Mode Of Action Of Centella Asiatica In Wound Healing Using Immunohistochemical Studies

Article *in* Egyptian Dental Journal · January 2011

CITATIONS
0

READS
773

3 authors:

Yousry Elhawary
Mansoura University
31 PUBLICATIONS **84** CITATIONS
SEE PROFILE

Lobna Rs Radwan
MBNSOURA UNIVERSITY, EGYPT- DELTA UNIVERSITY FOR SCIENCE AND TECHNOL…
9 PUBLICATIONS **12** CITATIONS
SEE PROFILE

Farid Badria
Mansoura University
30 PUBLICATIONS **162** CITATIONS
SEE PROFILE

MODE OF ACTION OF CENTELLA ASIATICA IN WOUND HEALING USING IMMUNOHISTOCHEMICAL STUDIES

Lobna RS Radwan*; Youssry M El-Hawary** and Farid A Badria***

ABSTRACT

Introduction: Centella asiatica (C A) used to support faster healing of small wounds, chaps and scratches, surgical wounds, superfacial burns and varicose ulcers and as oral preparation for atonic wounds. as It has been used for treatment of various diseases, the mechanism of action remains markedly unknown.

Aim of the work: was to investigate the possible mode of action C A and / or dexamethasone in induced wound on rats tongue by immunohistochemical detection of alpha smooth muscle actin (α-SMA) and vimentin as markers for wound healing.

Materials and Methods: thirty two male rats were subjected to V-shaped surgical incision on the dorsal surface of the tongue using scalpel No 11 then were divided into four equal groups. Group A was left without treatment. Group B received C A extract (800 mg / kg) daily by oral route. Group C was injected I M by dexamethasone (0.3 mg / kg) at the day of injury, then half the dose day after day. Group D received C A and dexamethasone. Four rats from each group were euthanized in the fourth day and the remaining in the seventh day. Specimens were prepared for detection of α-SMA and vimentin.

Results: C A increased α-SMA and vimentin expresion in the wound region a head of the other groups and improved the healing in dexamethasone group.

Conclusion: C A improved wound healing and can overcome the suppressing effect of dexamethasone in wound healing.

* Lecturer, Oral Biology Department, Faculty of Dentistry, Mansoura University, Mansoura, Egypt.
** Ass. Prof, Oral Biology Department, Faculty of Dentistry, Mansoura University, Mansoura, Egypt.
*** Prof. of Pharmacognosy Department, Faculty of Pharmacy, Mansoura University, Mansoura 35516 Egypt.

INTRODUCTION

Legend goes that the tigers used to rub themselves with the Tiger's herb (Centella plant), in order to healtheir scars [1]. Perhaps the legend is not without truth, nowadays the selected triterpenes of Centella asiatica is the principle active in a range of specialties for the management of dermatological conditions including post operative scarring. The use of Centella asiatica in the management of dermatological conditions has a long tradition in its native areas, such as India and Sri Lanka, where it is used to support faster healing of small wounds, chaps and scratches, surgical wounds, superficial burns, varicose ulcers and as an oral preparation for atonic wounds and hypertrophic healing. Centella has also been used traditionally as an antiinflammatory, particularly for eczema, and also for minor itching and insect bites [2-4].

In spite it has been used for treatment of various diseases, the mode of action remains largely unknown [5]. Naturopathic physicians recommended taking Centella as a tea and applying Centella extracts externally for treatment of wounds as they stimulate collagen synthesis a key element of skin repair [6].

Animal studies have consistently shown topical application of Centella asiatica to a sutured wound significantly increased the breaking strength of the wound. Asiaticoside, a saponin extracted from Centella asiatica, is thought to be one of its active constituents. It was showed that a 0.2% asiaticoside solution applied topically twice daily for seven days to punch wounds in guinea pigs resulted in 56% increase in hydroxyproline, 57% increase in tensile strength, increased collagen content, and better epithelialization compared to control [3].

It is well known that Glucocorticoids markedly alter most aspects of wound healing process when administered directly after injury since they will delay the appearance of inflammatory cells and fibroblasts, deposition of ground substance and collagen, regenerating capillaries, contraction, and epithelial migration [7,8]. Dexamethasone is a potent synthetic member of the glucocorticoid class of steroid drugs. It acts as an anti-inflammatory and immunosuppressant. Its potency is about 20-30 times that of the naturally occurring hormone hydrocortisone and 4-5 times of prednisone [9].

Wound Healing requires the collaborative efforts of many different tissues and cell lineages. It includes clotting, inflammation, granulation tissue formation, epithelialization, neovascularization, collagen synthesis and wound contraction [10]. The fibroblasts for wound repair are derived from two sources, the division of undamaged fibroblasts at the wound periphery and the differentiation and proliferation of undifferentiated perivascular cells. The resulting daughter cells from both sources migrate into the wound defect to form collagen [11].

Cell possesses a cytoskeleton of microtubules and filaments that provide support for the cell, facilitates intracellular transport, supports cell junctions and permits mobility. Filaments are of two types: microfilaments and intermediate filaments. Microfilaments are composed of the contractile protein actin and are less than 8nm in diameter. Intermediate filaments are so – called because they are intermediate in size 8 – 10 nm between microfilaments and the thick filament of muscle cells. In the fibroblast, intermediate filament is composed of the protein vimentin [11].

Vimentin is a widely expressed intermediate filament protein thought to be involved mainly in structural process, such as wound healing. Activated human macrophages secret vimentin into the extracellular space [12]. It is generally assumed that the vimentin intermediate filament network in most mesenchymally part responsible for the

strength and integrity of these cells and necessary for any tissue movement that require the generation of significant tractional forces. Fibroblastic and myofibroblastic cells always expressed vimentin and may also vimentin and alpha smooth muscle actin (α – S M A) [13]. An increased α – S M A expression is sufficient to enhance fibroblast contractile activity. A correlation between the level of α–S M A expression and fibroblast contraction [14]. The actin isoform is typical of vascular smooth muscle cell [15].

Centella asiatica was used in most clinical studies either in an undefined alcohol or aqueous extracts. The extracts TECA (titrated extract of Centella asiatica) and TTFCA (total triterpenoid fraction of Centella asiatica) are combinations comprised of asiatic acid (30%), madecassic acid (30%), and asiaticoside (40%). The Centella extract TTF (total triterpenic fraction) is comprised of asiatic acid and madecassic acid (60%) in a riot not clearly defined, in combination with asiaticoside (40%) [2].

So this study was conducted in an attempt to verify the possible mode of action of T E C A and / or Dexamethasone in induced wound healing of rates tongue using immunohistochemical detection of α – S M A and vimentin as markers.

MATERIALS AND METHODS

Thirty two adult males Sprague-Dawley rats (200 ±10 gm) were used in this study were allowed commercial standard diet and water ad-libitum. Rats were housed in the animal house of Faculty of Pharmacy, Mansoura University under standard laboratory conditions (room temperature 22 ± 2 °C, humidity 55 ± 5L, 12 hours light/dark cycle).

All rats were subjected to standerdised V shaped surgical incision in anterior two third of the dorsal surface of the tongue using scalpel No 11; then they were divided into four equal groups.

Group A: the rats were left without any treatment, either with C A or Dexamethasone, for seven days.

Group B: the rats received C A extract (800 mg / kg body weight) daily by oral route using a syringe without needle.

Group C: the rats were injected intramuscular by Dexamethasone (0.3 mg / kg) at the day of injury, then half the dose was injected day after day until the end of the study.

Group D: the rats received C A extract (800 mg / kg body weight) daily by oral route and Dexamethasone injection as the same protocol of the group C.

Four rats from each group were euthanized at the fourth day after surgery and the remaining four rats at the seventh day. Tongue specimens were taken from the rats, immersed in 10 % formal saline and processed for immunohistochemical study for α smooth muscle actin, and vimentin.

Immunolabeling of α-SMA was performed using the Dako kit. Immunohistochemical procedures were performed according to the manufacturer's recommendations. Briefly, Paraffin sections were deparaffinised in xylene and rehydrated in ethanol and water. The sections were incubated for 30 minutes in 0.1 % H_2O_2 thus avoiding endogenous peroxidase activity. After washing with phosphate-buffered solution (PBS), the sections were incubated (if necessary) in sodium citrate or ethylenediaminetetraacetic acid (EDTA) buffer for 10 minutes at 95°C and cooled to room temperature. In order to avoid the background activity, sections were incubated in 10 % normal goat serum for 20 minutes. The sections were incubated with primary antibodies for (α – S M A) after applying the primary antibodies, the sections were incubated

with mouse or rabbit secondary antibody for 30 minutes at room temperature. The sections were then washed in PBS, stained with diaminobenzidine tetrahydrochloride solution (DAB), and counter-stained with hematoxylin. Immunolabeling of vimentin was performed using Doko kit with the same procedure of α – S M A except using anti-vimentin primary antibodies [16,17].

RESULTS

The immunohistochemical positive results were detected as brown deposits using α-SMA and also vimentin intermediate filament stain. The qualitative of the intensity of the staining were weak (+), moderate (++) and strong (+++) [18].

The immunohistochemical sections of the injured rat tongue revealed the followings:

I- α-SMA Detection:

a) 4th Day:

In the control group (group A); the expression of α-SMA was confined only to the smooth muscles of the blood vessel walls and there were scanty weak staining reaction in the wound region (Fig 1 A).

Centella asiatica treated group (group B); revealed strong reaction in the wound region and the smooth muscles of the blood vessels walls (Fig 1B).

Dexamethasone treated group (group C); revealed weak reaction to α-SMA antibodies in the small blood vessel walls (Fig 1C).

Dexamethasone and Centella asiatica treated group (group D); the reaction for α-SMA antibodies was strong in the smooth muscle of the walls of obvious neovascularization and scanty weak reaction in granulation tissues (Fig. 1D).

b) 7th day:

There were positive reactions to α-SMA antibodies in the blood vessels walls in the four groups A, B, C and, D with increased blood vessels in the group C (Fig 2).

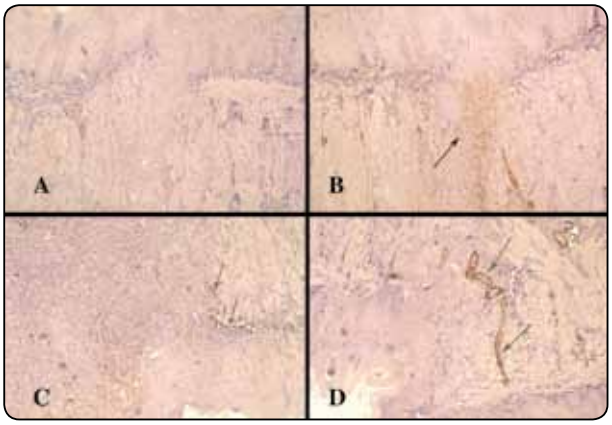

Fig. (1) photomicrographs of sections in rats tongue in the 4th day after surgery showing: Positive reaction of α-SMA in the blood vessel walls and scanty weak reaction in granulation tissue (injured control group A), strong reaction in wound region and blood vessel walls (injured centella asiatica treated group B), weak reaction in blood vessels wall in granulation tissue (injured dexamethasone treated group C), strong reaction in neovascularization and scanty weak in wound region in dexamethasone and centella asiatica treated group D).

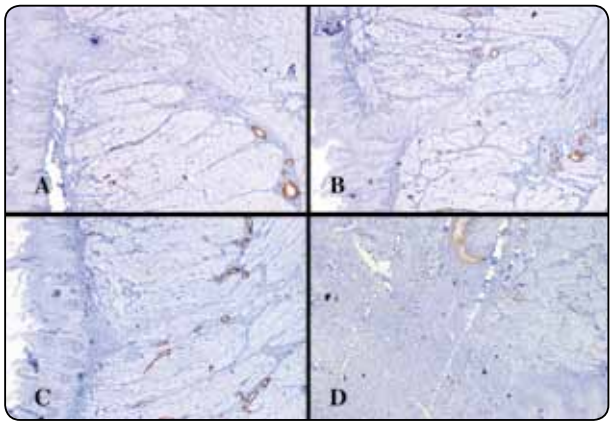

Fig. (2) photomicrographs of sections in rats tongue in the seventh day after surgery showing, positive reaction to α-SMA antibodies in the blood vessels walls of the wound A, B, C, D.

II- vimentin detection:

a. 4th day:

In the control group (group A); revealed strong reaction for vimentin antibodies in the wound region (Fig 3 A).

Centella asiatica group (group B); there was strong reaction in the periphery of the wound region and decreased toward the center (Fig 3 B).

Dexamethasone group (group C); there was moderate reaction in the granulation tissue of the wound (Fig 3 C).

Dexamethasone and Centella asiatica group (group D); there was strong reaction in granulation tissue of the wound region (Fig. 3 D).

b. 7th day:

There were strong reaction to vimentin antibodies in the wound region in group A, B, and, D but it was less intensity reaction in group C in the wound region (Fig 4).

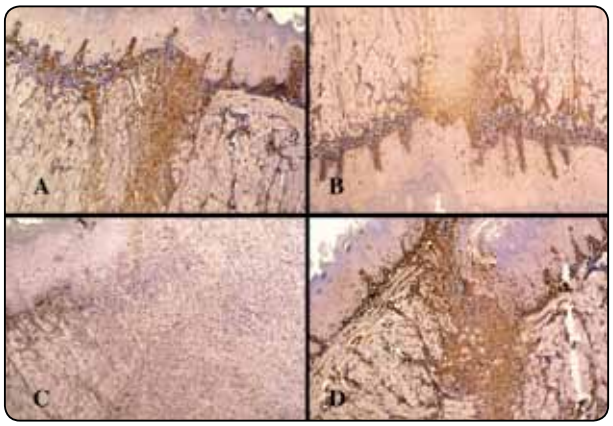

Fig. (3) photomicrographs of sections in rats tongue in the 4th day after surgery showing A) strong reaction to vimentin in control group. B) Strong reaction to vimentin in wound region in centella asiatica treated group. C) Moderate reaction to vimentin in the wound in dexamethasone treated group. D) Strong reaction to vimentin in injured dexamethasone and centella asiatica treated group.

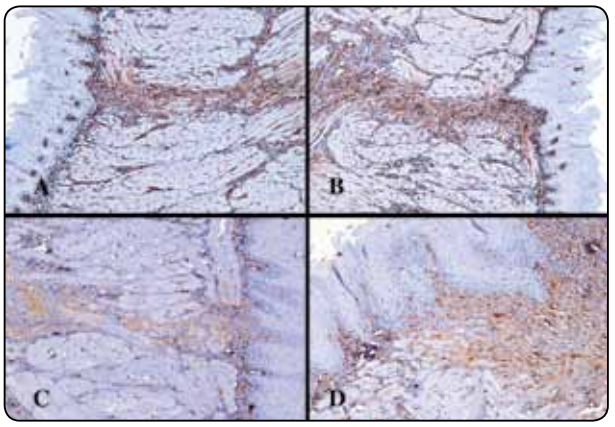

Fig. (4) photomicrographs of the sections in the rats tongue after surgery showing strong reaction to vimentin antibodies in groups A, B, and, D but Dexamethazone group C the reaction was less intense than the other groups.

DISCUSSION

Healing is a physiological process. Efforts are being made all over the world to discover agents that can promote healing and thereby reduce discomfort and other complications [19].

In the present study; the expression of α-SMA and vimentin in granulation tissue during induced wound healing of rats tongue was evaluated using specific antibodies directed against these proteins at the light microscope level.

Myofibroblasts develop gradually from granulation tissue fibroblasts and temporary express a marker of smooth muscle differentiation, these cells may be relevant for the understanding of the mechanisms of normal and pathologic wound healing [20]. α-SMA is a well accepted marker of myofibroblast differentiation and it has been suggested to play a role in the production of contractile force during wound healing [21].

In this study, there was strong reaction to α-SMA antibodies in the 4th day after surgery in group B (CA treated group) and weak in other groups; this coula be attributed to the promoting effect of CA in fibroblastic proliferation, collagen formation,

neovascularization and increasing antioxidant levels [22,23].

The weak reaction to α-SMA in group C (dexamethasone group) could be attributed to dexamethasone effect which decrease the mRNA and protein expression in both the amount and assembly of α-SMA in cells [24]. This is in compliance with **Gold Smith et. al** [25]., who found that dexamethasone reduce human airway smooth muscle α-SMA expression and its incorporation into contractile filaments as well as contractile function.

Important amount of α-SMA were always expressed in pericytic and / or smooth muscle cell of neoformed small vessels. In fibroblastic cells, microfilaments were absent 4 days after wounds but accumulated gradually [20].

In group D (dexamethasone and C A group) the reaction of α-SMA antibodies was strong in the walls of blood vessels in obvious neovascularization and in the granulation tissue of the wound. Faster healing was noted in comparison to dexamethasone injected group, this could clarify that C A is able to overcome the suppressing action of dexamethasone in wound healing [26].

In the 7th day after surgery, the reaction of α-SMA antibodies was confined to the walls of blood vessels and apparent blood vessels were seen in the dexamethasone group indicating delayed healing because, as healing progresses, vascularization diminishes and granulation tissue is replaced by newly formed connective tissue components which is more cellular and has many immature collagen fibers. Many of blood vessels disappear as a result of apoptosis [27]

Vimentin is necessary for any tissue movement that require the generation of significant tractional forces [28]. In this study, in the 4th day after surgery, the reaction to vimentin was strong in group A (control group) which is most probably resulted from inflammatory cells as normal expression of vimentin on the surface of activated macrophages, platelets, apoptotic T-lymphocytes and aged neutrophilis [29].

In C A group there was strong reaction to vimentin at the periphery of the wound. This could indicate the proliferation of fibroblasts at the periphery of the wound thus CA promote and increase the proliferation of fibroblasts [6, 30, 31]. This aids in accelerating the healing this is in agreement with the results of **Eckes et, al** [28] who revealed that; wounds in vimentin deficient animals showed delayed migration of fibroblasts into the wound site and subsequently retarded contraction that correlated with a delayed appearance of myofibroblasts in the wound site.

In group D (C A and dexamethasone group) the reaction of vimentin is marked with strong reaction than in dexamethasone alone with faster healing indicating that administration of C A diminished the suppressing effects of dexamethasone.

In the 7th day after surgery, in this study; reaction of vimentin was strong in groups A, B, and, D indicating progression of the inflammatory phases. This might be because vimentin is required for many vital cell functions like cell motility, chemotactic migration and wound healing [28,32]. But in group C, the reaction was less intense than other groups, this may be attributed to the dexamethasone had no effect on redistribution of vimentin this is in agreement with **Lieber and Evans** [33]; who concluded that dexamethasone had no discernible effect on vimentin organization in undifferentiated cells or during adipose conversion with absence of vimentin filament network with impairment of all functions depending upon mechanical stability [28].

In this study, as C A accelerates the wound healing and counteracts the suppressing effect

of dexamethazone, this proved by increasing the expression of α-SMA and vimentin which stimulate wound closure. This could be one of the possible explanations of the mode of action of C A in soft tissue healing

Further researches are needed at the molecular biology level to confirm the previous results

CONCLUSION

Centella asiatica C A increases the formation of α-SMA and vimentin and this may be one of the possible explanation of the faster healing of soft tissue wound upon administration of C A systemically.

ACKNOWLEDGMENTS

We thank Prof. Dr. Ahmed Ragheb Zaher for helpful suggestions and revision regarding the realization of this study.

REFERENCES

1- Council of Europe. Plants preparations used as ingredients of cosmetic products. Vol.I. Strasbourg Cedex: Council of Europe Publishing, 1994; p: 116-117 (61*8 PAT).

2- MacKay, D. Hemorrhoids and Varicose Veins: A Review of Treatment Options. Alternative Medicine Review, 2001; 6 (2): 126-140

3- MacKay, D. & Miller, A.L. Nutritional Support for Wound Healing. Alternative Medicine Review, 2003; 8 (4): 359-377

4- PDR for Herbal Medicines. Medical Econimics Company. Monvale (New Jersey), 1998; p: 729-731 (ref.633.8 (ENC)).

5- Punturee K, Wild CP, Kasinrerk W, Vinitketkumnuen U.: Immunomodulatory activities of Centella asiatica and Rhinacanthus nasutus extracts. Asian Pac J Cancer Prev. 2005 Jul-Sep;6(3):396-400.

6. Suguna L, Sivakumar P, Chandrakasan G.: Effects of Centella asiatica extract on dermal wound healing in rats. Indian J Exp Biol. 1996 Dec;34(12):1208-11.

7. Askar I, Bozkurt M.: Protective effects of immunosuppressants and steroids against ischemia-reperfusion injury in cremaster muscle flap at microcirculatory level. Microsurgery. 2002;22(8):361-6.

8. Witte MB, Barbul A.: General principles of wound healing. Surg Clin North Am. 1997 Jun;77(3):509-28.

9. Schmelzeisen R, Frölich JC: Prevention of postoperative swelling and pain by dexamethasone after operative removal of impacted third molar teeth. Eur J Clin Pharmacol. 1993;44(3):275-7.

10. Luo JD, Chen AF.: Nitric oxide: a newly discovered function on wound healing. Acta Pharmacol Sin. 2005 Mar;26(3):259-64.

11. Ten cate AR: oral Histology, Development, structure, and Function. 5th ed., Saint Louis, Baltimore, Bostone, Chicago, London, Mosby Company, 1998,50 – 68.

12. Mor-Vaknin N, Punturieri A, Sitwala K, Markovitz DM.: Vimentin is secreted by activated macrophages. Nat Cell Biol. 2003 Jan;5(1):59-63.

13. Skalli O, Schürch W, Seemayer T, Lagacé R, Montandon D, Pittet B, Gabbiani G.: Myofibroblasts from diverse pathologic settings are heterogeneous in their content of actin isoforms and intermediate filament proteins. Lab Invest. 1989 Feb;60(2):275-85.

14. Hinz B, Celetta G, Tomasek JJ, Gabbiani G, Chaponnier C.: Alpha-smooth muscle actin expression upregulates fibroblast contractile activity. Mol Biol Cell. 2001 Sep;12(9):2730-41.

15. Skalli O, Ropraz P, Trzeciak A, Benzonana G, Gillessen D, Gabbiani G.: A monoclonal antibody against alpha-smooth muscle actin: a new probe for smooth muscle differentiation. J Cell Biol. 1986 Dec;103(6 Pt 2):2787-96.

16. Liu S, Kapoor M, Leask A.: Rac1 expression by fibroblasts is required for tissue repair in vivo. Am J Pathol. 2009

May;174(5):1847-56. Epub 2009 Apr 6.

17. Gülkesen KH, Erdoğru T, Sargin CF, Karpuzoğlu G.: Expression of extracellular matrix proteins and vimentin in testes of azoospermic man: an immunohistochemical and morphometric study. Asian J Androl. 2002 Mar;4(1):55-60.

18. Mărgăritescu C, Mogoantă L, Mănescu P, Simionescu C, Pirici D, Streba L, Mercuţ D: The immunohistochemical profile of the adenocarcinoma of upper gastric pole. Rom J Morphol Embryol. 2007;48(3):215-35.

19. Shukla A, Rasik AM, Dhawan BN.: Asiaticoside-induced elevation of antioxidant levels in healing wounds. Phytother Res. 1999 Feb;13(1):50-4.

20. Darby I, Skalli O, Gabbiani G.: Alpha-smooth muscle actin is transiently expressed by myofibroblasts during experimental wound healing. Lab Invest. 1990 Jul;63(1):21-9.

21. Serini G, Gabbiani G.: Mechanisms of myofibroblast activity and phenotypic modulation. Exp Cell Res. 1999 Aug 1;250(2):273-83.

22. Lu L, Ying K, Wei S, Fang Y, Liu Y, Lin H, Ma L, Mao Y.: Asiaticoside induction for cell-cycle progression, proliferation and collagen synthesis in human dermal fibroblasts. Int J Dermatol. 2004 Nov;43(11):801-7.

23. Suguna L, Sivakumar P, Chandrakasan G.: Effects of Centella asiatica extract on dermal wound healing in rats. Indian J Exp Biol. 1996 Dec;34(12):1208-11.

24. Tsai WC, Tang FT, Wong MK, Pang JH.: Inhibition of tendon cell migration by dexamethasone is correlated with reduced alpha-smooth muscle actin gene expression: a potential mechanism of delayed tendon healing. J Orthop Res. 2003 Mar;21(2):265-71.

25. Goldsmith AM, Hershenson MB, Wolbert MP, Bentley JK.: Regulation of airway smooth muscle alpha-actin expression by glucocorticoids. Am J Physiol Lung Cell Mol Physiol. 2007 Jan;292(1):L99-L106. Epub 2006 Sep 15.

26. Shetty BS, Udupa SL, Udupa AL, Somayaji SN.: Effect of Centella asiatica L (Umbelliferae) on normal and dexamethasone-suppressed wound healing in Wistar Albino rats. Int J Low Extrem Wounds. 2006 Sep;5(3):137-43.

27. Avery JK: Oral development and histology. 3rd edition, thieme Medical Publishers NewYork, Francisco RH wound healing 2001, P 390 – 409.

28. Eckes B, Colucci-Guyon E, Smola H, Nodder S, Babinet C, Krieg T, Martin P.: Impaired wound healing in embryonic and adult mice lacking vimentin. J Cell Sci. 2000 Jul;113 (Pt 13):2455-62.

29. Moisan E, Girard D.: Cell surface expression of intermediate filament proteins vimentin and lamin B1 in human neutrophil spontaneous apoptosis. J Leukoc Biol. 2006 Mar;79(3):489-98. Epub 2005 Dec 19.

30. Punturee K, Wild CP, Kasinrerk W, Vinitketkumnuen U.: Immunomodulatory activities of Centella asiatica and Rhinacanthus nasutus extracts. Asian Pac J Cancer Prev. 2005 Jul-Sep;6(3):396-400.

31. Widgerow AD, Chait LA, Stals R, Stals PJ.: New innovations in scar management. Aesthetic Plast Surg. 2000 May-Jun;24(3):227-34.

32. Eckes B, Dogic D, Colucci-Guyon E, Wang N, Maniotis A, Ingber D, Merckling A, Langa F, Aumailley M, Delouvée A, Koteliansky V, Babinet C, Krieg T.: Impaired mechanical stability, migration and contractile capacity in vimentin-deficient fibroblasts. J Cell Sci. 1998 Jul;111 (Pt 13):1897-907.

33. Lieber JG, Evans RM.: Disruption of the vimentin intermediate filament system during adipose conversion of 3T3-L1 cells inhibits lipid droplet accumulation. J Cell Sci. 1996 Dec;109 (Pt 13):3047-58.