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Research Article

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Effects of border plant (Red Bush) and curcumin on incised wounds in rabbits

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Abstract

The present investigation was carried out on rabbits to find out the comparative effects of border plant (Red Bush) and curcumin on morphological changes in wound healing. General anaesthesia was done before creating experimental wounds of 20 mm length and 5 mm depth on either side of the paralumber region. Rabbits were randomly divided into three groups consisting of four animals in each. Fresh curcumin powder was applied in one group, while fresh Red Bush paste was applied in another group. In the third group, methyl extract of Red Bush was applied. All treatments were applied topically. Clinical features including exudation, redness, dryness, cicatrization, pigmentation etc. were compared with all groups during the period of healing. Morphological features including elevation of suture line from the skin surface, swelling area of wound, contraction length per week and width of the sutured area were recorded to determine the healing progress. A higher swelling area (12.78 ± 0.19 mm) was seen in wounds of the Red Bush paste treated group. Elevation of wound surface area was greater (3.62 ± 0.003 cm) in wounds of the curcumin-treated group and lesser (3.51 ± 0.003 cm) in wounds treated with methyl extract of Red Bush. Methyl extract of Red Bush showed the best results within 8 days for complete healing. Curcumin also had a stimulating effect on wound healing activity but was less effective than border plants. Methyl extract of Red Bush is a better topical therapy for wound management in rabbits compared to curcumin.

Keywords: Curcumin, Incised wound, Rabbits

INTRODUCTION

Wounds are interruptions to the steadiness of tissues and are caused by chemical, physical, thermal, immunological or infectious injury to the skin and mucous membrane. Wound healing is the orchestration of cellular, humoral, and molecular events. Wound healing attempts to conserve the normal anatomy and function of the biological system. Wound healing is hastened by antibiotics, analgesics, and herbal drugs (Wang *et al.*, 2017; Pastar *et al.*, 2014). Proper wound restoration is attained by the inflammatory stage characterized by satisfactory initiation and intrusion of inflammatory cells, neutrophils and macrophages (Rodero and Khosrotehrani, 2010; Han *et al.*, 2001). Neutrophils begin coming to the site of injury within hours of the injury due to the effect of platelet-derived growth factors (PDGF), fibroblast growth factor (FGF), and TGF-β which are effective chemotactic agents for neutrophils (Portou *et al.*, 2015). The wound is also infiltrated by Natural killer (NK) cells in primary inflammation along with neutrophils and control the making of important monocyte cytokines (Schneider *et al.*, 2011). Pro-inflammatory cytokines including TNF and IL-1 are released by macrophages (Rodero and Khosrotehrani, 2010).

Phytochemicals related to plant-based products and customary remedies are imperious (Yamini *et al.*, 2016;

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Sharma *et al.*, 2013). About 80% of the world depends on traditional therapies and phytochemicals for their sicknesses (Pathania *et al.*, 2015). Among the phytochemicals, *Curcuma longa* and Loropetalum species are considered as growth-promoting agents in wound healing (Guntas *et al.*, 2015). Herbal phytochemicals have shown efficacies in *In vitro* models (Bahramsoltani *et al.*, 2014). Most of these herbal phytochemicals had undergone human trials (Bahramsoltani *et al.*, 2014). A herbal therapeutic regimen might be cost-effective in livestock practices (Wang *et al.*, 2017). *Curcuma longa* is used as phyto drug to lessen inflammation (Venkatasubbu and Anusuya, 2017). Curcumin is the active component in Turmeric rhizomes. Curcumin shows antioxidant and antimicrobial properties (Venkatasubbu and Anusuya, 2017; Sorg *et al.*, 2017). It enhances deposition of collagen, granulation tissue formation, contraction of wound and remodelling of tissue (Akbik *et al.*, 2014; Miah *et al.*, 2017). There are many pieces of research on curcumin in the wound healing process. However, no such work on *Loropetalum chinense var. rubrum* and curcumin on open wound healing in the rabbit. Hence, this research was designed to compare the effects of curcumin (*Curcuma longa*) and Red Bush plant (*Loropetalum chinense var. rubrum*) on incised wounds in rabbits through the evaluation of the physical specifics of wound healing.

MATERIALS AND METHODS

The research work was done at the experimental research shed of the Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh during July-October, 2017.

Experimental Animals

With the ethical approval of the Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, 12 seemingly healthy rabbits were used for this experiment. Their body weight ranged between 1.5 to 2.2 kg. They were kept in typical laboratory settings and veterinary observation with no restrictions on food and water.

Preparation of Curcumin Powder

Fresh powder of turmeric root (*Curcuma longa*) bought from KR Market of Bangladesh Agricultural University and directly applied on wounds.

Preparation of Red Bush Leaves Paste

The Red Bush leaves were taken from the Botanical Garden, Bangladesh Agricultural University and from Horticulture centre, Kewatkhali, Mymensingh. The fresh leaves were properly cleaned with water. Then these were grinded thoroughly with a pestle in a mortar and directly applied on wounds.

Preparation of Methyl Extract of Red Bush

The fresh leaves were properly cleaned with water and dried in the sun after collection. Then methyl extract was prepared with the help of Soxhlet Apparatus at the Department of Pharmacology, Bangladesh Agricultural University.

Experimental Design

Two precise wounds were prepared on the paralumber skin of each rabbit. Rabbits were allocated in three groups with four rabbits in each.

Group-C: Fresh curcumin powder was administered daily topically to two wounds prepared in each rabbit. Rabbits were carefully looked after to escape interfering with the formation of granulation tissues.

Group-R: Fresh paste of Red Bush was daily administered topically to two wounds prepared in each rabbit. The treatment schedule was the same as in Group-C.

Group-M: Methyl extract of Red Bush was administered in the same process described above.

All wounds were apposed with the simple interrupted pattern using silk. Antihistaminic, antibiotics, or anti-inflammatory drugs were not used to alleviate their consequences on the healing course. The wounds were observed up to day 15 post-operation. Morphological features (exudation, reddening, dryness, cicatrisation, pigmentation, swelling and width of the sutured area were noted to detect wound healing. The width of the sutured area was measured at days 3, 6, 9, 12 and 15 to determine wound contraction length.

Wound Creation

Premedication was done with an intramuscular injection of xylazine hydrochloride (Rompun 20 mg/ml, Bayer Korea Ltd., South Korea) dosed at 2 mg/kg before injecting the anaesthetic ketamine hydrochloride (G-Ketamine 50 mg/ml ketamine hydrochloride USP, Gonoshasthaya Pharmaceuticals Ltd., Bangladesh) dosed at 20 mg/kg.

The paralumber area was clean-shaven and painted

with 10% Povidone Iodine solution (Viodin 10%, Square Pharmaceuticals Ltd., Bangladesh). A surgical wound of 20 mm length and 5mm depth was prepared through a vertical incision in that area. The skin was separated from the underlying tissues by blunt dissection. Wounds were apposed with a simple interrupted pattern placed 6 mm apart using silk. 5 mm distance was kept between the needle placement and the border of the cutting edge.

Local Treatment

Group-C was treated with curcumin powder, group R was treated with a paste of Red Bush and methyl extract of Red Bush was administered onto the sutured area of group M. According to our treatment protocol, curcumin powder, Red Bush paste and methyl extract of Red Bush were smeared on wounds once daily. These applications were repeated every day. The wounds in all groups were observed clinically every day. Suture removal was done on day 5 post-operation.

Observation of Morphological Changes

The elevation of suture line (mm), swelling area (mm), the width of the sutured area of wound (mm) and wound contraction were measured with a slide caliper. The swelling was noted up to day 3 post-operation because swelling started a gradual decline from day 3 (D3). The altitude of the sutured line was documented during suture removal on day 5 post-surgery. The width of the sutured area was noted from day 1 (D1), day 3 (D3), day 6 (D6), day 9 (D9), day 12 (D12), day 15 (D15) to find the length of the wound contraction.

Assessment of Morphological Changes

Wound morphology was assessed according to the swelling area of the wound, exudation, reddening, dryness, cicatrisation, pigmentation. A score +++ : Massive occurrence, ++ : Moderate occurrence and + : Mild occurrence.

Evaluation of Wound Healing

Every wound was monitored clinically and photos were captured using a digital camera (Sony© Cyber Shot, Sony Corp, Tokyo, Japan) every two days up to day 15 post-operation. Wounds were noted healed when noticeable epithelium enclosed the wound and pigmentation and cicatrisation were found. The days to healing in rabbits were documented and the meantime was calculated in each group.

Statistical Analysis

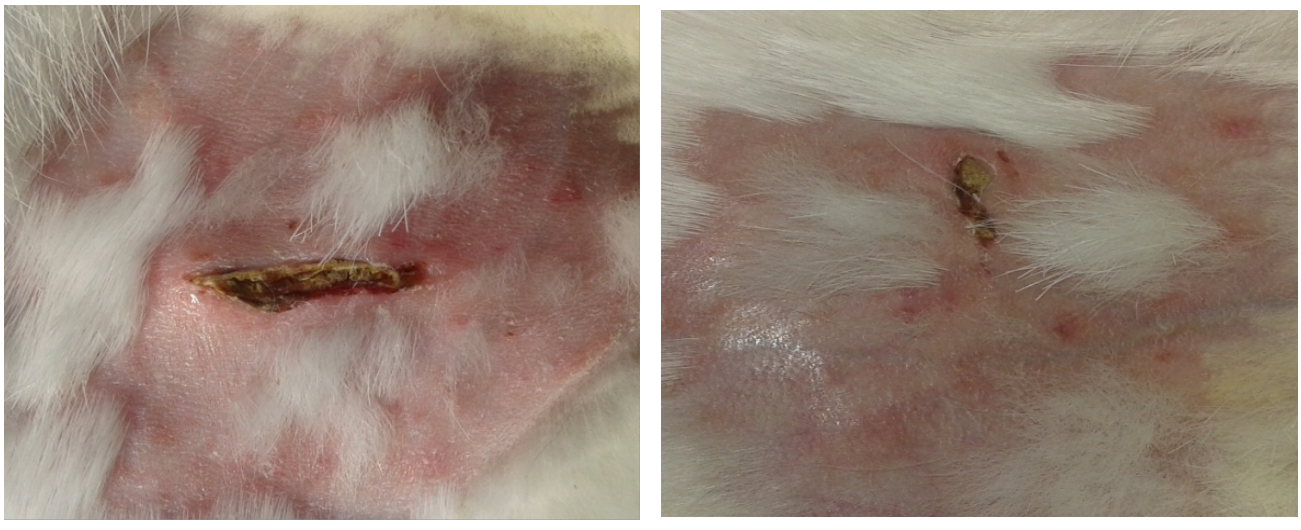
Data were collected from every rabbit before and after the operation was performed. Data were expressed as mean $\pm$ SEM. One way ANOVA (Analysis of Variance) was executed to compare among groups. Data were analyzed with SPSS statistics 17.0 software. Probability at $P < 0.01$ was significant statistically.

RESULTS

Morphological changes during wound healing

The wounds were observed daily for identifying the morphological changes during wound healing. Ranges of granulation and complete healing time of the wounds after treating with Red Bush paste, methyl extract of Red Bush and curcumin in rabbits are shown in Table 1. Methyl extract of Red Bush was seemed to be effective for healing of wounds taking 8 days more than the Red Bush paste where healing was completed in 11 days. In the case of the curcumin treated group, more time was taken for complete healing of wounds compared to Red Bush and methyl extract of Red Bush groups.

Table 2 and Table 3 show clinical alterations during various wound healing stages with different preparations. Mild exudation was seen on day 1 in all groups. The healing process started from the third day characterized by scab formation due to the drying of the exudates on the wound surface. On the third day, reddening was moderate in the curcumin group while mild in all other groups. The wounds were mild dry in Group-R and Group-M except for Group-C at day 3 of wounding. On the 5th day, mild reddening was observed in Group-C. Dryness was present in Group-C on day 5. Cicatrization and pigmentation were observed in all groups but a prominent response was found in the M group at day 7 of wounding (Figure 1). Pigmentation was massive in Group-R and Group-M at day 12 but moderate in Group-C. On the 15th day of wounding, pigmentation was massive in Group-C. Complete filling of the cavity of the wound was found earlier in Group-M compare to other groups.



(a) (b)

Figure 1: (a) Red Bush treated wound at 7 days and (b) Methyl extract of Red Bush treated wound at 7 days

Table 1: Range of granulation and complete healing time in different treatment groups of rabbits

Groups	**Treatment used**	**Range of granulation time(days)**	**Complete healing time(days)**
Group-R	Red Bush paste	3-7	11 ± 0.46^{b}
Group-M	Methyl extract of Red Bush	3-5	8 ± 0.46^{c}
Group-C	Curcumin	3-9	14 ± 0.46^{a}

Values with different superscripts (^{a,b,c}) differ significantly ($p < 0.01$)

Table 2: Distinctive clinical signs at various wound healing stages in groups treated with Red Bush, methyl extract of Red Bush and curcumin

Days	**Changes**	**Groups**		
		R	**M**	**C**
1^{st}	Exudation	+	+	+
3^{rd}	Reddening	+	+	++
	Dryness	+	+	+
5^{th}	Reddening	-	-	+
	Dryness	++	+++	+
7^{th}	Dryness	+++	+++	++
	Cicatrization	++	+++	++
12^{th}	Cicatrization	+++	+++	+++
	Pigmentation	+++	+++	++
15^{th}	Cicatrization	+++	+++	+++
	Pigmentation	+++	+++	+++

+ = Mild, ++ = Moderate, +++ = Massive

Table 3: Swelling of suturing areas of wounds (mm; Mean ± SEM)

Groups	Swelling of suturing areas of wounds (mm)		
	Day-1	**Day-2**	**Day-3**
Group-R	12.78 ± 0.19^{a}	5.247 ± 0.19^{a}	0.4913 ± 0.05^{a}
Group-M	11.80 ± 0.15^{b}	4.460 ± 0.20^{b}	0.4150 ± 0.05^{b}
Group-C	12.44 ± 0.27^{a}	4.839 ± 0.19^{ab}	0.5225 ± 0.04^{a}
Mean±SEM	12.34 ± 0.13	4.85 ± 0.11	0.476 ± 0.02
P value	0.0028	0.0116	0.0099

Values with different superscripts (^{a,b,c}) differ significantly ($p < 0.01$)

Table 4: Contraction of wound surface area (cm Mean ± SEM) for different treated groups on different days of post-surgery

Groups	Days to Wound Contraction				
	Day 1-Day 3	**Day 3-Day 6**	**Day 6-Day 9**	**Day 9-Day 12**	**Day 12-Day 15**
R	3.62±0.003	2.39±0.005	1.217±0.004	0.250±0.019	
M	3.51±0.003	2.26±0.003	0.621±0.015	0.150±0.019	
C	3.62±0.004	2.45±0.003	1.54±0.005	0.930±0.008	0.33±0.31

Wound Contraction

Wound surface area was calculated and expressed in millimeter (mm) as shown in Table 3. There was a noteworthy reduction in the surface area of the wound of the Red Bush and methyl extract of Red Bush treated lesions on day 6 to day 9 and day 9 to day 12 compared to those of curcumin-treated ones (Table 4).

DISCUSSION

This study compares topical Red Bush, methyl extract of Red Bush and curcumin concerning their therapeutic effects on the surgical wound. The results of this study show the differences and importance of choosing the appropriate topical medication for a wound.

In this study, the swelling was observed up to day 3 post-operation as swelling gradually declined from day 3 (D_3). Less inflammation in the wound might be indicated by the elevated suture line and lower swollen area. The thickness of sutured area was noted from day 0 (D_0) to day 15 (D_{15}) post-operation to comprehend wound contraction. The centripetal movement of wound edges facilitating apposition of a wound is contraction and it is the highest at 5-15 days post-injury (Chopra *et al.*, 1999). In our study, contraction length significantly varied ($p < 0.01$) between groups.

Results direct inflammatory phase of wound healing up to day 3 post-operation. Wounds cured with methyl extract of border plants showed the lowest diameter of suture area compared to the other two groups up to day 3 and the disparity was significant ($P < 0.01$) up to day 15 statistically. Wounds and inflammation are treated with Curcumin (Venkatasubbu and Anusuya, 2017). It enhances collagen deposition, granulation tissue formation, wound contraction and tissue remodelling (Akbik *et al.*, 2014).

In our study, the rate of healing time of wound varies from 8 to 14 days with different plant extracts, which are in contrast to Mallick *et al.* (2017) who state that the wound healing time is 22-26 days in ewes. A higher rate of healing was observed in methyl extract of border plant treated group followed by Red Bush plant and curcumin which is an agreement with Hossain *et al.* (1992); Eurides *et al.* (1998); Alam *et al.* (2005). The granulation tissue appeared from the 3rd day onwards in all treatment groups. This finding corresponds with Hossain *et al.* (1992). Pigmentation and cicatrization were found in all treated groups from the 7th day onwards, while Hossain *et al.* (1992) found these features on 11 days. Thus, methyl extract of Red Bush presented improved outcomes on the activity of wound healing in rabbits than Red Bush paste and curcumin.

The contraction of a wound is defined as a proportion of wound area from the highest wound size suc-

ceeding wound creation at 5-15 days post-injury (Slatter, 2002). There was an insignificant difference in terms of diminishing contraction length per week in wounds of all groups. The contraction of the wound depends on the periphery myofibroblast, its link to the constituents of the extracellular matrix and the spread of myofibroblast (Rohrich, 1990). Our results are in line with this notion. In this study, the highest swelling and altitude of the suture line were seen in group C where wounds were treated with curcumin compared to other groups.

In this study, no negative effect from either border plant (Red Bush) or curcumin paste was detected. Either the border plant or the curcumin can be clinically administered for wound healing. But wounds of the group treated with methyl extract of Red Bush showed early descending of inflammation, enhanced infection control and earlier wound healing in comparison to the other two groups.

CONCLUSIONS

Methyl extract of the Red Bush plant shows impressive outcomes in the curing of incised wounds in rabbits. It shows a good result than fresh Red Bush plant leaves' paste and curcumin. Wound care could be improved and simplified by the topical administration of fresh paste of Red Bush plant leaves as it is readily available and affordable, and when expediently administered, is an effective and affordable treatment for the wound.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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