JTCM 中医杂志 Journal of Traditional Chinese Medicine

Online Submissions: http://www.journaltcm.com
info@journaltcm.com

J Tradit Chin Med 2014 December 15; 34(6): 716-724
ISSN 0255-2922

EXPERIMENTAL STUDY

Effect of volatile oil from Blumea Balsamifera (L.) DC. leaves on wound healing in mice

Yuxin Pang, Dan Wang, Xuan Hu, Hui Wang, Wanjin Fu, Zuowang Fan, Xiaolu Chen, Fulai Yu

Yuxin Pang, Dan Wang, Xuan Hu, Xiaolu Chen, Fulai Yu, Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences, Danzhou 571737, China; Medicinal Efficacy Evaluation Laboratory, Hainan Provincial Engineering Research Center for Ainaxiang (*Herba Blumeae Balsamiferae*), Danzhou 571737, China; Key Laboratory of Tropical Crop Gene Resources and Germplasm Enhancement of Ministry of Agriculture, Danzhou 571737, China.

Hui Wang, Wanjin Fu, College of Traditional Chinese Medicine, Guangdong Pharmaceutical University, Guangzhou 510000, China

Zuowang Fan, College of Traditional Chinese Medicine, Guangdong Pharmaceutical University, Guangzhou 510000, China; Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences, Danzhou 571737, China; Key Laboratory of Tropical Crop Gene Resources and Germplasm Enhancement of Ministry of Agriculture, Danzhou 571737, China

Supported by the Fundamental Scientific Research Funds for Chinese Academy of Tropical Agricultural Sciences — Tropical Crops Genetic Resources Institute [The Evaluation and Mechanism Research for the Treatment of Ainaxiang (*Herba Blumeae Balsamiferae*) Oil on Skin Burns Based on Cytokines, No. #1630032014016], the Natural Science Fund of Hainan Province [the Evaluation and Mechanism Research of Ainaxiang (*Herba Blumeae Balsamiferae*) Oil on Healing and Repairing of Skin Wounds, No. #312022], and the Natural Science Fund of China (Study on Variety Classification of "Nalong" and It's Ethanopharmacology of Li in Hainan, No. # 81374065)

Correspondence to: Dr. Yuxin Pang, Laboratory of Tropical Medicinal Plants Resources, Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences, Danzhou 571731, China. blumeachina@126.com
Telephone: +86-898-23300268

Accepted: September 5, 2014

Abstract

OBJECTIVE: To assess the effectiveness of violate oil from Blumea Balsamifera (L.) DC. leaves (BB oil) on wound healing in mice.

METHODS: Undiluted BB oil and its diluted solutions with olive oil to 1/5 and 1/10 to yield BB $oil_{1/5}$ and BB $oil_{1/10}$ were applied to the wounded skin before wound healing conditions were assessed by healing rate, histopathology, and contents of collagen, hydroxyproline, and Neuropeptide Substance P (SP). All above results were compared with the efficacies of the control, pure olive oil, basic fibroblast growth factor (BFGF), and cream of Jing Wan Hong (JWH).

RESULTS: BB $oil_{1/5}$ and BB $oil_{1/10}$ improved wound contraction and closure. Histopathology study further confirmed a desirable histological organization of wound tissues. BB $oil_{1/5}$ and BB $oil_{1/10}$ reduced the number of inflammatory cells, increased wound-healing rates, and significantly increased the hydroxyproline content. Both BB $oil_{1/5}$ and BB $oil_{1/10}$ improved formation of collagen, and reduced the frequency of fibroblasts. Moreover, BB $oil_{1/5}$ and BB $oil_{1/10}$ markedly promoted SP expression. However, undiluted BB oil may induce skin thickening and hardening, inhibite collagen synthesis and delay complete skin wound healing.

CONCLUSION: The BB $oil_{1/5}$ and BB $oil_{1/10}$ promoted capillary regeneration, blood circulation, collagen deposition, granular tissue formation, epithelial deposition, and wound contraction. The mechanism underlying the action might be related to induction of SP secretion, and the proliferation and differentiation of mesenchymal cells.

Key words: Wound healing; Neuropeptides; Blumea Balsamifera (L.) DC.; Borneolum Synthciticum

INTRODUCTION

Traditional medicine practitioners have described the therapeutic efficacies of many traditional and indigenous plants against diseases.[1] Natural products that are safe, and possess physiological properties are excellent sources of new therapeutics for the treatment of conditions like mechanical damage of the skin.[2,3] Some researchers therefore have shifted their focus to the potential wound healing properties of plants.[4]

Wound healing is a process of restoring damaged cells and tissues.[5-7] The phases of wound healing occur in a precise and regulated order. Firstly, it involves hemostasis and inflammation; secondly, mesenchymal cells differentiate, proliferate, and migrate to the wound site; thirdly, angiogenesis and epithelial deposition at the wound surface site. Finally, synthesis, cross-linking and alignment of collagen is required to provides structural strength to the wound healing tissue.[8] The wound also undergoes physical contraction, which might be mediated by contractile fibroblasts.[9] Neuropeptide Substance P (SP) is a pro-inflammatory neuropeptide, and modulates inflammatory responses of skin wounds. SP also promotes the synthesis and metabolism of fibroblast and increases accumulation of collagen in the proliferative phase of mesenchymal cell growth and dynamics.[10] In addition, SP is an important medium in the process of wound repair and scar healing.[11,12]

Ainaxiang (*Herba Blumeae Balsamiferae*), also named Sambong in some tropical countries, is an herb with a rich constitution of essential oils that are used in Traditional Chinese Medicine (TCM).[13] In China, Blumea Balsamifera (L.) DC. normally grows in Hainan and Guizhou.[14,15] Its leaves have been used for healing many conditions including eczema, dermatitis, skin injury, skin bruises, beriberi, lumbago, menorrhagia, rheumatism, and some other diseases.[16] Recently, the extracts of the leaves have been verified to display physiological activities on plasmin-inhibitory,[17,18] anti-fungal,[19,20] free radical scavenging and anti-obesity functions.[21,22] The Records of Seeking Herbal Medicines in Lingnan, an ancient Chinese medical work, described that the extract from Ainaxiang (*Herba Blumeae Balsamiferae*), was used to treat snakebite injury, and skin wounds and itch. It documented that external application of the mashed fresh leaves or leaf water washings decoction could treat traumatic injury, carbuncle and skin pruritus.[23]

The main active ingredient in the violate oil from Blumea Balsamifera (L.) DC. leaves (BB oil) is l-borneol. However, little systematic study has been found on the pharmacologic use of BB oil. Herein, we have evaluated the potential for, and possible mechanisms of action of, BB oil to promote wound healing.

METERIALS AND METHODS

PLant and its volatile oil preparation

Blumea Balsamifera (L.) DC. was obtained from Luodian, Guizhou, China. It was identified by Dr. Yuxin Pang, associate researcher fellow at the Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences, Danzhou, Hainan, China. The herbarium voucher specimen (TCGRI 10011) was saved. BB oil was obtained by distilling its air-dried leaves with steam at Guizhou Ai-Yuan Ecological Medicine Development Industry, Guizhou, China. A yield of 0.1 mL of BB oil was obtained from each Kg of fresh Blumea Balsamifera (L.) DC. leaves.

Determination of l-borneol content in BB oil

The standard l-borneol (98%, Batch No. 10147015) was produced by Alfa Aesar, Heysham, Lancs, UK. The content of l-borneol of BB oil was determined by gas chromatography (GC, Agilent 7890, Santa Clara, CA, USA), using ethyl acetate as the solvent. The separation was performed on a HP-5 quartz capillary column (30 m×320 μm, 0.5 μm) equipped with a flame ionization detector (FID) detector. The injection volume was 0.6 μL and was carried out by nitrogen without splitting. The injector temperature was maintained at 250℃ and detector, 280℃. The oven temperature was programmed from 60℃ for 1 min, raised to 100℃ at a rate of 5℃/min, and to 260℃ at a rate of 20℃/min, and isotherm at 260℃ for 5 min. The number of theoretical plates was more than 50 000 and the separation degree was greater than 1.5. The external standard method was used.

Animals

Ninety eight healthy Kun-Ming mice of specific pathogen-free (SPF) grade (No. SPF2012005) weighing (19±1) g were supplied by the Experimental Animal Center of Guangzhou University of Chinese Medicine [Certificate of quality No. SCXK (Yue) 2008-0020], Guangzhou, China. Forty nine female and forty nine male mice were maintained under 20℃-25℃ with a relative humidity of 40%-70% and normal photo periods (12 h light/12 h dark). The mice were individually housed and fed a normal diet and allowed access to water ad libitum in the Laboratory of Tropical Medicinal Plants Resources, Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences, Danzhou, China. This study was registered at and authorized by Guangdong Pharmaceutical University, Guangzhou, China and Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences, Danzhou, China (Trial registration: current controlled trials: GDPU 2012005). The experiment was conducted in accordance with the UK Animal Care and Control Act of 1986, and the National Institutes of Health guide for the care and use of laboratory animals.

Animal modeling and grouping

Totally 98 mice were randomly divided into seven groups by random number table method: Control (*n*=14), solvent control (*n*=14), basic fibroblast growth factor (BFGF) (*n*=14), cream of Jing Wan Hong (JWH) (*n*=14), undiluted violate oil from Ainaxiang leaves (Blumeae Balsamiferae Folium) (BB oil) (*n*=14), BB oil diluted in olive oil to v/v=1/5 (BB $oil_{1/5}$) (*n*=14), and BB oil diluted in olive oil to v/v=1/10 (BB $oil_{1/10}$) (*n*=14).

Mouse wound model and drug treatments

Mice were anesthetized in advance by intraperitoneal injection with 5% chloral hydrate (0.1 mL per 10 g). The model skin was depilated with electric clippers. The part of each mouse that was at the back of the front leg, and located along the spine on the right side of the mouse at a distance of 1.0 cm, was perforated with a sterilized puncher. The diameter of the wound was 1.5 cm. Then the wound was trimmed with a scalpel to the depth of the fascia. Hemostasis was carried out with a piece of sterile gauze. By doing these, the mechanically induced wound animal model was formed.[26]

Approximately 0.05 mL of the different drug preparations was applied to the wound once daily. Three groups, undiluted BB oil, BB $oil_{1/5}$ and BB $oil_{1/10}$, were set up respectively. The BB $oil_{1/5}$ and BB $oil_{1/10}$ were BB oil diluted in olive oil to 1/5 and 1/10 (v/v), respectively. There was no drug treatment in the control group, and 0.05 mL olive oil (Shanghai Lingfeng Chemical Reagent Co., Ltd., Shanghai, China, 20120816) was applied in the solvent control group. There were two positive groups. A prepared solution of BFGF (basic fibroblast growth factor, Zhuhai Essex Bio-Pharmaceutical Co., Ltd., Zhuhai, Guangdong, China, No. S10980077) was applied in the BFGF group. The cream of JWH (Jingwanhong, Tianjin Darentang Jingwanhong Pharmaceutical Co., Ltd., Tianjin, China, No. Z12020440) was applied in the JWH group.

Observation of wound healing

The number and relative production of the exudates, contraction, and the scab and skin colors of the wound were observed on days 1, 3, 5, 7 and 10 following treatments.

Wound healing rate determination

On days 1, 3, 5, 7, and 10, the wound healing rate (WHR) was measured by weighs. The wound was covered with transparent film and labeled along the wound edge. Then, the required area was cut off and weighed. The wound healing process was considered to be completed once the WHR reach 95%.[27]

$WHR\ (\%)=[(W_o - W_u)/W_o]\times 100$

W_o: Original wound area weight; W_u: Unhealed wound area weight.

Histopathological observation

On days 3, 7 and 10, the skin specimens of the wound were cut out and immediately fixed in 10% v/v neutral- formaldehyde for 24 h. The specimens were embedded in paraffin and 4 μm sections were sliced for hematoxylin and eosin (HE) staining (HE staining kit, Nanjing Jiancheng Bioengineering Institute, Nanjing, China, batch No. 20120926). The slices were observed under a microscope for histological evaluation of fibroblast proliferation, angiogenesis, epithelial regeneration, and collagen deposition.

Determination of collagen

On days 3, 7 and 10, the skin specimens were stained by using the Masson staining kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China, batch No. 20121119) to determine the content of collagen. Ten random views of each slice were observed under microscope (× 40). Then the optical density (OD) and area of each view were assessed by using the software Image-Pro plus 6 to determinate collagen deposition.

Determination of hydroxyproline

On days 3, 7 and 10, the basal skin tissues were cut from the center of the wounds. The determination of hydroxyproline was carried out according to the hydroxyproline alkali hydrolysis kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China, batch No. 20121023).

Determination of SP

SP content in the wound was determined by using the SABC (Streptavidin-Biotin Complex kit, Wuhan Boster Bioengineering Co., Ltd., Wuhan, China, batch No. 07J16BM). Five random views of each slice were observed under microscopy (× 40). The OD of each view was assessed by using the software program Image-Pro plus 6 to determinate SP content.

Statistical analysis

Results were expressed as mean ± standard deviation (*SD*) and statistical analysis was performed by using Duncan's New Multiple Range Test. Data analysis was performed by using the software SPSS 21 (SPSS Inc., Chicago, IL, USA). The statistical significance was set at $P<0.05$.

RESULTS

Content of l-borneol

The GC maps of the standard and samples are shown in Figure 1. L-borneol showed a good linear relationship under 0.05-0.78 mg/mL. The regression equation was Y=13012X-61.963, with an average recovery of 100.4% and a relative standard deviation (RSD) of 0.93% . The content of l-borneol in BB oil in this study was 22.7%.

Situation of wound healing

All mice grew well and their weights increased steadily.

No unusual drinking or urination was observed. The wound tissues in BFGF, JWH, BB oil$_{1/10}$ and BB oil$_{1/5}$ groups were ruddy, and arranged in order and no skin scrapings on the edge. On day 2, the new epidermis covered the wound edge, and the inflammatory response yet undetected. Then, the wound of the control mice and solvent control groups were rather black and dry. On day 3, wound edges of some mice were epithelized. However, in the undiluted BB oil groups, the wounded skin was dark and some wounds dehiscence and hemorrhage were observed. After the wound healed, the skin was partially thickened and hardened. Some mice in undiluted BB oil group depilated in a large area, and the depilated skin was dark red.

Wound healing rate

By day 3, the BB oil$_{1/5}$ and BB oil$_{1/10}$ groups showed significant high WHRs than control (P<0.05). On days 5 and 7, these two groups healed the wounds significantly. The wounds in the BB oil$_{1/5}$ and BB oil$_{1/10}$ groups had achieved more than 60.0% wound contraction. However, the WHR in the undiluted BB oil group were low by day 10 (Table 1).

Histopathological evaluation

On day 3, inflammatory cells had infiltrated the wound, and a few capillaries had formed. The infiltration with inflammatory cells were light, and new capillaries and fibroblast were significantly more evident following treatments with BFGF, JWH, BB oil$_{1/5}$ and BB oil$_{1/10}$ as compared the other treatments. The collagen depositions in these four groups were better. The epidermis in the undiluted BB oil group showed significant thickening, and the fibroblasts were smaller and in a disordered state. The wounds in this group healed much worse than did the other groups (Figure 2).

Effects on content of collagen

We evaluated the collagen contents, fibrous proteins and constituent of non-contractile connective tissues,

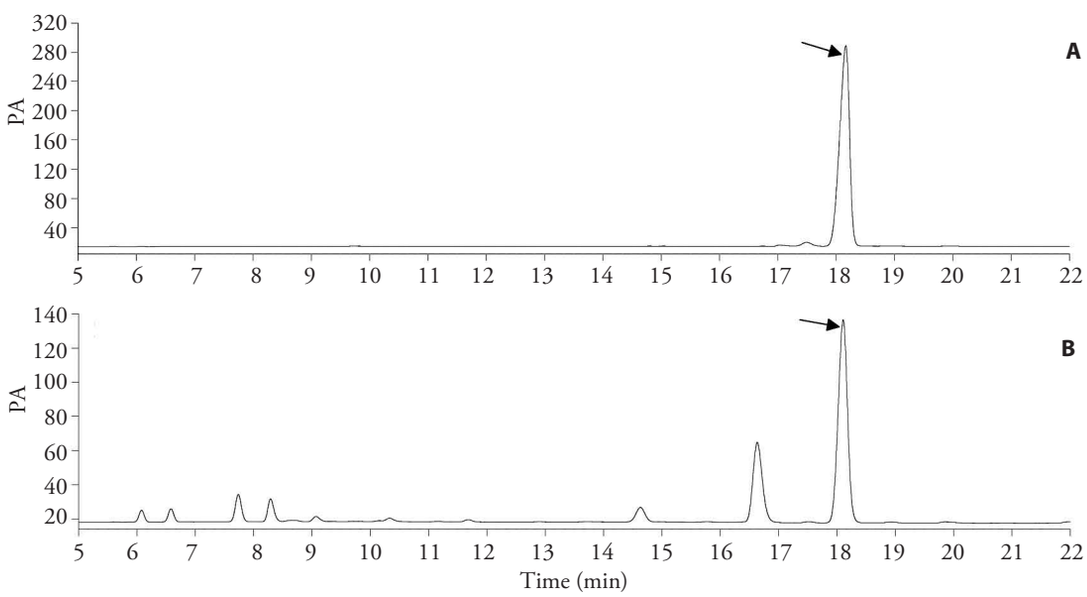

Figure 1 Gas chromatogram maps of the standard and samples
A: standard substance; B: violate oil of Blumea Balsamifera (L.) DC. leaves (BB oil) samples for determination of l-borneol (arrow).

Table 1 Effect of BB oil on wound healing rate (%)

Group	Day 3 (n=14)	Day 5 (n=14)	Day 7 (n=14)	Day 10 (n=10)
Control	32.4±5.0	52.4±9.0	78.8±6.5	97.1±2.5
Solvent control	27.3±9.1	57.1±8.5	82.9±4.5	96.4±3.2
BFGF	37.4±6.3^{a,b}	60.1±6.9^{a}	85.8±5.6^{d}	97.9±1.2
JWH	36.8±8.7^{b}	60.6±8.3^{a}	84.5±5.1^{d}	98.5±0.4
BB oil	30.2±7.4	46.0±10.2^{b}	72.0±7.3^{a,b}	97.5±0.5
BB oil$_{1/5}$	35.1±9.6^{c}	64.3±8.5^{d,c}	90.3±7.2^{d,b}	98.6±0.7^{c}
BB oil$_{1/10}$	34.3±7.6^{c}	62.2±9.1^{a}	83.5±5.5^{a}	98.0±0.4

Notes: control: no drug treatment; solvent control: olive oil; BFGF: basic fibroblast growth factor; JWH: the cream of Jingwanhong; BB oil: undiluted violate oil of Blumea Balsamifera (L.) DC. leaves; BB oil$_{1/5}$: BB oil diluted in olive oil to v/v=1/5; BB oil$_{1/10}$: BB oil diluted in olive oil to v/v=1/10. Approximately 0.05 mL of each drug preparation was applied once a day to the wound of rats in each group respectively except the control group. Compared with control group, ^{a}P<0.05, ^{d}P<0.01; compared with solvent control group, ^{c}P<0.05, ^{b}P<0.01.

by surveying the OD of the stained blue slide of the wound.[28] On day 3, there were few collagen fibers in the proliferating granulation tissue. The average OD in the BB oil$_{1/5}$ and BB$_{1/10}$ groups were 0.303 and 0.322, which were higher than other groups (P<0.01, Table 2).

Effects on hydroxyproline content

On day 3, there were statistically significant differences in hydroxyproline content of each treatment group as compared with the control and solvent treatment groups (P<0.01). The contents of hydroxyproline in undiluted BB oil, BB oil$_{1/5}$ and BB oil$_{1/10}$ groups were 1.64, 2.00 and 2.30 μg/mL respectively, which were markedly higher than that seen in the control groups with 59.22%, 94.18% and 123.30%, and were 1.76, 2.15, 2.47 times than that seen in the solvent control group. However, on day 10, the hydroxyproline content in BB oil group (2.87 μg/mL) was the lowest (Table 3).

Effect on the content of SP

SP shows the most positive reaction appearing on slice specimens as a yellow-brown coloration. The cells in the negative control group were stained blue without a positive reaction. The average OD represents the content of SP (Table 4). On day 3, the average OD of SP in the BB oil group was 0.0212, which was higher than was seen for the other groups, especially in the context of the control and solvent control groups (P<0.01). On day 7, SP in the undiluted BB oil and BB oil$_{1/5}$ groups increased highly significantly as compared with that seen in the control and solvent control groups (P< 0.01).

DISCUSSION

In the present study, the effect of BB oil was investigated. The major active ingredient of BB oil, l-borneol, induces resuscitation, relieves pain, has an anti-inflammatory effect, activates blood, and inhibits sympathetic nerve conduction. [24, 25, 30] Our findings revealed that BB oil$_{1/5}$ and BB oil$_{1/10}$ improved wound contraction and closure, and the effects were extremely and distinctly strong. Histopathological evaluation of the wound provided additional evidence of a more desirable histological organization. Our results revealed that, with the concentrations tested in the study, BB oil improved the formation of collagen and fibroblasts, and reduced the number of inflammatory cells that were required to accelerate effective wound healing and granulation of

Table 2 Effect of BB oil on collagen content ($\bar{x} \pm s$)

Group	Day 3	Day 7	Day 10
Control	0.128±0.056	0.327±0.027	0.467±0.086
Solvent control	0.155±0.094	0.299±0.106	0.452±0.080
BFGF	0.210±0.081[a]	0.309±0.065	0.464±0.064
JWH	0.192±0.044[a]	0.524±0.078[b,c]	0.599±0.026[b,c]
BB oil	0.190±0.048[a]	0.304±0.055	0.459±0.030
BB oil$_{1/5}$	0.303±0.049[b,c]	0.430±0.034[b,c]	0.562±0.068[b,c]
BB oil$_{1/10}$	0.322±0.044[b,c]	0.423±0.060[b,c]	0.546±0.040[b,c]

Notes: control: no drug treatment; solvent control: olive oil; BFGF: basic fibroblast growth factor; JWH: the cream of Jingwanhong; BB oil: undiluted violate oil of Blumea Balsamifera (L.) DC. leaves; BB oil$_{1/5}$: BB oil diluted in olive oil to v/v=1/5; BB oil$_{1/10}$: BB oil diluted in olive oil to v/v=1/10. Approximately 0.05 mL of each drug preparation was applied once a day to the wound of rats in each group respectively except the control group. Compared with control group, [a]P<0.05, [b]P<0.01; compared with solvent control group, [c]P<0.01.

Table 3 Effect of BB oil on hydroxyproline content (μg/mL, $\bar{x} \pm s$)

Group	Day 3	Day 7	Day 10
Control	1.03±0.36	1.87±0.61	3.91±1.09
Solvent control	0.93±0.30	1.54±0.31	3.43±0.27
BFGF	1.42±0.33[a,c]	1.95±0.40[d]	3.96±0.57[d]
JWH	1.85±0.47[b,c]	1.89±0.40[d]	4.02±0.71[d]
BB oil	1.64±0.38[b,c]	2.33±0.77[c]	2.87±0.72[a,d]
BB oil$_{1/5}$	2.00±0.32[b,c]	2.11±0.34[c]	3.91±0.61[d]
BB oil$_{1/10}$	2.30±0.73[b,c]	2.05±0.38[c]	4.13±0.89[d]

Notes: control: no drug treatment; solvent control: olive oil; BFGF: basic fibroblast growth factor; JWH: the cream of Jingwanhong; BB oil: undiluted violate oil of Blumea Balsamifera (L.) DC. leaves; BB oil$_{1/5}$: BB oil diluted in olive oil to v/v=1/5; BB oil$_{1/10}$: BB oil diluted in olive oil to v/v=1/10. Approximately 0.05 mL of each drug preparation was applied once a day to the wound of rats in each group respectively except the control group. Compared with control group, [a]P<0.05, [b]P<0.01; compared with solvent control group, [c]P<0.01, [d]P<0.05.

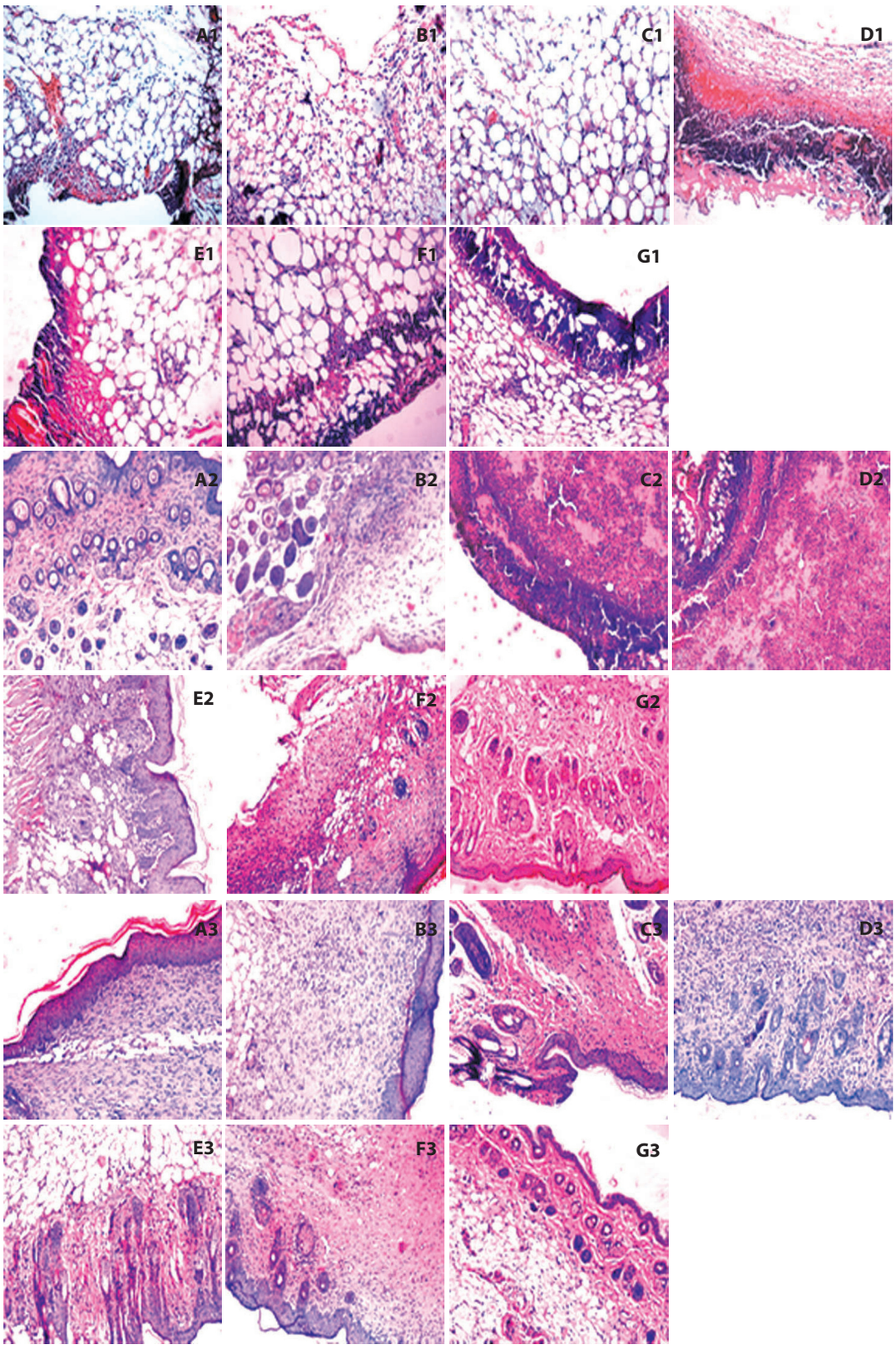

Figure 2 Effect of different treatments on histopathological changes (HE, ×40)
A1-G1: the expressions on day 3; A2-G2: the expressions on day 7; A3-G3: the expressions on day 10. A1, A2 and A3: the expression of control (with no drug treatment); B1, B2 and B3: the expression of solvent control (olive oil treated); C1, C2 and C3: the expression of BFGF (basic fibroblast growth factor treated); D1, D2 and D3: the expression of JWH (the cream of Jing Wan Hong treated); E1, E2 and E3: the expression of BB oil [undiluted violate oil of Blumea Balsamifera (L.) DC]; F1, F2 and F3: the expression of BB $oil_{1/5}$ (BB oil diluted with olive oil, v/v=1/4,); and G1, G2 and G3: the expression of BB $oil_{1/10}$ (BB oil diluted with olive oil, v/v=1/9). HE: hematoxylin-eosin.

new tissue in the proliferative phase.

The healing process depends on the biosynthesis and deposition of collagens and their maturation. Filling of the wound with granulation tissue serves to heal the

Table 4 Effect of BB oil on SP content ($\bar{x} \pm s$)

Group	Day 3	Day 7	Day 10
Control	0.0023±0.0022	0.0018±0.0009	0.0040±0.0010
Solvent control	0.0022±0.0016	0.0028±0.0019	0.0028±0.0017
BFGF	0.0090±0.0025[a,c]	0.0043±0.0017[a]	0.0038±0.0020
JWH	0.0055±0.0025[b]	0.0033±0.0025	0.0050±0.0038
BB oil	0.0212±0.0057[a,c]	0.0164±0.0030[a,c]	0.0021±0.0031
BB $oil_{1/5}$	0.0033±0.0022	0.0070±0.0026[a,c]	0.0037±0.0035
BB $oil_{1/10}$	0.0044±0.0028	0.0054±0.0028[a]	0.0029±0.0015

Notes: control: no drug treatment; solvent control: olive oil; BFGF: basic fibroblast growth factor; JWH: the cream of Jingwanhong; BB oil: undiluted violate oil of Blumea Balsamifera (L.) DC. leaves; BB $oil_{1/5}$: BB oil diluted in olive oil to v/v=1/5; BB $oil_{1/10}$: BB oil diluted in olive oil to v/v=1/10. Approximately 0.05 mL of each drug preparation was applied once a day to the wound of rats in each group respectively except the control group. SP: substance P. Compared with control group, [a]P<0.01; compared with solvent control group, [b]P<0.05, [c]P<0.01.

wound. The granulation tissue is comprised of fibroblasts, collagen, edematous fluids, and small neovascularized blood vessels. The increase in granular tissue dry weight suggests an increase in proteins. Thus, the healing process depends predominantly on the subsequent maturation of collagen.[31,32] Collagen is comprised of amino acids (e.g., hydroxyproline), and it is an important component of strengthening and supporting extracellular tissues. Breakdown of collagen liberates free hydroxyproline and peptides.[26,4,33] Therefore, the content of hydroxyproline is an index of collagen turnover.[34]

In this study, BB $oil_{1/5}$ and BB $oil_{1/10}$ demonstrated a significant increase in the hydroxyproline content of the granulated tissue, indicating an increase of collagen turnover. Both these two diluted BB oils stimulated wound healing more potently and more effectively than did the others. These findings implied that BB $oil_{1/5}$ and BB $oil_{1/10}$ promoted wound healing by influencing the proliferative and remodeling phases of wound healing, and do so *via* the pathways of angiogenesis, collagen deposition, granular tissue formation, epithelial deposition, and wound contraction. This result was also relevant in the context of other traditional medicinal plants. Topical applications of many traditional medicinal plants for skin wounds have been proved to have certain positive effects. These findings made these folk herbs potential skin wound healing agents.[29,35,36]

Peripheral nerves that conduct wounded tissues, also release SP, and then SP participates in the regulation of wound healing. SP plays an important role in communicating the nervous system with the wounded tissues.[37,38] In this experiment, determination of SP content showed that BB oil, BB $oil_{1/5}$ and BB $oil_{1/10}$ markedly promoted the expression of SP in the proliferative phase. The wound healing mechanism, under conditions of optimized concentrations of BB oil might be mediated, at least in part, by an increase in SP secretion. In addition, BB $oil_{1/5}$ and BB $oil_{1/10}$ increased the formation of new blood capillaries in the proliferative phase. This result could verify the efficacy of BB oil of its claimed Chinese medical efficacy "it can improve blood circulation and eliminate extravasated blood," and this efficacy might be associated with SP secretion. Moreover, the effect of BB $oil_{1/5}$ and BB $oil_{1/10}$ on capillary regeneration, blood circulation and accelerated wound healing might also be due to SP secretion. Possible mechanisms contributing to wound healing might be associated with SP secretion and the proliferation and differentiation of regulated cells.

However, the results in the undiluted BB oil group suggest that undiluted BB oil was not an optimum solution for accelerating wound healing. It should be diluted before being used to heal wounds. Further research and critical analysis on this filed is needed.

In summary, BB $oil_{1/5}$ and BB $oil_{1/10}$ promoted capillary regeneration, blood circulation, collagen deposition, granular tissue formation, epithelial deposition, and wound contraction. The BB $oil_{1/5}$ and BB $oil_{1/10}$ tested in this study can promote and enhance wound healing. These results may provide significant evidence for BB oil in clinical practice.

ACKNOWLEDGEMENTS

We would like to thank Dr. James Morris in English Teaching Laboratory, College of Foreign Languages, Hainan University, China and Dr. Dengfeng Wang in Tropical Resources and Environmental Research Laboratory, Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences, Danzhou, China for modifying the spelling and grammar mistakes and making valuable comments.

REFERENCES

1 **Natarajan V**, Venugopal PV, Menon T. Effect of Azadirachta indica (neem) on the growth pattern of dermatophytes. Indian J Med Micro 2003; 21(2): 98-101.

2 **Bhathena SJ**, Velasquez MT. Beneficial role of dietary

phytoestrogens in obesity and diabetes. Am J Clin Nutr 2002; 76(6): 1191-1201.

3 **Singh A**, Singh DK. Molluscicidal activity of Lawsonia inermis and its binary and tertiary combinations with other plant derived molluscicides. Indian J Exp Biol 2001; 39 (3): 263-268.

4 **Nayak BS**, Pinto Pereira LM. Catharanthus roseus flower extract has wound-healing activity in Sprague Dawley rats. BMC Complement Altern Med 2006; 6: 41.

5 **Diegelmann RF**, Evans MC. Wound healing: an overview of acute, fibrotic and delayed healing. Front Biosci 2004; 9: 283-289.

6 **Lau K**, Lai K, Liu C, et al. Synergistic interaction between Astragali Radix and Rehmanniae Radix in a Chinese l formula to promote diabetic wound healing. J Ethnopharmacology 2012; 141(1): 250-256.

7 **Peppa M**, Stavroulakis P, Raptis SA. Advanced glycoxidation products and impaired diabetic wound healing. Wound Repair Regen 2009; 17(4): 461-472.

8 **Gosain A**, DiPietro LA. Aging and wound healing. World J Surg 2004; 28(3): 321-326.

9 **Guo S**, DiPietro LA. Factors affecting wound healing. J Dent Res 2010; 89(3): 219-229.

10 **Jia ZG**, Fang Y, Yao M, et al. Molecular mechanisms of substance P-induced monocyte chemoattractant protein-1 secretion in fibroblasts under high glucose culture condition. Shanghai Jiao Tong Da Xue Xue bao 2012; 10(32): 1302-1306.

11 **Lai X**, Wang Z, Wei L, Wang L. Effect of substance P released from peripheral nerve ending on endogenous expression of epidermal growth factor and its receptor in wound healing. Zhong Hua Chuang Shang Za Zhi 2002; 5(3): 176-179.

12 **Muangman P**, Tamura RN, Muffley LA, et al. Substance P enhances wound closure in nitric oxide synthase knockout mice. J Surg Res 2009; 153(2): 201-209.

13 **Chen M**, Jin HZ, Zhang WD, Yan SK, Shen YH. Chemical constituents of plants from the genus Blumea. Chem Biodivers 2009; 6(6): 809-817.

14 **Pang YX**, Wang WQ, Zhang YB, Mo TH, Yuan Y. Clonal diversity and structure in natural populations of Blumea balsamifera. Guangxi Zhi Wu 2010; 30(2): 209-214.

15 **Yuan Y**, Pang Y, Wang W, Zhang Y, Yu J. Investigation on the plants resources of Blumea DC. in China. Re Dai Sheng Wu Xue Bao 2011; 1(2): 78-82.

16 **Chen M**, Qin J, Fu J, et al. Blumeaenes A – J, sesquiterpenoid esters from Blumea balsamifera with NO inhibitory activity. Planta Med 2010; 76(9): 897-902.

17 **Noor Rain A**, Khozirah S, Mohd Ridzuan MA, et al. Antiplasmodial properties of some Malaysian medicinal plants. Trop Biomed 2007; 24(1): 29-35.

18 **Osaki N**, Koyano T, Kowithayakorn T, Hayashi M, Komiyama K, Ishibashi M. Sesquiterpenoids and plasmin-inhibitory flavonoids from Blumea balsamifera. J Nat Prod 2005; 68(3): 447-449.

19 **Li J**, Zhao GZ, Chen HH, et al. Antitumour and antimicrobial activities of endophytic streptomycetes from pharmaceutical plants in rainforest. Lett Appl Microbiol 2008; 47(6): 574-580.

20 **Ragasa CY**, Co AL, Rideout JA. Antifungal metabolites from Blumea balsamifera. Nat Prod Res 2005; 19(3): 231-237.

21 **Nessa F**, Ismail Z, Mohamed N, Haris MRHM. Free radical-scavenging activity of organic extracts and of pure flavonoids of Blumea balsamifera DC leaves. Food Chem 2004; 88(2): 243-252.

22 **Kubota H**, Kojima-Yuasa A, Morii R, et al. Anti-obesity effect of Blumea balsamifera extract in 3T3-L1 preadipocytes and adipocytes. Am J Chin Med 2009; 37(05): 843-854.

23 State Administration of Traditional Chinese Medicine ed. Volumn 21 in: Chinese Materia Medica (Book 7). Shanghai, China: Scientific and Technical Publishers, 1999: 738-740.

24 **Guan LL**, Pang YX, Wang D, Zhang YB, Wu KY. Research progress on Chinese minority medicine of Blumea balsamifera L. DC. Zhi Wu Yi Chuan Zi Yuan Xue Bao 2012; 13(4): 695-698.

25 **Shao ML**, Ma XW, Wu Z, Yu WM, Chen XN. Pharmacopoeia of the People's Republic of China (Part I). Beijing: Chemical Industry Press, 2010: 82.

26 **Miao HM**, Xiao XH, Sun TT, et al. Study on effect of antimicrobial peptides dybowskin-1ST from skin of Rana dybowskii on Wound Healing Skin of Mice. Zhong Guo Shi Yan Fang Ji Xue Za Zhi 2011; 17(23): 38.

27 **Sadaf F**, Saleem R, Ahmed M, Ahmad SI. Healing potential of cream containing extract of Sphaeranthus indicus on dermal wounds in Guinea pigs. J Ethnopharmacology 2006; 107(2): 161-163.

28 **Kumar R**, Katoch SS, Sharma S. Beta-adrenoceptor agonist treatment reverses denervation atrophy with augmentation of collagen proliferation in denervated mice gastrocnemius muscle. Indian J Exp Biol 2006; 44(5): 371-376.

29 **Chen X**, Peng LH, Li N, et al. The healing and anti-scar effects of astragaloside IV on the wound repair in vitro and in vivo. J Ethnopharmacol 2012; 139(3): 721-727.

30 **Wang D**, Fu WJ, Pang YX, Wang H, HuX, Nie H. The Study of skin allergy and acute toxicity of Blumea Balsamifera oil. Re Dai Zuo Wu Xue Bao 2013; 12(34): 2499-2502.

31 **Hajiaghaalipour F**, Kanthimathi MS, Abdulla MA, Sanusi J. The Effect of Camellia sinensis on wound healing potential in an animal model. Evid Based Complement Alternat Med 2013; 2013: 386734.

32 **Nagappan T**, Segaran TC, Wahid MEA, Ramasamy P, Vairappan CS. Efficacy of carbazole alkaloids, essential oil and extract of Murraya koenigii in enhancing subcutaneous wound healing in rats. Molecules 2012; 17(12): 14449-14463.

33 **Nayak BS**, Nalabothu P, Sandiford S, Bhogadi V, Adogwa A. Evaluation of wound healing activity of Allamanda cathartica L. and Laurus nobilis L. extracts on rats. BMC Complement Altern Med 2006; 6(1): 12.

34 **Tang T**, Yin L, Yang J, Shan G. Emodin, an anthraquinone derivative from Rheum officinale Baill, enhances cutaneous wound healing in rats. Eur J Pharmacol 2007; 567 (3): 177-185.

35 **Duan QF**, Li Q, Lin Q, et al. Protection effect of Moringa Oil on skin injury of rabbit. Tian Ran Chan Wu Yan

Jiu Yu Kai Fa 2011; 1(23): 159-162.

36 **Nayak BS**. Cecropia peltata L (Cecropiaceae) has wound-healing potential: a preclinical study in a Sprague Dawley Rat model. Int J Low Extrem Wounds 2006; 5 (1): 20-26.

37 **Chiu IM**, Von Hehn CA, Woolf CJ. Neurogenic inflammation and the peripheral nervous system in host defense and immunopathology. Nat Neurosci 2012; 15(8): 1063-1067.

38 **Tellechea A**, Leal E, Veves A, Carvalho E. Inflammatory and angiogenic abnormalities in diabetic wound healing: role of neuropeptides and therapeutic perspectives. Open Circ Vasc J 2010; 2010(3): 43-55.