Available Online at http://www.journalajst.com

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN: 0976-3376

Asian Journal of Science and Technology
Vol. 08, Issue, 11, pp.6807-6811, November, 2017

RESEARCH ARTICLE

CLINICAL EVALUATION OF *Terminalia arjuna* ON WOUND HEALING IN CAPRINE

***Acharya, P. R., Aher, V. D., Gangane, G. R. and Rajurkar, S. R.**

College of Veterinary and Animal Sciences, Parbhani 431402

ARTICLE INFO

Article History:
Received 16th August, 2017
Received in revised form
29th September, 2017
Accepted 07th October, 2017
Published online 30th November, 2017

Key words:
Wound healing,
Terminalia arjuna,
Caprine,
Biochemical & Haematological,
Histopathology & Histochemistry.

ABSTRACT

The study was undertaken on the clinical cases of wounds in caprine presented at Teaching Veterinary Clinical Complex, Parbhani and area around the vicinity of Parbhani city. A total of 12 clinical cases of lacerated wounds in caprine were studied for the evaluation of 5% ointment of *Terminalia arjuna* compared with 5% povidone ointment. These clinical cases were divided in to two groups comprised of six cases in each group, irrespective of the type of wound, location, age, sex, breed and body weight. All the cases were observed carefully for the effect of both the treatment in each group. Clinico-pathological observations were recorded on 0, 5th and 10th day, respectively. Haematological and biochemical estimations were carried out by collecting blood samples and serum on 0, 5th and 10th day in each group. Microbiological estimation was carried out by collecting wound swab on 0, 5th and 10th day, respectively. Histopathological and histochemical study was carried out by collecting tissue samples of wound on 0, 7th and 21st day, respectively. The quality of wound healing was measured on the basis of reduction in wound area and wound contraction percentage. The mean values of percentage wound contraction in all cases of both the groups were recorded. Reduction in wound area percentage and rate of wound contraction percentage in group B was found statistically highly significant on day 5 and 10 on compared group A. Haematological values such as Hb, PCV, TLC, TEC and ESR were within normal physiological range, except eosinophil count was significantly increased on day 5 and day 10 in group B while monocyte count was decreased on day 5. However, in biochemical study the total serum protein values were decreased on day 5 which were within normal physiological range. Antimicrobial activity of *T. arjuna* ointment showed that there was decrease in microbial load in wounds treated on 0, 5th and 10th day compared to wounds treated with povidone ointment. Histopathological and histochemical observations revealed comparatively better results in wound healing with epithelialization, neovascularisation and collagen development, than povidone ointment treated group. From the above observations it concluded that 5% methanolic extract of bark of *T. arjuna* had better wound healing ability in caprine compared to 5% povidone ointment.

INTRODUCTION

Now a day goat industry is developing in our country. A considerable number of surgical affections are found in goats and associated with defects that affect leather quality, performance or cause death (Bhuyan *et al*., 2008). Plants and their extracts have great potential in the management and treatment of wounds. Herbal medicine is based on the fact that plants contains natural substances that promote health and making illness less severe. Due to their traditional applicability, affordability and safety plants gained a reputed position in the area of wound management. There are many such herbal plants which promote wound healing. One such of that is *Terminalia arjuna,* large evergreen tree which is reputed for wound healing property. *Terminalia arjuna* can be used in the treatment of wounds, inflammation, coronary artery diseases, heart diseases and diabetes. Improvement in cardiac muscle function of the heart is the primary benefit of *T. arjuna*. The flavonoid provides free antioxidant activity and vascular strengthening. The tannins provide significant increase in the tensile strength of incision wounds and increase in the percent reduction in wound size of excision wound (Rane and Mengi, 2003). Hence the present study was planned to find out wound healing activity of *Terminalia arjuna* in caprine.

Corresponding author: **Acharya, P. R.**
College of Veterinary and Animal Sciences, Parbhani 431402.

MATERIALS AND METHODS

The present study was conducted on 12 clinical cases of wound in caprine presented at Teaching Veterinary Clinical Complex, College of Veterinary and Animal Science, Parbhani as well as in and around vicinity of Parbhani city. In each group A and group B, 6 cases irrespective of type of wound, age, sex, breed, body weight, etc. In Group A, the wound area was flushed with normal saline and cleaned with cotton and then topically 5 % povidone ointment[1] was applied on 0, 5th and 10th day respectively. In Group B, the wound area was flushed with normal saline and cleaned with cotton and then

topically 5 % methanolic extract ointment of *Terminalia arjuna* was applied on 0, 5th and 10th day, respectively. *Terminalia arjuna* extract ointment was prepared in 5% (w/w) by mixing 5 gm. bark extract and 95 gm. of petroleum jelly.

Quality of healing

Clinical observations of type of wound, etiology and location of wound with wound area changes, wound contraction, epithelization and scar formation were recorded on 0, 5th and 10th day of treatment. Quality of healing was assessed by recording wound contraction by tracing of graph paper on wound on 0, 5th and 10th day and it was calculated in Cm^2. Percent wound contraction in both groups was calculated in all animals using following formula.

$$\% \text{ of wound contraction} = \frac{X - Y}{X} \times 100$$

Where,

X = maximum wound area on 0 day

Y = maximum wound area on 5th and 10th day

Haematological estimations

Blood samples were collected in sterilized EDTA vials on 0th, 5th and 10th days of treatment. Haematological parameters such as Haemoglobin, Packed Cell volume, Total leukocyte count, Total erythrocyte count, Differential leukocyte count and Erythrocyte sedimentation rate were studied.

Biochemical estimation

The total serum protein (TSP) estimation was carried by collecting fresh blood samples on day 0, 5th and 10th of treatment.

Evaluation of antimicrobial activity

Swabs from wound surface were taken on 0th, 5th and 10th day for microbial studies. Total Viable Count (TVC) was calculated by using standard formula. TVC of swab samples was expressed as log CFU/cm^2. Colonies were counted with the help of bacteriological colony counter and TVC of swab sample was calculated by using the following formula:

$$\text{CFU/sq.cm} = \frac{SC}{[n_1+(0.1\times n_2)]\times D}$$

Where,

SC	=	Total number of colonies counted
n_1	=	No. of plates of lower dilution
n_2	=	No. of plates of higher dilution
D	=	Dilution factor

Histopathological and Histochemical Studies

On day 0, 7th and 21st from both group A and group B wound tissue samples were collected for histopathological and histochemical studies. For histopathological changes taking place during the healing process, sections were subjected to routine Haematoxylin & Eosin (H & E) stain while Periodic Acid Schiff (PAS) and Van Gieson's (VG) staining protocol for observation of histochemical changes to assess the wound healing.

RESULTS AND DISCUSSION

During the study period it was observed that from both the group A and group B, all of 12 cases were lacerated wounds. Five wounds were located on thigh region, two wounds on thorax region, two wounds on neck region, one wound close to stifle joint, one wound on knee joint and one wound on fetlock joint. Etiology of wounds in 9 cases were due to dog bite, 2 cases were of trauma and 1 was caused by accident due to automobile injury. Marked reduction in size of fresh wounds were observed compared to infected wounds. The involvement of deeper tissue in wounds like muscle and joint were responsible for delayed healing compared to superficially located wounds. Similar findings were reported by Bhat (2012) while studying the clinical evaluation of *Cissus quadrangularis* on wound healing in caprine and Singh (2016) while studying the effect of platelet rich fibrin on wound healing in caprine. The quality of wound healing was measured on the basis of reduction in wound area % and percentage wound contraction in both group A and group B on day 0, 5th and 10th post treatment. Reduction in wound area % and rate of wound contraction in group B was found statistically highly significant on day 5 and day 10 compared to group A. Devi *et al.* (2012) while studying wound healing activity of *Terminalia arjuna* in albino Wistar rats observed that groups of rats pre-treated with ethanolic extract of *T. arjuna* showed more significant difference from control group. The Mean ± S.E. values of haemoglobin, packed cell volume, total leukocyte count, total erythrocyte count, erythrocyte sedimentation rate does not shown any significant difference in both group A and group B on day 0, 5 and 10 post treatment. In differential leukocyte count it was observed that neutrophil and lymphocyte count were not shown any significant difference while eosinophil and monocyte count were changed significantly on 5th and 10th day. Anumol *et. al.,* (2012) studied haemato-biochemical alterations in goats infected with coccidiosis and observed non significant increase in oesinophil in coccidia infected goats. No basophils were observed in both group A and group B on 0, 5th and 10th day post operative. The obtained results were within normal physiological range, which were similar to observations recorded by Thosar (2011), Bhat (2012) and Singh (2016).

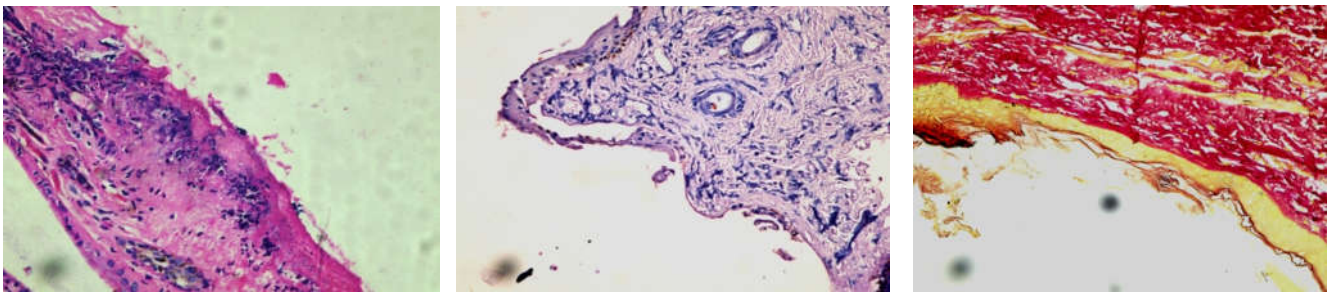
The TSP (gm/dl) values of animals from both group A and group B were decreased on day 5 with high significance and again it was increased non significantly on day 10 in group A while steady non significant decrease were observed in group B. All the values were within normal physiological range except in group A on day 5 which was decreased below the normal TSP range in goat. Similar findings of TSP were encountered by Thosar (2011) while studying wound healing in bovine and he commented that it could be due to the disturbances in protein metabolism. Ahmed *et al.* (2015) observed the TSP levels in goats suffering from gastrointestinal parasitism in Jaipur district of Rajasthan. Results indicated that there was significant decrease in total protein and albumin level was observed in infected goats in comparison to healthy animals. The mean ± S.E. values of TVC (log CFU/cm^2) in animals of group A and group B were observed consistent decrease on day 5 followed by day 10.

Table 1. Mean ± S.E. values of parameters in animals of group A and group B on Day 0, 5 and 10

S. No.	Parameters	Day 0		Day 5		Day 10	
		Group A	Group B	Group A	Group B	Group A	Group B
1.	Wound area (%)	0±0.00	0±0.00	79.68±5.10	57.22±4.58	56.94±7.82	38.14±11.80
2.	Wound Contraction (%)	0±0.00	0±0.00	20.30±5.10	42.76±4.58	42.21±8.03	61.85±11.80
3.	Haemoglobin (g%)	8.83±0.20	9.10±0.36	8.86±0.20	9.03±0.38	8.86±0.20	8.96±0.38
4.	PCV (%)	27±1.43	26±1.03	27.33±1.33	25±1.12	27.33±1.33	25±1.12
5.	TLC ($\times10^3$/ µl)	9.31±0.44	9.4±0.25	10.41±0.48	10±0.45	8.6±0.84	8.78±0.39
6.	TRC ($\times10^6$/ µl)	11.19±1.20	12.11±0.90	11.31±1.01	12.05±1.06	11.33±1.04	12.09±0.99
7.	ESR (mm/24hr)	2.23±0.08	2.16±0.10	2.21±0.06	2.23±0.09	2.23±0.07	2.26±0.09
8.	DLC (%)						
a.	Neutrophil	43±0.96	41.5±0.71	43.16±0.60	41.83±0.30	42.5±0.67	41.66±0.49
b.	Lymphocyte	50.83±0.65	52±0.81	51.16±0.30	50.83±0.54	51.16±0.40	51.33±0.42
c.	Eosinophil	4.16±0.40	4.33±0.33	4.16±0.47	5.33±0.21	4.33±0.21	5±00
d.	Monocyte	2.16±0.30	2.16±0.16	1.5±0.22	2±0.25	2±0.25	2.16±0.30
9.	TSP (gm/dl)	6.65±0.17	6.79±0.40	4.89±0.39	6.30±0.38	5.19±0.34	5.44±0.43
10.	TVC(logCFU/cm^2)	4.45±0.01	4.46±0.03	4.37±0.01	4.26±0.05	3.29±0.00	3.21±0.05

Group A on day 0, 5 and 10

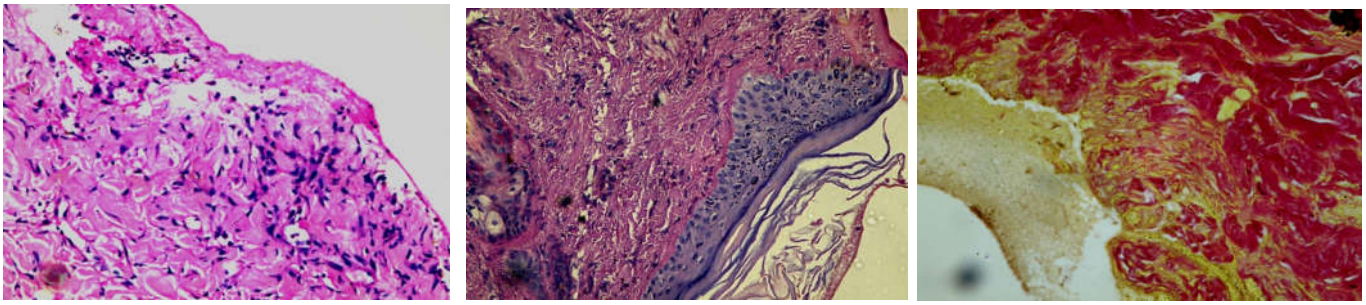
Group B on day 0, 5 and 10



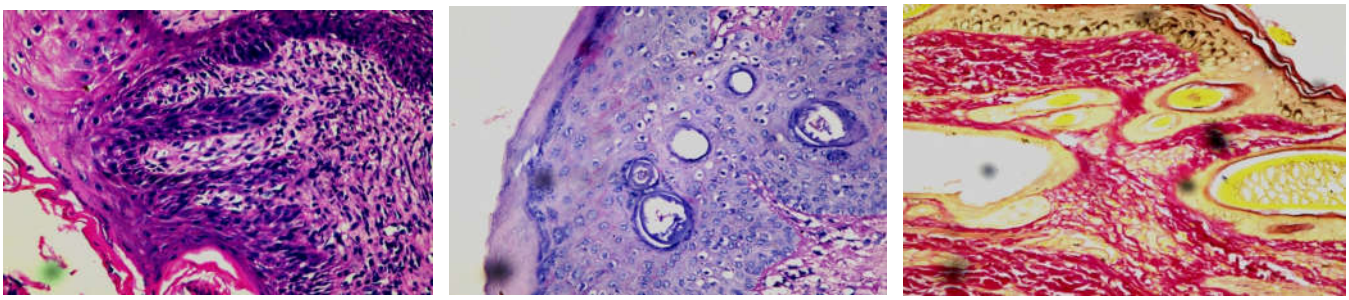
a. **b.** **c.**

a. Note the distorted epithelium with infiltration of inflammatory cells (×100 H&E), b. Note the distorted epithelium with infiltration of inflammatory cells (×400 PAS), c. Note the broken and distorted superficial epithelial lining (×400 VG)

Showing histopathological and histochemical observations on Day 0



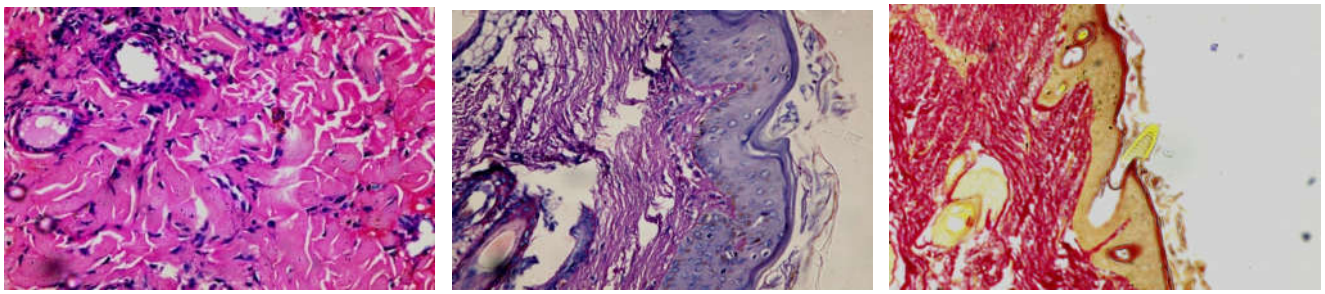
a. b. c.



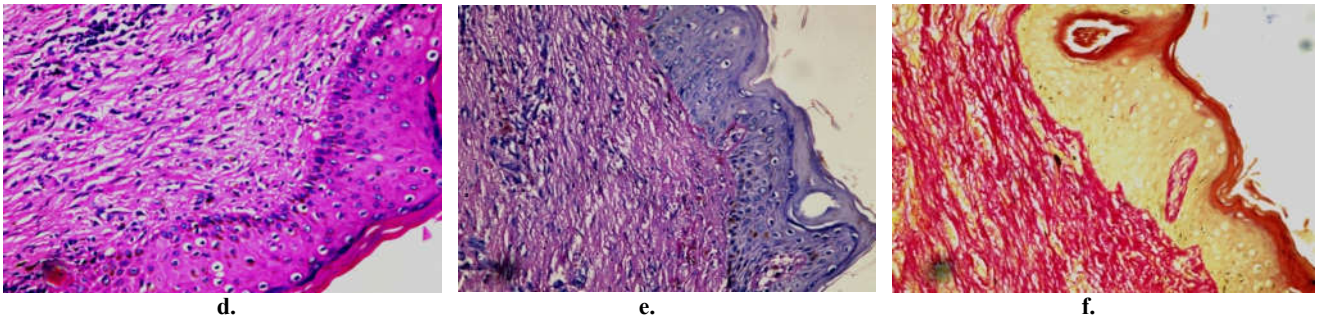
d. e. f.

Group A: a. Note the minimal infiltration of inflammatory cells along with epithelialisation, granulation and fibrous tissue proliferation (×400 H&E), b. Note the minimal infiltration of inflammatory cells along with epithelialisation, granulation and fibrous tissue proliferation (×400 PAS), c. Note the minimal deposition of collagen and elastic fibre (×400 VG). Group B: d. Note infiltration of inflammatory cells along with epithelialisation, granulation and fibrous tissue proliferation (×400 H&E), e. Note the granulation and fibrous tissue proliferation and contraction with epithelisation (×400 PAS), f. Note the epithelial lining regeneration with mild to moderate amount of collagen fibre and elastic fibre (×400 VG).

Showing histopathological and histochemical observations on Day 7



a. b. c.



d. e. f.

Group A: a. Note the inflammatory cell infiltration with neovascularisation and fibrous tissue proliferation (×400 H&E), b. Note the reparative organization of epithelium (×400 PAS), c. Note the completely developed epithelium in a healed wound (×400 VG).

Group B: d. Note the inflammatory cell infiltration in the newly developed portions of the wound with epithelisation (×400 H&E), e. Note the properly established epithelialisation with development of major layers of epithelium (×400 PAS), f. Note the properly developed epithelialisation with development of collagen and elastic fibres (×400 VG).

Showing histopathological and histochemical observations on Day 21

But the difference between mean TVC of group A and group B was non significant. This indicated the consistent antimicrobial activity of 5% methanolic extract of bark of *Terminalia arjuna.* The decrease in microbial load in wounds of group A can by attributed to application of povidone ointment on wound surface. Mandal *et al.* (2013) determined the antimicrobial activity of the methanolic *T. arjuna* bark extract using agar well diffusion method. The extract were tested against two Gram-positive *S. aureus, S. mutans*and two Gram-negative *E. coli and K. pneumonia,* human pathogenic bacteria. The results showed that the remarkable inhibition of the bacterial growth was against the tested organisms.

On day 0, cases from both the groups showed severe infiltration of inflammatory cells with minimal exudation at places, disrupted superficial epithelial lining, indicating lacerated wound involving major part of epithelium and partial dermal involvement. Histochemical observations did not reveal any evidence of epithelial lining regeneration with broken superficial epithelial lining. On 7th day, tissue biopsy samples from *T. arjuna* group revealed moderate inflammatory cells infiltration, granulation and fibrous tissue proliferation and contraction with epithelialisation. Initiation of epithelial lining regeneration, mild to moderate amount of collagen fibre and elastic fibre were observed with neovascularisation.

Tissue biopsy samples collected from *T. arjuna* group on day 21st showed properly established epithelialisation, which was evidenced by development of major layers of epithelium. The healed portions treated wound was with marked neovascularisation and minimal inflammatory cell infiltration. In group A, organization of epithelium was observed. There was neoangeogenesis with inflammatory cell infiltration in the newly developed portions of wound. Rane and Mengi (2003) recorded similar observations using *T.arjuna* bark on incision and excision wounds in rats and revealed that tannins were the important phytoconstituent responsible for wound healing mainly due to their astringent and antimicrobial property. Sayed (2016) observed the histochemical changes on wound healing in goats on day 0 and 21st using VG staining protocol and revealed that there was abundant, well arranged collagen formation with complete epithelization exhibiting better healing in *Euphorbia hirta* treated group compared with *Balanites aegyptica* group showing greater efficacy in wound healing.

Summary and Conclusion

The *T. arjuna* ointment on wound had a significant effect on rate of wound healing and contraction of wound as compared to povidone ointment treated group. Location of wound and wounds involving deeper tissue like muscle appeared to be the main factor for healing process. Reduction in wound area % and rate of wound contraction in group B was found statistically highly significant on day 5 and day 10 compared to group A. The values of haemoglobin, packed cell volume, total leukocyte count, total erythrocyte count, erythrocyte sedimentation rate and differential leukocyte count except eosinophil and monocyte does not shown any significant difference both group A and group B on day 0, 5 and 10 post treatment. The TSP (gm/dl) values were decreased on day 5 with high significance and again it was increased non significantly on day 10 in group A while steady non significant decrease was observed in group B. The values of TVC were shown consistent decrease on day 5 followed by day 10. Histopathological and histochemical changes were observed by collecting tissue biopsy samples on day 0, 7th and 21st and evaluated for wound healing activity on wounds treated with *T. arjuna* ointment and povidone ointment. The wounds treated with 5% *T. arjuna* ointment were showed comparatively better results in wound healing with epithelialization, neovascularisation and collagen development, than povidone ointment treated group.

REFERENCES

Ahmed A., M. A. Dar, A. A. Bhat, B. Jena, G. K. Mishra and R. P. Tiwari, 2015. Study on haemato-biochemical profile in goats suffering from gastrointestinal parasitism in Jaipur district of Rajasthan, *Livestock Science*, 6: 52-55.

Anumol J., P. V. Tresamol, K. Vinodkumar and M. R. Saseendranath, 2012. Haemato biochemical alterations in goats infected with cocccidiosis, *Tamilnadu J. Veterinary & Animal Sciences*, 8(3), 163-165.

Bhat M. A. 2012. Clinical evaluation of *Cissus quadrangularis* on wound healing in caprine. Unpublished M. V. Sc. thesis submitted to Maharashtra Animal and Fishery Sciences University, Nagpur.

Bhuyan A. A. M., M. M. Rehman, M. A. Hashim, M. K. Islam, M. N. Islam and A. Islam, 2008. Role of omentum in wound healing of goats. The Bangladesh Veterinarian 25(1): 1-8.

Devi H., S. Jothi, I. Singh, V. Subramaniyan, G. Silvarajah, S. Kaur and Y Annamalai, 2012. Wound healing activity of *Terminalia arjuna* in albino wistar rats. *International Journal of Phytopharmacology*, 3(3), 234-240.

Mandal S., A. Patra, A. Samanta, S. Roy, A. Mandal, T. D. Mahapatra, S. Pradhan, K. Das and D. K. Nandi, 2013. Analysis of phytochemical profile of *Terminalia arjuna* bark extract with antioxidative and antimicrobial properties, *Asian Pac J. Trop Biomed*., 3(12), 960-966.

Rane M. M. And S. A. Mengi, 2003. Comparative effect of oral and topical application of alcoholic extract of *Terminalia arjuna* bark on incision and excision wounds in rats. *Fetoterapia*, 74, 553-558.

Sayed M. A. 2016. Clinical efficacy of *Euphorbia hirta* and *Balanites aegyptica* ointments on wound healing in caprine. Unpublished M. V. Sc. thesis submitted to Maharashtra Animal and Fishery Sciences University, Nagpur.

Singh A. K. 2016. Clinical evaluation of platelet rich fibrin on wound healing in caprine. Unpublished M. V. Sc. thesis submitted to Maharashtra Animal and Fishery Sciences University, Nagpur.

Thosar S. R. 2011. Clinical evaluation of silver sulfadiazine and acriflavin on wound healing in bovine. Unpublished M. V. Sc. thesis submitted to Maharashtra Animal and Fishery Sciences University, Nagpur.

****