Original Article

Estimation of the healing effects of the topical use of MEBO and hyaluronic acid gel in the burned rats

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ABSTRACT

Exposing the dorsal superficial skin of rats to partial-depth burn leads to bacterial and microbes Invasion. Topical treatment is required in most superficial burn cases Moist exposed burn ointment (MEBO) protects wounds from infection and enhances healing without any harmful effects of purified chemicals is caused. The topical using of HA gel in rat models with full and partial thickness surgical wounds shows enhancement in wound repair. In this study, we compared the healing efficacy of topical use of hyaluronic acid gel products with MEBO as standard management in rats that were exposed to a partial-thickness burn. The experiment included twenty-four (24) adult albino rats of male sex with weight (150-220 gm) of 3 months' age divided into four groups. Partial-thickness burn wounds are applied on their shaved dorsal skin by a hot metal plate for 4 seconds. Group I: control group received topically normal saline as a vehicle placebo, group II: are treated topically with MEBO, Groups III & IV: are treated topically with hyaluronic acid gel (Afta Med and Gum Afta Clear) respectively twice daily for 14 days. The burn wound area was daily examined and measuring the diameter of the lesion area was until day 14 and photographs of the lesion area and wound repairs were taken at different time intervals (0,3,5,7, 10, and 14). % of wound contraction also detected. The rats were sacrificed on days 11 and 14, then elevates 5 mm of diameter full-thickness flap of healed and unhealed wound areas. Wound healing occurs faster when using HA gel products topically for treatment of the induced partial-thickness burn when compared with using MEBO and control group which was supported with histological examination and statistical analysis.

Keywords: MEBO, Hyaluronic acid gel, Partial-thickness burn, Wound-healing, Re-epithelialization

Introduction

The definition of a burn is coagulative necrosis or damage to the skin caused by excessive exposure to heat or caustic chemicals [1]. Burns is a common and hard healthcare issue [2]. The possibility of healing of a burn wound and/or its depth is considered the crucial determining factor in the management protocol of burns [3]. A burn that influences the epidermis and dermis which are the top two layers of skin, is called the partial depth burn (a second-degree burn). This type of thermal injury can continue to change over time and can progress to a full-thickness burn (third-degree), even after initial management. Partial-thickness burns can be serious and life-threatening and have a high risk of mortality and morbidity.

The healing of a burn wound is a dynamic and active operation that commences from the beginning of an injury. Additionally, the major difficulties for health care are delayed healing of burned wounds and this led to a lowered quality of life for patients who have this kind of deep wound [4, 5]. The delay in the initiation of proper burn management can elongate the healing process [6]. Infection is a serious complication of burn injuries and is responsible for 50–75% of the burn mortality rate [7]. So, prophylactically several antimicrobial agents are described to prevent the development of infection, at the same time other medications are intended to kill proliferating microbial cells that

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are within the burn when an infection has been developed [8]. The selection of the topical treatment should be depended on the ability of an agent to inhibit the recovery of microorganisms from burn wound surveillance cultures [9].

Moist exposed burn ointment (MEBO) is an oil-based natural pure preparation of herbal generally utilized in the Middle East and Asia [10]. In 1989; It was developed at China National Science and Technology Centre. Then, formulated in the USA as a burn ointment since 1995 [11]. It is used to treat different burn thicknesses and accomplish good results in clinical pieces of research [12]. In Chinese literature, several experimental and clinical research has demonstrated that the MEBO markedly decreases the evaporation of water from the surface of burn wounds [13]. MEBO consists of sesame oil, berberine oil and beta-sitosterol (is a plant steroid), and other Chinese herbal ingredients. The oils relieve pain, retain moisture and soften the wounds. Beta-sitosterol stimulates epithelialization [10]. Many studies express that β-sitosterol has anti-inflammatory effects [14] and berberine shows antimicrobial effects [15]. Different experimental and clinical researches explain the antimicrobial and analgesic effects of MEBO, which also accelerates the timing of burning treatment and wound healing in patients [12, 16, 17]. Additionally, MEBO promotes epithelial repair and debridement effect, with improved quality of scar and decreased treatment costs for patients [12, 17]. Similarly, it exhibits chronic ischemic and neurogenic ulcer healing [18, 19].

Hyaluronic acid (HA), is an essential physiological polysaccharide substance in the body composed of the glycosaminoglycan group which consists of N-Acetyl-D-glucosamine and D-glucuronic acid monosaccharide molecules. It is mostly found in a huge amount of synovial fluid, including cartilages, joints, eyes, and skin tissues [20]. During wound healing, HA has a crucial role in the formation of healthy connective tissues and enhances inflammatory response and attenuating inflammation by decreasing inflammatory cell infiltration and possibly stabilizing granulation tissue, regeneration and angiogenesis, also exhibits scavenging of free radicals which form during wound healing [21-23].

The topical using of hyaluronic acid gel in rat models with full and partial thickness surgical wounds shows enhancing in wound repair and facilitates re-epithelization which led to healthy tissue formation with good elasticity and high microvascular density [24] also by decreasing abscess formation, necrosis, and neutrophil infiltration as well as reduced inflammation in skin wounds in rats. Additionally, the synthesis of collagen, the formation of fibroblasts, and vascularization are also enhanced by HA treatment [25, 26].

The HA action happens through a cluster of differentiation (CD) 44 cell receptors. CD44 and intercellular adhesion molecule-1 are adhesive cell molecules that play a role in cell migration [21, 27]. The molecular weight of the HA plays a vital role in the appearance of these HA effects in wound healing [28]. The healing of wounds of soft tissue is more rapid than bone wounds healing [29] and HA with high molecular weight shows effective healing for infected bone wounds by developing bone formation and enhancing regeneration of bone defects [30]. hyaluronic acid brings moisture to the surface of skin because of the ability of HA to hold a thousand times its weight in water, and not only keep that moisture in skin and joints, but it is also preventing evaporation of all the moisture into the air [31].

The present study aims to compare the healing effect of the topical treatment between MEBO and hyaluronic acid gel products in rats exposed to a partial-thickness burn.

Materials and Methods

Materials

Normal saline (0.9% N.S), lidocaine (1ml/200 gm), Povidone-iodine**,** MEBO: Gulf Pharmaceutical industries (Juiphar), U.A.E. was purchased from Iraqi Medical Center Pharmacy, Hyaluronic acid gel products (Gum Aftaclear and Afta med). In addition, Sunstar Europe S.A. Made in Italy, Afta med with high molecular weight hyaluronic acid 240 mg/100 g gel is marketed by Pharmaniaga Sdn Bhd, Malaysia, and Gum AftaClear gel.

Experimental animals

Twenty-four male albino rats with a weight of (150-220 g) of age 3 months were obtained by the Animal House of the College of Pharmacy/ University of Baghdad. They are homed under standardized conditions of temperature, humidity, and light/dark cycle in the same location and individual cages about 2 weeks before the experiment. They were nourished with a standard rodent pellet diet and supplied with free water. The local Research Ethics Committee in the College of Pharmacy, University of Baghdad, approved the research protocol [32].

Method

Induction of burn wound

The back of each rat was shaved and washed with povidone-iodine solution. Then, all rats were anesthetized subcutaneously with lidocaine (1ml/200 gm), and 1 by 1.5 cm of partial-thickness burn wounds (2^{nd}- degree burn wounds) < 20% total body surface area were induced on a shaved area of the animals' dorsal skin, by Smahel method. A steel plate 2 mm thick (1 cm diameter) was heated in boiling water for 7 min and applied firmly and perpendicularly to the skin for 4 seconds, then the medical therapies were applied topically twice a day until 14 days .and then all wounds were exposed to air and checkout daily for evidence of clinical infection [33].

They were divided into 4 groups each one supplies with 6 rats as a following:

Group I: (Negative control) they received topically normal saline solution (as a placebo vehicle) on skin burn twice/day for 14 days.

Group II: (Positive control) they are treated topically with MEBO twice/day for 14 days.

Group III and **IV**: They are treated with topical hyaluronic acid gel products (AftaMed and Gum AftaClear) respectively twice/day for 14 days.

Measurement of burned wound area

Photographics of each burned lesion area were obtained regularly each day by a camera phone (Canon-Japan) to observe wound surface areas healing and constriction as shown in **Figure 1**. also percent (%) of burned wound contraction was calculated according to this formula [34], as shown in **Table 1**.

$$\% \text{ Wound contraction} = (\text{Current wound area/wound area at the beginning}) \times 100 \quad (1)$$

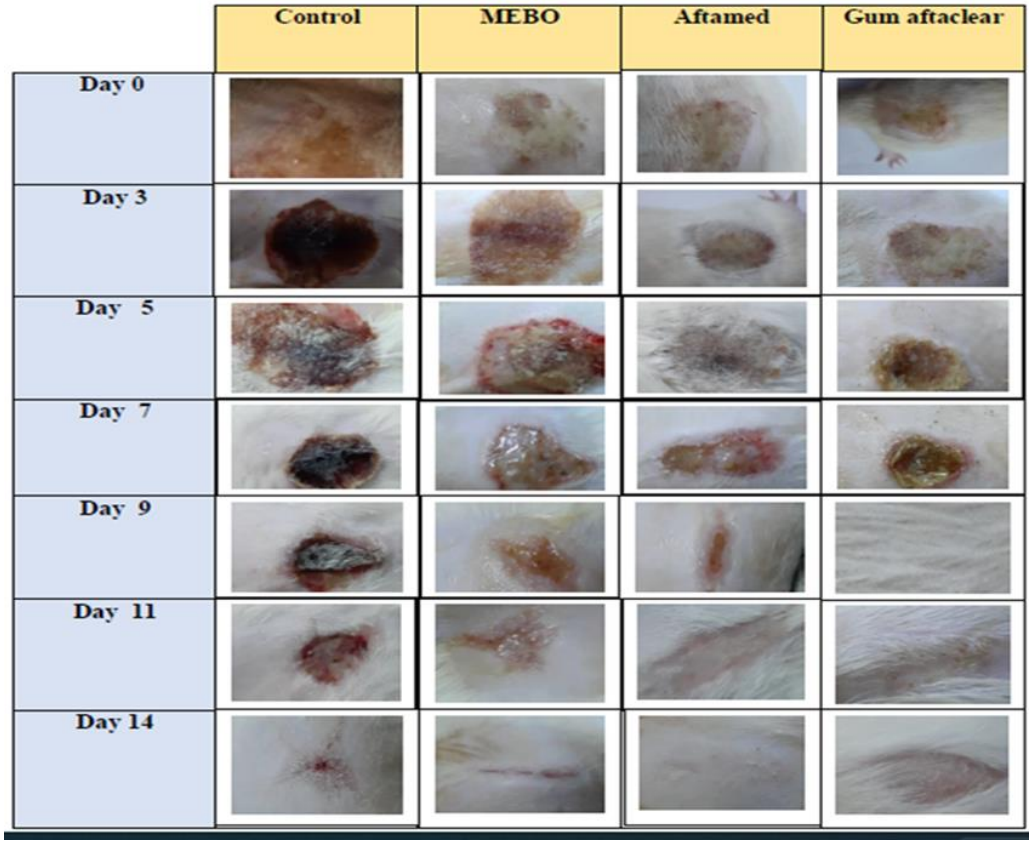

Figure 1. Gross appearance of wound healing skin in burned rats

Results and Discussion

Statistical analysis

Data were expressed as mean ± standard deviation (SD). The Statistical significance of the differences between various groups was determined by an unpaired student t-test. Differences were considered statically significant for p-value< 0.05.

Table 1. Wound healing by AftaMed hyaluronic acid gel and Gum AftaClear gel and their effects on percentage wound healing in rats

	C % of burn wound contraction (Mean±S.E.M)	MEBO % of burn wound contraction (Mean±S.E.M)	AftaMed % of burn wound contraction (Mean±S.E.M)	Gum aftaClear % of burn wound contraction (Mean± S.E.M)
Day 0	----------	----------	----------	----------
Day 3	124.159±0.767	132.625±1.804	117.269±1.368	121.673±1.613
Day 5	138.845±0.967	124.777±1.203	123.935±1.138	101.589±2.399
Day 7	127.270±0.488	111.522±0.985	91.723±0.955	79.847±0.269
Day 9	112.984±0.656	97.189±0.487	76.070±0.186	71.225±0.211
Day 11	91.564±0.137	82.977±0.219	63.695±0.163	52.709±0.156
Day 14	71.959±0.0913	47.189±0.063	27.946±0.051	19.314±0.099

Table 2. Statistical studies of MEBO and different hyaluronic acid gel products on the burned rat.

	C Mean of burned area (cm^2) ± SD	M Mean of burned area (cm^2) ± SD	AF Mean of burned area (cm^2) ± SD	GUM Mean of burned area (cm^2) ± SD
Day 0	5.0915 ± 0.75499	5.1708 ± 0.49202	5.05466 ± 0.5834	5.0471 ± 0.3031
Day 3	6.3216 ± 0.5797	6.8578 ± 0.8882	5.9275 ± 0.7987	6.141 ± 0.4895
Day 5	7.0693 ± 0.7305	6.452 ± 0.5924	6.2645 ± 0.66492	5.1273 ± 0.7275
Day 7	6.48 ± 0.36832	5.7666 ± 0.48555	4.6363 ± 0.55729	4.03 ± 0.0797
Day 9	5.75266 ± 0.4954	5.0255 ± 0.24036	3.8452 ± 0.10939	3.5948 ± 0.0648
Day 11	4.662 ± 0.10469	4.2906 ± 0.10815	3.21966 ± 0.0951	2.6603 ± 0.04753
Day 14	3.6638 ± 0.06897	2.44016 ± 0.0312	1.4126 ± 0.03046	0.9748 ± 0.03007

Histopathology

The skin specimen from wounds healed areas of rats were sacrificed and the healed skin was assessed by taking a 5 mm^2 section and removed on days 11 and 14. Then the sections were fixed in 10% buffered formalin and processed by a paraffin tissue processing machine and dyed with Hematoxylin & Eosin for examination under light microscopy [35].

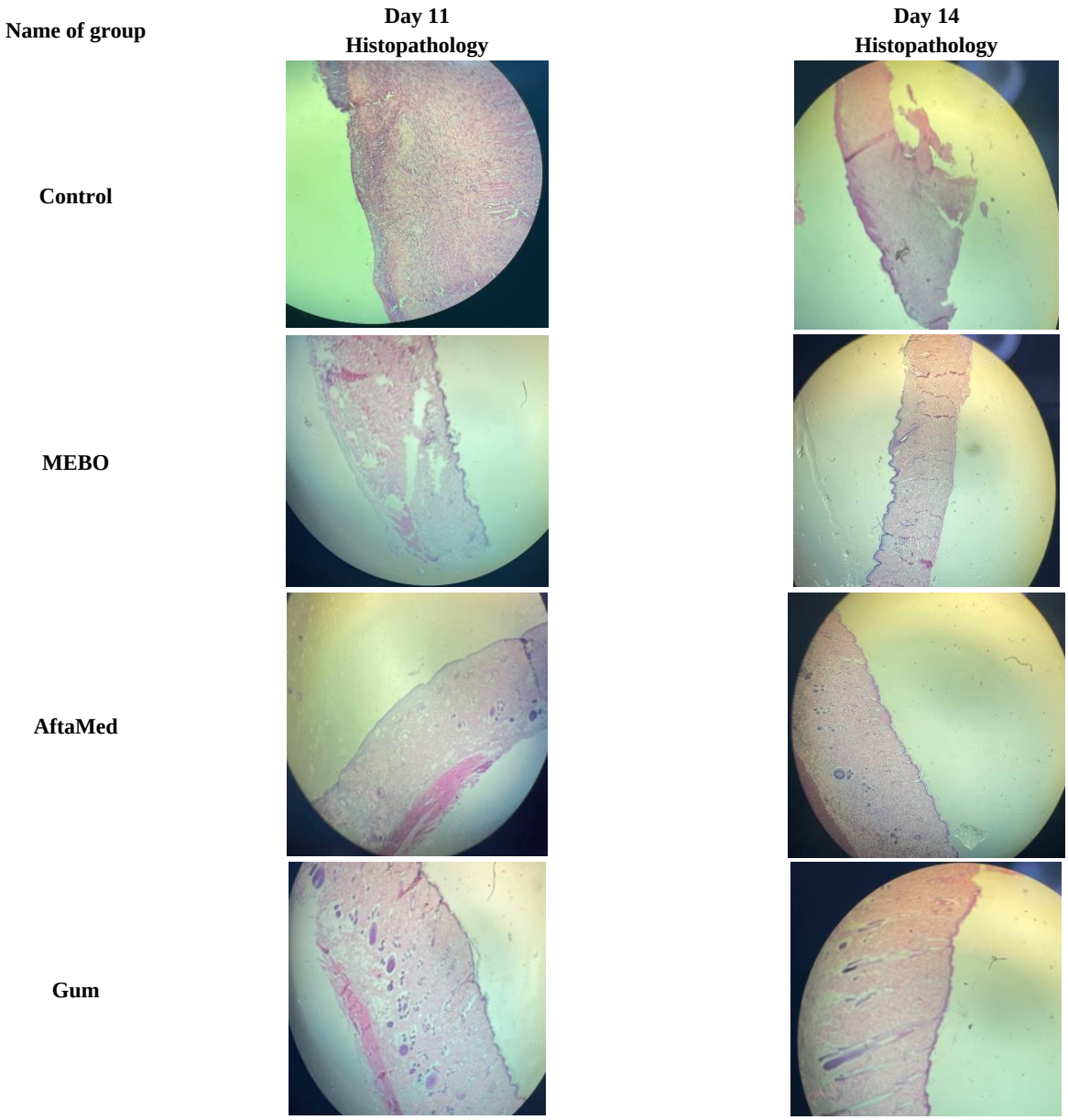

Figure 2. histopathological evaluation of healing burn wounds skin in rats.

Even partial depth of the burned wound is still a difficult clinical problem especially if the involved surface is comprehensive sufficiently [36]. Several compounds were discovered in a plant, and now become used in allopathic medicine but still contrast between herbal and modern medicine because the last depends on a single molecule [37]. So, topical treatment is required in most superficial burn cases. The healing of the burned wound is a biological event of a complex series of processes including; hemostasis, cellular proliferation, and differentiation, inflammation, connective tissue formation, neovascularization, epithelialization, collagen synthesis, and wound contraction, and participation of each process in healing differs in proportion to the kind of wound [38].

The present study was designed to increase the therapeutic field of hyaluronic acid products and to estimate the efficacy of hyaluronic acid gel products as a burned wound treatment in comparison with MEBO topical agent and with a non-treated group in a rat model. The results of the study suggest that the healing capacity of hyaluronic acid formulations is significantly higher as compared to MEBO topical agent and control groups as illustrated by a photographic assessment of the wound healing rate in the rats' model **(Figure 1)**. Furthermore, Statistically the results show that there is shrinkage in the percentage of burned surface area at 5,7 9,11 and 14 post-burn days when dealing with partial-depth burn wounds in rats **(Table 1)**.

Morphological analysis shows that HA gel products significantly accelerate the repairing of the damaged area through reepithelization and proliferation of granulation tissue, fibroblasts, and collagen synthesis **(Figure 2)**. Histologically, HA resulted in thicker regenerated skin, and accelerate quantity of small and newly formed blood vessels. MEBO-treated animals show rapid re-epithelialization on the 11th day which is statistically significant and consistent with previous findings **(Table 2)**, also on the 11th day of a topical GumAftaClear and Afta Med gel treatment show a rapid re-epithelialization and provided an adhesion barrier for burned skin area in comparison with the untreated control group. Several roles in wound healing occurred by HA, it enhances wound healing through regeneration of dermis and dermal and epidermal cell fractions. Although inflammation is crucial to granulation tissue formation, stabilization of this tissue matrix is important, relieving inflammation to maintain normal tissue repair. The role of HA in scavenging free radicals may beneficially impact inflammatory activation and it protects against oxidative stress and proteolytic damage to cells and extracellular matrix [20]. The effectiveness of HA in reducing epithelial cells (as lymphocytes and fibroblasts) proliferation is depending on its molecular weight and concentration, several pieces of research showed that hyaluronic acid with high molecular weight diminishes the inflammation in periodontal disease patients through this mechanism [39]. Considering, the HA molecules play an essential role in the normal epidermis, where it exists as a high concentration in the basal layer [40] and because of the extracellular space 3-dimensional architecture of HA, hydration of nutrient flow, and involvement in proliferation and migration of keratinocyte, makes the use of HA as a one of the developed medical substance in skin wound healing investigations [41]. Differently, MEBO soothes or moist wounds through the moisture-retaining oil, and berberine action and also inhibits the proliferation of microbes and enhances rapid re-epithelialization. This is due to the action of B-sitosterol, MEBO reduces exposure to the burning surface [42].

Conclusion

The hyaluronic acid gel is a suitable and effective agent with a faster capability of burn wound healing when compared with other topical agents (MEBO) that are used for local burn wound care.

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