Explor Anim Med Res,
Vol.6, Issue - 1, 2016, p. 107-112

ISSN 2277- 470X (Print), ISSN 2319-247X (Online)
Website: www.animalmedicalresearch.org

Research Article

BIOCHEMICAL CHANGES IN THE WOUNDS OF GOATS FOLLOWING TREATMENT OF SUNFLOWER OIL AND OLIVE OIL

Abhishek Anand, L.L. Dass, A.K. Sharma*, K.K. Singh

Received 15 January 2016, revised 13 May 2016

ABSTRACT: Experimentally created forty eight wounds of similar size and shape were randomly divided in to three groups, of 16 wounds in each group. Sunflower seed oil impregnated gauze were subjected to the wounds of goats belonging to group I, while Olive oil (*Olea europaea)* impregnated gauze were subjected to the wounds of goats of group II and normal saline solution (control) soaked gauze to the wounds of goats of group III. Healing tissues were collected from the junction of wound and intact skin from all the experimental wounds in each of the three groups on 3, 10, 15 and 25 days. Biochemical examinations of healing tissue were done for collagen, elastin, hexosamine and hydroxyproline. The level of collagen, elastin, hexosamine and hydroxyproline were significantly higher in group I followed by group II and then group III. It can be concluded that both sunflower oil and olive oil are effective for acceleration of wound healing and sunflower oil is more effective than olive oil.

Keys word: Goat wound, Sunflower oil, Olive oil, Biochemical cahnges.

INTRODUCTION

The alternative of the use of antibiotics should be searched, as these may not perform properly in controlling of infections in near future (Garg and Chauhan 2003, Chauhan 2005).

Since time immemorial, human race has sought various means to facilitate wound healing with the goal of speeding the healing process, preventing infection, maximizing wound strength, minimizing scarring and preventing disability. It is well known that the rate of wound healing can be enhanced by providing best possible environment *i.e.* complete asepsis, removal of devitalized tissue, apposition of wound edges and regular dressing. Besides these basic measures, using certain herbs, which possess antiseptic, astringent, anti-inflammatory, antimicrobial and bio-stimulatory property, can also enhance the rate of healing. The plant derived antimicrobial wound healing agents may work through different pathways than commonly used antibiotics and chemotherapeutic agents and

Department of Veterinary Surgery and Radiology, College of Veterinary Sciences and Animal Husbandry, Birsa Agricultural University, Kanke, Ranchi-834006, Jharkhand, India.
**Corresponding author. e - mail: arsham10@rediffmail.com*

thus may be an additional or alternative way to combat the problem (Pattanayak *et al*., 2014). Several plants are reported to possess qualities for prevention and cure of common health and production related problems of animals (Pattanayak *et al*., 2013). Herb based medicines are cheaper and safer than allopathic drugs and so may be very useful in veterinary practice, especially in India where these are found in abundance. The present paper deals the biochemical alterations in the healing tissue treated with sunflower oil and olive oil topically.

MATERIALS AND METHODS

The study was conducted on forty eight surgically created wounds in twelve goats of either sex and aged between one and half years to two years [as per approval of Animal Ethical committee, Ranchi Veterinary College, Jhankhand, India]. The goats were grouped randomly into three of four animals each. Feed and water were withheld for 24 and 12 hours respectively before starting the experiment.On each goat, two skin depth wounds equal to the size of a metal template measuring 2cm by 2 cm were produced aseptically under field block at the proposed site on either side of the dorso-median plane, thus a total of 4 wounds were created in each animal. A distance of 20 cm was kept between the two wounds of same side and 7.5 cm between the contralateral wounds.

Sunflower seed oil impregnated gauze were subjected to the wounds of animal belonging to group I while Olive oil (*Olea europaea)* impregnated gauze were subjected to the wounds of animal of group II and normal saline solution (control) soaked gauze to the wounds of animal of group III. Gauze was maintained in position with micropore bandages. The dressings were changed daily. All the dressing were then covered with multiple tail bandages. Healing tissues were collected from the junction of wound and intact skin from all the experimental wounds in each of the four groups on 3, 10, 15 and 25 days. Tissues were collected in such a manner that it did not affect significantly the process of healing. All the biopsy tissues were preserved in 10% neutral buffer formalin. After proper fixation in formalin, the preserved tissues were processed routinely and microscopic sections of 5µ thickness were prepared for histopathological examination.

Biochemical examinations of healing tissue were done for collagen (Neumanand Logan 1950), elastin (Neumanand Logan 1950), hexosamine (Rondle and Morgan 1955) and hydroxyproline (Neumanand Logan 1950).

Estimation of collagen (Hydroxyproline) Healing tissue collected at 3,10,15 and 25 days intervals were dried in hot air oven at 60°C for 12 h. The dried granulation tissues were added with 6 N HCl at the rate 1ml for 100 mg sample and then kept at 110°C for 24 h in sealed tube. Tube containing 1 ml of hydrolysate sample was mixed with 1ml each of 0.01 M copper sulfate solution, 2.5 N sodium hydroxide, and 6% hydrogen peroxide. Then it was mix properly and incubated for 80°C for 5 minutes. After this step, the tubes were chilled in an ice water bath and added 4 ml of 3 N sulfuric acid with agitation. Finally , 2 ml of p-dimethylamino-benzaldehyde (5%) solution was added and mixed thoroughly. The tubes were placed in a water bath at 70°C for 15 minutes and then chilled in an ice water bath. The absorbance was measured at 540 nm (Neuman and Logan 1950).

Estimation of Hexosamine: Healing tissue were collected and dried at 60°C for 24 hrs. Out of this, 50 mg was taken in the standard flask and added 5 ml of 2N HCL and kept at

Table 1: Level of collagen, elastin, hexosamine and hydroxyproline at different periods after treatment of wound with some herbal plants (Mean±SE).

Parameters	Groups	Periods in days			
		3 (16)	10 (16)	15 (16)	25 (16)
Collagen (%)	I	5.99 ± 1.31^{aA}	10.26 ± 0.36^{aA}	11.27 ± 0.47^{aA}	12.33 ± 0.34^{aA}
	II	5.24 ± 1.14^{aB}	8.19 ± 0.46^{abBC}	9.13 ± 0.59^{bBC}	10.24 ± 0.32^{bBC}
	III	4.48 ± 1.10^{aB}	7.71 ± 0.26^{abC}	8.46 ± 0.67^{bcC}	9.95 ± 0.45^{cC}
Elastin (%)	I	5.10 ± 0.31^{aA}	6.28 ± 0.36^{bA}	8.31 ± 0.30^{cA}	11.33 ± 0.27^{dA}
	II	4.06 ± 0.33^{aB}	4.75 ± 0.32^{bB}	5.36 ± 0.33^{cB}	9.83 ± 0.31^{cA}
	III	3.77 ± 0.33^{aC}	4.09 ± 0.34^{bC}	4.59 ± 0.32^{cC}	6.66 ± 0.31^{cB}
Hexosamine (mg/gram of weight tissue)	I	43.26 ± 1.65^{aA}	33.08 ± 1.74^{bA}	17.55 ± 0.75^{cA}	6.59 ± 0.43^{dA}
	II	32.66 ± 1.67^{aB}	25.46 ± 1.67^{bB}	10.71 ± 0.71^{cB}	4.64 ± 0.43^{dB}
	III	27.25 ± 1.77^{aC}	21.86 ± 1.61^{abC}	8.05 ± 0.73^{bC}	3.19 ± 0.35^{cC}
Hydroxyproline (mg/gram of weight tissue)	I	7.64 ± 0.39^{aA}	32.52 ± 1.43^{abA}	39.87 ± 1.38^{bcA}	47.98 ± 1.35^{cA}
	II	6.00 ± 0.37^{aB}	25.76 ± 1.39^{aB}	31.16 ± 1.50^{bB}	36.96 ± 1.15^{cB}
	III	5.45 ± 0.32^{aC}	20.87 ± 1.37^{bC}	22.07 ± 1.45^{cC}	27.61 ± 1.32^{dC}

Figures in parentheses are number of observations.
Values bearing same superscripts (small letters) in a row and (capital letter) in a column did not differ significantly($p>0.05$).

100°C for 6 hrs for hydrolysis. Hydrochloric acid was then removed by evaporation, and then the residue was dissolved in distilled water and made up to a known volume up to 10 ml. Then it is treated with 1ml of freshly prepared 2% acetyl acetone in 0.5M sodium carbonate and kept in boiling water bath for 15min. After cooling in tap water, 5 ml of 95% ethanol and 1ml of Ehrlich's reagent were added and mixed thoroughly. The purple red colour developed was read after 30 min at 530 nm.

Statistical analysis of data were done by one way analysis of variance (ANOVA) as per method described by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

A significant alteration could be observed during the process of collagenation within a group, which increased with the increase in period of observation, with highest intensity seen on day- 25 of observation (Table.1). On day- 3 of observation, group II and III differed significantly with that of group I. However, group II and III did not differ significant among themselves. The values recorded on day- 10, 15 and 25 of observation, group II differed significantly as compared to group I. Group I excelled in collagenation followed by group II and III. The intensity of collagen in group I and II were found to be more which may be due to anti- inflammatory activity of sunflower oil and

olive oil during early period of observation and Superiority of group I over group II might be due to presence of certain valuable ingredients such as vitamin A and ascorbic acid more in sunflower oil than in olive oil. This process results in stimulation of fibroblasts for synthesizing collagen fibers (Kumar *et al.*, 1998). The increase in collagen may be due to anti-inflammatory activity of Sunflower oil (Baskhi *et al.*, 1998, Iqbal and Bhanger 2007) and Olive oil (Elisa *et al.*, 2005). Al Sadi (1976) observed the role of vitamin A in fibroplastic proliferation and formation of collagen in wound healing. Vegad (2007) also reported the importance of vitamin A and C in collagen formation.

The values of elastin recorded from day- 3 and 10 of observation, group I, II and III differed significantly with each other. On day- 15 of observation, group I differed significantly as compared to group III. However, the values recorded in group I did not differ significantly between them. Group II differed significantly as compared to group I and III. However, the values recorded in group II did not differ significant between them. On day- 25 of observation, group I and II differed significantly to that of group III (Table-1).

The quantum of elastin was relatively higher in group I followed by group II and III. The increase in elastin may be due to anti-inflammatory activity of sunflower oil (Iqbal and Bhanger 2007) and olive oil (Elisa *et al.*, 2005). Superiority of group I over group II might be due to presence of certain valuable ingredients like vitamin A, copper, calcium and ascorbic acid etc. more in sunflower oil than olive oil. More elastin observed in group I, II and III might be due to intense fibroplasia in these groups. Bhargava *et al.* (1988) also observed a gradual increase in elastin content from day-3 to 30. Levenson *et al.* (1965) reported that elastin plays no definite role in wound healing, whereas, Katiyar (1999) reported the role of copper to maintain the copper frame work of elastin and collagen.

The quantum of hexosamine decreased with the increase in observation time and the maximum value could be recorded on day-3. On day- 3, 10, 15 and 25 of observation, group I, II and III differed significantly with each other (Table 1). Superiority of group I over group II might be due to presence of higher concentration of certain valuable ingredients in sunflower oil than olive oil. The decrease in hexosamine may be due to more activity of vitamin A and C in healing tissue. Baskhi *et al.* (1998) and Iqbal and Bhanger (2007) reported that vitamin A and C are more in Sunflower oil as compared to Olive oil. The gradual decrease in the level of hexosamine in the healing tissue consequent to formation of mature collagen was observed by Dunphy and Udupa (1965) and Ghani *et al.*(1981) in calves.

The process of hydroxyproline production increased with the increase in period of observation, with highest intensity seen on day-25 of observation. On day- 3, 10, 15 and 25 of observation, group I, II and III differed significantly with each other (Table 1).The quantum of hydroxyproline were relatively higher in group I followed by group II and III. The increase in hydroxyproline may be due to anti-inflammatory activity of sunflower oil (Lezcano *et al.*, 2007, Boskou *et al.*, 2004, Jaipuriar 2007) and Olive oil (Eduardo *et al.*, 2007). The excellent formation of hydroxyproline in group I over group II might be due to presence of certain valuable ingredients in higher concentration in sunflower

oil as compared to olive oil like as vitamin A, niacin, copper, calcium, ascorbic acid etc. Vitamine C (Ascorbic acid) has been linked to collagen synthesis and fibroblastic proliferation (Guo and Di Pietro 2010).Vitamin A produced antioxidant activity, increased fibroblastic proliferation, increased collagen and hyaluronidase synthesis (Burgess 2008). Copper is required for optimum cross linking of collagen (Campos *et al.*, 2008). Burgess (2008) reported that topical vitamins *viz*. vit A, C, E and B_3 (niacin) on wounds act as antioxidant and anti-inflammatory properties which might be helpful in more hydroxyproline formation. Calcium has an established role in normal homeostasis of wound (Lansdown 2002) to facilitate good environment for proliferation and cross linking of collagen fibers. Hydroxyproline is basic constituent of collagen (Subalakshmi *et al.*, 2014).

CONCLUSION

It is concluded that both sunflower oil and olive oil was found to be effective for enhancement of wound healing. However, sunflower oil was superior over olive oil.

ACKNOWLEDGEMENT

Authors are thankful to Dean, College of Veterinary Science and Animal Husbandry and Director Research, Birsa Agricultural University for providing financial assistance to carry out the research work.

REFERENCES

Al Sadi H I (1976) Studies on inflammation and repair in the dog; glucocorticoid and vitamin A antagonist as a modifying factor. Dissertation Abstracts Internat 37B(6): 2702.

Baskhi DN, Sensarma P, Pal DC (1998) A lexicon of medicinal plants in India. Nayaprokash, 206, Bidhansarani, Culcutta. 326-327.

Bhargava M K, Singh H, Kumar A, Singh GR (1988) Evaluation of *Annona squamosa* (sitaphal) as a wound healing agent in buffaloes – clinical, biochemical and mechanical studies. Indian J Vet Surg 9(1): 27.

Boskou D, Bentley R, Trimen H (2004) Antioxidant activities of alkanin and shikonin root extracts in oil substrates. Food Chemistry. 84 (2): 423-429.

Burgess C (2008) Topical vitamins. J Drugs Dermatol 7(7 suppl): s2- s6.

Campos AC, Groth AK, Branco AB (2008) Assessment and nutritional aspects of wound healing. Curr Opin Clin Nutr Metab Care 11: 281-288.

Chauhan P (2005) PanchGavya – The complete Aurvedic pharmacy. The Indian Cow 4 : 59-61.

Dunphy JE, Udupa KN (1955) Chemical and histochemical sequence in the normal healing wound. New England J Med 253: 847-851.

Eduardo M, Concepcion R, Manuel B, Antonio De C (2007) Antimicrobial activity of olive oil, vinegar, and various beverages against foodborne pathogens. J food protection 70(5): 1194-1199.

Elisa T, Giammanco M, Tabacchi G, Majo DD, Giammanco S, Guardia LM (2005) The phenolic compounds of Olive oil: structure, biological activity and bene?cial effects on human health. Nutrition Res Rev 18: 98–112.

Garg N, Chauhan RS (2003) Cow therapy as an alternative to antibiotics. Indian Science Congress, 3-7 January, 2003, Banglore, India.

Ghani A, Pandey SK, Quadri MA (1981) Biochemical evaluation on certain local medicaments as accelerator of wound healing. Indian J Med 2(1): 11-25.

Guo S, DiPietro LA (2010) Factors affecting wound healing: Critical reviews in oral biology and medicine. J Dent Res 89 (3): 219-229.

Iqbal S, Bhanger M (2007) Stabilization of sunflower oil by garlic extract during accelerated storage. Food Chemistry 100 (1): 246-254.

Jaipuriar MK (2007) *Helianthus annus* L. – Asteraceae. Herbs of Tribal Land, Jharkhand, India. 189-190.

Katiyar P (1999) Studies on the properties of *Azadirachta indica*, *Aleo vera* and *Psidium guyava* Linn. On coetaneous wounds in Buffaloes. M.V.Sc. thesis subsmitted to the C.S.A. University of Agriculture and Technology, Kanpur (U.P.).

Kumar S, Parmashwaraih S, Shivkumar HG (1998) Effect of topical formulations of aqueous extract of *Centella asiatica* on open wounds in rats. Indian J Expt Biol 36: 569-572.

Lansdown AB (2002) Calcium: a potential central regulator in wound healing in the skin. Wound Repair Regen 10(5): 271-285.

Levenson SM, Greever EP, Croneley LV, Oats JF, Bernard CW, Rogan H (1965) Healing of rats skin wounds. Ann Surg 161: 293-308.

LezcanoI, SechiL A, Nunez N, Espim M, Dupre I, Pinna A, Molicotti P, Fadda G, Zanetti S (2001) Antibacterial activity of ozonized sunflower oil (Oleozon). J Applied Microbiol 9(2): 279-284.

Neuman RE, Logan MA (1950) The determination of collagen and Elastin in tissue. J Biol Chem 186(2): 549-56.

Pattanayak S, Pal S, Mandal TK, Debnath PK, Bandyopadhyay SK (2014) A comparative study of extract of succulent leaves of living plant with methanolic and aqueous extract of *Berleria lupulina* Lindl. against pathogenic microbes by disc diffusion and spectrophotometry. Explor Anim Med Res 4(2): 148-157.

Pattanayak S, Maity D, Mitra S, Debnath PK, Mandal TK, Bandyapadhyay SK (2013) Use of fresh parts of medicinal plants for health and production in livestock - a new concept of farming. Explor Anim Med Res 3(1): 7-16.

Rondle CJ, Morgan WT (1955) The determination of glucosamine and glactosamine. Biochem J 61(4): 586-589.

Snedecor CW, Cochran WG (1994) Statistical Methods. 6thedn., Iowa State Univ. Press, Ames, Iowa USA, 258-298.

Subalakshmi M, Saranya A, Uma Maheswari M, Jarina A, Kaviman S (2014) An overview of the current methodologies used for the evaluation of drugs having wound healing activity. Int J Experi Pharmacol 4(2): 127-131.

Vegad JL (2007) Tissue repair (Healing). In: Textbook of Veterinary General Pathology. Vikas Publishing House. Jangpura, New Delhi. 80-133.

***Cite this article as:** Anand A, Dass LL, Sharma AK, Singh KK (2016) Biochemical changes in the wounds of goats following treatment of sunflower oil and olive oil. Explor Anim Med Res 6(1): 107-112.