Bangladesh Veterinary Journal (2021) 55(1-4): 38-47
pISSN 0378-8113 •eISSN 2708-6054
https://doi.org/10.32856/BVJ-55-2021.04

Research Article

OPEN ACCESS

Wound healing by border plant and green grass (Durba) in Black Bengal goats: A comparative study

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Abstract

This investigation was done on 12 Black Bengal goats to find out the comparative effects of border plant and green grass (durba) on histo-morphological changes in the cutaneous wounds at Veterinary Teaching Hospital, Bangladesh Agricultural University. Ten experimental cutaneous wounds for each group (n=04) were made in the goats following standard procedure. In Group A 100% plant extracts of Border plant (*Aerve sanguinolenta*), in Group B Green grass (durba) (*Cynodon dactylon*) and in Group C control 0.85% normal saline were applied. Clinical investigation revealed that border plant enhances wound healing within 11 days of treatment, which however takes 14 days for durba grass and 18 days for saline treatment. Clinical features revealed reddening was lower in Group A and B following day 5 of treatment compared to a high level of reddening in the wound areas of control skin. The level of cicatrization and pigmentation of wounds was higher in Group A and B until day 12 of treatment. On day 15 of treatment complete healing was seen in wounds treated with Border plant and durba grasses. Microbiological studies show the microorganisms isolated from the wounds were *Staphylococcus spp*, *E. coli* and *Bacillus spp*. On histopathological studies, the highest degree of inflammation and tissue response was seen in Group A goats trailed by Groups B and C goats. Complete keratinization was only seen over the injured skin of Group A goats following 7 days of treatment compared to incomplete keratinization in Group B and lack of keratinization in Group C. The results indicate that Border plant is an effective topical therapeutic agent followed by green grass (durba) extract for treatment of wounds in Black Bengal goats.

Keywords: Cutaneous wound; Wound healing, Histopathological study

INTRODUCTION

A cutaneous wound is defined as any type of physical injury involving with the breakdown of the continuity of the skin (Douglas and Alan, 2003). Generally, wound is created by the diseases and many type of accidental mechanical injuries. Due to impecunious condition, our farmers cannot bear the treatment cost. So, most often the owner tries with various types of inexpensive plant sources for the treatment of cutaneous wound without paying a visit to a veterinarian. These remedies usually retard wound healing. Many types of costly therapeutic agents are used for the treatment of wounds (Bojrab, 1982). On the other hand, use of antibiotics may have adverse effects on the safety of food and public health if the antibiotic residues enter the food chain (Hossain *et al.*, 1992). Worldwide, above 80% people prefers traditional medicines for several skin problems (Annan and Houghton, 2008). Around 33% of all traditional medicines are used for the management of wounds and skin conditions in contrast to only 1-3% of modern drugs (Houghton *et al.*, 2010). Phytochemicals related to plant-based products and traditional therapies

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Article history: Received: 17 October 2020; Accepted: 23 September 2021.

are essential to promote the significance of all therapies (Sharma *et al.*, 2013; Yamini *et al.*, 2016). Herbal phytochemicals have shown efficiency in animal prototypes (Bahramsoltani *et al.*, 2014). Therapeutic regimen by natural means could be cost-effective for veterinary practices (Wang *et al.*, 2011). In modern biomedical sciences, plant based wound healers are studied for treating burns and wounds (Kumar *et al.*, 2007). The *Cynodon dactylon* (L.) Pers. family Poaceae known as doob ghas, or Durva is extensively used in customary medical knowledge (Ayurvedic, Unani, Nepalese, and Chinese) and ethnomedicinal practices. Traditional wisdom is the basis of herbal preparations made from this grass (Mishra, 2016). Wound infection was successfully treated with curcumin (*Curcuma longa*) (Miah *et al.*, 2017) thankuni plant (*Centella asiatica*) and border plant (*Aerva sanguinolenta*) in goats (Amin and Hashim, 1997). In Bangladesh, experiments on the effect of herbal product in wound healing are inadequate. Therefore, this study was designed to evaluate and compare effects of border plant and durba on the healing of surgically induced wounds in Black Bengal goats.

MATERIALS AND METHODS

Studied Animals

Twelve Black Bengal goats (*Capra hircus*) were used in this experiment to know the comparative efficacy of Border plant and Durba extract for the treatment of wounds. Their body weight ranged 7-8 kg and age ranged 10-15 months. The goats were physically fit and devoid of any infections or infestations to carry out the experiment. The experimental goats were acquired from the live animal market. They were kept on chained grazing the day. The animals were kept in a closed stall with concrete floor at night. The goats were allowed to graze 6-8 hours a day. Some bran and concentrate were supplied in the late afternoon. All the experimental goats were dewormed before the experiment with albendazole (Almex®, Square Pharmaceuticals Ltd. Bangladesh) @7.5 mg/kg body weight.

Plants used in the Experiment

Two types of plants were used, Border plant (*Aerva sanguinolenta*) (Figure 1a) and Durba grass (*Cynodon dactylon*) (Figure 1b).

Preparation of the Herbal Extracts

Fresh herbal extracts were prepared from the leaves of Border plant and Durba grass. The leaves were collected from Botanical Garden of Bangladesh Agricultural University. They were properly cleansed and air dried. The leaves were grinded properly in a ceramic pestle. These crude extracts were used directly.

(a) (b)

Figure 1: Border plant (a) and Durba grass (b)

Preparation of the Wound

At first, the operative sites were washed with soap and water, prepared with povidone's iodine. At the lumbar region, 2% lidocaine hydrochloride (Jasocaine^{R}, Jayson pharmaceuticals Ltd.) was infiltrated at the site of operation. Then wounds were produced, one on both sides of the lumbar midline and were sutured by simple interrupted suture with nylon.

Experimental Design

The goats were clustered in random into 3 groups with 4 animals in each group.
GROUP-A: 100% extract of Border plant was locally applied daily to the three wounds made in each of the three goats and also one wound which was made in another goat in the same group. These goats were monitored carefully to avoid intervention with granulation tissue growth.
GROUP-B: 100% extract of the Durva grass was locally applied daily to the wounds. The treatment regime was similar as in Group-A.
Group-C: In this group 0.85% normal saline was applied. This is the control.

Gross Observations

The progress of wound healing was monitored daily in each group. Before complete healing the

changes were recorded daily. Cicatrisation and pigmentation of the wound was marked as healed. Gross morphological changes of wound were observed using magnifying glass and comparative histopathological study of wound healing was done.

Microbiological Investigation

Samples were collected directly with sterile cotton bud into test tubes with cotton cap having nutrient broth from wounds of goats to isolate and identify bacteria by morphology, staining and cultural characteristics. After collection, incubation of samples was done in nutrient broth at 37°C overnight. The samples were then inoculated in nutrient agar and incubated again at 37°C overnight to stimulate bacterial growth. The colonies on primary culture were repetitively subculture by streak plate method. Nutrient agar, mannitol salt agar, eosin methylene blue was used as media for subculture and incubated at 37°C for 24 hours.

Gram's staining method was performed to observe the morphology and staining features of bacteria and to presumptively identify bacteria (Cowan, 1979).

Biopsy and Histopathology

Biopsies (1.5×1 cm) were taken from wounds of each investigational goat on the 3rd day after the creation of wounds following standard surgical procedure. Dermis and epidermis werepresent in the wound tissue. 10% neutral buffered formalin solution was used to fix samples for 48 hours for histopathology.

Tissue Processing

The tissue was cut properly using a surgical scalpel and taken in histo cassette. The samples were rinsed in Phosphate Buffer Saline (PBS) for 30 minutes. Tissues were dehydrated in alcohol (ascending grades) using two changes in 50% alcohol, one change in 70% alcohol, one change in 80% alcohol, two changes in 90% alcohol and two changes in absolute alcohol for one hour each and finally samples were kept in HPLC grade absolute alcohol for overnight for complete dehydration of tissue. Tissue sections were cleaned in Xylene by three changes for one hour each. Then histo cassettes containing samples were immersed in molten wax at 60°C for two changes, each for one hour and then paraffin blocks were prepared. The prepared blocks were kept at –20°C for further use. Tissues were sectioned with a microtome (Histoline, USA) at a thickness of $4-\mu$m. For a better adhesion of the section to the slide, a trivial amount of gelatin was introduced to the water bath. Sections were permitted to extend on warm water bath (40°C) and taken on oil and grease free glass slides. Slides containing sections were dried in air and kept on hotplate (at 40°C) overnight. Routine hematoxylin and eosin staining was performed after tissue sectioning. After staining, sections were observed under compound light microscope to compare tissue reactions between experimental and control groups.

Microscopy

Stained slides were examined to appraise differences of tissue reaction between experimental and control lesions. Microphotography was taken using photomicrographic camera.

Statistical Analysis

Mean $\pm$ SEM of the data were calculated. Data were analyzed with one way analysis of variance (ANOVA) system using IBM SPSS Statistics, Version 20. The level of significance was at P value < 0.05.

RESULTS

Morphological Changes During Wound Healing

The wounds were observed daily for identifying the morphological changes during wound healing. Ranges of granulation time and complete healing time of the wounds after treating with Border plant extract, Durba extract and 0.85% Normal saline in Black Bengal goats are shown in the Table 1. Border plant extract was seemed to be effective for healing of wounds taking 11 days better than Durba extract where healing was completed in 13 days. In case of control group more time was taken for complete healing of wounds compared to the Border plant extract and Durba extract. Table 2 and Table 3 depict clinical changes during various wound healing stages. The exudation, reddening, dryness of wound, pigmentation and cicatrization were observed. Mild exudation was seen on the first day of wound creation in all experimental groups. The healing process started from the third day characterized by scab formation due to drying of the exudates on the wound surface. On the third day reddening was moderate in control group while mild in other groups. The wounds were mild dry in Group-A at day three of wounding. On the 5th day mild reddening was observed in Group-C. Dryness was present in Group-B on day 5. On the 7th day

cicatrization was moderate in Group-A, but mild cicatrization was found in Group-B. Pigmentation was massive in Group-A, but moderate in Group-B at day 12. At the day 15 of wounding massive cicatrization and pigmentation were found in both Group-A and Group-B. Complete filling of the cavity of the wound was found earlier in Group-A compare to other groups.

Wound Contraction

Wound surface area was calculated and expressed in centimeter (cm) as shown in Table 4. There was a significant reduction in the wound surface area of the Border plant and Durba extract on day 7 to day 9 and day 10 to day 12 compared to those of 0.85% Normal saline treated ones.

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

Groups	**Treatment used**	**Range of granulation time (days)**	**Complete healing time (days)**
Group-A	Border Plant extract	3-7	11 ± 0.39
Group-B	Durba extract	3-9	14 ± 0.29
Group-C	Normal saline (0.85%)	5-11	18 ± 0.42

Table 2: Distinctive clinical signs at various stages of wound healing in groups treated with Border Plant extract, Durba extract and 0.85% saline

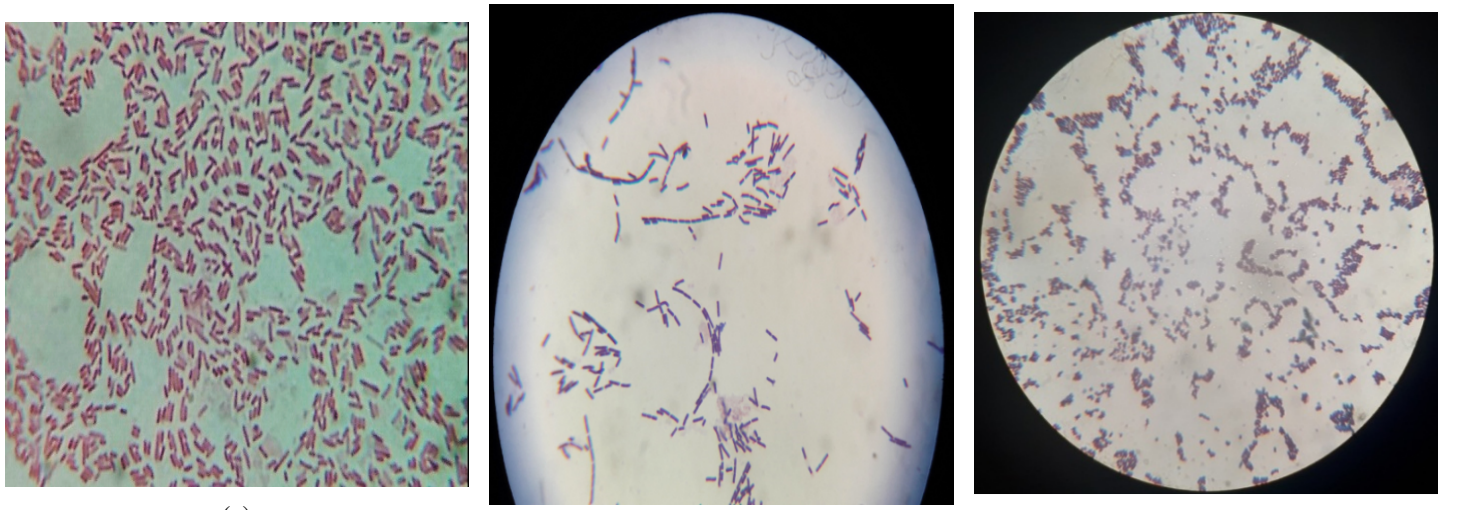
Days	**Changes**	**Group-A**	**Group-B**	**Group-C**
1^{st}	Exudation	++	++	++
3^{rd}	Reddening	+	+	++
	Dryness	+	-	-
5^{th}	Reddening	-	-	+
	Dryness	++	+	-
7^{th}	Dryness	+++	++	+
	Cicatrization	++	+	-
12^{th}	Cicatrizaton	+++	++	+
	Pigmentation	+++	++	+
15^{th}	Cicatrisation	+++	+++	++
	Pigmentation	+++	+++	++

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

Table 3: Swelling of suturing areas of wounds (mm; Mean $\pm$ SEM) observed

Groups	Swelling of suturing areas of wounds (mm)		
	Day-1	**Day-2**	**Day-3**
Group-A	$11.78 \pm .14^{a}$	$4.49 \pm .19^{a}$	$0.39 \pm .04^{a}$
Group-B	$12.44 \pm .22^{a,b}$	$4.91 \pm .15^{a,b}$	$0.48 \pm .04^{a}$
Group-C	$12.83 \pm .37^{b}$	$5.42 \pm .07^{b}$	$1.78 \pm .01^{b}$

Values with dissimilar superscript letter(s) in a column vary significantly ($p < 0.05$)



(a) (b) (c)

Figure 2: *E. coli* (a), *Bacillus spp.* (b), *Staphylococcus spp.* (c) in gram staining

(a) (b) (c)

Figure 3: *E. coli* in EMB agar (a), *Bacillus spp.* in Nutrient agar (b), *Staphylococcus spp* in Mannitol salt agar (c)

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

Groups	**Days to Wound Contraction**					
	Day 1-Day 3	**Day 4-Day 6**	**Day 7-Day 9**	**Day 10-Day 12**	**Day 13-Day 15**	**Day 16-Day 18**
Group-A	$3.63 \pm .012^{a}$	$2.38 \pm .100$a	$1.23 \pm .016^{a}$	$0.24 \pm .016^{a}$	0.0^{a}	0.0^{a}
Group-B	$3.62 \pm .003^{a}$	$2.44 \pm .003$b	$1.54 \pm .006^{b}$	$0.93 \pm .007^{b}$	$0.31 \pm .027^{b}$	0.0^{a}
Group-C	$3.62 \pm .003^{a}$	$2.88 \pm .021$c	$2.45 \pm .005^{c}$	$1.54 \pm .009^{c}$	$0.94 \pm .007^{c}$	$0.36 \pm .022^{b}$

Values with dissimilar superscript letter(s) in a column vary significantly ($p < 0.05$).

Table 5: Identification of the bacteria by staining and cultural characteristics from direct pus samples

Morphology of the bacteria by staining characters			**Cultural characters**			**Identified organisms**
Shape	Arrangement	Gram staining reaction (+)/(-)	Nutrient agar	Mannitol Salt Agar	Eosin Methylene Blue agar	
Small cocci	Chains or pairs	Gram positive	Small, circular, discrete, translucent, convex colonies	-	-	*Streptococcus spp.*
Cocci	Clusters	Gram positive	Gray white or yellowish colony	Yellow White Colonies	-	*Staphylococcus spp.*
Rod with square ends	Pairs and also in chains	Gram positive	Thick cream or grayish white colored colony with irregular surface	-	-	*Bacillus spp.*
Short plump rods	Singly, pairs or in short chains	Gram negative	Circular, smooth, white to grayish white colonies. Peculiar fetid odor	-	Moist, circular colonies with dark centers yellow green, metallic sheen	*E. coli*
Cocci, oval	Singly or in pairs	Gram positive	Scanty growth	Small, smooth discrete, glistening, translucent colony	-	Gram positive cocci
Rod shape	Pair or single	Gram negative	Poor growth	-	Poor growth	Gram negative rods

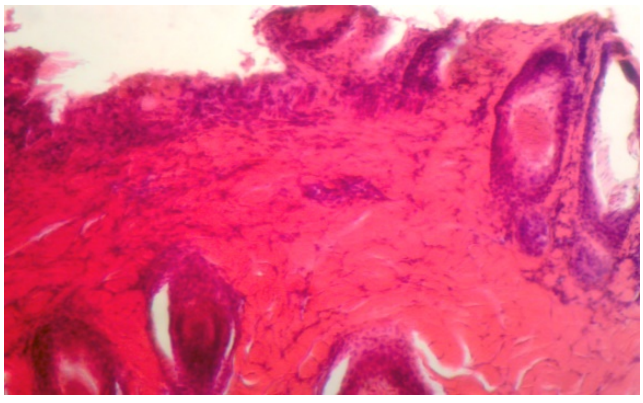


Figure 4: Biopsy collection of skin from border plant treated group at day 7 and stained with H&E (10x). Arrow indicating huge infiltration of reactive cells in the epidermis and dermis of injured tissue

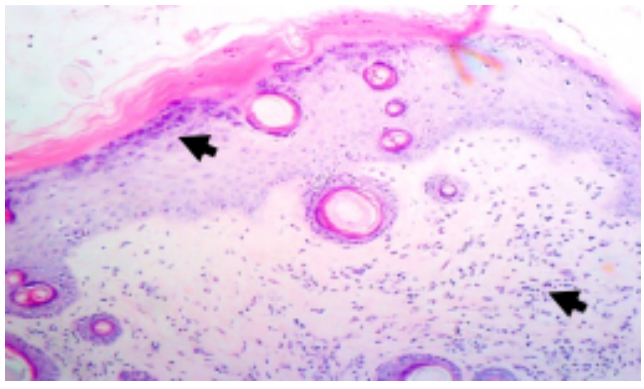


Figure 5: Biopsy collection of skin from durba plant treated group at day 7 and stained with H&E (10x). Arrow indicating moderate infiltration of reactive cells in the epidermis and dermis of injured tissue

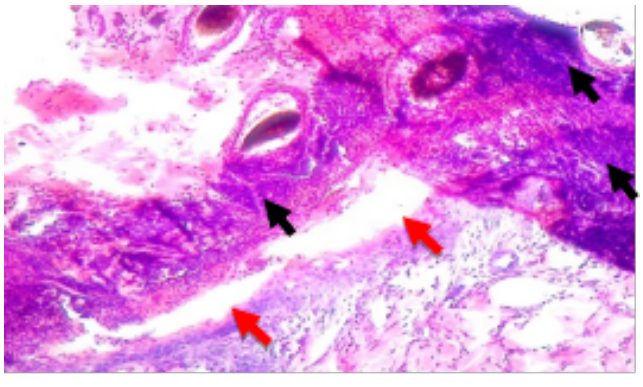


Figure 6: Biopsy collection of skin from 0.85% normal saline treated group at day 7 and stained with H&E (10x). Arrow (black) indicating deposition of tissue debris in the injured areas. The healed tissues containing wider areas of hemorrhages and congestion (red arrows) with infiltration of reactive cells predominantly neutrophils.

Cultural Characteristics of the Isolated Organisms

Cultural appearances of every type of bacteria secluded from samples were studied for the determination of shape, size, colony features and pigment of the bacteria in several media. The staining property of each samples directed the presence of numerous types of bacteria in the same smear. The pure culture of the organism from every mixed sample was acquired by streak plate method by means of different sample, enriched and selective solid media to study the individual cultural characteristic of the isolates and the results of the cultural features of bacterial isolates are shown in Table 5. Organisms were found as gram-negative rods of various shape and size, which organized singly, pair, or in short chain indicating *E. coli* (Figure 2a). Organisms were found as gram-positive rods with spores settled in pair and moreover in long chain demonstrating *Bacillus spp* (Figure 2b). Stained smear of the slide revealed gram-positive cocci organized in cluster specifying *Staphylococcus spp* (Figure 2c). The organisms found as gram-positive cocci arranged singly or in pairs indicating unidentified gram-positive cocci and gram-negative rods settled singly or in pairs indicating unidentified gram-negative rods. *Strertococcus spp.* isolated from samples revealed following cultural characteristics on nutrient agar and other media. The isolated *E. coli* created circular, smooth and white to grayish white colony with irregular fetid odor in nutrient agar (Figure 3a). *Bacillus spp.* formed thick, cream or grayish white colored colony with irregular surface in nutrient agar (Figure 3b). The species of *Staphylococcus spp.* secluded from samples exhibited grey–white to yellowish colony in nutrient agar and white to golden yellow on mannitol salt agar (Figure 3c).

Histopathological Changes

Histopathological evaluation of biopsies collected from various groups was focused on the regeneration of epidermis, fibroblast, proliferation of blood vessels

and fibrous connective tissue; proliferation, migration and differentiation of inflammatory cells. Inflammatory wounds in regenerating tissues were assessed on the basis of infiltration of reactive cells comprising lymphocytes, macrophages and neutrophils. The border plant extract treated wounds showed early stage of healing which is categorized by huge intrusion of reactive cells in the epidermis and dermis of injured tissue compared to other treated groups (Figure 4). The Durba extract treated wounds healed early than the control group characterized by moderate infiltration of reactive cells in the epidermis and dermis of the injured tissue (Figure 5). In the control group deposition of tissue debris were found in the injured area. The healed tissues contain wider areas of hemorrhages and congestion with infiltration of reactive cells predominantly neutrophils (Figure 6). On day 7 of wounding border plant extract treated wound appeared relatively free from tissue debris and exudates. Complete keratinization was only seen over the injured skin of border plant extract treated wounds following 7 days of treatment compared to incomplete keratinization in Durba extract treated group and lack of keratinization in control group.

DISCUSSION

In this study, morphological changes, swelled area, wound contraction and width of the sutured area were observed postoperatively to compare the effect of Border plant and Durba in the treatment of wound healing. The augmented swelling and width of sutured area after treatments were recorded up to day 3 (D_3) post operation. The result of this study showed differences and importance in choosing the appropriate topical medication for a wound.

It is observed that diameter of the wounds treated by border plant decreased significantly faster in comparison to that of durba extract treated group and the diameter of the wounds treated by durba extract decreased faster in comparison to that of control group. Normally, the increased inflammation of wound area after treatments was observed up to day 4 (D_4) post operation. It is due to inflammatory response of the reactive cells featured by the infiltration of macrophages, neutrophils and lymphocytes in sequence (Gosain and Dipietro, 2004; Broughton *et al.*, 2006; Campos *et al.*, 2008). Similar changes are found in our research. The inflammatory cells come within 2 to 4 days and it is the indication of the inflammatory stage which is an index of accelerating wound healing process. These inflammatory cells engulf the bacteria and other cells to make the wound environment fresh. Fresh wound is prerequisite for wound healing.

Mallick *et al.* (2017) found the healing time of wound in 22-26 days. In this research work, the healing time of wound varies from 11 to 18 days. The higher rate of healing was observed in border plant which is an agreement with Alam *et al.* (2005). The granulation tissue appeared from 3rd day onwards in all treatment groups. This finding corresponds with Hossain *et al.* (1992). Pigmentation and cicatrization was higher in Group A and Group B until day 12 treatment, while Hossain *et al.* (1992) found these features on 11 days. This could be due to the use of younger goats in the present study because healing is rapid in the younger animals. Redness was found in all treatment groups from 3rd day of wounding. This may be due to extravasation of ruptured blood vessels.

The marked decrease in the width of sutured line in all wounds from day 10 regardless of treatment might be due to the antiseptic properties of border plant and durba grass which help the centripetal movement of wound edges. The variation of diminishing contraction length per week in wounds of all groups was insignificant. This outcome is in conformity with the statement that the contraction of wound depends on the myofibroblast positioned at the wound periphery, its association to extra cellular matrix components and the proliferation of myofibroblast (Rohrich, 1991).

The microbiological study showed that bacterial colonies were present in all samples obtained from border plant and durba grass treated group at day 3 of treatment. In this study, mainly *Staphylococcus spp, E. coli, Bacillus spp* and some unidentified organisms found in the wounds in all group which is in agreement with Trengove *et al.* (1996). These findings are also in conformity with the results of Bowler *et al.* (2001); Smith *et al.* (2003). It is reported by Trengove *et al.* (1996) that no single microbe or collection of microbes was more injurious to wound healing than any other (inclusive of *P. aeruginosa, S. aureus*, beta-hemolytic streptococci, coliform and anaerobic bacteria). However, a markedly lesser possibility of healing was seen if four or more bacterial groups were existent in any wound and this designates the microbial interaction could have encouraged an enhanced pathogenic consequence. But the microorganisms found in this study do not hamper the healing process of the wound. It may be due to the antibacterial activity of border plant and durba grass.

Histopathological evaluation of biopsies collected from various treatment groups was concentrated on

the regeneration of epidermis, fibroblast, proliferation of blood vessels and fibrous connective tissue; proliferation, migration and differentiation of inflammatory cells. The inflammatory changes in the regenerating tissues were assessed on the basis of reactive cell infiltrations including lymphocytes, macrophages and neutrophils. Neutrophils are the first inflammatory cells to act during healing. They apparently regulate the microbial growth and sepsis (Silver, 1982). However, in border plant treated group, the degree of proliferation of fibrous connective tissue was the maximum.

The border plant extract treated wounds showed early stage of healing which is characterized by huge intrusion of reactive cells in the epidermis and dermis of injured tissue compared to other treated groups. On day 7 of wounding border plant extract treated wound appeared relatively free from tissue debris and exudates. Complete keratinization was only seen over the injured skin of border plant extract treated wounds following 7 days of treatment compared to incomplete keratinization in Durba extract treated group and lack of keratinization in control. The lymphocyte and macrophage were found disappeared from the border plant extract treated group on day 7 post operation. It indicates that border plant extract promotes healing of wound compare to the others.

CONCLUSIONS

Both the border plant and durba can be clinically effective in wound healing. However, border plant treated wounds showed early subsiding of inflammation, faster wound healing and improved infection control than that of Durba extract. The broadcasting of these outcomes among the veterinarians may benefit the livestock health and thus can inhibit skin problems due to wound complications. Besides, clinicians should be aware of the beneficial effects of border plant and durba extract and consider it as a practical choice for wound care.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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