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Original Article

Clinical evaluation of ethanolic extract of curcumin (*Curcuma longa*) on wound healing in Black Bengal goats

Md Abu Haris Miah[1], Moinul Hasan[1], Yousuf Ali Sarker[2], Md Mahmudul Alam[1] and Nasrin Sultana Juyena[1,#]

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AFFILIATIONS

[1]*Department of Surgery and Obstetrics, Bangladesh Agricultural University. Mymensingh-2202, Bangladesh.*

[2]*Department of Pharmacology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.*

CORRESPONDENCE:

[#]Nasrin Sultana Juyena, PhD, *Professor, Department of Surgery and Obstetrics, Bangladesh Agricultural University. Mymensingh-2202, Bangladesh.*
E-mail: juyenahabib@yahoo.com

ABSTRACT

Objective: This study was aimed at clinical evaluation of surgical wound healing in goats treated with ethanolic extract of turmeric (*Curcuma longa*) rhizomes through topical route.
Materials and methods: Eighteen surgical wounds were made in nine goats. The goats were divided into three groups; Group 1 (test group) was treated with ethanolic extract of turmeric, Group 2 (standard group) was treated with Povidone iodine, and the Group 3 was kept as untreated control. Follow up information was recorded from day 0 to day 21 postoperatively. Some morphological characters such as swelling area of wound, elevation of suture line from the skin surface, width of the suture area and contraction length per week were considered to determine the healing process. Bacteriological evaluation was done by conventional bacteriological techniques, and the tissue biopsies were stained by hematoxylin and eosin stains for histopathological studies.
Results: Swelling of suturing area (11.51±0.36 mm) and elevation of suture line (2.65±0.41 mm) were lowest in wounds treated with ethanolic extract of turmeric. In histopathological studies, it was seen that tissue debris and hemorrhages disappeared and a thin line of keratin layer reappeared on the epidermal surface of the wound treated with ethanolic extract of turmeric.
Conclusion: Ethanol treated turmeric enhances wound healing process in goats. This result could help the veterinarian and the researchers to consider herbal product especially ethanolic extract of turmeric for the treatment and better healing of surgical wounds with minimal complications.

KEYWORDS

Curcumin; Ethanolic extract; Surgical wound; Wound healing

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INTRODUCTION

Wounds are major cause of physical disabilities. Healing is a survival mechanism and represents an attempt to maintain normal anatomical structure and function (Wang et al., 2017). Restoration of damaged tissue is essential for the basis of all surgical manipulations (Majumder and Kamath, 2005). Immunosuppressant, cytotoxins and non-steroidal anti-inflammatory drugs suppress the wound healing. However, antibiotic, analgesic, and herbal drugs hasten wound healing. Povidone iodine (PVI) is mainly used as an antimicrobial agent. It has been found that 0.5% PVI can attenuate congestion, edema and pain induced by pressure sores (Wang et al., 2017).

In Bangladesh, many farmers cannot afford the cost of treatment of their animals. So, before taking the animal to the veterinarian, the owner try to treat their animal with various local remedies, of which plant sources are mostly common. Antimicrobial agents derived from plants have received much attention recently (Shirazi et al., 2008; Cutter, 2011). Moreover, efforts are being made to discover agents that can promote wound healing decreasing hospitalization cost (Shukla et al., 1999). In Indian subcontinent, herbal plants are found in plenty. These plants are cheaper and safer than allopathic drug. Thus, treatment by natural ways could be economic in farm animal practices (Wang et al., 2017).

Curcuma longa (turmeric) is a plant that belongs to the family Zingiberaceae (Chopra et al., 1999). *C. longa* has been used as medicine since long past in the treatment for inflammatoin (Venkatasubbu and Anusuya, 2017). Turmeric rhizomes are mainly used for this purpose. The main active ingredient of turmeric is curcumin. Curcumin is used for treatment of wound and inflammation. It has antimicrobial and antioxidant properties (Venkatasubbu and Anusuya, 2017). It has also been found that curcumin helps in open wound healing process when applied to cattle (Mamun, 2012). Curcumin accelerates reepithelization and migration of cells such as myofibroblast, fibroblast and macrophages necessary for wound healing (Goel et al., 2008).

Curcumin minimizes pain and inflammation and supports the homeostasis by selectively inhibiting the arachidonic acid cascade through the lipoxy and cyclooygenase (COX) pathway (Majeed, 1995). There are no available researches on ethanolic extract of turmeric in Bangladesh. Therefore, the present research has been designed to exploit the therapeutic effect of ethanolic extract of turmeric on wound healing.

MATERIALS AND METHODS

Ethical statement: All the wounds were created aseptically considering animal welfare. AVMA (American Veterinary Medical Association) Animal Welfare Principles were followed to provide minimal discomfort to the experimental goats. Local analgesia was done before creation of the wounds to minimize pain sensation.

Experimental location: The research work was conducted at the Department of Surgery and Obstetrics, Bangladesh Agricultural University (BAU), during January to May 2016. Nine apparently healthy goats were used for this experiment. The animals were kept under standard conventional housing system and veterinary monitoring with no restrictions on water and food. Before conducting the study, the goats were quarantined for three weeks. Deworming was done, and vaccination against peste des petits ruminants virus was done.

Experimental design: Goats were divided into three groups consisting of three animals in each group:
Test group (Group 1): Ethanolic extract of turmeric ointment was applied daily on surgical wounds.
Standard group (Group 2): PVI cream was applied daily on surgical wounds.
Control group (Group 3): No medicine was applied on surgical wounds. Only normal saline was used to wash the wound.

Two surgical wounds (2.0 cm length and 0.5 cm depth) were made on the skin of each goat. Wounds were sutured with simple interrupted suture using silk before application of desired medicine. Morphological (swelling area of wound, elevation of sutured line from normal skin surface, width of sutured area), microbial and histopathological characters were studied to evaluate the effects of the treatment. Follow-up information was obtained from day of surgical operation up to day 21 after surgery. Elevation of sutured line was recorded during suture removal after 7 days of surgery. Width of sutured area were measured on day 0 (D_0), day 3 (D_3), day 7 (D_7), day 14 (D_{14}), day 21 (D_{21}) to determine wound contraction length.

Extraction and drug formulation: Fresh turmeric of good quality in size, shape and color was collected from the KR (Kamal Ronjit) market in BAU. After careful washing with distilled water, turmeric was peeled off and washed again with distilled water. Turmeric was cut into small slices and kept in hot air oven over night for drying. Dried turmeric was powdered by mechanical grinder and

passed through sieve number 60 to get powder of desired coarseness. Powder material was kept in an air tight container. 550 gm of the coarsely powdered, dried rhizomes of *C. longa* were mixed with 3500 mL of ethanol and allowed to stand for 7 days. It was filtered and distilled under vacuum to get concentrated ethanolic extract. The ethanolic extract was stored in the desiccator for further chemical and pharmacological screening. A 5% (w/w) ointment formulation was prepared by incorporating the ethanolic extract with simple ointment base, petroleum jelly for topical application on incised wound.

Observation of wounds: Slide calipers was used to measure swelling area (mm), elevation of suture line (mm), wound contraction and width of sutured area of wound (mm). The progress of healing in animals of each group was monitored daily. Healing score was classified as:
Excellent: Absence of inflammation, exudation, infection, dehiscence and gradual decrease of cutting edge width.
Good: Minimum inflammation with minimum exudation, no dehiscence, gradual decrease of cutting edge width.
Fair: Marked inflammation, infection and exudation.

Wounds were observed daily to find any complication such as swelling (mm), wound dehiscence, suture abscess, local infection and exudation.

Bacteriological study: Swabs were collected on Day 0, Day 1 and Day 2 after operation and cultured aseptically from the wound of goats. Then Gram's staining method was performed for presumptive bacterial identification.

Biopsy and histopathology: The biopsies (1.5 cm x 1 cm) were collected from the wound areas of each goat on day 1, 3 and 7 after wounding using standard surgical procedure. Histopathological slides were prepared at the Department of Pathology, Faculty of Veterinary Science, BAU.

Statistical analysis: All data were presented as mean±SEM. To compare data among the groups One Way ANOVA (Analysis of Variance) factor analysis was performed. The data obtained in the present investigation were analyzed with SPSS statistics 20.0 software. Probability $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

This experiment was designed to study and compare the effects of ethanolic extract of turmeric, PVI cream and normal saline on healing of surgical wounds in goats. Morphological, microbial and histopathological characters were studied to evaluate the effects of these three treatments on surgical wound healing.

Swelling was seen up to three days of post-operation as it decreased gradually from day 3 (D_3). On day 7 (D_7) skin suture was removed and elevation of suture was recorded. Swelling of the wound edges was observed in experimental animals (**Table 1**). Lowest swollen area (11.51±0.36 mm) and elevation of suture line (2.65±0.41 mm) were observed in wounds of ethanolic extract of turmeric (**Table 1**). Lower swollen area and elevated suture line could indicate less inflammation in wound.

The width of suture area was recorded from day 0 (D_0) to day 21 (D_{21}) postoperatively to understand wound contraction process morphologically. Contraction is defined as the centripetal movement of wound edges that facilitates closure of a wound defect and is maximum at 5-15 days after injury (Chopra et al., 1999). Contraction length varied insignificantly ($P>0.05$) between the groups (**Table 1**). Width of sutured area increased up to day 3 and then decreased gradually in all wounds irrespective of treatment (**Table 2**). Furthermore, there was no significant variation in wounds of all groups in terms of decreasing contraction length after day 14 (**Table 2**). These results indicate inflammatory phase of wound healing that is marked up to day 3 of operation. Epithelialization is essential for wound healing. A wound cannot heal without re-epithelialization (Pastar et al., 2014). Wound treated with ethanolic extract of turmeric had lowest diameter of suture area in comparison to the other two groups up to day 3 and variation was statistically significant ($P<0.05$) up to day 21. Skin wound healing is a complex process dependending on many cell types and mediators interacting in a highly sophisticated temporal sequence (Sorg et al., 2017).

Table 1. The effects of ethanolic extract of turmeric ointment, povidine iodine cream and normal saline seal on wound healing in goats

Groups	Swelling of suturing area (mm)	Elevation of Suture line (mm)	Average contraction length (mm/week)	Healing score
Test Group	11.51±0.36[a]	2.65±0.41[a]	1.65±0.31	Excellent
Standard group	11.60±0.46[a]	2.81±0.50[a]	1.70±0.35	Good
Control Group	12.46±0.73[b]	3.7±0.35[b]	1.88±0.60	Fair

a, b indicate significant ($P<0.05$) difference between groups

Table 2. Width (mm) of suture area of wounds treated with ethanolic extract of turmeric ointment, povidine iodine cream and normal saline seal on wound healing in goats

Groups	D_0	D_3	D_7	D_{14}	D_{21}
Test Group	6.63±0.41[a]	7.51±0.29[a]	7.05±0.44[a]	6.11±0.37	4.45±0.52
Standard Group	6.67±0.47[a]	7.60±0.41[a]	7.38±0.44[ab]	6.23±0.29	4.46±0.53
Control Group	7.58±0.51[b]	8.61±0.98[b]	8.01±0.89[b]	6.61±0.37	5.20±0.47

a, b indicate significant (P<0.05) difference between groups

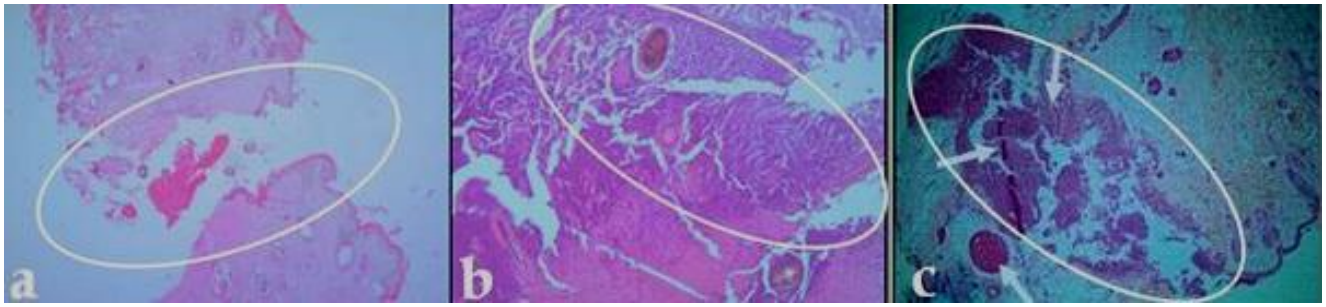

Figure 1. Histological analysis of healing process of skin in turmeric treated group stained with H and E (4x). Biopsy collected on day 1(a), day 3(b) and day 7(c) showed the tissue reactions during healing processes. On day 1, presence of tissue debris and much of hemorrhages in the experimental wounds was seen (a, white circle). On day 3, there was hemorrhages and huge infiltration of inflammatory cells in incised tissues (b, white circle). On day 7 of experiment, much of the debris and hemorrhages were disappeared and cells present were mostly inflammatory cell type (c, white circle). A thin line of keratin layer found to reappear on to the epidermal surface of the wound.

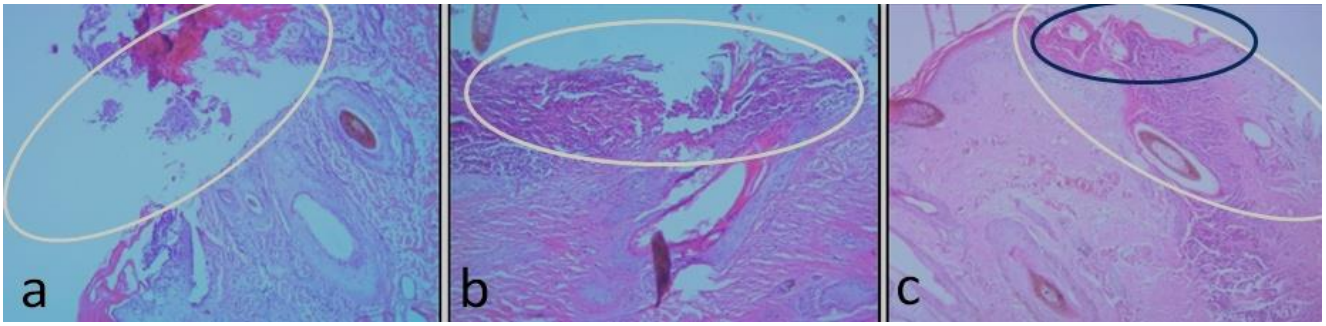

Figure 2. Histological evaluation of the sections of wound from povidone iodine treated group animals and stained with H and E (4x). On day 1(a, white circle) tissue debris and hemorrhages was seen in the wound. The wound was accompanied by the infiltrations of reactive cells with moderate hemorrhages and congestion (b, white circle). On day 7(c, white circle) the wound appeared in healing stage characterized by infiltration of fewer reactive cells and formation of keratin layer (c, white circle) over the wound surface.

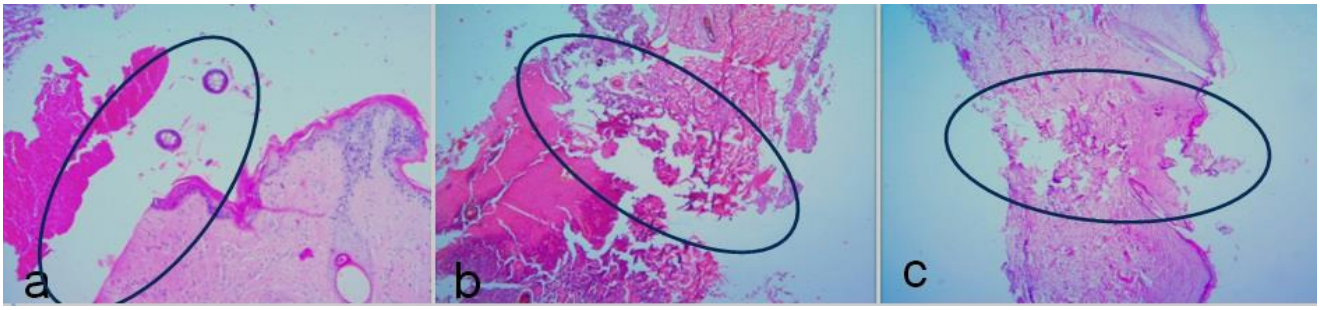

Figure 3. Histological evaluation of the sections of wound from untreated goats and stained with H and E (4x). On day 1(a, green circle) tissue debris and hemorrhages was seen in the wound. The wound was accompanied by the infiltrations of fewer reactive cells with wide spread hemorrhages and congestion (b, green circle). On day 7(c, green circle) the wound found to contain less inflammatory cells, there was gap between the edge of wound and showed less tendency of healing (c, black circle).

Although, results did not show any significant difference in the morphological parameters of wounds treated with ethanolic extract of turmeric and PVI cream, histo-pathological study showed some variations in their action in wound healing. Histopathological study revealed the infiltration of inflammatory cells in all groups at Day 1, 3 and 7 (**Figure 1-3**). Marked infiltration of round cells or macrophages were seen in samples collected from test and standard groups from D_3 to D_7 (**Figure 1b-c**, **2b-c**), but less macrophages infiltration was seen in the control group (**Figure 3b-c**). The variations in histopathological features were due to differences in sampling occasions

and treatment strategies adopted for different experimental groups. More infiltration of macrophages indicated inflammatory phase and early events of wound healing (Gosain and Dipietro, 2004; Broughton et al., 2006; Campos et al., 2008). Curcumin is used for treatment of wound and inflammation (Venkatasubbu and Anusuya, 2017). Curcumin enhanced formation of granulation tissue, deposition of collagen, remodeling of tissue and contraction of wound (Akbik et al., 2014). Thus, ethanolic extract of turmeric showed better results on wound healing activity in goats than PVI cream.

Microbiological studies revealed that bacterial colonies were present in culture of Day 0 samples collected from test and standard groups, whereas bacterial colonies were present in culture of samples of control group at Day 0, 1 and 2. Gram staining confirmed the presence of *Staphylococcus spp.* The bacteria in infected wounds occur in the form of biofilms, which are complex communities of aggregated bacteria embedded in a self-secreted extracellular polysaccharide matrix (Edwards and Harding, 2004). *Staphylococcus spp.*, *Pseudomonas aeruginosa*, and 13-hemolytic *Streptococci* are common bacteria in infected and clinically non-infected wounds (Edwards and Harding, 2004).

PVI disinfectant is the most commonly used topical agent for sterilization of skin, including in surgical settings (Mimoz et al., 2015). PVI can be promoted in prevention and treatment of infection (Flynn, 2003), and the present study shows that it does not interfere with wound healing. Topical application of 0.5% PVI could promote acute skin wound healing though increased expression of TGF-β leading to enhanced formation of granulation tissue, even in the absence of obvious infection (Wang et al., 2017).

Various disinfectants constituted with iodine have been reported to produce effects on wound healing in skin via multiple mechanisms including prevention of bacterial growth and acceleration of granulation tissue formation (Gulati et al., 2014; Mitani et al., 2016). Kanno et al. (2016) demonstrated that 1% polyvinylpyrrolidone-iodine could promote healing of contaminated wounds as it reduced bacterial counts in wounds, and enhanced re-epithelialization. These reports supported our findings where the test group showed significantly ($P<0.05$) faster wound closure and wound contraction.

CONCLUSION

Ethanolic extract of turmeric shows excellent healing score without any postoperative complication, and plays a beneficial role in surgical wound healing. As it is readily available, it can be considered as an effective and affordable treatment for wounds. Further study needs to observe specific inflammatory cells and different percentage of ethanolic extract of turmeric on every event of wound healing.

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CONFLICT OF INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

AUTHORS' CONTRIBUTION

MAHM designed the experiment and was involved in surgical procedures, ethanolic extraction of Curcumin and sample collection. MH participated in surgical procedures, sample collection and drafted the manuscript. YAS helped in ethanolic extraction of Curcumin (*Curcuma longa*) and analysis of data. MMA contributed in critical checking of this manuscript. NSJ supervised the research work. All the authors read and approved the manuscript before submission.

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