

Original Article

Wound-Healing Effects of Topical Oxymetholone on Second-Degree Burn Injury in Rats

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Abstract

Background and Objective: Burn trauma is a life-threatening condition that causes disability and the deaths of thousands of people worldwide every year. Previous studies have revealed that anabolic steroids have the potential to improve wound healing. This study aimed to investigate the effect of topical oxymetholone, an anabolic steroid, on second-degree burn wound healing in an animal model.

Methods: This study included 20 male and 20 female Wistar rats aged 8-12 weeks and weighing 200-250 g. Animals were randomly divided into four male and four female groups (n=5). Group 1 received no treatment; group 2 was treated with Eucerin (the ointment base vehicle for oxymetholone); group 3 was treated with topical oxymetholone 1%; and group 4 was treated with topical silver sulfadiazine 1% (SSD) cream as a positive control. Two second-degree burns were created on the dorsal skin of each rat using a heated metal plate. The burn wounds were assessed for 14 days. The percentages of wound closure and the tissue contents of hydroxyproline were measured. Histopathological studies were also performed on tissue samples.

Results: The results of this study showed that topical oxymetholone significantly ($P<0.05$) accelerated burn wound healing compared with the untreated and Eucerin-treated groups. Moreover, hydroxyproline contents of tissue samples collected from the oxymetholone group were significantly ($P<0.001$) higher than those of the untreated and Eucerin-treated groups. Histopathological studies also confirmed the wound healing activity of oxymetholone. However, SSD was more effective than oxymetholone in the treatment of burn wounds.

Conclusion: Oxymetholone has the potential to be used as a topical treatment for burn wounds.

Keywords: Anabolic steroids, Burn, Oxymetholone, Rat

Introduction

Burns occur when the skin or other tissues are damaged by thermal injury (the most common cause), chemicals, electricity, or radiation. Burns are traditionally divided by degree based on the depth of

injury: first (superficial), second (superficial partial-thickness), third (full-thickness), and fourth degree. Fourth degree describes burns that extend beyond the skin into the subcutaneous tissues [1]. Burn injuries are one of the most devastating accidents



and one of the major causes of disability and death worldwide [2]. After traffic collisions, burn injuries are the second most common cause of death [3]. Burn wounds have characteristics, such as slow healing, pain, skin fragility, and abnormal scar formation [4]. Despite many advances in regenerative medicine, burn treatment, and pharmacotherapy, the treatment of wounds remains a major medical challenge [5, 8].

Both minor and severe burn wounds need to heal. The wound-healing process occurs in several highly regulated, overlapping phases, including: hemostasis, inflammation, proliferation, and tissue remodeling. In addition to local wound healing, severe burns trigger a pathological stress response and a hypermetabolic-catabolic state [5]. During the hypermetabolic state, glycogenolysis, proteolysis, and lipolysis are increased. The hypermetabolic-catabolic response leads to lean body mass erosion, protein breakdown, and poor wound healing. One strategy to modulate the metabolic response is the use of anabolic steroids [6]. Anabolic steroids are a class of drugs that have been produced by modifying testosterone to increase its half-life while reducing androgenic side effects [1]. Anabolic steroids have been successfully used in the treatment of burn wounds to reduce the loss of lean mass in the acute phase of injury and also to restore the lost lean tissue faster in the recovery phase [6, 8]. The stress response to injury increases catabolism and decreases anabolism. Anabolic steroids, in addition to their anabolic activity that increases protein synthesis and new tissue formation, also have anti-catabolic activity, as a result of which they prevent protein degradation by catabolic hormones [9].

The mechanism of action of anabolic steroids in wound healing is thought to be through the activation of androgenic receptors, which are mainly found in dermal fibroblasts, myocytes, and epithelial cells [10]. However, several studies have shown that androgens play a vital role in the immune response and are responsible for differences in outcomes based on sex, including sepsis susceptibility, atherosclerosis, and parasite infection [11]. Androgens have been associated with both pro-inflammatory and anti-inflammatory states by modulating systemic and cellular levels of cytokines, such as interleukin (IL)-1, IL-2, and IL-6

[12]. Moreover, a significant decrease in systemic levels of the pro-inflammatory tumor necrosis factor (TNF)-alpha after castration of rats following burn injury has been reported in recent studies [11], and it has been revealed that *in vitro* production of IL-1 and TNF- α by macrophages is inhibited by androstenetriol [13]. This study aimed to evaluate the healing effects of topical oxymetholone on second-degree burn wound healing in rats.

Materials and Methods

Animals

This study included 20 male and 20 female Wistar rats weighing 200-250 g and aged 8-12 weeks, which were used as control and test groups for two weeks. They were randomly divided into four male and four female groups: [1] negative control (untreated), [2] vehicle group (treated with Eucerin), [3] test group (treated with topical oxymetholone 1%), and [4] positive control group (topical treatment with silver sulfadiazine 1% (SSD) cream). Topical treatment was applied twice a day.

Animals were housed in cages (20°C–25°C, 55%–60% humidity, 12 h light/dark cycle) in the animal house and allowed free access to food and water. All experiments were performed according to the instructions of the Animal Ethics Committee of Hamadan University of Medical Sciences (ethical approval: IR.UMSHA.REC.1401.760).

Burn Induction and Treatment

To create second-degree burns, the hair in the designated area was shaved and disinfected with povidone-iodine 10%. Animals were anesthetized by intraperitoneal injection of ketamine (60 mg/kg) and xylazine (10 mg/kg). Then, a heated (100°C) metal plate with a diameter of 1 cm was placed in direct contact with the dorsal skin of the animal for 15 seconds [14]. Second-degree burns were confirmed by microscopic and macroscopic evaluation after 24 hours of burn induction, after which the treatments were administered. The oxymetholone 1% ointment was prepared by dispersing 1 g oxymetholone powder (Sigma-Aldrich, USA; CAS number: 434-07-1) in 100 g Eucerin base.

Wound Contraction Percentage Assessment

The wounds were photographed every three days, and wound areas were calculated using ImageJ^R (Fiji) software. The percentage of wound contraction was calculated using the following equation [15]:

$$\text{Healing percent} = \frac{(\text{wound area on the first day} - (\text{wound area at day X}))}{(\text{wound area on the first day})} \times 100$$

Sampling

The tissue samples from animals in different experimental groups were collected on days 7 and 14 after burn induction. The animals were anaesthetized with ketamine (60 mg/kg) and xylazine (10 mg/kg). After disinfecting the sampling area with ethanol, the wound tissue and a small part of the adjacent intact area were removed. Each sample was divided into two parts: one part was maintained in 10% formalin for histopathological studies, and the other part was frozen at -80°C for the hydroxyproline assay.

Hydroxyproline Content assay

The hydroxyproline contents of tissue samples were measured using a hydroxyproline assay kit (KIAZIST life sciences, Iran) according to the manufacturer's instruction.

Histopathological Examination

The tissue samples were fixed in 10% buffered formalin, embedded in paraffin, and cut to a thickness of 5 µm. Then, the sections were stained with hematoxylin and eosin and Masson's trichrome.

Finally, the stained sections were examined using light microscopy.

Statistical Analysis

Statistical analyses were performed using SPSS software (version 16.0; IBM, ARMONK, NY, USA) and GraphPad Prism 8.00 software (LAJOLLA, CA, USA). For multiple comparisons between groups, statistical tests were performed using two-way analysis of variance (ANOVA) followed by Tukey's post hoc test, with a *P* value < 0.05 indicating statistical significance.

Results

Wound Contraction Percentage

Figures 1 and 2 show representative images of wounds from different groups, indicating improved wound healing in the SSD and oxymetholone groups comparing to the untreated and Eucerin groups. On day 4, the mean percentage of wound closure in the oxymetholone group did not differ significantly from that in the untreated and Eucerin-treated groups, but was significantly (*P* < 0.001) lower than that observed in the SSD group. On day 7, the wound closure rate in the oxymetholone group was significantly (*P* < 0.001) higher than that in the untreated group, however, no significant difference was observed, compared with the Eucerin-treated group, and the rate remained significantly (*P* < 0.001) lower than that of the SSD group.



Figure 1. Representative images of burn wounds of female rats in different experimental groups on day 1, 7 and 14 after induction of burn wound

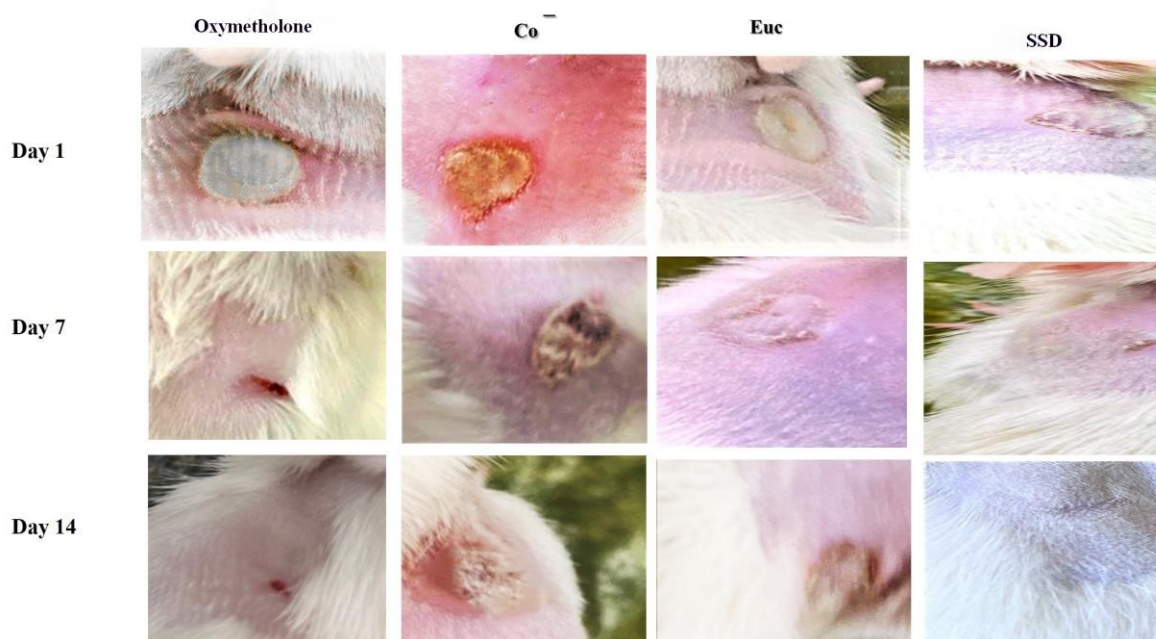


Figure 2. Representative images of burn wounds of male rats in different experimental groups on day 1, 7 and 14 after induction of burn wound.

On days 11 and 14, wound closure rates in the oxymetholone group were significantly ($P<0.001$) higher than those in the untreated and Eucerin groups; however, they were significantly ($P<0.01$) lower than those in the SSD group (Figures 3 and 4). The pattern of wound closure rates across the four experimental groups was consistent between the two independent studies performed in male and female animals.

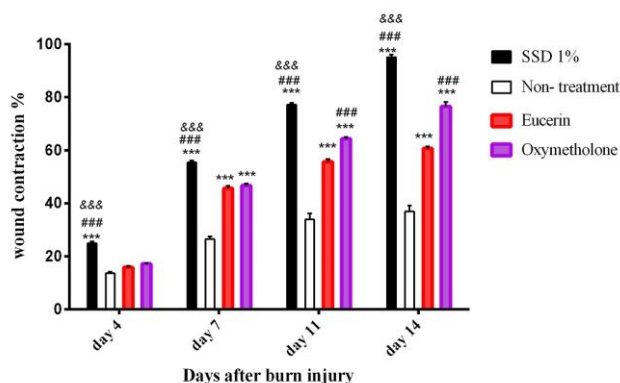


Figure 3. Percentages of wound closure in female animals on day 4, 7, 11, and 14 post burn injury. Data are shown as mean±SD.

*** ($P<0.001$) indicates significant difference from untreated group, ### ($P<0.001$) indicates significant difference from Eucerin group, and &&& ($P<0.001$) indicates significant difference from oxymetholone group

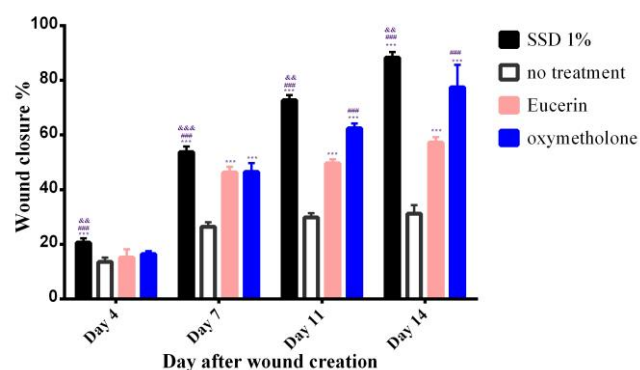


Figure 4. Percentages of wound closure in male animals on day 4, 7, 11, and 14 post burn injury. Data are shown as mean±SD.

*** ($P<0.001$) indicates significant difference from untreated group, ### ($P<0.001$) indicates significant difference from Eucerin group and, &&& ($P<0.001$), && ($P<0.01$) indicate significant difference from oxymetholone group.

Hydroxyproline Content in Tissue Samples

Figures 5 and 6 show the hydroxyproline concentrations in female and male animals on day 14, respectively. The SSD-treated group had the highest concentrations of hydroxyproline in both male and female animals. The hydroxyproline content in the oxymetholone-treated group was significantly ($P<0.001$) higher ($P<0.001$) than that in untreated Eucerin-treated groups.

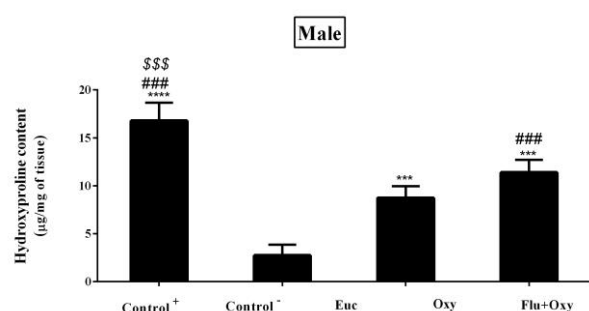


Figure 5. Hydroxyproline concentrations in tissue samples of female animals in different experimental groups. Data are shown as mean±SD.

*** ($P<0.001$) indicates significant difference from untreated group, ### ($P<0.001$) indicates significant difference from Eucerin group, and, \$\$\$ ($P<0.001$) indicates significant difference from oxymetholone group

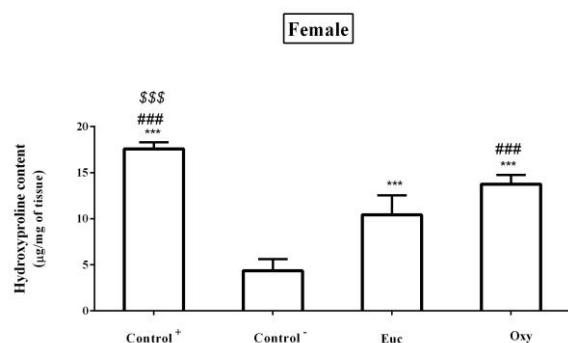


Figure 6. Hydroxyproline concentrations in tissue samples of male animals in different experimental groups. Data are shown as mean±SD.

*** ($P<0.001$) indicates significant difference from untreated group, ### ($P<0.001$) indicates significant difference from Eucerin group, and \$\$\$ ($P<0.001$) indicates significant difference from Oxymetholone group.

Histopathological Findings

Figures 7 and 8 show micrographs of tissue samples on days 7 and 14 post-injury, respectively. Microscopic evaluation of tissue samples from the experimental groups revealed that the SSD and oxymetholone-treated groups exhibited reduced inflammatory cell infiltration, enhanced epithelialization, and increased collagen deposition on day 7 post-burn injury. Histopathological studies on day 14 revealed a significantly thicker epidermis in the SSD and oxymetholone-treated groups than in the untreated and Eucerin-treated groups. In addition, the collagen fibers appeared more regularly arranged and exhibited improved structural organization in the SSD and

oxymetholone groups compared with those observed in untreated and Eucerin-treated groups.

Discussion

This study was conducted to evaluate the healing effects of topical oxymetholone on second-degree burn wound in rats. Our findings revealed that treatment of burns with oxymetholone accelerated wound closure and re-epithelialization and increased tissue collagen content. In addition, this study was conducted in both male and female animals, and the results showed that topical oxymetholone was effective in both sexes.

Oxymetholone is an anabolic steroid that has been used for various therapeutic purposes, such as hypogonadism and delayed puberty. It is also used for breast carcinoma management, correct hereditary angioneurotic edema, support nitrogen balance following injury and surgery, and stimulation of red blood cell production [16].

Various hormones are involved in protein synthesis and energy production, or so-called anabolism, and some hormones also play a role in catabolism and protein degradation. The balance between anabolic and catabolic hormones plays a role in wound healing both indirectly through protein synthesis and degradation and by directly affecting the wound healing process [9, 17]. Following tissue injury, the stress response decreases the activity of anabolic hormones and increases the activity of catabolic hormones; as a result, protein synthesis is reduced and lean mass is lost [9]. Therefore, reducing the response of catabolic hormones to stress and enhancing anabolic activity are two processes that lead to a net increase in protein synthesis, which is required for wound healing [18]. One of the ways to increase anabolic activity and decrease catabolic activity is the use of anabolic steroids (modified derivatives of testosterone) for burn wound treatment.

Oxandrolone is the anabolic steroid that has been most extensively studied for the treatment of wounds and burns [8, 19]. The findings of a study conducted by Demling et al. (2000) indicated that oral oxandrolone decreased wound closure time and increased hydroxyproline content and tissue tensile strength [20]. These results are consistent with our findings in this study.

The wound healing effects of steroids appear to be due to stimulation of anabolic activity. However, increasing evidence shows that these agents can stimulate all phases of wound healing [19, 20]. Several mechanisms account for the actions of anabolic steroids, including modulation of androgen receptor expression and interference with the expression of glucocorticoid receptors, which lead to anti-catabolic effects [21].

In this study, SSD was used as a positive control. The results of the present study showed that SSD significantly reduced wound closure time and enhanced the collagen content of tissue samples. SSD is a broad-spectrum antimicrobial agent that is effective against common pathogens causing infection in burn wounds [22]. Although the findings of the present study demonstrated that SSD was effective in the treatment of burn wounds in rats, previous studies have reported that it may delay

wound healing; therefore, its use should be subject to certain limitations [23].

Eucerin was used as the oxymetholone ointment vehicle in this study. The results showed that Eucerin significantly increased wound healing compared with the untreated group. In contrast, our previous studies [15, 23] showed that Eucerin had no significant effect on wound healing. The discrepancy may be attributed to differences in experimental conditions, including the wound model, treatment duration, and observation period. As an occlusive agent, Eucerin maintains a moist wound environment, which under certain conditions may promote wound healing [24]. Therefore, the effect of Eucerin on wound healing appears to be context-dependent and influenced by the experimental design.

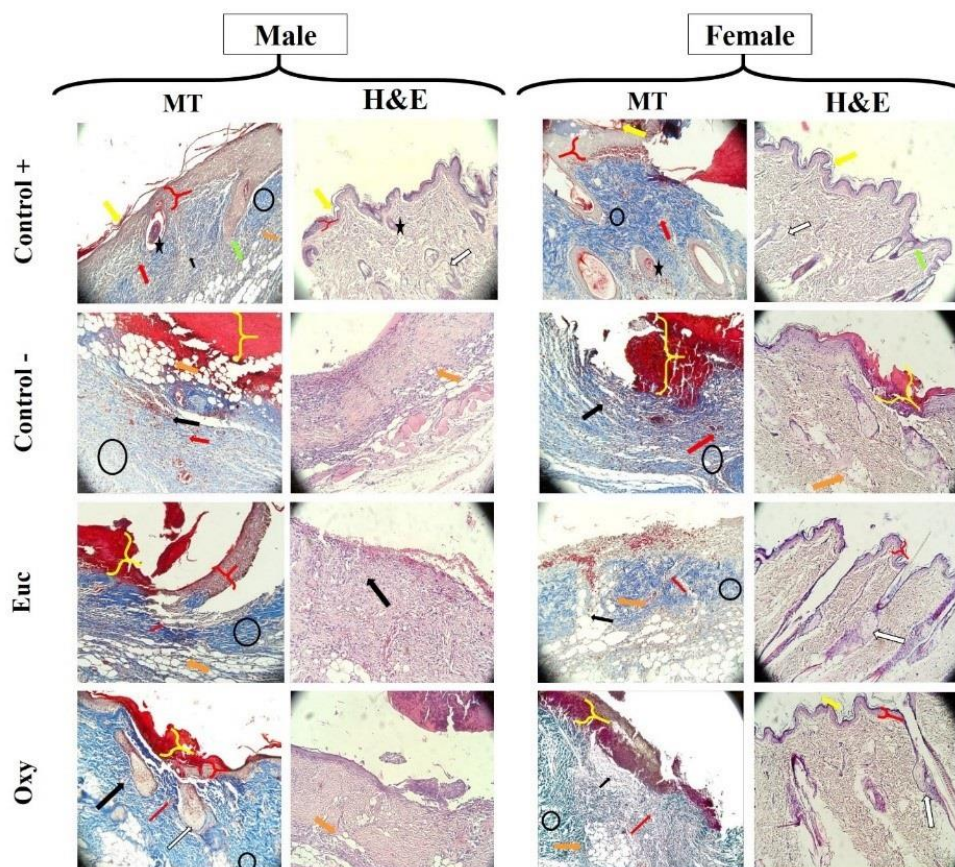


Figure 7. Microscopic images of tissue samples collected from different experimental groups on day 7.

Yellow arrow = keratin fibers on the epidermis, red bracket = epidermal layer, red arrow = fibroblasts, white arrow = sebaceous glands of the hypodermis, green arrow = rete ridge, black star = Hair follicles and black circle = collagen fibers and black arrow = macrophage cells (20x objective lens). Control+: group receiving silver sulfadiazine 1%, Control-: untreated group, Euc: group receiving Eucerin, Oxy: group receiving topical oxymetholone

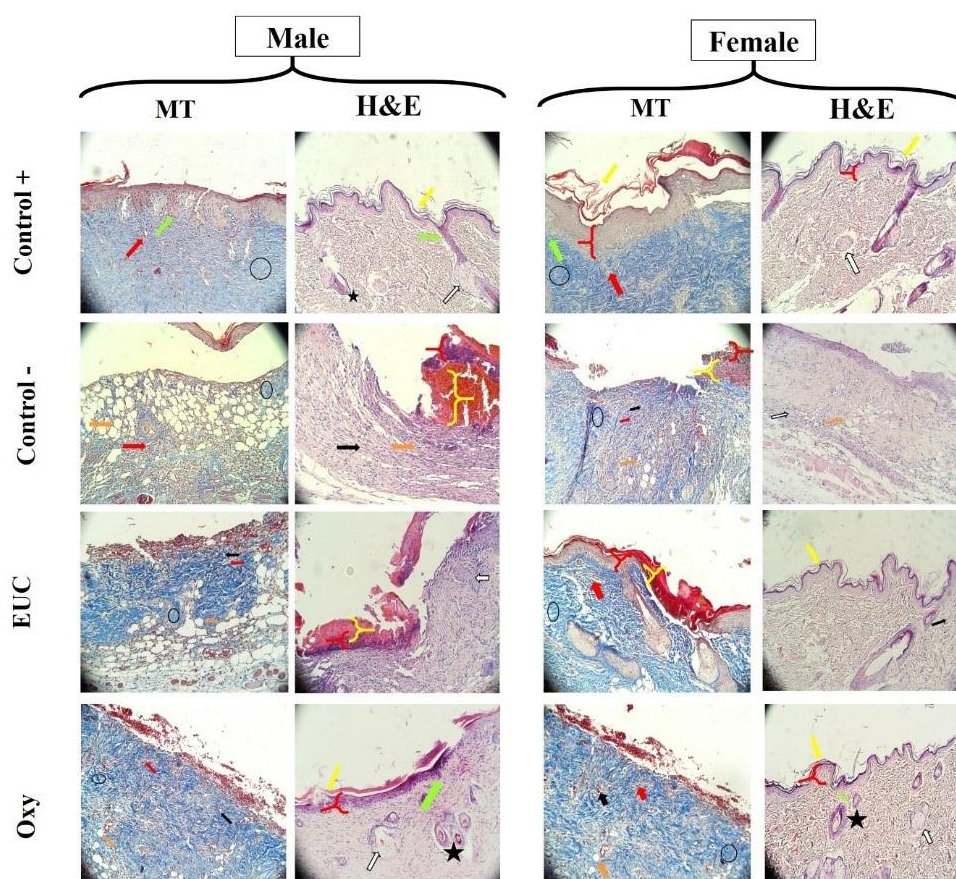


Figure 8. Microscopic images of tissue samples collected from different experimental groups on day 14.

Yellow arrow = keratin filaments on the epidermis, red bracket = epidermal layer, red arrow = fibroblasts, white arrow = sebaceous hypodermis, green arrow = rete ridge. Black star = hair follicles and black circle = collagen fibers and black arrow = macrophage cells (20x objective lens). Control+: group receiving silver sulfadiazine 1%, Control-: untreated group, Euc: group receiving Eucerin, Oxy: group receiving.

Conclusion

The results of the present study demonstrated that topical oxymetholone significantly improved wound healing, as evidenced by an increased wound contraction rate, hydroxyproline content, and histopathological findings. However, SSD showed superior efficacy compared with oxymetholone in promoting overall wound healing outcomes. Despite the beneficial effects observed, this study has several limitations. First, the experiments were conducted in an animal model, and the results may not fully translate to clinical settings. Second, the sample size was limited, which may affect the generalizability of the findings. Finally, molecular mechanisms underlying the effects of oxymetholone on wound healing were not investigated. Further studies, including mechanistic analyses and clinical

trials, are warranted to better clarify the therapeutic potential of oxymetholone in wound management.

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