Indian Journal of Experimental Biology
Vol. 49, January 2011, pp. 50-55

Effect of Bis [benzyl *N'*-(indol-3-ylmethylene)-hydrazinecarbodithioato]-zinc(II) derivatives on wound healing in Sprague Dawley rats

Fathi Farag Mughrabi[1*], Harita Hashim[1], Mahmood Ameen[2], Hamid Khaledi[3], Hapipah Mohd. Ali[3] & Salmah Ismail[2]
[1]Department of Biology, Faculty of Applied Science University Teknologi MARA, 40450, Shah Alam, Malaysia
[2]Department of Molecular Medicine, Faculty of Medicine, University of Malaya, 50603, Kuala Lumpur, Malaysia
[3]Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

Received 25 March 2010; revised 2 August 2010

Effects of topical application of Bis[benzyl *N'*-(indol-3-ylmethylene)-hydrazinecarbodithioato]-zinc(II) (BHCZ) on wound healing and histology of healed wound were assessed. Sprague Dawley rats were experimentally induced wound in the posterior neck area. Tween 20 (0.2 ml of 10%) was applied to rats in Group 1 (negative control). Intrasite gel (0.2 ml) was applied topically to rats in Group 2 as reference. BHCZ at the concentrations 0.2 ml of 25, 50 and 100 mg/ml were applied to Group 3, 4 and 5, respectively. Wound dressed with BHCZ significantly healed earlier than those treated with 10% Tween 20. Also wound dressed with 100 mg/ml BHCZ accelerated the rate of wound healing compared to those dressed with intrasite gel and, 25 mg/ml and 50 mg/ml BHCZ. Histological analysis of healed wound with BHCZ showed comparatively less scar width at wound enclosure and the healed wound contained less macrophages and large amount of collagen with angiogenesis compared to wounds dressed with 10% Tween 20. Results of this study showed that wounds dressed with 100 mg/ml of BHCZ significantly enhanced acceleration of the rate of wound healing enclosure, and histology of healed wounds showed comparatively less macrophages and more collagen with angiogenesis.

Keywords: BHCZ derivatives, Histology, Intrasite gel, Wound healing

Metal complexes of Schiff bases derived by condensation of heterocyclic aldehydes with S-benzyldithiocarbazate have been shown to exhibit significant biological activities[1,2]. Biological activity of these Schiff bases and their metal complexes may be due to their interaction with potential donors of biological heterocycles *in vivo*[3,4]. In many cases, the pharmacological activity has been found to be highly dependent on the identity of the metal and the donor sequence of the ligands, as different ligands show widely different biological activities although they may vary only slightly in their molecular structure[5,6]. Indole derivatives have also been reported to exhibit antidepressive[7], antiallergic[8], antifungal[9] antioxidant[10, 11] and antiulcer activities[12].

Wound healing, or wound repair, is an intricate process in which the skin (or some other organ) repairs itself after injury[13]. Dermal wound healing is a coordinated process of tissue remodeling involving an inflammatory response, re-epithelialization, and revascularization and the process is mediated by soluble cytokines and growth factors, which act on multiple cell types including keratinocytes, dermal fibroblasts, and vascular cells. Activated inflammatory cells secrete various matrix proteinases to facilitate breakdown of the extracellular matrix (ECM), which aids in the migration of keratinocytes and fibroblasts into the wound bed. Deposition of provisional matrices such as fibronectin provides a permissive environment for angiogenesis to occur, which ultimately leads to the healing of the wound and restoration of dermal function[14].

Intrasite gel is a colorless transparent aqueous gel containing a modified carboxymethylcellulose (CMC) polymer together with propylene glycol as a humectants and preservative. Intrasite gel gently re-hydrates necrotic tissue, facilitate autolytic debridemen, loosen and absorb slough and exudates, cleaning the way for effective wound healing. It is also designed for wounds that are granulating and epithelialising and can also be used to provide the optimum moist wound management environment during the later stages of wound closure. It is non-adherent and does not harm viable tissue or the skin surrounding the wound thereby making it ideal for every stage in the wound management process[15].

*Correspondent author
Telephone: 603-42704382/ Mobile: 0176409136
Fax: 603- 55444560
E-mail: fathi.faraz@yahoo.com

Since there are no reports available regarding wound healing of this derivative compound, the present study was undertaken to evaluate the rate of wound healing properties of BHCZ in experimental rats. Intrasite gel was used as a positive control and Tween 20 was used as a negative control.

Materials and Methods

Preparation of S-benzyldithiocarbazate—This compound was synthesized as reported previously[8]. Briefly, mixture of hydrazine hydrate (10 g, 0.2 mol) and potassium hydroxide (11.4 g, 0.2 mol) in 90% ethanol (70 ml) was cooled in an ice bath. Carbon disulphide (15.2 g, 0.2 mol) was then added drop-wise with vigorous stirring. The temperature of the reaction mixture was not allowed to rise above 5°C during the period of addition of carbon disulfide. To the mixture, 40% ethanol (60 ml) was added and the solution was cooled in ice. Benzyl chloride (25.3 g, 0.2 mol) was then added slowly with vigorous stirring and the white product was separated by filtration, washed with water and dried in air. The crude product was recrystallized from absolute ethanol and the yield was 23 g (58%).

Preparation of the ligand benzyl N'-(indol-3-ylmethylidene) - hydrazinecarbodithioate—Indole-3-carbaldehyde (4.35 g, 0.03 mol) and S-benzyl dithiocarbazate (5.94 g, 0.03 mol) were heated in methanol (300 ml) for 3 h. The solution was set aside till a yellow precipitate formed. The precipitate was filtered off, washed with cold ethanol and dried over silica gel and the yield was 8.3 g (85%).

Preparation of the complex Bis[benzyl N'-(indol-3-ylmethylene)-hydrazinecarbodithioato]- zinc(II)—The ligand (3.25 g, 0.01 mol) was dissolved in hot ethanol (500 ml) in the presence of triethylamine (1 ml). An ethanol solution of zinc acetate dihydrate (1.1 g, 0.005 mol) was added to this solution and the clear mixture was refluxed for 2 h and a pale yellow product precipitated. The solid was filtered off, washed with ethanol and dried over silica gel; yield, 3.24 g (91%). The purity of the product was confirmed by 1HNMR spectrum (Fig. 1).

Intrasite gel—Intrasite gel was purchased from University Malaya Medical Center Pharmacy. Intrasite gel (0.2 ml) was applied topically to the wound of Group 2 rats (Intrasite gel is a trademark for Smith and Nephew Ltd)[15].

Lignocaine HCl (2%, 100 mg/5 ml)—The local anesthesia was purchased from experimental animal house, Faculty of Medicine, University Malaya. Lignocaine (1 ml) was used to inject each rat subcutaneously.

Experimental animals—Adult male Sprague Dawley rats were obtained from the experimental animal house, Faculty of Medicine, University of Malaya, and Ethic No. PM/27/07/2009/MAA (R). Rats were divided randomly into 5 groups of 6 rats each. Each rat that weighted between 200-220 g was housed separately (one rat per cage). Animals were maintained on standard pellet diet and tap water. The study was approved by the ethics Committee for Animal Experimentation, Faculty of Medicine, University of Malaya, Malaysia. Throughout the experiments, all animals received human care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals", National Institute of Health, Bethesda, MD, USA.

Experimentally induced wounds—Animals were anesthetized by diethyl ether. The skin shaved by electrical shaver, disinfected with 70% alcohol and injected with 1 ml of lignocaine HCl (2%, 100 mg/5 ml). An area of uniform wound 2.00 cm in diameter was excised from the nape of the dorsal neck of all rats with the aid of round seal as described by Morton and Melone[16], with slight modification (using transparency papers and permanent marker) incision of the muscle layer has been avoided and tension of skin was kept constant during the procedure. The wound area was measured immediately under light diethyl ether anesthesia as described by Nayak and Pinto Pereira[17].

Topical application of vehicles—Wounds of Group 1 animals were dressed with 0.2 ml of 10% Tween 20 solution as a negative control twice daily. Wounds of Group 2 rats were dresses topically with 0.2 ml of Intrasite gel as a reference, twice daily. 0.2 ml of 25 mg/ml, 50 mg/ml and 100 mg/ml of compound derivative were applied topically twice daily to the

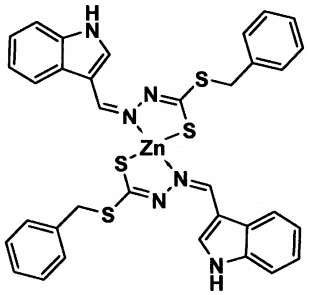

Fig. 1—Bis [benzyl *N'*-(indol-3-ylmethylene)-hydrazinecarbodithioato]-zinc(II)

wound of Group 3, 4 and 5 (experimental animals), respectively as described by Chah *et al*., [18] with slight modification. The wound was observed daily until complete epithelization and the wound closure rate was assessed by tracing the wound on days 1, 5, 10 and 15 post-wounding using transparency papers and permanent marker as described by Nayak and Pinto Pereira[17], with slight modification. The wound areas recorded were measured using a graph paper. Number of days required for falling of scar without any residual raw wound gave the period of epithelization. The percent wounds healing on these days are determined.

Histological evaluation of healed wounds—The skin specimen from wounds healed areas were fixed in 10% buffered formalin and processed by paraffin tissue processing machine. The healed skin was assessed by taking a 5 µ section, stained with hematoxylin and eosin.

Statistical analysis—All values are reported as mean ± SEM and the statistical significance of differences among groups were assessed using one-way ANOVA. A value of $P<0.05$ was considered significant.

Results and Discussion

Wounds dressed with BHCZ showed considerable signs of dermal healing and significantly ($P<0.05$) healed earlier compared to wounds dressed with 0.2 ml of 10% Tween 20 (negative control). Animals in the 100 mg/ml of compound derivative treated group showed significantly better healing and healed faster compared to animals in the 25 mg/ml and 50 mg/ml compound-treated groups (Fig. 2). There were no significant differences between wounds dressed with Intrasite and 25 mg/ml and 50 mg/ml of the compound in the term of duration of wound healing enclosure (Table 1). Histological, wound dressed with the T1 compound contained comparably less scar at wound enclosure, and healed wound contained few macrophages, and more collagen and proliferating blood capillaries compared with wound dressed with 10% Tween 20 solution (Fig. 3).

In the present study, topical application of compound derivative significantly enhanced the rate of wound healing and the healed wound contain less macrophages, more collagen and angiogenesis. Wound-healing processes could include cell proliferation, suppression of inflammation and contraction of the collagen tissue[19]. Wound healing effects may be due to up-regulation of collagen

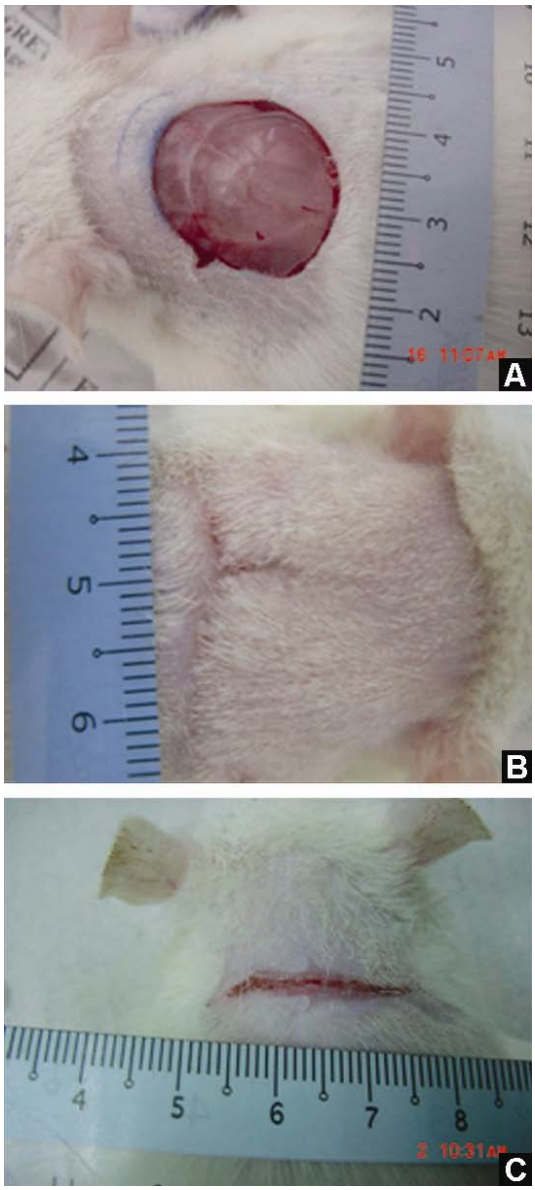

Fig. 2—(A): Excision skin wound (2.0 cm) on day 0 before application of vehicle; (B): complete wound healing on day 12 with 0.2 ml of 100 mg/ml compound derivative; (C): incomplete wound healing on day 17 in animal treated with 0.2 ml of 10% Tween 20 (negative control).

Table 1—Time required for wound healing by BHCZ in experimental animals

[Values are mean ± SE of 6 animals]

Treatment groups	Type of dressings (0.2 ml)	Healing time (days)
Group 1	10% Tween 20 (negative control)	19.58 ± 0.57^{a}
Group 2	Intrasite gel (positive control)	14.71 ± 0.27^{b}
Group 3	25 mg/ml BHCZ	14.04 ± 0.22^{b}
Group 4	50 mg/ml BHCZ	13.38 ± 0.19^{b}
Group 5	100 mg/ml BHCZ	11.58 ± 0.22^{c}

Values with different superscripts are significantly different ($P<0.05$)

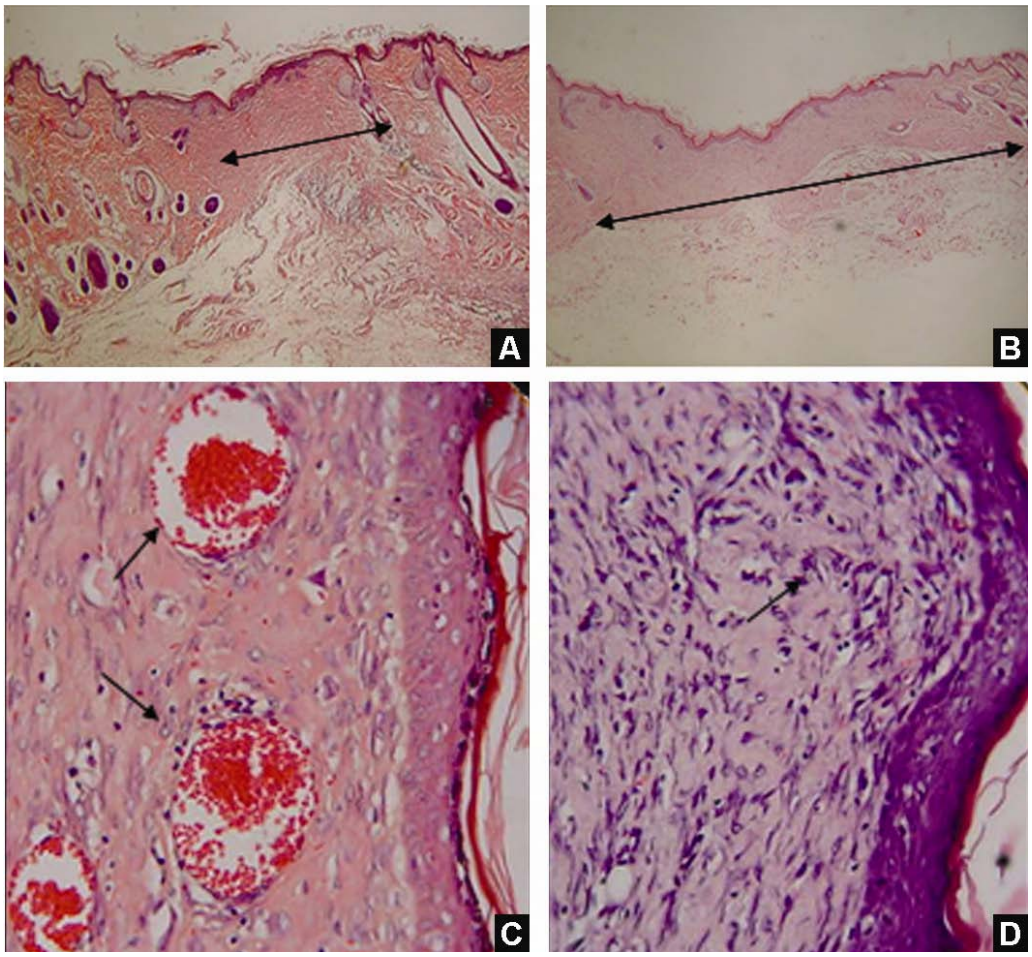

Fig. 3—(A): Healed wound dressed with 0.2 ml of 100 mg/ml compound derivative showing comparatively narrow scar at the wound closure, H & E stain 10×; (B): Histological section of healed wound dressed with 0.2 ml of Tween 20 (negative control) showing comparatively wide scar at the wound closure, H & E stain 10×; (C): Healed wound dressed with 0.2 ml of 100 mg/ml compound derivative. Granulation tissue contains comparatively more collagen, fibroblast and blood capillaries (indicated by ↑), and few or absence of inflammatory cells, H & E stain 40×; (D): Healed wound dressed with 10% Tween 20 (negative control). Granulation tissue contains less collagen, fibroblast and blood capillaries, and more inflammatory cells (indicated by ↑), H & E stain 40×.

expression[17], and an increase in tensile strength of the wounds[20], and an increase in tensile strength of the wounds[21]. Similarly, enhanced healing activity has been attributed to increased collagen formation and angiogenesis[22,23]. Collagen played a central role in the healing of wounds and it is a principal component of connective tissue and provides a structural framework for the regenerating tissue[24]. Angiogenesis in granulation tissues improves circulation to the wound site thus providing oxygen and nutrients essential for the healing process[25], that include-re-epithelization. Stimulate epithelial cell proliferation and angiogenesis are important for wound healing process[26]. In the agreement with a previous study[27], the result of present study indicates that histological analysis of the treated healed wound group contained a large amount of fibroblast proliferation, collagen synthesis, and neovascularization, which resulted in an increased wound tensile strength and accelerated wound healing. However, Nascimento and Costa[28] demonstrated that histology of wound healed in overweight rats induced by a high-fat diet increased the inflammatory infiltrate and delayed myofibroblastic differentiation, collagen deposition, epithelial and connective tissue cells proliferation, and angiogenesis.

Molecular oxygen plays a central role in the pathogenesis and therapy of chronic wounds. Overproduction of reactive oxygen species (ROS) results in oxidative stress thereby causing cytotoxicity and delayed wound healing. Therefore, elimination of ROS could be an important strategy in healing of chronic wounds[29]. Wound healing mechanisms may

be contributed to stimulate the production of antioxidants in wound site and provides a favorable environment for tissue healing[23]. Indole derivatives have shown antioxidant activity[10,11]. Antioxidants have been reported to play a significant role in the wound healing process[23]. Topical applications of compounds with antioxidant properties significantly improve wound healing and protect tissues from oxidative damage[30]. In conclusion, results of the study showed that topical application of BHCZ to wounds significantly enhanced the wound healing process.

Acknowledgement

Financial support Universiti Teknologi MARA from DANA (600-RMI/ST/DANA 5/3/Dst) 289/2009) and FRGS (600-RMI/ST/FRGS 5/3/Fst (34/2010) grants and University of Malaya are acknowledged.

References

1 Singh H L & Varshney A K, Synthetic, structural, and biochemical studies of organotin (IV) with Schiff bases having nitrogen and sulphur donor ligands, *Bioinorg Chem Appl,* (2006) 1.

2 Tarafder M T H, Chew K B, Crouse K A, Ali A M, Yamin B M & Fun H K, Synthesis and characterization of Cu(II), Ni(II) and Zn(II) metal complexes of bidentate NS isomeric Schiff bases derived from S-methyldithiocarbazate (SMDTC): Bioactivity of the bidentate NS isomeric Schiff bases, some of their Cu(II), Ni(II) and Zn(II) complexes and the X-ray structure of the bis[S-methyl-beta-N-(2-furylmethyl) methylenedithiocarbazato] zinc(II) complex, *Polyhedron,* 21 (2002) 2683.

3 Chan S C, Koh L L, Leung P H, Ranford J D & Sim K Y, Copper (II) complexes of the antitumour-related ligand salicylaldehyde acetylhydrazone (H_2L) and the single-crystal X-ray structures of [{Cu (HL) H2O} $_2$] · 2(NO_3) and [{Cu (HL) (pyridine) (NO_3)} $_2$], *Inorg Chim Acta,* 236 (1995) 101.

4 Zhu X H, Chen X F, Ren X M, You X Z, Raj S S & Fun H K, Unambiguous *cis*-coordination of mono- and bi-dentate Lewis bases to Ni (SN) $_2$ Schiff-base complexes derived from *S*-alkyl dithiocarbazate, *Polyhedron,* 18 (1999) 3683.

5 Hossain M E, Alam M N, Akbar A M, Nazimuddin M, Smith F E & Hynes R C, The synthesis, characterization and bioactivities of some copper(II) complexes of the 2-acetylpyridine schiff bases of s-methyl- and s-benzyldithiocarbazate, and the X-ray crystal structure of the nitrato(s-benzyl-β-n-(2-acetylpyridyl) methylenedithiocarbazato) copper(II) complex, *Polyhedron,* 15 (1996) 973.

6 Hossain M E, Alam M N, Begum J, Akbar A M, Nazimuddin M, Smith F E & Hynes R C, The preparation, characterization, crystal structure and biological activities of some copper (II) complexes of the 2-benzoylpyridine Schiff bases of *S*-methyl- and *S*-benzyldithiocarbazate, *Inorg Chim Acta,* 249 (1996) 207.

7 Krishna C, Joshi K C & Chand P, Biologically active indole derivatives, *Pharmazie,* 37 (1982) 1.

8 Unangst P C, Connor D T, Stabler S R, Weikert R J, Carethers M E, Kennedy J A, Thueson D O, Chestnut J C, Adolphson R L & Conroy MC, Novel indole carboxamidotetrazoles as potential antiallergy agents, *J Med Chem,* 32 (1989) 1360.

9 Canoira L, Gonzalo R J, Subirats J B, Escario J A, Jimenez I & Martinez-Fernandez A R, Synthesis, structure and anti-fungal activity of 3-(2′-nitrovinyl) Indoles, *Eur J Med Chem,* 24 (1989) 39.

10 Kaneko S, Okumura K, Numaguchi Y, Matsui H, Murase K, Mokumo S, Morishima I, Hira K, Toki Y, Ito T & Hayakawa T, Melatonin scavenges hydroxyl radical and protects isolated rat hearts from ischemic reperfusion injury, *Life Sci,* 67 (2000) 101.

11 Liu F & Ng T B, Effect of pineal indoles on activities of the antioxidant defense enzymes superoxide dismutase, catalase, and glutathione reductase, and levels of reduced and oxidized glutathione in rat tissues, *Biochem Cell Biol,* 78 (2000) 447.

12 Bell M R, Zalay A W, Oesterlin R, Clemans S D, Dumas D J, Bradford J C & Jr J R, Experimental antiulcer drugs- 1-Indole-1-alkanamides and pyrrole-1-alkanamides, *J Med Chem,* 20 (1977) 537.

13 Nguyen D T, Orgill D P & Murphy G F, The pathophysiologic basis for wound healing and cutaneous regeneration in *Biomaterials for treating skin loss* (CRC Press, US & Woodhead Publishing, UK, Boca Raton/Cambridge) 2009, 25.

14 Midwood K S, Williams L V & Schwarzbauer J E, Tissue repair and the dynamics of the extracellular matrix, *Int J Biochem Cell Biol,* 36 (2004) 1031.

15 Williams C, Intrasite Gel: A hydrogel dressin, *British J of Nurs,* 3 (1994) 843.

16 Morton J J & Molane M H, Evaluation of vulnerary activity by an open wound procedure in rats. *Archives Internationales de Pharmacodynemiie et de Therapie,* 196 (1972) 117.

17 Nayak B S & Pinto Pereira L M, Catharanthus roseus flower extract has wound-healing activity in Sprague Dawley rats, *BMC Complement Altern Med,* 6 (2006) 41.

18 Chah K F, Eze C A, Emuelosi C E & Esimone C O, Antibacterial and wound healing properties of methanolic extracts of some Nigerian medicinal plants, *J Ethnopharmacol,* 104 (2006) 164.

19 Houghton PJ, Hylands PJ, Mensah AY, Hensel A & Deters AM, *In vitro* tests and ethnopharmacological investigations wound healing as an example, *J Ethnopharmacol*, 100 (2005) 100.

20 Bonte F, Dumas M, Chadgne C & Meybeck A, Influence of Asiatic acid, madecassic acid, and asiaticoside on human collagen I synthesis, *Planta Medica*, 60 (1993) 133.

21 Suguna L, Sivakumar P & Chandrasan, Effect of centella asiatica extract on dermal wound healing in rats, *Indian J Exp Bol*, 34 (1996) 1208.

22 Trabucchi E, Preis-Baruffaldi F, Baratti C & Montorsi W, Topical treatment of experimental skin lesions in rats: macroscopic, microscopic and scanning electron-microscopic evaluation of the healing process, *Int J Tissue React,* 8 (1986) 533.

23 Shukla A, Rasik A M & Dhawan B N, Asiaticoside-induced elevation of antioxidant levels in healing wounds, *Phytother Res,* 13 (1999) 50.

24 Cohen I K, Diegelmann R F & Lindblad W J, *Wound healing: Biochemical and clinical aspects*, (Saunders, Philadelphia) 1992.

25 Szabo S, Kusstatscher S, Sakoulas G, Sandor Z, Vincze A & Jadus M, Growth factors: New "endogeneous drug" for ulcer healing, *Scand J Gastroenterol,* 210 (1995) 15.

26 Buntrock P, Jentzsch K D & Heder G, Stimulation of wound healing. Using brain extract with fibroblast growth factor (FGF) activity. II. Histological and morphometric examination of cells and capillaries, *Exp Pathol,* 21 (1982) 62.

27 Habibipour S, Oswald T M, Zhang F, Joshi P , Zhou X C, Dorsett-Martin W & Lineaweaver WC, Effect of sodium diphenylhydantion on skin wound healing in rats, *Plast Reconstr Surg,* 112 (2003) 1620.

28 Nascimento AP & Costa AM. Overweight induced by high-fat diet delays rat cutaneous wound healing, *Br J Nutr*, 6 (2006) 77.

29 Dissemond J, Goos M & Wagner SN. The role of oxidative stress in the pathogenesis and therapy of chronic wounds, *Hautarzt,* 53 (2002)718.

30 Martin A, The use of antioxidants in healing, *Dermatol Surg,* 22 (1996) 156.