

Original Article

Comparative Efficacy of Grotto Cream with Fucidin Cream on Normal and Diabetic Wound Models in Rats

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Abstract

Wound healing is a process of well-recognized orchestrated and predictable events, in which there are four distinct inter-related phases: haemostasis, inflammation, proliferation and remodeling. The present study was carried out to evaluate comparative efficacy of Grotto cream (combination composed of, bees wax, D-panthenol, Lavender oil, glycerin, Vitamin E, allantoin and dimethicone) with fucidin cream in normal and diabetic rats. Circular wound was excised on the dorsal side of each rat either control healthy or diabetics rats. Grotto cream or fucidin cream were topically applied to the wounds on alternating days for 21 days in normal rats and 30 days in diabetic ones. Streptozotocin (50 mg/kg b.wt) single intra-peritoneal injection was used to induced diabetic rat model. Wound contraction and epithelialization period were measured following excision wound model and were used to evaluate wound healing effect of either Grotto or fucidin cream. The obtained results indicated that topical application of Grotto creams accelerated wound healing when compared to control non treated rats. The rate of wound contraction was significantly increased on days 3–21 for normal rats and days 3 to 30 for diabetic rats in Grotto and fucidin creams treated animals respectively. The duration of wound epithelialization was decreased in wounds treated with the reference standard and Grotto creams than the vehicle-treated group. It has been concluded that Grotto cream promoted wound healing in normal and hyperglycemic rats and its effect was more efficient than wound treated with reference drug (fucidin cream).

Introduction

Wound healing is a process of well-recognized orchestrated and predictable events, in which there

are four distinct inter-related phases: haemostasis, inflammation, proliferation and remodeling. A wound is defined as loss or breaking of cellular, anatomical, or functional continuity of living tissues (1). Wound healing are comprehensive process of well-recognized orchestrated and predictable events which the activity of an intricate network of blood cells, cytokines, and growth factors that leads to the restoration of the injured skin to its normal condition. The normal

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wound healing process is divided into three sequential phases: Inflammatory phase (hemostasis and inflammation), proliferative phase (granulation, contraction, and epithelialization), and remodeling phase, which organizes structure with increased tensile strength (2). The normal healing process may be impaired by several factors such as diabetes mellitus, immune disorders, ischemia, and venous stasis. Abnormalities associated with diabetic wounds which induced primarily by hyperglycemia include prolonged inflammation phase, impaired neovascularization, decreased synthesis of collagen, increased levels of proteases, and abnormal macrophage function. Moreover, diabetic wounds are also prone to infection because of impaired granulocytic function and chemotaxis (3, 4, 5).

Therefore, new era in wound healing researches are required, including new strategies to deal with this emerging issue. One of these is the use of natural products or medicinal plants to explore new therapeutic tools to be used for diabetic wound management and treatment. Grotto cream is a product composed of bees wax, D-panthenol, Lavender oil, glycerin, Vitamin E, allantoin and dimethicone.

Honey is one of the oldest known medicines. It has been valued highly in the Middle East and was in the Holy Quran since 1436 years ago. It has been used for treatment of diseases of respiratory, urinary, and digestive systems as well as skin diseases including ulcers, wounds, eczema, psoriasis and dandruff (6). Honey, an ancient remedy rediscovered during the 1990s, is now being utilized for wound care in the world as Australia (7), New Zealand (8) and in the US (9). Honey reduces inflammation, edema and exudation, promotes healing, diminishes the scar size and stimulates tissue regeneration (10-11-12). The basis of using beeswax in the mixture was derived from the observation that beeswax has antibacterial properties (13)

Panthenol, the biologically active alcohol analogue of pantothenic acid, is a pro-vitamin of the B-complex group, which is a normal constituent of skin and hair. When applied topically, it is converted to pantothenic acid, which is necessary to normal

epithelial function (14). Clinical observations have reported that topically applied panthenol accelerate wound healing in burns, corneal lesions, and allergic dermatitis, with minimal risks of skin irritancy (15). Lavender essential oil is expected to have a beneficial effect on wound healing because a few evidences for its effect were already reported (16-19). Topical treatment with lavender oil on ulceration showed a significant ulcer size reduction as compared to control in both an animal experiment and a clinical study (20). In addition, Morim et al., (21) reported that wound closure progressed more rapidly as a result of topical application of lavender oil promote wound healing in the early phase by acceleration of formation of granulation tissue, tissue remodeling by collagen replacement and wound contraction through up-regulation of transforming growth factor- β (TGF- β) which are growth factors playing important roles in wound healing process such as tissue remodeling and re-epithelialization.

The aim of the present work is to evaluate the wound healing effects of the tested cream named Grottoin rats using excision wound model and comparing this activity with that of fucidin cream in normal and experimentally induced diabetic wounds in rats. The effect of grotto cream on the rate of wound healing was assessed by the rate of wound closure and period of epithelialization in normal and diabetic rats.

Material and Methods

Materials

Grotto cream was supplied by Pharma International Pharmaceutical Industries (Pico Egypt). It is composed of beeswax, D-panthenol, Lavender oil, glycerin, Vitamin E, allantoin and dimethicone while fusidic acid was obtained as 2% under trade name fucidin cream manufactured by Mina Pharm Egypt for Leo France.

Experimental animals

Wister male rats weighing 150-170 g were obtained from the Lab Animal Care Unit, Faculty of Veterinary medicine, Cairo. All animals were housed in polypropylene cages under standard experimental

conditions with $26\pm 2^\circ\text{C}$ ambient temperature and 12 h light dark cycle. The animals were fed standard diet and were provided water *ad libitum*. All studies were carried out using ten rats in each group.

Safety Evaluation (Skin Irritation Study)

To evaluate the safety of Grotto cream, skin irritation test was conducted on albino rats as per OECD guidelines number 404 (22). Twenty-four hours before application of the tested sample, back of the albino rats was shaved carefully. Grotto cream was applied on the skin patches of albino rats, and the application site in terms of erythema and/or edema was examined at 24, 48, and 72 h for changes in any dermal reactions. The irritation index was calculated to assess the irritation potential of Grotto cream and fucidin according to Draize Test (23).

In vivo Evaluation of Wound Healing Activity

Effect on Wound Healing in Normal Rats

Thirty rats were allocated into 3 groups each of 10 rats as follows :

Group 1: Control group (treated topically with cream base).

Group 2: Treated topically with *Grotto cream*.

Group 3: Treated topically with fucidin cream.

Effect on Wound Healing in Diabetic Rats

Experimental induction of diabetes was carried out by a single intraperitoneal injection of streptozotocin (50 mg/kg b.wt.) dissolved in citrate buffer (0.1 M, pH 4.5). Fasting blood glucose level was measured 3 days post injection to confirm the induction of diabetes of the tested animals. Measurement of blood glucose level was done via withdraw of blood samples from the tail vein. Rats having blood sugar level above 250 mg/dl, were selected for study. Two weeks after induction of diabetes, rats with high blood glucose level, more than 250 mg dL⁻¹ were deemed diabetic and used for the experiment. The diabetic rats were allocated into three groups comprising ten rats in each group as given below :

Group I: diabetic control rats (treated topically with cream base).

Group II: rats treated with *Grotto cream*.

Group III: diabetic rats treated with Fucidin cream.

All animals in each group either normal or diabetic rats were anaesthetized via intraperitoneal injection of ketamine and xylazine (5 and 2 mg/kg, respectively). Skin of the dorsal area of each rat was shaved and disinfected with 70% alcohol. A uniform circular wound of approximately 100 mm² area was excised on the dorsal side of each rat as described by Mughrabi *et al.*, (24) Care was carried out to avoid damaging the muscle layer, and the tension of skin was kept constant during the process. The wounding day was considered as day 0. The wounds were treated with the topical application of the vehicle (cream base), reference standard or Grotto cream till the complete healing of wounds. The wounds were observed and the area of wounds was measured on 3, 6, 9, 12, 15, 18 and 21 post-wounding day for normal rats and on 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 post-wounding day for diabetic ones.

Parameters evaluated for wound healing

a. Measurement of wound contraction:

The percentage of wound contraction was assessed by tracing the wound on days 0, 3, 6, 9, 12, 15, 18 and 21 after wounding or till the wound gets healed using transparent paper and a permanent marker. The areas of wounds were measured against scale graph paper (mm²). The rates of wound contraction were calculated (25).

$$\text{Wound contraction (\%)} = \frac{\text{Wound area on day 0} - \text{Wound area on days } n}{\text{Wound area on day 0}} \times 100$$

Where n is the number of days: 3rd, 6th, 9th, 12th, 15th, 18th and 21st days.

Epithelialization period

The epithelialization period was calculated as the

duration in days required for falling of the dead tissue remnants without any residual raw wound (26).

Statistical analysis

Results were expressed as mean ± standard error of the mean (SE). Data analysis was carried out using one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison tests. Results obtained in groups exposed to Grotto and fucidin creams were compared with those of the control groups. In addition, the results related to Grotto cream were compared with those related to fucidin cream. P<0.05 was considered statistically significant.

Results

Safety Evaluation (Skin Irritation Study)

There were no signs of redness and itching when Grotto cream or fucidin cream was applied on the shaved back of albino rats. The primary skin irritation index of the creams was calculated as 0.00. This indicates the safety of Grotto and fucidin creams.

In vivo Evaluation of Wound Healing Activity

Topical application of Grotto cream showed significant promotion of wound healing in normal rats (Table I & Fig. 1) and hyperglycemic ones (Table II & Fig. 2), as compared to their corresponding control groups. Daily topical application of experimentally induced wound with Grotto cream caused a significant reduction in wound area of both normal and hyperglycemic rats compared to their corresponding control groups. In addition, daily topical application of fucidin cream on wound induced experimentally in normal and diabetic rats produced significant reduction in the wound area compared to the wounds of the corresponding control groups.

Period of wound epithelialization was reduced in group treated with either Grotto cream or with fucidin cream than vehicle. In control rats, wound takes more than 26 days in normal rats (Table I) and more than 36 days in hyperglycemic ones (Table II) to heal completely unlike with Grotto cream in which wounds heal almost around days 16-17 in normal rats and almost around days 24-25 in hyperglycemic rats.

TABLE I: Effect of topical application of Grotto or fucidin creams on wound contraction % and period of epithelialization in normal rats (n=10).

Treatment	Wound contraction %							Period of epithelialization (days)
	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21	
Control	11.2±1.04	22.8±2.13	34.2±2.7	45.5±3.4	54.4±2.17	64.9±3.8	74.8±3.4	25.3±0.57
Grotto cream	24.9±1.2*	42.7±2.1	63.2±3.7	79.4±2.9	92.7±3.7	100±0.0*	100±0.0*	17.1±0.73*
Fucidin cream	21.4±1.2*	40.5±3.7	59.8±4.2	71.5±2.9	85.9±2.17	100±0.0*	100±0.0*	18.7±0.36*

*Significantly different from the values of the control rats at P<0.05.

TABLE II: Effect of topical application of Grotto or fucidin creams on wound contraction % and period of epithelialization in diabetic rats (n=10).

Treatment	Wound contraction %										Period of epithelialization (days)
	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21	Day 24	Day 27	Day 30	
Diabetic control	0.0±0.0	5.4±0.04	9.2±0.47	15.7±1.27	27.8±2.17	39.0±1.23	41.2±2.28	54.7±2.49	64.2±2.48	68.8±1.44	36.4±1.57
Grotto cream	10.9±0.57*	18.7±1.61*	30.5±2.63*	41.8±3.51*	53.8±3.37*	68.4±2.11*	78.4±0.94*	93.1±1.46*	100±0.0*	100±0.0*	24.2±0.27*
Fucidin cream	12.3±0.97*	19.4±1.47*	33.1±3.47*	40.85±3.1*	52.64±1.7*	66.8±1.97*	76.4±0.67*	91.7±2.17*	100±0.0*	100±0.0*	25.6±0.49*

*Significantly different from the values of the control rats at P<0.05.

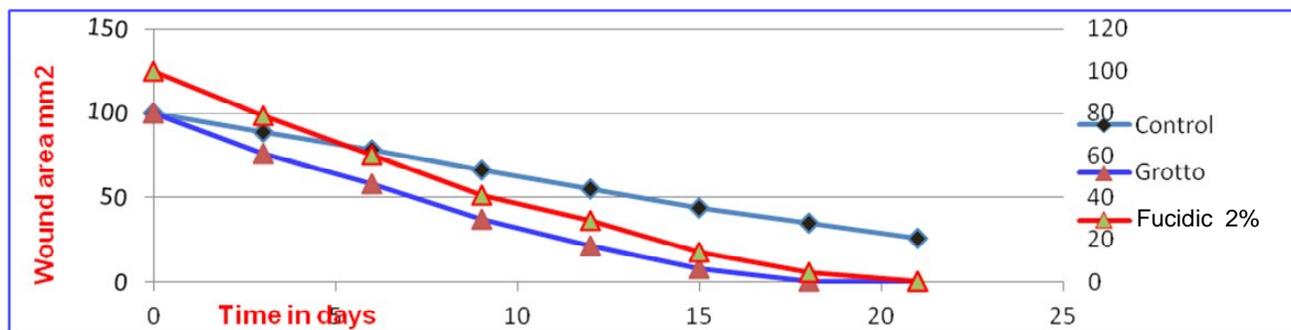


Fig. 1: Effect of topical application of Grotto and Fucidin creams on wound area (mm²) in normal rats (n=10).

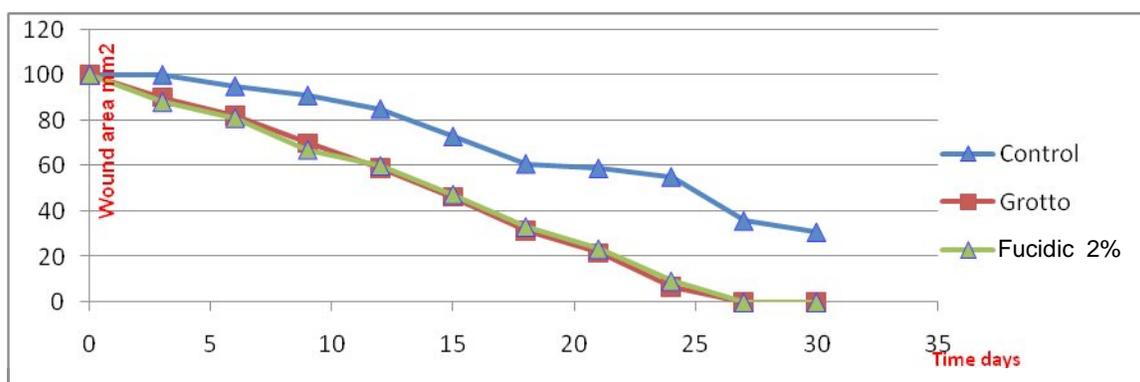


Fig. 2: Effect of topical application of Grotto or fucidin creams on wound area (mm²) in diabetic rats (n=10).

Discussion

Wound healing is a physiological process occurred naturally, that consists of four overlapping stages; hemostasis, inflammation, proliferation, and tissue remodeling or resolution (27-28). These processes, especially new tissue formation and tissue remodeling, consist of sequential coordinated stages including angiogenesis, cellular proliferation, collagen synthesis followed by formation of granulation tissue, matrix degradation followed by replacement of collagen, wound contraction, and scar tissue formation (29-30-31.32.33). The processes of wound healing are controlled by several factors, including cytokines, mitogens and chemotactic factors. These factors include, insulin like growth factors (IGFs), platelet-derived growth factors (PDGFs), epidermal growth factors (EGFs), and fibroblast growth factors (FGFs) which played an important role in wound healing possesses as these factors controlled cell migration and proliferation and the formation of

extracellular matrix proteins, which are essential for formation of granulation tissue (34, 35, 36). The edges of excised wounds are not in contact with each other, so contraction and epithelization steps are necessary for the repairing process. The obtained results revealed that daily topical application of Grotto cream promoted contraction and reduced the period of epithelization of experimental wounds in normal and diabetic rats. Contraction of wounds area processes occurring at the healthful skin surrounding the wounds which coats or covers the naked area. These process may be attributed to the action of myofibroblasts while epithelialization or epithelial regeneration following damage, includes the proliferation and immigration of epithelial cells to the center of wounds (37). Therefore, the effect of the combination of bees wax, D-panthenol, Lavender oil, glycerin, Vitamin E, allantoin and dimethicone (Grotto cream) on the contraction and epithelialization of wounds suggest their possible promoting or enhancing effect of either component of Grotto cream on the migration and proliferation of epithelial cells,

as well as the formation and the action of myofibroblasts. In this respect, several authors reported that the use of honey and beeswax in promoting or accelerating wound healing process as measured by the thickness of granulation tissue, epithelialization of the periphery of the wound and the size of the induced wound (9, 10, 11, 12, 13). In addition, Gore and Akolekar (38) reported that, a mixture of high molecular weight alcohol which isolated from beeswax, induced a significant reduction of exudates volume of the inflammation occurred by carrageenan. Furthermore, panthenol when applied topically, is an aid of wound healing, in burns, corneal lesions, and allergic dermatitis (17) as panthenol is converted to pantothenic acid, a component of coenzyme A and holo-fatty acid synthase that is essential to normal epithelial function (14). In addition, several clinical trials suggest a beneficial effects of lavender oil on wound healing and they were reported that topical application of lavender oil on ulceration showed a significant ulcer size reduction as compared to control (18-19). Furthermore, Morim *et. al.*, (21) demonstrated that wound closure progressed more rapidly as a result of topical application of lavender oil as compared to the control, accompanied by increased expression of some growth factors (PDGF-A and EGF) which played an important role in wound healing process including tissue remodeling and re-epithelialization steps. Furthermore, Araújo, *et. al.* (39) proved the

mechanism of allantoin action on wound healing as allantoin occurred via the regulation of inflammatory response and stimulus to fibroblastic proliferation and extracellular matrix synthesis. In addition, Cheng-San, *et al* (40) concluded that application of silicone gel on wounds decreases inflammatory reaction and improves recovery index. Glycerine, lanolin, and dimethicone are common emollients acts by forming an oily layer on the top of the skin that traps water in the skin. The increased capability of accelerating wound healing effects with combination of beeswax, D-panthenol, Lavender oil, glycerin, Vitamin E, allantoin and dimethicone in a pharmaceutical preparation named Grotto could be explained on the basis of the anti-inflammatory effects of beeswax, an increased expression of growth factors which played important roles in wound healing process including tissue remodeling and re-epithelialization steps.

Conclusion

In conclusion, the present study indicated that Grotto cream mixture promoted wound healing in normal and hyperglycemic rats and its effect was comparable to that produced by the reference drug (fucidin cream). However, further work is required to evaluate each of the components in Grotto cream for their beneficial effects on different degree of burns.

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