

RHEUM EMODI (RHUBARB): AN EFFECTIVE WOUND HEALING AGENT IN COW CALVES

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ABSTRACT

Two full thickness skin wounds were created on either side of the median plane of thoraco lumbar area in 10 healthy calves of 9-12 months of age. One wound on either side of the vertebral column i.e. R1 & L1 were washed with normal saline and served as control. Other two wounds, i.e. RII & LII were washed with normal saline and applied with rhubarb powder. Healing was evaluated on the basis of clinical observations, histopathological studies and bacteriological examination. The healing, epithelialisation and wound contraction was significantly faster in wounds treated with rhubarb as compared to control group. Inflammatory reaction on 5th post treatment day was marked in control wounds as compared to treated wounds. Proliferation of fibroblasts and angioblasts on 10th post operative day was moderate in control wounds and moderate to marked in rhubarb treated wounds. Similarly, proliferation of blood capillaries and proliferation and maturation of connective tissue was earlier in wounds treated with rhubarb powder. The healing was more or less complete in rhubarb treated wounds as compared to control ones where it was totally incomplete. The bacterial viable count at different intervals was lesser in rhubarb treated wounds than in control ones. Rhubarb proved effective in creating favourable and desirable conditions for accelerating the wound healing process without any complications.

Keywords: Calf, *Rheum emodi*, wound healing.

INTRODUCTION

Wound healing has got higher priority among body functions as it plays a vital role in life for survival of the animal. The main objective of a surgeon is thus to stimulate healing of a wound without any associated complications like septicaemia and toxemia, which in turn may result in deterioration of health condition of the animal and consequent economic loss (O' Connor, 1982; Singh and Singh, 1993). Sometimes pyemia and infective foci that are inaccessible to treatment may lead to the death of the patient (Frank, 1964). To prevent infection and to accelerate early healing, various antibacterial agents, antiseptics and vulnerary are

being used topically. These anti-infective agents although check the infection and thus promote early healing, yet they also suffer from certain limitations, like tissue damage by means of irritation or allergy (Nagesh *et al.*, 1999) developing resistant strains at the site and thus high cost & non-availability of antibacterials. To avoid these limitations, emphasis is laid on the use of various biological dressing materials for the sake of faster and uncomplicated wound healing viz; Skin graft, fascia, placenta and cartilage powder (Jadon and Kumar, 1984; Mbithi and Barley, 1991; Schumacher *et al.*, 1996 and Saika *et al.*, 1998). Similarly various indigenous medicinal plants and

herbs have also been reported to possess wound healing, anti-inflammatory, anti-bacterial, anti-fungal and analgesic properties and have been used for therapeutic purposes (Rao and George,1949 ; Bhatnagar *et al.*, 1961 ; Shukla,1967 ; Kumar *et al.*, 1996). *Rheum emodi* (Rhubarb) a medicinal herb (Fig. 1) has been reported to possess early ulcer healing property (Chada ,1982) and also possesses anti inflammatory, analgesic and wound healing property (Hakeem, 1929) and antifungal activity (Agarwal *et al.*, 2000). The present study was thus undertaken to assess the efficacy of *Rheum emodi* in the healing of wounds in cow calves.

MATERIAL AND METHODS

The present study was conducted on ten clinically healthy cow calves of either sex aged 9-12 months using rhubarb (*Rheum emodi*) root powder. The roots of *R. emodi* were purchased from the market. Roots were dried properly in shade and ground to fine powder, sieved and kept in plastic bottle for use. The calves were kept under identical managemental conditions. Dorsal median plan of

thoraco-lumbar region on either side of vertebral column was used for creation of wounds after aseptic preparation and infiltration with 2% Xylocaine solution @ 1ml /cm. Four full thickness skin wounds, 2 on either side of the median plan of the thoraco lumbar region of size 3 x 3 cms and 5 cms apart from one another were created on each calf (Fig.2) so that in all 40 wounds were created. Starting from anterior to posterior the wounds were numbered as R I and R II and LI and LII on right and left side of the vertebral column respectively. All wounds were cleaned with normal saline. After cleaning R II and LII wounds were treated with *R emodi* root powder medicament. R 1 and L1 wounds were not medicated and served as control. The medicament was applied daily for 15 days and then on alternate days till the biopsy specimens were taken. The biopsy specimens were collected on 5th, 10th, 15th, 20th and 30th day. These specimens were processed for histopathological studies and for demonstration of collagen and reticular fibre.

The healing of wound was evaluated on the basis of



Fig. 1. *Rheum emodi* herb a wound healing agent

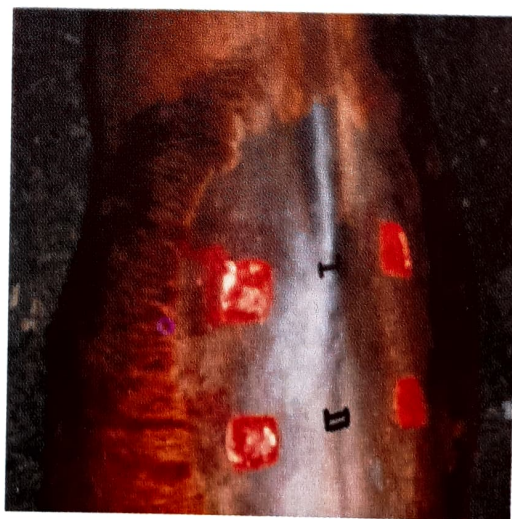


Fig. 2. Surgical wounds created on dorsal paramedian plan of thorac-lumbar region

clinical studies, histopathological and bacteriological observation.

Rectal temperature, respiration rate and heart rate were recorded daily. Physical condition of the wound, degree of inflammation and cicatrisation of the wound was evaluated subjectively. Percent healing was calculated by the method described by Kumar and Tyagi (1972).

The biopsy specimens were collected and preserved in 10% buffered neutral formalin solution for histological studies. The tissues were processed by routine paraffin embedding technique and 5-6 μ thick sections were cut. The sections were stained with Haematoxylin and Eosin method with Harts method (for collagen and elastic fibres) and with Gomori's method (for reticular fibres) as described by Luna (1986) for histopathological studies.

For total bacterial viable count, each wound on the day of creation and then at day 5, 10, 15, 20 and 30 was washed with 5 ml of NSS and the washings were collected in sterile test tubes. One ml of each washing was used for serial dilution in NSS for estimating total bacterial count. One ml of the each dilution was spread with the help of a sterile bent

glass rod on nutrient agar plates in duplicate. Agar plates were then incubated for 24 hours at 37 degree C. Bacterial colonies were counted using colony counter and the total viable count was calculated by multiplying the average number of colonies in the required dilution with the dilution factor.

Statistical analysis of the data was done using analysis of variance test as outlined by Snedecor and Cochran (1967)

RESULTS AND DISCUSSION

The daily rectal temperature, respiration rate and heart rate of test animals ranged from 101.4°F to 103°F, 24-44 breaths/min and 72 - 100 beats/min respectively. These physiological parameters though increased slightly but remained almost in normal range throughout the study period. This indicates that the medicament applied on the wound did not allow the micro-organism to cause systemic effects. Besides, Rhubarb is also reported to possess anti-inflammatory activity (Hakeem and Arshi, 1929).

The summary of wound condition recoded during study period is depicted in (Table 1).

Table 1. Tabulated summary of wound condition during study period

Group	Appearance of contraction On 2 nd day	Peripheral swelling			Wound condition				Healing on 30 th day	
		Marked	Moderate	No swelling	Slight	Granulation tissue formation moderate	Marked with organisation	Peripheral contraction Slight Moderrate		
Control	Dry and reddish	2 nd day	5 th day	-	7 th day	10-15 day	-	10 th day	15th	Incomplete
Rhubarb traeted	Dry and pinkish with haemorrhagic spots	-	-	5 th day	4 th day	6 th day	10 th day	-	12th	More or less complete

The control wounds appeared dry and reddish on day 2nd while as treated wounds appeared dry with some haemorrhagic spots. The periphery of the control wounds was markedly swollen and tender to touch on day 2. The peripheral swelling and tenderness was mild on day 2 in treated wounds. On day 5 after wounding the peripheral swelling and tenderness was moderate in control wounds, and had completely subsided in treated wounds. Peripheral contraction and epithelialisation of edges was slight on 10th day after wounding but on day 15 onwards progressive epithelialisation and peripheral contraction was clearly evident in control wounds. Granulation was slight on day 7 and moderate from day 10-15 in control wounds. In treated wounds granulation was slight on day 4, uniform and moderate on day 6, and marked and well organised on day 10. The peripheral contraction and epithelialisation was clearly evident on day 12 and a thick and firm scab was observed by day 20 in treated wounds. The control wounds did not heal completely even up to 30th day and were covered with thin, pliable scab. The treated wounds healed more or less completely by day 30. Rhubarb is reported to possess anti-inflammatory activity (Hakeem and Arshi, 1929).

The anti-inflammatory property of the dressing material has been reported to contribute towards early wound healing (Bhargava *et al.*, 1988; Zama *et al.*, 1988; Kumar *et al.*, 1993 and Ansari *et al.*, 1997). Rhubarb also contains an astringent compound gallotannins in addition to tannins and gallic acid. Tannins are non nitrogenous plant constituents having an astringent action on tissue cells. Tannins are also capable of precipitating proteins, resulting in shrinkage of cells. This precipitated protein forms a coagulum. Underneath the coagulum quicker regeneration of tissue may take place. (Zama *et al.*, 1988).

Percent healing increased in both the groups, thus substantiating the observations of Bhargava *et al.*, (1988). The healing percentage was significantly ($P < 0.05$) higher in treated group as compared to control group at all the intervals i.e. from day 5 till the end of the study period (Table 2). Tracing of wound boundaries on cellophane paper gave actual data regarding shape and size of wound. Similar observations have also been reported by other workers (Kumar and Tyagi, 1972; Bhargava *et al.*, 1988; Zama *et al.*, 1991 and Tugnaiyat *et al.*, 2000).

Bacterial infection can delay the wound healing

Table 2. Mean + SE values of per cent healing of different treated group

Period (Days)	Treatment Groups	
	Control wounds	Treated wounds
5	7.4226 +0.3199 ^{Aa}	10.2835+0.3823 ^{Ba}
10	29.0719+1.1834 ^{Ab}	38.9213+1.0279 ^{Bb}
15	57.7633+1.5718 ^{Ac}	62.5600+0.9575 ^{Bc}
20	75.5625+1.5789 ^{Ad}	86.5925+1.9553 ^{Bd}
30	96.1900+0.2500 ^{Ac}	98.0525+0.2775 ^{Be}

Capital letter indicate significant difference between groups

Small letter indicate significant difference between days

Values with same superscript do not differ significantly

process (Smith and Enquist, 1967 and Bucknall, 1980). It is as such necessary to keep the bacterial load in a wound at a minimum. At day Zero the bacterial viable count from all the wounds did not show significant difference. The bacterial count progressively increased in control group up to 10th day post wounding where the value was significantly higher than the 3rd day value. After 10th

day though the value decreased significantly up to end of the study period, yet the bacterial viable count was significantly higher than the zero day value. In treatment group the bacterial viable count showed progressive and significant decrease such that all the values were significantly lower than any previous value and also than corresponding value of control group (Table 3).

Table 3. Mean +SE of bacterial viable count of different wound groups (Values were expressed in log)

Inteval	Treatment Groups	
	Control	Treated
0	4.1762+0.00042 ^{Aa} (15010)	4.1789+0.0030 ^{aA} (15100)
5 th	6.0114+0.0002 ^{Ab} (1027000)	3.9220+0.0101 ^{Bb} (8356)
10 th	6.0146+0.0003 ^{Ab} (1034000)	3.8585+0.0098 ^{Bc} (7219)
15 th	5.8012+0.0055 ^{Ac} (632700)	3.6809+0.0272 ^{Bd} (4794)
20 th	5.0884+0.0005 ^{Ad} (122600)	3.4712+0.0294 ^{Be} (2959)
30 th	5.0853+0.0005 ^{Ad} (121700)	3.3959+0.0359 ^{Bf} (2488)

Capital letter indicate significant difference between groups

Small letter indicate significant difference between days

Values with same superscript do not differ significantly

Mineo *et al.* (1988) attributed this antibacterial activity to anthroquinones present in Rhubarb, whereas Lewis and Elvin - Lewis (1977) attributed this activity to Rhein (casic acid) present in Chinese Rhubarb.

Histological studies are important tool to know the process of healing at cellular level. A definite pattern of cellular and molecular events lead to the repair of the injured tissue (McMinn, 1969). On 5th day control wounds showed presence of blood clots and fibrial material with marked infiltration of neutrophils and slight proliferation of spindle shaped fibroblasts from the sides and base of the

clot. On 5th day, treated wounds revealed presence of blood clot, fibrial material, few neutrophils and oedematous fluid (Fig. 3). There was marked proliferation of fibroblasts from the side of clot which were loosely arranged. Angiogenesis was noted. Proliferating blood vessels were perpendicular to the base surrounded by oedematous fluid (Fig.4).

These results indicate that severity of inflammatory changes were slight in treated wounds as compared to control ones as was evident by presence of comparatively low infiltration of inflammatory cells. Ansari *et.al*,

(1977) has reported similar changes in wounds in buffalo calves.

On 10th day, the control wounds showed mild proliferation of fibroblasts which were more loosely arranged. Angiogenesis was also evident. The clot at places had become homogenously pink (Fig.5). Oedema was also evident. On 10th day, treated wounds revealed comparatively lesser fibroblasts but fibres were superseding the cells and were horizontally arranged towards the base. There was marked angioblastic proliferation which were vertically arranged. The fibres are comparatively closely arranged (Fig. 6). From results it was noticed that neovascularisation and formation of mature connective tissue (collagen) was marked in treated wounds as compared to control ones. Besides fibroblasts and angioblasts were well organised as compared to control wounds.

On 15th day in "Rhubarb" treated wounds there was

formation of mature connective tissue (Collagen) along with angiogenesis (Fig 7).The fibroblasts and angioblasts were well organised as compared to control wounds, which slowed progressive proliferation of fibroblasts and a few inflammatory cells. On 20th day, control wound showed less mature connective tissue than the treated wounds. In Rhubarb treated wound the fibres were more densely arranged and the epithelium covering the whole wound surface. On 30th day there was fully grown connective tissue with epithelium covering the wound surface fully in treated group as compared to control group where there was a thin layer of contracted epithelium over the wound surface. These observations are in accordance with those of Bhargava *et al.*, (1988) Kumar *et al.*, (1993) and Ansari *et al.* (1997).

The amount of reticular fibres and collagen fibres are shown in Table -4 .

Table 4. Extent of reticular fibres, fibroblasts and collagen fibres in different treatment groups at different intervals

Interval (Days)	Treatment groups					
	Control wounds			Rheum emodi treated wounds		
	Reticular fibres	Fibroblasts	Collagen fibres	Reticular fibres	Fibroblasts	Collagen fibres
5 th	(++)-(+++)	±	±	+++	+++	(+)-(++)
10 th	++	+	(+)-(++)	++	(++)-(+++)	++
15 th	(+)-(++)	++	++	(+)-(++)	++	+++
20 th	(+)-(++)	(++)-(+++)	++	+	+	+++
30 th	+	++	++	±	±	++++

± = Slight; + = Mild; ++ = Moderate; +++ = Marked; ++++ = Extensive

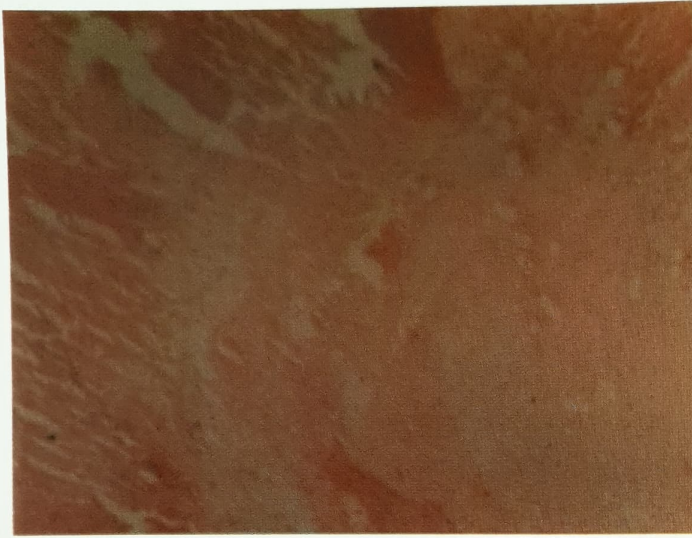


Fig 3. *Rheum emodi* (Rhubarb) treated wounds on day 5, showing blood clot, fibrial material, oedema and neutrophil infiltration. H&Ex20

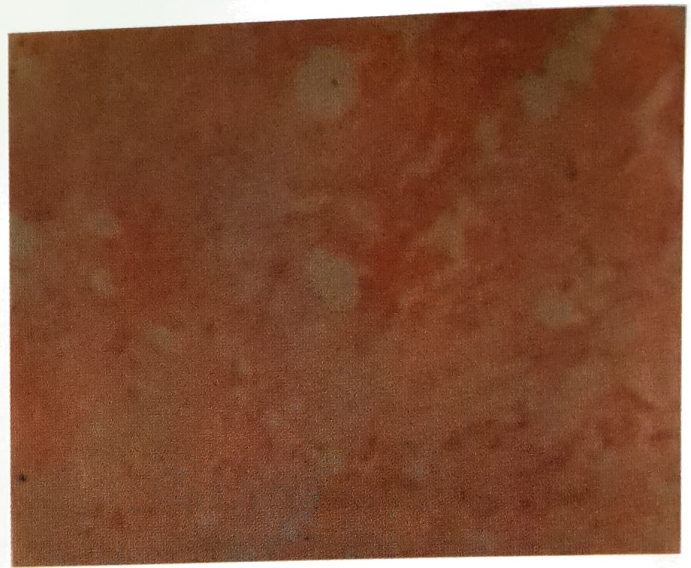


Fig 4. *Rheum emodi* (Rhubarb) treated wounds on day 5, showing neovascularisation and oedema. Proliferating vessels are perpendicular to base. H&Ex20



Fig 5. Control wounds on day 10, showing homogenous blood clot and mild proliferation fo fibroblasts and angioblasts around the clot. H&Ex20



Fig 6. *Rheum emodi* (Rhubarb) treated wounds on day 10, showing less fibroblasts as compared to fibres. The collapsed vessels are still evident perpendicular to base. H&Ex20

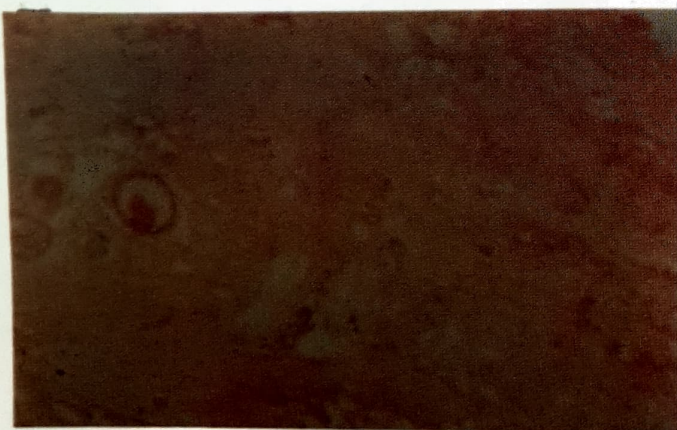


Fig 7. *Rheum emodi* (Rhubarb) treated wounds on day 15, showing fibres super ceding fibroblasts. H&Ex10

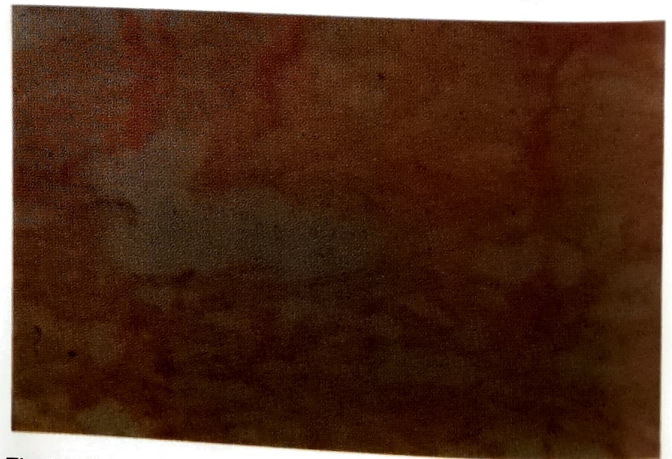


Fig 8. Control wounds on day 5, showing less amount of collagen and elastic fibres. Harts method X 10



Fig 9. *Rheum emodi* (Rhubarb) treated wounds on day 5, showing moderate collagen and elastic fibres. Harts method X 20

The control wounds as well as treated wounds revealed moderate to marked number of reticular fibres on 5th and 10th day, their number decreased gradually from 15th day onwards (Figs. 8&9). It may be due to the fact that the fine argyropillic fibres which appear early in healing wounds are subsequently replaced by coarse fibres of collagen (Greep, 1966). Collagen gradually increased in its extent and was greater in treated wounds and by 30th day maximum contents were observed. These observations are in corroboration with the observations of Ghani *et al* (1981) in calves and Bhargava *et al* (1988) in buffalo calves.

CONCLUSIONS

It can, therefore, be concluded that Rhubarb root powder resulted in most favourable and desirable effects because “Rhubarb” produces mild inflammation early onset and better organisation of granulation tissue.

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