EVALUATION OF WOUND HEALING POTENTIAL OF SPIDER SILK USING MICE MODEL

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ABSTRACT

Spider silk is an excellent biocompatible material with strong regenerative capability that make it extremely attractive candidate for cutaneous wound repair. In this study we evaluated the wound healing potential of silk recovered from three different spider species (i.e., *Pholcus phalangioides*, *Crossopriza lyoni* and *Araneus diadematus*) and compared with the standard povidone-iodine, honey and turmeric. Animals were maintained in the animal house. Full thickness excisions were created on the dorsum of each animal with sterilized scalpel. All test materials were applied topically on respective wounds according to standard protocol. Results of study showed that silk of *Pholcus* and *Crossopriza* tremendously accelerated the skin reconstruction process and significantly reduced the wound surface area in 14 days compared to more than 20 days of natural untreated healing. The pace of wound contracture and reepithelialization was nearly the same in groups which received silk solution of *Araneus diadematus*, honey and povidone-iodine treatment. Turmeric hardly succeeded in making minute difference from natural healing in untreated group but remain exceptional in its scar inducing potential. The present work furnishes scientific proofs to revalidate the previously underappreciated role of spider silk as a potent wound healing agent. The outcomes of this study strongly recommend harnessing of spider silk components in the formulation of commercial dermatological ointments for wound repair.

Keywords: spiders, silk, wound healing, biomaterials.

INTRODUCTION

Wound healing is a multifactorial, and complicated physiological process. Cellular and biochemical components as well as enzymatic pathways play vital roles during the patch-up and recovery of a wound tissue (Kordestani, 2014). Successful cutaneous wound repair occurs in a series of tightly coordinated and overlapping phases; inflammatory phase, proliferative phase, and maturation phase (McIntire, 2014). The inflammatory phase is characterized by two portions, the hemostatic portion and the cellular portion. Hemostasis ends in the formation of a plug of platelets and proteins at the site of tissue trauma (Richetta *et al.,* 2011). The cellular portion causes influx of neutrophils and macrophages to the injured area. These cells then destroy bacteria and eliminate debris from dying cells and damaged matrix so that the repair processes can proceed (Kloth *et al*., 1990).

The proliferative phase comprises three sub-phases; the re-epithelialization portion, the neo-vascularization portion and the collagen deposition portion. Re-epithelialization refers to the process of epithelial renewal in response to injury (Arnoux *et al*., 2005). Neo-vascularization portion is marked by migration of micro-vessel endothelial cells to the spot for the formation of new blood vessel networks (Cumming, 2006). Collagen deposition is essential for wound repair as it bestows the wound with high tensile strength and the ability to heal faster (Broughton and Rohrich, 2005). The maturation phase involves phenotypic differentiation of the preexisting fibroblasts into myofibroblasts (Sarrazy *et al*., 2011), which control the synthesis and degradation of extracellular matrix [ECM] proteins (Mu *et al.,* 2014).

Spider silk has fascinating combination of properties that makes it an extremely attractive candidate for numerous applications in medicine and industry (Brown *et al.*, 2015). The growing need for new therapeutics for wound healing has fueled the initiative to explore the potential of spider silk in medicine (Hu *et al.*, 2012). Intensive efforts are underway to discover natural biochemical agents that can promote wound healing. On account of its fine sized fiber, spider webs are thought to have great clotting potential in addition to bactericidal properties (Vollrath and Knight, 2001). Silk is rich in vitamin K, which plays a direct role in clotting of the blood (Lateef *et al.*, 2015). Silk-derived biomaterials being biocompatible to the human tissues are routinely used in surgery (Gellynck *et al*., 2008).

In spite of traditional use of spider silk in wound healing, no systematic attempts have been made to exploit its capability in modern medicine. Keeping in view the great potential of spider silk in cutaneous tissue repair and regeneration, the aim of this study was to test the applicability of spider web to treat dermal lesions on a pre-clinical model. Current study would prove the suitability of spider silk components for wound healing.

MATERIALS AND METHODS

Web collection: Insect free spider webs were recovered by winding them around ethanol treated sterilized glass rods. Webs of three spider species (i.e., *Pholcus phalangioides*, *Crossopriza lyoni* and *Araneus diadematus*) were collected from Sahiwal, Sargodha. Spiders were indentified with the help of key provided by Barrion and Litsinger (1995). Species specific webs were stored separately in clean sampling bottles. The collection was repeated several times until an ample amount of webs was obtained. The collected webs were washed twice in 50 ml distilled water at room temperature before use (Higgins *et al*., 2001).

Ointment preparation: Sodium Hydroxide (NaOH) crystals (2.5gm) were dissolved in 100 ml distilled water at room temperature to obtain 2.5% NaOH solution. Spider web (100 mg) was gradually added in NaOH solution and stirred gently to get uniform suspension. Acetic acid was also poured into the suspension in adequate amount to counter high alkalinity. NaOH-povidone mixture was prepared by mixing equal quantity of both the solutions (50 ml of 2.5% NaOH + 50 ml povidone). Turmeric powder (10 gm for single preparation) was mixed with few drops of distilled water to make the paste for topical application.

Experimental animals: Swiss Webster albino mice (*Mus musculus*) ranging from 35 to 40 gm weight and about 3-4 months old were used as experimental models. Mice were purchased from the animal husbandry of National Institute of Health (NIH), Islamabad. The animals were housed in well-ventilated steel cages with clean paper pieces and sawdust as bedding. The mice were offered with uniform basal diet comprising wheat grains and fresh vegetables with water at free access all the time. Mice were maintained under controlled conditions of 20°C temperature ± 2°C, and 50% humidity± 5%. The holding room was illuminated for 12 hours light/dark cycles throughout the experiment. Before the onset of experiment, the mice were acclimatized for 10 days to make them habitual of the laboratory conditions.

Lesion induction: Under general anesthesia, predetermined skin area of 2 cm^2 was prepared for excision by removing hair with the help of a clean razor. The cutaneous lesions were induced on the dorsal surface to limit the animal's own access to the wound. Each mouse was subjected to a single rounded cut of 600-800 mm^2diameter and 2 mm depth on the dorsal thoracic surface; 1 cm away from the vertebral column and 4 cm away from ears. The skin was excised to the full thickness using sterile scalpel piercing epidermis and dermis.

Treatment: The mice were randomly allocated to eight groups of five animals each. Animals of group I were left untreated on the mercy of natural healing. Mice of group II received standard povidone-iodine ointment. Silk solution of *Pholcus phalangiodes* and *Crossopriza lyoni* webs was applied to the wounds of the group III and IV, respectively. Wounds of group V were bestowed with prepared orb-web solution from *Araneus diadematus* as healing agent. Topical treatment with povidone-orb web mixture was performed on group VI. The wounds of group VII were dressed with honey and those of group VIII were covered with turmeric paste.

The respective therapeutic material was administered topically on the respective wound in Groups II to VIII until complete epithelialization. Treatment was performed once in two days regularly and wound area was then covered with a fresh sterile bandage. The healing progress of dermal wounds was monitored and picturized with two day interval every time before the treatment. Animals were periodically weighed and observed closely for any infection throughout the experiment.

Measurement of wound area: The gradual reduction in wound area was quantified after every two days. The area was measured by tracing the wound boundaries on a sterilized transparency paper sheet placed on the wound. Wound contraction rate was expressed in cm^2 decrease in original wound area. The period of epithelialization corresponded to the time taken by the wound to shed the scar (dead-tissue remnants) leaving no residual raw wound. Each measurement was repeated three times at the same sitting to minimize the possibility of errors. The average value of the measurements was considered in data processing. Data are presented as means ±standard error (Mean±SE) of means using SPSS.

RESULTS

The study clearly exhibited significant improvement in wound healing activity with the prepared spider silk ointments, compared to that of the reference standard and control group of animals. The outcomes of excision wound repair model with topical application of spider silk solution and comparative biomaterials are presented in Table (1).

Group III (G-III/silk of *Pholcus phalangiodes*) and IV (G-IV/silk of *Crossopriza lyoni*) shared the victory of wound healing contest with fastest healing pace, closing the wound completely in 14 days as compared to more than 20 days of normal healing in Group I (G-I/without any treatment). The healing process touched the end with comparatively less efficiency on 16th day in Group II (G-II/povidone treatment), Group V (G-V/Orb web of *Araneus diadematus* and Group VII (G-VII/Honey treatment) at more or less equal rates. Group VI (G-VI/Orb web-povidone mixture) took 18 days to wind up the healing task exhibiting slightly slower repair than povidone-iodine and all the spider silk treated groups. Slowest healing progress among all the treatment groups was presented by Group VIII (G-VIII/Turmeric treatment) which took more than 18 days to rebuild the lost skin.

The webs of *Pholcus* and *Crossopriza* found to be more operative with respect to skin regeneration as well as improved wound condition during the whole period of wound repair. The rate of wound closure and epithelization in silk solution of *Araneus diadematus* and honey treated mice runs parallel to the synthetic povidone-iodine in compensating the skin damage and antiseptic properties. The pure orb web solution of *Araneus* showed slightly better healing progress than the orb web-povidone mixture. The silk-povidone mixture however exceeds the healing process than negative control and turmeric treated group. Despite fast healing progress, silk solutions remain second to all in maintaining good wound health. Turmeric remained prominent due to its unparalleled scar-inducing potential which minimizes the risk of possible infection at wound area. The images of lesions of representative animals from each group at the day of wound infliction [G-I to G-VIII (A)] and the day 16thofhealing [G-I to G-VIII (B)] can be seen in Figures (1 and 2).

Table 1. Tabulated representation of the impact of spider silk on wound closure in excision wound model (wound area in cm^2).

	G-I	**G-II**	**G-III**	**G-IV**	**G-V**	**G-VI**	**G-VII**	**G-VIII**
	Negative control	*Positive control*	*Pholcus phalangiodes*	*Crossopriza lyoni*	*Araneus diadematus*	*Web + povidone*	*Pure Honey*	*Turmeric paste*
Days	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
0	0.98±0.06	0.98±0.05	0.76±0.08	0.76±0.10	0.82±0.05	0.82±0.02	0.76±0.04	0.74±0.05
2	0.91±0.06	0.90±0.07	0.58±0.05	0.68±0.09	0.72±0.05	0.75±0.02	0.64±0.02	0.64±0.05
4	0.84±0.07	0.80±0.08	0.48±0.05	0.54±0.08	0.58±0.06	0.65±0.02	0.56±0.04	0.56±0.05
6	0.79±0.09	0.66±0.07	0.39±0.07	0.40±0.07	0.46±0.04	0.57±0.02	0.46±0.04	0.48±0.03
8	0.72±0.07	0.44±0.05	0.27±0.03	0.26±0.05	0.36±0.04	0.45±0.02	0.32±0.05	0.34±0.04
10	0.67±0.07	0.32±0.04	0.18±0.03	0.15±0.04	0.26±0.05	0.32±0.02	0.28±0.05	0.30±0.05
12	0.57±0.06	0.18±0.03	0.10±0.02	0.09±0.02	0.22±0.03	0.25±0.05	0.22±0.05	0.24±0.04
14	0.50±0.04	0.08±0.03	0.02±0.02	0.03±0.04	0.06±0.02	0.15±0.08	0.14±0.02	0.20±0.04
16	0.43±0.04	0.06±0.02	------	------	0.06±0.02	0.10±0.07	0.06±0.02	0.12±0.04
18	0.35±0.04	0.04±0.03	------	------	0.04±0.02	0.08±0.05	0.04±0.02	0.10±0.03
20	0.24±0.05	------	------	------	------	------	------	0.08±0.07

Note: The values after ± in above table are representing standard error.

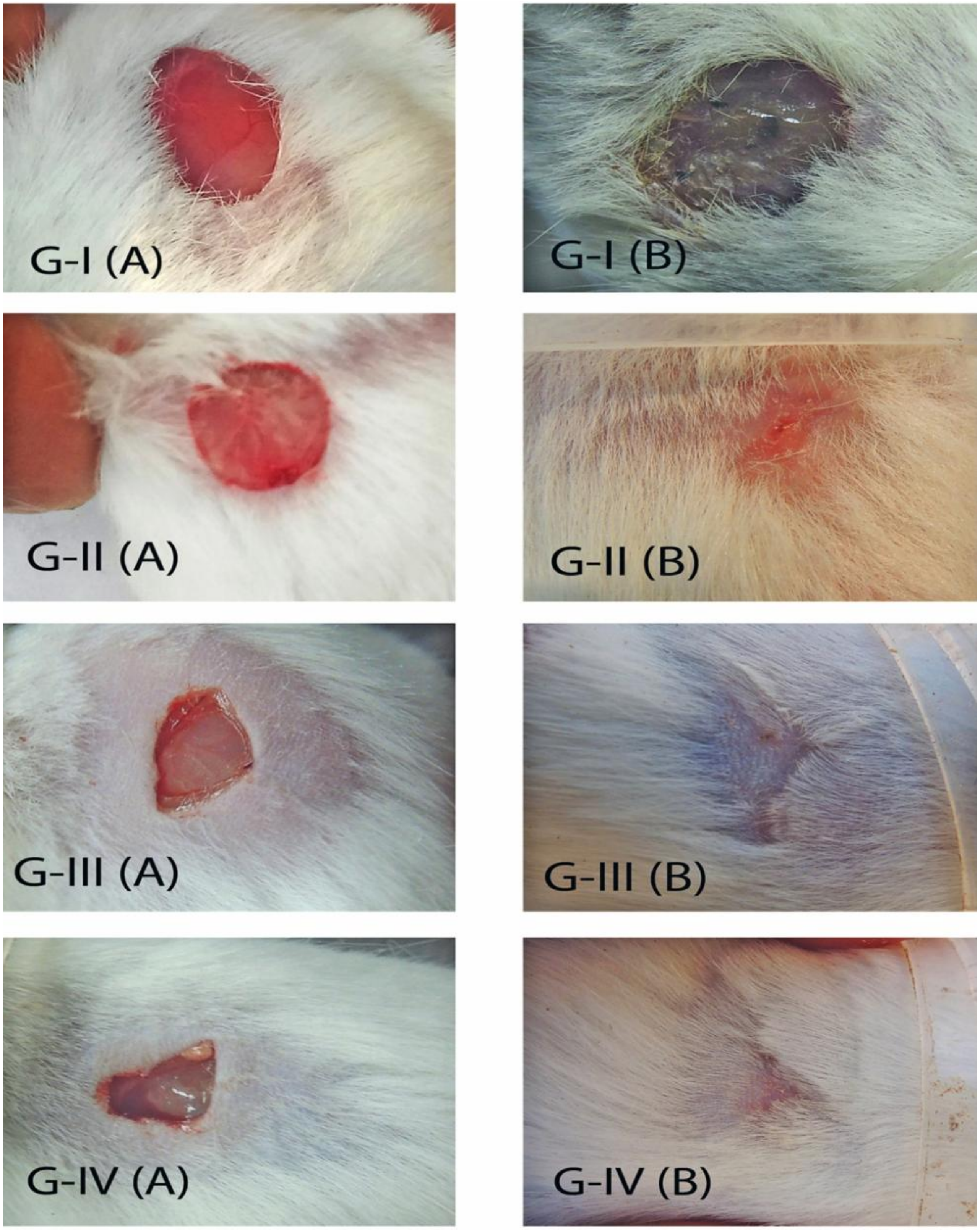

Figure: 1. Pictorial demonstration of comparative effect of spider silk and other biomaterials on wound contraction and re-epithelialization in excision wound model. ['A' represents the initial wound infliction at day 0 and 'B' pictures out 16th post-wounded day (except G-III-B and G-IV-B which depicts 14th day)].

Figure: 2. Pictorial demonstration of comparative effect of spider silk and other biomaterials on wound contraction and re-epithelialization in excision wound model. ['A' represents the initial wound infliction at day 0 and 'B' pictures out 16th post-wounded day].

DISCUSSION

Wound repair is a spontaneous and self-sustaining response to tissue damage (Dunn, 2005) but when tissue has been disrupted so severely that it cannot heal naturally on its own without disfigurement, the wounded area must be treated with appropriate material to augment the innate healing mechanism. A wide range of treatment strategies for effective wound healing are available including; wound dressings, compression bandaging, debridement, negative pressure wound therapy, ultrasound, electrical stimulation, phototherapy and skin substitutes.

Despite the multitude of treatment options, none of the currently available options combine all the requirements necessary for quick and optimal cutaneous regeneration (Andreu *et al*., 2015). The search for efficient therapeutic approaches seems to be essential in order to avoid the aggravation of cutaneous wounds. Natural biomaterials of both plant and animal origin own good medicinal characteristics for situations where synthetic materials have not met clinical expectations (Yoo *et al*., 2011). Considering the drawbacks of synthetic biomaterials, the use of spider silk proteins as natural biomaterial could be of great value.

In the present study, the influence of topical application of the various spider webs incorporated into sodium hydroxide solution on the excision wounds in albino mice has been evaluated. The obtained results demonstrated that silk of *Pholcus* and *Crossopriza* web caused a higher rate of wound healing and reduced the epithelialization period significantly than the natural untreated healing. Both of the species turned out to be functional in improving the overall wound conditions and epidermal cell proliferation due to blood clotting and antibacterial action of silk proteins. Spider silk proteins have strong antimicrobial action against a broad spectrum of pathogenic bacteria and are capable of sustaining the proliferation of mammalian cells (Gomes *et al*., 2011).

The time taken by the orb web silk of *Araneus diadematus* to restore the wound was equal to that of povidone-iodine and honey. The orb web is a specific purpose built web and spiders construct it by piecing together different types of silk proteins. Each protein is specialized to perform specific function such as mechanical strength, flexibility and scaffold during web construction (Brunetta& Craig, 2010). Hence, the orb web exhibited less regenerative potential than the *Pholcus* and *Crossopriza* web because it is especially secreted for strength and stability which serves to entangle prey rather than self repair. However, the orb web proteins have antibacterial and antifungal properties indicating the potentials for preventing the wound from infection.

The mixture of orb web solution and povidone-iodine showed slightly slower wound repair than all other silk treatments. It is probably because of the harsh nature of sodium hydroxide whose high alkalinity is devastating for newly regenerated cells. Sodium hydroxide is a strong corrosive material that can cause irreversible damage to the living cells (Starr, 2005). It occupies the peak of alkalinity having pH 14 while the wound healing process is accelerated under acidic conditions probably due to enhanced antimicrobial activity (Gethin, 2007). High osmolarity and antiseptic property of honey and strong antibiotic effect of povidone-iodine make them good wound healers. Honey is a highly viscous material that can kill the many potent pathogens by subjecting them to a strong dehydrating environment. Turmeric, the representative of natural plant extracts, showed poor results as compared to all other testing materials. The possible reasons of the slow progress may be the anticoagulant property of turmeric components and its mode of application. Curcumin shows anticoagulant activity by inhibiting collagen and adrenaline-induced platelet aggregation *in vitro* as well as *in vivo* in rat thoracic aorta (Srivastava *et al*., 1985). Moreover, oral treatment of curcumin is found to be more potent than topical treatment for angiogenesis at wound site (Sidhu *et al*., 1999).

According to Kumari *et al*. (2013) spider silk treatment caused dramatic reduction in wound closure and re-epithelialization duration taking 24 days as compared to more than 36 days of normal healing and 32 days of povidone-iodine treatment. Our results are not in close accordance with them owing to the quality of silk recovered from selected species of spider. Also, the quantity of silk used and the protocol to dissolve it were quite different. Our results fall in agreement with Maheshwari *et al*. (2015) who observed significant difference in healing progress by *Crossopriza lyoni* spider web as compared to normal healing and ointment control. The orb web healing in our experiment, however, diverged probably because of different class of the spider web. Our results are concurrent with Siyam and Abushama (2008) who observed significant epidermal cell recovery by *Pholcid* spider web than normal healing and panderm ointment control. In context of cell proliferation, this study generated outcomes parallel to those of Shahbuddin *et al*. (2015). They discovered the potency of *Pholcu sphalangioides* silk to ignite the division and subsequent migration of teeth pulp stem cells and human keratinocytes.

Conflict of interest: It is hereby declared that authors have no conflict of interest.

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