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## Effect of *Rheum emodi* and its extracts on wound healing: A qualitative and quantitative histological study

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**Abstract**

In the present investigation, the efficacy of *Rheum emodi* and its different extracts in the healing of full-thickness skin wounds of rabbits was evaluated with a focus on qualitative and quantitative histopathological changes. 30 clinically healthy rabbits of either sex, randomly divided into 5 equal groups, of 6 animals each. In group 1, povidone iodine ointment was used, which served as a control. The animals of group 2 were treated with crude dust powder of *R. emodi*, group 3 with 10% ethanolic extract ointment, group 3 with 10% aqueous extract ointment, group 4 with 10% petroleum ether extract ointment of *R. emodi*. General anesthesia was induced using xylazine and Ketamine Hydrochloride. Two full thickness cutaneous wounds (1.5 x 1.5cm) were created on either side of dorsal spine in the thoraco-lumbar region. Present work suggested that *Rheum emodi* and its extracts accelerate the inflammatory reaction and initiate early phase of healing. Extract treated groups showed better and faster healing than dust treated and control group.

**Keywords:** *Rheum emodi*, wound, excisional wounds, wound healing, rabbits, histology

**1. Introduction**

Skin-wound healing starts immediately after injury and consists of three phases: inflammation, proliferation, and maturation. These phases proceed with complicated but well-organized interaction between various tissues and cells<sup>[1, 2]</sup>.

Healing can be accelerated using wound healing agents which accelerate early completion of inflammation phase and early initiation of proliferation phase. Research on wound healing agents is one of the developing areas in modern biomedical sciences. Many medicinal plants have been used since centuries for treatment of many ailments and affections esp, wound healing. Herbal drugs being much less expensive than their synthetic counterparts, also have better cultural acceptability, better compatibility with the human body and minimal side effects<sup>[3, 4]</sup>. *R. emodi* is one such herbal plant. Rhubarb (*Rheum emodi*), family Polygonaceae, has been traditionally used as diuretic, liver stimulant, purgative/cathartic, stomachic, anticholesterolaemic, antitumor, antiseptic and tonic. The healing efficacy of *Rheum* sp is known from long<sup>[5]</sup>. Keeping this as a prospective, randomized study was designed to observe the effects of *R. emodi* and its different extracts on histopathological findings during wound healing.

**2. Materials and Methods****2.1 Selection and preparation of animals:**

Thirty (30) clinically healthy rabbits of either sex, 9-15 months age with their body weight ranging between 2-3 kg were used for the study. The rabbits were randomly divided into 5 equal groups, of 6 animals each. All the animals were reared under identical managemental conditions. The dorsal thoraco-lumbar portion was shaved, cleaned and prepared for aseptic surgical procedures.

**2.2 Anesthesia**

General anesthesia was induced in animals for creation of wounds. Each animal was given xylazine @ 10 mg/kg I/M, left in calm environment for 5 minutes and then administered Ketamine Hydrochloride @ 50 mg/kg I/M.

### 2.3 Experimental Design

Full-thickness cutaneous wounds were created (1.5× 1.5 cm) on dorsal spine in the thoraco-lumbar region, one on the right side (R) and two on the left side (L1 & L2) with 2.5 cm distance in between. The wounds were named cranio-caudally

Group	No. of animals (No. of wounds)	Treatment
Group 1	6 (12)	Flushing of wound with Normal saline solution + Povidone Iodine Ointment.
Group 2	6 (12)	Flushing of wound with Normal saline solution + Dusting the wound with <i>Rheum emodi</i> powder.
Group 3	6 (12)	Flushing of wound with Normal saline solution + 10% Ethanolic Extract Ointment ( <i>Rheum emodi</i> ).
Group 4	6 (12)	Flushing of wound with Normal saline solution + 10% Aqueous Extract Ointment ( <i>Rheum emodi</i> ).
Group 5	6 (12)	Flushing of wound with Normal saline solution + 10% Petroleum Ether Extract Ointment ( <i>Rheum emodi</i> ).

The medicines were applied daily for 12 days and then on alternate days till complete healing of wound. The biopsy samples were collected on 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and on 28<sup>th</sup> day after wounding and preserved in 10 % formalin solution followed by routine procedure for histopathological examination. Paraffin embedding technique was used and 5-6 $\mu$  thick sections were cut and stained using H & E stain [6] and Mason's Trichrome stain.

The Abramov's histological scoring system (modified Greenhalgh's scoring system) was used for scoring epithelialization, fibrosis, angiogenesis, and collagen level; the number of macrophages under this system was modified<sup>7</sup>. The Abramov's system assessed each parameter independently and gave a score of 0-3.

- The collagen level was graded as: 0 (none), 1 (scant), 2 (moderate), and 3 (abundant).
- Epithelialization was graded as either: 0 (none), 1 (partial), 2 (complete, but immature or thin), and 3 (complete and mature).
- Angiogenesis was graded as either: 0 (none), 1 (up to 5 vessels per high power field (HPF)), 2 (6–10 vessels per HPF) and 3 (more than 10 vessels per HPF).
- Fibrosis was graded as: 0 (none to minimal fibroblasts), 1 (few fibroblasts), 2 (more fibroblasts), 3 (predominantly fibroblasts).
- The number of macrophages was scored as 0–25 = 1, 26–50 = 2 and > 51 = 3

### 3. Results

At day 7 in control group as well as dust group (Plate 1) scant collagen deposition with few and moderate fibroblastic proliferation was evident respectively. There was no dehiscence, infection, or exudate seen on the surface of wounds in the treated groups. Among extract treated groups, ethanolic extract treated group revealed more fibroblastic proliferation with scant collagen deposition. Aqueous extract group, showed abundant collagen associated with fibroblastic proliferation and infiltration of admixture of mono nuclear and poly morpho-nuclear cells (Plate 2 & Plate 3) whereas, petroleum ether extract group revealed moderate collagen deposition associated with more fibroblastic proliferation (Plate 4). Angiogenesis was evident in all extract treated groups. On day 14, in control group moderate to abundant collagen deposition with predominant macrophages were seen. However, heterophils were also observed (Plate 5). In dust treated group histological picture simulated as of day 7 (Plate 6). Among extract treated groups in ethanolic extract group predominant amount of collagen deposition with fibroblastic proliferation varying from moderate to predominant was found. In aqueous extract group collagen was more with moderate fibroblastic proliferation whereas, petroleum ether extract group showed moderate collagen

as R-1 and L-1 on right and left side of vertebral column respectively. L-1 was used for histopathological evaluation.

All wounds were flushed with normal saline solution followed by treatment with medicaments, as shown below:

deposition with more fibroblastic proliferation (Plate 7 & Plate 8). At day 21, partial epithelialization was noted in both control (Plate 9) as well as dust treated group (Plate 10 & 11). Among extract treated groups, in ethanolic extract treated group partial to complete but immature epithelialization was noted (Plate 12). In aqueous extract treated group moderate collagen with few fibroblastic proliferation, marked epithelialization which however appeared immature (Plate 13 & 14) was observed, whereas in petroleum ether treated group collagen was more compact with more fibroblastic proliferation. Vascularization being prominent (Plate 15) with partial to complete epithelialization (Plate 16). Angiogenesis was noted to be better in ethanolic extract treated group. This result was subsequently quantified using Abramov's histological scoring system (Table 1- Table 5).

### 4. Discussion

Macrophages play an important role in augmenting the inflammatory response and tissue debridement [8]. In present study the macrophages showed a decreasing trend in all the groups towards the end of study period. However in control and crude dust treated groups the macrophage number remained higher on day 14 and 21 although comparatively lower in crude dust treated group. Present study suggests that rhubarb treated groups showed better healing than control. This difference may be due to the anti-inflammatory effect of Rhubarb [9]. Extract treated groups showed better healing than the crude dust treated and control group. This may be attributed to the fact that the anti-inflammatory effect of rhubarb is obtained higher in extract groups than in crude dust form [10, 11].

Angiogenesis, fibroplasia and epithelialization mark the proliferative and maturation phase of wound healing. In present study the amount of fibroplasia and angiogenesis remained higher in extract treated groups followed by crude dust treated and control group on day 7 and 14 post wounding. Also Nisbet *et al.* (2010) gave similar findings reporting that treated groups showed higher amount of fibroplasias and angiogenesis earlier as compared to control group [12]. The last stage of wound healing involves the gradual involution and regeneration of dermis. Collagen content is one of the necessary parameter for determining the pharmacological effects of potential wound healing agents. In present study the collagen content of extract treated groups remained highest throughout the study period followed by crude dust treated groups and then by control group. Epithelialization showed an increasing trend towards the end of study period, this trend being highest in extract treated groups then in crude dust treated groups with control group showing the least amount of epithelialization. These findings corroborate with that of Aakhoon *et al.* (2001), who reported mature collagen content in Rhubarb treated groups on day 15 post wounding. Also

Aakhoon *et al.* (2001) reported epithelium covering the wound surface at the end of the study period.

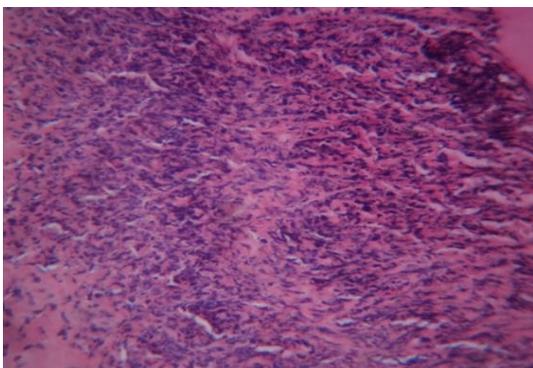
### 5. Conclusion

The quantitative as well as qualitative results suggest that the full-thickness wounds treated with *R. emodi* and its different extracts showed increased epithelialization, angiogenesis, and fibroplasia in the early phases of healing, the highest being in all treated groups on day 7. Extract treated groups showed better healing than control and dust treated group. At the end of study period angiogenesis was noted better in ethanolic extract treated group than aqueous extract and petroleum ether extract treated group.

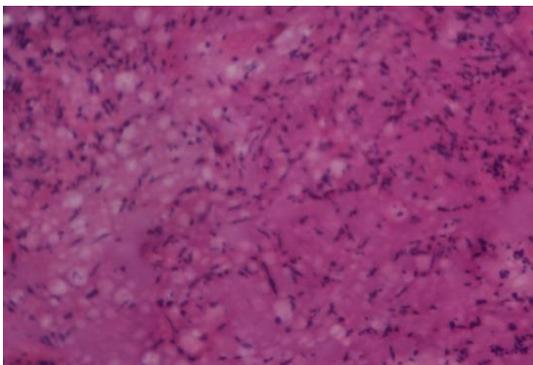
### 6. Acknowledgment

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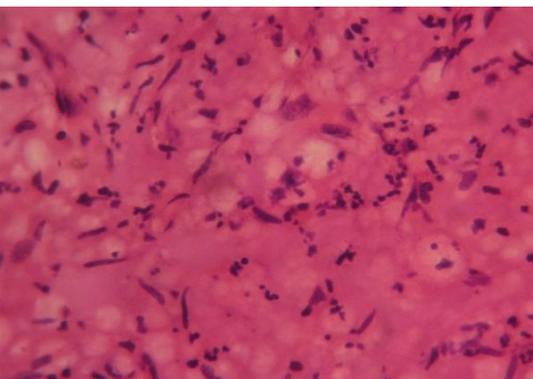
### 7. Images



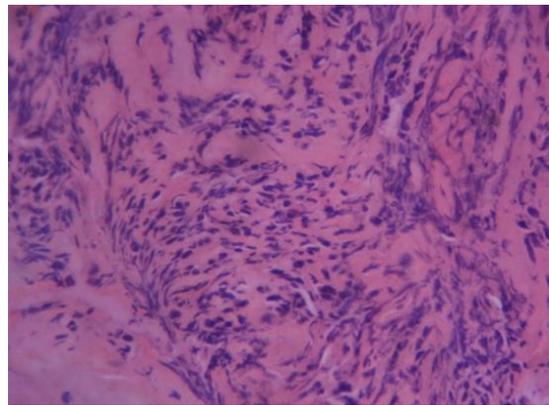
**Plate 1:** On day 7, control group showing scant collagen deposition with few and moderate fibroblastic proliferation (H&E x 100)



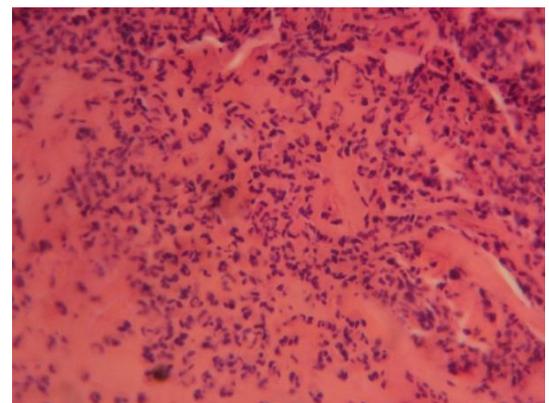
**Plate 2:** On day 7, aqueous group, showing abundant collagen associated with fibroblastic proliferation (H&E x 100)



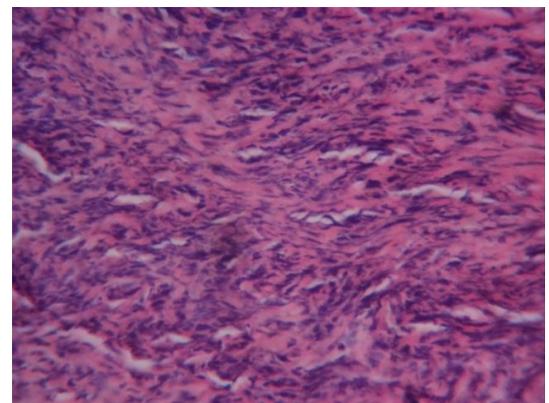
**Plate 3:** On day 7, aqueous group showing infiltration of admixture of mono-nuclear and poly morpho-nuclear cells (H&E x 400)



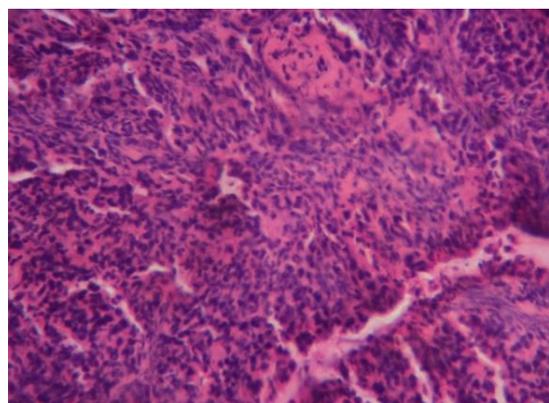
**Plate 4:** On day 7, petroleum ether group revealing moderate collagen deposition associated with more fibroblastic proliferation. Also large number of macrophages were observed (H&E x 400)



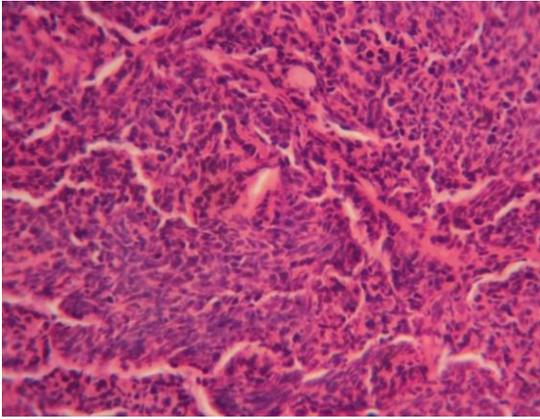
**Plate 5:** On day 14, control group showing heterophils (H&E x 400)



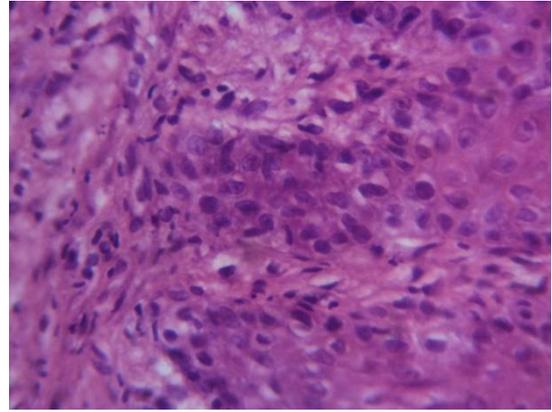
**Plate 6:** On day 14, dust group showing scant collagen deposition with few and moderate fibroblastic proliferation (H&E x 400)



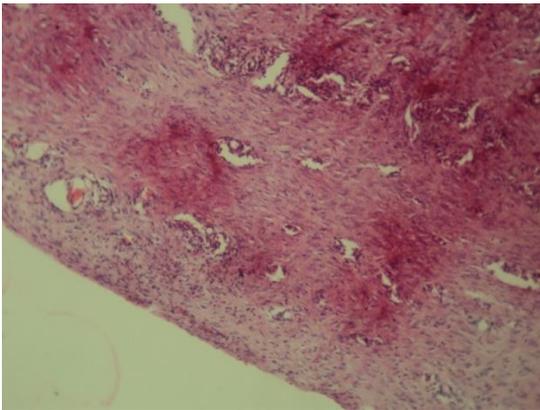
**Plate 7:** On day 14, aqueous group showing more collagen with moderate fibroblastic (H&E x 100)



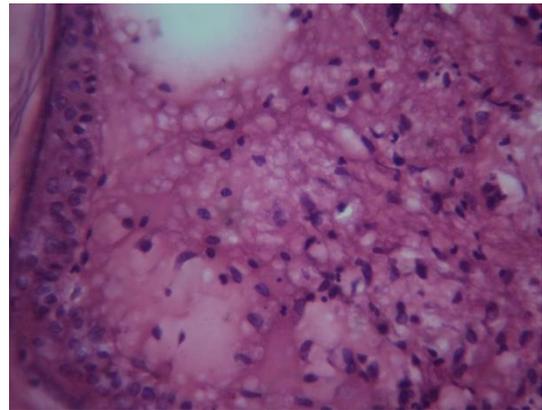
**Plate 8:** On day 14, petroleum ether group showing moderate collagen deposition with more fibroblastic proliferation (H&E x 100)



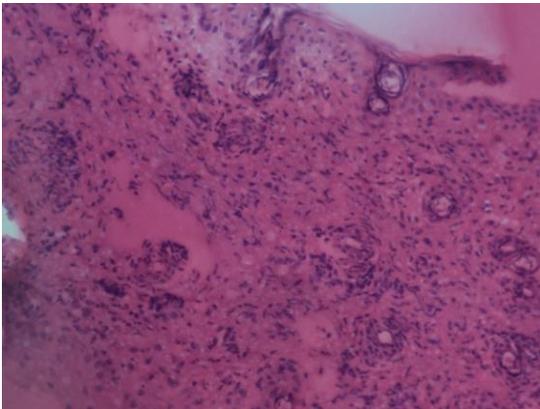
**Plate 12:** On day 21, ethanolic treated groups showing partial to complete but immature epithelialization (H&E x 400)



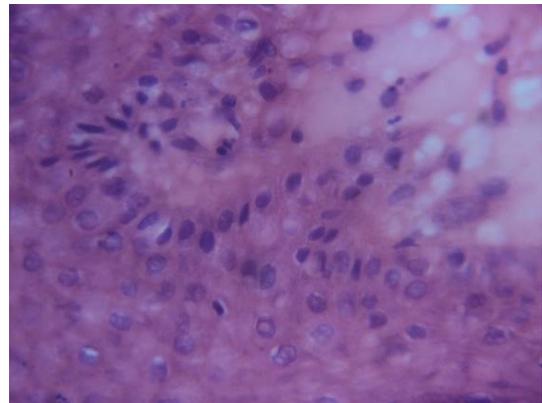
**Plate 9:** On day 21, control group showing partial epithelialization (H&E x 100)



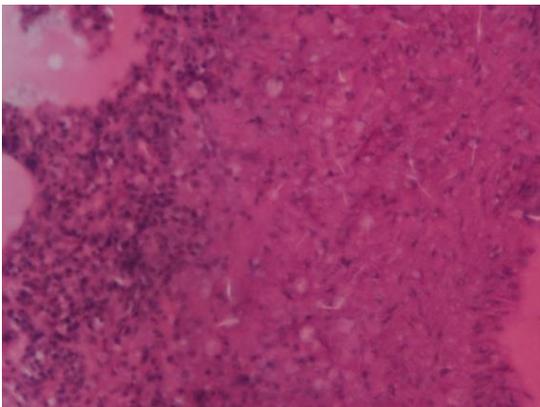
**Plate 13:** On day 21, aqueous treated group showing moderate collagen with few fibroblastic proliferation (H&E x 400)



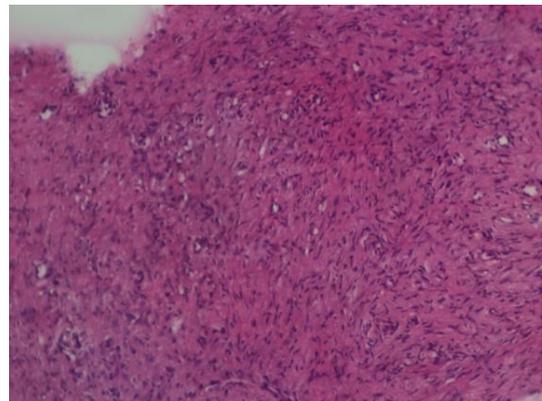
**Plate 10:** On day 21, dust treated group showing moderate collagen deposition (H&E x 100)



**Plate 14:** On day 21, aqueous treated group showing marked epithelialization which however appeared immature (H&E x 400)



**Plate 11:** On day 21, dust treated group showing few fibroblastic proliferation (H&E x 100)



**Plate 15:** On day 21, petroleum ether treated group showing prominent vascularization with partial to complete epithelialization (H&E x 100)



**Table 5:** Percent Score of Epithelialization in different animals on different observation intervals

Group No.	Score No.	Observation Intervals (Days)		
		7	14	21
1	0	66.66		
	1	33.33	16.67	
	2		16.67	33.33
	3		66.66	66.66
2	0	66.66		
	1	33.33		
	2		16.67	16.67
	3		83.33	83.33
3	0	66.66		
	1	16.67		
	2	16.67		
	3		100	100
4	0	66.66		
	1	16.67		
	2	16.67		
	3		100	100
5	0	66.66		
	1	16.67		
	2	16.67		
	3		100	100

**Epithelialization**

0 = None

1 = Partial

2 = Complete, but immature or thin

3 = Complete and mature

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