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Letter to the Editor

Inhibitory effects of *Ganoderma lucidum* pharmacopuncture on atopic dermatitis induced by capsaicin in rats

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Atopic dermatitis (AD) is a chronic relapsing inflammatory skin disease with a worldwide prevalence, and causes itch-scratch cycles that injure skin and exacerbate problems [1]. The diagnostic criteria in the pathophysiology of many atopic skin diseases have been established, and include, the hyperproduction of immunoglobulin E (IgE) [2], the infiltration of mast cells [2], and decreased expressions of keratinocytes differentiation markers (filaggrin, involucrin) [3]. In addition, AD is characterized by poorly defined erythema with edema, vesicles, and weeping in the acute stage and by skin thickening in the chronic stage [4]. However, its pathology has not been fully clarified. For a long time, it was often poorly dominated by currently available medications, in the case of long-term use of steroids, which are a favored treatment for AD, however, abuse and the presence of many side effects exist [5]. Thus, the development of effective and safe therapies for AD is required.

Recently, the efficacy of electroacupuncture as a remedy for capsaicin-induced model of AD in rats has been proved [6]. Moreover, the well known acupoints, LI11 (Gokji) and ST36 (Joksamli) have been established to be involved in the alleviation of AD [6].

Pharmacopuncture, a recent innovation of traditional acupuncture, is based on two major traditional Asian medical theories, meridian and herbal medicine. It is introduced to combine the benefits of acupuncture and herbal medicines, and legally considered an accepted alternative medical practice in Asian countries [7]. Furthermore, it has been reported that *Rehmanniae radix* pharmacopuncture has anti-allergic and anti-inflammatory effects on AD in mice [8]. Most importantly, the methanol extract of *Ganoderma lucidum* (hereafter referred to as GL extract), has been reported to have inhibitory effects on acute itch-associated responses in mice [9].

Thus, in the study, we asked whether *G. lucidum* pharmacopuncture (GLP) at bilateral LI11 and ST36 acupoints suppresses AD-related symptoms.

AD-like lesions were induced by subcutaneously injecting 10 μ l/g body weight of capsaicin (50 mg/kg), into rat pups within 48 h of birth [10]. GL pileus were washed, cut into pieces, and submerged in 4l of 25% alcohol for 10 h at room temperature. The alcohol extract so obtained was condensed using a rotary

evaporator to 500 ml, filtered at 0.22 μ m, heat sterilized, and stored under the temperature of 20 °C. Before administration, the extract was dissolved in ethanol and diluted with 5% dextrose water (DW) to keep a GL extract concentration of 10% (w/v). GLP therapy was performed at acupoints (LI11, ST36) by slowly injecting GL extract (50 μ l/60 g body weight). The depth of needle insertion was about 5 mm in LI11 and 10 mm in ST36.

New-born male rats were randomly divided into four groups: NAIVE group, the CAP (capsaicin) group, the CAP+DW group, or CAP+GLP group. Animals in the CAP, CAP+DW, and CAP+GLP groups were administered capsaicin as neonate. Animals in NAIVE group were not treated with capsaicin. On alternate days from postnatal day 21 (PND 21) to 42, animals in the CAP+GLP group were administered GLP, whereas animals in the positive control group, named as CAP+DW group, were administered DW rather than GL. Animals in the CAP group were administered capsaicin only.

The reduction of itching, and thus, scratching, is the most effective therapeutic strategy for improving the quality of life of AD patients. Once a week from the PND 21 to 42, scratching behavior was evaluated by video-recording for 1 h. On the other hand, dermatitis score, represents the accumulated effects of scratching, and thus, provides a more objective evaluation criterion than scratching behavior. Severities of dermatitis were evaluated weekly. Cutaneous lesions were inspected and assessed by scoring as described previously [10]. Summed scores of three different regions (face, ears, and back) are referred to as final dermatitis scores. On PND 21, mild dermatitis appeared in the CAP+DW and CAP groups, and became more severe with time, and peaked at PND 35. Dermatitis scores were consistently lower from PND 28 to 42 in the CAP+GLP group than in the CAP+DW or CAP group (Fig. 1B). Scratching behaviors followed similar patterns, but a significant difference between the CAP+GLP and CAP groups was only observed at PND 42, that is, after 3 weeks of GLP treatment, suggesting GLP had a cumulative effect (Fig. 1A).

Body weights were measured on alternate days from PND 21 to 42. The body weights of all experimental rats increased with age. No significant difference was found up to PND 42 (Fig. 1C), which elucidated that GLP application did not show evident adverse effects in the rats.

On PND 43, that is, immediately after GLP, skin tissues and blood samples were obtained from rats per group under anesthesia. Histopathologic changes of skin were assessed using hematoxylin and eosin (HE) and toluidine blue (TB) stainings. And changes in epidermal differentiation marker protein expressions were also assessed using immunofluorescence (IF). Serum IgE levels were quantified using the Rat IgE ELISA quantitation kit. The CAP and CAP+DW groups showed significant histopathological changes, such as, epidermal hyperplasia, hyperkeratosis (Fig. 2A,B,D,E), increased numbers of infiltrating mast cells (Fig. 2B,C), and no

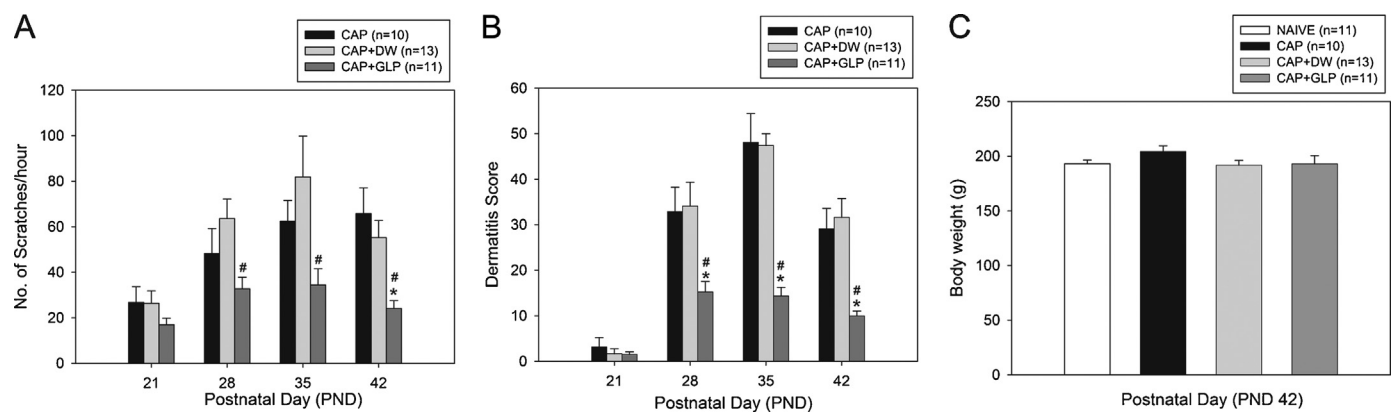


Fig. 1. Effect of GLP on spontaneous scratching behavior and atopic dermatitis-like skin lesions induced by neonatal capsaicin treatment. GLP was applied every other day from PND 21 to 42. The effect of GLP on scratching behavior (A) and skin lesions (B). * $P < 0.05$, the CAP+GLP vs. the CAP group, # $P < 0.05$, the CAP+GLP vs. CAP+DW group, One way ANOVA. (C) Effect of GLP on body weight on PND 42. No difference was observed between the NAIVE, CAP, CAP+DW, and CAP+GLP groups. Data are presented as means $\pm$ SEMs. The statistical analysis was carried out using SigmaStat version 11.0 (Systat Software, San Jose, CA, USA).

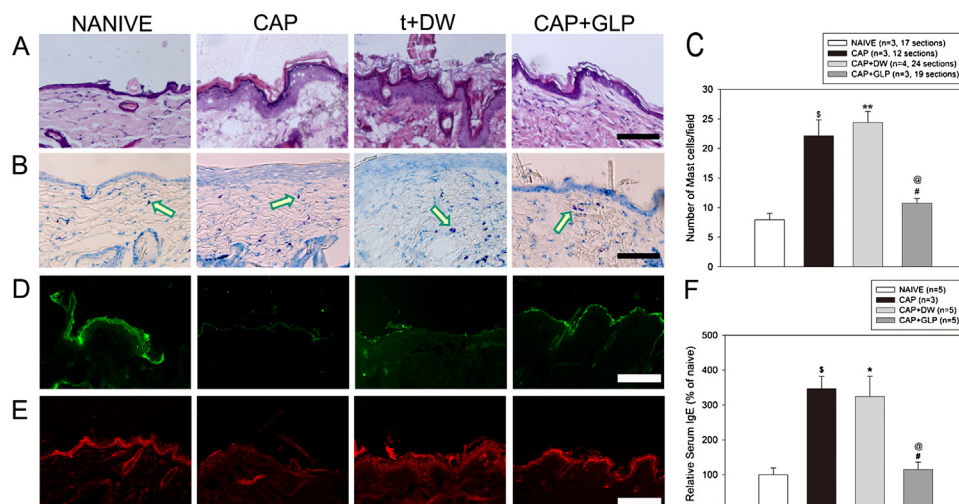


Fig. 2. Effect of GLP on morphological changes (A–C) and immunofluorescent results (D & E) of rostral back skin as well as the serum IgE levels (F) at PND 43 after neonatal treatment with capsaicin. Hematoxylin and eosin (HE) (A) and toluidine blue (TB) (B) staining of skin samples. Compared to naïve rats, histopathologic changes, such as epidermal hyperplasia, hyperkeratosis and increased number of infiltrating mast cells, were readily detectable in the CAP and CAP+DW groups. However, these changes were relieved by GLP treatment. Arrows indicate mast cells. Scale bar = 100 μ m. (C) Changes in mast cell numbers. \$ $P < 0.05$, CAP vs. NAIVE group; ** $P < 0.001$, CAP+DW vs. NAIVE group; # $P < 0.05$, CAP+GLP vs. CAP+DW group; @ $P < 0.05$, CAP+GLP vs. CAP group; One way ANOVA. Mast cells were counted in microscopic fields at a magnification of 10 $\times$. Representative images immunofluorescence labeling of involucrin (D) and filaggrin (E) (both markers of keratinocyte differentiation). Reductions in the expressions of epidermal involucrin (D) and filaggrin (E), and histopathologic changes were readily observed in CAP and CAP+DW groups. The expressions of involucrin and filaggrin were greater in the CAP+GLP group than in the CAP and CAP+DW groups. Scale bar = 100 μ m. (F) Serum IgE levels in the various groups were plotted as percentages of level in the naïve groups. Serum IgE levels were greater in the CAP and CAP+DW groups than in the naïve group. Furthermore, the hyperproduction of serum IgE observed in the CAP and CAP+DW groups was decreased by GLP. \$ $P < 0.05$, CAP vs. NAIVE group; * $P < 0.05$, CAP+DW vs. NAIVE group; # $P < 0.05$, CAP+GLP vs. CAP+DW group; @ $P < 0.05$, CAP+GLP vs. CAP group; One way ANOVA. Data are presented as means $\pm$ SEMs. The statistical analysis was carried out using SigmaStat version 11.0 (Systat Software, San Jose, CA, USA).

immunoreactivity for epidermal involucrin (Fig. 2D) or filaggrin (Fig. 2E). However, these changes were relieved by GLP treatment. In addition, dramatic increases in serum IgE levels relative to naïve group (set at 100 %) in the CAP and CAP+DW groups were also decreased as a result of GLP treatment (Fig. 2F).

In capsaicin induced AD model, EA did not induced neither immunologic nor histologic changes [6], but the changes were induced as a result of GLP on LI11 and ST36 acupoints. The data implies that GLP has more advantage in management of AD, although the locations of injected acupoints may not be the same in human.

In conclusion, the study shows that GLP ameliorates the capsaicin-induced atopic dermatitis-like phenotype in rats, and suggests a new therapeutic approach to reduce the symptoms of AD. Future work should be conducted to determine the mechanism underlying the effects of GLP on AD.

Conflict of interest

The authors have no conflict of interest to declare.

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