facilitates wound healing via multiple mechanisms, such as enhancing re-epithelialization, angiogenesis, keratinocyte migration, and activating hypoxia-inducible factor-1 (HIF-1), which leads to the generation of endogenous wound healing stimulants [25]. Bardaa et al. (2016) in their in vivo investigation demonstrates that pumpkin seed oil significantly surpassed the control group treated with Cicaflora cream, which contains 10% Mimosa tenuiflora extract, in the healing of skin wounds. The cohort receiving pumpkin oil achieved complete re-epithelialization [26]. This study illustrates that cold-pressed pumpkin seed oil provides a substantial source of many advantageous constituents, including antioxidants and antibacterial compounds. The presence of tocopherols, sterols, and polyunsaturated fatty acids in pumpkin oil makes it a remarkable medicinal and cosmetic substance, perhaps providing protection against dermatological conditions, such as skin wounds [27]. Our results indicated that wound healing in rabbits administered pumpkin oil extract was superior to that in the untreated or reference groups, as evidenced by macroscopic, morphometric, and histological data, corroborating the findings of Wargala et al [28].

Conclusion

Pumpkin oil (Cucurbita pepo L.) is a potential therapeutic agent for wound healing in animal models. Overall, pumpkin oil is recommended for nutritional and therapeutic objectives.

Conflicts of interest

The authors declare no competing interest.

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