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WOUND HEALING ACTIVITY OF AQUEOUS EXTRACT OF *RADIX PAEONIAE* ROOT

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Abstract: Aqueous extract of the roots of *Radix paeoniae* (Paeonaceae) was screened for wound healing by excision, incision and dead space wound models on Wistar rats. The parameters studied were breaking strength in case of incision wounds, epithelialisation and wound contraction in case of excision wound and granulation tissue dry weight, breaking strength and hydroxyproline content in case of dead space wound. The Nitrofurazone ointment treated group showed a significant ($p < 0.001$) reduction in the wound breaking strength when compared to control group in incision type of wound model. The results obtained indicated that *Radix paeoniae* root extract accelerates the wound healing process by decreasing the surface area of the wound and increasing the tensile strength. The histological examination of the granulation tissue of treated group showed increased cross-linking of collagen fibers and absence of monocytes.

Keywords: *Radix paeoniae,* excision wound, incision wound, dead space wound

Wound healing is the process of repair that follows injury to the skin and other soft tissues. Following injury, an inflammatory response occurs and the cells below the dermis begin to increase collagen production. Later, the epithelial tissue is regenerated (1). *Radix paeoniae* is the dried root of *Paeonia lactiflora* Pallas (Paeonaceae), synonyms: *Paeonia albiflora* Pallas., *P. edulis* Salisb., *P. officinalis* Thunb. (2). It is reported for various activities including antipyretic, hepatoprotective, antifertility, antispasmodic, antiinflammatory and antifibrinolytic (3). Traditionally, *Radix paeoniae* is used for the healing of wounds. So far no scientific evidence was found during literature survey for that activity. So, the present study was focused on wound healing activity of *Radix paeoniae* root aqueous extract to justify its traditional use. Wound healing activity of the root was evaluated in different wound models using Wistar rats.

MATERIALS AND METHODS

Animals

Wistar albino rats and mice of either sex were used for the study of the crude extracts. Institution Animal Ethics Committee has approved the project (Registration No. 1227/AC/08/CPCSEA)**.** The animals were kept at 27 ± 2°C, relative humidity 44–56% and light and dark cycles of 10 and 14 h, respectively, for 1 week before and during the experiments. Animals were provided with standard diet (Lipton, India) and water *ad libitum* and the food was withdrawn 18–24 h before the start of the experiment. All the experiments were performed in the morning according to current guidelines for the care of the laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals.

Plant resources and preparation of crude drug extract

The roots of *Radix paeoniae (RP)* were collected from local market of Indore district of Madhya Pradesh (MP) state, India and identified at the Agriculture College, Indore (M.P.). The herbarium specimen has been submitted to Pharmacognosy department of the college (Voucher specimen no. 001 / R). Roots were shade dried, coarsely powdered and extracts were prepared by maceration method. The extract was filtered and vaccum dried.

Phytochemical studies

Extract was subjected for phytochemical study (4).

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Acute toxicity study (ALD_{50})

The acute toxicity study for aqueous extract of *RP* roots was performed using albino mice. The animals were fasted overnight prior to the experiment and maintained under standard conditions. The extract was administrated orally in increasing doses and found safe up to a dose of 2000 mg/kg. The fixed dose (OECD Guideline no. 420) method of CPCSEA was adopted for toxicity studies.

Wound healing activity

Adults Wistar albino rats of either sex weighing 180-200 g were used for the study. The effect of the extract was evaluated on excision, incision and dead space granuloma wound models in rats. Nitrofurazone ointment (0.2% w/w) was used as a standard drug for comparing the wound healing potential of the extract in different animal model. The wound-healing activity was assessed by the rate of period of epithelialisation and skin-breaking strength. Histological study of the granulation tissue was carried out to know the extent of collagen formation in the wound tissue.

Excision wound model

The wound site was prepared following the excision wound model. Three groups of five animals each were used. The rats were anesthetized prior to and during infliction of the experimental wounds. The surgical interventions were carried out under sterile conditions using diethyl ether. Wound of 500 sq. mm on dorsal thoracic region was made. Animals were closely observed for any infection and those which showed signs of infection were separated and excluded from the study and replaced. The animals were observed for wound closure at 0, 5, 10 and 15th day and for period of epithelialisation (5).

Incision wound model

Albino rats (150-200 g) were taken for studies and three groups of five animals each were used. The rats were anesthetized prior to and during creation of the wounds, with diethyl ether. The dorsal fur of the animals was shaved with an electric clipper. A longitudinal paravertebral incision of 6 cm long was made through the skin and cutaneous tissue on the back (6). After the incision, the parted skin was sutured 1 cm apart using a surgical thread and curved needle. The wounds were left undressed (7). The extract was topically applied to the wound once a day. The sutures were removed on 10th post wound day and the application of the extract was continued. The skin-breaking strength was measured by the method of Lee (8) on the 15th day evening after the last application.

Dead space wound model

Dead space wounds were created under light ether anesthesia, by subcutaneous implantation of sterilized cylindrical glass piths (2.5×0.3 cm) in the region of groin on both sides. The granulation tissues formed around the grass pith were harvested on the tenth day post wounding and subjected to breaking strength and histopathological study (9, 10).

Histopathological study

The healing tissues obtained on the 15th day from all three groups of animals of the incision wound model were processed for histological study. The amount of collagen was quantified using Vangeison stain.

Statistical analysis

The data are expressed as the mean ± S.E.M. The difference among means has been analyzed by one-way ANOVA. A value of $p < 0.05$ was considered as statistically significant.

RESULTS

Preliminary phytochemical screening

Phytochemical investigations of root extract showed the presence of glycosides, flavonoids, tannins, resins and terpenoids.

Acute toxicity study

Before the study of wound healing activity, preliminary toxicity studies of the tested extracts were carried out. The tested extracts did not cause any mortality when administered up to a dose of 2000 mg/kg body weight orally.

Excision wound model

Percentage closure of original wound area was calculated at different time intervals. The measurement on 5th and 10th day showed that the percentage closure of the original excision wound area was found to be 41.85 and 63.77 (standard ointment treated group), 38.27 and 61.38 (*RP* roots aqueous extract). The tested extracts significantly ($p < 0.001$) promoted wound closure compared to controls. On 15th day the extent of percentage wound closure was 98.21 (standard ointment treated group), 97.69 (*RP* roots aqueous extract) (Fig. 1, Table 1).

Incision wound model

The mean tensile strength in the control group was 277.86 ± 03.19 g whereas in standard ointment

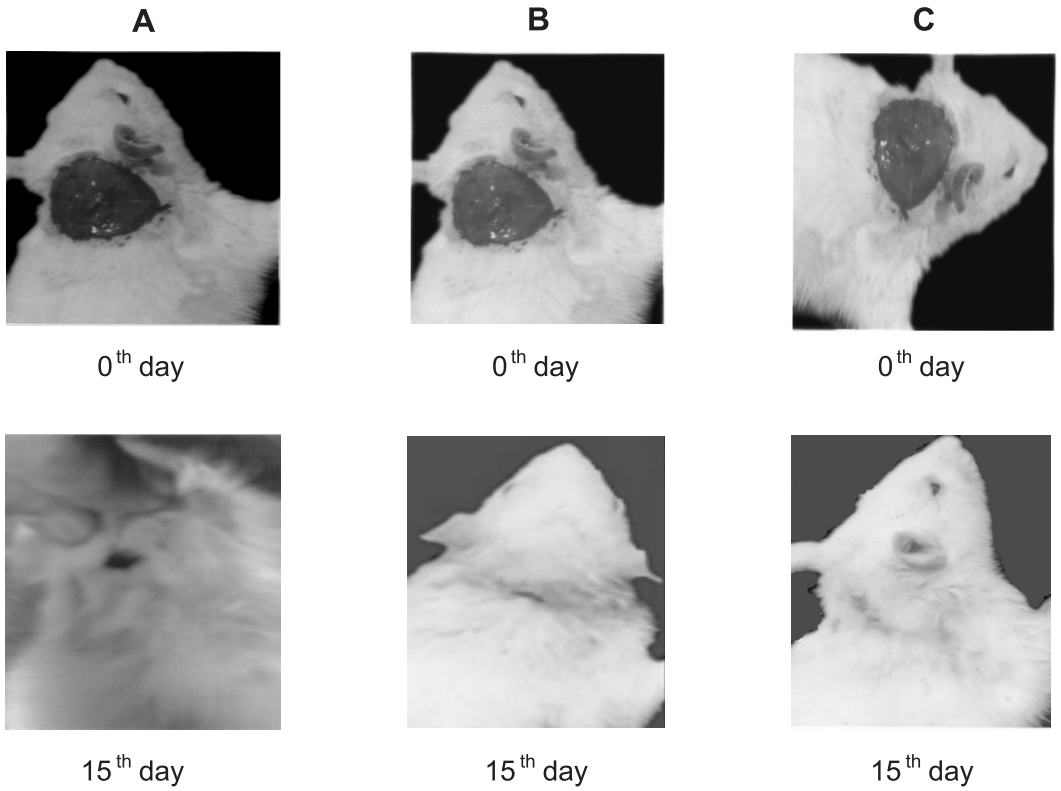

Figure 1. Comparison of wound site by excision wound model in control (A), standard ointment treated group (B) and extract treated group (C).

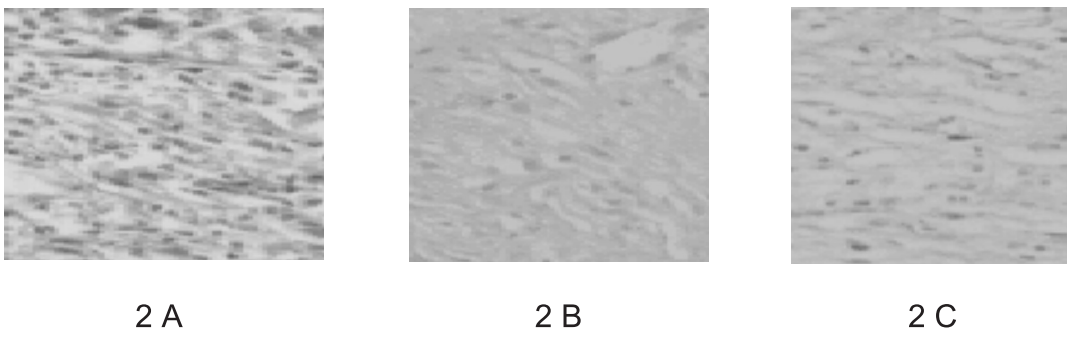

Figure 2. Histopathological characteristics of healed tissue treatment with Control, Standard and Test groups. 2 A: Granulation tissue of control group animal showing with less collagen and more macrophages (Vangeison stain). 2 B**:** Granulation tissue of standard ointment treated group animal showing moderate deposition collagen (Vangeison stain). 2 C: Granulation tissue of extract treated group animal showing more collagen and less macrophages (Vangeison stain).

treated group and in extract treated group it was 497.13 ± 06.06 and 420.33 ± 5.92, respectively (Table 2). These observations of incision wound model confirms prohealing effect of standard ointment treated group and in extract treated group as observed in excision wound model.

Dead space wound model

The mean breaking strength of granulation tissue in the control group was 311.86 ± 03.13 g. A marked increase in breaking strength was observed (399.33 ± 5.41 g) in the extract treated group when compared to the control group. The breaking

Table 1. Effect of the extract on healing of excision wound model

Group	Wound area (mm^2) Post wounding days				Period of epithelialisation
	0th	**5th**	**10th**	**15th**	
Control	502.6 ± 5.37 (0.0)	412.83 ± 15.05 (17.86)	284.66 ± 10.82 (43.36)	181.83 ± 9.803 (63.82)	25.26 ± 0.40
Standard	506.5 ± 5.51 (0.0)	294.5 ± 17.30*** (41.85)	183.46 ± 6.02*** (63.77)	09.03 ± 0.82*** (98.21)	16.03 ± 0.39
Extract Treated	503.3 ± 4.6 (0.0)	310.66 ± 6.86*** (38.27)	194.33 ± 7.75*** (61.38)	11.60 ± 4.41*** (97.69)	18.17 ± 0.54

Values are the mean ± SEM, one way ANOVA, n = 6 *** $p < 0.001$ vs control; in parenthesis values showing percentage closure of original excision wound area.

strength in the standard ointment treated group was 447.13 ± 06.06 g. The mean dry weight of granulation tissue in control group was 43.31 ± 01.24 mg which significantly ($p < 0.05$) increased to 73.45 ± 01.19 mg and 62.51 ± 02.32 mg in groups treated with the standard ointment and *R. paeoniae* root extract (Table 3). An increase in dry weight in the standard ointment treated group could be due to fibroblasts and other inflammatory cells.

Histopathological study

Histological studies of the tissue obtained from the extract treated group (Fig-2 C) showed significant increase in collagen deposition, few macrophages, tissue edema and more fibroblasts. It was more or less equal to the animals treated with 0.2% w/w nitrofurazone (Fig-2 B).

Table 2. Effect of the extract on tensile strength of incision wound model

Group	Tensile strength (g)
Control	277.86 ± 03.19
Standard Ointment Treated	497.13 ± 06.06***
Extract Treated	420.33 ± 5.92***

Values are the mean ± SEM, one way ANOVA, n = 6; *** $p < 0.001$ *vs*. control.

DISCUSSION

The repair of wounds involves different phases including contraction, formation of epithelialisation and fibrosis (11). The biological response regulating the body's own cellular defense mechanisms contributes to the wound and its repair. The use of single model is inadequate and no reference standard exists that can collectively represent the various phases of wound healing. Hence, three different models have been chosen in our study to assess the effect of *RP* on wound healing. Topical application of root aqueous extract at wound site in excision wound healing model produced significant ($p < 0.001$) wound healing activity. Treated excision wounds showed an increased rate of wound contraction, leading to faster healing as confirmed by the increased healed area when compared to the control group. Tensile strength was measured to confirm the wound healing activity claimed for this plant. An increase in tensile strength of treated wounds may be due to an increase in collagen concentration and stabilization of the fibers facilitating wound healing. In our study, dead space wound model *RP* root

Table 3. Effect of the extract on dead space wound model

Group	Granulation dry weight (mg/100g)	Breaking strength (g)
Control	43.31 ± 01.24	311.86 ± 03.13
Standard Ointment Treated	73.45 ± 01.19***	447.13 ± 06.06***
Extract Treated	62.51 ± 02.32***	399.33 ± 5.41***

Values are the the mean ± SEM, one way ANOVA, n = 6; *** $p < 0.001$ *vs*. control.

extract treated group showed significant increase in breaking strength, hydroxyproline concentration and dry weight of the granulation tissue. The wound breaking strength is determined by the rate of collagen synthesis and more so, by the maturation process where there is covalent binding of collagen fibrils through inter and intra molecular cross linking. The *RP* root extract treated group enhanced wound contraction, it would have either enhanced contractile property of myofibroblasts or increased number of myofibroblasts recruited into the wound area. The *RP* root extract was also promoting epithelialisation either by facilitating the proliferation of epithelial cells or by increasing the viability of epithelial cells.

In conclusion, the observations and results obtained in this study indicated that the root extract of *Radix paeoniae* significantly stimulated wound contraction. The breaking strength of the treated excision wounds increased in the treated groups compared with the control group. It showed remarkable wound healing activity and it may be suggested 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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