WOUND HEALING ACTIVITY OF HEMOLOK (SEPGARD) GEL IN SPRAGUE DAWLEY RATS

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Wound healing activity of 3% homolok gel was evaluated in Sprague Dawely rats. Each animal was applied with the positive control, Placebo gel as well as the 3% Hemolok formulation on separate wounds for a duration of 24 days and the progress of wound healing was monitored every 4^{th} day. Wounds applied with the test gel showed a 20.9%, 12.94% and 13.47% reduction in wound area when compared to the normal control, positive control and placebo. This was also seen in the histopathogical section in Hemolok gel treated versus povidone iodine treated and untreated (normal control) skin section. It was observed that the Hemolok Gel proved to be a highly potent and better wound healing activity.

INTRODUCTION

A wound is a loss or breaking of cellular and anatomic or functional continuity of living tissue under the action of any external agency, including surgery (Patil et. Al., 2001). In this general definition many subdivisions are possible, taking into account and grouping together the various forms of violence or tissue damage. Destruction of skin by a burn or a trauma can cause severe loss of the very important protective functions of skin. This may result into functional interferences or even fatal manifestation in serious cases. Therefore, it is required that the process of wound healing should be executed immediately after the initiation of a wound. The process of wound healing primarily involves an acute inflammatory phase, collagen and other macromolecular synthesis, tissue remodeling and finally scar formation (Chithra et. Al., 1998)

This process is primarily characterized by three mechanisms. In each wound one of the three mechanisms predominates. The three mechanisms of wound healing are contraction, Epithelialization, and connective tissue deposition. The main stages of healing are (1) Inflammation (2) Proliferation and repair (3) Remodeling (Del Maestra, 1984). In each of these stages, specific components play a part through several mediators. However, with respect to gross morphological changes a thin liner red scar is formed at first which is raised above the level of the surrounding skin but this gradually fades until it is considerable paler than the surrounding skin. For many weeks after the scar forms, the process of contracture continues as is observed by the gradual shortening of the wound.

It has been reported that wound healing is more efficient under moist wound environment. Hence, currently a treatment encompassing of the application of gel/cream based synthetic/natural agents in and around the wound area is preferred. A typical treatment encompasses of cleaning the wound are with any antiseptic compound and then application of a wound healing agent that quickens the pace of natural wound closure. Such an agent may be a synthetic or herbal compound with established activity (Patil et. Al., 2001; Shirwarkar et. Al., 2003; Fulzele al. 2003) In this paper the wound healing activity of a formulation has been studied.

MATERIAL AND METHODS

The 3% Hemolok gel used was acquired from Themis Medicare Limited, Mumbai. 5% Povidone iodine solution w/v made by ICI India Limited was diluted to 3% using sterile water for injection and used as a positive control.

Composition of 3% Hemolok (sepgard) Gel: The test formulation used contained 3% feracrylum, 5% carbopol, 12.5% propylene glycol, 0.2% butylated hydroxyl anisole, 4% triethanol amine and water q.s. to 100%.

Animals: Sprague Dawley rates (n=6) weighing 350-450 g of either sex bred in the animal colony at the B.V. Patel PERD, Center, Ahmedabad, from the original stock obtained from National Institute of Nutrition were used for the study.

Housing Conditions: Initially the animals were housed 3 per cage in polypropylene cages and were moved to the experimental room where they were allowed to acclimatize for a day before treatment. The environmental conditions of the animal room were as per a specific design. A 10 % air exhaust in the air conditioning unit was maintained along with a relative humidity to of 60±5% and a temperature of 25±3^{0}C was stabilized. A 12 hour light/dark cycle was also regulated for the experimental animals.

Amrut certified rodent diet (Maharastra Chakan Oil Mills Ltd.) and tap water (boiling hot water cooled to room temperature) was provided ad libitum to the experimental animals. All experimental protocols were reviewed and accepted by the Institutional Animal House Ethics Committee (IAEC) prior to the initiation of the experiment.

Treatment : The excision wound model (shirwaikar et., 2003) was used to study the wound healing activity of the test gel. One day before the study, animals were removed from their cages and were put in a holding bucket for randomization and were divided into groups and numbered. After allocation of the numbers to animals, the cages were given respective identification numbers and a single animal was housed per cage.

Application of 3% Hemolok gel : On the day of initiation, the animals were removed from the cage and four circular areas of approximately 100 sq.mm. were marked out with Indian Ink on either side of the inter-scapular region and its full thickness were excised with a scalpel and scissors under ether anesthesia. The wounds in each animal were randomly marked as normal control, positive control, placebo and test and according the normal control wound was left as such, the positive control was applied with 3% Povidone –Iodine, the placebo was applied with

the placebo gel and the test wound was applied topically with the 3% hemolok gel.Application was carried out once in a day (early morning hours) and was repeated daily for the next 24 post operative days. However, as test wounds showed complete healing by the 12th post operative day, the experiment was terminated on the 16th day after the initiation.

Dosage: The amount of the test, positive control and placebo gel applied daily per wound was approximately 0.138 ± 0.003 g, 0.1 ml and 0.137 ± 0.004 g respectively.

Data evaluation: The progressive decrease in the wound area was monitored every four days tracing the wound margin on a tracing paper and the area was assessed using a graph paper (Fulzele et. Al. 2003). The wound contraction was measured as a percentage reduction in the wound area in each animal. The damaged skin prior to treatment and the regenerated skin after treatment were examined instopathologically.

RESULTS AND DISCUSSION

Percent reduction in the wound area in normal and gel control and test gel is presented in table 1. Our result that the wounds applied with the test get showed a 20.9%, 12.94% and 13.47% reduction in wound area when compared to the normal control, positive control and placebo (table1). The test gel demonstrated a similar highly efficient wound healing activity throughout the experiment displaying a significantly more reduction in wound area as compared to not only the normal control but also the positive control treated wounds. Hence, we can infer that the test gel not only is actively promoting faster wound contraction, but is also acting as a potent agent in aiding the process of tissue granulation and remodeling in the first and second weeks of the healing process. The significant healing outcome can also be visualized in the histopzthological sections in Hemolok gel treated versus povidone iodine treated and untreated (normal control) skin section.

Table 1: Excision wound studies showing percentage reduction in wound size when treated with test compound

Group	Day4	Day 8	Day 12	Day 16
Normal Control	43.00±6.37	48.84±5.41	97.39±2.33	100.00±0.00
Positive Control	50.96±9.23	62.96±6.73	88.11±4.45	100.00±0.00
Placebo	50.43±2.96	64.05±4.66	89.28±3.19	99.33±0.67
Test	63.90±5.20	77.32±4.34*	100.00±0.00	100.00±0.00

Values are expressed as Mean ±Sem; * = $p<0.01$; n=6

Furthermore, the process of wound healing is currently believed to be more effective under moist wound conditions. Thus, the active component 3 % feracrylum in a gel suspension shows a highly potent wound healing agent. Further investigation may essential to establish its usefulness in initiating early healing in superficial as well as invasive wound surfaces.

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