

Sanpao herbs inhibit development of atopic dermatitis in Balb/c mice

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Summary

Objective: To explore the effects of SANPAOCAO (SPC), a compound traditional Chinese folk medicine, on chronic dermatitis/eczema in mice induced by 2, 4-dinitrochlorobenzene (DNCB).

Methods: Thirty-three Balb/c mice were randomly divided into a negative control group, a positive control group, a prednisolone treatment group, an SPC ethanol extract treatment group, a *Cardiospermum halicacabum* ethanol extract treatment group, a *Physalis minima* ethanol extract treatment group, and a *Jussiaea repens* ethanol extract treatment group. Mice in the six treatment groups had twenty-five microliters of 0.1% DNCB in acetone/olive oil (3: 1) applied to each side of their right ears and dorsal skin three times a week, over a 5 week period. They were treated with prednisolone or the various kinds of ethanol extract after each challenge. The weight difference between the two ears, pathological changes in the right ears, dermal inflammatory cell numbers, and total serum Ig E levels were used to assess the effects of the drugs.

Results: after the 5 weeks of challenges, the weight differences of the ears in the SPC group and the prednisolone group were significantly less than those in the other groups. There was evidence of significant suppression of the development of dermatitis, as determined by a histological examination and the serum Ig E levels.

Conclusion: SPC has beneficial effects when used in the treatment of chronic dermatitis-eczema in mice. (*Asian Pac J Allergy Immunol* 2014;32:140-4)

Key words: anti-dermatitic activity, atopic dermatitis, Sanpaocao, Chinese herbs, cytokines

Introduction

Atopic dermatitis (AD) is a clinical syndrome that is characterized by pruritic skin lesions that are distinguished by infiltrating lymphocytes, macrophages and granulated mast cells.^{1,2} AD is a common disease with an increasing incidence in industrialized countries and is a genotypic diathesis in which minor skin stimulation is perceived as an itching and, when scratched or rubbed, elicits a heightened immune response.^{3,4} The heightened immune response leads to the development of eczema, which is the clinical syndrome of the eczematous dermatitis group of inflammatory skin conditions characterized by pruritus, pale, erythematous and violaceous hues, vesiculation, erosion, scaling, exudation, crusting, lichenification, and excoriations.⁵⁻⁶ Although topical steroid therapy is used to manage this disease, many patients suffer from serious side effects of steroid ointments.⁷⁻⁸ Hence, a great deal of effort has been directed toward identifying safer compounds or herbal remedies that can inhibit the development of atopic dermatitis.

Some traditional Chinese herbal prescriptions, such as luteolin and white peony root, have been clinically applied as curative agents against incurable chronic diseases.⁹⁻¹⁰ Sanpaocao (SPC) is widely used in the Hainan province of China for the treatment of dermatitis. SPC is a decoction consisting of three tropical medicinal plants, *Cardiospermum halicacabum*, *Physalis minima* and *Jussiaea repens*. This study investigated the protective effect of SPC with regard to the development of dermatitis in Balb/c mice that had been topically challenged with 1-chloro-2, 4-dinitrobenzene (DNCB). The effect of Sanpaocao on thymus activation regulated chemokine (TARC) production in the keratinocyte cell line, HaCaT, was

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also examined to elucidate a possible mechanism for the anti-dermatitic effects of SPC.

Methods

Mice

The Institutional Animal Care and Utilization Committee of Hainan Medical University approved all animal procedures. Male Balb/c (weighing 20g) were purchased from Experimental Animals Co.(Guangdong, China), placed in cages at a temperature between 20 and 23°C with a 12 hour light and dark cycle and a relative humidity of 50%. Animals were given commercial mouse chow (Hainan, China) and water *ad libitum*. Twenty-five microliters of 0.1% DNCB in acetone and olive oil (3: 1) was applied to each side of the right ears and dorsal skin three times per week (Monday, Wednesday and Friday) for 5 weeks. Mice were then housed for 3 days without any further treatment. There were seven treatment groups: a negative control group (DNCB (-)), a positive control group (DNCB (+)), a prednisolone group (DNCB (+) prednisolone), an SPC ethanol extract group (DNCB (+)SPC), a *C. halicacabum* ethanol extract group (DNCB (+) *C.halicacabum*), a *Ph. minima* ethanol extract group (DNCB (+)*Ph.minima*) and a *J. repens* ethanol extract group (DNCB (+) *J.repens*). Each ethanol extract treated group was treated orally at a dose of 100mg/kg/d and the prednisolone group was given a dose of 30mg/kg/d (3 times per week for 5 weeks) in their drinking water. Control animals received the vehicle only. Mice were sacrificed 38 days after first applying the DNCB) and blood was collected from the *vena cava*. Skin tissues from the backs of the mice and their ears were excised and subjected to histological examination.

Preparation the ethanol extract

Three medicinal plants were collect from the outskirts of Haikou, Hainan Province, China, and was authenticated by Doctor Kainian Cen, Hainan Medical University. The air-dried and ground aerial parts of Sanpao (*C. halicacabum* 200 g *Ph. minima* 200 g *J. repens*. 120g), *C. halicacabum* (200 g), *Ph. minima* (200 g) , and *J. repens* (200 g) were extracted with 70% ethanol (EtOH, 3 times W/V) using maceration for 3 days with 90rpm shaking at 40°C, repeated three times. The extract was filtered through a cotton bed followed by Whatman No. 1 filterpaper. The filtrate was evaporated under reduced pressure at 45°C using a Buchii Rotaroy Evaporator to leave a gummy concentrate and

through vacuum drying to produce Sanpao ethanol extract (73.54g), *C. halicacabum* ethanol extract (23.88g), *Ph. minima* ethanol extract (31.21g), and *J. repens* ethanol extract (16.59g).

Enzyme Linked Immunosorbent Assay (ELISA)

Total serum Ig E levels were measured by ELISA. Blood samples were collected 24 hours after the fifth application of DNCB and Ig E concentrations in serum were quantified using BD OptEIA™ Set Mouse Ig E ELISA kits (BD Pharmingen, CA, U.S.A). In brief, a 1: 250 dilution of anti-mouse Ig E monoclonal antibody in PBS was placed in each well of an immunoplate (Corning 3590 96-well EIA/RIA plate, Corning, NY, U.S.A.) and maintained overnight at 4 °C. After washing the wells with PBS containing 0.05% Tween 20 (washing buffer) 3 times, 200ml of PBS containing 1% bovine serum albumin (BSA in PBS) was placed in each well. After 1 h at room temperature, wells were washed three times with washing buffer and 100ml aliquots of serum samples diluted 30—50 fold with BSA in PBS were placed in the wells. After further incubation for 1 h at room temperature, wells were again washed five times with washing buffer, 100ml of streptavidin- horseradish peroxidase-conjugated detection antibody (SAv-HRP) diluted 250-fold with washing buffer was then added, and the plate left for one hour at room temperature. After washing 7 times with washing buffer, the enzyme reaction was initiated by adding 100m l of substrate solution (0.1 M citric acid, 0.2 M Na₂HPO₄, o-phenylene diamine and H₂O₂), and the plate was left for 30 minutes at room temperature. The reaction was terminated by adding 50ml of 1 M H₂SO₄ to each well and absorbance at 450 nm was measured immediately using an ELISA reader (EL800) (Bio-Tek, VT, U.S.A.).

Histopathologic Examination

Ear tissues were isolated from each mouse and fixed in 10% formalin in 50mM of a phosphate buffer (pH 7.0) for 24 hours at 4°C. Ear tissues were subsequently embedded in paraffin, sectioned (4 mm), stained with Hematoxylin and Eosin, and examined by optical microscopy (Olympus, Tokyo, Japan). A certified pathologist analyzed and scored the samples in a blinded manner. A minimum of 2 sections per experimental animal were examined for the presence and degree of incrustation, thickening of the epidermis, epidermal necrosis, bleeding, hyperkeratosis and inflammation of the epidermis and dermis.



Statistical Analysis

Results were expressed as means±S.E. (S.E.M.). The significances of any differences were evaluated using Student's *t*-test. Differences between experimental groups were evaluated using analysis of variance. Values of $p < 0.05$ were accepted as significant.

Results

Ear Swelling Inhibition by SPC or Prednisolone

After the fourth and fifth DNCB applications, right ear swelling and thickening was evident, different degrees. It was most obvious in the DNCB (+) groups, but the daily administration of SPC or prednisolone significantly inhibited the degree of swelling (Figure 1). Table 1 shows that the SPC group and prednisolone group mice right ear weight were 2.80 g and 3.07 g, respectively, whereas the positive controls weighed as much as 11.23 g.

Histological analysis

Balb/c mice have been previously shown to develop AD-like skin lesions after the repeated topical application of DNCB under SPF conditions, and in the present study Balb/c mice were used to investigate the preventative effect of AM in this context. Symptom severity in Balb/c mice was

Table 1: Weight differences between the two ears of mice in different groups

	number of mice	weight difference between ears ($\bar{X} \pm s$, mg)
DNCB(-)	3	1.30±0.21
DNCB(+)	5	11.23±0.17
DNCB(+) Prednisolone	5	3.07±0.20
DNCB(+) SPC	5	2.80±0.16
DNCB(+) <i>Ph.minima</i>	5	8.53±0.23
DNCB(+) <i>C.halicacabum</i>	5	10.75±0.21
DNCB(+) <i>J.repens</i>	5	8.80±0.17

found to increase gradually with time during the 5-week DNCB challenge period. Oral SPC (100 mg/kg/d) suppressed AD-like skin lesion development, and, as expected, prednisolone (30 mg/kg/d) significantly inhibited the development of skin lesions, but in the other groups there were no obvious treatment effects. Photographs showed that SPC and prednisolone effectively treated AD-like lesion induced by DNCB on mice. These groups showed broader recovery from AD-like lesions and hair growth on the dorsal skin (Figure 1). Histological examination of the skin lesion showed no abnormal changes in the control group, whereas in the DNCB (+) group, the *Ph.minima* group, the

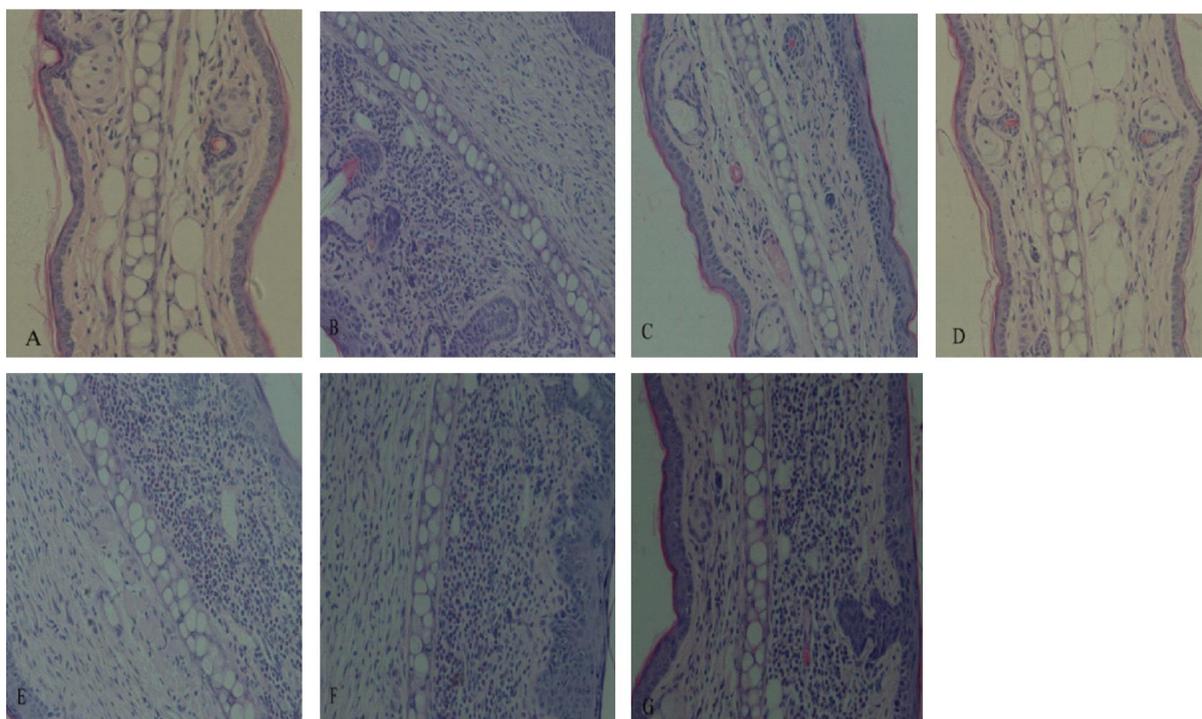


Figure 1. Pathological changes in the right ears of mice observed by light microscopy (HE staining, ×200), A: DNCB (-) group, B: DNCB(+) group C: DNCB(+) prednisolone group, D: DNCB(+) SPC group, E: DNCB(+) *Ph.minima* group, F: DNCB(+) *C.halicacabum* group, G: DNCB(+) *J.repens* group

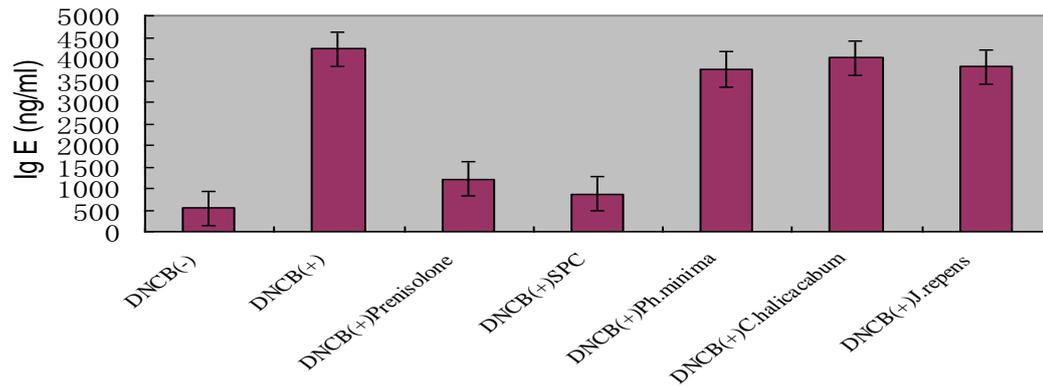


Figure 2. Serum Ig E levels in mice in the different treatment groups, measured 24 hours after the final administration

C.halicacabum group and the *J.repens* groups showed significant inflammatory changes, including hemorrhage, hypertrophy, hyperkeratosis of the epidermis, such as an increased number of mast cells with mild degranulation, and infiltration of numerous eosinophils and a small number of mononuclear cells. The epidermis was thickened by moderate hyperplasia with elongation of the rete ridges and prominent, hyperkeratosis with areas of parakeratosis.

SPC Did Inhibit DNCB-Induced Serum Ig E Level Elevation

After completing DNCB treatment, serum samples were obtained and total Ig E levels were determined by ELISA. Serum Ig E levels were markedly increased by DNCB (Figure 2). Oral SPC inhibited this DNCB-induced serum Ig E increase, as did prednisolone.

This study shows that SPC can reduce the severity of dermatitis-like skin lesions induced by topical DNCB in Balb/c mice. DNCB-treated Balb/c mice developed skin lesions characterized by erythema, edema, excoriation, and scaling, and histologically by massive inflammatory cell infiltration, which are similar to those reported for Balb/c mice maintained under conventional conditions.¹ Moreover, oral SPC, like prednisolone, administered to DNCB-treated Balb/c mice clearly inhibited the development of AD-like skin lesions.

The upregulation of total serum IgE is a hallmark of atopic dermatitis. Th2-type cytokine IL-4 increases the switching of B cells from Ig M to Ig E production.⁴ Accordingly, total serum Ig E was found to be significantly increased following repeated DNCB treatment in Balb/c mice (Figure 2). These results highlight the protective effects of SPC on the development of dermatitis in DNCB- applied

mice. The topical application of DNCB on the backs of mice was an efficient tool for inducing severe and controlled dermatitis within 5 weeks.

The three plant materials used contain several chemicals with anti-allergic and anti-inflammatory activity.¹¹⁻¹³ These include physalin, flavonoids, lignans and catechins in the plant sources of SPC. However, it is impossible to isolate all the active constituents from SPC. Through this experiment, we found that only three plants crude extract mixed together of have a beneficial effect in inhibiting chronic dermatitis. Therefore, SPC contain many compounds from three medicinal plant sources, and the structures of components might change into new or modified compounds during the mixed ethanol extraction. Alternatively, several compounds from different plants may need to be combined together to have the treatment effect. It is uncertain changes are induced by ethanol extraction, and which elements elements play a role in the treatment process, and so more research is needed.

Balb/c mice topically treated with DNCB were used to determine the anti-dermatitic effect of SPC *in vivo*. The oral administration of SPC effectively prevented development of dermatitis, as evidenced by histology examination. Moreover, ELISA assay results confirmed that SPC treatment inhibited the increase in serum IgE level normally induced by DNBC. SPC is often prescribed to patients with atopic dermatitis in Chinese folk medicine, and these results provide the necessary scientific evidence of its clinical efficacy.

In conclusion, the oral administration of SPC extracts was found to inhibit the development of AD-like skin lesions in Balb/c mice treated repeatedly with DNCB.

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Conflict of interest

The authors declare that they have no conflicts of interest concerning this article.

References

1. Soter NA. Morphology of atopic eczema. *Allergy*. 1989; 44:16–9.
2. Garnacho SG, Salido VR, Moreno GJC. Atopic Dermatitis: Update and Proposed Management Algorithm. *Actas Dermosifiliogr*. 2013;104:4-16.
3. Allam JP, Bieber T, Novak N. Recent highlights in the pathophysiology of atopic eczema. *Int Arch Allergy Immunol*. 2005;136:191 - 7.
4. Claudia S, Wolf-Dietrich FD, Zollner TM, Röse L. Chronic mouse model of TMA- induced contact hypersensitivity. *J of Inv Derm*. 2009;129:899–907.
5. Mina Y, Takayo H, Kiyoshi Y, Takahashi H, Iduhara M, Takaki S, et al. A novel atopic dermatitis model induced by topical application with dermatophagoides farinae extract in NC/Nga mice. *J Allerg Int*. 2007;56:139-48.
6. Wollenberg A, Bieber T. Proactive therapy of atopic dermatitis-- an emerging concept. *Allergy*. 2009;64:276-8.
7. Wollenberg A, Reitamo S, Girolomoni G, Lahfa M, Ruzicka T, Healy E, et al. European Tacrolimus Ointment Study Group. Proactive treatment of atopic dermatitis in adults with 0.1% tacrolimus ointment. *Allergy*. 2008;63:742-50.
8. Leung DY. Atopic dermatitis: immunobiology and treatment with immune modulators. *Clin Exp Immunol*. 1997;107:25–30.
9. Wu WW, Liu BL, Tan N, Jing CF, Wang Y. Topical Application of Luteolin Inhibits Dry Skin Pruritus in Mice. *Chin J Nat Med* . 2005;3:173-7.
10. Li CY, Wang C, WEI W. Effects and sectional mechanisms of total glucosides of paeony on mice with chronic dermatitis/eczema. *Chin Pharm acological Bulletin*. 2008;24:1366-9.
11. Kawai M, Yamamoto T, Makino B, Yamamura H, Araki S, Butsugan Y, et al. The structure of physalin T from *Physalis alkekengi* var. *franchetti*. *J Asian Nat Prod Res*. 2001;3:199-205.
12. Ragupathy S, Steven GN, Gopinadhan P, Candice B. Exploring ethnobiological classifications for novel alternative medicine: A case study of *Cardiospermum halicacabum* L. (Modakathon, Balloon Vine) as a traditional herb for treating rheumatoid arthritis. *Ethnobotany*. 2007;19:1-18.
13. Marzouk MS, Soliman FM., Shehata IA, Rabee M, Fawzy GA. Flavonoids and biological activities of *Jussiaea repens*. *Nat Prod Res*. 2007;21:436–43.

Erratum

Author names correction

In the article Pediatric Asthma Quality of Life Questionnaire: Validation in Children from Singapore (*Asian Pac J Allergy Immunol*. 1999 Sep;17(3):155-61), the names of authors should be changed from ‘Elizabeth Clarke, Suzanna Sulaiman, Fook Tim Chew, Lynette Pei-Chi Shek, Mital R. and Bee Wah Lee’ to ‘**Clarke Elizabeth, Sulaiman Suzanna, Chew Fook Tim, Shek Lynette Pei-Chi, R. Mital and Lee Bee Wah**’

