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Wound Healing Potential/Activity of Polyherbal Ointment Containing *Salvadora persica*, *Azadirachta indica* and *Calendula officinalis* Extracts: An Experimental Study

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Abstract. The present study explore the wound healing potential of a novel herbal ointment formulation, fast wound healing ointment (FWHO) composed of 10% w/w crude ethanolic extracts of *Salvadora persica* (sticks), *Azadirachta indica* (leaves) and *Calendula officinalis* (whole plant) on excision wound model in albino rats. The FWHO was applied topically once daily on the wound to evaluate the rate of contraction and period of epithelialization in rats. Complete contraction and epithelialization of wound was observed at 19 day ($P<0.0005$) in test group animals compared with standard (polyfax skin ointment) at 23 day ($P<0.0005$) and control group (blank petroleum jelly) at 29 day respectively. It was observed that 10% FWHO has potent wound healing capability as evidenced by decrease in the period of epithelialization and increase in the rate of wound contraction in test group as compared to the standard and control groups. The test group illustrates 34.4%, while the standard group shows 20.6% decrease in the time of epithelialization as compare to control group. The study revealed that test ointment (10% FWHO) has a potential to promote wound healing by accelerating the rate of epithelialization and scar formation as compare to standard (polyfax skin ointment) and control (blank petroleum jelly) groups.

Keywords: extracts of *S. persica, A. indica, C. officinalis*, excision wound model

Introduction

Wounds and healing of wounds are two vital events in the life of animals and human. Wound is a physical trauma, where the skin is torn, cut, burnt or punctured due to disruption of normal anatomic structure and function. The most common symptoms of wounds are bleeding, loss of function, heat, redness around the wound, painful or throbbing sensation, swelling of local tissue and oozing from wound. The healing process is the survival mechanism representing an attempt to maintain normal anatomical structure and function. It is a natural body reaction against the injury which starts immediately after wounding and occurs in four phases i.e. hemostasis, inflammation, proliferation and maturation (Kumaran *et al*., 2016; Gupta *et al*., 2015). A curative agent should ideally improve one or more phases of healing process without causing harmful effects. The side effects of synthetic medicine are alarming and produce to numerous undesirable effects despite their strong pharmacological action (Parasuraman *et al*., 2014). Today there is widespread interest is developed in producing plant derived drugs. The use of plants as a whole or extracts of their certain parts to accelerate the process of healing has been in use since ancient times due to their widespread availability, minimal toxicity and their effectiveness as crude preparations (Gautam *et al*., 2014).

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Although many traditional healer have successfully used hundreds of medicinal plants to manage various forms of health conditions (Wambugu and Waweru, 2016). Henceforth it was aimed to find out wound healing effects of FWHO which was composed of 10% w/w crude extract of *Salvadora persica* (sticks), *Azadirachta indica* (leaves) and *Calendula officinalis* (whole plant) to establish the scientific base for its use (Maan *et al*., 2017; Imran *et al*., 2015; Emeka *et al*., 2013).

Salvadora persica (*Salvadoracea*) commonly known as Miswak abundantly found in Asian countries, contains important phyto-constituents such as vitamin C, salvadorine, salvadourea, alkaloids, trimethylamine, cyanogenic glycosides, tannins, saponins and salts mostly as chlorides etc. Medicinally possesses anti-oxidant, antiseptic, antimicrobial, hemostatic, anti-plaque, analgesic, anti-inflammatory, anti-pyretic, astringent and diuretic properties. It is well documented that the promising wound healing activity of *S. persica* is due to the synergistic effect of both antimicrobial and antioxidant activities which increase wound contraction

and accelerate the wound-healing process (Tatke *et al*., 2017; Imran *et al*., 2015; Ahmad *et al*., 2011). *Calendula officinalis* L. (Asteraceae) known as pot marigold or common marigold is the herb of ancient time with medicinal repute used in traditional and homeopathic medicine. Commonly it is used in first-aid treatment of wound for reducing inflammation, as antiseptic to clean wound, as disinfectant to prevent infection and helps to promote wound healing (Parente *et al*., 2012; Paul *et al*., 2017). It also increases blood flow and oxygen to wounds and affected areas which in turns helps the body to grow new tissue and healing of wound more rapidly. The high content of flavonoids acts as antioxidants and protects the body cells from damage caused by oxidation that may suppress the immune function (Bernatoniene *et al*., 2011). *Azadirachta indica* (Meliaceae) known as Neem is a well reputed useful medicinal plant with wide spectrum of biological activities. The active ingredients like nimbidin, nimbin and nimbidol are sufficiently found with anti-inflammatory, antibacterial, antifungal and antiviral properties (Alzohairy, 2016; Chhibber and Sharma, 2014). It is considered valuable in various skin problems like itching, burning and skin ulcer. *A. indica* has an excellent amount of amino acids, vitamins and minerals that are playing very important role in the proliferation phase of wound healing process (Chundran *et al*., 2015; Kumari and Keshri, 2015). At present more than 50% of clinically used drugs are plant origin (Vamsi *et al*., 2014). This interest primarily stems from the belief that green medicines are commonly safe and dependable as compared to costly synthetic drugs. These herbal drugs are considered as a therapeutic weapon to fight against various ailments/diseases in birds, humans and animals with less or without any side effects. Herbal drugs are boon to our society. This present study is majorly aimed to determine the effect of FWHO on the rate of wound healing (percent wound contraction and period of epithelialization) in excision wounds in Wistar albino rat model.

Material and Methods

Preparation of extracts. The specified portion of the plants i.e. *S. persica* (twigs) was purchased from local medicinal market of Karachi, whereas *A. indica* (leaves) and *C. officinalis* (whole plant) were collected from garden of PCSIR Labs. Complex, Karachi, Pakistan. All parts were air dried under the shade at room temperature in controlled room environment until the material gets completely dried. Each plant sample was cut into small pieces and then finally chopped to make coarse powder. The extract was prepared by soaking the equal weights i.e. 2 Kg of each material into 6 liters of commercial ethanol and caped in screw tight separate bottles for one week with occasional shaking. After filtration through muslin cloth and through Whatman no. 1 filter paper, the filtrates were then evapourated under reduce pressure (-760 mmHg) at 35-40 °C in a rotary evapourator. The thick semi-solid crude extracts were collected in Amber glass bottles and stored in refrigerator till use.

Preparation of fast wound healing ointment (FWHO). The test sample (FWHO) was prepared by incorporating the active ingredients (ethanolic extracts of *S. pesica*, *C. officinalis* and *A. indica*) in the base petroleum jelly by trituration using mortar and pestle in a concentration of 10% (w/w). The prepared ointment was filled in sterilized airtight containers and stored at room temperature.

Selection of animal. Eighteen albino male rats (180-200 g) reared at animal house of PCSIR Labs Complex, Karachi were selected and grouped accordingly for wound healing activity. Animals were housed and maintained under standard conditions and fed with standard diet and water *ad libitum*. They were kept carefully following acclimatization period of 7 days to ensure their suitability for the study. Animals showing any signs of illness were excluded from the study.

Wound healing activity. Excision wound model was used for the study of rate of contraction and epithelialization of wound. Animals were anaesthetized with diethyl ether and a circular wound of about 150 mm^2 was made on depilated dorsal thoracic region of rats. Wounded areas were measured immediately by means of Vernier Caliper and consider as initial wound area reading. Treatment with the ointments started immediately after wound creation. Animals of Group I were treated with thin layer of (10% FWHO) as test drug, Group II was treated with thin layer of (polyfax skin ointment) as reference drug, while Group III was treated with thin layer of blank petroleum jelly as control respectively. The entire wound was left open. All samples were applied topically once daily until the achieving of complete healing of wound. The observation was made every 3rd day of post wounding day. The wound contraction was calculated as percentage reduction in wound area with respect to initial wound area, while the epithelialization time was noted as the number of days after wound required for scar to fall off leaving

no raw wound behind. Rate of wound contraction was calculated by the formula given below (Nguyen *et al*., 2017; Imran *et al*., 2015; Kodati *et al*., 2011);

$$\text{Percent wound contraction} = \frac{\text{wound area at day 0 - wound area at day } n}{\text{wound area at day 0}} \text{x100}$$

Statistical analysis. All the results obtained were analyzed statistically by using Student's *t* test and P<0.05 were considered significant.

Results and Discussion

The studies on excision wound healing model reveals that all three groups showed decrease in wound area from day one to last day. Rate of wound contraction in control rats was 33.3% to 68.8% from day 3 to day 9, 77.7% to 92.4% from day 12 to day 18 and 96% to 98.2% from day 21 to day 27. The mean time for complete normalization of skin in control group animals was noted on day 29 shown in Fig. 1. FWHO showed time dependent healing effect on wound surface area. Increase in wound contraction in treated group was 43.1% to 84% from day 3 to day 9 and 94.6% to 100% from day 12 to day 18, whereas in standard group 41.7% to 81.3% from day 3 to day 9 and 92% to 97.7% from day 12 to day 18, while 100% contraction was noted on day 19 (P<0.0005) respectively as compared to control group. The data obtained indicates that there is significant promotion in wound-healing activity in the animals of test group treated with FWHO which showed faster rate of epithelialization than in the animals of standard and control groups (Table 1, Fig. 2). FWHO also demonstrated the better healing capacity as it completely healed the wound within the period of 19 days (P<0.0005) in animals of test group whereas wounds treated with Polyfax skin ointment showed similar effects within 23 days (P<0.0005) in standard group as compared to control group which showed complete wound healing and epithelialization within 29 days. During the excision wound healing study, it was observed that FWHO not only effectively and promptly heals wounds by inducing fast epithelialization but also prevents infection because of its antiseptic effects like with polyfax skin ointment.

Fig. 1. Excision wound on back of neck of albino rat on day 1st.

Wound healing is a complex process aiming at the re-construction of damage tissue which requires precise coordination of connective tissue repair, re-epithelialization and angiogenesis. Healing of wound and generation of new tissue require the proliferation of fibroblasts not only to increase cell numbers but also to produce several extra cellular matrix proteins and growth factors (Jettanacheawchankit *et al*., 2009). Plants are more potent healers as they promote the repair mechanism in the natural way and function as efficacious natural antibiotics. The plant base materials are used in first aid for wound wash. They act as antiseptic, antibiotics, coagulants, vasodilators and antioxidants by enhancing the healing of acute as well as chronic wounds due to the presence of phytochemical agents and vitamins (Pereira and Bártolo, 2016). Properly prepared herbal formulations/products act as potent and effective medicines and generate the desired effects without numerous adverse side effects generally associated with synthetic antibiotics (Wachtel-Galor and Benzie, 2011). The aim of present study is to formulate and evaluate a new successful poly herbal remedy containing necessary natural supplements and other physiological active compounds that are essential for healthy skin, for its nourishment and to promote prompt wound healing in short period of time (Ahmad *et al*., 2014; Meena *et al*., 2009). Fast wound healing ointment (FWHO) is a poly herbal preparation containing plant extracts of *S. persica*, *A. indica* and *C. officinalis* as active agents, while petrolatum jelly is used as a base. Petroleum jelly is a mixture of natural hydrocarbons and mineral oils obtained from petroleum. It locks skin moisture and act as lubricant that softens and soothes the skin (Park and Song, 2010). The preparation formulated by using these herbs because they have strong evidences to have anti-inflammatory,

immuno-modulatory, antimicrobial, anti-oxidantand and analgesic activities respectively which are complementary to wound healing process (Talha Bin Emran, 2015; Khatkar *et al*., 2014; Ahmad *et al*., 2011; Mariod *et al*., 2009; Biswas *et al*., 2002). Polyfax skin ointment was used as a standard ointment, contains (1 g contains: polymyxin B 10000 IU, bacitracin 500 IU). Polymyxin and bacitracin ointment (Polyfax®) is used to treat skin infections caused by bacteria. It act as a topical antibiotic commonly used to treat wounds, burns, skin grafts, skin ulcers, itching and rashes (Glaxosmithkline Pakistan Limited).

In the excision wound model, animals treated with FWHO showed better and fast healing as compared to the standard and control groups (Fig. 1). Also there was significant decrease in the epithelialization period. It was observed that FWHO treated group showed considerable wound healing activity from the third day onwards, which was comparable to the standard drug polyfax skin ointment and blank petroleum jelly for control groups. The percentage of wound contraction was found much more in FWHO treated group (19 days for 100% contraction) which was more than that of standard group and control group animals i.e. 23 and 29 days for 100% contraction respectively (Table 1, Fig. 2). The healing time of test and standard group was also compared with control group and it was observed that the test group had 34.4% ability to decrease the time of epithelialization, while 20.6% decrease time of epithelialization was observed in standard verses control group. On the basis of the result obtained in the present investigation, we can conclude easily that the FWHO has remarkable wound healing activity. The better activity of polyherbal formulation may be due to the synergistic action of the plants constituents present in the formulation like alkaloids, saponins, tannins, flavonoids, curcumin and triterpinoids (Vamsi *et al*., 2014). *S. persica* is a well reputed plant with properties that are needed for wound healing like analgesic, anti-inflammatory, antioxidant, antiseptic and antimicrobial along with hemostatic activities. Hemostasis plays a vital role in healing process as it arrest the bleeding from damaged blood vessel, initiate tissue repair and prevents tissue death through hemorrhage (Imran *et al*., 2015; Ahmed *et al*., 2011). It is also reported that phytochemicals (flavonoids, saponin, sterol, tannins) and mineral salts (copper, manganese, zinc, vitamins and amino acids etc.) found in *S. persica* which also help to promote wound repair by increasing the rate of

Table 1. Percent wound healing activity of FWHO in excision wound model

Animal groups	% of wound healing									
	3rd day	6th day	9th day	12th day	15th day	18th day	21st day	24th day	27th day	30th day
Test group	43.5	64	85	95	98.9	99.7	100	100	100	100
Standard group	42.1	67.1	81.3	91.9	95.7	98.2	99.1	100	100	100
Control group	30.3	49.4	65.1	76.5	85.3	92	96	98.5	99.7	100

Number of animals=6

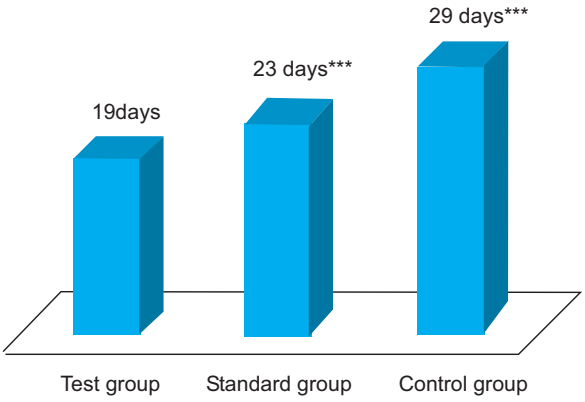

Fig. 2. Mean healing time in days of FWHO in excision wound model.

epithelialization in shortest period of time (Talekar *et al*., 2017; Imran *et al*., 2015; Khatak *et al*., 2010). *A. Indica* is reported for fibroblast proliferation, collagen synthesis and neo-vascularization that resulted in an increased wound tensile strength and accelerate wound healing (Rajasree *et al*., 2012). The amino acids, vitamins and minerals present in *A. indca* also play important role in proliferation phase of wound healing processes (Emeka *et al*., 2013). Its antimicrobial, antioxidant and anti-inflammatory properties accelerates healing process by preventing the infection and speeding up the proliferation phase in the entire wound healing process (Chundran *et al*., 2015; Raina *et al*., 2008). A study conducted by Shafeie *et al*. (2015) reported that topical application of *Calendula officinal* is gel contributions to the reduction of edema and the regulation of micro circulation. The polysaccharides in *Calendula* flowers also demonstrate strong bio adhesive or mucilaginous effect may not only help to decrease local inflammation by shielding tissues from irritants, but also facilitate

tissue hydration (Ahmed *et al*., 2003). *Calendula* may also facilitate wound healing by increasing wound angiogenesis and collagen, nucleoprotein and glycoprotein metabolism leading to improvements in both local circulation and in the formation of granulation tissue. Several other experimental studies also support to these claims and demonstrate that the daily application of *calendula* cream or a 1:10 alcoholic extract of *Calendula* to paravertebral incisions in rats facilitates collagen maturation and epithelialization within 10 to 25 days (Leach, 2008). All these studies strongly support our findings.

The results of this study indicate that daily topical application of FWHO significantly accelerated the rate of wound healing. The potency and powerful healing action of FWHO could be due to the combined action of phyto-constituents that accelerates cell reproduction, tissue regeneration and simultaneously depressing the inflammatory process along with providing local analgesic, antibacterial, antifungal and astringent effects (Thangapazham *et al*., 2016; Manjunatha *et al*., 2006). Furthermore, the wound healing effect can be attributed to free radical scavenging activity of flavonoids and triterpenoids. They are known to reduce lipid peroxidation, not only by preventing or slowing the onset of cell necrosis, but also improving vascularity. It is documented that any drug that inhibits lipid peroxidation can increase the viability of collagen fibrils, which in turn results in increase in the strength of collagen fiber by increasing the circulation, preventing the cell damage and promoting DNA synthesis (Rekha *et al*., 2015). Roy *et al*. (2012) reported a clinical study that topical application of compounds with free radical scavenging properties showed improve wound healing significantly and protect tissues from oxidative damage. In epithelialization of wound and chemotaxis in fibrosis alkaloids are also known to play a pivotal role (Fetse *et al*., 2014). All these reports strengthen our results. On the basis of wound healing studies it can be summarized that prepared ointment possesses superior wound healing contracting ability than the standard and control groups. Thus, the prepared topical ointment is effective in terms of acceptability and possesses a versatile approach in healing the wound within short period of time.

Conclusion

The aim of present investigation is to formulate a natural, safe, indigenous herbal wound healing remedy. The present formulation is found very effective in wound healing by showing quick response following its application on wounds. It can be concluded that the FWHO is a valuable herbal product which may be effectively used for the treatment and management of wounds due to its promising quick healing property.

Conflict of Interest. The authors declare no conflict of interest.

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