IJPSR (2020), Volume 11, Issue 3 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 09 March 2019; received in revised form, 07 January 2020; accepted, 20 February 2020; published 01 March 2020

FORMULATION AND EVALUATION OF DERMA HEAL CREAM AGAINST WOUND AND BURN HEALING ACTIVITY IN STREPTOZOTOCIN-INDUCED DIABETIC WISTAR ALBINO RAT

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Keywords:

Diabetes mellitus, Incision wound, Excision wound, Burn heal, Dermas heal cream

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ABSTRACT: Delayed wound healing is producing a high economic burden on the society and is generally associated with Diabetes mellitus or a related complication thus needs safe treatment like herbal formulation. Dermas heal cream (an anti-diabetic ayurvedic formulation) was evaluated for wound and burn healing potential in normal and diabetic rats. The effect of Derma heal cream on wound healing has been studied in diabetic and normal animals and compared with standard using the excision wound model. Diabetes was induced by a single dose of Streptozotocin 55 mg/kg i.p. Rats with blood glucose levels >250 mg/dl are considered as diabetic and included in the study for incision, excision and burn heal model. Rats treated with derma heal cream (DHC) at doses 1000 mg/kg dose per day were applied topically in the excision wound model, treated DHC shows significant effect reduction in period of 21st day and wound area is (5 ± 1.15 mm)and normal group wound area is (1.66 ± 2.12 mm) as compared to diabetic control group (25 ± 5.90 mm). Dermas heal cream in the incision wound model shows significantly reduced on 14th day treatment period the wound concentration in normal group (7 ± 1.7 mm) and DHC group (6.33 ± 1.15 mm) as compared to diabetic control group (25.10 ± 3.40 mm). Derma heal cream a showing healing of the open, closed and burn wound in negative group animals along with diabetic animals as compared to diabetic control group.

INTRODUCTION: Wound can be defined as a disruption of the normal cellular, anatomical, and functional continuity of a structure. Thus, wound healing is a complex process were aims to restore the structural and functional integrity of the wounded tissue. Wound healing can be divided into 3 stages, inflammation, proliferation, remodeling and maturation phases which involved the interaction of various cells, cytokines, and growth factors.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(3).1380-85

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(3).1380-85

In some pathological disorders like diabetes mellitus, renal failure, malnutrition, wound healing is greatly impaired. In diabetic patients, the prevalence of diabetic foot ulcers was 4–10% and the treatment of foot ulcers are expensive and extensive. Previous research study has shown that free radical inhibits the wound healing process. Thus, the wound healing process can be accelerated by using antioxidants.

Herbal medicines have been enjoying revitalization among clients all over the world. There are hundreds of medicinal plants that have a long history of curative properties against various diseases and ailments. However, screening of herbs for their activity is very crucial and needs imperative attention in order to know the value of the herbs.

Recently, research has focused on the use of natural antioxidants like an herbal extract on wound healing. The beneficial effects of natural source on wound healing have mainly been studied using animal models. Oral and topical application of natural source has been shown to enhance healing in diabetic, open and closed wound animal models. The topical application of natural source was more effective in accelerating wound healing compared to oral administration. Honey is one of the oldest known medicines. Some effective methods, such as recombinant been valued highly in the Middle East and were mentioned in the Holy Quran since 1436 years ago. It has been used for the treatment of respiratory diseases, urinary diseases, gastro-intestinal diseases and skin diseases including ulcers, wounds, eczema, psoriasis and dandruff [1].

Honey reduces inflammation, edema, and exudation promotes healing, diminishes the scar size and stimulates tissue regeneration [2-4]. The basis of using beeswax in the mixture was derived from the observation that beeswax has antibacterial properties [5]. One such potential burn dressing is olive oil, which was selected for several reasons: when destruct of the skin occurs, as happens with burns, one of the first reactions of the cells in the stratum corneum is to secrete fatty acids were ordered to restore the permeability barrier [6, 7]. On the other hand, they are more resistant to oxidative stress, which occurs in the burn area, than polyunsaturated fatty acids. Finally, fatty acids have antimicrobial properties [8], which can potentially reduce wound contamination. Olive oil also contains vitamin E 9 and phenol compounds such as hydroxytyrosol, tyrosol, oleuropein, 1-acetoxypinoresinol and (+)-pinoresinol, which are known to have powerful antioxidant potential [9-10].

Because free radicals play an important role in the pathophysiology of burns [11-14], both locally and systematically, using an antioxidant as a topical burn therapy may help in the recovery process. Honey, beeswax and olive oil are natural materials that contain flavonoids, antioxidants, antibacterial ingredients, and effects cytokines production by skin cells when applied topically [15, 16]. Hence, in this study, we formulate the Derma healed cream (DHC) and aimed to evaluate the effect of DHC topical application in the form of cream on wound healing of streptozotocin-induced diabetic rats and open and closed wound and second-degree burn heal activity on a rat.

MATERIALS AND METHODS:

DHC Mixture: DHC cream was prepared by thoroughly mixing natural honey, olive oil and beeswax (v/v/v, 1:1:1). Natural unprocessed beeswax was obtained from farmhouse, Dhaith, Sharjah, UAE. The natural, pure mono Samar honey was obtained from farmhouse, Dhaith, Sharjah, UAE and it was supplied directly from the company without heating or irradiation and stored in dark containers at room temperature for use in the study. The honey was subjected to analysis of physical characters and chemical composition. The olive oil used in the mixture was an extra virgin olive oil.

Formulation of DHC Cream: The cream was prepared by using a natural wax cream base. The standard method of fusion was used, where the natural wax was melted and mixed by continuous triturating. The required quantity of the natural wax was weighed and melted at a temperature of about 70 ºC in a hot water bath. The designated quantity of the content (s) was respectively added to the melted base at 70 ºC and the mixed honey and olive oil, stirred gently and continuously until a homogenous dispersion is obtained. Formulations were stored at 4, 25 and 40 ºC for two weeks and then the stability was evaluated.

Evaluation of DHC: pH 1.0 g cream was weighed and dispersed in 100 ml water. Using a digital pH meter, the pH of the dispersion was calculated. The pH meter was calibrated before use with a standard buffer solution at 4.0, 7.0 and 9.0. The readings of pH were done in triplicate and average values were calculated.

Spreadability: One of the important criteria for a topical formulation is that it should possess good spreadability. It is the term used to denote the extent of the area to which formulation readily spreads when applied to the skin or affected part. The therapeutic efficacy of a formulation depends upon its spreading value. To determine the spreadability of DHC formulations, 0.5 g of DHC was placed in a circle of 1 cm diameter pre-marked on a glass plate of 20 × 20 cm, over which a second glass plate was placed. A weight of 500 g allowed

resting on the upper glass plate for 5 min. The increase in the diameter due to DHC spreading was noted.

Homogeneity: The developed formulations were tested for homogeneity by visual inspection after the DHC had been filled in the container. They were tested for the appearance of DHC and the presence of any aggregates in DHC.

Animals: Healthy male rats (weighing 250-300 g) bred in Laboratory Animal Resource Unit were used throughout the experimental period. They were housed under controlled environmental conditions with free access to rat pellets and clean water, caged individually. Prior ethical approval was obtained from the Animal Ethics Committees. The experimental protocol (Reg. no: DMU/AEC/ DIER-2019-9/245) was approved by Dubai Medical University Animals Ethic Committee

Grouping of Animals: Groups of animals containing three per model (n = 6) in each were used for excision and incision wound models. The animals of groups were considered as the control, DHM cream respectively.

Diabetes Induction: Streptozotocin (STZ, Sigma, Germany) was dissolved in normal saline. Following this, 45 mg/kg dose of STZ was injected to overnight fasted rats via a tail vein under mild diethyl ether anesthesia. Three days later, blood samples were drawn from the tail of these rats to determine fasting blood glucose levels using a glucometer (Advantage, Germany). The rats with fasting blood glucose levels of more than 120 mmol/L were labeled diabetic and were included for the experiment [17, 18].

***In-vivo* studies:**

Excision Wound Model: The diabetic animals were divided into 3 groups with six in each were anesthetized by an open mask method with anesthetic ether before wound creation. The particular skin area was shaved 1 day before to experiment. An excision wound was inflicted by cutting away a 30 mm full thickness of skin from a predetermined shaved area. The wounds were left undressed to the open environment. The DHC Cream was applied topically to the diabetic group and normal. The diabetic control group was no treatment respectively, till the wound was completely healed. In this model, wound contraction was monitored. Wound contraction was measured using Vernier caliper scale as in 2 days after wound formation and the wound area was evaluated using a ruler in 1, 3, 7, 14, 21, 31 days [19].

Incision Wound Model: In the incision wound model, all the diabetic animals of each 3 group were anesthetized under light ether anesthesia. Two full-thickness paravertebral long incisions were made through the skin at the distance of about 1 cm from the midline on each side of the depilated back of a rat. After the incision was made on both edges of skin kept together and stitched with black silk surgical thread (no. 000) and a curved needle (no. 11) was used for stitching. The continuous threads on both wound edges were tightened for good closure of the wound. After stitching, wound was left undressed then control without cream application and test sample DHC cream and normal group was applied daily up to cured periods; the wound area was evaluated using a ruler in days, when wounds were cured thoroughly the sutures were removed on the 1, 3, 7, 14, 21, 31 day was measured using Vernier caliper scale [18].

Burn Wound Healing: Deeply anesthetized rats were kept in a prone position. A deep second-degree burn wound was induced by a hot metallic device (diameter: 5 × 2.5 cm^2) warmed for 5 minutes within boiling water and put for 10 seconds on the dorsum of rat skin with equal weight and pressure. All diabetic animals and normal animals were resuscitated with an injection of 5 ml normal saline after burning.

They burned animals were randomly divided into 3 groups of six rats. Group 1 (NS) was control and rats were only washed with normal saline during dressing without any topical treatment. Group 2 and 3 normal and diabetic group was treated with DHC cream without any effective agent. After the topical application of creams, the wound was covered with the sterile plain gauze for 24 h. The wound area was daily washed with normal saline in all groups and then was dressed in cream for each group. In order to quantify the rate of wound healing, the wound area was evaluated using a ruler in 1, 3, 7, 14, 21, 31 days after burn injury. The area of wounds at each day was measured using a Vernier caliper scale.

Statistical Analysis: All results were expressed as mean ± standard error (SEM). Data were analyzed by using one way ANOVA followed by Tukey's multiple comparison tests using Graph Pad InStat. P<0.05 was considered as statistically significant.

Observation:

TABLE 1: EFFECT OF DERMA HEAL CREAM ON DIABETIC RAT INCISION WOUND HEAL

Group	1st day	3rd day	7th day	14th day	21st day
Control Diabetic	24.52 ± 0.22	25.23 ± 1.77	25.84± 1.54	25.10 ± 3.40	22.12 ± 1.22
Normal + DHC	20.33 ± 3.51	20 ± 3.60	15.66 ± 2.08	7 ± 1.73*	0.33 ± 0.57*
Diabetic + DHC	23.66 ± 3.78	23 ± 3.60	19.6 ± 2.88	6.33 ± 1.15*	0.01 ± 0.07*

Values are mean ± SEM (n = 6) * p < 0.05 statically significant value. DHC (Derma heal Cream)

TABLE 2: EFFECT OF DERMA HEAL CREAM ON DIABETIC RAT BURN HEAL

Group	1st day	3rd day	7th day	14th day	21st day	31st day
Control Diabetic	25.63 ± 1.10	26.12 ± 0.03	26.71 ± 1.10	27.15 ± 2.12	25.45 ± 2.20	22.13 ± 4.02
Normal +DHC	29.5 ± 2.12	25.5 ± 2.12	24.5 ± 0.70	22 ± 7.07	10.5 ± 3.53	4.5 ± 3.53*
Diabetic+ DHC	23 ± 1.41	25 ± 0	23.5 ± 0.70	21.5 ± 3.5	18 ± 1.41	11.5 ± 0.70*

Values are mean ± SEM (n = 6) * p < 0.05 statically significant value. DHC (Derma heal Cream)

TABLE 3: EFFECT OF DERMA HEAL CREAM ON DIABETIC RAT EXCISION WOUND HEAL

Group	1st day	3rd day	7th day	11th day	14th day	21st day
Control Diabetic	27.55 ± 2.31	28.05 ± 1.11	28.55 ± 2.64	28.08 ± 0.80	25 ± 5.90	20.81 ± 3.57
Normal + DHC	31.33 ± 4.93	29.66 ± 3.78	20.66 ± 5.03	12 ±3.60	5 ± 4.0*	1.66 ± 2.12*
Diabetic +DHC	29.66 ± 4.72	27.33 ± 5.50	24 ± 5.56	15.33 ± 3.21	13 ± 5.65	9 ± 4.24*

Values are mean ± SEM (n = 6) * p < 0.05 statically significant value. DHC (Derma heal Cream)

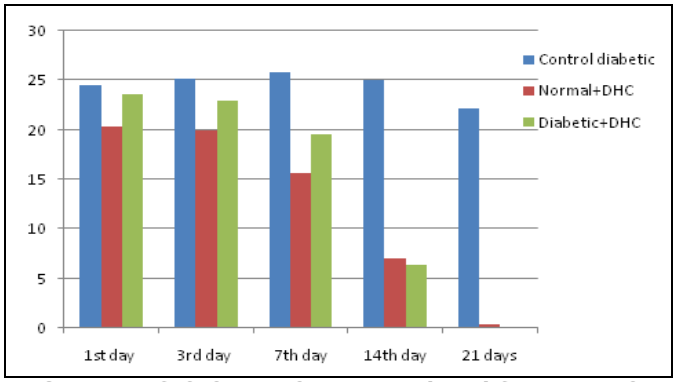

FIG. 1: INCISION WOUND HEAL ACTIVITY OF EXPERIMENTAL RATS (VALUES ARE EXPRESSED AS MEAN OF RESPECTIVE GROUP OF RATS (N=6). DHC (DERMA HEAL CREAM). All values are expressed as mean ± SEM (n=6); * represents significance (p<0.05) as compared to control

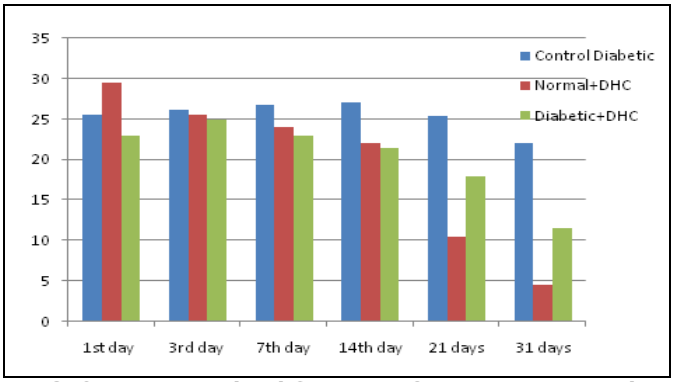

FIG. 2: BURN HEAL ACTIVITY OF EXPERIMENTAL RATS (VALUES ARE EXPRESSED AS MEAN OF RESPECTIVE GROUP OF RATS (N=6). DHC (DERMA HEAL CREAM). All values are expressed as mean ± SEM (n=6); * represents significance (p<0.05) as compared to control

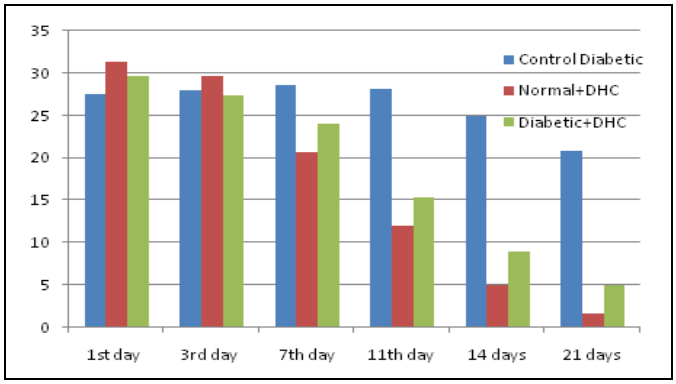

FIG. 3: EXCISION WOUND HEAL ACTIVITY OF EXPERIMENTAL RATS (VALUES ARE EXPRESSED AS MEAN OF RESPECTIVE GROUP OF RATS (N=6). DHC (DERMA HEAL CREAM) all values are expressed as mean ± SEM (n=6); * represents significance (p<0.05) as compared to control

RESULTS:

Evaluation of Derma Heal Cream: The pH was found to be neutral pH, thus the formulations can be used without the risk of skin irritancy. By the results, we can infer that the selected ingredients for cream formulation did not alter the pH of the formulation. The values of spreadability for DHC were found out to be 8.4, 8.5, 8.6 cm indicating that the cream is easily spreadable by less amount of shear. The results concluded that the formulation can be applied easily without being runoff. This assures that the formulation maintains a good wet contact time when applied to the targeted site. DHC formulations were good in appearance and homogeneity.

Incision Wound Model: In this wound model, the incision wound was measured. The mean area of wound heal on 1st day in the normal group was 20.33 ± 3.51 mm and in a diabetic group, it was 23.66 ± 3.78 mm. After the 14th day, the mean area of Derma heal cream treated groups of the normal group was 7 ± 1.7 mm; it was a highly significant effect. The diabetic animal groups mean area of treated of 6.33 ± 1.15 mm, when compared with the control group 25.10 ± 3.40 mm. It has a significant difference with a p-value <0.05 compared to diabetic control and DHC treated group and nondiabetic normal groups **Fig. 1** and **Table 1**.

Excision Wound Model: In this excision wound model, the wound was measured. The mean area of wound heal on 1st day in the negative group was 31.33 ± 4.93 mm and in the diabetic group, it was 29.66 ± 4.72 mm. After 21st day the mean area of Derma heal cream treated groups of the negative group was 1.66 ± 2.12 mm, it has a highly significant effect. The diabetic animal groups mean area of treated of 5 ± 1.15 mm when compared with the control group animals 25 ± 5.90 mm. It has a significant difference with a p-value <0.05 compared to diabetic control and DHC treated group and nondiabetic normal groups **Fig. 2** and **Table 3**.

Burn Heal Model: In this burn heal model, the second-degree burn was measured. The mean area of second-degree burn on 1st day in the normal group was 29.5 ± 2.12 mm and in the diabetic group, it was 23 ± 1.41 mm. After 21st day the mean area of Derma heal cream treated groups of the normal group was 10.5 ± 3.53 mm; it has a highly significant effect.

The diabetic animal groups mean an area of treated of 18 ± 1.14 mm when compared with the control group animals 10.5 ± 3.53 mm. It has a significant difference with a p-value <0.05 compared to diabetic control and DHC treated group and non-diabetic normal groups **Fig. 3** and **Table 2**

DISCUSSION: Diabetic Wound healing is a complex and natural response of the body that results in the achievement of the restoration of the normal physiological function and integrity of the injured/damaged tissues and also produces several extracellular matrix proteins and growth factors. The burn is one of the most widespread injuries in the world. The pathophysiology and histopathology of thermal burns in animals is very similar to that in human. The use of natural products such as honey, beeswax and olive oil to treat diabetic wounds and burns has great appeal, especially in developed countries. A verity of natural products has been reported for the treatments. These results indicate that DHC is able to accelerate the rate of the diabetic wound, normal wound and burn to heal. The present study shows decrease the diabetic wound and normal wound and burns are faster as compared to other natural products. In conclusion, DHC promoted diabetic wound, normal wound and burns healing better. However, further work was need to analyze each of the components in DHC for their beneficial effect on healing.

CONCLUSION: In present work we have found that Derma Heal cream showed dose dependent enhancement in % wound contraction as it exhibited significant ($p<0.05$) and consistent wound healing activity at to diabetic groups when compared to the normal and diabetic control rats. A major component of antioxidant, Phenol and protein content was found to be increased significantly ($p<0.05$) effect in both normal and diabetic rat. Derma Heal showed direct co-relation between dose and antioxidant activity, when given for 21 days. From the above results it can be concluded that the Derma Heal Cream possesses the significant wound healing activity in diabetes along with antioxidant profile. So, this finding should be confirmed further for more precision with large number of animals and small

progression of doses and be studied in future for clinical trials, as delayed wound healing is major issue in diabetic patients.

ACKNOWLEDGEMENT: The first author (Dr. Rawoof Khan) is thankful to the Chairman, Board of Trustees of Dubai Institute for environmental research and laboratory analysis for providing and guiding, all facilities to carry out the research work.

CONFLICTS OF INTEREST: We authors declare no conflict of interest in this study/manuscript.

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How to cite this article:
Khan RG, Jumaan A and Taliab A: Formulation and evaluation of derma heal cream against wound and burn healing activity in streptozotocin-induced diabetic Wistar albino rat. Int J Pharm Sci & Res 2020; 11(3): 1380-85. doi: 10.13040/IJPSR.0975-8232.11(3). 1380-85.