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Research Article

WOUND HEALING POTENTIAL OF SUNFLOWER OIL, *MORINGA OLEIFERA* (SAIJANA) AND OLIVE OIL IN GOATS: A HISTOMORPHOLOGICAL STUDY

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ABSTRACT

The study was conducted on sixty four surgically created wounds in sixteen goats of either sex, aged between one and half years to two years. Sunflower seed oil impregnated gauze was subjected to the wounds of animal belonging to group I while Saijana (*Moringa oleifera*) root bark extract impregnated gauze were subjected to the wounds of animals of group II. Olive oil (*Olea europaea*) impregnated gauze were subjected to the wounds of animal of group III and normal saline solution (control) soaked gauze to the wounds of animal of group IV. Epithilialization was significantly higher ($P<0.05$) in group I and II with that of group III and IV on day-15 of observation. Wounds treated with sunflower seed oil (group I)exhibited maximum infiltration, neovascularization and fibroplacia followed by wounds of animal treated with saijana (group II) and olive oil (group-III). It is concluded that sunflower oil, saijana root bark extract and olive oil are quite effective in enhancement of healing process of surgical wound on topical application. However, sunflower oil is recommended over saijana and olive oil because of its cost effective, easy availability and early healing property.

Keywords: Goats, Histomophological changes, Olive oil, Sunflower oil, *Moringa oleifera*, Wound healing

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Despite tremendous advancement, an effective wound management continues to be a challenge to the clinicians (Singh and Singh, 2004). Herbal plants increase the rate of tissue healing by providing different essential substances, required at various steps of regeneration and proliferation. These herbs being cheaper and safer than allopathic drugs may be very useful in veterinary practice, especially in India where these are found in abundance.

The clinical and histopathological aspects of cutaneous application of sunflower seed oil with high concentration of linoleic acid (65%) on the healing of surgically created open wounds have been shared by Marques *et al.* (2004).The root barks of Saijana plant (*Moringa oleifera* Lam.) contain alkaloids, viz., moringine, moringinine and pterygospermin. They help to prevent inflammation and possess circulatory stimulant effect (Kumar *et al.*, 2007). The compound pterygospermin dissociates in to two molecules of benzyl isothiocyanate which have antimicrobial properties (Anwar and Banger, 2003). Olive oil is considered as biogenic stimulant and it has anti-inflammatory property. It causes faster epithelialization, wound contraction and early suppression of inflammation and thus helps in wound healing (Singh *et al.*, 2001). Olive oil is reported to be effective in ulcer healing (Nasiri *et al.*, 2015).The present paper dealt with the histomorphological evaluation of wound after topical application of given above herbal plants.

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MATERIALS AND METHODS

The study was conducted on sixty four surgically created wounds in sixteen goats of either sex and aged between one and half years to two years. The goats were grouped randomly into four groups of four animals each. Feed and water was 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 2 cm × 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 gauzes were subjected to the wounds of animal belonging to group I while Saijana (*Moringa oleifera*) root bark extract impregnated gauzes were subjected to the wounds of animals of group II. Olive oil (*Olea europaea*) impregnated gauzes were subjected to the wounds of animal of group III and normal saline solution (control) soaked gauzes to the wounds of animal of group IV. Gauzes were maintained in position with micropore bandages. Healing tissues were collected from the junction of wound and intact skin from all the experimental created 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. From each tissue, slides were prepared in quadruplet, one for histomorphology and the other three for histochemistry. For histomorphological changes taking place during the process of healing, sections were subjected to routine Heamatoxyline and Eosine (H&E) stain. Histomorphological evidences of progression of wound healing in terms of inflammatory cell proliferation, fibroplasias, epithelialization, neovascularization and collagen were visually quantified on the scales, viz., 0,1,2,3 and in some cases up to 4.

Leucocytic infiltration:

0 : Absent

1 : 0-5 cells/HPF*

2 : 6-30 cells/HPF*

3 : More than 30 cells/HPF*

Neovascularization:

0 : Absent

1 : 1-5 new blood vessels/HPF*

2 : 2-10 new blood vessels/HPF*

3 : 11-15 new blood vessels/HPF*

Fibroplasia:

0 : Absent

1 : Small number of fibroblasts towards surface.

2 : Diffuse fibroblastic proliferation towards surface extending down to deeper areas.

3 : Dense fibroblastic proliferation over the whole area.

4 : Dense fibroblastic proliferation over the whole area and fibroblasts laying down collagen fibres.

Epithelialization:

0 : Absent

1 : Upto 20% epithelialization.

2 : 20% epithelialization.

3 : 80% or more epithelialization.

Collagen formation:

0 : Absent

1 : Small amount of thin collagen.

2 : In between 1 and 3.

3 : Diffuse thick collagen.

4 : Wavy collagen.

STATISTICAL ANALYSIS

Data obtained for each set of wound healing models were expressed as Mean±SE and analyzed by one way analysis of variance (ANOVA) as per method described by Snedecor and Cochran (1994). Level of significance was set at $P<0.05$.

RESULTS AND DISCUSSION

Epithelialization :

With the increase in period of observation, the epithelialization process also progressed significantly in all the groups (Table 1). On day- 10 of observation, the values recorded in group I, II and III differed significantly with that of group IV. However, the values recorded in group I, II and III did not differ significantly among themselves on corresponding period of observation. On day-15 of observation, group I and II differed significantly with that of group III and IV . However, group I and II did not differ significantly between themselves. The values recorded in group III were significantly higher ($P<0.01$) as compared to group IV on 15th day observation. A progressive increase in epithelialization could be noticed at different intervals of observation in all the groups. However, the increase was of faster rate in group I followed by group II, III and IV at corresponding intervals. Certain important ingredients of Sunflower oil, *Moringa oleifera* and Olive oil like minerals and vitamins might be responsible for early and better epithelialization in group I, II and III as it has been reported that vitamin A and C are required for epithelial formation and cellular differentiation (Mackey *et al.*, 2003; Singh and Singh, 2004 and Vegad, 2007). Bhargava *et al.* (1988) also reported more distinct epithelialization in treated wounds with *Annona squamosa* as compared to control. Formation of granulation tissue provides a surface for the epithelial cells to migrate over and confers resistance to infection (Katiyar, 1999). In support of our finding, Lambole and Kumar (2012) also reported that the root bark extract of *Moringa oleifera* treated wound epithelize faster with significant wound contraction as compared to wound treated with povidone iodine.

Fibroplasia: A significant alteration could be observed during the different periods of observation within the groups (Table 1). The process of fibroplasias increased with the increase in period of observation, with highest intensity seen on day- 25 of observation in all the groups. The values recorded on day- 3 in group II, III and IV differed significantly as compared to the value of group I. However, group II, III and IV did not differ significantly among themselves. On day- 10 of observation, group I and II differed significantly with that of group III and IV.

Table 1

Mean±SE of epithelialization, fibroplasias, neovascularization, leucocytic infiltration and collagen as observed histomorphologically during different periods of observation after treatment of wound with some herbal plants.

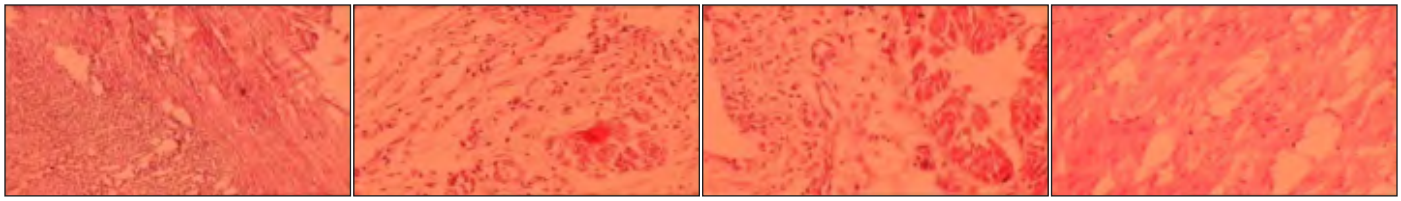
Parameters	Groups	days			
		3(16)	10(16)	15(16)	25(16)
Epithelialization	I	0.00^{aA}	1.79 ± 0.13^{bA}	2.53 ± 0.11^{cA}	2.93 ± 0.05^{dA}
	II	0.00^{aA}	1.56 ± 0.12^{bAB}	2.26 ± 0.11^{cA}	2.89 ± 0.06^{dA}
	III	0.00^{aA}	1.48 ± 0.14^{bAB}	1.87 ± 0.12^{cB}	2.61 ± 0.05^{dB}
	IV	0.00^{aA}	0.79 ± 0.13^{bC}	1.41 ± 0.12^{cC}	2.39 ± 0.06^{dC}
Fibroplasia	I	2.53 ± 0.13^{aA}	2.93 ± 0.11^{bA}	3.13 ± 0.07^{bA}	3.59 ± 0.06^{cA}
	II	1.28 ± 0.14^{aB}	2.63 ± 0.12^{bA}	2.83 ± 0.05^{bcB}	3.11 ± 0.06^{cB}
	III	1.08 ± 0.12^{aB}	2.10 ± 0.12^{bB}	2.53 ± 0.05^{cC}	2.93 ± 0.04^{dC}
	IV	0.94 ± 0.09^{aB}	1.98 ± 0.13^{bB}	2.16 ± 0.08^{bD}	2.53 ± 0.06^{cD}
Neovascularization	I	2.92 ± 0.14^{aA}	2.87 ± 0.02^{aA}	3.26 ± 0.04^{bA}	2.23 ± 0.07^{cA}
	II	2.67 ± 0.12^{aAB}	2.76 ± 0.04^{aAB}	2.67 ± 0.08^{aB}	1.88 ± 0.03^{bB}
	III	2.52 ± 0.11^{aB}	2.72 ± 0.04^{aB}	2.67 ± 0.03^{aB}	1.73 ± 0.07^{bB}
	IV	0.81 ± 0.13^{aC}	2.25 ± 0.03^{bC}	2.28 ± 0.08^{bC}	1.51 ± 0.05^{cC}
Leucocytic infiltratration	I	2.99 ± 0.10^{aA}	2.80 ± 0.11^{aA}	2.34 ± 0.11^{bA}	1.16 ± 0.10^{cA}
	II	2.67 ± 0.07^{aB}	2.39 ± 0.07^{bB}	2.05 ± 0.12^{cAB}	0.85 ± 0.07^{dB}
	III	2.59 ± 0.06^{aB}	2.30 ± 0.07^{bB}	1.85 ± 0.11^{cB}	0.63 ± 0.04^{dC}
	IV	1.64 ± 0.07^{aC}	1.44 ± 0.08^{aC}	0.94 ± 0.09^{bC}	0.56 ± 0.06^{cC}
Collagen	I	1.54 ± 0.07^{aA}	2.38 ± 0.08^{bA}	3.13 ± 0.13^{cA}	4.06 ± 0.10^{dA}
	II	1.26 ± 0.08^{aB}	1.80 ± 0.12^{bB}	2.79 ± 0.10^{cB}	3.58 ± 0.11^{dB}
	III	0.86 ± 0.06^{aC}	1.57 ± 0.10^{bB}	2.78 ± 0.11^{cB}	3.21 ± 0.12^{dC}
	IV	0.71 ± 0.05^{aC}	1.23 ± 0.08^{bC}	1.85 ± 0.13^{cC}	2.81 ± 0.15^{dD}

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($p>0.05$).

However, group I and II did not differ significantly. The values recorded in group I, II and III on day- 15 and 25 of observation were significantly higher as compared to the value recorded in group IV. The fibroplasia was more marked in group I followed by group II, III and IV at different intervals of observation. The values recorded on day- 25 were significantly higher ($P<0.01$) in all the groups as compared to values recorded on day 3, 10 and 15. However, the values recorded on day- 10 and 15 showed non significant alterations to each other in group I, II and IV. Group-wise analysis of data for fibroplasias showed a significant variation ($P<0.01$) for different groups at different periods of observation (Table 1).

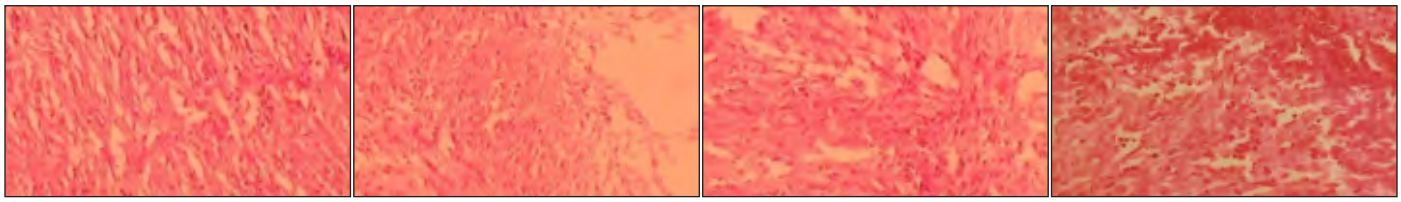
Vitamin A is present in higher concentration in the Sunflower oil followed by *Moringa oleifera* and Olive oil. Increased fibroblastic proliferation after administration of vitamin A has been observed by Al Sadi (1976).

Neovascularization: A significant alteration could be observed during the process of neovascularization (Table 1 and Fig. 1-4). In all groups, the intensity of neovascularization increased from day- 3 and 10 except in group IV, where neovascularization process increased on day- 10 of observation. This increase was followed by a decrease commencing from day- 15 except in group I where intensity of neovascularization could be seen decreasing on day- 25 of observation. A significant decrease in values on day- 25 were recorded in all the groups as compared to values on day 3, 10 and 15 in group I, II and III, whereas, the values on day- 10 and 15 in group IV were significantly higher as compared to value on day- 3. Group-wise analysis of data for neovascularization showed significant variation ($P<0.01$) for different groups at different periods of observation (Table 1). On day- 3 and 10 of observation, group I and II showed significantly higher values as compared to group III and IV. However, the values of group I and II did not differ significantly between themselves. Group II, showed a non significant alteration as compared to group III. On day- 15 of observation, group II and III differed significantly with



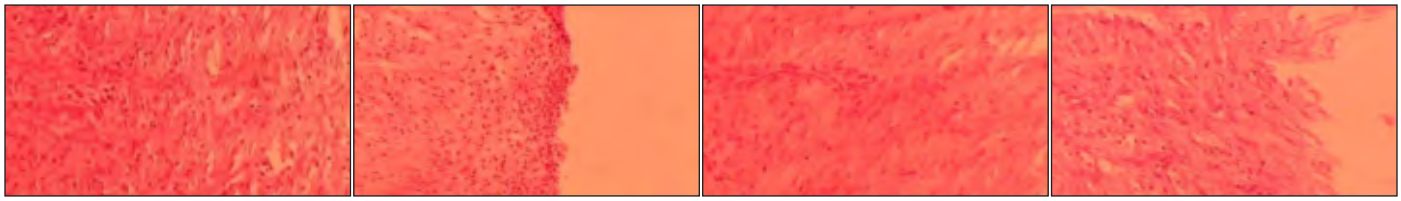
Group I Group II Group III Group IV

Fig. 1. The microscopic section of tissue collected from wound healing in different groups on day-3 showing long fibroblastic cells, leucocytic infiltration and neovascularization (HEx100)6



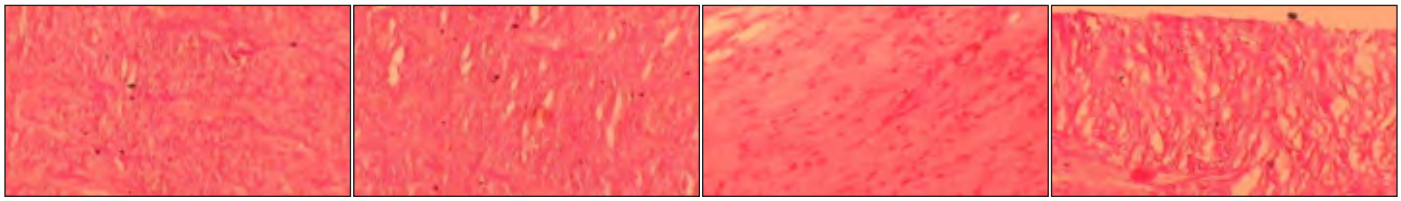
Group I Group II Group III Group IV

Fig. 2. The microscopic section of tissue collected from wound healing in different groups on day-10 showing long fibroblastic cells, leucocytic infiltration and neovascularization (Hex100)



Group I Group II Group III Group IV

Fig. 3. The microscopic section of tissue collected from wound healing in different groups on day-15 showing long fibroblastic cells, leucocytic infiltration and neovascularization (HEx100)



Group I Group II Group III Group IV

Fig. 4. The microscopic section of tissue collected from wound healing in different groups on day-25 showing long fibroblastic cells, leucocytic infiltration and neovascularization (HEx100)

that of group I and IV. However, group II and III did not differ significantly between themselves. On day- 25 of observation, group II and III differed significantly with that of group I and IV. However, group II and III did not differ significantly between themselves.

All groups exhibited intensive neovascularization from day-3 to 15 but the intensity was less on day-25. Group IV showed minimum neovascularization as compared to group I, II and III at any period of observation. The nutrient contents like vitamin A and C, calcium, cooper and iron of Sunflower oil, *Moringa oleifera* and Olive oil might have contributed in the growth of granulation tissues in group I, II and III, respectively. The regression of vascularity appeared on day-15 in group I, II and III, which marked the beginning of maturation phase (Heinze, 1976).

Leucocytic Infiltration: Infiltrative changes exhibited a significant variation ($P<0.01$) at different periods of observation in all the four groups (Table 1). Infiltrative changes were more pronounced on day- 3 and thereafter reduced gradually by day- 25 in all the groups. On day- 3 of observation, group- II and III differed significantly with that of group I and IV. However, group II and III did not differ significantly between themselves. On day- 10 of observation, group II and III differed significantly with that of group I and IV. However, group II and III did not differ significantly between themselves. On day- 15 of observation, group I differed significantly with that of group III and IV. However, group I and II did not differ significantly between themselves and also on same day of observation, group II differ significantly with that of group IV. On day- 25 of observation, group I and II differed significantly with that of group III and IV. However, group

III and IV did not differ significantly between themselves. Group-wise analysis showed that the alteration in infiltrative changes was highly significant among groups (Table 1).

Infiltrative changes were pronounced till day-3 and macrophages outnumbered neutrophils in group I, II and III as compared to group IV. On day-10, the infiltration of these cells appeared to be decreased and it further decreased progressively at different intervals of observation. Comparatively, there was less infiltration of these cells in group IV at any period of observation. From the study, it is apparent that these treatments somehow mediate the inflammatory process. Bisht *et al.* (1999) and Kumar *et al.* (1998) observed similar finding, wherein they noticed more infiltrative changes in early phase followed by a significant decrease in later phase of healing in treated wounds due to anti-inflammatory properties of agents in three groups as compared to control.

Collagen: The wounds of all groups exhibited increasing trend in the quantum of collagen fibres with maximum intensity observed on day-25. Group I excelled in collagenation significantly ($P<0.01$) followed by group II, III and IV. The intensity of collagen fibre in group I, II and III were found to be more which may be due to intense neovascularization and infiltrative response in these groups during early period of observation. This process results in stimulation of fibroblasts for synthesizing collagen fibres (Kumar *et al.*, 1998). Excellency of group I over group II and III might be due to more valuable ingredients like vitamin A and C, Cu, Ca in Sunflower oil than *Moringa oleifera* and least in Olive oil. Vegad (2007) also reported the importance of vitamin C in collagen formation. Ascorbic acid is required to hydroxylate proline and lysine to synthesize hydroxyproline and hydrolysine, the two constituents of collagen fibres (Vegad, 2007). During collagen synthesis, vitamin C forms extra bonds between collagen fibers that increase stability and strength of collagen matrix (Harris and Fraser, 2004).

From the present study, it can be concluded that Sunflower oil, Saijana root bark extract and Olive oil are quite effective in enhancement of healing process of surgical wound on topical application in goats. However, Sunflower oil is recommended over Saijana and Olive oil because of its cost effective, easy availability and early healing property for early wound healing in goats.

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