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WOUND HEALING ACTIVITY OF *SALVIA OFFICINALIS (SAGE)*

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ABSTRACT

Medicinal plants have played an essential role in the development of human culture. Medicinal plants are resources of new drugs. Plants are a source of large variety of potent drugs to alleviate suffering from diseases. Sage has one of the longest histories of use of any culinary or medicinal herb. Ancient Egyptians used it as a fertility drug. In the first century C.E. Greek physician Dioscorides reported that the aqueous decoction of sage stopped bleeding of wounds and cleaned ulcers and sores. He also recommended sage juice in warm water for hoarseness and cough. It was used by herbalists externally to treat sprains, swelling, ulcers, and bleeding. It is considered a useful medicine in typhoid fever and beneficial in biliousness and liver complaints, kidney troubles, haemorrhage from the lungs or stomach, for colds in the head as well as sore throat, quinsy, measles, for pains in the joints, lethargy and palsy. It has been used to check excessive perspiration in phthisis cases, and is useful as an emmenagogue. A cup of the strong infusion will be found good to relieve nervous headache. In the present study ethanolic extract of *salvia officinalis* leaves were used to evaluate its wound healing activity in rats. Rate of wound contraction were taken as parameters. The wound healing activity of topically applied extract of leaf of *salvia officinalis* was evaluated in albino rat by excision wound model for a period of 16 days. Extract of *salvia officinalis* showed marked reduction in wound area in comparison to control group from 4th day onwards. The result obtained indicates that ethanolic extract of *salvia officinalis* accelerates the wound healing process by decreasing the surface area of the wound. The present study gives the evidence for wound healing activity of *salvia officinalis.*

Keywords: *Salvia officinalis (SO),* Excision wound healing, contraction period.

INTRODUCTION

A wound occurs when the integrity of any tissue is compromised (e.g. skin breaks, muscle tears, or a bone fractures). A wound may be caused as a result of a fall, or a surgical procedure; or by an underlying condition. The phases of wound healing are:

1. Hemostasis
2. Inflammation
3. Proliferation or granulation
4. Remodeling or maturation

Hemostasis

Once the source of damage to a house has been removed and before work can start, utility workers must come in and cap damaged gas or water lines. So too in wound healing damaged blood vessels must be sealed. In would healing the platelet is the cell which acts as the utility worker sealing off the damaged blood vessels. The blood vessels themselves constrict in response to injury but this spasm ultimately relaxes. The platelets secrete vasoconstrictive substances to aid in this process but their prime role is to form a stable clot sealing the damaged vessel. Under the influence of ADP (adenosine diphosphate) leaking from damaged tissues the platelets aggregate and adhere to the exposed collagen. They also secrete factors which interact with and stimulate the instrinsic clotting cascade through the production of thrombin, which in turn initiates the formation of fibrin from fibrinogen. The fibrin mesh strengthens the platelet aggregate into a stable hemostatic plug. Finally platelets also secrete cytokines such as platelet-derived growth factor (PDGF), which is recognized as one of the first factors secreted in initiating subsequent steps. Hemostasis occurs within minutes of the initial injury unless there are underlying clotting disorders [6].

Inflammation phase

Clinically inflammation, the second stage of wound healing presents as erythema, welling and warmth often associated with pain, the classic "rubber et tumor cum calore et dolore". This stage usually lasts up to 4 days post injury. In the wound healing analogy the first job to be done once the utilities are capped is to clean up the debris. This is a job for non-skilled laborers [6] .These non-skilled laborers in a wound are the neutrophils or PMN's (polymorphonucleocytes).

The inflammatory response causes the blood vessels to become leaky releasing plasma and PMN"s into the surrounding tissue. The neutrophils phagocytized debris and microorganisms and provide the first line of defense against infection. They are aided by local mast cells. As fibrin is broken down as part of this clean-up the degradation products attract the next cell involved [2]. The task of rebuilding a house is complex and requires someone to direct this activity or a contractor.

The cell which acts as "contractor" in wound healing is the macrophage. Macrophages are able to phagocyte bacteria and provide a second line of defense. They also secrete a variety of chemotactic and growth factors such as fibroblast growth factor (FGF), epidermal growth factor (EGF), transforming growth factor beta (TGF-and interleukin-1 (IL1) which appears to direct the next stage.

Proliferative Phase (Proliferation, Granulation and Contraction)

The granulation stage starts approximately four days after wound and usually lasts until day 21 in acute wounds depending on the size of the wound. It is characterized clinically by the presence of pebbled red tissues in the would base and involves replacement of dermal tissues and sometimes sub dermal tissues in deeper wounds as well as contraction of the wound. In the wound healing analogy once the site has been cleared of debris, under the direction of the contractor, the framers move in to build the framework of the new house. Sub-contractors can now install new plumbing and wiring on the frame work and siders and roofers can finish the exterior of the house. The "framer" cells are the fibroblasts which secrete the collagen framework on which further dermal regeneration occurs. Specialized fibroblast is responsible for wound contraction. The 'plumber' cells are the pericytes which regenerate the outer layers of capillaries and the endothelial cells which produce the lining. This process is called angiogenesis. The "roofer" and "sider" cells are the keratinocytes which are responsible for epithelialization. In the final stage of epithelialization, contracture occurs the keratinocytes differentiate to form the protective outer layer or stratum corneum.

Remodeling or Maturation Phase

Once the basic structure of the house is completed interior finishing may begin. So too in wound repair the healing process involves remodeling the dermal tissues to produce greater tensile strength. The principle cell involved in this process is the fibroblast. Remodeling can take up to 2 years after wounding and explains why apparently healed wounds can break down so dramatically and quickly if attention is not paid to the initial causative factors.

MATERIALS AND METHODS

Plant material:

The *Salvia officinalis* leaves were identified and authentified by Dr. K. Madhava Chetty, Department of botany, S.V. University, Tirupati, Chittoor (dist), Andhra Pradesh.

Preparation of extract:

The leaf of *Salvia officinalis* was collected, washed, dried in shade and pulvarized to obtain a coarse powder and then was subjected to solvent extraction by soxhlet apparatus. About 100g of powdered drug was extracted sucessively with ethanol using soxhlet apparatus. The extraction was carried out for 72 hours until the extract becomes colourless. Then the solvent was completely removed by evaporating in rotatory flask evaporator. The dried extract thus obtained was kept in desicator and was used for further experiment. Percentage yield of ethanolic extract of leaves of *Salvia officinalis* was found to be 4.98% w/w.

ACUTE ORAL TOXICITY STUDY

Acute oral toxicity study was followed by using OECD 423 (Acute Toxic Class Method) and was done in rats (150-200g) which were fasted overnight. They were divided into 5 groups of three animals each. The ethanolic extract of leaves of *Salvia officinalis* was administered orally through the feeding tube to the pair of rats of each group in ascending and widely spaced doses viz., 10,30,100,300,1000, 2000mg/kg. The animals were observed continuously for overnight, mortality was recorded. No signs of toxicity were observed even with 2000mg/kg of *Salvia officinalis.* So, the dose of the extract chosen for the study was 200mg/kg, 400mg/kg. This is corresponding to the 1/10th of the maximum tolerated dose (2000mg/kg).

EXPERIMENTAL ANIMALS

Abino wistar rats (150-200g) of either sex were obtained from the animal house in Sree Vidyanikethan College of Pharmacy, Tirupati. The mice were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. Standard pellet feed (Hindustan Lever Limited., Bangalore) and drinking water was provided *ad libidum through* out experimentation period. Mice were acclimatized to laboratory conditions one week prior to initiation of experiments. Ethical committee clearance was obtained from IAEC (Istitutional Animal Ethics Committee) of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Excision wound

The rats were inflicted with excision wounds as described by[6] Shivananda Nayak *et al*., The rats were anaesthetized prior to creation of the wounds, with anesthetic ether. The dorsal fur of the animal was shaved with an electric clipper and the area of the wound to be created was outlined on the back of the animals with methylene blue using a circular stainless steel stencil. A full thickness of the excision wound of 2.5 cm in width (circular area = 5.0 cm^2) and 0.2 cm depth was created along the markings using toothed forceps, a surgical blade and pointed scissors. The entire wound left open. The animals were divided into four groups of 6 each. The group 1 animals were left untreated and considered as the control. Group 2 animals served as reference standard and treated with Soframycin 2% ointment. Animals of groups 3 and 4 were treated with formulation 1 and 2 respectively for 15 days. The parameters studied were wound contraction and epithelialisation time. The measurements of the wound areas of the excision wound model were taken on 3^{rd}, 6^{th}, 9^{th}, 12^{th} and 15^{th} day following the initial wound using transparent paper and a permanent marker. The recorded wound areas were measured with graph paper. The period of epithelialisation was calculated as the number of days required for falling of the dead tissue remnants without any residual raw wound [6].

The percentage protection was calculated on the 15^{th} day by using the following formula and tabulated in table [1].

$$\text{Percentage protection} = \frac{100 - (\text{Final} \times 100)}{\text{Initial}}$$

STATISTICAL ANALYSIS

The relative wound area results were compared using one- way analysis of variance (ANOVA) followed by Dunnet's tests. *P* values less than 0.05 were considered as indicative of significance.

RESULTS

The percentage of wound contraction includes by recording the changes in wound area at fixed intervals of time, Viz., 0^{th}, 3^{th}, 6^{th}, 9^{th} and 12^{th} day after treated with ethanolic extract of SO. However, on 12^{th} post wounding day, Group I animal showed 84.98% of healing, which may be due to self immunity of animal whereas the extract treated group(Group II) showed 91.76% healing, the obtained result compared with control, the activity of the extract was found to be highly significant ($P<0.001$).

Percent wound contraction on different days

	Percent wound contraction on different days				
	0th	**3rd**	**6th**	**9th**	**12th**
Control	14.34+1.53	28.58+3.26	45.69+3.57	73.98+7.16	84.98+7.16
Ethanolic extract of SO	23.06+3.06	54.72+4.04	80.05+3.27**	90.76+0.92**	91.76+0.92**

Value expressed in mean ± SEM, n=6, not significant (P>0.005), * significant (P>0.01), ** Significant (P>0.001).

DISCUSSION

Wounds are referred to as disruption of normal anatomic structure and function. Skin wounds could happen through several causes like physical injuries resulting in opening and breaking of the skin (Gerald *et al*; 1994). The most common symptoms of wounds are bleeding, loss of feeling or function below the wound site, heat and redness around the wound, painful or throbbing sensation, swelling of tissue in the area and pus like drainage [14]. Wound healing is a very complex, multifactor sequence of events involving several cellular and biochemical processes. The aim in these processes is to regenerate and reconstruct the disrupted anatomical continuity and functional status of the skin. Healing process, a natural body reaction to injury, initiates immediately after wounding and occurs in four stages. The first phase is coagulation which controls excessive blood loss from the damaged vessels. The next stage of the healing process is inflammation and debridement of wound followed by re-epitheliasation which includes proliferation, migration and differentiation of squamous epithelial cells of the epidermis. In the final stage of the healing process collagen deposition and remodeling occurs within the dermis [13]. Study on animal models showed enhanced rate of wound contraction and drastic reduction in healing time than control, which might be due to enhanced epitheliasation. The flavonoids which are present in the extracts responsible for the free radical scavenging activity were believed to be one of the important components in wound healing. Alkaloid is known to assist in epithelization of wound and chemo taxis in fibrosis [3, 13]. Saponin on the other hand stimulates angiogenesis by modifying the balance of protease/protease inhibitor secretion in human endothelial vascular cells [10, 11, 12]. Tannin is believed to have Haemostatic activity, arresting bleeding from damaged or injured vessels by precipitating proteins to form vascular plugs [12].

CONCLUSION

The process of wound healing has two components, first is formation of new tissue and other is protections from microbial invasion during the healing process. The observations and results obtained in this study suggest that these plants for treating various types of wounds in human beings. Further studies with purified constituents are needed to understand the complete mechanism of wound healing activity.

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