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Histological Evaluation of Local Application of Cress Seeds Oil on Wound Healing in Facial Skin Rat

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Abstract

Background: The process of skin healing necessitates the restoration of the body's structural integrity. This physiological process includes the extracellular matrix, blood cells, cytokines, and growth factors. Cress seed oil is a widely utilized herbal remedy that is believed to have medicinal activities as an antioxidant, antimicrobial, and anti-inflammatory.

Objective: Assess the influence of applying cress seeds oil directly to facial cutaneous wounds in rats to determine its effect on healing.

Materials and Methods: Sixty male Albino rats, with an average weight 200 -300 g were studied. Full thickness surgical incisional wounds were made in the cutaneous surface of each rat's cheek. The rats have been divided into the subsequent groups using random assignment: **Control group:** (n=30), where wounds were allowed to heal without any intervention**. Experimental group:** (n=30), wounds were treated daily with topical application of cress seed oil. Assessment of wound contraction, epithelial thickness, inflammatory cells and blood vessels quantification. The animals had scarification at the healing periods (days three & seven).

Results: There is a noticeable rise in wound contraction and epithelial thickness over time with a significant difference observed in the experimental group at day 7. The number of inflammatory cells reduced over time. The experimental group exhibited the highest mean values for blood vessel count on day 7.

Conclusion: The results of the present study demonstrate that the use of cress seeds oil applied directly to the skin wounds of rats enhances the healing process.

Introduction:

The superficial integumentary apparatus is the greatest organ in the human body, occupying an enormous surface area of over twenty square feet. The skin is the body's biggest organ, comprising approximately 15% of the body weight. It is also connected to the mucous membrane that covers the body's surface (1). The layers of the skin are the epidermal layer which is located superficially and beneath it the dermal layer which contains the circulation vessels and connective tissue. The third layer beneath the dermis is the subcutaneous fatty layer. The skin tissue aids in protecting the body and internal organs against outside risk factors such ultraviolent rays from the sun and from any chemical or physical hazardous elements. Since the skin serves as the front defense against any potential harm, the process of wound healing is a mandatory step for survival of any living organism (2). The process of cutaneous wound healing is an important physiological and biological process that consist of numerous types of cells along with their chemical products. At the end of this process, the tissue must be repaired and healed (3). There are four different and Subsequent phases of the healing process and in order to accomplish an optimal wound healing, these phases must be conducted with precision and deliberate restraint (4).
The four phases of the healing process include: Hemostasis, inflammatory stage, proliferative stage that involves differentiation of mesenchymal cells and their migration to the injury site, blood vessels formation and re-epithelialization and the last stage is the remodeling which involve synthesis and differentiation of collagen fibers (5). Recent treatment methods that include medical, chemical, herbal and biological technologies are utilized in order to facilitate the invention of new products that aid in the healing of wounds and chronic wounds. Recently, herbal remedies have been widely employed for the healing of chronic wounds. The effect of these remedies are well known historically (6). Lepidium sativum or commonly known as garden cress that belongs to the plant family Brassicaceae. This plant is native to the Mediterranean region and is utilized for ethnobotanical uses throughout the Middle East, West Asia, and India. The seeds and leafs of garden cress has been used to treat disorders of the respiratory system, rheumatologic uses, in cardiovascular system, in several metabolic diseases, and in some gastrointestinal disorders (7). Several earlier studies have reported that the extract obtained from garden cress exhibited multiple biological effects such antioxidant, antidiarrheal, antispasmodic, antibacterial, anti-inflammatory and several properties as protection of the liver tissue against oxidative damage (8). The oil derived from cress seeds has been assessed in order to study its antimicrobial properties, the oil showed remarkable activity against several bacteria and fungi that been used in the study and thus the study highlighted a wide-range activity of cress seeds oil as an antimicrobial. The oil also shown antioxidant and anti-inflammatory properties. The findings indicated that the oil produced from the seeds contains chemical substances that could serve as excellent sources of bioactive chemicals with significant biological properties (9). It has been demonstrated that the modulatory effect of cress seed oil May have therapeutic potential to treat inflammatory disorders because it is rich in α-linolenic acid through direct effects on the lipid compositions, spleen lymphocyte proliferation, and inflammatory mediator production by peritoneal macrophages in female wistar rats (10). Although the general health promoting effects of lepidium sativum and its seeds were documented well, the current study attempted to evaluate the cellular regenerative activity of wounds healing including wound closure, re-epithelization, inflammatory cells and formation of new blood vessels in skin wounds treated with cress seeds oil in rats.

Materials and Methods

The experiment's procedures adhered to the ethical approval of animal

experimentation of Baghdad University\ Dentistry faculty (Reference Number: 870 on 3/12/2023). A group of sixty male Albino rats, all in good health, with an average weight ranging from 200 to 300 grams were used, all animals were examined by veterinarian staff in a private animal house to exclude unhealthy animals. The rats kept under controlled temperature, ventilation conditions, housing and feeding, has convenient access to potable water. The rats were housed in individual containers for a duration of two weeks in the same suitable conditions for adaptation before the surgical procedure were done. The animals were randomly subdivided into two main groups: experimental and control groups (thirty rats each). From each group,15 rats were sacrificed according to the healing periods (3&7 days). This procedure was done in a private animal house in Najaf city, Iraq from mid-October 2023 to early November 2023.

Prior to the surgical procedures, the weight of each animal was measured in order to identify the appropriate dosage of anesthesia needed. General Anesthesia was induced by giving xylazine 2% (0.4 mg/kg body weight) and ketamine HCl (50 mg, 40 mg/kg body weight) through intramuscular administration. Depilation was performed on the facial area. A solution of ethanol with a concentration of 70% was employed for the purpose of cleansing the skin. Surgical incisional wounds with full skin thickness of 2 cm length were made in the cheek of each rat. Local daily application of cress seed oil (30 ul) was take place for all the animals of experimental group and nothing applied to the control group. Full thickness excisional biopsies done in the scheduled healing periods (day 3 & day 7) after sacrificing the animals. The specimens were put in a 10% solution of newly synthesised formalin for a duration of 24 hours. After Dehydration, samples washed by running water over night, then the samples pass through the serial steps of slide tissue preparation technique (11). Slides of skin tissue were stained with the usual method of staining with haematoxylin and eosin (H&E) for microscopical evaluation to evaluate the number of inflammatory cells, as well as measure the thickness of the epithelial layer and count the blood vessels. This procedure was done from early November 2023 until mid-December 2023 in the histopathology laboratory of oral diagnosis department at Baghdad university, and the operator was blinding about all groups. A quantification of inflammatory cells in five fields was performed using a light microscope fitted with a square grid in one eyepiece. The counting was conducted using a microscope with a magnification of x40. The average cell count was subsequently documented for each healing duration (12).

Statistical analysis

A statistical analysis was conducted to examine the effect of using cress seed oil on the healing of rat skin wound. The data was created using the Statistical Package for Social Sciences (SPSS) version 26. Data was expressed using simple and cluster chart bar, frequency, percentage, minimum, maximum, mean, standard deviation. Shapiro Wilk test for normality, Repeated Measure ANOVA with Bonferroni posthoc test, Paired T test, Two Independent Sample. P-value less than or equal to 0.05 with a confidence level of 95%, has been considered significant.

Results

Clinical finding: The percentage of wound closure was calculated using the formula: ((R1- R2) / R1) x 100, where R1 is the initial length of the wound on day 0, and R2 is the length of the wound on the day of observation. The analysis of the percentage wound contraction provided descriptive statistics that showed wound contraction in control higher than that in cress seed oil after 3 days but with no significant difference while the wound contraction is higher in cress seed oil than that in control after 7 days with significant difference. The results are presented in the Table (1).

Histological finding

Three days duration

Control group: The histological examination of the wound site showed the presence of necrotic tissue on the surface of the wound, infiltration of inflammatory cells, follicles of hair, several blood vessels enclosed by granulation tissue, and irregularly distributed collagen fibers and fibroblasts. Figures (1) and (2).

Experimental group:

The microphotograph of wound area reveals the presence of a new epithelium on the surface, which is located beneath the remaining necrotic tissue. Additionally, fibroblast and collagen fibres are visible, along with several blood vessels and a small number of inflammatory cells in the dermis. Figures (3) and (4).

Seven days duration

Control group: The histological assessment of the wound site reveals the presence of newly formed epithelium that is sealing the surface of the wound. Additionally, there is granulation tissue consisting of collagen fibers that are undergoing remodeling, along with the presence of fibroblasts and blood vessels. Figure (5).

Experimental group:

The histological analysis of the wound after 7 days reveals complete epithelialization, with the wound site being filled with granulation tissue, blood vessels, collagen fibers, and fibroblasts. Figures (6) and (7).

Inflammatory cell parameter

Although mean values of the count of inflammatory cells in control higher than that in cress seed oil after 3 days but with no significant difference while mean values of the inflammatory cell count is lower in cress seed oil than that in control after 7 days with significant difference., in both groups there is a decrease in inflammatory cell count from 3 days to 7 days with significant change but this change is higher in cress seed oil than that in control, These results are illustrated in figure(8) as well.

Epithelial thickness evaluation

The epithelial thickness was measured using a power x40 lens. This measurement involved determining the distance between the outermost layer of the keratin and the innermost basal layer of the epidermis at the wound edges. Two readings were taken and the mean was calculated using Image J computer software. The findings demonstrated that although mean values of thickness in control group less than that in cress seed oil after 3 days but with no significant difference while the thickness is higher in cress seed oil group than that in control after 7 days with significant difference, in both groups there is increase in thickness from 3 days to 7 days with significant change but this change is higher in cress seed oil than that in control, these results are illustrated in the Figure (9).

Blood vessel count

The process was performed via Image J software. The numerical density of blood vessels in a 45µm2 area was assessed using a light microscope with a magnification of x40, in 3 fields. Mean values of blood vessels count were higher in experimental group than that in control group at days 3 and 7. Significant difference was recorded at day 7 and the difference was non-significant between two study groups in at day three, these findings also illustrated in figure (10).

Discussion

Rats are commonly chosen as subjects for research on skin wound healing, mainly because they are easily accessible, cost-effective, and tiny in size. These advantages provide a more efficient and economical utilization of limited laboratory space and housing facilities (12). Therefore, rats were chosen as the experimental model to conduct this research. The efficacy of the wound healing process is contingent upon the inherent reparative capacity of the tissue, the nature and extent of tissue damage, and the overall health status of the individual. An optimal therapeutic approach for wound healing would ideally involve a safe treatment, described as a

medicinal substance, specifically created to improve the generation of new tissues while minimizing any undesirable adverse effects (13). The process of wound healing is an intriguing mechanism. Skin wound healing is a crucial process for survival, as it serves as a barrier against physical, chemical, and microbiological threats, ultimately resulting in the closure of the wound. It is a complex process, which is dependent on many cell types and mediators interacting in a highly sophisticated temporal sequence. It consists of hemostasis, tissue inflammation, proliferation, and remodeling that must occur in the proper sequence and time frame (14). Botanical extracts represent promising candidates for facilitating wound healing, primarily owing to their widespread availability, non-toxic attributes, absence of undesirable side effects, and efficacy in their crude forms. A therapeutic agent selected for wound healing should ideally contribute to the enhancement of at minimum, one phase of the healing process without undesirable reactions (15). Lepidium sativum seed oil commonly known as cress seed oil, has been documented for its reported efficacy in alleviating anemia, stimulating appetite, blood purification, and in treating hepatitis. Furthermore, it is used in treating various health issues, including liver disturbances, jaundice, diabetes mellitus, spleen disorders, menstrual irregularities, sexual debility, gastrointestinal disorders, abdominal colic, arthritis, pleurisy, asthma, fractures, dropsy, and some other inflammatory conditions (9). The oil derived from lepidium sativum seeds has undergone investigations for its antibacterial and antioxidant properties, revealing notable efficacy against the tested bacterial strains. Additionally, the oil demonstrated heightened antioxidant activity (16). Tissue repair is facilitated by wound contraction. It is essential to achieve the optimal level of contraction during the healing process, striking a delicate balance between insufficient contraction that might result in non-healing injuries and excessive contraction that can lead to contractures (17). The results of our present study, showed that the statistical percentage of wound contraction increased with time and were highest in experimental group with significant difference at day 7, in agreement with the findings of (18) who showed a significant reduction of wound diameter by application of lepidium sativum seed oil for the management of diabetic skin wounds in rats.

Histological and histomorphometrical assessment

The process of wound healing is subjected to a sequential cascade of physiological responses. These responses include the processes of inflammation, cellular proliferation and migration, angiogenesis, matrix formation, reepithelialization, and the development of granulation tissue. The newly formed tissues comprise fibroblasts, collagen, edema, and freshly formed capillaries (13). The findings of current study revealed that the mean values of inflammatory cell count were less in the cress seed oil group compared to the control group after 7 days, with a statistically significant difference. This finding illustrates the effectiveness of the herbal extract that was used. In contrast, the control group had a more pronounced inflammatory response, possibly attributed to bacterial invasion and the absence of immunomodulatory substances. This observation is consistent with the findings of an earlier study carried out by (19). Re-epithelialization and granulation tissue development are essential stages in the healing mechanism as they contribute to the repair of integrity of the skin, reducing susceptibility to infection (20). The findings of this study reveal that the group that received cress seed oil exhibited a much thicker epithelium compared to the control group after 7 days. The find is consistent with prior research carried out by Amer et al., which showed that lepidium sativum seed has favorable characteristics for nanofibrous wound dressings (21). These features entail the capacity to facilitate the regrowth of epithelial tissue as well as the formation of collagen. Angiogenesis comprises an essential phase in the healing cascade, as it facilitates the delivery of nutrients and

maintains oxygen homeostasis. This, in turn, enhances cell proliferation and fosters tissue regeneration (22). Our study showed that in both groups there is increase in blood vessels count from 3 days to 7 days with significant change but this change is higher in cress seed oil group than that in control whereas maximum mean value of blood vessels was recorded in experimental group at day 7. This study result ran in agreement with Amer et al. and Kamel et al. who showed that local application of cress seed oil enhanced the healing process, re-epithelialization and angiogenesis (18, 21).

Conclusions

The results obtained indicate that the use of Cress Seeds Oil has the potential to enhance skin wound healing in rats.

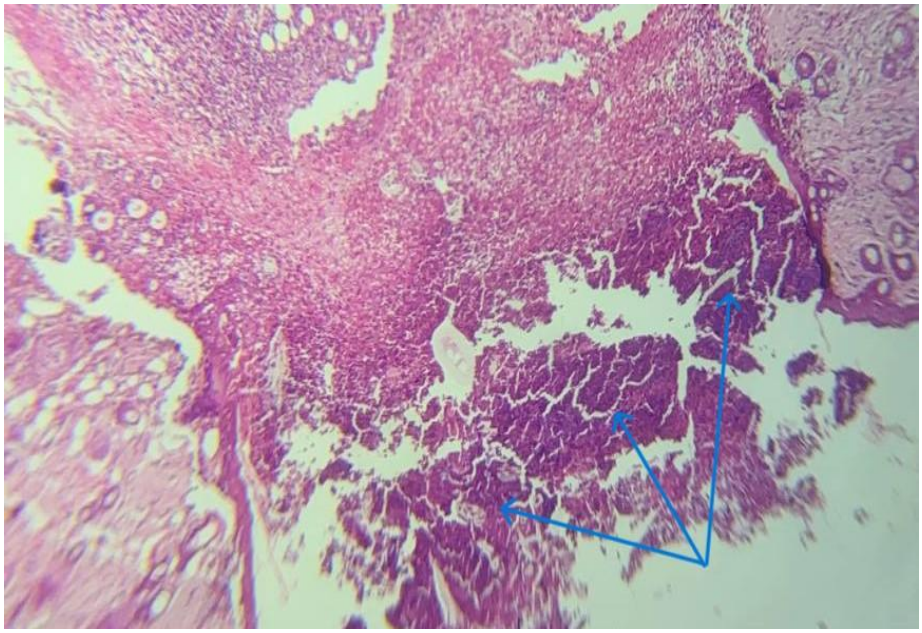

Figure (1) Blue arrows show necrotic tissue at wound surface. H&E X4.

Figure (2) Wound site: Blue arrows show necrotic tissue, white arrows show congested blood vessels, yellow arrows show inflammatory cells infiltrate, purple arrows show hair follicles. H&E X10.

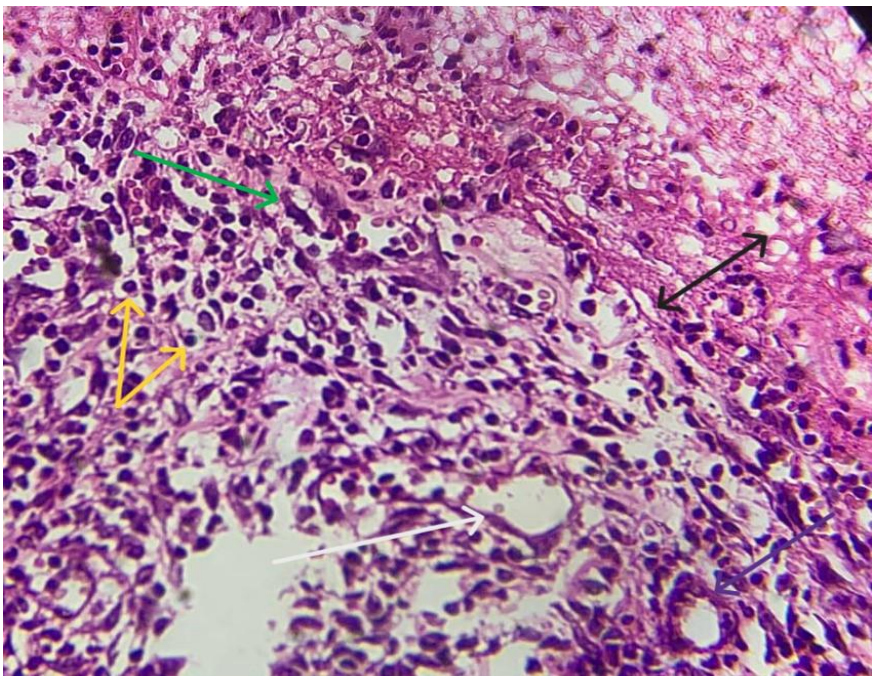

Figure (3): View shows newly formed epithelium (black arrow), fibroblasts (green arrow), inflammatory cells (yellow arrows), blood vessel (white arrow), new hair follicle (purple arrow). H&E x40

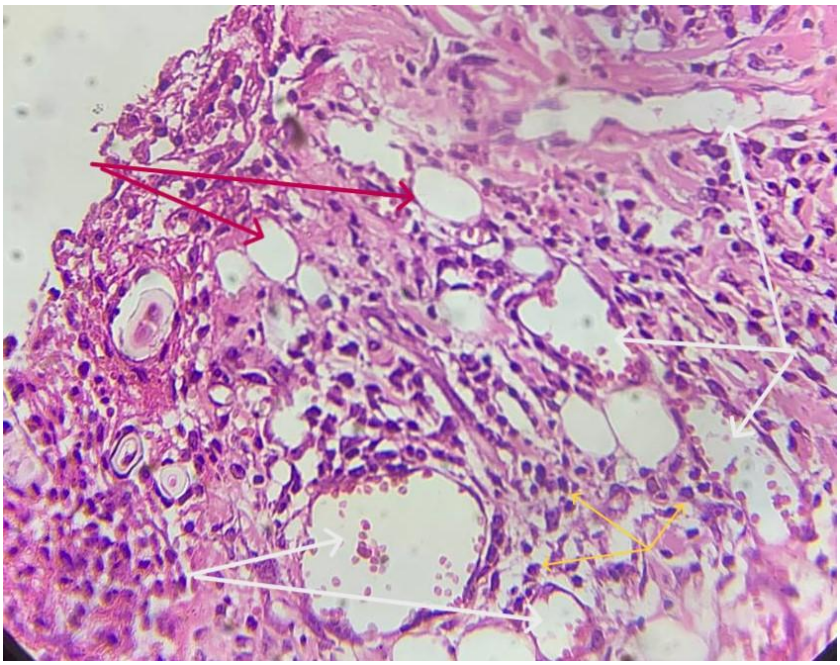

Figure (4): View shows inflammatory cells (yellow arrows), blood vessel (white arrows), fatty pads (red arrows). H&E x40

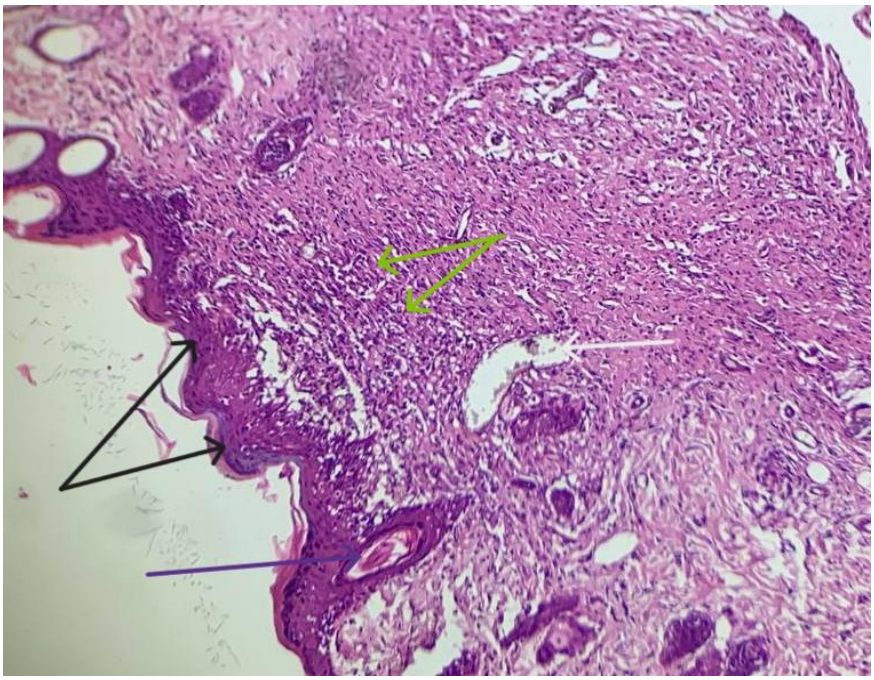

Figure (5): View shows surface sealed by new epithelium (black arrows), granulation tissue (light green arrows), new hair follicles (purple arrows), new blood vessel (white arrow).H&E x10.

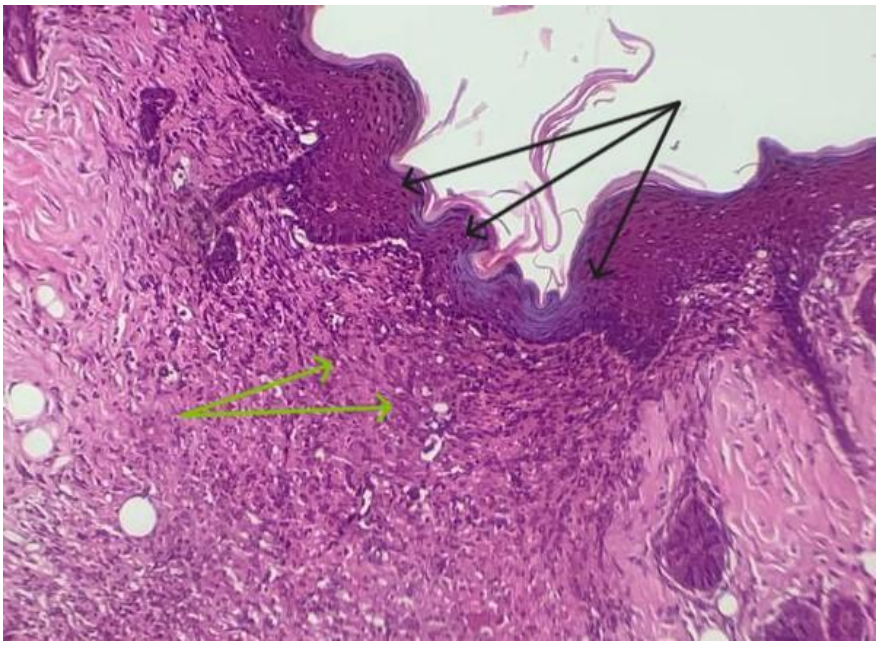

Figure (6): Wound site filled by granulation tissue (light green arrows), new epithelium at surface (black arrows). H&E x10

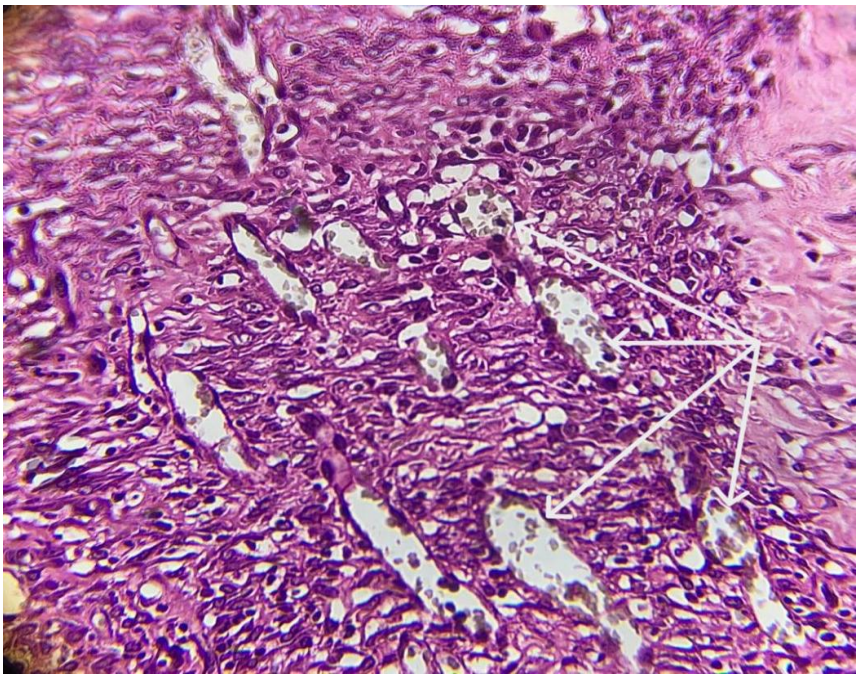

Figure (7): View of wound site shows numerous blood vessels (white arrows).H&E x40

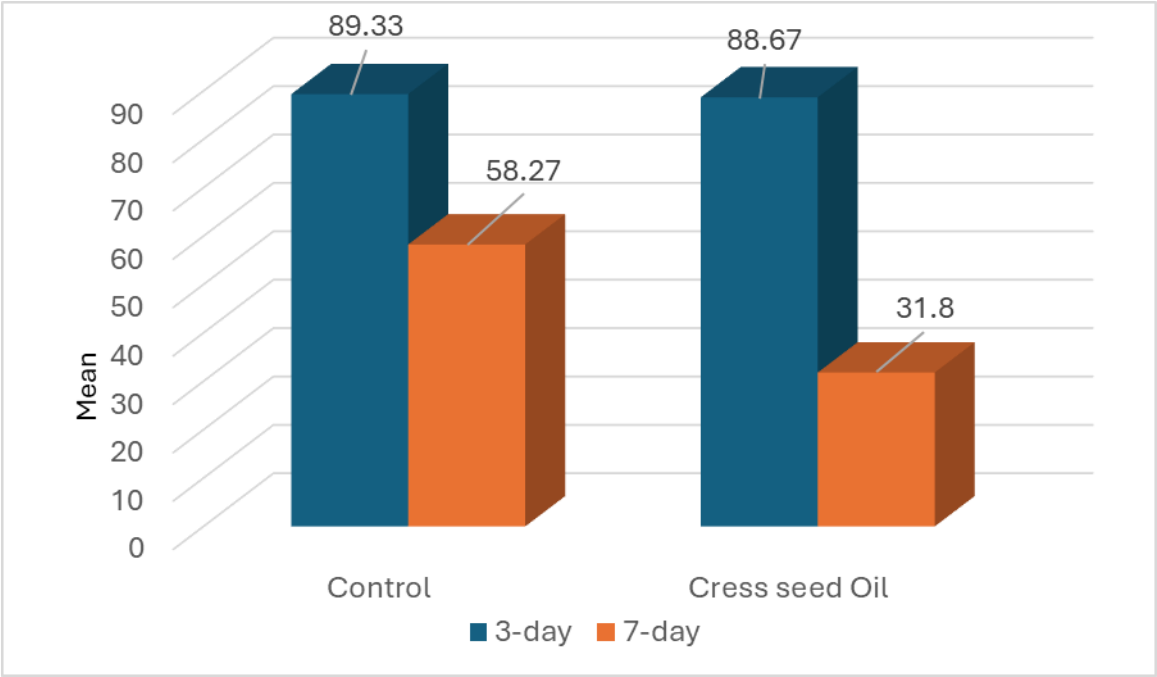

Figure (8): Inflammatory cells count among both groups in each healing period.

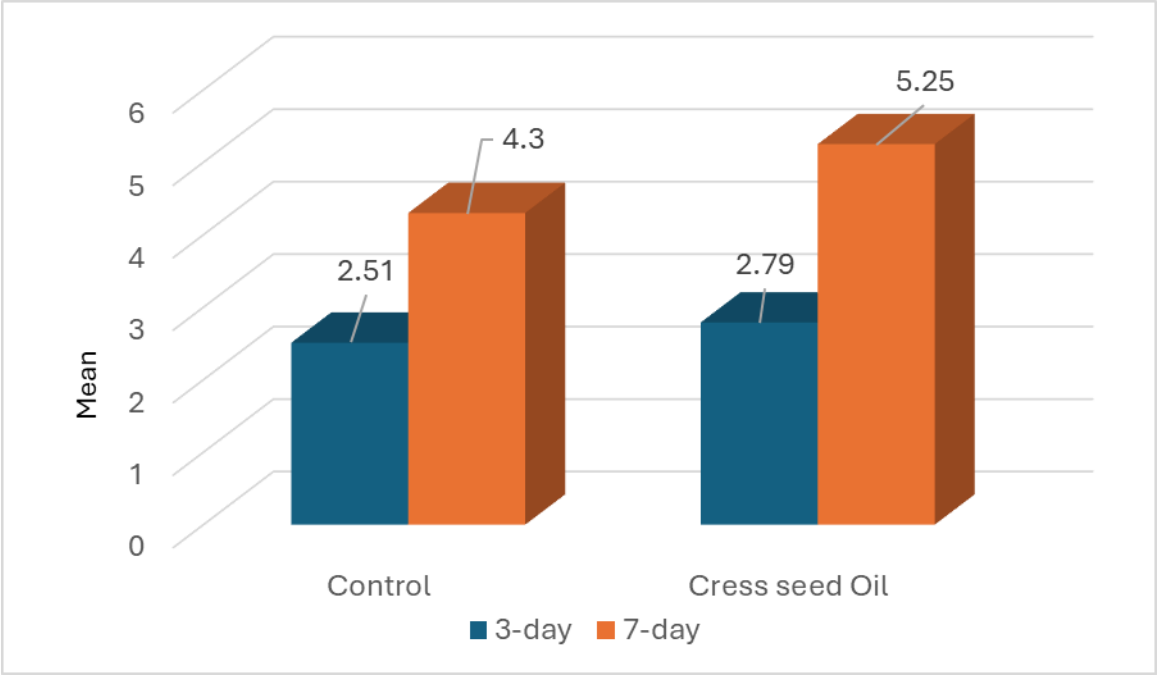

Figure (9): Epithelial thickness among both groups in each healing period.

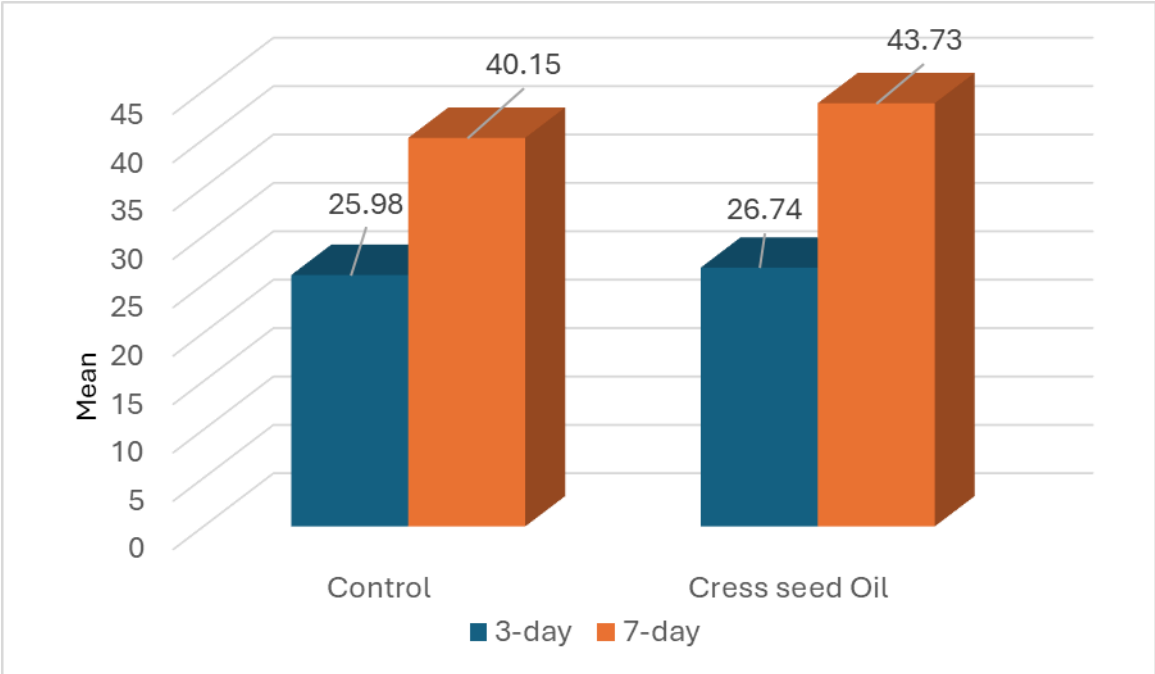

Figure (10): blood vessels count among both groups in each healing period.

Table 1: presents the descriptive statistics of the percentage wound contraction during each healing interval for both study groups.

Groups		Baseline	3-day	7-day	F	P value	Effect size
Control	Minimum	19.00	13.80	.00	3465.12	0.000*	0.990
	Maximum	20.00	18.70	2.50			
	Mean	19.93	15.75	1.28			
	±SD	0.26	1.22	0.97			
Cress seeds oil	Minimum	19.00	13.40	.00	3776.818	0.000*	0.996
	Maximum	20.00	18.60	1.70			
	Mean	19.93	15.42	0.48			
	±SD	0.26	1.40	0.33			
T test		0	0.677	2.676			
*P-value less than 0.05		1.00^{NS}	0.504^{NS}	0.012^{*}			

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