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Original Research Article

Hemostatic, anti-inflammatory and antibacterial effects of Sanqixiantao dressing in vivo and in vitro

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Abstract

Purpose: *To study the hemostatic, anti-inflammatory and antibacterial effects of Sanqixiantao dressing.*
Methods: *Sanqixiantao dressing was prepared by mixting with sanqixiiantao extract (8 %) with membrane-forming matrix (5:4:9:2 volume ratio of polyvinyl alcohol: Na CMC: gelatin: glycerol). Rats with local surface wounds were used to evaluate the effects of Sanqixiantao dressing on hemostatic time, wound healing time and infection rate. Serum levels of tumor necrosis factor (TNF)-α and interleukin (IL) 6 were determined. The anti-inflammatory and analgesic effects of Sanqixiantao extracts were assessed by dimethylbenzene-induced ear edema and acetic acid-induced abdominal writhing tests. In in vitro studies, the effect of the extract on blood clotting time, and its antibacterial activities against six pathogenic bacteria (Escherichia coli, Staphylococous aureus, Pseudomonas aeruginosa, Staphylococcus epidermidis, Clostridium perfringens and Clostridium tetani) were evaluated.*
Results: *Sanqixiantao dressing significantly decreased hemostatic time ($p < 0.01$), wound healing time ($p < 0.01$) and infection rate (10 vs 100 %), when compared to control rats. Sanqixiantao extract significantly shortened blood clotting time in vitro ($p < 0.01$), and showed antibacterial activities against E. coli (minimum inhibitory concentration, MIC: 0.4 mg/mL, MBC: 1.6 mg/mL), S. aureus (MIC: 0.8 mg/mL, minimum bacterial concentration, MBC: 3.2 mg/mL), P. aeruginosa (MIC: 0.8 mg/mL, MBC: 3.2 mg/mL), S. epidermidis (MIC: 1.6 mg/mL, MBC: 3.2 mg/mL). Besides, Sanqixiantao extracts (100, 200, 400 and 600 mg/kg) dose-dependently decreased dimethyl-benzene-induced ear edema and acetic acid-induced abdominal writhes in mice ($p < 0.05$, $p < 0.01$, $p < 0.01$, $p < 0.01$).*
Conclusion: *The results demonstrate that Sanqixiantao dressing has significant hemostatic, anti-inflammatory and antibacterial effects in vivo and in vitro, and thus provide some support for the therapeutic application of Sanqixiantao dressing for treating skin wounds.*

Keywords: *Sanqixiantao dressing, Acute skin wound, Hemostatic, Anti-inflammatory activity, Antibacterial effect, Herbal medicine*

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INTRODUCTION

The skin is the largest organ of human body and provides physiological barrier against infections [1,2]. Acute skin wounds usually occur in field battles, traffic accidents and in daily life [3]. Normally, small wounds heal without much medical intervention, but large wounds require timely medical attention [4]. Most of the available drugs currently used for wound healing are synthetic chemicals [5]. These drugs are expensive and are associated with some side-effects [6,7]. Thus, there is needed to evolve natural, newer and safer methods for treating acute wounds.

In recent years, herbal therapy have been shown to be beneficial in curing various diseases [8,9]. In addition, these alternative herbal remedies are reliable, inexpensive and of low toxicities [9,10].

Sanqixiantao dressing is a preparation in Teaching Hospital of Chengdu University of Traditional Chinese Medicine (Chengdu, PR China) and it is used in the hospital for treating acute wounds. The dressing is derived from six herbs (*Panax notoginseng*, *Veronica peregrine*, *Rhus chinensis*, *Bletilla striata*, *Rheum palmatum*, *Glycyrrhiza uralensis);* polyvinylalcohol, carboxymethylcellulose sodium (CMC-Na), gelatin, and glycerol. Although *Sanqixiantao* dressing is an effective alternative clinical remedy for treating acute skin wounds, there are so far, no experimental data regarding its pharmacological activities.

In this study, the hemostatic, anti-inflammatory and antibacterial effects of *Sanqixiantao* dressing were investigated.

EXPERIMENTAL

Plant materials

The herbs that make up *Sanqixiantao* extract (*Panax notoginseng*, *Veronica peregrine*, *Rhus chinensis*, *Bletilla striata*, *Rheum palmatum*, *Glycyrrhiza uralensis*) were obtained from the dispensary of Traditional Chinese Medicine of Teaching Hospital of Chengdu University of Traditional Chinese Medicine (Chengdu, China).

Chemicals

Tumor necrosis factor (TNF) α and interleukin (IL) 6 ELISA kits were purchased from the Biosource International Co. (Camarillo, CA, USA). *Yunnanbaiyao* powder was purchased from the Yunnanbaiyao Co. (Kunming, China). Polyvinyl alcohol, CMC-Na, gelatin and glycerol were purchased from the Sigma China Co. (Shanghai, China). All other used regents were analytical grade.

Bacterial strains and animals

Bacterial strains used in this research were obtained from ATCC (MD. USA). SD rats, KM mice and rabbits were obtained from the Shanghai Laboratory Animal Research Center (Shanghai, China). The animal protocols were approved by the Animal Care and Use Committee of Teaching Hospital of Chengdu University of Traditional Chinese Medicine (approval no. 201607-JA-034). The animals were handled according to the standard protocols for the use of laboratory animals [11].

Preparation of Sanqixiantao extracts and Sanqixiantao dressings

The six herbal were ground and decocted 3 times by deionized water. Thereafter, the extracts were pooled and filtrated, and the filtrate was dried by vacuum drying apparatus under 50 °C to yield the *Sanqixiantao extract*.

The *Sanqixiantao dressing* was prepared by mixing the extract (at 8 % incorporation) with forming membrane matrix (5:4:9:2 volume ratio of Polyvinyl alcohol: CMC-Na: gelatin: glycerol).

Determination of therapeutic effects of *Sanqixiantao* dressing on localized surface wound in rat

A total 30 SD rats were divided into 3 groups namely: control groups, positive group and *Sanqixiantao* dressing group (n = 10). For the positive group, *Yunnanbaiyao* powder, a well-known traumatic drug in China [12], was used as the positive drugs.

Rats with local wound rats were prepared as follows: The rats were anesthetized by intraperitoneal injection (*i.p.*) of 1 % pentobarbital sodium at doses of 40 mg/kg. Thereafter, a 1.5 × 1.5 cm bleeding wound was made on the backside of the rat. Subsequently, drugs were administered topically on the wounds, and hemostatic time, wound healing time and infection rate were evaluated in over a period of 10 days [13]. During the study, blood sample from each rat was collected 5 days after surgery by orbital blood sampling, and the serum levels of TNF-α and IL-6 were determined by commercial ELISA kits.

Determination of blood coagulation time *in vitro*

Rabbit blood sample was collected form the ear vein using EDTA vacuum anticoagulant tubes. Then, the anti-coagulated plasma was prepared by centrifugation (3000 rpm, for 20 min).Subsequently, clotting time was determined *in vitro* using plate method for blood coagulation and test tube method according to the previous reports [13].

Antibacterial assay

The antibacterial activities of *sanqixiantao* extracts against 6 pathogenic bacteria that cause wound infection were determined. These bacteria

include *Escherichia coli* (ATCC 35218), *Staphylococous aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus epidermidis* (ATCC 12228), *Clostridium perfringens* (ATCC 13124), and *Clostridium tetani* (ATCC 19406). The final concentrations of the extract in bacterial culture medium were 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 and 12.8 mg/mL. The MIC and MBC values were determined as described earlier [14].

Dimethylbenzene-induced ear edema test

The effect of *sanqixiantao* extract on dimethyl-benzene-induced ear edema test in mice was according to the method outlined previously [15]. *Sanqixiantao* extracts and positive drugs (Indometacin) were administered orally. After 40 min of drug treatment, dimethyl-benzene was topically applied to the right ear of mice, and the mice were sacrificed under anesthesia (1 % sodium pentobarbital, 40 mg/kg, *ip*). The degree of ear edema was determined.

Acetic acid-induced abdominal writhing test

The analgesic effect of *Sanqixiantao* extract was evaluated by acetic acid-induced abdominal writhing test according to previously reported method [16]. The *sanqixiantao* extract and positive drug (indometacin) were administered orally. After 40 min of the drug treatment, acetic acid (0.75 %, 10 mL/kg) was intraperitoneal administered to mice. The number of writhes was counted, starting 3 min after acetic acid treatment over a period of 12 min.

Statistical analysis

Data are expressed as mean ± SD. One way analysis of variance (ANOVA) was used to compare the means between two groups. Data analysis was carried out by SPSS 17.0 software. $P < 0.05$ was considered statistically significant.

RESULTS

Effect of *Sanqixiantao* dressing on localized wound surface

As shown in Table 1, in control rats, the hemostatic time was 95.83 ± 5.74 s; after treatment with *sanqixiantao* dressing, the hemostatic time significantly decreased (47.31 ± 4.85 s, $p < 0.01$) when compared with rats in the control group. Furthermore, would healing time was significantly decreased by treatment with *sanqixiantao* dressing (3.8 ± 0.80 day *vs*. 5.8 ± 1.32 day, $p < 0.01$). In addition, *sanqixiantao* dressing significantly decreased infection rate of the wounds (10 *vs* 100 %).

Results from ELISA (Table 2) show that serum levels of TNF-α (932.18 ± 374.49 pg/mL *vs* 217.81 ± 65.75 pg/mL, $p < 0.01$)and IL-6 (596.92 ± 147.36 pg/mL *vs* 132.86 ± 28.35 pg/mL, $p < 0.01$) were significantly decreased after treatment with *Sanqixiantao* dressing, when compared with control rats. This suggests that the dressing possesses significantly anti-inflammatory effect, which was better than that of the positive drug (*Yunnan Baiyao* powder).

Sanqixiantao extract shortened blood clotting time *in vitro*

The results of the plate method and tube method (Table 3) revealed that *sanqixiantao* extracts (0.2, 0.4, 0.8, 1.6, 3.2 and 6.4 mg/mL) had significantly higher blood coagulation effects ($p < 0.01$) when compared with control. This effect was also concentration-dependent.

Table 1: Effect of *sanqixiantao* dressing on hemostatic time, wound healing time and infection rate on local wound surface in rat

Treatment	Hemostatic time (s)	wound healing time (day)	Infection rate (%)
Control	95.83±5.74	5.8±1.32	100
Positive	80.96±4.92*	4.4±1.35*	30
Sanqixiantao dressing	47.31±4.85**	3.8±0.80**	10

*Yunnan Baiyao was used as the positive drug; *p < 0.05, **p < 0.01, vs normal control*

Table 2: Effect of *sanqixiantao* dressing on serum levels of TNF-α and IL-6 in local wounded rat

Treatment	TNF-α (pg/mL)	IL-6 (pg/mL)
Normal control	932.18 ± 374.49	596.92 ± 147.36
Positive control	549.54 ± 144.38**	276.87 ± 59.34**
Sanqixiantao dressing	217.81± 65.75**	132.86 ± 28.35**

Yunnan Baiyao was used as positive control; *$p < 0.05$, **$p < 0.01$, *vs* control

Table 3: Effect of *sanqixiantao* extracts on clotting time of plasma in rabbit

Treatment	Plate method (s)	Tube method (s)
Normal control	264.77 ± 13.64	294.81 ±16.34
Sanqixiantao extracts		
0.2 mg/mL	206.21 ± 13.17**	221.02 ± 14.52**
0.4 mg/mL	164.28 ± 10.03**	175.39 ± 9.84**
0.8 mg/mL	142.07 ± 9.13**	150.63 ± 10.35**
1.6 mg/mL	129.38 ± 8.03**	132.04 ± 8.97**
3.2 mg/mL	90.03 ± 8.12**	100.46 ± 7.26**
6.4 mg/mL	77.62 ± 7.39**	79.18 ± 8.07**

*$P < 0.05$, **$p < 0.01$, vs control

Table 4: Anti-bacterial effects of *sanqixiantao* extracts *in vitro*

Microorganism	Concentration (mg/mL)	
	MIC	MBC
*Escherichia coli*ATCC 35218	0.4	1.6
*Staphylococous aureus*ATCC 25923	0.8	3.2
Pseudomonas aeruginosa ATCC 9027	0.8	3.2
Staphylococcus epidermidis ATCC 12228	1.6	3.2
Clostridium perfringens ATCC 13124	1.6	6.4
Clostridium tetani ATCC 19406	0.8	3.2

Table 5: Anti-inflammatory and analgesic effects of *sanqixiantao* extracts in mice

Treatment		Weight of ear edema (mg)	Number of writhings
Control		13.71 ± 3.53	31.6 ± 6.22
Positive		10.02 ± 3.31*	25.8 ± 4.78*
Sanqixiantao extract			
	100 mg/kg	10.11 ± 3.66*	25.7 ± 5.48*
	200 mg/kg	9.14 ± 2.76**	23.8 ± 5.35**
	400 mg/kg	7.76 ± 2.54**	20.4 ± 2.59**
	600 mg/kg	6.29 ± 2.71**	18.3 ± 3.09**

Indomethacin was used as the positive drug; *$p < 0.05$, **$p < 0.01$, vs control

In vitro antibacterial activity of *sanqixiantao* extract

The antibacterial activities of the *sanqixiantao* extract are presented in Table 4. The extract produced appreciable antibacterial activities against the six tested organisms viz *E. coli* (MIC: 0.4 mg/mL, MBC : 1.6 mg/mL); *S. aureus* (MIC: 0.8 mg/mL, MBC: 3.2 mg/mL); *P. aeruginosa* (MIC: 0.8 mg/mL, MBC: 3.2 mg/mL); *S. epidermidis* (MIC: 1.6 mg/mL, MBC: 3.2 mg/mL); *C. perfringens* (MIC: 1.6 mg/mL, MBC: 6.4 mg/mL) and *C. tetani* (MIC: 0.8 mg/mL, MBC: 3.2 mg/mL).

Anti-inflammatory and anti-analgesic activities of *sanqixiantao* extract

The results of anti-inflammatory and anti-analgesic properties of the extract which were evaluated by dimethyl-benzene-induced ear edema test and acetic acid-induced abdominal writhing test, are shown in Table 5. *Sanqixiantao* extract showed significant dose-dependent effects on ear edema at 100, 200, 400 and 600 mg/kg, when compared with mice in control group ($p < 0.05$, $p < 0.01$, $p < 0.01$, and $p < 0.01$, respectively). In addition, the extract (at 100, 200, 400 and 600 mg/kg) dose-dependently decreased abdominal writhes induced by acetic acid, relative to the control group ($p < 0.05$, $p < 0.01$, $p < 0.01$, and $p < 0.01$, respectively). These results suggest that *sanqixiantao* extract possesses potential anti-nociceptive anti-inflammatory properties.

DISCUSSION

From time immemorial, herbal derived medicines have been applied in the treatment or prevention of various diseases. Herbal medicines are known to produce reliable pharmacological effects [9,17,18]. In the present study, hemostatic, anti-inflammatory and antibacterial effects of *sanqixiantao* dressing were demonstrated *in vivo* and *in vitro* for the first time.

Studies have indicated that medical dressings have beneficial for wound healing, especially drug-loaded dressings [19,20]. These drug-loaded dressings not only inhibit the wound

infections, but also promote the healing of wounds [20]. In the treatment of acute skin wounds, rapid hemostasis is a very important property of the good wound dressings [21,22]. In the present study, *sanqixiantao* dressing and its extract had significant blood coagulation activities *in vivo* and *in vitro*.

Infection and inflammatory reactions are the most common complications of skin wounds, and might result in high fever and even sepsis [22,23]. Therefore, controlling inflammatory reactions and infections are essential for wound healing. The present study revealed that *sanqixiantao* extracts alleviated the inflammation and significantly decreased the serum levels of pro-inflammatory cytokines such as TNF-α and IL-6 [24]. Bacterial infections usually result in the exacerbation of wounds and severe inflammatory reactions [25]. The results also demonstrated that *sanqixiantao* extract had significant antibacterial activities against six pathogenic bacteria that cause wound infection (*E. coli*, *S. aureus*, *P. aeruginosa*, *S. epidermidis*, *C. perfringens* and *C. tetani*). In addition, the present investigation indicated that *sanqixiantao* extract possesses strong analgesic activity. *Sanqixiantao* dressing shortened wound healing time of local surface wound in rats. These results suggest that *sanqixiantao* dressing is beneficial for treatment of skin wounds.

CONCLUSION

The findings of this study demonstrate that *Sanqixiantao* dressing has significant hemostatic, anti-inflammatory and antibacterial effects *in vivo* and *in vitro*, and thus lends some support for its therapeutic application in the treatment of skin wounds.

DECLARATIONS

Acknowledgement

None.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

REFERENCES

1. *Sadowski T, Klose C, Gerl MJ, Wójcik-Maciejewicz A, Herzog R, Simons K, Reich A, Surma MA. Large-scale human skin lipidomics by quantitative, high-throughput shotgun mass spectrometry. Sci Rep 2017; 7: 43761.*
2. *Mieremet A, Rietveld M, Absalah S, van Smeden J, Bouwstra JA, El Ghalbzouri A. Improved epidermal barrier formation in human skin models by chitosan modulated dermal matrices. PLoS One 2017; 12: e0174478.*
3. *Chae DS, Han S, Son M, Kim SW. Stromal vascular fraction shows robust wound healing through high chemotactic and epithelialization property. Cytotherapy 2017; 19(4): 543-554.*
4. *Kim M, Lee HJ, Randy A, Yun JH, Oh SR, Nho CW. Stellera chamaejasme and its constituents induce cutaneous wound healing and anti-inflammatory activities. Sci Rep 2017; 7: 42490.*
5. *Boateng JS, Matthews KH, Stevens HN, Eccleston GM. Wound healing dressings and drug delivery systems: a review. Pharm Sci 2008; 97(8): 2892-2923.*
6. *Jørgensen B, Friis GJ, Gottrup F. Pain and quality of life for patients with venous leg ulcers: proof of concept of the efficacy of Biatain-Ibu, a new pain reducing wound dressing. Wound Repair Regen 2006; 14(3): 233-239.*
7. *Kim J, Lee CM. Wound healing potential of a polyvinyl alcohol-blended pectin hydrogel containing Hippophae rahmnoides L. extract in a rat model. Int J Biol Macromol 2017; 99: 586-593.*
8. *Pu J, Fang FF, Li XQ, Shu ZH, Jiang YP, Han T, Peng W, Zheng CJ. Matrine Exerts a Strong Anti-Arthritic Effect on Type II Collagen-Induced Arthritis in Rats by Inhibiting Inflammatory Responses. Int J Mol Sci 2016; 17(9):e1410.*
9. *Patwardhan B. Ethnopharmacology and drug discovery. JEthnopharmacol 2005; 100 (1-2): 50-52.*
10. *Li JW, Vederas JC. Drug discovery and natural products: end of an era or an endless frontier? Science 2009; 325(5937):161-165.*
11. *National Institute of Health, USA. Public health service policy on humane care and use of laboratory animals; 2002.*
12. *Frederick J, Boysen S, Wagg C, Chalhoub S. The effects of oral administration of Yunnan Baiyao on blood*

coagulation in beagle dogs as measured by kaolin-activated thromboelastography and buccal mucosal bleeding times. Can J Vet Res2017; 81(1): 41-45.

13. *Wang XQ, Wu SH, Su YY, Luo XD. Preliminary definition of the effective fraction of a herb and its procoagulant activity. Acta Acad Med Milt Tert 2004; 26(20): 1841-1844.*
14. *Peng W, Han T, Xin W, Zhang X, Zhang Q, Jia M, Qin L. Comparative research of chemical constituents and bioactivities between ether extracts of the aerial part and the rhizome of Atractylodes macrocephala. Med Chem Res 2011; 20(2): 146-151.*
15. *Yang L, Li L, Chen L, Li Y, Chen H, Li Y, Ji G, Lin D, Liu Z, Qiu Y. Potential analgesic effects of a novel N-acylethanolamine acid amidase inhibitor F96 through PPAR-α. Sci Rep 2015; 5: 13565.*
16. *Iniaghe L, Okpo S, Olung J, Eguae A. Analgesic effect of methanol leaf extract of AlstoniaBoonei De Wild (Apocynaceae). Trop J Pharm Res 2012; 11(5): 793-798*
17. *Rahman A, Chakma J, Bhuiyan N, IslamS. Composition of the essential oil of Clausena Suffruticosa leaf and evaluation of its antimicrobial and cytotoxic activities. Trop J Pharm Res 2012; 11(11): 739-746.*
18. *Wu L, Jia M, Chen L, Zhu B, Dong H, Si J, Peng W, Han T. Cytotoxic and Antifungal Constituents Isolated from the Metabolites of Endophytic Fungus DO14 from Dendrobium officinale. Molecules 2016; 21(1): e14.*
19. *Guo CL, Deng HY, Qu HL. A comparative study on application effect of different silver dressings in treatment of patients with chronic wounds. Chin Nurs Res 2015; 29: 1170-1175.*
20. *Zhou ZX, Wei XL, Yao JR, Zhang J, Bian G, Li JH. Evaluation of biological hemostatic dressings and gauzes in surgical wounds. J Clin Reha Tis Engineer Res 2010; 14: 9635-9638.*
21. *Shi L, Li CQ, Lian TT, Xu QT, Kang WY. Effect of toddalia asiatica on bleeding time and clotting time in mice. Chin Pharm 2010; 21: 4424-4425.*
22. *Lawrence JC. Dressings and wound infections. Am J Surg 1994; 167(167): 21-24.*
23. *Xia F, Yan Q, Zhou ZH. Risk factors of wound infections after open trauma surgery in hand surgery department and prevention countermeasures. Chin J Nosocomiol 2013; 23(14): 3657-3668.*
24. *Wang Q, Kuang H, Su Y, Sun Y, Feng J, Guo R, Chan K. Naturally derived anti-inflammatory compounds from Chinese medicinal plants. J Ethnopharmacol 2013; 146(1): 9-39.*
25. *McFarland AP, Luo S, Ahmed-Qadri F, Zuck M, Thayer EF, Goo YA, Hybiske K, Tong L. Woodward JJ. Sensing of Bacterial Cyclic Dinucleotides by the Oxidoreductase RECON Promotes NF-κB Activation and Shapes a Proinflammatory Antibacterial State. Immunity 2017; 46(3): 433-445.*