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Original Research Article

Assessment of ameliorative effect of Aab-e-Shifa polyherbal formulation in experimentally-induced wound in rabbits

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Abstract

Purpose: *The aim of the current study is to evaluate the wound healing potential of a polyherbal formulation (PHF) Aab-e-Shifa in normal and diabetic albino rabbits.*
Methods: *The activity of PHF application was evaluated in comparison to tetrachlorodecaoxide (TCDO) on experimentally-induced excision wound in the thigh of normal and diabetic rabbits under ketamine anesthesia. Preliminary phytochemical analysis, total phenolic contents, wound contraction, as well as toxicological and histopathological studies were also investigated.*
Results: *PHF exhibited parallel ($p < 0.05$) activity for initial wound healing in both normal (48.07 %) and diabetic groups (36.32 %), when compared to their respective control groups. Phytochemical analysis showed the presence of high levels of total phenolic contents in Allilum sativum L. (54.25 ± 0.15 GAE mg/g), Curcuma longa L. (25.45 ± 0.48 GAE mg/g), Zingiber officinale Rosc. (29.08 ± 0.35 GAE mg/g) and some phytochemicals such as flavonoids, couramins, terpenoids in these plants. No adverse sign of PHF was observed when applied at a dose of 2000 - 3000 g on rabbit skin.*
Conclusion: *Aab-e-Shifa has great potential in wound healing and may be used as an alternative treatment for the healing of wounds in animals. The phytochemicals present in PHF might play a role in the wound healing activity possibly due to their antioxidant potential. However, further detailed studies are required to buttress this.*

Keywords: *Skin damage, Wound repair, Foot ulcer, Tetrachlorodecaoxide*

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INTRODUCTION

A wound refers to an injury to living tissue when skin is cut or broken, and the dermis is exposed to invaders. Wound healing is a multifaceted, complicated process which involves a series of events that take place in a well-organized manner [1]. The first process involves the vasoconstriction and formation of fibrin clot on the affected skin in order to stop the bleeding.

Fibrin clot on the neighboring injured cells serve to release a number of pro-inflammatory cytokines and various growth factors that includes transforming growth factor (TGF), platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and fibroblast growth factor (FGF). Inflammatory cells proceed into the wound and triggers the inflammatory phase, characterized by the penetration of lymphocytes, neutrophils and macrophages [2]. Neutrophils are associated with the clearance of cellular debris and invading microbes in the wound area, and they produce some necessary substances like reactive oxygen species (ROS) and some enzymes such as proteases.

Tetrachlorodecaoxide (TCDO) is a relatively new, more potent and efficacious topical wound dressing solution. Tetrachlorodecaoxide is chemically rich in oxygen, and as a result, increases the partial pressure of oxygen in the wound, which leads directly to the activation of macrophages [3]. This is a good technique for treating pressure ulcers, especially the chronic ulcers associated with diabetes [4]. Being an aqueous bio-activated oxygen carrier, tetrachlorodecaoxide breaks the brutal cycle of hypoxia in a wound. Its oxygen richness fulfills the increased oxygen demand, which is needed for the phagocytic activity of macrophages. The mitogenic potential of tetrachlorodecaoxide on fibroblasts adds extra strength to it [3]. The curative effects of tetrachlorodecaoxide has been described in three ways, strengthening the wound cleaning process, nurturing the formation of new tissue granulation, and modulating the immunological response to the injury [4].

Aab-e-Shifa is a hot water decoction of *Allium sativum* (bulb) *Zingiber officinale* (rhizome) and *Curcuma longa* (rhizome) in an optimized ratio of 1:1:2. Most likely, the ratio and composition of the three herbs used were deployed to previous animal experimental studies to ascertain their effectiveness against diabetes mellitus and pressure ulcers [5,6]. Moreover, antibacterial activity of these plants have also been reported [7]. Given the overlapping, but complementary, activities of these three herbs, we decided to evaluate polyherbal formulation *Aab-e-Shifa* on skin physiology and pathophysiology of normal and diabetic wounds.

EXPERIMENTAL

Drugs and chemicals

All the drugs used in this experiment were of pharmaceutical grade. Tetrachlorodecaoxide solution was bought from Brooks Pharmaceuticals Pvt. (Ltd.) Pakistan. Lignocaine gel was purchased from Atco Pharmaceuticals. Ketamine and streptozotocin were purchased from Indus Pharmaceuticals and *Aab–e-Shifa* was purchased from Muhammad Dawakhana, Nasheman Colony, Multan.

Animals

Rabbits of both sexes with an average weight of 1.5 kg were purchased from the pet market Husain Agahi, Multan, Pakistan. Rabbits were kept under standard laboratory conditions at 27 ^{0}C room temperature. The experiments were performed in accordance with the guidelines provided by the National Institute of Health Guide for the Care and Use of Laboratory Animals [8], and approved by the Institutional Animal Ethical Committee of Muhammad Institute of Medical and Allied Science, Multan, Pakistan (03/pharma/times/Nov/16).

Induction of diabetes

After the rabbits were kept on an overnight fast, they were injected streptozotocin 50 mg/kg (intraperitoneal) after titrating it with an ice-cold citrate buffer (pH 4.5). Rabbits were given 5% dextrose solution to drink to overcome the hypoglycemic shock. After 48 h, the development of diabetes was confirmed with blood glucose level over 200mg/dL [9].

Experimental design

All animals were divided into 4 groups, each group containing 6 rabbits as follows:

Group-1: Treated for normal wounds with TCDO solution (1mL) (Normal control group)

Group-2: Treated for normal wounds with *PHF* (1mL)

Group-3: Treated for diabetic wounds with TCDO solution (1mL) (Diabetic control group)

Group-4: Treated for diabetic wounds with *PHF* (1mL).

Wound healing activity

The rabbits were anesthetized with ketamine prior to the creation of wounds [9]. Back surface hairs of rabbits were shaved with blade and lignocaine gel (local anesthetic) was applied. A circular wound of 20 mm^2 was created on the thigh of each rabbit using a scalpel. The percentage wound contraction was calculated on day 0, 3rd, 6th and 9th post wound infliction. The

areas of wound contraction were calculated in mm. It was meant as the initial wound healing area. In all the experimental groups, topical treatment was done once daily at 10:00 am. On the 9th day post wound infliction, by using the following formula, the percentage wound closure was estimated.

Percent wound closure = Initial area of wound – nth day of wound/Initial area of wound × 100

Estimation of total phenol contents

Phenolic contents were calculated using previously described protocol [10]. For each replicate, 1 mL of plant extract (0.5 g/20 mL), prepared in sterile distilled water was added to 4 mL of Folin-Ciocalteu's reagent (Sigma, USA). After 7 minutes, 5 mL of 20 % sodium carbonate was added to each solution. The resultant solutions were incubated in darkness for 2 h at room temperature. The absorbance was measured at 740 nm in a spectrophotometer (UV 3000, ORI. Germany). Gallic acid (5, 10, 25, 50, 75 and 100 mg/L) was used as a standard chemical for the calibration curve. Quantification of TPC was expressed in terms of gallic acid equivalent (GAE) mg/g of dried fraction. All samples were analyzed in triplicate.

Histopathological examination

Excision biopsies were taken from the wounded skin from each group on 3rd, 6th and 9th day post wound infliction. Samples were put in formaldehyde solution (10%), and then subjected to various steps of tissue processing. Slides from each sample were stained with Hematoxylin and Eosin. Pictures were taken from all slides using a microscope equipped with camera and results were interpreted.

Acute dermal toxicity evaluation

The acute dermal toxicity of PHF was evaluated by fixed dose method in accordance with the guidelines of OECD (Organization for Economic Co-operation and Development). The dorsal areas of the rabbits were shaved and the PHF was applied topically on rabbits at a dose of 2000-3000 mg and observed for 48 h for irritation, redness and inflammation [11].

Statistical analysis

Data from Group-2 was compared to normal control Group-1 by using Student's T test. Similarly, Group-4 was compared to Group-3 (Diabetic Control Group) with the help of Student's T test. $P \leq 0.05$ was considered as significant.

RESULTS

The effect of PHF on the wound healing process in comparison with their control groups are depicted in Table 1. It is clear from the data that the values of the PHF treated group in normal and diabetic groups were parallel to the groups treated with TCDO for an initial 3 days. However, 68% and 80% wound healing was observed on the 6th and 9th day in the PHF treated group in normal rabbits. These values were 54% and 72% respectively in the PHF treated group in diabetic rabbits. These values were significantly low ($p < 0.05$) when compared to their respective control groups.

The phytochemical analysis of crude extracts of *A. sativum, C. longa* and *Z. officnale* are presented in Table 2.

Table 1: Effect of *PHF* on normal and diabetic excision wound model

Post wounding days	Normal rabbits		Diabetic rabbits	
	Group-1 (Control)	Group-2 (PHF-treated)	Group-3(Control)	Group-4 (PHF-treated)
0	80.57 ± 12.5 (0%)	79.32 ± 12.8^{NS} (0%)	78.57 ± 12.5 (0%)	79.32 ± 12.8^{NS} (0%)
3	38.50 ± 7.3 (52.21 %)	41.19 ± 8.4^{NS} (48.07 %)^{NS}	47.19 ± 6.8 (39.93 %)	46.19 ± 8.4^{NS} (36.32 %)^{NS}
6	21.76 ± 9.3 (72.99 %)	28.97 ± 10.5* (68.51 %)*	31.28 ± 6.6 (60.18 %)	35.97 ± 10.5* (54.65 %)*
9	7.2 ± 2.7 (91.06 %)	15.19 ± 3.1* (80.84 %)*	15.44 ± 1.5 (80.34 %)	22.19 ± 3.1* (72.02 %)*

Value is mean ± SEM of six animals (n=6) in each group. The number in parenthesis indicates the percentage of wound contraction. *Values are significant when compared to their respective control group. ^{NS}Non significant values

Table 2: Phytochemical analysis of crude extracts of *A. sativum, C. longa and Z. officinale*

Phytochemicals	*Allilum sativum L.*	*Curcuma longa L.*	*Zingiber officinale*
Flavonoid	+	+	+
Coumarin	+	+	+
Terpenoid	+	+	+
Saponin	+	-	-
Steroids	+	-	+
Tannins	-	-	-
Phlobatanins	+	-	-
Cardiac glycoside	+	-	-

Presence = (+) absence = (-). Each value is the average of two independent determinations

Total 3: Polyphenol contents of *A. sativum, Z. officinale* and *C. longa* extracts

Sample	Phenol contents (GAE mg/g)*
Allilum sativum L.	54.25 ± 0.15
Curcuma longa L.	25.45 ± 0.48
Zingiber officinale Rosc.	29.08 ± 0.35

*Values are expressed as means ± standard deviation (n = 3)

Histopathological evaluation

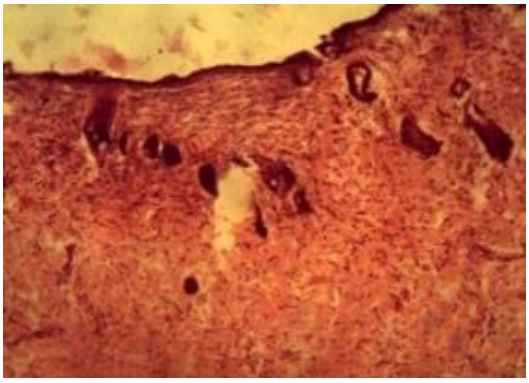
Both of the diabetic groups treated with TCDO and PHF showed significantly decreased in the inflammatory cells as the study progressed, high rate of granulation, re-epithelization, fibroblast, collagen and neovascularization (Figure 1).

DISCUSSION

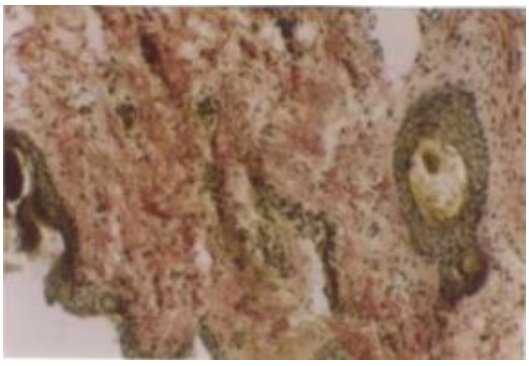
Plants are extensively used in the treatment of different ailments in animals. They have some advantages over the other remedies, such as, the least toxicity, easy availability, good therapeutic action and minute adverse reactions. In recent years, a number of different phytoconstituents having wound healing activities are screened out from various plants and their pharmacological actions are well documented [12,13].

Phytoconstituents exert their pharmacological action in a number of ways. One possible explanation of the wound healing activity of plants may include the involvement and stimulation of antioxidants which provide a favorable environment for tissue repair [6].

All the three plants have shown excellent antioxidant activity in previous studies [14,15]. Antioxidant properties may serve to promote healing at the wound site by enhancing cell proliferation and migration. It has been reported that antioxidants may play vital part in the wound healing process and may be an important contributory factor in providing a healthy environment for wound healing [6,9].



(a) Dermis of the healing skin treated with TCDO



(b) Dermis of the healing skin treated with PHF

Figure 1: Microscopic picture of PHF and TCDO treated groups

Antimicrobial activity is another important aspect in the wound healing process. It is evident from the previous studies that plants having antimicrobial activity respond well to the wound healing process [16]. The antimicrobial action of *A. sativum* and *Z. officinale* have been reported in the previous studies [7]. In addition, the anti-fungal and anti-inflammatory actions of these plants are also well documented [17]. These properties aid in the wound healing potential of these plants. Tannins and flavonoids also play a supporting role in the wound healing process. They are supposed to have astringent and antimicrobial potential which may contribute to the increase in the rate of epithelization and wound contraction process [18]. The three plants used in the current study are reported to have significant amount of tannins and flavonoids in recent studies [5,6].

Glycosides play an important role in the wound healing process [19]. These compounds are suggested to be involved in the stimulation of collagen fibroblast for wound healing process [20]. The presence of glycoside in two tested plants, *A. sativum* and *C. longa* has also been reported previously [5] which could be one of the possible reasons for the wound healing potential of these plants.

β-sitosterol is an important phytophenol, and has a vital role in the angiogenesis process of wound healing [21]. Previous studies have demonstrated the presence of β-sitosterol in all of the three tested plants [22,23]. The generation of new vasculature is crucial for wound healing phenomenon especially in the diabetic rabbits [4].

TCDO/PHF has great potential to treat chronic and resistant wounds, and it shows a very prompt response in treating diabetic foot [24]. The possible reason for the efficient response may be due to the presence of bio-activated oxygen carriers, which readily break the vicious cycle of hypoxia in wounds and increases the phagocytic activity [25].

CONCLUSION

The wound healing ability of PHF may be due to the phytoconstituents present in *Allium sativum, Zingiber officinale* and *Curcuma longa*. Their cumulative effect may be responsible for their quick and effective wound healing. However, further detailed studies are required before its clinical application.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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