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ORIGINAL ARTICLE

Invivo and ex-vivo evaluation and characterization of wound healing effect of topical calendulaofficinalis and turmeric cream in diabetic rats.

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ABSTRACT… Objective: To evaluate wound healing by topically administration of calendulaofficinalis and tumeric cream to diabetic rats. **Study Design:** Observational Cross-sectional study **Setting:** Department of Pharmaceutics, Faculty of Pharmacy, BahaUddin Zakariya University, Multan, Pakistan **Period:** October 2020-march 2021. **Method:** Different batches of hydrophilic cream bases (C1-C6) were developed and characterized. The optimized base composition (C6) was loaded with calendula Officinal is extract and turmeric powder as an active ingredient (C7) and evaluated the rate at which wounds heal in diabetic rats. Calendula officinal is and turmeric were combined in a cream, as different stability parameters of the creams were analysed at different temperature. Microbiological action of the cream was also examined. **Results:** The formulation was homogenous, stable with pH 6.29±0.03, viscosity 381± 6.96 Cps and the microbiological quality met the requirements of European pharmacopeia. The total flavonoid content was less than 1. The formulation showed accelerated wound healing with more than 95% wound contraction after tenth day. **Conclusion:** The result were favorable and suggested that topical administration of Calendula officinal is and turmeric powder combination would enhance wound healing in diabetic rats with potential for diabetic foot ulcer treatment. Moreover, the designed formulation poses good quality emulsion system stabilizing the active ingredient as well as appropriate release of medicinal agents.

Key words: Calendula Officinal is, Diabetic Rat, Turmeric Powder, Topical Cream, Wound Healing.

INTRODUCTION

Wound healing is a multi-phased process with three distinct phases: inflammation, proliferation, and remodeling or maturation. The interplay of different cells, cytokines, and growth factors is required for wound healing.[1] Diabetes mellitus, a metabolic disorder arising from perpetual hyperglycemia and associated with various micro-vascular convolution and macro-vascular intricacy, is one of the chronic diseases which causes poor wound healing leading to a poor standard of living, significant health-care costs and a high death rate.[2,3]

Wound healing failure is caused by a variety of pathologic abnormalities, ranging from disease-specific intrinsic deficiencies in blood flow, angiogenesis, and matrix replacement to extrinsic factors such as infection and repetitive trauma.[4] Appropriate treatments are necessary to improve patients' overall quality of life. For treatments developed to treat chronic wounds and skin ulcers, the capacity to keep adequate moisture in the wound bed is essential.[5] Treatment for diabetic ulcers includes wound debridement, infection management, revascularization treatments if needed, ulcer off-loading, hyperbolic oxygen therapy, and negative pressure wound therapy.[6,7]

Herbal therapy has recently gained popularity in the treatment of skin disorders. In the field of wound healing management using medicinal plants, extensive study has been conducted. To support the creation of a favourable environment for the natural healing process, herbal treatments for wound healing include debridement, disinfection, and maintaining a moist atmosphere.[8] Calendula, scientifically known as Calendula officinalis

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(Asteraceae), is an annual herbaceous plant found in Europe, China, and Asia with bright green oblong leaves and huge orange blooms. It's also known as "African marigold" and various chemical and pharmacological investigations have been conducted on it. It has a variety of medical applications, including wound healing, jaundice treatment, blood purification, and antispasmodic. Anti-inflammatory, antioxidant, antitumoral, antifungal, immune-modulatory, antimicrobial, antibacterial, and hypoglycemic properties have been identified for C. officinalis. Various classes of substances, such as tri-terpenoids, flavonoids, quinines, volatile oil, coumarins, carotenoids, and amino acids, have been discovered through chemical investigations.[10] The safety of C. officinalis hydro glycolic extract, as well as its clinical efficacy and effectiveness in the topical treatment of diabetic foot ulcers, were validated in a randomized, controlled research.[11]

Turmeric, obtained from the dried rhizomes of Curcuma longa, contains anti-inflammatory, anti-diabetic, antibacterial, and cytotoxic qualities in addition to wound healing.[12] Turmeric accelerates the wound healing process by provoking the movement of monocytes, fibroblast cells and eosinophils. Turmeric is found to exhibit various biological activities in treating diabetic foot ulcers via various mechanisms such as inhibition of the activity of matrix metalloproteinase and proinflammatory cytokines.[13]

Various cream bases (B1-B6) were formulated. Thus, the product was showed to be o/w emulsion. Calendula officinalis and turmeric will be combined in a cream, as different stability parameters of the creams were analysed at different temperature. Microbiological action of the cream was also examined. Cream administered topically to diabetic rats to see if they may speed up wound healing. Wound closure was observed and photographs were taken after 2, 5 and 10 days. The untreated rats were used as control group. The percentage of wound closure were checked. It exhibited the increased injury treatment in diabetic rats. The result suggested an antidiabetic similar process for the injury treatment action.

MATERIAL

Quercetin, Carbopol 940 (polymer), dry Calendula extract (active), and Turmeric powder (active) were purchased from (Sigma Ireland), Ethanol, stearic acid, and aluminium chloride were obtained from (Merck USA). White petrolatum, Triethanolamine, liquid paraffin and isopropyl alcohol were purchased from (Fluka, UK) were of Ph. Eur quality.

Preparation of C. Officinalis extract and turmeric powder loaded cream

Table-I shows the composition of the various produced cream formulations.

Determination of the total flavonoids content

0.5 g of each of the resulting cream formulations was extracted six times using a combination of isopropanol and ethanol (2:8 v/v). The extract solutions were mixed, and the volume was made up to 100 ml using an extractant. The total flavonoid concentration of the extracts was then measured using the aluminium chloride colorimetric technique.

Organoleptic characteristics, homogeneity, and presence of foreign particles

Visual inspection was performed on all of the prepared formulations with or without active ingredient to check for physical appearance of cream including color, smell, phase separation of cream, homogeneity, and the presence of foreign particles.

Spreadability

Cream spreadability was determined by measuring the cream sample's spreading diameter between two horizontal glass slides.

METHODS

This study was conducted at Bahauddin Zakariya University, Multan from October 2020 to March 2021 after approval from ethical committee (188/AEC/2021) (30-9-2020). Healthy male Wister albino rats (weighing 250-300g) were obtained from the animal section of Pharmacology Research laboratory, Faculty of Pharmacy Bahauddin Zakariya University, Multan Pakistan.

Ethical Committee for Utilization of Laboratory Animals was reviewed and approved the study protocols. The animals were kept in a natural light cycle and at constant temperature 25°C. Alloxan was used for the induction of diabetes mellitus in rats, which served as a pathological bio-model for in vivo testing. All the Rats were injected with single dose of Alloxan monohydrate (150mg/kg) dissolved in normal saline. After 48 hours, A glucometer was used to measure the rats' blood glucose levels. Rats that had blood glucose levels more than 250 mg/dl were labeled as diabetic and included in the study. The blood glucose level of the rats was kept stable throughout the procedure by feeding the rats with glucose continuously. The diabetic rats were divided into two groups each group containing six rats. Group A was a diabetic control group that received only cream base with active ingredient, whereas Group B was a diabetic treatment group that received cream containing medicinal substances. All rats had their backs shaved and their skin cleaned with a cotton swab, after which a wound was created on the dorsum of each rat. The wounds were kept naked for 10 days and treated with 0.1 g cream applied topically once day. At 0, 6, and 10 days following wounding, the size of the wound was measured and photographed. The wound closure rate was estimated using the formula below.[22]

$$\text{Rate of wound closure} = \frac{W_o - W_d}{W_o} \times 100 \quad (1)$$

Where W_0 represent the area of wound and W_d is the area of wound on day 1st, 6th, or 10th

Statistical Analysis

The experiments in this study were carried out three times. The software programme was used to perform the statistical analysis (SPSS 17). P values having less than 0.05 value were used to determine relevance in statistics. The outcomes of In-vivo wound healing were compared using the Student's t test.

RESULTS

The therapeutic effectiveness of calendula and turmeric powder cream on wound healing in the diabetic rat model observed for 10 days. The rate of wound contraction was significantly higher in the treatment group in comparison to the control group ($p < 0.05$). In Group A (control group) rate of the wound contraction was 20.8% ± 2.64, 45.6% ± 2.42, and 68% ± 2.25 on Day 2nd, 5th, and 10th respectively. On the other hand, the rate of wound contraction for the treatment group was 39.1% ± 2.31, 80.5% ± 2.4, and 95% ± 3.31 on day 2nd, 5th, and 10th, respectively. (Figures-3). Calendula officinalis and turmeric are rich in active medicinal agents having anti-inflammatory and wound healing activity.[13,15] Thus, a cream containing medicinal agents has shown significantly improved healing of wounds in comparison to cream vehicles.

	Ingredients	Amount of ingredient (% W/W), Formulation coded from C1 to C7						
		C1	C2	C3	C4	C5	C6	C7
A	White petrolatum	6	6	6	6	6	6	6
	Liquid paraffin	-	6	6	6	6	6	6
	Stearic acid	3.65	2.5	-	3.3	3.4	2.8	2.8
B	Triethanolamine	-	-	1.4	1.6	1.8	2	2
	Purified water	15	16	16	16	16.5	16	16
C	Carbopol 940	-	0.1	0.2	0.1	0.1	0.2	0.2
	Purified water	18	18	17	17	16	17	17
D	Calendula extract	-	-	-	-	-	-	0.4
	Turmeric powder	-	-	-	-	-	-	0.1
	Purified water	-	-	-	-	-	-	3.5

Table-I. Composition of topical creams

Formulation/Parameters	C4	C5	C6	C7
Physical appearance	Opaque	Opaque	Opaque	Opaque
Color	White	White	White	Yellow
Texture	Smooth	Smooth	Smooth	Smooth
Phase separation	No	No	No	No
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous
pH (mean ± SD)	6.0 ± 0.02	6.23± 0.03	6.5± 0.04	6.29± 0.03
Viscosity (Cps) (mean ± SD)	320 ± 3.24	356± 4.56	425± 7.52	381± 6.96
Spreadability (gram.cm/s) (mean ± SD)	5.89± 0.31	5.64± 0.24	4.16± 0.36	5.23± 0.41

Table-II. Physicochemical evaluation of selected topical creams

Days	Physical Appearance	Colour	Texture	Homogeneity	Phase Separation
1st day	Opaque	yellow	Smooth	Homogeneous	No
8th day	Opaque	yellow	Smooth	Homogeneous	No
16th day	Opaque	yellow	Smooth	Homogeneous	No
30th days	Opaque	yellow	Smooth	Homogeneous	No
90th days	Opaque	yellow	Smooth	Homogeneous	No
180th days	Opaque	yellow	Smooth	Homogeneous	No

Table-III. Stability Studies of C7 (Organoleptic characteristics)

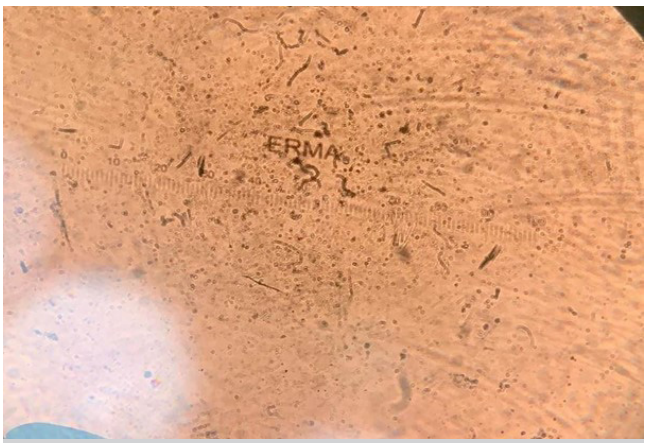

Figure-1. Microscopic image of the cream with Calendula extract along with Turmeric powder

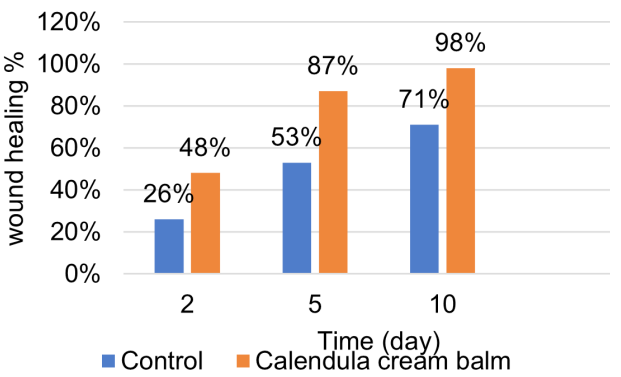

Figure-2. Rate of wound closure in diabetic wound rat model on Day 2, 5 and 10. (A) Control group, (B) Treatment group indicates statistically significant (p <0.05)

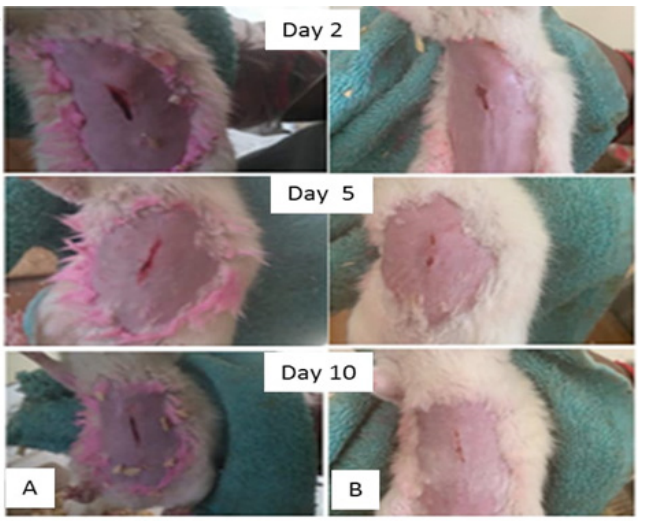

Figure-3. Photographs taken on the excision wounds made on the experimental rats. All groups showed complete wound closure on day 10

DISCUSSION

The affective wound healing properties of Calendula officinalis and turmeric have been reported in several studies (13-15). It is important to design aesthetically appealing and therapeutically effective topical cream for appropriate delivery of these medicaments to the skin. In current study, a hydrophilic cream base has been successfully developed to evaluate the improved diabetic wound healing effectiveness of flavonoids present in natural medicinal agents. Externally hydrophilic creams are miscible with

water and skin secretions, providing an elegant and cosmetically appealing topical application technique.[23]

A cream containing 0.4% Calendula officinalis extract and 0.1 % turmeric powder showed total flavone and flavanol content less than 1 % which is appropriate for wound healing.[24]

Results of the organoleptic evaluation are presented in Table-II. All cream bases (C4-C6) were opaque, odorless, white in color, and smooth in texture. However, The incorporation of active ingredients imparts a yellow color to cream (C7) with a characteristic odor. All formulations showed good homogeneity with no grittiness.

In terms of appearance, texture, homogeneity and effectiveness, the compositions was ideal. The results of the organoleptic evaluation of selected creams suggested that the formulated cream had cosmetically appealing and acceptable characteristics. The measured pH values of all formulations were in the range of 6.31±1.89 to 6.54 ± 1.76, which are in the acceptable range for preparations applied to the skin the ideal pH of cream should be (5.5-6.6).[25]

The viscosity of creams is an important parameter for determining the stability and flowability of creams. The viscosity of creams was found to be in the range of 320 ± 3.24 cps to 425± 7.52 cps (Table-II). It is noticed that increasing the concentration of triethanolamine and carbopol will increase the viscosity of cream; however, the incorporation of active ingredients has slightly reduced the viscosity. The viscosity of creams was found to be appropriate for the physical stability of the creams.

Spreadability plays a vital role in terms of look, texture, and uniformity of a standard dose of the medicinal agent. The Cream base should spread easily without too much drag and should not produce greater friction in the rubbing process. Results of spreadability (Table-II) indicated that the formulation can easily spread. Formulation with the highest viscosity showed lowest spreadability.

In emulsions, the particle size of the dispersed phase is a significant parameter that manifests the value and consistency of the formulation in addition to efficacy and exterminating procedure. Under the light microscope, a homogenous mixture was observed at a magnification power of 100x. At 400x magnification, it was discovered that the majority of the particles were small, with a maximum particle size of less than 10μm (Figure-1). The mean diameter of the particles in the formulations increased from 0.128 to 7.152micron meter, with the cream base C6 having a mean diameter of 1.18 m and the cream (C7) with calendula extract (0.4%) and Turmeric powder (0.1%) having a mean diameter of 1.102 m. 1.56104 and 1.33104 were the degrees of dispersion, respectively. As a result, the internal phase's particle size was small, and the droplets were uniformly disseminated in the external phase.

Creams were found to be safe with no signs of skin irritation, lesion, or information. Microbiological quality met the pharmacopeial specifications.[26] Pseudomonas aeruginosa and Staphylococcus aureus were found to be absent from the formulation both immediately after manufacture and after 6 months of storage.

The goal of this research was to assess the short-term stability of prepared cream upon six months of storage at 25°C. This was achieved by the comparison of the flavonoid content, pH, and viscosity, organoleptic properties including color, texture, homogeneity, and phase separation of freshly prepared cream against that after storage. The pH and viscosity remained consistent throughout the storage duration. Moreover, the flavonoid content didn't show any significant change after the storage ($P > 0.05$). The organoleptic properties including colour, texture, homogeneity and phase separation were the same throughout the storage period (Table-III). The presence of triethanolamine stearate emulsifier and Carbopol gel in the formulation might have positively contributed towards the good stability of the cream. These data concluded that the prepared cream is both chemically and

physically stable over six months.

CONCLUSION

Topical application of a combination of C. Officinalis and turmeric incorporated in hydrophilic cream showed accelerated wound healing in a diabetic rat model. The developed formulation was physically and chemically stable, free from microbial contamination and having pH suitable for skin application. The cream has suitable viscosity and good spread ability for ease of application. The formulated hydrophilic cream shows potential for appropriate topical application for wound healing and diabetic foot treatment.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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2	Bushra Nasir	Worked on formulation stability, Article setting.	
3	Aroosa Sharif	Worked on result complication tabulation and applicaiton of statistics on results.	
4	Maria Shah	Experimental work and data analysis.	
5	Furqan Iqbal	Organoleptic an microscopi analysis.	