Efficacy of Herbal Cellulose Gelatin Foam on 2nd Degree Cutaneous Burn Healing

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Abstract

A lot of researchers try many types of therapies for burns in Veterinary medicine in the last decades. This project aims to compare the efficacy of novel therapy, herbal cellulose gelatin foam on 2nd degree cutaneous burn healing. Fifty female white albino rats, who were housed in plastic boxes divided randomly into two groups 25 rats for each. Control group G1 had no treatment, second group G2 treated with herbal cellulose gelatin foam. Sample from cellulose gelatin foam was examined under SEM for the morphology of the foam at 100 µm.

Under routine surgery with general anesthesia all rats had thermal injuries at the left side of their backs. The thermal injuries were then created using a solid aluminum bar with a diameter of 2 cm in diameter had previously been heated to 100°C in boiling water. The bar is kept in touch with the rat's skin in the dorsal proximal region of the back. Hyperemia, edema, scar tissue, and scab were recorded for morphological exam. The therapies were applied topically once daily for 10 days. At the time of burn and on the appropriate days for the biopsies at 7th, 14th, 21st and 28th day, sterile "swabs" were taken and sent to laboratory for bacterial contamination. Biopsies were collected for histologic evaluation of healing. The morphologic appearance were recorded at 1 day, 7th day, 14th day,21th day, and 28th day, Histologic evaluation showed the superiority of herbal cellulose gelatin foam than control group for 2nd degree burn healing and this result accompanied with the other parameters. Histological changes post burn injury in herbal cellulose gelatin treatment showed complete healing of all layers of skin including epidermis, papillary dermis, reticular dermis , and hypodermis, hair follicles, sweat glands, and sebaceous glands were obvious. The study concluded that herbal cellulose gelatin foam speed up the healing of 2nd degree burn.

Keywords: *Herbal, Cellulose, Gelatin, Foam, Burn, Cutaneous, Healing.*

Introduction

Burns are one type of wound that is thought to present a significant health risk to society. Numerous wars and calamities involving tourists in the preceding few decades exposed countless Iraqis to burns.

The use of herbal medicines and nutraceuticals supplements continues to expand rapidly, worldwide, in particular in last few years in which herbal remedies have become more and more popular as alternatives to conventional pharmaceuticals because of its lesser side effects compared to chemical pharmaceutics

(Chakotiya, 2014). Cellulose is the natural polymer with β (1, 4)-linked d-glucose which has high water maintenance and fine microfibrillar mesh structure that acts as a microbial barrier. It was reported that cellulose is nontoxic and biocompatible with the human skin. This water-insoluble polymer can be extracted from plants and surface of some bacterial culture media (Pourali et al., 2014). Although cellulose has an acceptable wound-healing activity, the combination of this polymer with some natural and traditional substances such as some types of herb extracts with anti-inflammatory, anti-oxidant and/or anti-microbial activities made it a good candidate for wound-healing purposes. Create new herbal cellulose gelatin foam and research its effectiveness in healing second-degree burns.

Materials and methods

Ethical approval

This study was approved by the Scientific Committee of the College of Veterinary Medicine in the University of Al-Qadisiyah, Iraq.

Study animals

Fifty female white albino rats, who were housed in plastic boxes divided randomly into two groups 25 rats for each. Control group G1 had no treatment, second group G2 treated with herbal cellulose gelatin foam.

Preparation of cellulose gelatin foam:

Gelatin of BioReagent grade was obtained from Sigma Aldrich in powder form. Ultrapure water obtained from a Millipore purification system (Milli-Q) was first heated to 40 °C. Gelatin was added and manually stirred until dissolved. Cellulose was added to the solution. Upon dissolution at 40 °C, stirring was continued for 10 additional minutes. Solutions were cast into plastic 52 ± 3 mm diameter petri dishes and allowed to dehydrate at ambient conditions for at least six days to form dehydrated films. Dehydrated gelatin films aged in ambient conditions were foamed using a microwave oven at wattage of 700 W and a frequency of 2450 MHz for 30 to 50 seconds then kept at – 20°C (Frazier, et al., 2018). Sample from cellulose gelatin foam was examined under SEM for the morphology of the foam at 100 µm.

Under routine surgery with general anesthesia all rats had thermal injuries at the left side of their backs. The thermal injuries were then created using a solid aluminum bar with a diameter of 2 cm in diameter had previously been heated to 100°C in boiling water. The bar is kept in touch with the rat's skin in the dorsal proximal region of the back. Hyperemia, edema, scar tissue, and scab were recorded for morphological exam. The therapies were applied topically once daily for 10 days. At the time of burn and on the appropriate days for the biopsies at 7th, 14th, 21st, and 28th day, sterile "swabs" were taken and sent to laboratory for bacterial contamination. Biopsies were collected for histologic evaluation of healing. The morphologic appearance were recorded at 1 day, 7th day, 14th day, 21st day, and 28th day,

Statistical Analysis

The significance between groups was determined using statistical analysis of the morphometric data using the ANOVA test and Least Significant Difference (LSD) to the level of $P \leq 0.05$ (Razooqi et al., 2022; Gharban, 2023).

Results

Table-1 showed the clinical signs of the burn along the period of the study 0 day, 7th day, 14th day, 21th day, 28th day. The Clinical signs were hyperemia, edema, crust, and scar tissue.G1 showed sever hyperemia and edema at 0 day while moderate hyperemia and mild edema and crust at 7th day. At 14th day there were mild hyperemia, mild crust, and scar tissue. At 21st day, moderate crust and mild scar tissue. At 28th day there were sever crust and mild scar tissue.G2 showed mild hyperemia and crust at 7th day. At 14th and 28th day showed absent of clinical signs of the burn. At 21th day showed mild scar tissue.

Table 1: Clinical parameters recorded in the experimental rats at different periods.

Groups	periods	Clinical signs			
		Hyperemia	Edema	Crust	Scar tissue
G1 (n=25)	0 day	+++	+++	-	-
	7^{th} day	++	+	+	-
	14^{th} day	+	-	+	+
	21^{st} day	-	-	++	+
	28^{th} day	-	-	+++	+
G2 (n=25)	7^{th} day	+	-	+	-
	14^{th} day	-	-	-	-
	21^{st} day	-	-	-	+
	28^{th} day	-	-	-	-

The intensity of clinical signs were scored as - :absent, present: +:mild, ++:moderate, +++: severe

Wound contraction

Wound contraction parameter was an essential for burn healing evaluation. Table-2 showed the mean ± SE of the diameters values of the burn contraction along the period of the study 7th day, 14th day, 21st day, 28th day. The G1 recorded significant 1.81±0.02, 1.67±0.03, 1.48±0.04, 1.17±0.02 respectively. G2 recorded highly significant 1.37±0.03, 1.27±0.03, 0.66±0.04, 0.20±0.02 respectively.

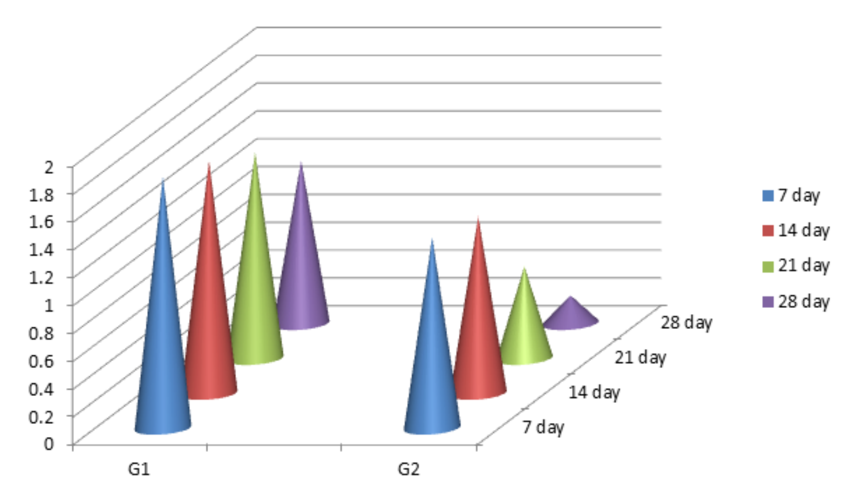
Table 2: Results of the diameters of Burn contraction

Groups	Diameters of burn (mm.)			
	7^{th} day	14^{th} day	21^{st} day	28^{th} day
G1 (n=25)	1.81±0.02 Aa	1.67±0.03 Ab	1.48±0.04 Ac	1.17±0.02 Ad
G2 (n=25)	1.37±0.03 Ba	1.27±0.03 Cb	0.66±0.04 Cc	0.20±0.02 Cd

* Capital letters refers to the vertical statistical comparison, whereas small letters refer to the horizontal statistical comparison.

* Different letters denote to the significant difference at $P<0.05$, whereas similar letters refer to the no significant difference.

Figure 1: Diagram of wound contraction showed the differences between the experimental groups



Bacterial contamination

Bacterial contamination might affect the burn healing. Table-3 showed the Mean± SE bacterial colonies counts (CFU/ml.) post the treatments of the experimental rats along the period of the study 3rd day, 7th day, 14th day, 21st day, 28th day. The bacterial colonies count of G1 recorded 15.2±1.42, 107.1±2.05, 117.1±1.16, 133.5±2.68, 141.2±3.07, respectively were significantly along the period of the study. the G5 recorded 9.4±1.03, 7.6±13.5, 6.7±1.10, 7.7±0.55, 6.6±0.70, respectively were significantly at 3 and 7 day while non-significant at 14, 21, and 28 day.

Table 3: Mean± SE bacterial colonies counts (CFU/ml.) post the treatments of experimental rats

Groups	Post treatment period				
	3rd day	7th day	14th day	21st day	28th day
G1 (n=25)	15.2±1.42 Aa	107.1±2.05 Ab	117.1±1.16 Ac	133.5±2.68 Ad	141.2±3.07 Ae
G2 (n=25)	9.4±1.03 Aa	7.6±13.5 Cb	6.7±1.10 Da	7.7±0.55 Da	6.6±0.70 Ba

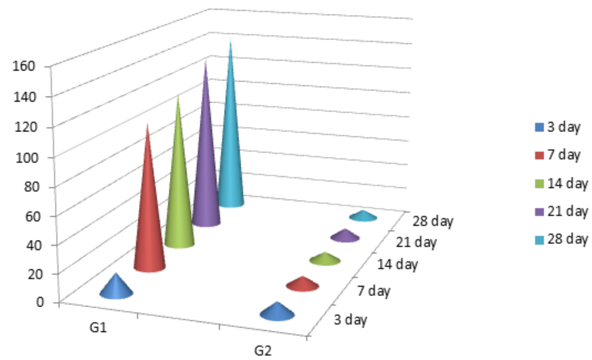
*Different letters mean the variances were significant at P≤ 0.05

*LSD = 9.59

Table 4: Bacterial contamination rate (%) of post burn wounds according to colonies count

Groups	Colonies count (%)				
	3rd day	7th day	14th day	21th day	28th day
G1	(1.5%)	(30%)	(15%)	(38%)	(50%)
G2	(0.9%)	(11%)	(14%)	(12%)	(8%)

Figure 2: Diagram of bacterial contamination showed the differences between the experimental groups

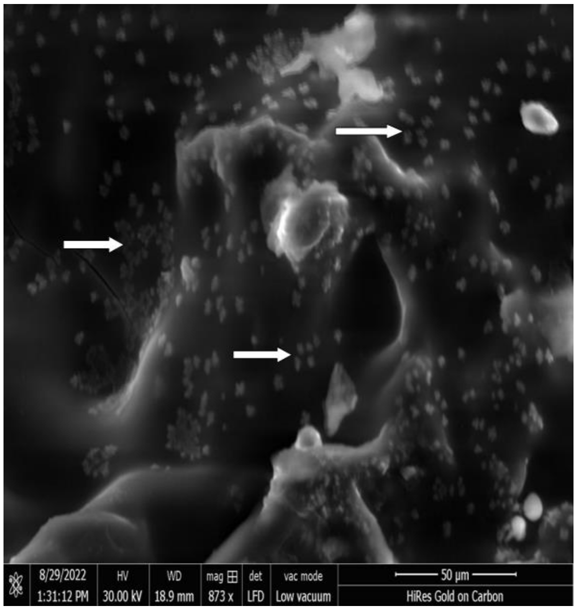


Morphological appearance showed different phases of burn healing at different study experimental days

Morphologic appearance of cutaneous burn of the animal groups showed at 0, 7, 14, 21, and 28th days. At 0 day there were pale sharp edge of the burn, at 7th day the burn still covered with scab with clear reduction of the burn size of G2 than G1 (Figures 3, G1) while G2 (Figure 1, G2) showed decrease in size. Complete epithelialization was seen with less scar tissue. At 28th day complete healing were noticed G2 so that it was difficult to distinguish the healed burn from the normal skin.

Figure 3: Morphological appearance of burn in groups at different periods

Figure 4: Scanning Electron Microscope analysis (SEM) image of cellulose gelatin foam showed extracted cellulose microfibers (white arrow)



Histological Assessment of Wound Healing

The histological assessment reveals the following changes of each group for four weeks, as the figures below.

Figure 5: w1. G1. Histological changes following burn injury showed the presence of remnants of the epidermis layer full of infiltrating inflammatory cells (red arrows), the presence of a gap between the epidermis layer and the dermis (blue arrows), the infiltration of a large number of inflammatory cells in the boundary between the two layers below the gap (black arrows) mostly lymphocytes, extravassated RBCs (EVRBCs), edema, accumulation of few fibroblast cells (pink arrow), and the absence of the characteristic hair follicles and sebaceous and sweat glands. Hand E, 40X, 100X, and 400X

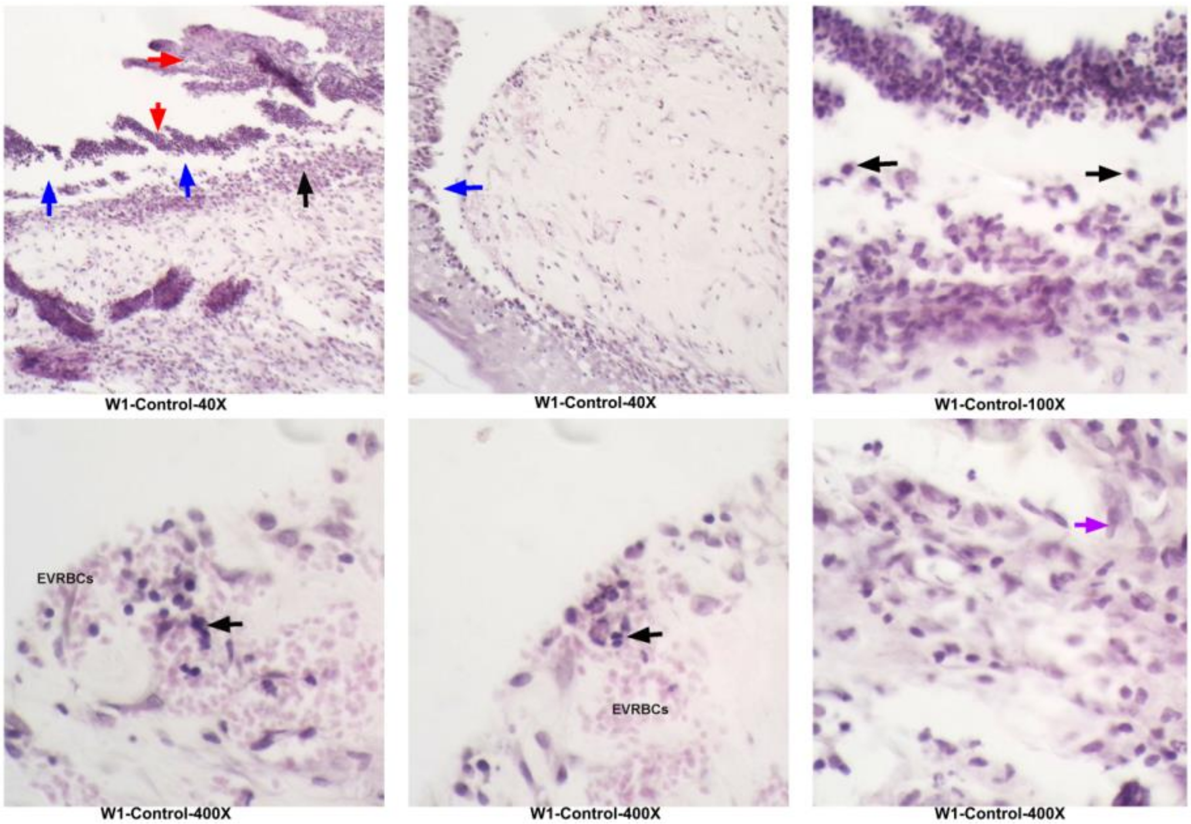


Figure 6: W2-G1. Histological changes following burn injury showed destructed epidermis (black arrows) and thick scab formation (blue arrows), subepidermal and dermal edema (red arrows) and severe inflammatory cells infiltration (INFLC). The inflammatory infiltrated cells are mostly epidermis neutrophils (green arrow) and macrophages (pink arrow) as well as extravassated RBCs were seen. Hand E, 40X, 100X, and 400X

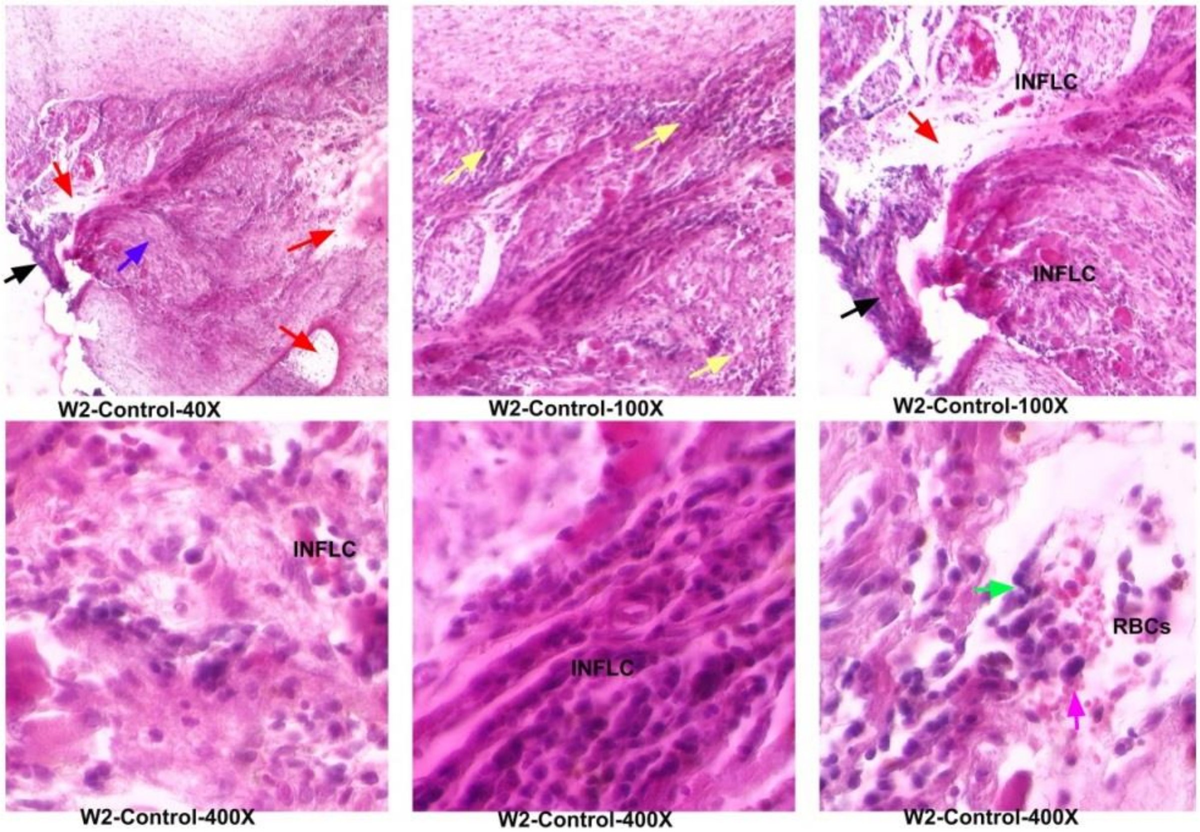


Figure 7: W3-G1. Histological changes following burn injury showed partial re-epithelization (blue arrow) of stratum basale (2) and stratum spinosum (1), subepidermal edema (ED) were evident. Hand E, 40X, 100X, and 400X

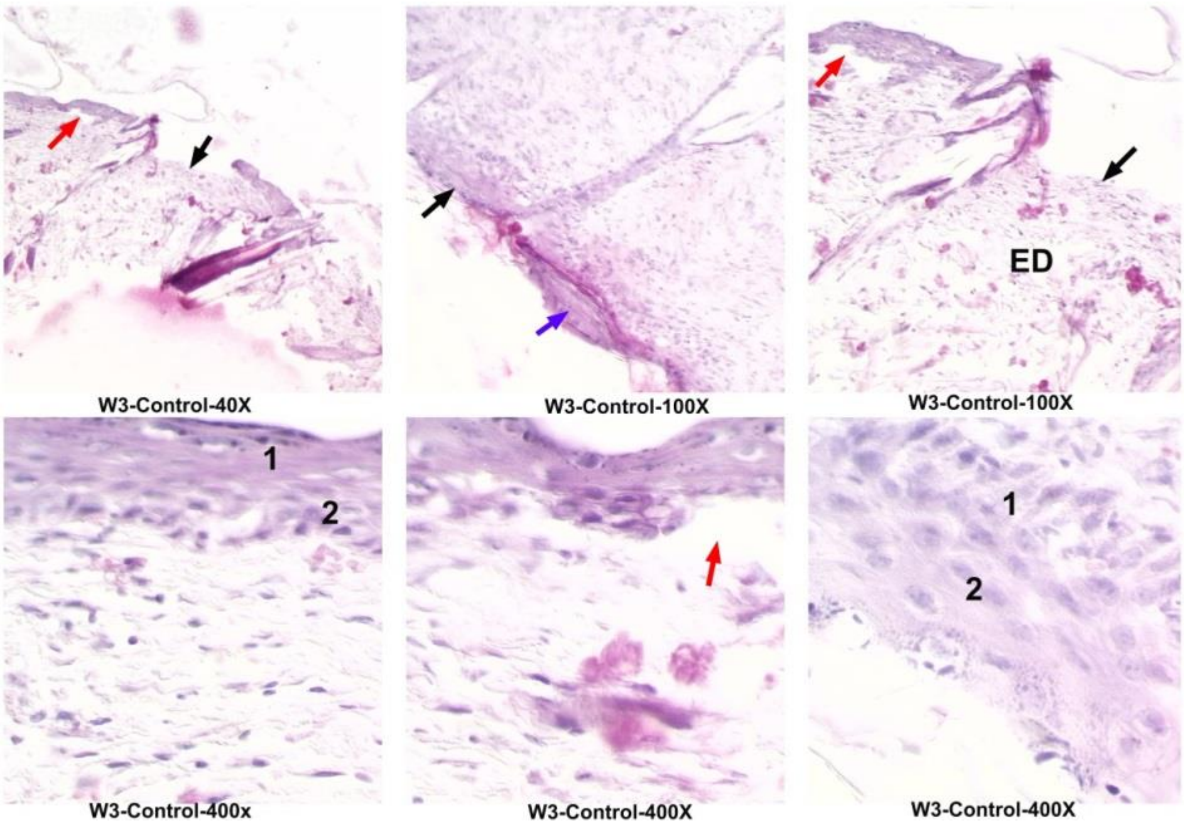


Figure 8: W4-G1. Histological changes following burn injury showed complete re-epithelization (black arrows) of epidermis (EP), but a subepidermal edema (red arrows) was evident, newly formed hair follicles (HF), sweat glands (SWG), and sebaceous glands (SBG) were seen, but subcutaneous edema (SCE) also present. Hand E, 40X, 100X, and 400X

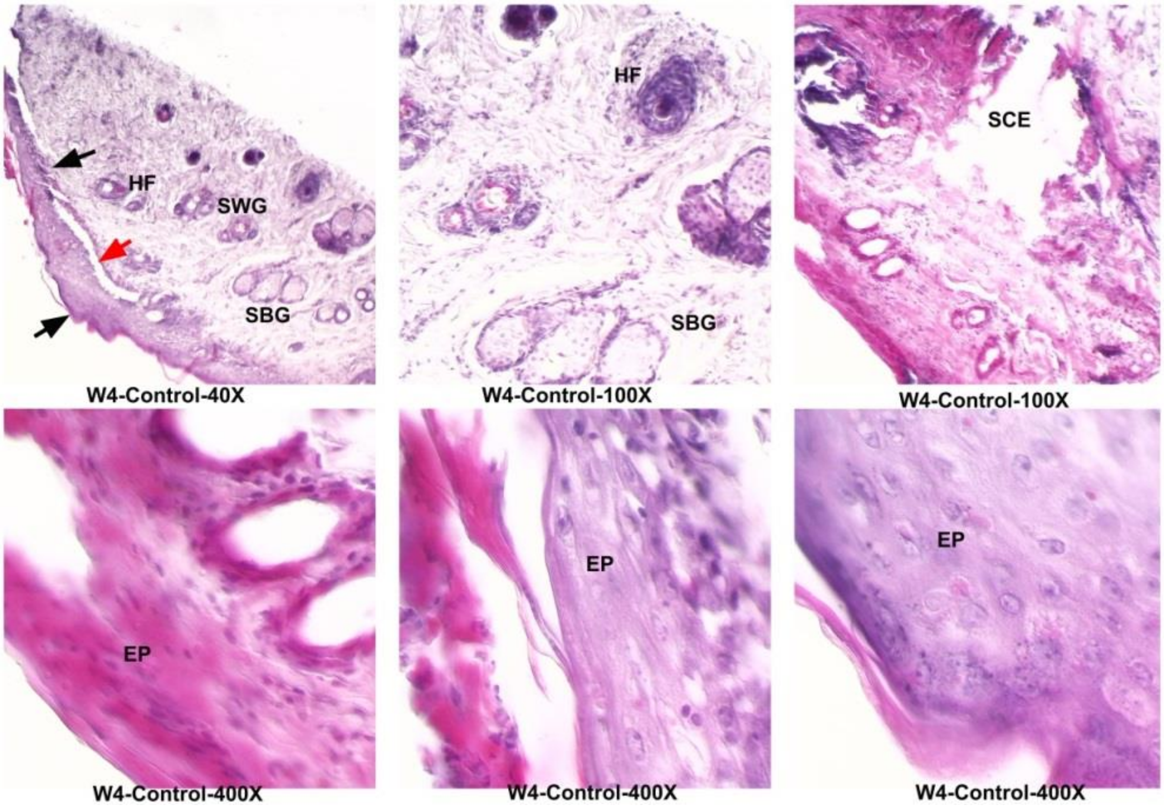


Figure 9: w1-G2. Histological changes following burn injury showed severe destruction and necrosis of the epidermis (black arrows) layer, presence of gap and edema between epidermis and dermis (red arrow), Loss of the normal microarchitecture of the epidermis and hair follicle (f), thickening of epidermis, angiogenesis (blue arrow). Hand E, 40X, 100X, and 400X

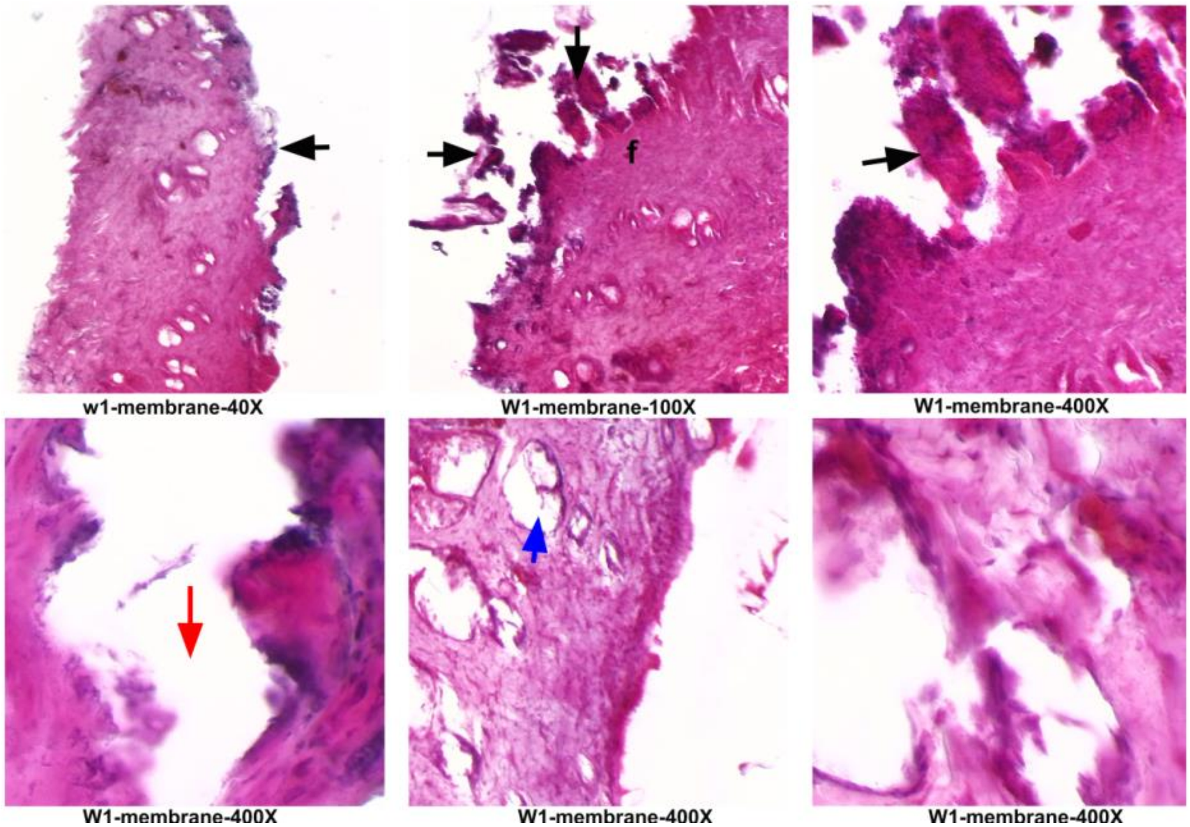


Figure 10: W2- G2. Histological changes following burn injury showed partial re-epithelization (black arrows) of two layers of epidermis including stratum basale (red arrow) and stratum spinosum (blue arrow) layers, subepidermal edema (ED), infiltration of the inflammatory cells (INFLC), and extravassated RBCs were evident. Hand E, 40X, 100X, and 400X

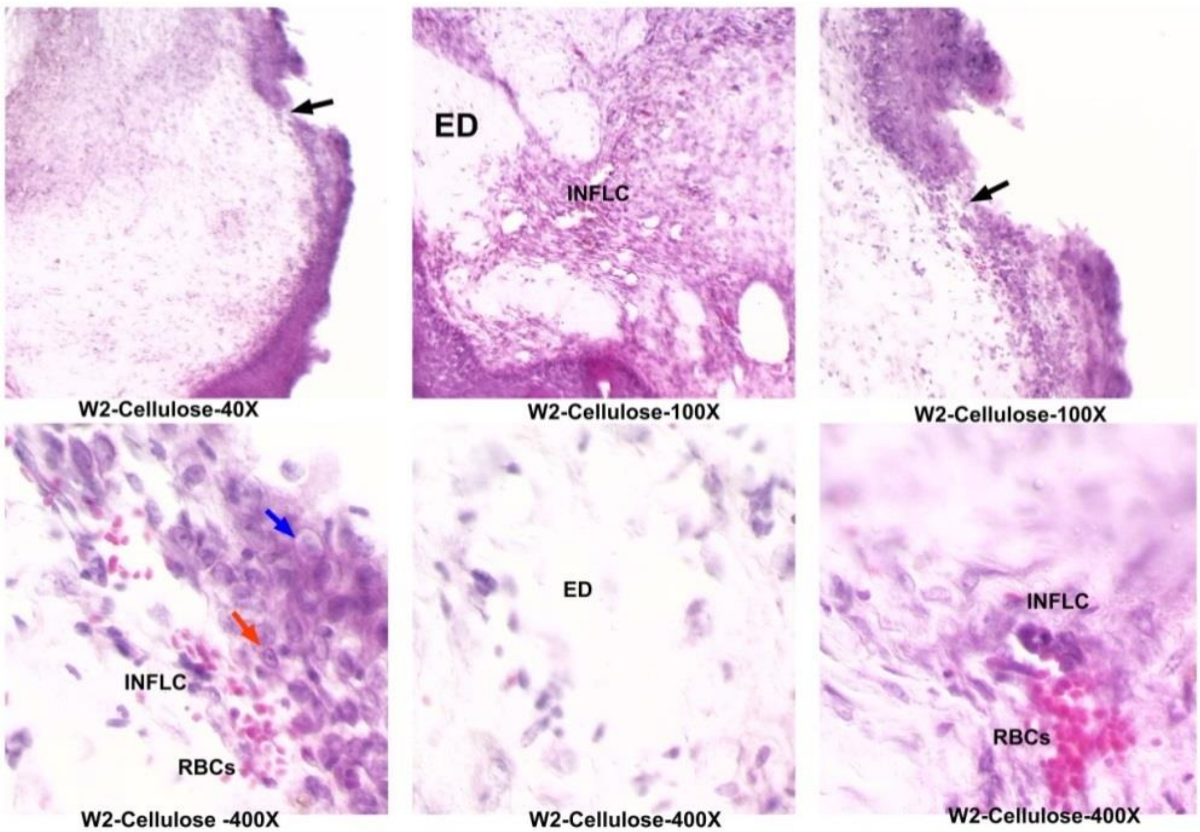


Figure 11: W3-G2. Histological changes following burn injury showed partial re-epithelization (black arrows) of two layers of epidermis including stratum basale (red arrow) and stratum spinosum (blue arrow) layers, large subepidermal (ED) and dermal edema (ED), infiltration of inflammatory cells in the epidermis and dermis, neutrophils (N) and lymphocytes (L), and extravassated RBCs were evident. Hand E, 40X, 100X, and 400X

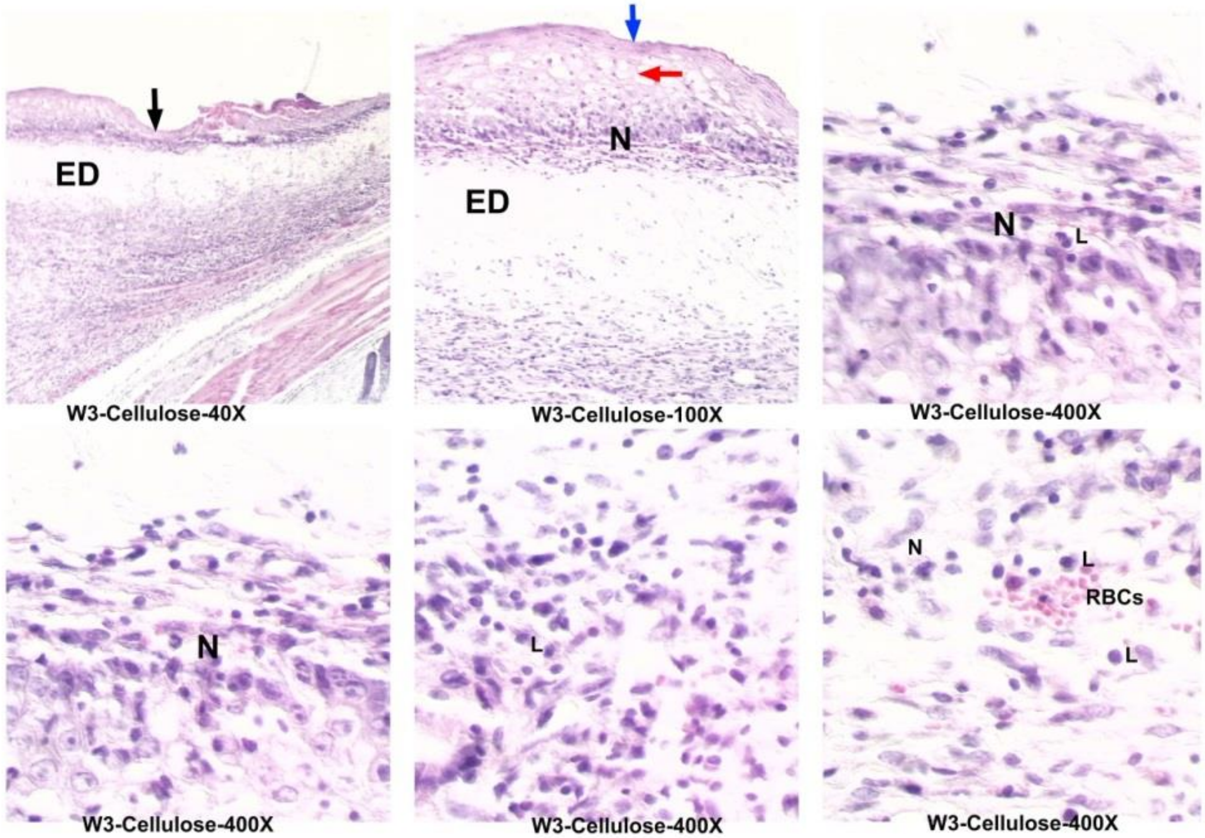
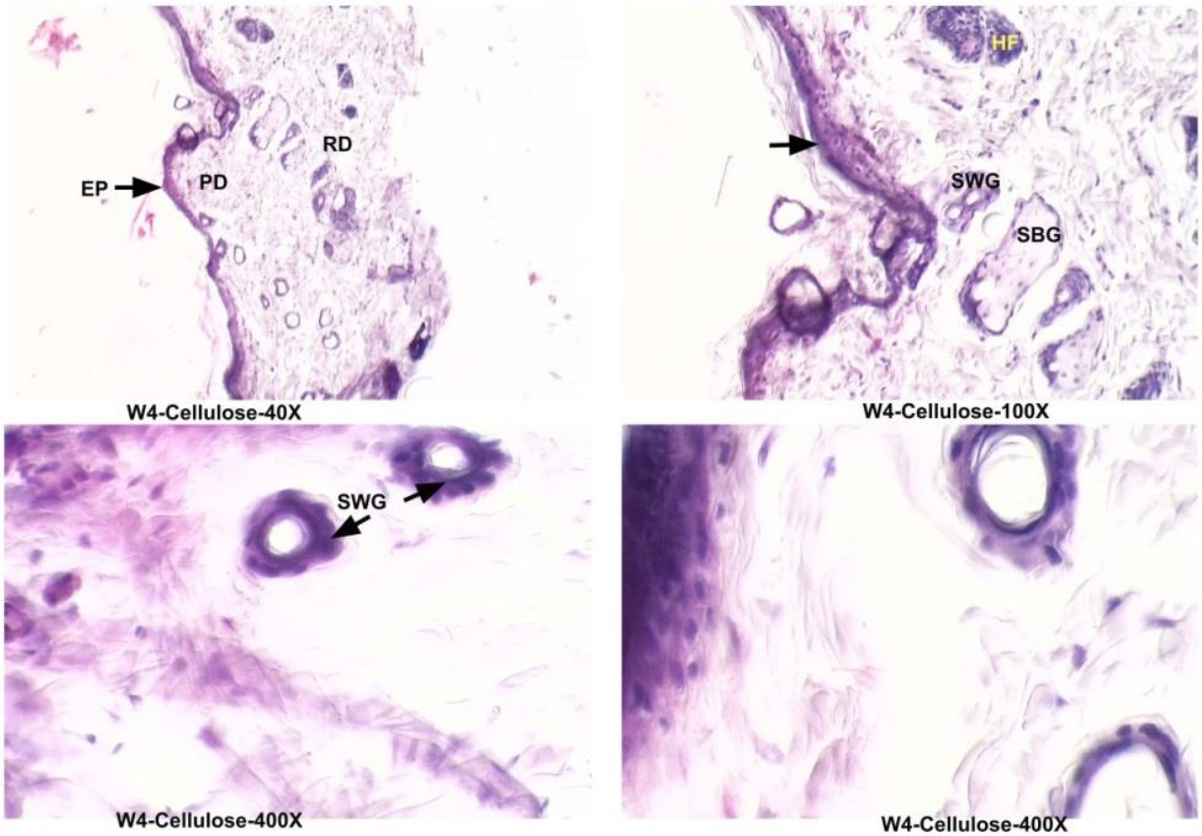


Figure 12: W4-G2. Histological changes following burn injury showed complete healing of all layers of skin including epidermis (EP), papillary dermis (PD), reticular dermis (RD), and hypodermis, hair follicles (HF), sweat glands (SWG), and sebaceous glands (SBG) were obvious. Hand E, 40X, 100X, and 400X



Discussion

The extent of the burns that were associated with the treatment groups exhibited a substantial decrease in inflammatory symptoms at all times of monitoring, revealing significant effects of the application of cellulose gelatin foam. Time-based decreases in burn size are not seen in group 1, however after 21 days, when compared to the other treatment groups, the later group did demonstrate such reductions in burn size.

Cellulose derivatives have the right and ideal applicability to develop novel wound dressings that can enhance wound healing, according to studies conducted primarily in the past ten years on cellulose derivatives-based wound dressings with various bioactive agents to accelerate the regeneration of skin tissue injuries (Lee and Yoo, 2021).

The creation of new biopolymeric materials with specific properties to create the ideal wound dressing is required for the development of dressings that enable quick healing with few visible scars on the body's surface. The cellulose microfibrils network accelerates burn healing by acting as a transport scaffold for fibroblasts.

It's important to note to be easily applied, to maintain local moisture, to ensure an appropriate exchange of gases (O2 and CO2), to absorb exudates that develop on the lesion site (Mishra et al., 2017), to stimulate angiogenesis, to protect against external pathogens, to clear the injured tissue, to eliminate nonviable tissues, to reduce the exposed area (Kamoun et al., 2015), to be easily removed and replaced (Patil et al., 2020), Additionally, materials for wound dressings must be elastic, sterile, non-adherent, and non-allergenic (Marin et al., 2018), as well as affordable and able to insulate heat (Akrami et al., 2020).

Microorganisms can thrive in an environment that is created by wounds. Staphylococcus aureus, Escherichia coli, Clostridium perfringens, Clostridium tetani, coliform bacilli, and enterococcus are among the typical microbiological pollutants of wounds (Bowler et al., 2001). Pseudomonas aeruginosaand Staph.aureus, two organisms frequently linked to chronic, non-healing wounds, were among the pathogenic bacteria that were evaluated in clinically isolated microbiological contamination of wounds (Bjarnsholt et al.2008). A more prominent acute inflammatory response brought on by microbial infection in the wound slows healing and increases the risk of secondary infection and tissue damage (Bowler and others, 2001). As a result, the extract's antibacterial activity on wound isolates may partially contribute to the wound healing effect by eradicating infection and so enabling the start of regular tissue regeneration processes. Therefore, by removing the existing infection, palm frond cellulose extract may be useful in hastening the healing of old wounds.

Due to maintaining a sufficient temperature, hyperemia as shown in table -1 was mild only after the seventh day. Edema was absent in G2 due to promoting blood circulation, and these results are consistent with (Dhivya et al., 2015). The crust was mild at day 7 and then disappeared at days 14, 21, and 28 in G2 as a result of maintaining local moisture, which is in accordance with (Mishra et al., 2017). Wound contraction, G2 showed a substantial superior contraction of 0.20 0.02. This result, which is consistent with (Dhivya et al., 2015), was caused by a speeding up of fibroblast growth, promotion of blood circulation, and stimulation of cell expansion, as shown in table-2. Bacterial contamination, as shown in table-3 also had the best effect on bacterial contamination in G2 due to act as antibacterial activity by protect against extraneous pathogens, to clear the injured tissue, to eliminate nonviable tissues, and to reduce the exposed area. These findings are consistent with those of (Kamoun et al. 2015) and (Bjarnsholt et al. 2008). Microscopic Findings of G2 showed that the histological changes post burn injury showed complete healing of all layers of skin including epidermis, papillary dermis, reticular dermis, and hypodermis, hair follicles, sweat glands, and sebaceous glands were obvious. The efficacy of the cellulose polymer that used for G2 was clear in speed up the angiogenesis and collagen fibers synthesis as mention by different studies (Nayak et al., 2006; Barua et al., 2009; Pilehvar-Soltanahmadi et al., 2018). The cellulose extract also might contain hemicellulose, and lignin as polymers with high water contents (99%) that preserve the moisture of the burn which help in healing process (Tanga et al., 2022). Zha et al. (2020) found that cellulose was excellent scaffold in tissue engineering because of its electro conductivity that accelerated the proliferation of fibroblasts.

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