**Original Article** 

# Wound healing by marigold (*Calendula officinalis*) and turmeric (*Curcuma longa*) paste: A comparative approach

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# ABSTRACT

**Objective:** This experiment was conducted to evaluate the therapeutic potency of marigold flower (*Calendula officinalis*) and turmeric (*Curcuma longa*) rhizome paste in wound healing.

**Materials and methods:** Thirty six aseptic surgical wounds were tooled in six non-pregnant black Bengal goats dividing them in 3 groups. Month long information and follow-up examinations along with complications such as edema, wound dehiscence, suture abscess, exudation etc. were studied. Wound healing was assessed by observing some morphological characters as well as histopathological changes of the wounded area.

**Results:** Results revealed that negligible elevation of suture line  $(1.17\pm0.11 \text{ mm})$  and significant (P < 0.01) reduction of sutured area were observed in goats of turmeric group at day 3 (D3) and 7 (D7) respectively. Moreover, histologic appearance of wound of the similar group displayed splendid development of keratin layer and collagen fiber than the others from day 3 (D3). However, therapeutic efficacy of marigold flower paste was not up to the mark as turmeric in this experiment. Control group treated with povidone iodine, showed good results but took long time.

**Conclusion:** This study could patronize veterinarians to consider the use of herbal plants specially turmeric as a great wound healer. It will also reduce toxicities created by haphazard use and applications of pharmaceutical products. The findings of this very experiment will not only prevent the skin degradation but also help to maintain the good health status of animals.

# **KEYWORDS**

Collagen; Keratin; Marigold; Toxicities; Turmeric

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#### INTRODUCTION

The role of livestock sub-sector is requisite for economic development of agro-based Bangladesh. Current contribution of livestock sub-sector to overall GDP is about 1.78% (BER, 2014). The export earnings from quality leather are 3.21% of the total export (BER, 2014). Though the warmer climate of Bangladesh is helping in producing superior quality leathers, all of its advantages are submerged by various defects (Jain et al. 2010) specially wound. Wound is defined as the disruption of the cellular and anatomic continuity of a tissue; it may be produced by physical, chemical, thermal, microbial or immunological insult to the tissue (Cherry et al., 2000). If not treated in due time, a wound may lead to serious consequences (Cherry et al., 2000). The skin or external wounds are more common in ruminants (Nooruddin and Dev, 1990). Animals can get wounded at the farm, during transportation, or by getting strike against some hard object, kicked by another animal or during different surgical interventions. Among economic losses of approximately US\$ 220.95 million due to wounded and diseased skin, US\$ 24.1 million was due to Black Bengal goats' skin defects in Bangladesh (Jain et al. 2010).

Most of the farmers in Bangladesh have no facility for modern treatment for their livestock as well as they cannot afford the cost of treatment. For this reason, the unauthorized persons get the chance rather than veterinarian and create complications in wound healing. Immediate complications include formation of hematoma secondary to improper homeostasis, development of a wound infection, and delayed complications include scar formation, which may be due to improper suturing with excess tension or to lack of eversion of the edges, stitch marks and wound necrosis (Gabrielli et al., 2001). For proper wound healing the healing agents should have certain specifications such as it should facilitate granulation and collagen formation; promote normal immunity; debride wound slough and necrotic tissue; minimize microbial colonization; alleviate pain; and facilitate angiogenesis and tissue perfusion. Again, wound dressing should also be cost effective, produce minimal patient discomfort, and be easily applied and removed. Though few dressings satisfy all these criteria, the plant and herbal paste and extract having wound healing efficacy also give splendid results.

In wound healing process different plant and herbal preparations are used traditionally. The therapeutic efficacies of many indigenous plants, for various diseases have been described by traditional herbal medicine practitioners (<u>Natarajan et al., 2003</u>). Antimicrobial substances derived from plants have received considerable attention in recent years (<u>Jain et al. 2010</u>).

There are limited researches on the use of herbal product to assist healing process in Bangladesh (Jain et al. 2010). Among many other medicinal attributes Calendula officinalis, commonly known as marigold flower has wound healing property (Preethi and Kuttan, 2009). Research has confirmed that curcumin the active ingredient of Curcuma longa aids in wound healing (Maheshwari et al., 2006). This research, efficacy of Calendula officinalis and Curcuma longa over wound healing will definitely help for proper healing of wound created by any means in animals. It will reduce harmful effects (toxicities) of pharmaceutical products by reducing their haphazard use and applications. Moreover, this research will be helpful for researchers to develop the most efficient ways of using these plant and herbal paste and extracts in wound healing of animals, to gather knowledge over stages of wound healing, preventing complications of healing process, histological analysis of cellular growth etc. Thus this will be a significant research for Bangladesh as it will satisfy the farmers both economically and therapeutically.

The aim of the research was to examine wound healing efficacy of marigold flower, turmeric rhizome pastes and povidone iodine; to find out the histopathological changes of wounded area during and after healing and to exploit the therapeutic potency of marigold flower and turmeric rhizome paste in 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 (Inj. Jasocaine<sup>®</sup>, Jayson, Bangladesh) was done before creation of the wounds to minimize pain sensation.

**Experimental animal:** Six apparently healthy nonpregnant Black Bengal goats (*Capra hircus*) were used for this research. The body weight and age of the animal was ranged from (18-20) Kg and (1-3) years respectively. The goats were kept on open grazing during the day and at night in a closed stall with concrete floor. They were served with concentrate (wheat bran, crushed wheat, pulses etc.) and water *ad libitum*. All of the experimental goats were dewormed with ivermectin (0.2 mg/kg body weight, SC; Inj. Vermic<sup>®</sup>, Techno Drugs, Bangladesh) before starting the experiment.

**Collection of agents and observation:** The marigold flower and turmeric rhizome were collected locally. Solution Povisep<sup>®</sup> (Jayson, Bangladesh) was collected as a source of povidone iodine (10%). The surgical wound

creation, herbal paste preparation and application, suturing of the wound edges, sample collection and histopathological study were performed in the operation theater of Surgery & Radiology and Histopathology Laboratory of Central Lab., FVMAS, BSMRAU, Gazipur-1706, respectively.

**Experimental design:** A total of 36 artificial fresh surgical wounds (30 mm length and 5.0 mm width) were made following aseptic measures in six goats divided into three groups with two goats in each group. Fresh paste of marigold flower and turmeric rhizome prepared by using mortar and pestle were applied locally daily for 5 days to 6 (six) surgical wounds made in each animal of respected group. Another group of animals were kept as control group treated by povidone iodine for 5 days.

All wounds were closed with cross mattress pattern using surgical needle (Cutting bodied half circle needle, 8×26; China) and silk (Black braided non-capillary waxed silk, no. 4-0; China) to mitigate the suture effects on wound healing. The application of antibiotic, antihistaminic and anti-inflammatory drugs was avoided.

Wound observation: Follow-up information was obtained since day of surgical operation ( $D_0$ ) up to day 30 (D<sub>30</sub>) after surgery. Some morphological characters such as swelling area of wound (mm), elevation of sutured line from the skin surface (mm), width of sutured area (mm) were recorded to determine the healing of the wounds by measuring scale and slide calipers. Elevation of sutured line was recorded up to 7 days of surgery. Width of sutured area was measured from day of surgical intervention day 0 ( $D_0$ ), day 3 ( $D_3$ ), day 7 ( $D_7$ ), and day 14  $(D_{14})$ . Wound length was recorded from day of surgical intervention day 0 (D<sub>0</sub>), day 3 (D<sub>3</sub>), day 7 (D<sub>7</sub>), day 14 (D<sub>14</sub>), day 21 (D<sub>21</sub>) and day 30 (D<sub>30</sub>) determine wound contraction and morphology. Healing score was categorized as:

*Excellent-* Early healing, relatively dry suture surface, no inflammation, high contraction of wound

*Good-* Moist suture surface, moderate inflammation, moderate healing *Fair-* Inflammation, edema, and presence of pus

All wounds were closely monitored daily to observe any complication. Tissue samples were collected from all treatment groups at  $D_1$ ,  $D_3$ ,  $D_7$  and  $D_{15}$  for histopathological study.

**Biopsy and Histopathology:** The biopsies  $(1.5\text{cm} \times 1 \text{ cm})$  were collected from the wound areas of each experimental animal on the 1<sup>st</sup>, 3<sup>rd</sup>, 7<sup>th</sup> and 15<sup>th</sup> days after wounding using standard surgical procedure. The biopsy sites were cleaned with saline solution and gauze. The samples containing dermis and epidermis were fixed in

10% buffered neutral formalin solution. Histopathological slides were prepared according to the method of Jain et al. (2010). The histologic slides of all wound samples were observed at 4X and 10X optic by inverted microscope (Primovert<sup>®</sup>, Compact inverted microscope, Germany) Histopathology Laboratory of Central Lab., FVMAS, BSMRAU, Gazipur-1706.

**Statistical Analysis:** Statistical analysis was done by using utilized SPSS statistical software. All data were presented as Mean $\pm$ SEM. To compare data among groups, one way ANOVA (Analysis of Variance) factor analysis was performed. Probability P < 0.05 was considered statistically significant

#### **RESULTS AND DISCUSSION**

All the six goats were well managed and showed good response throughout the experiment. Thirty six (36) incised skin wounds were created in goats and treated with marigold (*Calendula officinalis*) flower paste in marigold group, turmeric (*Curcuma longa*) rhizome pastes in turmeric group as well as povidone iodine in control group to evaluate their effect on wound healing.

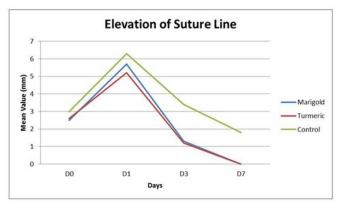


Figure 1. Elevation of suture line in three groups up to day 7 (D7).

In this experiment, three parameters of wound healing elevation of suture line (mm), width of sutured line (mm) length of wound (mm) were evaluated and postoperatively. Swelling was observed up to three days of operation because swelling started decreasing gradually from day 3 (D<sub>3</sub>). Elevation of suture line was recorded up to day 7 ( $D_7$ ) as suture material was removed after day 7. At day 3 ( $D_3$ ), among three groups negligible elevation of suture line indicating less wound swelling (1.17±0.11 mm) was observed in wounds treated with turmeric rhizome pastes (Figure 1). On the contrary, more swelled area (3.42±0.19 mm) indicating more inflammation in wounds was significantly (P < 0.05) higher in control group at day 3 (D<sub>3</sub>) (Table 1). There was insignificant (P>0.05) difference between turmeric  $(1.17\pm0.11 \text{ mm})$ 

and marigold  $(1.33\pm0.14$ mm) groups representing swelled areas of wounds at day 3 (D<sub>3</sub>).

Contraction of wound was evaluated by observing width of sutured area and was recorded from day of operation  $(D_0)$  to 14<sup>th</sup> day  $(D_{14})$  of post-operation. Width of sutured area of three groups started decreasing sharply after day 3  $(D_3)$  and then decreased gradually (**Figure 1**). These results indicated inflammatory phase of wound healing and was marked up to third day of operation (**Table 1**). Wounds of control group had higher diameter of sutured area (1.58±0.23 mm) in comparison to that of turmeric group (0.54±0.04 mm) which was highly significant (*P*<0.01) up to day 14. Moreover, (0.83±0.21 mm) elevation was found in marigold group at day 14 (D<sub>14</sub>).

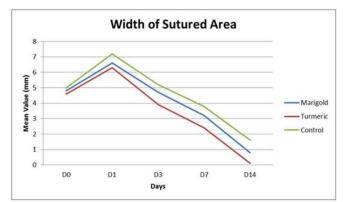


Figure 2. Width of sutured area in three groups up to day 14 (D<sub>14</sub>).

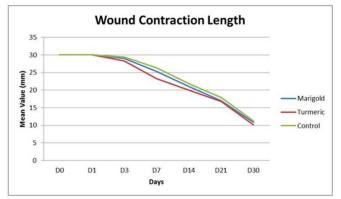


Figure 3. Wound contraction length in three groups up to day 30  $(D_{30})$ .

Insignificant variation (P > 0.05) was revealed when contraction length per week was considered among groups (**Figure 3**). Contraction length was recorded up to day 30 ( $D_{30}$ ) and it was observed that highest contraction length ( $11.33\pm0.61$  mm) in control group and lowest contraction length ( $10.08\pm0.43$  mm) in turmeric group whereas marigold group showed ( $10.75\pm0.63$  mm) (**Table 1**). Again, the variation of contraction length among the groups was insignificant (P > 0.05). Reactive cells are the marker of inflammation in the wounded area. The histological slides prepared from biopsies of wounds treated in three different groups represented some remarkable scenario of inflammatory process. Formation of epidermal layer, fibrous connective tissue proliferation, and arrangement of collagen fiber was considered as the status of healing in samples taken in four different days (Day 1, 3, 7 & 15). All samples showed a large accumulation of reactive cells at day 1 (D<sub>1</sub>) of wounding (Figure 4a, 4b, 4c). The presence of reactive cells started decreasing in wound of marigold and turmeric group from day 3 (Figure 5a, 5b) whereas, control group showed severe infiltration of reactive cells in the similar day (Figure 5c) as well as in day 7  $(D_7)$  of wounding (Figure 6c). A remarkable result was evidenced in wounds of turmeric group having spectacular development of collagen fiber from day 3 (Figure 5b) but it was very little in marigold group in the similar day of wounding (Figure 5a). In contrast, good collagen fiber formation was observed in wounds of marigold group at day 15 (D<sub>15</sub>) (Figure 7a). Newly formed keratinized layer of epidermis and presence of connective tissue in the dermis indicating complete healing was noticed at 15 day (D15) of wounding in marigold and turmeric group (Figure 7a, 7b) where splendid healing was observed in turmeric group. However, broken and developing keratin layer was evidenced at day 15 ( $D_{15}$ ) in control group (Figure 7c). Good healing results were observed in wounds of control group but it took longer time than the wounds of marigold and turmeric group.

Excellent healing was noticed in wound of turmeric group at day 7 (D<sub>7</sub>) (**Figure 8b**). Wound healed with good morphologic appearance was seen at day 14 (D<sub>14</sub>) in both marigold and turmeric group (**Figure 8a, 8b**). However, wound of control group showed more scar formation in the wounded area in similar day (**Figure 8c**).

In this study, few wounds treated with marigold flower paste showed presence of very little pus with moist sutured area at day 7 (D<sub>7</sub>) when sutures were removed. According to healing score scale, fair to good healing was observed in wounds of marigold group. In contrast, all sutured wounds of turmeric group maintained excellent healing score. Early healing, less inflammation and dry suture line was observed D<sub>3</sub> to D<sub>14</sub> in the turmeric group which is the indication of excellent healing. Comparatively moist suture surface and delay healing were observed in wounds of control group in between day 7 (D<sub>7</sub>) and day 30 (D<sub>30</sub>). No dehiscence was observed in any wound of three groups.

Table 1. Different parameters (mm) of wound in marigold, turmeric, and control group (Mean±SEM)

	Marigold Group (mm)			Turmeric Group (mm)			Control Group (mm)		
Da	ESL	WSA	WL	ESL	WSA	WL	ESL	WSA	WL
у	(Mean±SEM)	(Mean±SEM)	(Mean±SEM)	(Mean±SEM)	(Mean±SEM)	(Mean±SEM)	(Mean±SEM)	(Mean±SEM)	(Mean±SEM)
$\mathbf{D}_0$	$2.50 \pm 0.19$	4.83±0.17	30.00±0.00	$2.58 \pm 0.19$	4.58±0.15	30.00±0.00	$3.00 \pm 0.28$	$5.00 \pm 0.21$	30.00±0.00
$D_1$	$5.67 \pm 0.31$	$6.58 \pm 0.29$	$30.00 \pm 0.00$	$5.17 \pm 0.21$	6.33±0.19	$30.00 \pm 0.00$	6.25±0.33	7.17±0.24	$30.00 \pm 0.00$
$D_3$	1.33±0.14**a	4.67±0.19	29.00±0.21	1.17±0.11**a	$3.92 \pm 0.19$	$28.25 \pm 0.22$	3.42±0.19**	$5.17 \pm 0.24$	29.42±0.15
$D_7$	0.00	3.17±0.11	$25.23 \pm 0.30$	0.00	$2.42 \pm 0.29$	$23.25 \pm 0.37$	$1.83 \pm 0.21$	$3.75 \pm 0.33$	26.33±0.38
$D_{14}$	0.00	0.83±0.21**	$21.00 \pm 0.44$	0.00	$0.54 \pm 0.04 **$	$20.00 \pm 0.39$	0.00	1.58±0.23**	$21.75 \pm 0.49$
$D_{21}$	0.00	0.00	$17.00 \pm 0.60$	0.00	0.00	$16.75 \pm 0.43$	0.00	0.00	$17.92 \pm 0.48$
$D_{30}$	0.00	0.00	10.75±0.63 <sup>NS</sup>	0.00	0.00	10.08±0.43 NS	0.00	0.00	11.33±0.61 NS

ESL: Elevation of Suture Line; WSA: Width of Sutured Area; WL: Wound Length

NS indicates non-significant difference among three groups

\* indicates significant (P<0.05) difference among three groups

\*\* indicates highly significant (P<0.01) difference among three groups

<sup>a</sup> indicates non-significant (P>0.05) difference between marigold and turmeric groups

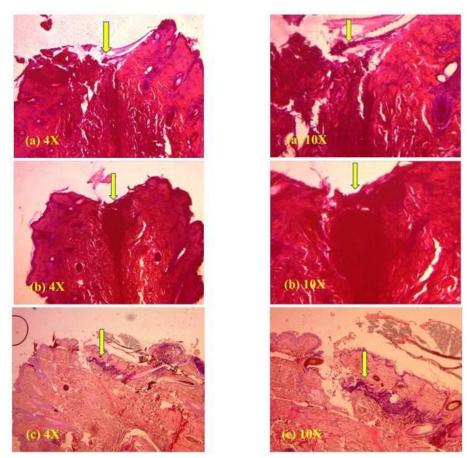


Figure 4. Presence of reactive cells (yellow arrows) beneath the keratinized tissues of epidermis of wounds of marigold (a), turmeric (b) and control (c) groups at  $D_1$  after treatment; (Primovert<sup>®</sup> Microscope, magnification 4X, 10X).

As a means of wound management plant and herbal preparations are spreading their incense with high significance. Though Bangladesh is a land of many herbal plants, the examples of research using those plants are quite limited. For this purpose, this experiment was designed to study and compare the effects of marigold flower paste, turmeric rhizome and povidone iodine on healing of surgical wounds in a goat model. The evaluation of the effects of these three treatments on wounds sutured with cross mattress suture using silk was done depending upon the morphological and histopatholoical changes. Elevation of suture line, width of sutured area and wound contraction were observed postoperatively to compare effects of three treatments on wound healing. In this study, the elevation of suture line and width sutured area following the surgical operation and treatment noted up to day 3 (D<sub>3</sub>) and day 14 (D<sub>14</sub>) respectively. Contraction of wound length was recorded up to day 30 (D<sub>30</sub>).

Healing is the end result of a complex event consisting of homeostasis, inflammation, differentiation, proliferation and migration of mesenchymal cell to the wound site, proper angiogenesis, prompt re-epithelialization, cross-

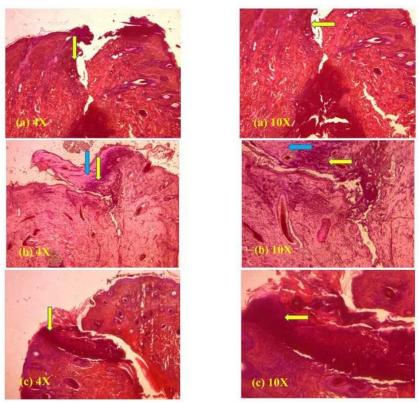


Figure 5. Presence of reactive cells (yellow arrows) and collagen fiber (blue arrow) beneath the keratinized tissues of epidermis of wounds of marigold (a), turmeric (b) and control (c) groups at D<sub>3</sub> after treatment; (Primovert<sup>®</sup> Microscope, magnification 4X, 10X).

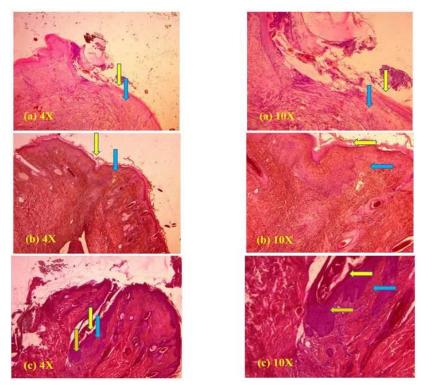


Figure 6. Newly formed keratinized layer of epidermis (yellow arrow) along with newly formed connective tissue (blue arrow) under the scab in wounds of marigold (a), turmeric (b) and control (c) groups at  $D_7$  after treatment; Presence of huge number of reactive cells in control group (orange arrow); (Primovert<sup>®</sup> Microscope, magnification 4X, 10X).

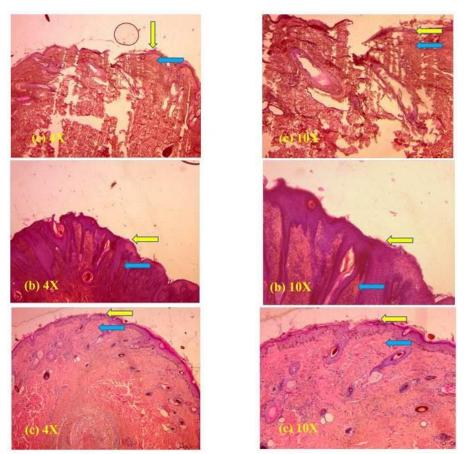


Figure 7. Epithelial tissues with a newly formed keratinized layer of epidermis and complete healing (yellow arrow), the dermis shows flabby connective tissue with organized interconnecting collagen fibers running parallel to each other (blue arrow) in wounds of marigold (a), turmeric (b) and control (c) groups at  $D_{15}$  after treatment; (Primovert<sup>®</sup> Microscope, magnification 4X, 10X).



Figure 8. Wound observation of wounds of marigold (a), turmeric (b) and control (c) groups at D7 and D15 after treatment.

linking and alignment of collagen (Mathieu et al., 2006). The samples of all groups indicating inflammatory response at day one  $(D_1)$  may be due to the result of traumatic tissue handling and suture placement (Runk et al., 1999). Again, as there is a presence of bleeding,

inflammatory cells migrate into the wound (chemotaxis) and promote the inflammatory phase, which is characterized by the sequential infiltration of neutrophils, macrophages, and lymphocytes (<u>Campos et al., 2008</u>). Histopathological study revealed the infiltration of reactive cells in all samples of three groups collected at D<sub>1</sub> (Figure 4a, 4b, 4c). Reactive cells decreased gradually in wounds of turmeric rhizome pastes group and marigold flower paste group respectively with in D<sub>3</sub> (Figure 5b). Reactive cells decreased markedly in turmeric treated wounds than that of marigold. Moreover, proliferation of fibrous connective tissue was observed notably in wounds of turmeric group at day 3 (D<sub>3</sub>). This result upholds the finding of Sidhu et al. (1999). It may be due to the anti-inflammatory ability of marigold flower (Hamburger et al., 2003) and turmeric (Aggarwal et al., 2003) respectively. However, reactive cells are seen in wounds treated with povidoine iodine till day 7 (D7) (Figure 6c). Iodine formulations such as povidoine iodine are highly recognized for their capacity of absorbing wound effusion and pus (Nakao et al., <u>2006</u>). The reactive cells are helpful for proper healing of wound.

Formation of keratinized layer and connective tissue was evident in all wounds at day 7 (D7). Noteworthy quantities of vascular endothelial cell growth factor are liberated by the wound epidermal cells (Brown et al., 1992). Granulation tissue formation which will take 4 to 7 days of wounding is largely depends on fibroblast growth factor (Nissen et al., 1998). An interesting finding was observed in samples of turmeric group and marigold group at day 7 (D7), where newly formed keratinized layer of epidermis along with newly formed connective tissue was found under the scab infiltrated with reactive cells (Figure 6a, 6b). Curcumin may be useful in conditions of impaired wound healing, as it appears to induce endogenous production of TGF-\$1 in the wound which can play an important role in improvement of wound repair (Mani et al., 2002). Other pro-aniogenic factors such as fibroblast growth factor (FGF), angiogenic cytokines as interleukin 8 (IL-8), tumor necrosis factoralfa (TNF-a), and transformation growth factor-beta (TGF- $\beta$ ) may be related to the angiogenic activity evidenced in the C. officinalis extract (Carmeliet, 2000, 2003) which is helpful for healing. Histological findings of control group at D7 postoperatively indicated comparatively delayed healing of wounds (Figure 6c).

Re-epithelialization is a must for wound healing (Pastar et al., 2014). Research has confirmed that curcumin aids wound healing by stimulating the migration of macrophages, neutrophils and fibroblasts to the wound, accelerates the wound contraction, formation of granulation tissue, neovascularization and reepithelialisation (Maheshwari et al., 2006). The cytotoxic as well as tumor reducing potential of marigold flower such as sesquiterpenes, alcohol, saponins, triterpenes, flavonoids, hydroxycoumarin, carotenoids, tannin, and volatile oils (Crabas et al., 2003) affect cutaneous wound

healing and also collagen production. Pharmacological studies have confirmed that *C. officinalis* exhibit a broad range of biological effects, such as antimicrobial (Radioza and Iurchak, 2007), antioxidant (Cetkovic et al., 2004), wound healing (Preethi and Kuttan, 2009) etc. Progressive wound healing was observed after day 14 in control group (Figure 7c). Topical application of iodine exerts cell proliferative effects (Hirsch et al., 2010). Recently, Kanno et al. (2016) demonstrated that 1% polyvinylpyrrolidone-iodine could promote healing of contaminated wounds as it reduces wound bacterial counts and enhances re-epithelialization and acceleration of granulation tissue formation (Gulati et al., 2014; Kanno et al., 2016).

Results represented that width of sutured line markedly decreased from day 7 (**Table 1**) in all wounds irrespective of treatment. It may supports <u>Gulati et al. (2014</u>), where it is indicated that contraction of wound is maximal 5-15 days after injury which is defined as the centripetal movement of wound edges that facilitates closure of a wound defect.

Furthermore, insignificant (P>0.05) variation was observed in wounds of all groups in term of diminishing contraction length per week (Table 1). This result supports the hypothesis of Nissen et al. (1998), which states that wound contraction depends on the myofibroblast located at the periphery of the wound, its connection to components of the extra cellular matrix and myofibroblast proliferation, and does not seem to depend on collagen synthesis. More swelling was evident in control group where wounds treated with povidone iodine in comparison to that of other two groups. This result hypothesizes that povidone iodine cause marked tissue reaction in wounds. Histological findings observed in this experiment confirm this hypothesis. It was found that marked infiltration of reactive cells was present in samples collected from control group from D1, D3 and D7 (Figure 4c, 5c, 6c). Results showed little difference in the morphological and histopathological parameters of wounds treated with marigold flower and turmeric paste, in their action in wound healing.

#### CONCLUSION

In this study, application of turmeric paste gives splendid result in comparison with marigold flower paste. Now, it can be easily recognized the beneficial effects of turmeric over cutaneous wound in goats. Moreover, this study will help the veterinarians to prescribe this herbal product for cutaneous wound healing with very minor complications. Cellular events created by turmeric and marigold flower can be studied in future to find out the actual healing capabilities of these two herbal plants. Extraction of specific biomaterials from turmeric, its molecular characterization and direct application in wound can also be studied in future. As turmeric showed a good result in proper healing and controlling infection in wounds, future study should be directed to explore active component responsible for its effects on infection control and wound healing.

#### ACKNOWLEDGEMENT

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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**

SP designed the experiment, completed surgical procedures, prepared marigold and turmeric paste, and collected sample aseptically. SP, MAAM & ARU participated in tissue processing, slide preparation and staining. SP & MAAM completed data collection and analysis. SP, ANMAR, ZCD and MAAM performed histological analysis of prepared slides. SP wrote the manuscript and all the authors read and approved the manuscript before submission.

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