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Comparative Efficacy of Two Medicinal Plant Extracts and an Antibiotic on Wound Healing

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Abstract: The present investigation was carried out to study the comparative efficacy of two medicinal plant extracts and an antibiotic on wound healing. This was conducted on 18 female goats at BAU Veterinary Clinic. Infiltration anesthesia was performed with 2% lignocaine hydrochloride (Jasocaine®, Reneta Pharmaceuticals Ltd.) at the site of operation on lumbar region. Four experimental skin wounds, two in either side of the midline plane at the lumbar region were made, each was of 2.5 cm length and 0.5 cm depth. The experimental animals were studied under 6 Groups. A series of 50 and 30% extracts of border plant (*Aerva sanguinolenta*), thankuni plant (*Centella asiatica*), antibiotic (Pronapen®) and saline were used in this experiment and these were applied twice daily to each wound. All extracts seemed to be effective for rapid healing. A 50% extracts of border plant showed the best result where healing time was 14 days whereas in antibiotic it took 16 days. In contrast more time (20 days) was required for control group. Clinical feature revealed exudation, scab formation, redness and other routine changes. Redness was more prominent in control and antibiotic treated groups compare to other groups on day 3 of wounding. Thus both border and thankuni plant extracts are very much effective for wound healing and can be applied instead of antibiotic.

Key words: Comparative efficacy, plant extracts, antibiotic, wound healing

INTRODUCTION

Livestock is an important sub-sector and contributes about 3.9% of total foreign income in Bangladesh^[1]. Among livestock goat is the second highest population, which accounted for 21.70 million heads^[2]. The goats rank second in term of meat and skin production. Black Bengal goats are highly preferred because of their high quality meat and skin in this country. However, it is worth mentioning that the devastating diseases especially various types of wounds affecting these two properties of goat and thereby degrading their quality every year. As a whole annual (1990-91) economic loss associated with leather defects in Bangladesh is over 220 million dollars^[3]. External wounds interfere with the normal food intake of the animals resulting in decrease in milk production and growth rate. The reproductive performance of the animal may also be decreased which eventually affects the national economy.

In Bangladesh most of the farmers do not have the facility for modern treatment for their livestock. In addition, many farmers cannot afford the cost of treatment. Under the circumstances, the rural farmers and owners tend to incline towards traditional systems of animal treatment, which are relatively less expensive and

available. So before taking the animal to the veterinarian, the owners try to treat their animals with various local remedies of which plant sources are mostly common.

Research work in this field is still limited in Bangladesh. So the present research was designed to investigate the effect of thankuni and border plant and its comparison with antibiotics in the healing of artificially produced aseptic wounds.

MATERIALS AND METHODS

Experimental animals: A series of experiments were performed in eighteen apparently healthy Black Bengal goats (*Capra hircus*). The body weight of the animal ranged from 7-8 kg, all the animals were female and age ranged from 8-12 months. The animals were replaced by new group, once the experiment was done to find out the effect of plant extracts on fresh wound. All of the experimental goats were dewormed with albendazole (Almax®, Square pharmaceuticals Ltd.) prior to experiment.

Plants used in the experiment: Two types of plants were used, Border plant (*Aerva sanguinolenta*) and Thankuni plant (*Centella asiatica*).

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Preparation of the herbal extracts: Fresh herbal extracts were prepared from leaves of border plant (*Aerva sanguinolenta*) and thankuni plant (*Centella asiatica*). The fresh leaves were collected from Bangladesh Agricultural University garden. The leaves were properly cleansed with water and air dried. The green leaves were taken in a mortar and grinded thoroughly with a pestle. Then the crude extracts were filtered with a small pore strainer. The leaves extracts were mixed with distilled water and 50 and 30% extracts were made.

Antibiotic used in the experiment: Procaine penicillin (Pronapen®, 4 lack IU vial, Renata Ltd.) was used as powder for local infiltration (after mixing with distilled water) in the wound.

Preparation of the wound: The operation sites were clipped, washed with soap and water, shaved and prepared with Iosan (Ciba Geigy, Switzerland Ltd.). The animal was then placed on the operation Table on left lateral recumbency. Infiltration anesthesia was performed using 2% lignocaine hydrochloride (Jasocaine®, Jayson pharmaceuticals Ltd.) at the lumbar region i.e. at the site of operation. Then four wounds, two on either side of the midline at the lumbar region were produced, each was of 2.5 cm length and 0.5 cm depth. The wounds produced in all experimental groups were left unsaturate.

Experimental design: After making wound, the goats were randomly divided into 6 Groups with 3 animals in each group.

Group A: 50% extract of the Border plant (*Aerva sanguinolenta*) was applied to four wounds made in each animal two times daily after washing the wounds with distilled water soaked sterile gauze. These animals were maintained carefully so as to avoid interference with granulation tissue formation.

Group B: 30% extract of Border plant (*Aerva sanguinolenta*) was applied to four wounds made in each animal. The treatment schedule was same as in Group A.

Group C: 50% extract of Thankuni plant (*Centella asiatica*) was applied to four wounds made in each animal. The treatment schedule was same as in Group A.

Group D: 30% extract of Thankuni plant (*Centella asiatica*) was applied to four wounds made in each animal. The treatment schedule was same as in Group A.

Group E: One vial of Pronapen® (4 lack IU) was applied to four wounds made in each animal. The treatment schedule was same as in Group A.

Group F: This group was kept as control. In this group normal saline (0.9%) was applied to four wounds made in each animal.

Gross observations: The progress of healing in animals of each group was monitored daily. The changes were recorded every 3 days until healing. A wound was assumed healed up when cicatrisation and pigmentation was found.

Biopsy and histopathology: The biopsies (1.5x1.5 cm) were collected from the wound areas in each experimental animal on the 3rd, 7th and 21st days after wounding using standard surgical procedure. The wound tissue contained dermis and epidermis, the samples were fixed in 10% buffered neutral formalin solution for histopathology. Histopathological slides were prepared according to the method of Luna^[4].

RESULTS

Morphological changes during wound healing: Border plant extract (in both strength 30 and 50%) and thankuni extracts (30 and 50%) seemed to be effective for healing of the wounds. but border plant extracts (50%) showed best results where healing was completed in 14 days. with border plant, both 50 and 30% extracts were more effective than those of same strength of thankuni plant extracts and antibiotics. In case of control group more time was taken for complete healing of the wound compared to extract treated group (Table 1).

The exudation, reddening, dryness of the wound, pigmentation and cicatrisation were observed. Exudation occurred on the first day of wounding in all experimental groups (groups). The healing process started from the second day characterized by thin scab formation due to drying of the exudates on the wound surface. On 3rd day

Table 1: Granulation and complete healing time in different treatment groups of Black Bengal goats

Groups	Preparations	Range of granulation time (days)	Complete healing time (days)
A	50% border plant extract	3-11	14
B	30% border plant extract	3-13	15
C	50% thankuni plant extract	4-13	15
D	30% thankuni plant extract	4-15	17
E	Antibiotic Pronapen® (4 Lack IU)	4-14	16
F	Saline (0.9%)	7-17	20
(control)			

Table 2: Characteristic clinical features at different stages of wound healing with different plant extracts and antibiotics

Days	Changes	Border plant extract		Thankuni plant extract		Antibiotic	Control (Saline)
		50%	30%	50%	30%		
1st	Exudation	+	+	+	+	+	+
3rd	Reddening	++	++	++	++	+++	+++
	Dryness	+	+	+	+	-	-
7th	Reddening	+	+	+	+	++	++
	Dryness	+	+	+	+	+	+
11th	Cicatrization	++	+	+	+	+	-
	Pigmentation	++	+	+	+	+	-
15th	Cicatrization	+++	++	++	++	++	+
	Pigmentation	+++	++	++	++	++	+

- = Absent; +++ = Massive; ++ = Moderate; + = Mild

reddening was prominent in control and antibiotic treated groups while moderate in all extracts treated groups. The wound was dry in all extracts treated groups but moist in saline and antibiotic treated groups at day 3 of wounding. On 7th day moderate reddening was observed in saline and antibiotic but mild in all extracts treated groups. Cicatrization and pigmentation were observed in all extracts treated groups but prominent respond was found in 50% border plant extract treated group at day 11 of wounding. In control group (saline) these changes were observed from the 15 day of wounding (Table 2).

Histopathological changes: Histological assessment of biopsies taken from various treatment groups were focused on the regeneration of epidermis, fibroblasts, proliferation of blood vessels and fibrous connective tissue. The inflammatory lesions in the regenerating tissues were evaluated based on the infiltration of reactive cells including macrophages, lymphocytes and neutrophils. The highest degree of inflammation was observed in the animals of control group, followed by antibiotic treated group. Mild to moderate inflammatory response was observed in all extract treated groups at day 3 of wounding. There was lowest degree of inflammation in all groups at day 21. In antibiotic treated groups the predominant tissue response was fibrous connective tissue proliferation while in other groups mild fibrous connective tissue proliferation response was observed.

The border plant extract (both) treated wound showed early stage of healing which is characterized by moderate infiltration of lymphocytes and macrophages compared to other treated groups. Subcutaneous tissue appeared less responsive to the reaction of artificial wound in presence of border plant extracts. On the other hand, in antibiotic treated group, the subcutaneous tissue showed inflammatory reaction characterized by deposition of reactive cells and congestion of blood vessels. There was marked thickening of the gap between the wound edge in antibiotic and control group due to marked deposition of inflammatory exudates.

The epithelial lining of the epidermis started regenerating from the edge of the wound but covered a little distance in border plant extract treated group at day 7 of wounding, which was absent in other groups. The healed tissue showed histological restoration of the collagen tissue of the dermis, but the response appeared moderate in border extract, little in thankuni extract and absent in antibiotic treated group. On day 7 of wounding border extract treated wound appeared relatively free from tissue debris and exudates. Disappearance of macrophage and lymphocyte as seen in the border plant treated wound which was uncommon in thankuni extract, antibiotic and control groups. The gap between the edges of the wound was also found sharply closure in the border treated group than other treated groups. Hemorrhagic blood was separated from the underlying tissue in extracts treated groups while a clotted blood was present over the healed tissue in antibiotic and saline group on day 7 of wounding. Subcutaneous tissue appeared entirely free from inflammatory reaction. The thickening of fibrous connective tissue getting reduced in the healed tissue in all the treated groups. Variable sizes empty spaces in the fibrous connective tissue were seen in antibiotic group.

On day 21, all the treated groups healed tissue showed same picture. In case of antibiotic treated group the regenerating epidermal cellular layers appeared thicker compared to extracts treated groups. There was marked thickening of the keratinized layer of epidermis in Groups A and B (50 and 30% border extract), mild in Group B and C (50 and 30% thankuni extract) while thin keratinized layer in Groups E (antibiotic) and F (control). The hair follicle started regenerating in the healed tissues in thankuni extract treated group.

DISCUSSION

Gross observations: The ability of the wound to heal is a biological process, which follows a definite pattern of cellular and molecular events, ultimately leading to complete repair of the injured tissues. High order animals possess very limited regenerative capabilities except for those of some organs e.g. liver, parenchyma or in simple tissue e.g. connective tissue, epithelium and outer surface of the skin. The healing rate differs among species and tissues and even between sites of the same tissue. However, in general the pattern of wound healing in domestic ruminants appears to be similar. The initial events of wound healing mechanism occurred smoothly under favourable condition. The normal process of wound healing may be disrupted. During this condition before using drugs, many herbal preparations are extensively used in the rural area.

In our study, the rate of healing time of wound varies from 14 to 20 day. The higher rate of healing was

observed in 50% border plant extract treated group followed by other extracts and antibiotic which is in agreement with Sivdas^[5], Santhanam and Nagarajan^[6], Hossain *et al.*^[7] and Eurides *et al.*^[8]. The granulation tissue appeared from 3rd day onwards in all treatment groups. This finding corresponds with Hossain *et al.*^[7]. Pigmentation and cicatrization was found in all treated groups from 11 day onwards, while Hossain *et al.*^[7] found these features on 12 and 15 days, respectively. This could be due to the use of younger goats in the present study because healing is rapid in the younger animals. Poor and high level of nutrition or in specific protein deficiency may cause delayed wound healing. Redness was found in all treatment groups from 3rd day of wounding. This may be due to extravasation of blood when blood vessels are ruptured.

Histopathological changes: Morphological changes in the regenerating tissue in different groups of animals were successfully studied. The degree of inflammation and proliferation of blood vessels in the regenerating tissue was higher in control group at day 3 post operation compared to treated groups. However, the highest degree of fibrous connective tissue proliferation was observed in antibiotic treated group. This result is in agreement with Yeasmin^[9].

Proliferation of fibrous connective tissue was moderate to antibiotic treated tissue mild in other extract treated group at day 3 post operation. Highest proliferation was found in antibiotic treated group. The first inflammatory cells to appear during healing are the neutrophils, which presumably control the microbial growth and sepsis^[10]. Neutrophilic infiltration in healed tissue was not observed in the present study. This may be due to fact that the tissues were collected on day 3 post operation by which time, the neutrophilic infiltration was replaced by macrophages and lymphocyte. The highest number of mononuclear cells (lymphocyte and monocyte) at the day 3 experiment was seen in the control group compare to other groups. Lymphocytes appear in greater percentage due to stimulation of foreign antigen. This result also correlates with Yeasmin^[9]. The lymphocyte and macrophage was found disappeared from the border plant extract treated group on day 7 post operation. It indicates that border plant extract promote healing of wound compare to others.

The thickening of fibrous connective tissue getting reduced and containing variable size and empty spaces in antibiotic group due to deposition of inflammatory fluid. There was no significant variation of healing stage in different groups on day 3 post operation. The gap of the wound appeared closure in border extract group than other treated groups indicated less irritation and reduce infiltration of exudate. The epithelium of the epidermis in the border extract treated group showed the healing tendency. The epithelial lining of the epidermis starts

regenerating from the edge of the wound but covered a little distance in border plant extract treated group at day 7 which, however, was not seen in other groups. There was marked thickening of the keratinized layer of epidermis in both strength of border extract treated groups, mild thickening in thankuni extract treated (50 and 30%) groups. While thin keratinized layer in antibiotic and control group. The hair follicle started regenerating in the healed tissues on 21 day of wound healing in the thankuni extract treatment group. At the end of study it did appear that the wounds in all the treated groups were healed by epithelial regeneration at the top and fibrous connective tissue proliferation at the bottom. However, the final fate of proliferating connective tissue in the dermis was not studied due to the study designs for short period of time. Lastly it may be concluded that instead of antibiotic extract of birder and thankuni plant (especially 50%) may be used for aseptic wound healing in Black Bengle goat.

REFERENCES

1. BBS, 2001. National Accounts Statistics (Gross Domestic Product, 2000-2001), Bangladesh Bureau of Statistics, Ministry of Planning, Government of the People's Republic of Bangladesh, Dhaka.
2. Hossain, M.M. and M.A. Islam, 2003. Infectious diseases of goats. Ninth BSVER Annual Scientific Conference held at Bangladesh Agricultural University, Mymensingh on 6-7 January 2003. BSVER Publication, 24: 20.
3. Dey, A.S. and M. Nooruddin, 1993. Economic impact of leather defect in Bangladesh. Bangladesh J. Training and Development, 6: 27-38.
4. Luna LGHT (ASCP), 1968. Manual of Histological Technique. 3rd Edn., McGraw/Hall Book Company.
5. Sivdas, C.G., 1980. Preliminary study on traditional systems of veterinary medicine. FAO Regular Programme, RAPA., 49: 49-58.
6. Santhanam, G. and S. Nagarajan, 1990. Wound healing activity of *Curcuma aromatica* and *Piper betle*. Fitoterapia, 61: 458-459.
7. Hossain, M.A., M.A. Hye and A.S.M. Bari, 1992. Evaluation of indigenous medicinal plants in the treatment of external wounds. Bangladesh Veterinarian, 9: 55-60.
8. Eurides, D., A. Mazzanti, G.F. Goncalves, M.E. Belletti, L.A.F. Silva, M.C.S. Fioravanti, N.S.T. Chaves, P.P. Bombonato, V.A. Campos and A.S. Ogata, 1998. Morphology, morphometrics and histology of wound healing of mice skin treated with copaiba oleoresin (*Copaifera langsdorfii*). Veterinaria-Noticias, 4: 77-82.
9. Yeasmin, F., 1992. Wound healing in Black Bengal goats. M.Sc Thesis, BAU, Mymensingh, Bangladesh.
10. Silver, I.A., 1982. Basic physiology of wound healing. Equine Vet. J., 14: 17.