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Research Article

Effect of Different Extracts of *Rheum emodi* for Wound Healing in Rabbit Model

Nida Handoo, Jalal-ud-Din Parrah, Bashir Ahmed Moulvi, Muhammad Moin Ansari*, Hakim Athar, Taziyun Imtiyaz, Neelofar Nabi, Gazalla Siraj and Zahid Rahim Malik

Division of Veterinary Surgery and Radiology, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Jammu and Kashmir- 190006, India
***Corresponding author:** drmoin7862003@gmail.com

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ABSTRACT

Use of herbal drugs has received greater attention in recent times, because of their diverse nature of curing diseases, safety and well-tolerated remedies compared to conventional drugs. The main objective of the present study was to evaluate the comparative wound healing activity of *R. emodi* and its different extracts. The study was conducted on twenty four (n=24) apparently healthy rabbits for a period of 21 days. Animals were randomly divided into four equal groups,of 6 animals each. In group A, povidone iodine ointment was used, which served as control. The animals of group B were treated with, 10% ethanolic extract ointment, group C with 10% aqueous extract ointment, group D with 10% petroleum ether extract ointment of *R. emodi*. Xylazine-ketamine combination was used for the introduction of general anaesthesia. Two full thickness square excisional cutaneous wounds of 1.5 x 1.5cm size, 2.5 cm apart from one another were created on either side of dorsal spine in the thoraco-lumbar region. A locally fabricated metal marker was used to demarcate the standardized wound margins. The efficacy of herb as a wound healing agent was evaluated on the basis of wound size and percentage healing. Extract treated groups showed complete and better healing. The percentage healing at the end of study period was 77.73% in control group and 100% in extract treated groups, which indicates that Rhubarb and its extracts accelerate wound healing process in rabbit model.

Key words: Different extract of *R. emodi,* Full-thickness wounds, Rabbit, Wound healing

INTRODUCTION

A wound may be defined as disruption of the cellular or anatomical continuity of the normal organ structure. Healing of wound involves migration, infiltration, proliferation, and differentiation of several cell types like keratinocytes, fibroblasts, endothelial cells, macrophages and platelets which were culminate in an inflammatory response, the formation of new tissue and wound closure (Barrientos *et al.,* 2008). Nature provides a wide variety of plants that contain medicinal properties. The powerful ingredients found in the stems, leaves, roots, flowers, and seeds of certain plants have natural healing properties that have been found to cure various ailments. Herbal medicinal plants (also called phyto medicines) can be administered as the whole plant or plant parts or by extracting one or more ingredients with solvents to yield tinctures, tea or other extracts. Herbal medicines are the outcome of therapeutic experiences of generations of practicing physicians of indigenous systems of medicine for over hundreds of years. Synthetic or conventional drugs are synthesized chemically in the laboratory. Herbal medicine is a triumph of popular therapeutic diversity. The utilization of herbal drugs is on the flow (Sharma *et al.,* 2008). According to the World Health Organization (WHO), the use of herbal drugs throughout the world has increased tremendously (Pal and Shukla, 2003).

Many medicinal plants have been used since centuries for treatment of many ailments and affections. Among these Rhubarb (*Rheum emodi*) has been cultivated for over 5000 years for its medicinal purposes, originating in the mountains of the North-western provinces of China and Tibet. *Rheum emodi* Wall Ex Meissn, locally known as "Pambchallan" (Kashmir) is a leafy perennial herb distributed in altitudes ranging from 2800 to 3800 m in the temperate and subtropical regions of Himalayas from Kashmir to Sikkim in India (Nautiyal *et al*., 2003). Rhubarb (*Rheum emodi*), family Polygonaceae, has been traditionally used as diuretic, liver stimulant, purgative/ cathartic, stomachic, anticholesterolaemic, antitumor,

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antiseptic and tonic. A number of anthraquinone derivatives including emodin, aloe-emodin, physcion, chrysophanol, rhein, emodin glycoside and chrysophanol glycoside occur as the main chemical constituents. Previously rat model was being used for xenogenic Mesenchymal stem cell for full thickness wound healing (Kumar *et al*., 2014). With this background, the present study was designed with the objective to evaluate potential effect of different extracts of *Rheum emodi* for full thickness wound healing in rabbit model.

MATERIALS AND METHODS

This study was permitted by Institute Animal Ethics Committee (IAEC), Sher-e-Kashmir University of Agricultural Sciences and Technology, Faculty of Veterinary Sciences and animal Husbandry, Jammu and Kashmir, India. Twenty four clinically healthy rabbits of either sex, 9-15 months age with their body weight ranging between 2-3 kg were used for the study. Each animal was and provided for free access to water and a standard diet. The animals were acclimatized to approaching, handling and animal house conditions for a period of 10-15 days prior to the study. They were randomly divided into 4 equal groups viz. group of 6 animals each (Table 1). All the animals were reared under identical managemental conditions. The animals were kept off-feed for 6 hours and off-water for 2 hours. The animals were weighed and subjected to thorough physical and clinical examination before wounding.

Surgical procedure

The animals were placed in sternal position on the operative table. Each animal was anaesthetized using xylazine hydrochloride dosed at 10 mg/kg intramuscularly, left in calm environment for 5 minutes and then administered ketamine hydrochloride dosed at 50 mg/kg intramuscularly. The dorsal thoraco-lumbar portion was shaved, cleaned and prepared for aseptic surgical procedures. Locally fabricated metal marker (Figure 1) was used to demarcate the wound margin. Two full thickness square excisional cutaneous wounds of 1.5 x 1.5cm size, 2.5cm apart from one another were created on either side of dorsal spine in the thoraco-lumbar region (Figure 2). The animals in each group were treated as mentioned in table 1.

Treatment of wounds

All wounds were flushed with normal saline solution followed by treatment with medicaments, as shown below (table 1).

Wound Size

For calculating wound size, sterile cellophane paper was employed. It was placed on the wound and its edges were marked on the paper using a permanent marker. Subsequently following day 0, the margin at the leading edges of the advancing epithelium in excision wound were traced on a cellophane paper on day 3^{rd} day 7^{th} and then on weekly basis, till the wounds got healed. The wound area was then measured by retracing the wound on a centimeter scale graph paper (Sadaf *et al.,* 2006).

Percentage healing

The evaluated surface area was used to calculate the percentage healing using the below formula (Sadaf *et al.,* 2006). Percentage healing was calculated on the day of creation and subsequently on day 3^{rd}, 7^{th}, 14^{th} and 21^{st}. Percentage healing was determined by the following formula:

$$H = \frac{A - B}{A} \times 100$$

Where,
H = Percentage healing.
A = Area of wound at the beginning of the experiment.
B = Area of wound at the end of particular period.

Statistical analysis

The data was analyzed using the suitable statistical program for Social analysis (SPSS) for windows (Snecador and Cochran, 1989). One way ANOVA (analysis of Variance) and multiple range tests were used to compare the means at different time intervals among different group. A value of $P<0.05$ was considered significant.

RESULTS

Wound size

The Mean±SE values of wound size (cm^2) in animals of different groups at different observation intervals are depicted in Table 2 and Figure 3. Post wounding a significant ($P<0.05$) decreasing trend in the area of wound size was noted in all the groups on all observation intervals. The complete closure of cutaneous excisional wounds was noted by day 14 in the animal of groups C and D, and by day 21 in the animals of group B, respectively. Comparison among the groups revealed significant ($P<0.05$) decrease in the wound size in the animals of treated groups (B, C and D) from those of control group (A) on all corresponding observation intervals. Furthermore the wound size value was significantly ($P<0.05$) lower in the animal of all treated groups as compared to control group on all corresponding interval of wound size.

Percentage Healing

The Mean±S.E values of percentage healing (%) in animals of different groups at different observation intervals are depicted in Table 3. Post wounding the percentage healing increased significantly ($P<0.05$) in all groups, at all observation interval with the result per cent healing was achieved in the animals of group C by the end of the observation period. The healing was still incomplete in the animals of group A and B by the end of the observation period. Comparison among the group showed significant ($P<0.05$) increase in percentage healing of all treated wounds as compared to control wounds. Among treated groups percent wound healing was significantly ($P<0.05$) faster in the animals of group C as compared to the animal of groups A and B.

DISCUSSION

The extracts of rhubarb contain alkaloids, flavanoid, proteins, amino acids (Aslam *et al.,* 2012), terpenes (Wani *et al.,* 2012). Tannins are capable of precipitating proteins,

Table 1: Treatment protocol in groups

Group	No. of animals (No. of wounds)	Treatment
A	6 (24)	Flushing of wound with normal saline solution + povidone iodine ointment
B	6 (24)	Flushing of wound with normal saline solution + 10% ethonolic extract ointment (*Rheum emodi*).
C	6 (24)	Flushing of wound with normal saline solution + 10% aqueous extract ointment (*Rheum emodi).*
D	6 (24)	Flushing of wound with normal saline solution + 10% petroleum ether extract ointment (*Rheum emodi).*

The medicines were applied daily for 12 days and then on alternate days till complete healing of wound

Table 2: Mean±SE of wound size (cm^2) in the animals of different groups at different observation intervals

Group No.	Observation Intervals (Days)				
	0	3	7	14	21
A	2.41 ± 0.05^{aA}	2.04 ± 0.07^{aB}	1.50 ± 0.08^{aC}	1.06 ± 0.08^{aD}	0.53 ± 0.06^{aE}
B	2.51 ± 0.08^{aA}	1.54 ± 0.04^{bcB}	1.04 ± 0.06^{bC}	0.41 ± 0.07^{cD}	0.00 ± 0.00^{cE}
C	2.43 ± 0.11^{aA}	1.43 ± 0.10^{cB}	0.72 ± 0.08^{cC}	0.00 ± 0.00^{dD}	0.00 ± 0.00^{cD}
D	2.47 ± 0.10^{aA}	1.30 ± 0.07^{cB}	0.72 ± 0.02^{cC}	0.00 ± 0.00^{dD}	0.00 ± 0.00^{cD}

Figures with different superscript (small letters) differ significantly between groups; Figures with different superscript (capital letters) differ significantly between days within the groups; n = 6 animals in each group

Table 3: Mean±SE of percentage healing (%) in the animals of different groups at different observation intervals

Group No.	Observation Intervals (Days)				
	0	3	7	14	21
A	0.00 ± 0.00^{aA}	15.53 ± 2.01^{aB}	37.71 ± 2.27^{aC}	55.73 ± 3.25^{aD}	77.73 ± 2.47^{aE}
B	0.00 ± 0.00^{aA}	38.21 ± 2.63^{bcB}	58.38 ± 2.54^{bC}	83.69 ± 2.46^{cD}	100.00 ± 0.00^{cE}
C	0.00 ± 0.00^{aA}	39.39 ± 6.88^{bcB}	70.35 ± 3.10^{cC}	100.00 ± 0.00^{dD}	100.00 ± 0.00^{cD}
D	0.00 ± 0.00^{aA}	47.19 ± 1.84^{cB}	70.77 ± 1.20^{cC}	100.00 ± 0.00^{dD}	100.00 ± 0.00^{cD}

Figures with different superscript (small letters) differ significantly between groups; Figures with different superscript (capital letters) differ significantly between days within the groups; n = 6 animals in each group

Fig. 1: Metal Marker.

Fig. 2: Nomenclature of the wounds induced on right and left side

resulting in shrinkage of cells. This precipitating protein forms a coagulum. Underneath the coagulum quicker regeneration of tissue takes place (Zama *et al.,* 1991). Anthraquinones are the most important constituents of rhubarb which consist of rhein, aloe emodin, emodin, physion, chrysophanol, chrysophanin (Malik *et al.,* 2010). The emodin is known to possess wound healing effect, as emodin obtained from rheum species has been found the wound healing activity in excisional wound model in rats (Tang *et al.,* 2007). Chrysophanol, topical application, has been found to improve wound contraction and closure, mostly by 4th post wounding day (Sheeba *et al.,* 2009). Flavenoids, besides having astringent and anti-microbial improves vascularity thereby enhancing wound contraction and increased rate of epithelization. Among the extract groups Aqueous and Petroleum ether extract groups showed better healing than Ethanolic group. The ethanolic extract has 5 anthraquinones namely chrysophenol, physion, emodin, chrysophenol 8-O-β-O glucopyranoids and emodin 8-O-β-O glycopyrenoids (N goc *et al.,* 2008). Among which chrysophenol has strong wound healing activity. Petroleum ether extract is reported to contain flavenoids, terpenoids, tannins, steroids and anthraquinones (Kumar *et al.,* 2013). Valecene-2, an sesquiterpene, is well known for its anti-inflammatory activity (Khan *et al.,* 2011) and selective cyto toxicity towards cancer cells (Compagnone *et al.,* 2010). Aqueous extract of rhubarb was reported to contain seven anthraquinones namely sennoside B, sennoside A, aloe emodin, rhein, emodin, chrysophenol and physion (Wang *et al.,* 2011). This extract is advantageous in traumatic brain surgery by implicating nerve protective effect and crossing blood brain barrier. The less healing efficacy of ethanolic extact on day 14 as compared to other extract groups could be probably due to deficiency of proteins and amino acids, as proteins and amino acids are needed in later stages (remodeling stages) of wound healing. (Aslam *et al.,* 2012). Kumar *et al.* (2014) reported that medical grade chitosan powder with xenogenic mesenchymal stem cell to accelerated wound healing in comparison to standard dressing material (dermafin) for repair of full thickness skin wounds in rat. In conclusion, the percentage healing at the end of study period was 77.73% in control group and 100% in extract treated groups, which indicates that Rhubarb and its extracts accelerate wound healing process in rabbit model.

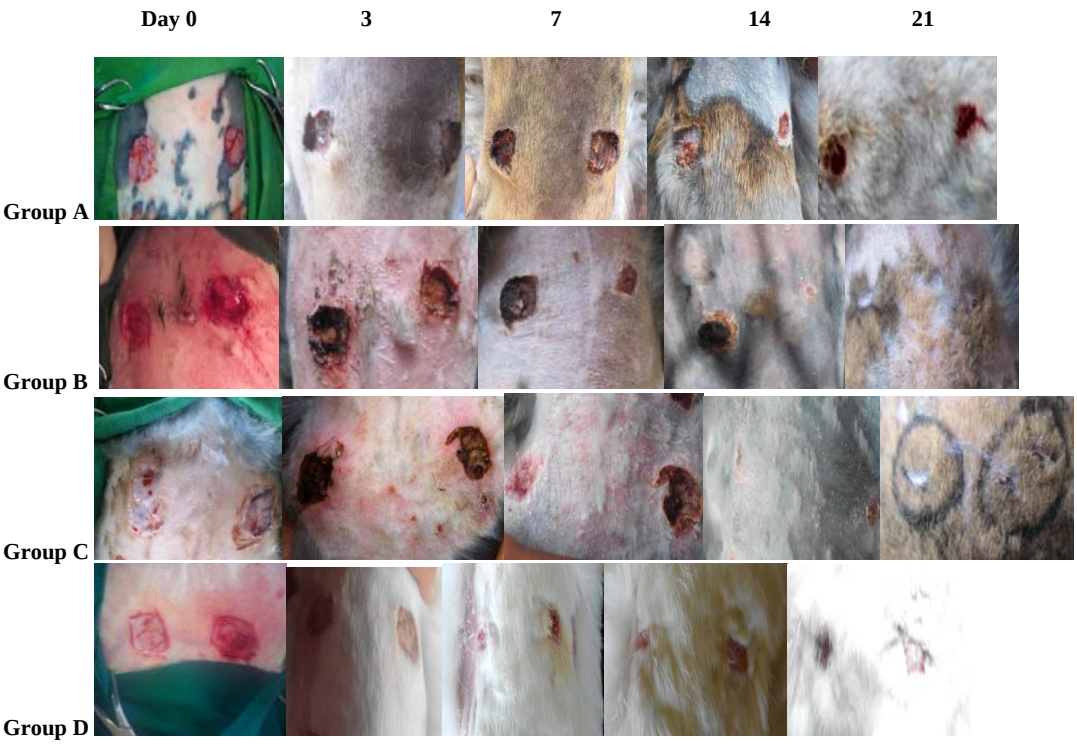

Fig. 3: Gross appearance of wounds in animals of different groups on day 0, 3, 7, 14 and 21.

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