

Medical Sciences

Old Technique – New Evidence: Topical agents for musculo-skeletal injuries

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The popular use of topical agents for the treatment of musculo-skeletal injuries has persisted for centuries but not much scientific evaluations have been done. Since medicinal herbs are particularly popular in Asia, we started a systematic exploration on their choices, and their pharmacological activities; whether transcutaneous transport of bioactive components occur and above all, whether quality clinical evidences could be generated. We found that a search on the vast literature pool would reveal the favourable choices of herbal agents. Biological screening of those selected herbs showed that they probably follow three major common pathways to help with healing after injury, viz. anti-inflammation, pro-angiogenesis and cellular proliferation. Using a simple formula of selected herbs with the ideal bioactivities, evidence based clinical trials could be organized to further prove the efficacy. We have created two such formulae to be put on clinical trial. Our early pilot clinical trials on two minor injuries on the foot and one chronic inflammatory condition have yielded positive data on the value of such topical agents on pain and oedema control, as well as functional maintenance. There was also suggestion of more rapid bone healing. Although limitations exist clear with the small number of study subjects, the positive data and safe application support more studies. *Journal of Nature and Science*, 1(3):e51, 2015.

Herbal treatment | Topical agent | Foot injury | Plantar fasciitis

Introduction

An ancient art of healing is being practiced in spite of advances in traumatology. Musculo-skeletal injuries are enjoying many effective means of treatment today, from sophisticated surgery to rehabilitation techniques. For the less severe situations and very often later, during the rehabilitation period, the majority of people would administrate their own choice of treatment viz. use various forms of topical treatment – pastes, rubs, balms, patches etc. – to relieve pain and swelling. This art of healing must have existed ever since there is human civilization. Users in the ancient days feel better after application. Users today also feel better after application. However, is there ever scientific evidence that those agents are really helping with the pain, and the swelling?¹

To give light to the centuries long queries we need to sort out a number of fundamental issues:-

- (I) Which herbs could be used for the Topical Applications?
- (II) What are the biological activities that the topical herbs generate?
- (III) Do the topical agents get across the skin surface to exert their biological effects?
- (IV) Are there clinical evidences of efficacy?

Since ten years ago, our research institution, Institute of Chinese Medicine (ICM) at The Chinese University of Hong Kong has started explorations on those issues and the following account is a summary of ten years' efforts.

I. Which herbs could be used for the Topical Applications?

One branch of Traditional Chinese Medicine since 3,000 years ago is called "Bone Setting" Experts in Bone Setting serve patients suffering from injuries which include fractures, sprains and bruises. Theories and techniques involved in fracture treatment like reduction of fracture fragments, re-aligning, and stabilization, were

well described. To supplement the techniques and to ensure secure healing, topical herbal applications were always used.² Over one hundred of medicinal herbs indicated for injury treatment have been described.³ It would be logical to screen out from the classical records, those items that have been most popular and are easily available today. In the past twenty years, a lot of popular medicinal herbs have been tested on the biological platforms to identify their pharmacological activities. Results of the testings would further guide the choice of herbs to be studied today.⁴

The areas of biological activities as being crucial for tissue healing must be emphasized during the screening process. Considering the healing process after any form of injury, three events are always involved in the repair process, viz. inflammation, vascularization and cellular regeneration. Clinically inflammation is leading to pain and swelling in the acute phase of injury while vascularization and cellular regeneration are related to symptom improvement and functional return. With the guidance of these fundamental considerations, the principle of herb selection could follow. We realize that ancient practices favour complex herbal formulae. However, modern biological research platforms would prefer the minimal number of herbs so that quality control and interpretation of activities could be easier. We need 5-6 popular herbs to be testified to be anti-inflammatory, angiogenic and promoting cellular regeneration in quality biological research platforms.

Eventually five herbs are selected, viz. *Flis Carthanic*, *Radix Dispac*, *Rhizoma Rhei*, *Angelica sineuses*, and *Achyranthis Bidentatae*.

II. What are the biological activities that the topical herbs generate?

In-vitro and *in-vivo* experiments will be required to illustrate the essential biological activities of the individual herbs and their combination.⁵ These are standard cell culture platforms and Table 1 shows the details.

Results of the *in-vitro* studies showed that the individual herbs and the combined formula had remarkable suppressive effects on the NO production in the RAW 264.7 cell line; and remarkable promotive effects on both the HUVEC and UMR-106 cell lines. These results were consistent with earlier observations reported.⁶⁻⁸ *In-vivo* experiments included zebra fish embryo studies to verify the pro-angiogenic effects of the herbs and animal models on soft tissues injury and bone fractures, where a topical paste was applied to promote healing. Using radiological, biochemical and biomechanical (on the long bone after fracture healing) testings, the topical paste was demonstrated to have promoted the fracture callus formation, increased bone-specific alkaline phosphatase level and stronger fracture union.^{5,9}

Conflict of interest: No conflicts declared.

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Table 1. *In-vitro* studies on the biological effects of the topical herbs

Purpose of in-vitro study	Cell Line	Observations
Inflammation	Mouse macro phage RAW 264.7	Nitric oxide release Anti-oxidation effects ^{13, 14}
Angiogenesis	Human Umbilical vein Endothelial cells HUVEC	Proliferations ^{15, 16}
Cellular proliferation	Rat Osteoblast UMR-106	Proliferations ^{17, 18}

Table 2. Three pilot studies on minor injuries or chronic inflammatory condition

Clinical Condition	Ankle Sprain	5 th Metatarsal Fracture	Plantar Fasciitis
Aim	To observe whether a herbal bath Formula I, helps to reduce pain & swelling	Whether Formula II helps to reduce pain/facilitate healing	Whether Formula II helps to reduce pain
Methodology	A pilot, open label, one arm self-controlled, observational study	A pilot, open label, one arm self-controlled, observational study	A pilot, open label, one arm self-controlled, observational study
Study Duration	Six weeks or pain/ swelling disappear	Six weeks or pain/ swelling disappear	Six weeks or pain/ swelling disappear
Number of Subjects	10	10	10
Inclusion Criteria	Ankle sprain, age >18 years	Acute traumatic fracture of 5 th metatarsal No displacement	Plantar fasciitis (Heel pain) 18-65 years. History over 4 weeks
Exclusion Criteria	Previous injury of ankle, sensitivity to drugs	Previous foot injury on same foot Open injury/ sensitivity to drugs	Ulceration/ Sensitivity/ Pregnancy/ Breastfeed
Study Product	Formula 1: 10gms of three herbs extract + Borneol into 2 litres of warm water	Formula 2: semi solid paste containing concentrate of 3 herbs extracts + Borneol	Formula 2 + Borneol
Assessments	Interview, orthotics <ul style="list-style-type: none"> American Orthopaedic Foot and Ankle Society + Ankle Hindfoot Scale (AOFAS) Foot/ Ankle Ability Measure (FAAM) Pain evaluation – visual analog Ultrasonic study Check blood for inflammation cytokine Water displacement test 3D Scan Gogh II imaging 	Interview, orthotics <ul style="list-style-type: none"> American Orthopaedic Foot and Ankle Society + Ankle Hindfoot Scale (AOFAS) Foot/ Ankle Ability Measure (FAAM) Pain evaluation – visual analog Ultrasonic study Check blood for inflammation cytokine Water displacement X ray site 3D Scan Gogh II imaging 	Interview, Map site of pain <ul style="list-style-type: none"> Foot Function Index Pain evaluation – visual analog Ultrasonic study Check blood for inflammation cytokine
Treatment	30 minutes of foot/ ankle emersion per day Data checking 0, 3 & 6 weeks	Apply patch over fracture site, change every 3 days until 6 weeks Data checking 0, 3 & 6 weeks	Apply patch over fracture site, change daily until 6 weeks Data checking 0, 3 & 6 weeks
Outcome measure	Swelling/ pain control Others	Swelling assessment/fracture healing	Pain relief Inflammation control
Safety/ Allergy	Report serious adverse effects	Report serious adverse effects	Report serious adverse effects

In-vivo experiments on the healing of soft tissues after injury under the influence of the herbal paste are still going on.

With the series of tests completed on inflammation, vascular regeneration and cellular proliferation, the five herbs selected for topical study could be considered suitable for further investigations.

III. Do the topical agents get across the skin surface to exert their biological effects?

Since transcutaneous delivery of medicinal material has become more popular, *in-vitro* and *in-vivo* testings of transcutaneous drug transport have become necessary. Special device like the Franz Diffusion cell gives *in-vitro* information about the diffusion of medicinal agent through a selected membrane, either artificial or prepared from the skin of an animal.¹⁰ *In-vivo* testing would involve the appearance of the medicinal agent, either within the subcutaneous tissue or in the circulation of the animal used for study. In order to facilitate the transcutaneous transfer, various enhancing material could be used. Ozone is a popular agent used for such purposes in commercial products. In traditional Chinese Medicine, Borneol has been popular.

When the topical agent consists of a simple chemical compound, diffusion study is not complicated because a direct analysis of the transfer of the compound would fulfill the requirement. To study the transport of herbal material across a membrane or skin,

nevertheless, would be far more complicated because of the complexity of chemicals involved. Authentication of commonly used medicinal plants is dependent on the identification of specific markers for a particular plant. This practice is obviously a compromise since any plant contains numerous chemicals rather than a single one. However, before a better method is found, the appearance of a known chemical marker of a specific medicinal plant across a membrane, could be considered an objective proof about the transfer, although it is only a qualitative and partial demonstration.

All the five medicinal plants chosen for this study have officially recognized chemical markers and five of them are selected for the *in-vitro* and *in-vivo* studies.¹¹ Using the Franz diffusion chamber and either an artificial or mouse skin membrane, the markers were identified in the receptor compartment, thus proving the across membrane transport.

Adding Borneol as the enhancer, the 5 herbs paste was applied to the rabbit leg over an artificially produced tibiae fracture. Sera were taken from the rabbit intermittently within 72 hours. The chemical markers were detected throughout with the peak concentration within the first 24 hours. It therefore demonstrated that the topical application of herbal paste did produce a transdermal transfer of chemicals across the skin barriers to the subcutaneous tissues, thence to the general circulation.

Table 3. Result of Topical Treatment

Clinical Condition	Ankle Sprain			5 th Metatarsal Fracture			Plantar Fasciitis		
Pain score	Significant reduction after 7 days Average 50% reduction by 6/52			Significant decrease in pain after 2 weeks Average 64% pain reduction after 6/52			Average decrease in pain 64%		
Ultrasonic study	baseline N= 10	Endpoint N=10	p-value	Pre-treatment N= 10	Post-treatment N=10	p-value	Reduction in plantar fascia thickness		
• *Ultrasound edema thickness (in cm)	1.766 (0.370)	1.395 (0.228)	0.008	0.622 (0.121)	0.496 (0.110)	0.002			
• Water displacement (in g)	1290.21 (197.837)	1237.11 (161.099)	0.282	1126.39 (221.64)	1154.37 (200.81)	0.504			
• 3D-imaging Volume (in cm ³)	706.05 (160.344)	696.46 (121.588)	0.777	733.99 (236.49)	673.46 (207.57)	0.047			
• FAAM ADL Subscale	52.20(14.729)	89.70(9.525)	0.001	33.34(23.368)	77.88(21.239)	<0.001			
• Sports Subscale	14.32(8.280)	64.11(24.084)	<0.001	13.51(13.991)	48.33(26.802)	0.002			
• AOFAS Total Score	63.6(12.331)	87.70(9.522)	0.001	50.2(13.522)	83.5(9.107)	<0.001			
**Foot Function Index							Pre (n= 9)	Post (n=9)	p-value
• Pain							53.22(21.554)	29.77(15.163)	0.001
• Difficulty							45.47(17.056)	20.68(16.877)	0.001
• Activity Restriction							28.81(19.492)	13.99(15.358)	0.018
• Total Score							42.49(16.933)	21.48(11.902)	0.001
Blood test (Inflammation cytokine)	Concentration too low for assessment			Concentration too low for assessment			Concentration too low for assessment		
Concomitant medications (analgesics anti-inflammatories)	Need for analgesic minimal			Need for analgesic minimal			Markedly reduced		
Safety	No sensitivity or serious adverse effects			No sensitivity or serious adverse effects			No sensitivity or serious adverse effects		

*Ultrasound edema thickness, FAAM (ADL, Sports) and AOFAS total score were significantly improved after treatment when compared with baseline (p<0.01). **Foot Function Index (FFI) including pain, difficulty, activity restriction and total score was significantly decreased after six-week treatment when compared with baseline (p<0.01)

IV. Are there clinical evidences of efficacy?

Clinical studies are essential for the proof of efficacy of any medication. Severe injuries would need hospitalization and proper modern managements. Studies on the topical use of herbal medicine should only be indicated for limited trauma cases or situations when standard treatment fails to give perfect results and symptoms persist.

We have chosen two acute minor injuries; viz. sprained ankle and fractured 5th metatarsal bone; and one chronic inflammatory condition for study. Two separate three herbs topical formulae were created:

Formula 1 *Flos Carthami, Angelica Sinensis and Achyranthis Bidentatae*

Foot and ankle bath for ankle sprain and swelling

Formula 2 *Flos Carthani, Rhizoma Rhei and Dipsaci Radix*

Herbal patch for metatarsal fracture and plantar tissue inflammation

Formula 1 is designed for better swelling and inflammation control, while Formula 2 has more inclination on tissue healing. Both formulae have the addition of Borneol to enhance transcutaneous penetration. The study details and results of the three trials are summarized in Table 2 and Table 3.

Discussion

The popular use of topical agents for musculo-skeletal injuries has been persistent. On the other hand, accusations about the adverse effects related continue. Advocates insist on the subjective feeling of comfort and improvement. Surgeons and medical experts firmly believe that topical agents are not only myths, but frequently lead to skin allergy, infection, and unnecessary delayed treatment, hence complications.

We seriously attempted to sort out the controversies and obtained the early answers. We found that among the many medicinal herbs traditionally used as topical agents, the appropriate ones could be selected according to their biological activities of anti-inflammation, pro-angiogenesis and cellular proliferation. Of the five herbs selected for our study, all of them are subsequently proven to possess such healing abilities. It is easy to demonstrate

that transcutaneous transport of bio-active chemicals is responsible for the healing capacity of topical agents, so that the assumptions that such agents are just counter-irritants producing psychological effects are totally groundless. Most importantly carefully planned clinical trials using topical agents on minor injuries and chronic inflammatory conditions showed impressive results on pain and swelling control. The clinical trials had the limitations of small patient groups and short durations of treatment, which could be overcome in future trials.

Topical applications of herbal extracts should have plenty of practical value because tissue injuries resulting from trauma commonly leave residual problems of pain and swelling. Moreover, injuries initially expected for quick spontaneous healing might instead experience chronicity and incomplete recovery. Many common inflammatory conditions in the upper limb e.g. de Quervain's disease, tennis elbow and tendonitis around the wrist, are good examples. Herbal medicine has the additional attraction of being a complex chemical formulation, which not only targets against pain or oedema, but works on multiple directions.

The speed and completeness of recovery after injury differ among individuals and not infrequently, injuries in the musculo-skeletal system are job-related, so that recovery might not be straightforward. The self-care component in the normal rehabilitation process under such circumstances would need special emphasis. Thorough understanding and utilization of topical agents therefore deserve extra attention.

In the present era of multi-disciplinary collaboration, the future research on topical agents for injury management could start with the identification of more effective multiple focal individual herbs and biophysical means to enhance the surface penetration and efficacy of biological activities. Many outstanding issues like the cellular and molecular mechanisms of tissue healing under the influence of topical agents, and the nature of bone healing, whether through the usual periosteal induction process or via stem cell activities, would all deserve thorough explorations.¹²

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