

EXPERT OPINION

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Anti-allergic drug discovery in China for eczema: current methods and future strategies

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Introduction: Eczema is a common childhood atopic disease associated with chronicity and impaired quality of life. As there is no cure for this disease, treatment relies on topical and systemic anti-allergic or immunomodulating therapies. Topical corticosteroids, macrolide immunosuppressants and oral immunomodulating drugs have been the mainstay for the treatment of recalcitrant disease. However, in China as well as throughout Asia, the use of traditional and proprietary topical and herbal medicines is also popular.

Areas covered: This article reviews the evidence for the anti-allergic effects of topical and oral forms of these medicinal. The authors also present and discuss the current methods of preparation and evaluation for these traditional and proprietary medications. Additionally, the authors present the novel delivery method of employing nanotechnology to deliver these herbal therapies.

Expert opinion: Many herbal concoctions have had trials which have claimed therapeutic benefits for eczema. However, the scientific methods in these trials are often flawed and often not objective. In fact, the majority of these trials do not provide proof of objective efficacy for the anti-allergic effects claimed. It is felt that the delivery of herbal medication by nanotechnology has several crucial challenges that certainly need to be overcome for the optimal design of therapies for eczema, through optimizing material properties, methods of delivery and understanding the mechanisms of action.

Keywords: allergy, atopic dermatitis, eczema, herbal medicine, traditional Chinese medicine

Expert Opin. Drug Discov. [Early Online]

1. Introduction

1.1 Eczema as a form of allergy

Eczema or atopic dermatitis (AD) is a chronically relapsing dermatosis characterized by pruritus, erythema, vesiculation, exudation, excoriation, crusting, scaling and sometimes lichenification [1-7]. The prevalence of AD has increased two- to three-folds over the past three decades in industrialized countries and there is evidence to suggest that this prevalence continues to increase [1,6,8,9]. AD affects 9 – 20% of children and 1 – 3% of adults in the USA and is more prevalent in children that belong to upper socioeconomic classes, smaller family sizes and families with overzealous hygiene [1,3,10-14]. In China, the prevalence was estimated to be approximately 5% [15]. The increase in prevalence may be due to increased access to medical care, improved recognition, better epidemiological reporting or increased environmental allergens due to industrialization and pollution. Conversely, the hygiene hypothesis postulates that the cause of eczema and other allergic diseases is an unusually clean environment. The hypothesis states that exposure to bacteria and other immune system modulators are important during development, and missing out on this exposure increases risk for allergy [16,17]. Secondary bacterial

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Article highlights.

- Eczema is a chronic relapsing dermatitis associated with allergy/atopy.
- There is no cure by western medicine and many parents and patients resort to oral or topical herbal medicine usage.
- Standardisation and manufacture of proprietary herbal medicine have become possible.
- There is a need for more well-design randomized control trials in herbal medicine research for eczema
- Novel drug discovery strategies for eczema should include objective scientific documentation of drug efficacy and adverse effects; and evaluation of the anti-allergic effects of herbal concoctions.
- Targeting mechanisms can be broadly against allergic processes, or specifically against individual humoral or cell-mediate hypersensitivity pathways.

This box summarizes key points contained in the article.

infection, most commonly with *Staphylococcus aureus*, is the main complication of AD [1,18].

Children with AD may suffer from lack of sleep, irritability, daytime tiredness, emotional stress, lowered self-esteem and psychological disturbance [19,20]. The disruption of school, family and social interactions can severely impair the quality of life and extends beyond childhood [19,21-23]. Parents may experience guilt, frustration, resentment, exhaustion and helplessness due to their child's condition [8].

The diagnosis of AD is predominantly clinical, based on a constellation of clinical features. Firm criteria to define AD were first established by Hanifin and Rajka [1,24]. The UK working diagnostic criteria are also concise and practical [25-27].

AD involves defective cell-mediated immunity related, in part, to an imbalance in two subsets of CD4-T cells that creates a predominance of T-memory cells in the T-helper 2 pathways and preferential apoptosis of interferon (IFN)-gamma producing T-helper 1 memory and effector T-cells [3-5]. T-helper 2 cells express a set of cytokines (interleukin-4, -5, -6, -10 and -13) [4,28]. These cytokines stimulate the proliferation and differentiation of B-lymphocytes, upregulate the expression of adhesion molecules on endothelial cells and contribute to the hypereosinophilia, high serum IgE levels, sustained cutaneous inflammation, histamine release and pruritus characteristic of AD [29]. Recently, we demonstrated that AD also involves many cellular and humoral immune mediators in addition to an aberration of cell-mediated immunity [30].

There is a strong genetic predisposition, as evidenced by the familial nature of the disease and the high concordance in monozygotic twins [1,31]. It has been shown that loss-of-function mutations in the filaggrin (filament-aggregating protein) (FLG) gene predispose to AD [32-36]. Filaggrin is a key component of the epidermal differentiation complex of the stratum corneum in the epidermal layer of human skin. It forms the natural moisturizing factor in the stratum corneum. Loss-of-function mutations in filaggrin are associated with an

increased risk of atopic sensitization in these individuals. AD is the first stage of the atopic march that describes the phenomenon of increased rates of allergic rhinitis and allergic asthma observed in individuals with early AD [1,37]. FLG, an epidermal barrier protein, plays an important role in the barrier function of the skin [38]. The protein is present in the granular layers of the epidermis. The keratohyalin granules in the granular layers are predominantly composed of profilaggrin [39]. FLG aggregates the keratin cytoskeleton system to form a dense protein-lipid matrix that is cross-linked by transglutaminases to form a cornified cell envelope [38,39]. The latter prevents epidermal water loss and impedes the entry of allergens, infectious agents and chemicals [38]. It is believed that defective epidermal function is related to the down-regulation of the *FLG* gene [40]. *FLG* has an important function in epidermal differentiation and barrier function [41-44]. Approximately 10% of people in European origin are carrying these variants. Recent findings have shown that the affected skin of atopic individuals is deficient in filaggrin degradation products [45].

In summary, AD is an atopic/allergic disease that involves complex interactions among susceptible genes, immunological factors, skin barrier defects, infections, neuroendocrine factors and environmental factors [1].

1.2 Concept about eczema and allergy in China

Although considered as a form of allergy or atopic disease in western medicine, the concept of allergy is different according to the Qi concept in Chinese medicine. According to Chinese medicine theory [46], Qi can be disrupted by 'wind', 'coldness', 'summer-heat', 'dampness', 'dryness' or 'fire evils'. Main pathogenic factors of eczema are thought to be 'wind', 'dampness' and 'heat' [46]. From a Chinese Medicine theory perspective, eczema is caused by a specific diathesis as a result of accumulated internal dampness-heat due to reduced function of the spleen. Symptoms can be triggered by exposure to wind, dampness and heat pathogens. Clinically, eczema is classified into the following patterns from a Chinese medicine viewpoint: accumulation of internal dampness; excess of dampness with the spleen deficiency or Yin deficiency with dryness of blood.

Chinese herbal medicines have been employed for treatment of eczema for many years [47,48]. They may be administered orally and/or topically. Oral applications of Chinese herbal medicines are guided by the pattern differentiation, known as 'individualized treatment' while topical administrations have been devised with little or no consideration on pattern differentiation [47]. In recent years, herbal medicine is converted to various concoctions under the broad category of proprietary Chinese medicine. One must realize that these products may contain non-herbal, animal ingredients or even western medicine [48,49].

Little is known about the anti-allergy patent situation in China. Zhang *et al.* summarized and analyzed anti-allergy patents issued in China from January 1988 to September

2008 [49]. A total of 789 anti-allergy patents have been granted in China during the last 20 years. China, European countries, USA, Japan and other countries possesses 44, 21, 19, 12 and 4% of all of these anti-allergy patents, respectively. Interestingly, 88% anti-allergy patents issued to Chinese are held by civilians, whereas vast majority of the patents issued to foreigners were held by pharmaceutical companies. All anti-allergy patents are focused on synthetic compounds, Traditional Chinese Medicines (TCM), combinations of synthetic compounds and TCM (CST), biological products and medical apparatus. The anti-allergy patents in China mainly focus on well-known targets, such as histamine receptor and leukotrienes, which consist of 93% of patents for validated targets. Approximately 93% targeting diseases are bronchial asthma, allergic rhinitis and AD. Their results indicate that there are great opportunities for application of patents on development of novel anti-allergic compounds and modernization of TCM in China, and eczema is one of such targets.

Among these anti-allergic compounds, herbs such as Cortex Moutan (Danpi), Radix Paeoniae Alba (Bai Shao), Herba Potentillae Chinensis (Weilingcai) and Radix Glycyrrhizae (Gan Cao) are common treatments for allergy [50]. Flos Lonicerae (Jingyinhua) and Herba Menthae (Bohe) clear 'damp-heat' from the exterior, Cortex Moutan (Danpi) clears 'heat' from blood while Rhizoma Atractylodis (Cangzhu) and Cortex Phellodendri (Huangbai) clear the 'damp-heat' from the interior. Pharmacological studies indicate that these herbs have anti-allergic, anti-inflammatory and sedative action for itchiness [46,51,52]. Herbs markers with anti-allergic activities include gallic acid, paeonol and paeoniflorin in cortex moutan, berberine in cortex phellodendri, luteolin and chlorogenic acid in flos lonicerae, emodin in herba menthae and atractylenolide I in rhizoma atractylodis. Specifically, gallic acid has been shown to inhibit histamine release and pro-inflammatory cytokine production in mast cells, suppress antigen-induced allergic reactions in RBL-2H3 mast cells, and is a G protein-coupled receptor-35 agonist [53-56]. Paeonol and paeoniflorin possess anti-inflammatory and anti-allergic effects [57-59]. Berberine suppresses proinflammatory responses through AMPK activation in macrophages and inhibits TPA-induced MMP-9 and IL-6 expression in normal human keratinocytes [60-62]. Luteolin possesses anti-oxidant, anti-inflammatory and anti-allergic activities which inhibits myelin basic protein-induced human mast cell activation and mast cell-dependent stimulation of Jurkat T cells [63-67]. Chlorogenic acid possesses allergy-preventive effects and has demonstrated better anti-inflammatory effect than indomethacin [68,69]. Herba menthae possesses anti-allergy activities and is a common ingredient used in cooking [70-72]. Emodin is a naturally occurring anthraquinone derivative that suppresses IgE-mediated anaphylactic reaction and mast cell activation [73-78]. Atractylodes japonica Koidzumi inhibits the production of proinflammatory cytokines through inhibition of the NF-kappaB/I-kappaB signal pathway in HMC-1 human mast cells [61,79]. Atractylenolide I inhibits

inducible nitric oxide production and iNOS protein expression in lipopolysaccharide-stimulated rat aorta and Raw 264.7 macrophages [80].

This article aims, using a global overview of putative novel topical and systemic drugs for eczema in China, to describe the clinical methods and strategies to develop new pharmacological agents that target allergy in eczema pathophysiology. Indeed, from a pharmacological point of view, these topical or systemic Chinese medicines act by immunomodulating the allergic process.

2. Methodology

We searched MEDLINE, Embase and the Cochrane Library for articles published using the following search terms: 'treatment', 'eczema', 'atopic', 'allergic', 'Chinese', 'China', 'herbal' and 'herbs'. We also searched for 'eczema' or 'atopic dermatitis' combined with the terms 'strategies', 'pathogenesis', 'genetic', 'pathophysiology', 'immunomodulant' and 'immunomodulating' [47,81]. We selected literature from mainly the past 20 years but did not exclude commonly referenced and highly cited older articles. We limited our search to include and describe randomized trials, case series and bench studies in Chinese medicines for eczema. In addition, the bibliographies of the retrieved articles and our departmental files were also hand searched.

TCM also incorporates the concept of meditation, Tai Chi and acupuncture. These non-drug complementary and alternative medicine (CAM) studies were not included. All controlled trials of homeopathy in patients with eczema were considered.

As of December 2010, we retrieved 47 articles from PubMed using the keywords "'Chinese herbal medicine' and ('atopic dermatitis' or 'eczema')" [47]. We also searched the Cochrane Database for Systematic Reviews. Using PubMed Clinical Queries, we retrieved 9, 2 and 9 references under Clinical Study Categories, Systematic Reviews and Medical Genetics, respectively. All RCTs and relevant case series and bench studies were included. Review articles that did not provide any information on eczema and Chinese medicine were excluded. The theme is about anti-allergic drug. Studies without evaluation of anti-allergic mechanisms were not evaluated in details.

3. Overview of anti-allergic drug discovery in China for eczema

3.1 Case series

A number of case series reported the efficacy of herbal medicine on childhood AD. None of these series specifically addressed the individual humoral- or cell-mediated anti-allergic effects.

Luo *et al.* reported about the 56 cases of 'stubborn' ('stubborn' in Chinese probably meant recalcitrance) eczema treated by oral administration and topical application of herbal medicine [82]. The study was flawed in that it combined

oral and topical treatments rendering the studying of individual effects impossible.

Salameh *et al.* assessed the effectiveness of the combination of Chinese herbal medicine and acupuncture for the treatment of AD [83]. Twenty mild-to-severe AD patients aged between 13 and 48 years were given a combined treatment of acupuncture and Chinese herbal medicine and were followed prospectively. The patients received acupuncture treatment twice a week and the Chinese herbal formula three times daily for a total of 12 weeks. Assessments were performed before treatment, and at weeks 3, 6, 9 and 12 of treatment. The primary outcomes were defined as the changes in the Eczema Area and Severity Index (EASI), Dermatology Life Quality Index (DLQI), and patient assessment of itch measured on a visual analogue scale (VAS). After 12 weeks of treatment, an improvement in EASI over the baseline was noted in 100% of patients. The mean EASI fell from 4.99 to 1.81; the median percentage of decrease was 63.5%. Moreover, 78.8% of patients experienced a reduction in DLQI and VAS, as compared with the baseline. The mean DLQI decreased from 12.5 to 7.6 at the end of treatment, with 39.1% improvement. Mean VAS decreased from 6.8 to 3.7, with 44.7% improvement. No adverse effects were observed. The authors concluded that the combination of acupuncture and Chinese herbal medicine had a beneficial effect (reduction of disease severity and improvement of quality of life) on AD patients, probably better than Chinese herbal medicine alone. As this study involved heterogeneous age groups of both children and adults and it was an open-label study, conclusion about the Chinese herbal medicine cannot be ascertained. Furthermore, there was no evaluation of any underlying anti-allergic mechanism.

Hon *et al.* performed a single-center open label trial to assess the efficacy and tolerability of a Chinese medicine syrup in younger children with AD [84]. Children aged 4–7 years with AD diagnosed according to Hanifin and Rajka's criteria [24] were recruited. The clinical severity of AD was evaluated according to the SCORing Atopic Dermatitis (SCORAD) index, [85], and all subjects had an objective SCORAD score of ≥ 15 (moderate-to-severe disease) at the entry into this study [86]. At baseline (visit 1), dietary intake, emollient, topical corticosteroid usage and information regarding the severity of AD were collected. They then received Chinese herbal medicine syrup 20 ml daily for 12 weeks. The physicians at the Institute of Chinese Medicine suggested that this dosage (equivalent to a bowl of herbal tea) would suit a wide range of age groups [87,88]. Enrolled subjects were followed up at 2 weeks (visit 2), 7 weeks (visit 3), 12 weeks (visit 4) and 4 weeks after completion of treatment (visit 5) for the control of their skin condition. Each patient was given an Eczema Diary for recording daily symptom during the period prior to each visit. The severity of AD and quality of life as assessed with the SCORAD index and Children's Dermatology Life Quality Index (CDLQI), respectively [20,85,86,89] at every visit were used as the primary

outcome measures. The individual components that constitute SCORAD, such as disease extent, intensity, pruritus and sleep loss [85] formed the secondary outcome measures. The potency of topical CS was not changed during the study period. The type of oral anti-histamines used by a particular subject remained the same throughout the study. Twenty-two Chinese children (10 boys and 12 girls), with a mean (SD) age of 5.8 (0.9) years, participated in this study. Their mean (SD) objective SCORAD was 36.6 (12.5), and mean (SD) CDLQI was 11.9 (6.0). There were significant improvements in the objective SCORAD, pruritus and CDLQI scores 4 weeks after study completion. There was no change in sleep score or amount of topical steroid consumption. No biochemical evidence of any adverse drug reaction was found during the study period. The Chinese herbal medicine syrup was generally palatable and well tolerated by the children. Adverse effects were mild although two patients with rash withdrew during this study. The authors concluded that further evaluations and dosage studies of the decoction was warranted for treating young children. The findings in quality of life improvement agreed with the previous randomized placebo-controlled trial with the same decoction in capsular preparation for older children [88]. The therapeutic effects persisted 1 month after treatment had stopped. The investigators did not specifically evaluate the anti-allergic effects of the concoction.

3.2 Laboratory studies

To delineate the actions of PentaHerbs on AD, the authors analyzed the effects of an extract of these herbs on interleukin 4 (IL-4)-induced CD23 expression on peripheral blood monocytes collected from non-atopic subjects. They found that PentaHerbs inhibited CD23 expression up to 60% ($p < 0.001$) whereas the placebo extract had no significant effect on CD23 expression. This inhibition was dose-dependent, and PentaHerbs was effective at a concentration of 250 $\mu\text{g/ml}$ ($p = 0.001$). If PentaHerbs or placebo was added after IL-4, the action of PentaHerbs was still observed at 12 h. This inhibition was not due to cell death, as peripheral blood mononuclear cells (PBMCs) cultured with PentaHerbs or placebo at a concentration used in these experiments had a similar cell viability to control cultures. Down-regulation of the low affinity receptors for IgE on antigen-presenting cells in patients with eczema may contribute to the benefit observed following treatment with PentaHerbs [90].

Laboratory studies also demonstrated favorable immunomodulatory effects [91,92]. Leung *et al.* investigated the immunomodulatory effects possibly induced by PentaHerbs treatment [91] on cytotoxicity and proliferation of phytohemagglutinin (PHA)- and staphylococcal enterotoxin B (SEB)-stimulated PBMCs isolated from buffy coat of blood donors. PentaHerbs-induced immunomodulation for five inflammatory mediators in cultured PBMC was measured by reverse transcription-polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay. The effects of a 3-month, open-label study of PHF treatment on circulating

inflammatory mediators in children with AD were also assessed. PentaHerbs at up to 1 mg/mL dose-dependently suppressed PBMC proliferation. The addition of PentaHerbs to cultured PBMC reduced supernatant concentrations of brain-derived neurotrophic factor (BDNF), IFN- γ and tumour necrosis factor (TNF)- α in response to PHA and BDNF and thymus and activation-regulated chemokine (TARC) following SEB stimulation. PentaHerbs increased epithelial cell-derived neutrophil activating peptide-78 levels in culture supernatants. At the RNA level, PentaHerbs suppressed the transcription of BDNF, TARC, IFN- γ and TNF- α . Twenty-eight children with AD were treated with PentaHerbs for 3 months, and their mean plasma concentrations of BDNF and TARC decreased significantly from 1798 pg/mL to 824 pg/mL at baseline to 1378 pg/mL and 492 pg/mL ($p = 0.002$ and 0.013 , respectively) on study completion. The investigators concluded that PentaHerbs possessed *in vitro* and *in vivo* immunomodulation that might mediate the clinical efficacy observed in AD treatment with PentaHerbs [91].

The actions of PentaHerbs on mast cell activation were also investigated [92]. Effects of aqueous extracts of PentaHerbs and individual component herbs on mediator release from rat peritoneal mast cells (RPMCs) and cytokine production from Human Mast Cell HMC-1 were investigated. PentaHerbs, Cortex Moutan and Herba Menthae significantly attenuated histamine release and prostaglandin D₂ synthesis from RPMC activated by anti-IgE and compound 48/80. While Flos Lonicerae and Rhizoma Atractylodis suppressed only mediator release from compound 48/80 activated RPMC, Cortex Phellodendri potentiated only anti-IgE induced mediator release. However, with the exception of Cortex Moutan, PentaHerbs and the other four component herbs failed to affect cytokine production in HMC-1. The investigators concluded that individual herbs of PentaHerbs modulate mast cells and inhibit the inflammatory mediators from mast cells thereby achieving the therapeutic efficacy of PentaHerbs. They illustrate that the anti-allergic effects of herbal products can and should be evaluated in the laboratory.

3.3 Clinical trials

The beneficial effects of Chinese medicine on children with AD have not been consistently demonstrated [93]. A limited number of Chinese medicine trials in children and adults with AD did not show convincing results [94-96]. Cochrane systematic review in 2005 on the topic of Chinese herbal medicine for AD included only four clinical trials [96]. Further meta-analyses have been performed by Cochrane reviews which show no conclusive evidence that oral intakes of most Chinese herbs or Chinese herbal formulae used in the included studies could improve eczema. Further, they could not find convincing evidence that topical application of Chinese herbal medicine whether used alone or in conjunction with oral administration of Chinese herbal formula could reduce the severity of eczema in children or adults. Even though the included studies claimed that there were

statistically significant differences in the outcome measures on Chinese herbal medicine treatment groups compared with those in the control groups, these claims could not be taken for granted due to a low strength of evidence and high risks of bias. Some of these formulae are discussed as follows.

3.3.1 Zemaphyte trial

In the early 1990s, a decoction (Zemaphyte, Phytopharm Plc, UK) was efficacious for the treatment of AD in both children [97,98] and adults [99] in the UK. Sheehan *et al.* carried out a randomized placebo-controlled double-blind trial of a specific prescription formulated for widespread non-exudative atopic eczema [97]. Forty-seven children were given active treatment and placebo in random order, each for 8 weeks, with an intervening 4-week wash-out period. Thirty-seven children received all the treatment and completed the study. Active treatment was more effective than the placebo. There was no evidence of hematological, renal or hepatic toxicity. The authors concluded that the Chinese medicinal herbs under their trial have a therapeutic potential in treating eczema and other skin diseases.

The opportunity to continue treatment was offered to the parents of 37 children who had completed a double-blind placebo-controlled trial of the same formulation of Chinese medicinal herbs for atopic eczema [98]. The parents selected continued treatment in all cases, and the progress of the children was monitored for 12 months. The aim was to reinforce the clinical improvement and to reduce treatment frequency progressively. At the end of the 12-month follow-up period, 18 were reported to have at least 90% reduction in eczema activity scores, and 5 showed moderate improvement. Fourteen children withdrew from the study. Seven of the children discontinued treatment without relapse; the other sixteen required treatment to maintain control of their eczema, but only four of these still required daily treatment. Asymptomatic elevation of serum aspartate aminotransferase (AST) to 7 – 14 times normal values was noted on one occasion in two children whose eczema after treatment was so well that the therapy was stopped. Liver function became normal after 8 weeks. The authors suggested that these Chinese medicinal herbs provided a therapeutic option for children with the extensive atopic eczema which did not respond to other treatments.

The adult patients with severe atopic eczema who had completed a similar double-blind placebo-controlled crossover trial were offered continued treatment for 1 year [99]. Out of the 31 patients who completed the placebo-controlled study and after a wash-out period and further treatment, 17 continued treatment (group 1), 11 chose not to continue treatment (group 2), 1 was lost to follow-up and 2 patients continued treatment but finally decided to stop treatment. At the end of the 12-month period, 12 patients in Group 1 had reduction greater than 90% and the remaining 5 had reduction greater than 60% in clinical scores from baseline values. Clinical scores of patients in Group 2 gradually deteriorated during the year. Difference between groups 1 and 2 in clinical

scores was highly significant ($p = 0.005$ and $p = 0.002$ for erythema and surface damage, respectively). At the end of the 12-month period, no patient in Group 1 discontinued treatment although eight patients were on an alternate-day regimen by 6 months and remained on this regimen until the end of the 12-month period, and seven were able to control their eczema with treatment throughout the period. The remaining two patients continued on daily treatments. Toxicology screening revealed no abnormalities in either blood counts or biochemical parameters in all patients under continued treatment. Improvement in disease was not associated with any significant change in the levels of serum IgE or peripheral blood lymphocyte subsets. These studies were different from many of other TCM studies in that the investigators tried to clinically evaluate the anti-allergic effects using serum IgE and peripheral blood lymphocytes albeit no objective effects were demonstrated.

Despite the efficacy reported in the UK trials, a subsequent randomized placebo-controlled, cross-over trial (RCT) of the same decoction in Hong Kong failed to demonstrate beneficial effects on Chinese patients with recalcitrant AD in Hong Kong [94]. Forty recruited patients were given Zemaphyte and placebo in random order, each for eight consecutive weeks with a 4-week wash-out period in between. Scores based on the severity and extent of four clinical parameters (erythema, surface damage, lichenification and scaling) were recorded at baseline and at 4-weekly intervals throughout the 20-week trial period. Thirty-seven patients completed the trial. There was general clinical improvement throughout the trial period in both patient groups, irrespective of whether they received Zemaphyte or placebo first. Zemaphyte, however, offered no statistically significant treatment effect over placebo for all four clinical parameters, except for lichenification at week 4. There were no significant carry-over effects. The results of blood tests for hematologic, renal and liver functions were all normal throughout the trial. The investigators concluded that Zemaphyte did not benefit the Chinese patients with recalcitrant AD in their study. This Hong Kong study did not evaluate the anti-allergic effects of the concoction.

3.3.2 PentaHerbs trials

In a pilot study, Hon *et al.* evaluated the clinical and biochemical effects of a Chinese medicine capsule (PentaHerbs capsule) in children with AD [87]. After a run-in period of 4 weeks, children old enough to manage oral medication were admitted and their disease severity was evaluated by the SCORAD index. Blood samples were taken for complete blood count, total and allergen-specific immunoglobulin E (IgE), biochemical studies and inflammatory markers of AD severity [serum cutaneous T cell-attracting chemokine (CTACK), macrophage-derived chemokine (MDC), TARC and eosinophil cationic protein (ECP)] prior to, and after 3 months of Chinese medicine use. Three PentaHerbs capsules twice a day were prescribed for 4 months. Patients were

followed up monthly to ensure compliance, and SCORAD scores were obtained at each visit. Five boys and four girls participated in the study. All patients had detectable food or inhalant-specific IgE in serum. There was significant improvement in the overall and component SCORAD scores. There were no significant differences between the pre- and post-treatment values of the serum CTACK, MDC, TARC and ECP levels but CTACK showed a decreasing trend ($p = 0.069$). No clinical or biochemical evidence of any adverse drug reaction was found during the study period. The PentaHerbs capsules were well tolerated by the children and apparent reduction of disease severity were noted clinically. The authors concluded that a larger, randomized placebo-controlled study is required to confirm the efficacy of this formulation for AD.

The PentaHerbs capsules were manufactured, packaged and labeled by the Chinese Medicine Industry Development Centre, the Hong Kong Institute of Vocation Education (Hong Kong, China), and fulfilled the Good Manufacturing Practice standard for proprietary Chinese medicines [100]. The composition of each herb in the formulation was standardized. The formula comprised 2 grams of Flos Lonicerae (Jinyinhua), 1 gram of Herba Menthae (Bohe), 2 grams of Cortex Moutan (Danpi), 2 grams of Rhizoma Atractylodis (Cangzhu) and 2 grams of Cortex Phellodendri (Huangbai). The dosage calculation was based on the standard Chinese medicine prescription for children (one bowl of herbal tea) [87] and is equivalent to a daily dose of 20 ml of syrup. This dosage is adequate for children of this age. The syrup was formulated and assessed for its quality and safety according to established procedures [87] under the supervision of the Clinical Trials Section of the Institute of Chinese Medicine (ICM) (Hong Kong, China). In particular, the syrup had been tested for heavy metals, microbial products and residual pesticides; the results met the safety standards of Chinese medicines in Hong Kong. A laboratory study found no corticosteroids in the five constituent herbs [101]. The investigators in this study tried to use specific immune serum markers of CTACK, MDC, TARC and ECP levels to demonstrate anti-allergic effects. Nevertheless, only a trend improvement of CTACK (a T helper cell marker) was demonstrated [87].

In a subsequent double-blinded randomized placebo-controlled trial, the researchers assessed the efficacy and tolerability of the decoction in children with AD [88]. Following a 2-week run-in period, children with long-standing moderate-to-severe AD were randomized to receive a 12-week treatment with a twice-daily dose of three capsules of either PentaHerbs or placebo. The SCORAD score, CDLQI, allergic rhinitis score and requirement for topical corticosteroid and oral antihistamine were assessed before and at weeks 4, 8, 12 and 16 of treatment. Adverse events, tolerability, hematological and biochemical parameters were monitored during the study. Eighty-five children with AD were recruited. Over 12 weeks, the mean SCORAD score fell from 58.3 to 49.7 in the PentaHerbs group ($n = 42$; $p = 0.003$) and from 56.9 to 46.9 in the

placebo group ($n = 43$; $p = 0.001$). However, there was no significant difference in the scores at the corresponding time points between the two groups. The CDLQI of PentaHerbs-treated group was more significantly improved than that of the placebo group at the end of the 3-month treatment and 4 weeks after stopping therapy ($p = 0.008$ and 0.059 , respectively). The total amount of topical corticosteroid used was also significantly reduced by one-third in the PentaHerbs group ($p = 0.024$). The formulation was palatable and well tolerated. No serious adverse effects were observed between the groups. The investigators concluded that the PentaHerbs decoction is efficacious in improving quality of life and reducing topical corticosteroid use in children with moderate-to-severe AD.

3.3.3 Shuangfujin

Bai *et al.* evaluated the effects and safety of Shuangfujin (SFJ) on acute eczema [102]. One hundred and twenty patients with acute eczema were randomly assigned to four groups of same size, namely the saline group, the boric acid group, the Pifukang lotion group and the SFJ group. After 4 days of treatment with the respective medicine, the symptom score was remarkably lower in the SFJ group than in the other three groups, score in the saline group was higher than that in the boric acid group and the Pifukang lotion group and difference between the latter two groups was insignificant. The effective rate and recovery rate were 46.4 and 14.3%, respectively in the SFJ group, which were equivalent to those in the Pifukang lotion group and significantly higher than those in the other two groups, and the saline group showed the lowest efficacy. The effects on itchiness in the SFJ and the boric acid group were matched, which was higher than those in the Pifukang lotion group, and the lowest was in the saline group. No skin irritation and other adverse reactions were found. The investigators only described symptomatic improvement and did not objectively evaluate anti-allergic effects of the treatment.

3.3.4 A Kampo formula

Hochu-ekki-to, a Kampo formula (consisting of 10 herbs, namely Radix Astragali, Panax ginseng, Rhizoma Atractylodis Macrocephalae, Radix Glycyrrhiza Uralensis, Radix Angelicae Sinensis, Citri Reticulatae Pericarpium, Rhizoma Cimicifugae, Radix Bupleuri, Rhizoma Zingiber officinale, Fructus Jujubae), is effective for patients with *Kikyo* (delicate, easily fatigable or hypersensitive) constitution. Previous case reports suggested that this herbal drug was effective for a subgroup of AD patients. Kobayashi *et al.* evaluated the efficacy and safety of *Hochu-ekki-to* in the long-term management of *Kikyo* patients with AD [103]. In a multicenter, double-blind, randomized, placebo-controlled study, 91 *Kikyo* patients with AD were enrolled. *Kikyo* condition was evaluated by a questionnaire scoring system. All patients continued their ordinary treatments (topical steroids, topical tacrolimus, emollients or oral antihistamines) before and after their protocol entry. *Hochu-ekki-to* or placebo was orally administered twice daily

for 24 weeks. The skin severity scores, total equivalent amount (TEA) of topical agents used for AD treatment, prominent efficacy (cases with skin severity score = 0 at the end of the study) rate and aggravated rate (more than 50% increase of TEA of topical agents from the beginning of the study) were monitored and evaluated. Seventy-seven out of ninety-one enrolled patients completed the 24-week treatment course (treatment: $n = 37$, placebo: $n = 40$). The TEA of topical agents (steroids and/or tacrolimus) was significantly lower in the *Hochu-ekki-to* group than in the placebo group whereas the overall skin severity scores were not statistically different. The prominent efficacy rate was 19% (7 of 37) in the *Hochu-ekki-to* group and 5% (2 of 40) in the placebo group ($p = 0.06$). The aggravated rate was significantly lower in the *Hochu-ekki-to* group (3%; 1 of 37) than in the placebo group (18%; 7 of 39). Only mild adverse events such as nausea and diarrhea were noted in both groups without statistical difference. This placebo-controlled study demonstrated that *Hochu-ekki-to* was a useful adjunct to conventional treatments for AD patients with *Kikyo* constitution. Use of *Hochu-ekki-to* significantly reduced the dose of topical steroids and/or tacrolimus used for AD treatment without aggravating AD. The investigator did not objectively evaluate the anti-allergic effects of *Hochu-ekki-to* with any immune tests.

3.3.5 Xiao-Feng-San

In a prospective, randomized, double-blind, placebo-controlled trial, 71 patients with severe intractable AD were given an 8-week treatment with oral Xiao-Feng-San (XFS; 47 patients) or placebo (24 patients) [104]. Total lesion score, erythema score, surface damage score, pruritus score and sleep score were measured at 4-week intervals. Fifty-six patients completed both the treatment and follow-up periods. The decrease in the total lesion score in the treatment group at 8 weeks was significantly greater than that of the placebo group ($79.7 \pm 5.8\%$ vs. $13.5 \pm 7.64\%$; $p < 0.001$). There was also a statistically significant difference between the treatment and placebo groups with regard to erythema, surface damage, pruritus and sleep scores. The difference between the two groups was still significant for all outcome measures except the erythema score at the 12-week follow-up, 4-weeks after the 8-week treatment had ended. Patients reported no side effects from treatment, although some commented on the unpalatability of the medication. The investigators suggest that the traditional Chinese herbal medicine XFS may be an alternative choice of therapy for severe, refractory, extensive and non-exudative AD. The investigators did not objectively evaluate the anti-allergic effects of XFS with any immune tests.

In summary, the majority of these clinical trials demonstrate only limited evidence that a Chinese herbal formula taken by mouth may provide benefit for patients with eczema. Symptomatology of eczema can be alleviated, and topical corticosteroid usage may be spared with some of these herbal medications. Nevertheless, there is no evidence that these medications can cure the disease.

4. Current methods

Eczema is often considered as a skin disease than a systemic allergic disease [105]. Parallel to the usage of oral TCM, topical TCM applications have been widely used in China and Asia. Many of these products are adulterated with western anti-allergic medications including steroidal and/or non-steroidal anti-inflammatory/anti-allergic medications, and few have undergone randomized control trials [48].

Although topical administration of steroidal creams is commonly used for management of eczema, the use of topical administration of Chinese herbal medicines (usually in paste form) is not so popular. This may be due to the color and smell of the Chinese herbal medicines, as well as the difficulties in applying the paste. Recent advances in patch development have certainly improved the ease of administration. However, there are still certain issues with the topical administration of Chinese herbal medicines, such as the transdermal bioavailability, and the safety (allergy) aspects. Up till now, very limited information on the transdermal absorption or bioavailability of the use of Chinese herbal medicines and this certainly requires more research work. For example, the inclusion of enhancers in Chinese herbal formulae for increasing the transdermal delivery, the measurement of appropriate herbal chemical markers in the plasma/serum to demonstrate the absorption, and the use of artificial skin model for studying the toxicity and allergy of the herbal formulae.

Hon *et al.* previously evaluated a wet-wrap garment and found that it was effective in the short-term improvement of itching, disease severity and quality of life in children with AD [106]. The investigators further tested the concept of impregnating herbal ingredient by nanotechnology in a garment to be worn by children with AD during sleep. It is important to understand how to apply microencapsulation technology into textile engineering for drug delivery. Microencapsulation is defined as a technology of packaging solids, liquids or gaseous materials in miniature sealed capsules that can release their contents at controlled rates under specific conditions. Microencapsulation technique is an effective and long-lasting method for the release of encapsulated drugs, and has drawn increasing attention due to its potential applications in the fields of food and drug delivery systems, cosmetics and biomedical engineering [107-111]. Chitosan and Sodium alginate have been widely used for the preparation of microcapsules as wall materials due to their properties including non-toxicity, good biodegradable and high biocompatibility. Sodium alginate is a natural anionic polysaccharide derived from marine kelp, mainly the brown sea algae and is thus mainly used to form hydrogel nanoparticles or microcapsules [111-114]. Chitosan, the only naturally cationic polysaccharide, can diffuse into three-dimensional alginate gel network. Under simultaneous electrostatic interaction between protonated amino groups of chitosan and carboxyl groups of alginate, polyelectrolyte complex microcapsule membrane is formed [115-118]. In preparation of microcapsules

encapsulated herbal extract for clinical trial, it is necessary to develop an appropriate method for maximizing the herb load inside the microcapsules and increasing encapsulation efficiency. An orthogonal experimental design is used for determination of the optimum level of each variable in the process to design an appropriate method for microcapsules preparation. In a recent trial (unpublished data), Chitosan-sodium alginate (CSA) microcapsules loaded with herbal ingredient were prepared using emulsion-chemical cross-linking method [108,119] and the size distribution, surface morphology, release behavior, cytotoxicity and grafting performance onto cotton fabrics were investigated.

The CSA microcapsules not only had regular surface morphology and uniform particle size, but also displayed good controlled release of drug over a long period of time. FTIR spectrum indicates that PentaHerbs was successfully microencapsulated. Cytotoxicity test showed that CSA microcapsule did not have toxic effect on cells. In addition, the CSA microcapsule loaded with PentaHerbs was grafted onto the cotton fabrics successfully.

4.1 Process of impregnation of microencapsulated capsule into the cotton fiber

Chitosan/sodium alginate microcapsule (CSA) was prepared according to emulsion-chemical cross-linking method. A given mass of chitosan was dissolved in 25 ml of 2% (v/v) aqueous acetic acid solution until the solution was transparent. The same mass of sodium alginate was added to the 25 ml of Cortex Moutan powder solution and stirred mechanically until sodium alginate was dissolved completely. The mixture was stirred mechanically until sodium alginate was dissolved completely. Then chitosan acetic solution was added to the mixture and stirred mechanically until the system was homogenized. The mixture was then dropped into 150 ml of liquid paraffin solution containing 2% (w/v) of surfactant span-80 when the temperature reached 55°C, and agitated mechanically for 20 min in 1100 revolutions per minute (rpm). After that, the hot plate was switched off and the water bath removed. The mixture was cooled to below 10°C in ice bath surroundings. Then 5 ml of glutaraldehyde (25%, water solution) was further added dropwise to the mixture and stirred for another 60 min to stabilize the microcapsules through cross-linking reaction. The microcapsules from centrifuge separation were repeatedly washed three times with isopropyl alcohol, prior to lyophilization. The process chart of encapsulation is shown in Figure 1.

First, CSA microcapsules containing PentaHerbs were diluted with deionized water at the liquor ratio of 1:10. The solution was stirred for 10 min using a magnetic stir plate at a speed of 1200 rpm to obtain a better dispersion of microcapsules. Textile binder (Devan Chemicals) was used to enhance the fixation of CSA microcapsules onto the cotton fabrics (100%, 20 × 20 cm). The fabric samples were then immersed into the microcapsule solution followed by padding

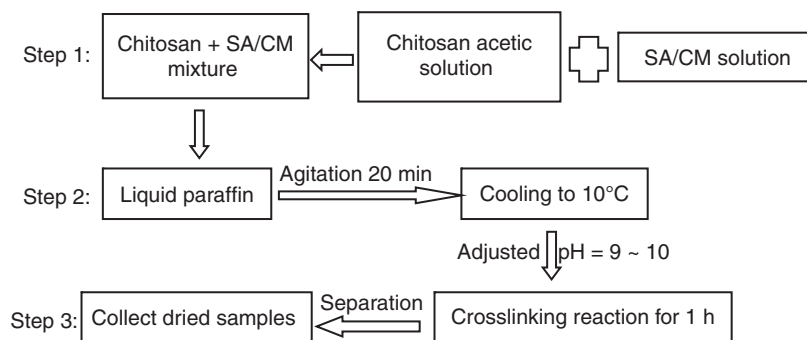


Figure 1. Process flow of encapsulation.

using a vertical padder at a constant pressure of 1.5 kg/cm^2 and a speed of 7.5 rpm. The wet uptake level of fabric samples was 70 – 80% followed by drying the fabric at 100°C in 2 min, then cooled to room temperature. The surface of samples was observed by scanning electron microscopy (SEM) and the SEM image of encapsulated capsules impregnated onto cotton fiber is shown in Figure 2.

The SEM results confirmed that microcapsules were successfully padded onto the fiber surface without obvious breakage during the padding process. The particle size of the CSA microcapsules ranged from $3 \mu\text{m}$ to $18 \mu\text{m}$ which was similar to that of the previous SEM evaluation. The Pentaherbs substance is released from the CSA microcapsules in the presence of humidity or due to the breakage of microcapsule wall shell during wear. A relatively small amount of water is sufficient to achieve the release because of the sensitivity of PentaHerbs to moisture, such as the water and sweat present on the surface of the skin. Hence, the released drug may be directly absorbed by the skin and, therefore, is against the disease, and the clinical use for AD treatment.

4.2 Study of the released pattern of the encapsulated drug from the cotton fiber

4.2.1 Methodology

In order to study the controlled-release of Pentaherb from the microcapsules, gallic acid was selected as the indicator, among the numerous components. Two samples of chitosan microcapsule containing Pentaherb (5.0 mg) were dispersed into 5 ml of phosphate buffered saline (PBS) with different pH values (pH 5.0 and 5.4) and incubated at 37°C in a shaking water bath at 100 rpm for various time periods of up to 144 h (6 days). The microcapsules suspension was centrifuged periodically at 4000 rpm to collect the supernatant for gallic acid analysis, followed by re-suspension of microcapsules in a fresh PBS. The amounts of gallic acid released from the microcapsules were evaluated by a High Performance Liquid Chromatography (HPLC), Hewlett Packard Agilent 1000 series, equipped with G1329A ALS Autosampler and G1315A Diode Array Detector (Agilent Technologies).

Sample solution was injected into an Ultrasphere ODS column ($250 \times 4.6 \text{ mm}$ i.d., particle size $5 \mu\text{m}$) (Beckman Instrument, Inc., Fullerton, CA, USA). All solvents were pre-filtered with $0.45 \mu\text{m}$ Millipore filter disk (Millipore) and de-gassed. The mobile phase systems consisted of acetonitrile (A) and double distilled water/phosphoric acid (99.0/1.0; v/v) (B). The flow rate was 1.0 ml/min and the wavelength was set at 260 nm. Each sample ($10 \mu\text{l}$) of the collected supernatant was injected into the column after filtration through a $0.45 \mu\text{m}$ filter disk. The system was monitored by a computer equipped with 32 Karat Software (Beckman Instrument Inc., Fullerton, CA, USA) for data collection, integration and analysis.

4.2.2 Released performance

The release behavior of Pentaherbs from CSA microcapsules under two simulated human skin conditions (PBS, pH = 5.4 and 5.0) in different time intervals was monitored over 144 h (6 days) (Figure 3). The two release curves in Figure 3 representing two simulated human skin conditions indicate that Pentaherb was released from CSA microcapsules at a faster rate during the initial 24 h but the release rate dropped steadily and reached a constant value. The release percentage of Pentaherb reached approximately 80.9% and 56% during the initial phase of first 24 h and eventually achieved a constant level of 96% and 70% for pH = 5.0 and pH = 5.4 PBS, respectively.

Figure 3 also reveals that the rate of release of Pentaherbs from microcapsules in pH = 5.0 PBS is significantly higher than that in pH = 5.4 PBS over the same period which indicates that the release kinetics are influenced by pH values of PBS. That is to say, higher pH value of PBS impedes the release of Pentaherb from CSA microcapsules. In addition, Pentaherbs release reached equilibrium at 72 h in both pH = 5.0 and 5.4 PBS.

Based on the results of the release experiment, the controlled-release of Pentaherbs from microcapsules is at a uniform rate in the first 48 h, which is generally acceptable for clinical use.

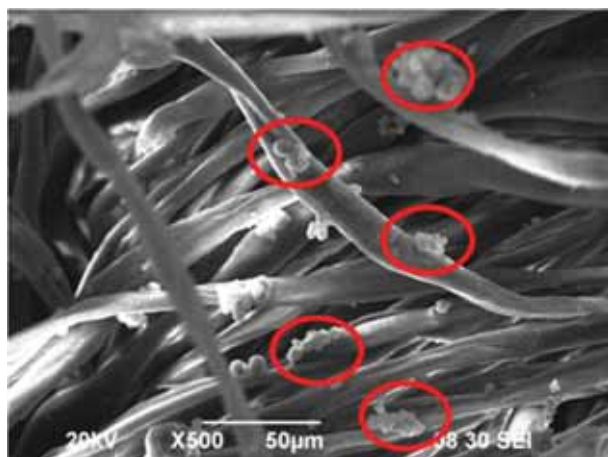


Figure 2. SEM image of impregnating microencapsulated capsule into the cotton fiber.

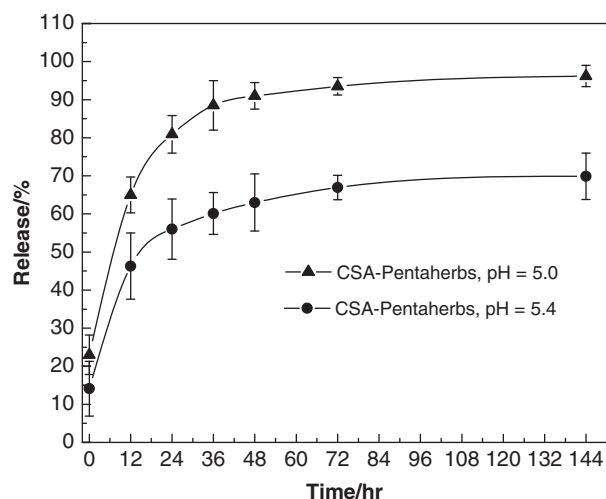


Figure 3. Release profiles of Pentaherbs (using gallic acid as marker) from encapsulated microcapsules immersed in PBS of pH = 5.0 and pH = 5.4.

In a preliminary trial (unpublished data), the TCM-garment was worn by 10 patients during sleep. There was no apparent improvement of AD severity in these patients. Further modifications of these garments are necessary to enhance patient acceptability. Nevertheless, the microencapsulation concept with TCM on garment provides an innovative concept TCM delivery.

For both topical and systemic administration of medications, most of the clinical trials have relied primarily on documenting clinical effects such as disease severity, quality of life, skin biophysiology or steroid sparing effects [28,88,89,106,120]. Very few of these clinical trials would document the anti-allergic effects of the trial medications. The studies by Leung and Chan *et al.* have demonstrated that the immunomodulating

effects of a trial medication should be objectively evaluated in the current methodology of drug evaluation [91,92]. Hon *et al.* proposed that documentation of therapeutic effects should be performed as thoroughly as possible using clinical methodology as well as immune or seromarkers of disease activities [28,88,89].

5. Future strategies

A number of oral and topical traditional Chinese herbal formulae have been used for the treatment of eczema [47,121]. However, systematic reviews have not demonstrated consistent clinical efficacy [96]. A recent Cochrane review by Gu *et al.* included 28 studies of herbal medicine for eczema; all were randomized controlled parallel clinical studies with oral or topical applications of Chinese herbal medicine (CHM) for eczema. To date, there was limited evidence that a Chinese herbal formula taken by mouth may provide benefit for children or adults with eczema [47]. There was no conclusive evidence that other Chinese herbs or Chinese herbal formulae used orally improved the condition, nor convincing evidence that topical application of CHM was of benefit to children or adults with eczema. The toxicity of CHM should also be studied in future research. CHM research for eczema should recruit participants with similar age group or severity to minimize heterogeneous outcomes within the study. Stratified randomization is recommended if the study recruits both children and adults. In addition, allocation concealment should be done to ensure that the participants are selected into two groups by chance rather than by error. Numbers of participants being randomized should be balanced in two groups. Methods for randomization must be clearly described on the published paper. Blinding method should always be used because outcome measures for eczema are subjective in the majority of cases. An open labeled design study could lead to risks of bias for outcome assessment. Quality control and quality assurance of Chinese herbs to be investigated should be vigorously performed prior to the conduct of clinical trials [101]. Assessment of effectiveness of the intervention should rely on data from both objective and subjective outcome measures assessed at baseline and at the endpoint of the trial [88,89]. Using published and validated scoring system as a primary outcome measure may help to achieve this and continuous data should always be reported or provided on request. Adverse events should be adequately reported. Liver and renal function tests should be used as one of the safety parameters in a RCT. Toxicity of CHM should be well addressed. In summary, Experts in the Cochrane reviews have suggested that well-designed, adequately powered trials are needed to evaluate the efficacy and safety of CHM for managing eczema [96,122]. There are great opportunities for application of patents on development of novel anti-allergic compounds and modernization of CHM in China [49]. Physicians should not be afraid to subject ancestral herbal formulae to bench and clinical trials.

6. Expert opinion

The delivery of anti-allergic herbal medicine orally or topically offers new research opportunities. Eczema is a chronic relapsing dermatitis associated with allergy/atopy. It is a systemic disease with skin manifestations during childhood but subsequent 'march' to airway atopies [30,105]. There is no cure by western medicine and many parents and patients resort to oral or topical herbal medicine usage. Even though western medicine is available to manage the disease, phobias (such as steroid and antibiotic phobias) and fallacies limit their efficacy [123]. Conversely, many patients in China and Asia accept the principles of herbal medicine and hence good compliance to herbal medicine usage is likely. Herbal medicine has been demonstrated to possess anti-allergic properties. Nevertheless, the major hurdle is that there have been no means to standardize delivery of the herbal ingredients either orally or topically. Worse still, the quality of trials on herbal medicine has largely been flawed. Fortunately, Good Manufacturing Practice (GMP) Regulations have been established in the manufacture of herbal products to ensure that the products are not adulterated with western medicine, heavy metals or bacterial contamination. There is a need, or rather,

an opportunity for more well-design randomized control trials in herbal medicine research for eczema. Novel drug discovery strategies for eczema should include objective scientific documentation of drug efficacy and adverse effects; and evaluation of the anti-allergic effects of herbal concoctions. Targeting mechanisms can be broadly against allergic processes, or specifically against individual humoral- or cell-mediate hypersensitivity pathways. Nanotechnology in the delivery of topical herbal ingredient represents a novel and innovative future. Combined with research tools for evaluation of clinical outcome measures for disease severity, pruritus, sleep disturbance, quality of life, steroid-sparing effects, skin biophysiology parameters, and serum immune markers offer accurate, objective and unbiased evaluations. Many of these evaluation methodologies are non-invasive and already well established.

Declaration of interest

The authors declare they have involved in TCM trials. They have also received a Hong Kong Government Health & Health Service Research Fund (HHSRF: 02030381) and an Innovation and Technology Fund (ITF) of Hong Kong (ITS/409/09) in the studying of TCM for eczema.

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