



Healing effect of *Adiantumcapillus veneris* on surgical wound in rat

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Abstract

The aim of this study was to investigate the potential effect of *Adiantumcapillus veneris* extract on the healing of surgical wound. Forty eight male Wister rats were divided into 3 groups of 16 rats each. Group I received ointment containing the *Adiantumcapillus veneris* extract, group II received simple ointment twice a day until the animals were euthanized. The control group did not receive any medication. Animals were kept under observation for a period of 21 days. Histological evaluation of wound healing was done on 3, 5, 14 and 21 day after surgery. For this purpose four animals in each group were euthanized at the set time. The tissues were stained with haematoxylin and eosin and the number of inflammatory cells, new fibroblast cells, new capillary formation, epithelial cells and collagen fibres formation were counted. Histological analyses revealed that in the group I and II a few of the inflammatory cells and fibroblast-like cells on day 3 were observed. On day 5, a continuous incomplete epidermal layer was observed under the crust, and the number of inflammatory cells decreased slightly, there was a distinct increase in fibroblast-like cells, collagen formation and new vessels formation from day 3. On day 14, a complete epidermal layer with multiple layers of spinous cells was observed, and the number of inflammatory cells decreased remarkably. On day 21, group I and II showed relatively well organized epidermal layer, increased number of matured fibroblasts, remarkable degree of neo vascularisation and epithelization. The score for inflammation was the highest on day 3, and thereafter, it decreased gradually up to day 14. The score for the proliferation of the fibroblast like cells increased from days 3 to 14, and a sharp increase in the score was observed from day 5 to 14. The score for collagen formation was same as proliferation of the fibroblast like cells. The score for the epidermal cell regeneration cells increased from days 5 to 14, and a sharp increase in the score was observed from day 14 to 21. The changes observed in these 5 parameters were significant. The *Adiantumcapillus veneris* extract showed remarkable wound healing and it may be suggested for treating various types of wounds in human beings.

Keywords: *Adiantumcapillus veneris*; wounds healing; histology; rat

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Introduction

A wound is described as a break in the continuity of tissue caused by violence or trauma (Imperato, 1982a). Injuries and wounds formation are still one of the major causes of disability around the world and different communities are inflicted on many physical, psychological and economic damages (Heimbach, 1999; Cakir and Yegen, 2004). Wound healing disorders present a serious clinical problem and are

likely to increase since they are associated with diseases such as diabetes, hypertension and obesity (Reddy et al., 2001).

Healing is a complex process that involves five stages of hemostasis, inflammation, proliferation, repair and maturation (MacKay et al., 2003). In this processes several groups of cells such as thrombocytes, platelets, neutrophils, macrophages, fibroblasts and keratinocytes and number of enzymes such as cytokines, growth factors, collagenase, elastase and plasmin (Gillitzer and

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Goebeler, 2001; Losordo and Isner, 2001). Though the healing process is self curing phenomenon but due to the slow process it may cause discomfort and increases the susceptibility of infection (Mokaddas et al., 1998).

Given the importance of wounds healing, finding new drugs and effective methods have always been considered (Balaino et al., 2005). Research on wound healing agents is one of the developing areas in modern biomedical sciences (Malan et al., 2001). Chemicals compounds are commonly used as antimicrobial and disinfectants agents. Mostly these chemical agents are expensive and pose side effects.

Herbs have few complications and low price. Hence, plant products are used as alternative solution to the problem of wound treatment in developing countries. Plant products are potential agents for wound healing, and are largely preferred because of their widespread availability and effectiveness as crude preparations (Sasidharan et al., 2010).

Adiantumcapillus veneris is a popular plant in traditional medicine in the orient. This plant with compounds such as fatty acids, flavonoids, acid ester and mucilage have been used anti-fever drug (Besharat et al., 2009). Some of the important compounds in this plant include tannins, saponins, phenolic, sulfates hydroxysynamic ester acid, kaempferol and fern (Imperato, 1982a&b; Redzic, 2010). *Adiantumcapillus veneris* has anti microbial, anti fungal and anti viral properties. Historically, *Adiantumcapillus veneris* is used to treat the diseases in different parts of the world (Haider et al., 2011; Jiang et al., 2011). The aim of this study was to investigate the potential use of *Adiantumcapillus veneris* extract as ointment on the surgical wound healing.

Materials and Methods

Adiantumcapillus veneris extract was prepared with percolation technique, then concentrated using rotary machine and dried with heating for 48 hours at 70°C in ban marry bath. In the next stage, 9 g dried extract was dissolved in 8 ml of distilled water and 92 mg Eucerin was added as preservative. The produced material was sterilized by ultraviolet and packed in 5 g tube.

This experimental protocol was reviewed and approved by Kahnnoj Institutional Animal Care and Use Committee. About 48 male Wister rats (mean weight of 250 ± 50 g) were randomly divided into 3 groups of 16 rats each. All animals had free access to food and water and were individually housed in a 12 hours light dark cycle. Ten rats were anesthetized with intramuscular injection of 10% ketamine hydrochloride (Alfasan, Holland) and xylazine 2% (Pantex-Holland) at the dose rate of 60 mg/kg and 10 mg/kg respectively. The back area was shaved, washed and disinfected with

povidone-iodine (10%). A 6 cm long and 3 mm deep incision was made parallel to the spine, on skin, underlying connective tissue and part of the muscles. Following incision, the skin was sutured with 5/0 nylon (Ethicon, Somerville, NJ, USA) with single simple pattern. Group I received ointment containing the *Adiantumcapillus veneris* extract, group II received simple ointment twice a day until the animals being euthanized at defined time period. The control group did not receive any treatment. Animals were kept during wound healing for a period of 21 days.

Histological evaluation of wound healing was done on 3, 5, 14 and 21 days after surgery. For this purpose, four animals in each group were euthanized at the set time. The total wound area was removed and submitted for microscopically analyses. Sample tissues were fixed in 10% formalin and were embedded in paraffin wax. Serial sections (5 µm thickness) of paraffin embedded tissues were cut. The tissues were stained by haematoxylin and eosin and the number of inflammatory cells, new fibroblast cells, new capillary formation, epithelial cells and collagen fibres formation were counted under high power fields (×100) over 3 separated sections. Assessment was performed by a blinded assessor while considering parameters in Table 1, with an Olympus BH-2 light microscope (Tokyo, Japan).

The data was analyzed using Stata 6. All results were presented as means and standard deviations of their respective groups. Significance was calculated using analysis of variance and Duncan multiple range test. P value less than 0.05 was considered as significant.

Results

Histological analyses

Histological analysis revealed that on day 3, in the group I and II, a few of inflammatory cells and fibroblast-like cells were present. On the sutured area, blood clots and inflammatory cells were also seen. Epidermal structures were absent in this area. The main inflammatory cells were neutrophils, and a few lymphocytes and macrophages were present.

On day 5, a continuous incomplete epidermal layer was observed under the crust, and the number of inflammatory cells had decreased slightly, there was a distinct increase in fibroblast-like cells, collagen formation and new vessels formation from day 3.

On day 14, a complete epidermal layer (comprising corneal to the basal layer) with multiple layers of spinous cells was observed, and the number of inflammatory cells decreased remarkably (Fig. 1C).

On day 21, group I and II showed relatively well organized epidermal layer, increased number of matured fibroblasts, remarkable degree of neo vascular-

Table 1: Histological scoring

| Parameter | Scores | 3 | 2 | 1 | 0 |
|---------------------------|----------------------|---|--|-------------------------------------|----------------------|
| Epithelial cells | More than 50 centres | | 10 to 50 centres | 5 to 10 centres | No appearance |
| Fibroblast cells | More than 50 centres | | 10 to 50 centres | 5 to 10 centres | No appearance |
| Inflammatory cells | very few scattered | | 5 to 10 centres | 10 to 50 centres | More than 50 centres |
| New capillary formation | | | Excessive formation of New capillaries aligned | Slight formation of new capillaries | No formation |
| Collagen fibres formation | | | Excessive formation of collagen fibres aligned | Slight formation of collagen fibres | No formation |

The numbers 0, 1, 2 and 3 are the scores for evaluations of quality of healing

Table 2: Skin repair histological scores

| Parameter (maximum score) | Days | | | 3 days | | | 5 days | | | 14 days | | | 21 days | | |
|---------------------------------|--------|------------------|------------------|------------------|------------------|------------------|-------------------|--------------------|------------------|------------------|-------------------|-------------------|------------------|--|--|
| | Groups | I | II | III | I | II | III | I | II | III | I | II | III | | |
| Epithelial cells appearance (3) | | 0.6 | 0.4 | 0.5 | 0.9 | 0.8 | 0.6 | 2.5 | 2.5 | 0.7 | 3 | 3 | 0.9 | | |
| Fibroblast cells appearance (3) | | 0.6 | 0.5 | 0.4 | 0.8 | 0.6 | 0.6 | 2.8 | 2.4 | 0.9 | 3 | 3 | 1.5 | | |
| Inflammatory cells Presence (3) | | 0.4 | 0.5 | 0.4 | 0.7 | 0.7 | 0.6 | 1.8 | 2.3 | 0.9 | 3 | 3 | 1.4 | | |
| New capillary formation (2) | | 0.4 | 0.4 | 0.3 | 0.6 | 0.7 | 0.5 | 1.5 | 1.4 | 0.6 | 1.9 | 1.8 | 0.8 | | |
| Collagen fibres formation (2) | | 0.7 | 0.6 | 0.3 | 0.8 | 0.7 | 0.7 | 1.4 | 1.1 | 1.1 | 1.8 | 1.7 | 1.4 | | |
| Total (13) | | 2.7 ^a | 2.4 ^b | 1.9 ^c | 3.8 ^a | 3.5 ^b | 3.00 ^c | 10.00 ^a | 9.7 ^b | 4.2 ^c | 12.7 ^a | 12.5 ^b | 6.0 ^c | | |

^{a,b,c}Different superscripts in a row differ significantly

rization and epithelization. The semi quantitative scores for inflammation and fibroblast like cell proliferation, new vessels formation, epidermal cell formation and new vessels formation are shown in Table 2.

The score for inflammation was the highest on day 3, and thereafter, it decreased gradually up to day 14. The score for the proliferation of the fibroblast like cells increased from days 3 to 14, and a sharp increase in the score was observed from days 5 to 14. The score for collagen formation was same as proliferation of the fibroblast like cells. The score for the epidermal cell regeneration cells increased from days 5 to 14, and a sharp increase in the score was observed from day 14 to 21. The changes observed in these 5 parameters were significant.

Discussion

Wound healing is a complex and dynamic process of restoring cellular structures in damaged tissue as closely as possible to its normal state (Imperato, 1982 a). Several materials have so far been used to affect healing differently. Wound healing researchers focus on the economic and efficacious agent that could obviate the long hospitalization of patients following surgery (Imperato, 1982b).

Medicinal plants have been used since time immemorial for treatment of various skin diseases (Reddy et al., 2001). Plant products are potential wound healing agents, and largely preferred because of their widespread availability, non-toxicity, absence of unwanted side effects, and effectiveness as crude preparations (Sasidharan et al., 2010).

The aim of this study was to investigate the potential effect of the extract of *Adiantumcapillus veneris* on the surgical wound healing. In the present study, the healing process was evaluated on histological factors such as collagen density, epithelialization, inflammatory cells infiltrates, angiogenesis and rate of fibroblast cells. Dermal regeneration in the treated group confirmed that the ointment containing the *Adiantumcapillus veneris* extract had a positive effect on cellular proliferation, granulation tissue formation and epithelialization. The positive effect may be due to the *Adiantumcapillus veneris* having useful flavonoids, tannins, saponins, phenolic compounds and kaempferol (Cano and Arnao, 2002).

Flavonoids are known to promote the wound healing process mainly due to their properties such as reducing lipid peroxidation to prevent or slow the onset of cell necrosis, increasing the viability of collagen fibrils by increasing the strength of collagen fibres, preventing the cell damage by promoting the DNA synthesis and increasing the circulation (Imperato, 1982b). Thus, wound healing property of *Adiantumcapillus veneris* may be attributed to the Flavonoids present in it. In a report, Cano and Arnao (2002) showed that kaempferol scavenges free radicals and protects cells against oxidative damage (Jiang et al., 2011). Halider et al. (2011) demonstrated that ethanol extract of *Adiantumcapillus veneris* prevents the release of nitric oxide and reduces the levels of tumour necrosis factor alpha and anti-inflammatory effects.

Similarly in this study, early skin regeneration in the treated group confirmed that the ointment containing *Adiantumcapillus veneris* leaf extracts had a

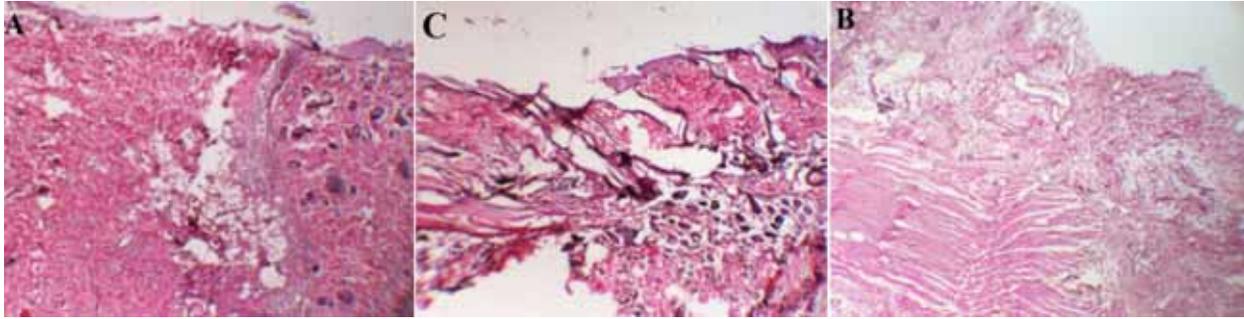


Fig. 1: Histological features of coetaneous wound healing in the rats on 3rd days. In groups I (A) and II (B) after 3 days few inflammatory cells, fibroblast activity and new capillary formation is evident, but in the in group III (c) after 3 days infiltrated inflammatory cells are seen and fibroblastic activity and new capillary formation were absent in the wound healing area (magnification 10×10, H&E staining).

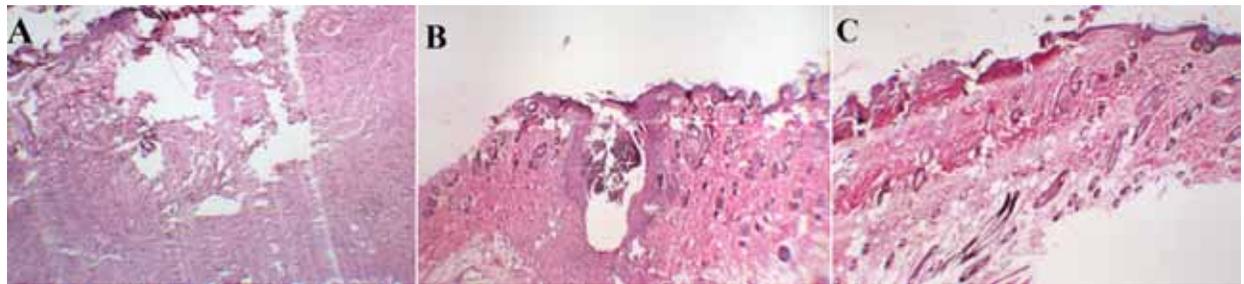


Fig. 2: Histological features of coetaneous wound healing in the rats on 5th days. In groups I (A) and II (B) after 5 days, partial formation of epidermal layer is evident, but in the in group III (c) after 5 days infiltrated inflammatory cells are seen and epidermal layer were absent in the wound healing area (magnification 10×10, H&E staining).

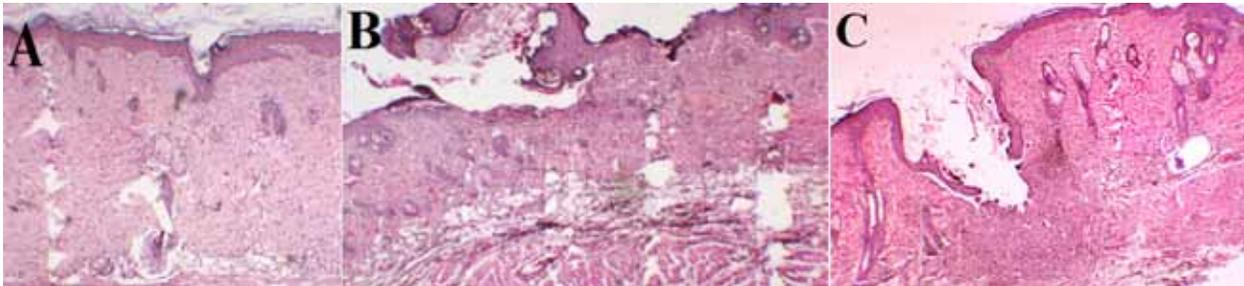


Fig. 3: Histological features of coetaneous wound healing in the rats on 14th days. In groups I (A) and II (B) after 14 days, complete formation of epidermal layer is evident and collagen fibres organization was oriented, but in the group III (c) after 14 days epidermal layer was incomplete in the wound healing area (magnification 10×10, H&E staining).

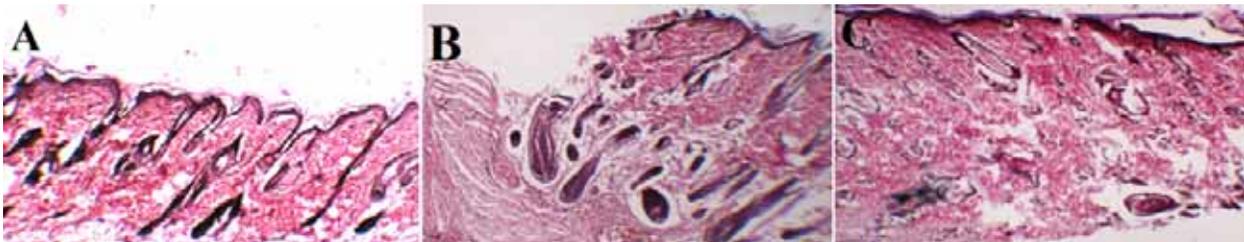


Fig. 4: Histological features of coetaneous wound healing in the rats on 21st day. In groups I (A) and II (B) after 21 days, complete formation of epidermal layer, but in the group III (c) after 21 days epidermal layer was incomplete, and collagen fibres organization was disoriented in the wound healing area (magnification 10×10, H&E staining).

positive effect on cellular proliferation and granulation (Reddy et al., 2001). The well-formed collagen bundles in the treated group shown in hematoxylin and eosin staining support the efficacy of *Adiantumcapillus veneris* on fibroblast proliferation and synthesis of extracellular matrix during healing (Fig. 5). Incomplete epithelialization with less extracellular matrix synthesis was observed in control rats (Sasidharan et al., 2010).

Leaf extracts of *Adiantumcapillus veneris* acts as a stimulant for wound healing because it has polyphenols and flavonoids as active constituents. A significant increase in collagen content due to enhanced migration of fibroblasts and epithelial cells to the wound site was observed during the wound healing process in the treated group (Reddy et al., 2001). At this stage, it is difficult to say which component (s) of the extracts is responsible for this wound healing activity. However, further phyto-chemical studies are needed to isolate the active compound (s) responsible for these pharmacological activities (Imperato, 1982b).

Conclusion

The leaf extracts of *Adiantumcapillus veneris* promoted wound healing activity and it may be suggested for treating surgical wounds in human beings.

References

- Balaino, C.A. Pereira, L.M. and Curi, R. 2005. Mecanismos envolvidos na cicatrizaÇão: umarevisão. *Brazilian Journal of Pharmaceutical Sciences*, 41: 27-51.
- Besharat, M., Rahimian, M. Ghaemi, E. and Besharat, S. 2009. Effect of ethanolic extract of *Adiantum capillus-veneris* in comparison with Gentamicine on 3 pathogenic bacteria in vitro. *Pharmaceutical Sciences*, 15: 49-52.
- Cakir, B. and Yegen, C. 2004. Systemic responses to burn injury. *Turkish Journal of Medical Sciences*, 34: 215-226.
- Cano, A. and Arnao, M.B. 2002. Superoxide scavenging by polyphenols: effect of conjugation and dimerization. *Redox Report*, 5: 379-383.
- Gillitzer, R. and Goebeler, M. 2001. Chemokines in cutaneous wound healing. *Journal of Leukocyte Biology*, 94: 513-521.
- Haider, S., Nazreen, S. and Alam, M.M. 2011. Anti-inflammatory and anti-nociceptive activities of ethanolic extract and its various fractions from *Adiantum capillus veneris* Linn. *Journal of Ethnopharmacology*, 3: 741-747.
- Heimbach, D. 1999. Burn patients, then a now. *Burns*, 25: 1-2.
- Imperato, F. 1982a. Kaempferol 3-sulphat in the fern *Adiantum Capillus-Veneris*. *Phytochemistry*, 8: 2155-2159.
- Imperato, F. 1982b. Sulphate esters of hydroxycinnamic acid-sugar derivative from *Adiantum Capillus-Veneris*. *Phytochemistry*, 11: 2717-2718.
- Jiang, M. Hui, Y. and Wen, Y. 2011. In vitro and in vivo studies of antioxidant activities of flavonoids from *Adiantum capillus-veneris* L. *African Journal of Pharmacy and Pharmacology*, 18: 2079- 2085.
- Losordo, D.W. and Isner, J.M. 2001. Estrogen and angiogenesis. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 21: 6-12.
- MacKay, D. Alan, L.M. and Miler, N.D. 2003. Nutritional support for wound healing. *Alternative Medicine Review*, 4: 359-377.
- Malan, R. Walia, A. Saini, V. and Gupta, S. 2011. Comparison of different extracts leaf of *Brassica juncea* Linn on wound healing activity. *European Journal of Experimental Biology*, 2: 33- 40.
- Mokaddas, E. Roimi, V.O. and Sanyal, S.C. 1998. In vitro activity of piperacillin/ tazobactam versus other broad-spectrum antibiotics against nasolacrimal gram-negative pathogens isolated from burn patients. *Journal of Chemotherapy*, 3: 208-214.
- Reddy, V.L., Ravikanth, V., Rao, T.P., Diwan, P.V. and Venkateswarlu, Y. 2001. A new triterpenoid from the fern *Adiantum lunulatum* and evaluation of antibacterial activity. *Phytochemistry*, 56: 173-175.
- Redzic, S. 2010. Wild medicinal plants and their usage in traditional human therapy (Southern Bosnia and Herzegovina, W.Balkan). *Journal of Medicinal Plants Research*, 11: 1003-1027.
- Sasidharan, S. Nilawaty, R. Xavier, R. Latha, L.Y. and Amala, R. 2010. Wound healing potential of *Elaeis guineensis* Jacq leaves in an infected albino rat model. *Molecules*, 15: 3186- 3199.