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PII: S2005-2901(17)30014-6

DOI: 10.1016/j.jams.2017.06.004

Reference: JAMS 357

To appear in: *Journal of Acupuncture and Meridian Studies*

Received Date: 15 February 2017

Revised Date: 3 June 2017

Accepted Date: 6 June 2017

Please cite this article as: Zhong Y, Liu X, Li Y, Liang H, Li J, Chen C, Li K, Liu X, Zhong S, Li L, Wang Y, Effects of Acupuncture on 1-chloro-2,4-dinitrochlorobenzene Induced Allergic Contact Dermatitis in Mice, *Journal of Acupuncture and Meridian Studies* (2017), doi: 10.1016/j.jams.2017.06.004.

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Effects of Acupuncture on 1-chloro-2,4-dinitrochlorobenzene Induced Allergic Contact Dermatitis in Mice

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Abstract

Allergic contact dermatitis (ACD) is a chronic inflammatory skin disease. Topical corticosteroids are the first-line therapy for ACD although their significant adverse effects. Acupuncture has been widely used in the treatment of various skin diseases, but its underlying mechanism remains unrevealed. In this study, we investigated the characteristics of acupuncture treatment based on effectiveness and mechanism. BALB/c mice received 1-chloro-2,4-dinitrobenzene (DNCB) application to build AD like model. Results showed that acupuncture treatment was a effective method in inhibiting inflammatory conditions, serum IgE levels, and expression of proinflammatory cytokineTh2 (*IL-4*, *IL-6*), Th2 (*IL-1β*,*TNF-α*) mRNA compared with DNCB group. Acupuncture treatment also inhibited NF-κB p65, phosphorylation of IκBα, and phosphorylation of occludin proteins expression. In addition, acupuncture treatment could improve EGF expression both in mRNA and protein levels. These results suggest that acupuncture, as an alternative therapy treatment for its no significant side effects, was effective in alleviating allergic contact dermatitis by reducing pro-inflammatory cytokines and changing proteins expression.

Keywords: Acupuncture; DNCB; Atopic Dermatitis; Mice

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1. Introduction

Allergic contact dermatitis (ACD) is a paradigmatic genetically complex disease involving in gene-gene and gene-environment interactions. Both skin barrier defects and aberrant immune responses are believed to drive cutaneous inflammation in ACD. The prevalence of ACD has increased 2-3 folds during the last century, particularly in industrialized countries[1,2].

Corticosteroids, such as mometasone, have been the mainstay of therapy for ocular inflammatory diseases. Their temporary effective anti-inflammatory effects are often accompanied with numerous of adverse effects including skin atrophy, characterized by a profound loss in skin thickness and elasticity combined with decreased barrier function [3,4]. Acupuncture is an ancient medical technic of China that can be traced back at least 2500 years[5].It is now widely used as a complementary and alternative medicine in many countries. Acupuncture is becoming a popular way to modulate diverse immune disorders because it can be used long-term with almost no sideeffects [6].In several studies, acupuncture has been widely used in the treatment of pain, wounds, and various skin diseases such as inflammation [7,8].However, acupuncture's mechanism ofaction remains poorly understood.

NF-κB is a transcription factor that binds to promoters of many pro-inflammatory mediators and is considered to be a crucial regulator of inflammatory responses [9,10].NF-κB dimers are kept inactive in the cytoplasm through association with IκBproteins, thus stimulating activation of the IκB kinase complex,leading to phosphorylation, ubiquitination and degradation of IκB proteins. Released NF-κB dimers translocate to the nucleus, binding specific DNA sequences and promote transcription of target genes, whereas some pro-inflammatory cytokines drive activation of NF-κB in turn [11-14].

Epidermal growth factor (EGF) has biologically active polypeptides comprising 53 different amino acids which are involved in cell growth, differentiation, proliferation, metabolism and skin regeneration [15,16].EGF has been linked to ACD

therapy, NF-κB signaling and the phosphorylation on Tyrosine residues of occludin which is required for its assembly into tight junctions.

Recent studies demonstrated that, as an integral part,NF-κB, EGF and occludin are required for skin barrier balance [17].In this study, therapeutic effects of acupuncture andmometasone were analyzed. This research revealed an advantage of acupuncture for skin-barrier protection and faster skinregeneration, using a 1-chloro-2,4-dinitrobenzene (DNCB)- induced mice model. This research also showed that acupuncture, combined with mometasone, could serve as the besteffective therapy for ACD.

2.Materials and methods

2.1 Reagents

1-chloro-2,4-dinitrobenzene (DNCB) was purchased from Sigma-Aldrich (Sigma St. Louis, MO, USA). Other reagents were purchased from Sinopharm Chemical Reagent Co. Ltd., China, unless otherwise specified.

Animals and Treatment

Adult female BALB/c mice were purchased from the animal center of Guangzhou University of Chinese Medicine.Both animal care and the study protocol were conducted according to the guidelines of the Committee on Care and Use ofLaboratory Animals of the animal center of Guangzhou University of Chinese Medicine. The mice were maintained for 7 days in pathogen-free conditions before the start of the experiment. Mice were kept at a constant temperature (23°C) andhumidity (55%), with a 12-hour light/dark cycle, and theywere provided with a laboratory diet and water *ad libitum*.

After the 10-day adaptation period, mice were randomly divided into 6 groups (each, n=10). Control group: mice without any stimulus and painted with phosphate buffer solution (PBS, pH7.4); DNCB group: mice sensitized with DNCB and painted with PBS. N-MP group: mice sensitized with DNCB and acupuncture at non-meridian point. MP group : mice sensitized with DNCB and acupuncture at meridian point

(BL17 (geshu), BL20 (pi shu) and ST36 (zusanli)) for 10 minutes every other day (Fig. 1). MO group: mice sensitized with DNCB and painted with mometasone on each dorsal skin every other day. MM group: mice sensitized with DNCB and painted with mometasone at once after acupuncture every other day.

For the induction of allergic contact dermatitis[18], the surfaces of bothdorsal skin of mice were stripped with an electric razor (Nichiban, Japan). After stripping, 20 μl of 1% 2, 4-dinitrochlorobenzene (DNCB) (Sigma Aldrich, St Louis, MO, USA) dissolved in acetone/olive oil solution (acetone : olive oil = 1:3) was painted on each shaved dorsal skin once a day for 1 week (days 1-7), and followed by a period of 0.5% DNCB applied repeatedly to the dorsal skin every other day for 3 weeks (days 8-28) (Fig. 2).In the second challenge, the MP, MO and MM groups were treated with acupuncture or mometasone 3 h prior to the application of 0.5% DNCB (days 8-28). Samples were collected and then fixed with 10% neutral buffered formalin solution or freezed in liquid nitrogen for histopathologic examinations, serum analysis, and expression pattern analysis on day 28 of the experiment.

2.3 Measurement of Scratch Times and Clinical Score

For scratch timesmeasurement,behaviors of mice were monitored according to observation methodology of Kobayashietal [19].The frequency of scratching occurring on facial or dorsal skins was counted with a 30-min visual observation on the first,s the 14st and the 28st day after DNCB application.

Forclinical scoremeasurement, all mice were photographed to show the clinical symptoms on the first, the 14st and the 28st day after DNCB application. Clinical symptoms of each mouse were evaluated as previously described [11]. Briefly, erythema, edema, excoriation and dryness on the dorsal surface was scored as 0 (not visible), 1 (mild), 2 (moderate), 3 (severe). Scoring was performed by three independent observers.

2.4 Histological Study

The fixed dorsal tissues were embedded in paraffin blocks, tissue sections (4-6 μm) were mounted on slides,deparaffinized with xylene, rehydrated through graded

alcohols, and stained with hematoxylin and eosin. Finally, observed with a Nikon Eclipse E600FN microscope (Nikon Instruments Inc., Melville, USA) at a fixed magnification of 200 X.

2.5 Measurement of IgE levels in Serum

Serum IgE levels were analyzed with mouse IgG ELISA kit (BETHYL, Montgomery, TX, USA) by following the manufacturer's protocol. Antigen specific IgE levels were indicated by OD value. Mean absorbance of antigen coated well minus mean absorbance of non-coated well was used as the OD value of the mite specific IgE levels.

2.6 Detection of mRNA Expression by Reverse Transcription Polymerase Chain Reaction (RT-PCR)

To determine genes expression in the dorsal skin, RT-PCR (Teal Time-PCR) was performed. Total RNA was extracted from dorsal skin using RNA Extraction Kit (Invitrogen) according to manufacturer's instructions. Concentration of total RNA was quantified by measuring the absorbance at 260 nm. One and half microgram of total RNA was used for cDNA synthesis using oligodT primers and Super-Script II reverse transcriptase (Invitrogen), and subsequently diluted with nuclease-free water to 10 ng/uLcDNA. qPCR was performed utilizing hot-start SYBR-green based method (Invitrogen). Gene fold changes were determined by utilizing $2^{-\Delta\Delta Ct}$ method. DNA was amplified withan initial denaturation at 94°C for 4 min, followed by 40 cycles of 94°C (15 s) and 60°C (15 s). Primers were exhibited in Table 1. All experiments were performed in triplicate and repeated twice.

2.7 Detection of Protein Expression by Western Blot Analysis

Western blotting was performed to study protein expression. Total protein was separated from each sample by electrophoresis on a 12% SDS-PAGE polyacrylamide gel and electrophoretically transferred onto polyvinylidene fluoride membranes (PVDF) (Bio-Rad Laboratories, Berkeley, California). The immunoblot was incubated overnight in blocking solution (5% skim milk) at 4°C followed by incubation with

primary antibody. Then the membranes were washed two times using 1x PBS and incubated with HRP-conjugated secondary antibodies (Santa Cruz Biotechnology, CA, USA) for 1-2 h. The proteins were then visualized using an enhanced chemiluminescence (ECL) detection reagent (Amersham Pharmacia, Piscataway, NJ, USA). The relative band density was determined using a computerized densitometry system and normalized to the β-actin signal from a blot developed under similar conditions.

2.8 Statistical Analysis

The data are presented as the mean ± standard and analyzed using Statistical Package for the Social Sciences (SPSS) 17.0 statistical software (SPSS Inc., Chicago, IL). Comparison among the groups was carried out by One-way ANOVA. The comparison between two groups was carried out by LSD. $P<0.05$ was considered as statistically significant differences.

3. Results

3.1 Effects of Acupuncture on the Symptoms of Experimental AllergicContact Dermatitis

First, it was determined if acupuncture treatment affectedsymptoms of experimental ACD. Results showed that the ACD symptoms of MM group, MP group and MO group were significantly alleviated compared with DNCB and N-MP group (Fig. 3A) including scratching frequency (Fig. 3B) and clinical scores (table 2). In addition, the scratching frequency of MM group was statistically significant lower than MO group. Interestingly, MO group and MM group, treatment containing mometasone, were hyperpigmented patches and hardly any furcovering. In the MM group, acupuncture treatment couldnot ease the ACD symptoms although there was a littleimprovement.

3.2 Effects of Acupuncture on Histopathological Changes

To explore the visual evaluation of ACD symptomsfurther, histological analysis on the dorsal skins were performed by microscope. The epidermis and dermis in the

DNCB and N-MP groups showed strong edema and hyperplasia, as wellas a massive infiltration of inflammatory cells (Fig. 4). The MO and MM groups had significantly reduced numbers ofinfiltrated immune cells and thickness of the epidermiscompared with the DNCB and N-MP groups.

3.3Effects of Acupuncture on Serum IgE levels

To further determine if suppression of ACD progressionwas associated with serum IgE levels, total serum IgE levels were measured. Compared to the control group, IgE levelswere increased significantly in the DNCB and N-MP groups(Fig. 5). Furthermore, IgE levels of all the treatment groupswere significantly decreased, compared with the DNCB andN-MP groups. In addition, IgE levels of the MM group werelower, compared to the MO group.

3.4 Effects of Acupuncture on Cytokine Expression levels

Furthermore, the current authors wondered if proinflammatory cytokines were involved in suppression of ACDprogression. Pathogenic cytokine expression levels weredetected using an RT-PCR method. The data indicated thatexpression of interleukin *(IL)-4*, *IL-6*, tumor necrosis factor *(TNF)-α* and *IL-1β* mRNA were induced by DNCB treatment (Fig. 6). *IL-4*, *TNF-α*, and *IL-1β* mRNA expression were downregulated in the dorsal skin of the MP, MM and MO-treated mice. In addition, the above cytokine mRNA in the MM group was significantly lower than that of the MO group.

3.5Effects of Acupuncture on the IκBα/NF-κB Pathway

To elucidate the underlying mechanism of inhibitory effect of different treatments on inflammatory reaction further, western blot analysis was performed. The data demonstrated thatcompared to the control group, all the treatedgroups showed suppression roles in the activation of NF-κB signaling (Fig. 7). However, the expression of NF-κB p65 and phosphorylation of IκB-α were weaker in the MP, MM, and MO groups than in the DNCB and N-MP groups. Inaddition, the MM group showed greater anti-inflammatoryefficacy than the MO group. These data indicate

that acupuncture plays an important role in the regulation of immune and inflammatory responses through inhibiting the NF-κB signaling pathway.

3.6 Effects of Acupuncture on the EGF and Occludin Expression

To investigate the anti-inflammatory mechanism of acupuncture treatment in ACD-like disorders, the effects of acupuncture on regulation of EGF and occludin expression were examined by RT-PCR and western blotting. The DNCB group could induce occludin expression (Fig. 8) and reducedEGF expression (Fig. 9) compared with the control group. Onthe contrary, acupuncture and mometasone enhanced EGF expression and decreased occludin expression, compared with the DNCB group. Of the three treatment groups, the MM group was the most effective for improving expression of EGF (Fig. 9) as well as inhibiting occludin.

4. Discussion

The skin is one of the most important and the largest mammalian organs. It serves as a barrier that protects from a wide variety of microbial, physical and chemical insults. It is also considered as a major factor in the innate host defense system. ACD is an inflammatory, chronically relapsing and intensely pruritic skin disease, and is caused by complex pathogenic factors including skin barrier dysfunction, bacterial infection, immune dysregulation genetic susceptibility and environment trigger.

IgE and cytokine expression have been known to cause both acute- and chronic-phase skin inflammations that are often associated with ACD [20]. Although generalized Th2-deviated immune response is closely linked to ACD, the skin disease itself is a biphasic inflammation with an initial Th2 phase and while chronic lesions harbour Th0/Th1 cells [21]. Patients with ACD always have higher IgE levels and tend to secrete more IL-4 spontaneously. Previous studies have suggested that IL-4 and IL-13 impair expression and function of hBD2 and hBD3 in human epidermal keratinocytes, which might account for the increased susceptibility to skin infections seen in patients with ACD [22]. Likewise, in specimens with epidermal atrophy,

intense IL-6 expression was detected. However, plasma was not elevated from patientswith localized or systemic scleroderma, which suggests that IL-6 may be related to the pathophysiology of dermatologic diseases characterized by epidermal atrophy [23].

NF-κB is a heterodimeric transcription factor of the Rel family that usually resides in the cytosol in an inactive form bound to the endogenous inhibitor of NF-κB (IκB) family proteins. IκB kinase phosphorylate serine residues at the NH2-terminus of IκB during various inflammatory responses. The phosphorylated IκB is immediately ubiquitinated and degraded in the 26S proteasome, leading to release of NF-κB and its translocation to the nucleus. The association of this released NF-κB with its specific DNA binding sequences drives target genes and leads to cytokine production and cell proliferation. Growing evidences had demonstrated the role of NF-κB signaling in the immunological disturbance that was observed in ACD. Martin et al. reported that topical application with a new NF-κB Inhibitor improves ACD in NC/NgaTnd mice [24]. Christopher et al. explored the possibility of using topical NF-kκB decoy as a novel therapeutic alternative for targeting Th1/Th2-driven skin inflammation in experimental ACD [25].

As an essential part of skin regeneration growth factor, EGF supports cell renewal by assisting in the synthesis of proteins, increasing circulation, metabolism, mitosis, cell growth, differentiation and blood-vessel formation [26]. It was reported that chronic skin damage induced by DNCB as well as their frequently scratch displayed a down-regulation expression of EGF and its mRNA in mice [15]. Down-regulation of EGF and a mis-localization of EGF receptor in the cytoplasm of keratinocytes probably contribute to an inhibition of epithelialization in chronic skin damage. Conversely, it appears to differ in acute skin damage. Present study showed that the healing process for ACD is satisfactory in the PPI group which a severe EGF immunohistochemical reaction could be observed [27]. Moreover, a previous non-clinical study concluded that the EGF vaccination in mice decreased the normal croton-oil-induced inflammation response without apparent impairment in tissue

healing [28]. EGF-dependent signaling pathways that facilitate cell growth and re-epithelisation via binding to the EGF receptor localised through the entire epidermis [29].

Tight junctions (TJs) form a selective barrier to the diffusion of toxins, allergens, and pathogens from the external environment. As an important component of TJs, occludin plays an important role in the regulation of epithelial TJs [30]. Tyrosine phosphorylation of occludinon specific residues results in loss of its interaction with ZO-1 and therefore disassembly of TJs [31]. Furthermore, a recent in vitro study demonstrated that Tyr phosphorylation of the C-terminal region of occludin reduces its ability to interact with ZO-1 [32,33]. Hydrogen peroxide-induced barrier dysfunction was attenuated by pretreatment of cell monolayers with EGF [34]. In PKC-mediated protection of TJs by EGF was also demonstrated in Mz-Ch1 cell monolayers, a human cholangiocyte cell line [35].

In this study, we aimed to investigate the roles of IgE, cytokine, NF-κB, EGF and occludin in ACD using DNCB induced model in mice. The results revealed that compared with control group, serum IgE level and cytokine expression were increased significantly in DNCB group. In addition, IκBα/NF-κB pathway was inhibited in DNCB induced group including higher expression of NF-κB-p65 and phosphorylation of IκBα proteins than control group. Furthermore, we also detected increased occludin expression in DNCB induced model through western blotting analysis. The current data indicated that the dysregulated proteins might play a central role in theprogression and maintenance of ACD. Therefore, therapies focusing on changing expression of the above proteinsmight be beneficial for patients with ACD.

As the first-line therapy for ACD, the beneficial therapeutic effects of topical corticosteroids (mometasone) are often accompanied by numerous adverse effects including skin atrophy, characterized by a profound loss in skin thickness and elasticity combined with decreased barrier function. Acupuncture is an ancient medical technique of China that can be traced back at least 2500 years. It is now widely used as a complementary and alternative medicine in many countries [36, 37].

The therapeutic efficacy ofacupuncture—with hardly any adverse effects—for treatingACD has been proven in many studies especially with respect to reducing experimentally induced itching, allergeninduced basophil activation, and eczema in ACD.

Although acupuncture treatment has been increasingly used for ACD, with several clinical studies demonstrating the effectiveness of acupuncture [38], preclinical studies-especially *in vivo* studies-investigating the mechanism of acupuncture for treating ACD are lacking.

In this study, therapeutic effects of acupuncture and mometasone were compared, using a DNCB-induced micemodel. First, the ACD symptoms of the MM and MP groupswere alleviated significantly, compared with the DNCB andMO groups; these symptoms included scratching frequencyand clinical scores. Second, the MM and MO groups hadsignificantly reduced numbers of infiltrated immune cells andthickness of epidermis, compared with the DNCB and MPgroups. Third, serum IgE levels of the MM and MO groups were decreased significantly, compared with the DNCB andMO groups. These results indicate that acupuncture treatmentcould markedly reduce symptoms induced by DNCB and is arelatively effective method for curing ACD.

Then, an analysis was conducted to determine the effectsof acupuncture on cytokine, NF-κB, EGF and occluding expression that were key factors for the progression for ACD. Western blot analysis revealed that cytokine, NF-κB-p65, and phosphorylation of IκBα expression were significantly increased in the MM and MP groups, compared with the DNCB and MO groups. In addition, occludin expression was also increased in the MM and MPgroups. However, the MM and MP groups had EGF expression, compared with the DNCB and MO groups. Theseresults demonstrated that acupuncture was an effectivetherapy for ACD.

There were some novelty mentions in our study compared to the research of Park [39]. First of all, we chose ACD as a research model, however, atopic dermatitis was their research field. What's more, we investigated the effects of acupuncture on regulation of EGF and occludin expression by RT-PCR and western blotting, which was not involved in Park's study.

In conclusion, these experimental results suggest that acupuncture treatment had anti-inflammatory effects that were produced by inhibiting proinflammatory activities. Acupuncture maybe also accelerated skin regeneration and skin-barrierprotection though promoting EGF secretion and inhibiting Tyr phosphorylation of occludin. Long-term use of topicalcorticosteroids for skin inflammation poses risks of systemic and local side-effects. Therefore, the current study's results suggest that acupuncture combined with mometasone might be a better choice for ACD treatment, at least inmice. However, cautious and deeper research is indispensable to assess the effect of acupuncture on ACD treatment inhuman beings.

Disclosure statement

The authors affirm that they have no conflicts of interest orno financial interests related to the material of thismanuscript.

Acknowledgments

The authors would like to thank the staff of Scientific Research Department of Hebei University of Chinese Medicine and Guangzhou University of ChineseMedicine for technical support.This research was supported by: (1) China Postdoctoral Science Foundation (NO. 2015M572294); (2) Science and Technology Program of Guangdong Province (No. 2014A020210024); (3)Project of Education Department of Hebei Province (No. QN2014098); (4) Scientific Research Project of Hebei Provincial Administration of Traditional Chinese Medicine (No. 2016001).

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Figure captions

Figure 1 Body Acupoints for Acupuncture. Mice were treated at meridian point (geshu, pishu, zusanli) using for 10 minutes every other day.

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Figure 2 Study design for mice treatment. 1% 1-chloro-2,4- dinitrobenzene (DNCB) was painted on each shaved dorsal skin of mice once per day (d) for 1 week (days 0-7), and followed by a period of 0.5% DNCB applied repeatedly to the dorsal skin every other day for 3 weeks (days 7-28). In the second challenge, the MP, MM, and MO groups were treated with acupuncture or mometasone 3 hours prior to the application of 0.5% DNCB (days 7- 28). MP group: mice sensitized with DNCB and treated at meridian points. MO group: mice sensitized with DNCB and painted with mometasone. MM group: mice sensitized with DNCB and painted with mometasone at once after acupuncture every other day.

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Figure 3 Effects of acupuncture on the symptoms of experimental allergic contact dermatitis. (A) The allergic contact dermatitis symptoms of different groups. (B) Scratching frequency of different groups. Data are the mean ± standard error of the mean (n = 3), Each bar represents the mean of three independent experiments carried out in triplicate. DNCB [1-chloro-2,4-dinitrobenzene] group: mice sensitized with DNCB. N-MP group: mice sensitized with DNCB and treated at non-meridian points. MP group: mice sensitized with DNCB and treated at meridian points. MM group: mice sensitized with DNCB and painted with mometasone at once after acupuncture every other day. MO group: mice sensitized with DNCB and painted with mometasone. ■P < 0.05 versus DNCB group; ◆P < 0.05 versus N-MP group; #P < 0.05 versus MO group; ***P < 0.0001 versus control group; ■■ P < 0.001 versus DNCB group; ◆◆ P < 0.001 versus N-MP group; # # P < 0.001 versus MO group; min, minutes.

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Figure 4 Effects of acupuncture on histopathologic changes by hematoxylin and eosin staining of epidermal and dermal hyperplasia. (A) Control group. (B) DNCB [1-chloro-2,4-dinitrobenzene] group. (C) N-MP group. (D) MP group. (E) MM group. (F) MO group. DNCB group: Mice sensitized with DNCB. N-MP group: Mice sensitized with DNCB and treated at non-meridian points. MP group: mice sensitized with DNCB and treated at meridian points. MM group: mice sensitized with DNCB and painted with mometasone at once after acupuncture every other day. MO group: mice sensitized with DNCB and painted with mometasone.

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Figure 5 Effects of acupuncture on serum immunoglobulin (Ig)E levels. Data are the mean ± standard error of the mean (n = 3). Each bar represents the mean of three independent experiments carried out in triplicate. DNCB [1-chloro-2,4-dinitrobenzene] group: mice sensitized with DNCB. N-MP group: mice sensitized with DNCB and treated at non-meridian points. MP group: mice sensitized with DNCB and treated at meridian points. MM group: mice sensitized with DNCB and painted with mometasone at once after acupuncture every other day. MO group: mice sensitized with DNCB and painted with mometasone. * $P < 0.05$ versus control group; ■$P < 0.05$ versus DNCB group; ◆$P < 0.05$ versus N-MP group; # $P < 0.05$ versus MO group.

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Figure 6 Effects of acupuncture on cytokine expression levels. Data are the mean ± standard error of the mean (n = 3), Each bar represents the mean of three independent experiments carried out in triplicate. DNCB [1-chloro-2,4-dinitrobenzene] group: mice sensitized with DNCB. NMP group: mice sensitized with DNCB and treated at non-meridian points. MP group: mice sensitized with DNCB and treated at meridian points. MM group: mice sensitized with DNCB and painted with mometasone at once after acupuncture every other day. MO group: mice sensitized with DNCB and painted with mometasone.* $P < 0.05$ versus control group; ■$P < 0.05$ versus DNCB group; ◆$P < 0.05$versus NMP group; # $P < 0.05$ versus MO group. IL, interleukin; TNF, tumor necrosis factor.

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Figure 7 Effects of acupuncture on the IκBα/NF-κB pathway. (A&B) Western blots analysis of IκBα/NF-κB expression. (C&D) Western blots analysis of phosphorylated of IκB-α expression. Data are the mean±standard error of the mean (n = 3), Each bar represents the mean of three independent experiments carried out in triplicate. DNCB [1-chloro-2,4-dinitrobenzene] group: mice sensitized with DNCB. N-MP group: mice sensitized with DNCB and treated at non-meridian points. MP group: mice sensitized with DNCB and treated at meridian points. MM group: mice sensitized with DNCB and painted with mometasone at once after acupuncture every other day. MO group: mice sensitized with DNCB and painted with mometasone. * $P < 0.05$ versus control group; ■$P < 0.05$ versus DNCB group; ◆$P < 0.05$ versus N-MP group; # $P < 0.05$ versus MO group.

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Figure 8 Effects of acupuncture on occludin expression. (A&B) Western blots analysis of phosphorylation of occludin (Poccludin) expression. (C) P-occludin and occluding ratio. Data are the mean ± standard error of the mean (n = 3), Each bar represents the mean of three independent experiments carried out in triplicate. DNCB [1-chloro-2,4-dinitrobenzene] group: mice sensitized with DNCB. N-MP group: mice sensitized with DNCB and treated at non-meridian points. MP group: mice sensitized with DNCB and treated at meridian points. MM group: mice sensitized with DNCB and painted with mometasone at once after acupuncture every other day. MO group: mice sensitized with DNCB and painted with mometasone.* $P < 0.05$ versus control group; ■$P < 0.05$ versus DNCB group; ◆$P < 0.05$ versus N-MP group; #$P < 0.05$, versus MO group.

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Figure 9 Effects of acupuncture on epidermal growth factor (EGF) expression. (A) DNA quantitative analysis of EGF expression (B&C) Western blots analysis of EGF expression. Data are the mean± standard error of the mean (n = 3), Each bar represents the mean of three independent experiments carried out in triplicate. DNCB [1-chloro-2,4-dinitrobenzene] group: mice sensitized with DNCB. N-MP group: mice sensitized with DNCB and treated at non-meridian points. MP group: mice sensitized with DNCB and treated at meridian points. MM group: mice sensitized with DNCB and painted with mometasone at once after acupuncture every other day. MO group: mice sensitized with DNCB and painted with mometasone. * $P < 0.05$ versus control group; ■$P < 0.05$ versus DNCB group; ◆$P < 0.05$ versus N-MP group; #$P < 0.05$ versus MO group.

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Table 1. Primers used for Quantitative Real-Time PCR analysis

Gene	Name	Primers (5' to 3')
IL-4	IL-4-F	CCCTTAAGAGGGATGCTGCC
	IL-4-R	AGGACGTTTGGCACATCCAT
IL-6	IL-6- F	GGCTAAGGACCAAGACCATCC
	IL-6-R	GCACTAGGTTTGCCGAGTAGA
IL-1β	IL-1β- F	TGCCACCTTTTGACAGTGATG
	IL-1β- R	ATGTGCTGCTGCGAGATTTG
	TNFα-F	GATCGGTCCCCAAAGGGATG
TNFα	TNFα-R	GTGGTTTGTGAGTGTGAGGGT
EGF	EGF- F	CAGGAGGTCCGCTAGAGAAATG
	EGF- R	CAGAACAAATCCTGTGGGGC
GAPDH	GAPDH-F	CCCTTAAGAGGGATGCTGCC
	GAPDH-R	TACGGCCAAATCCGTTCACA

Primers were designed using Primer Express version 2.0 software. Primer specificity was confirmed using Primer-BLAST web software (National Centre for Biotechnology Information; PCR, polymerase chain reaction; IL, interleukin; TNF, tumor necrosis factor; EGF, epidermal growth factor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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Table 2. Effects of acupuncture on the clinical score in different groups

	Day after induction		
Group	*0 days*	*14 days*	*28 days*
Control	0	0	0
DNCB	0	4.5±0.31*	5.5±0.42*
N-MP	0	4.7±0.42*	5.8±0.44*
MP	0	3.8±0.38■◆#	3.5±0.32■◆#
MM	0	3.7±0.22■◆#	3.3±0.31■◆#
MO	0	4.2±0.31	4.1±0.28

Note: Symbols used in this table match those used in several figures. Clinical scores of all the mice were recorded on the 1st day, the 14th day, and the 28th day after DNCB application. Scoring was performed by 3 independent observers. *P<0.05 , versus control group; ■P<0.05, versus DNCB group; ◆P<0.05, versus N-MP group; # P<0.05 versus MO group. DCNB, 1-chloro-2,4-dinitrobenzene; N-MP, non-meridian points; MP, meridian points; MM, mometasone (immediately after acupuncture every other day); MO, mometasone (every other day).

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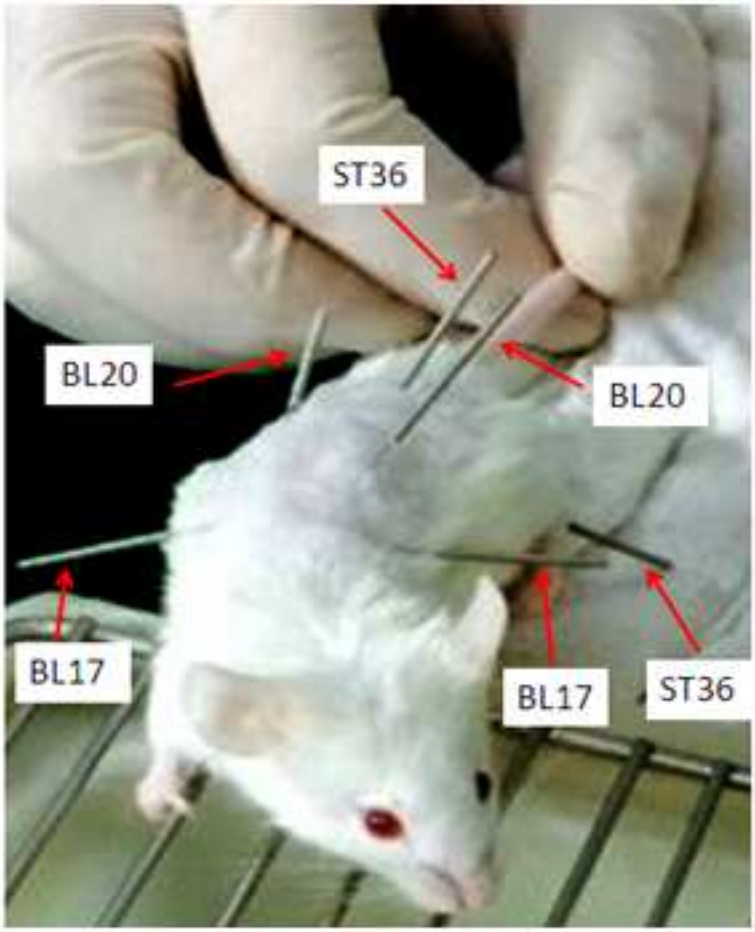

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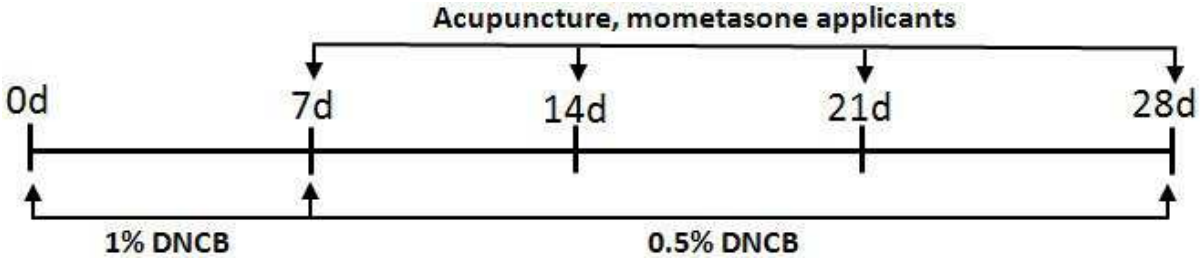

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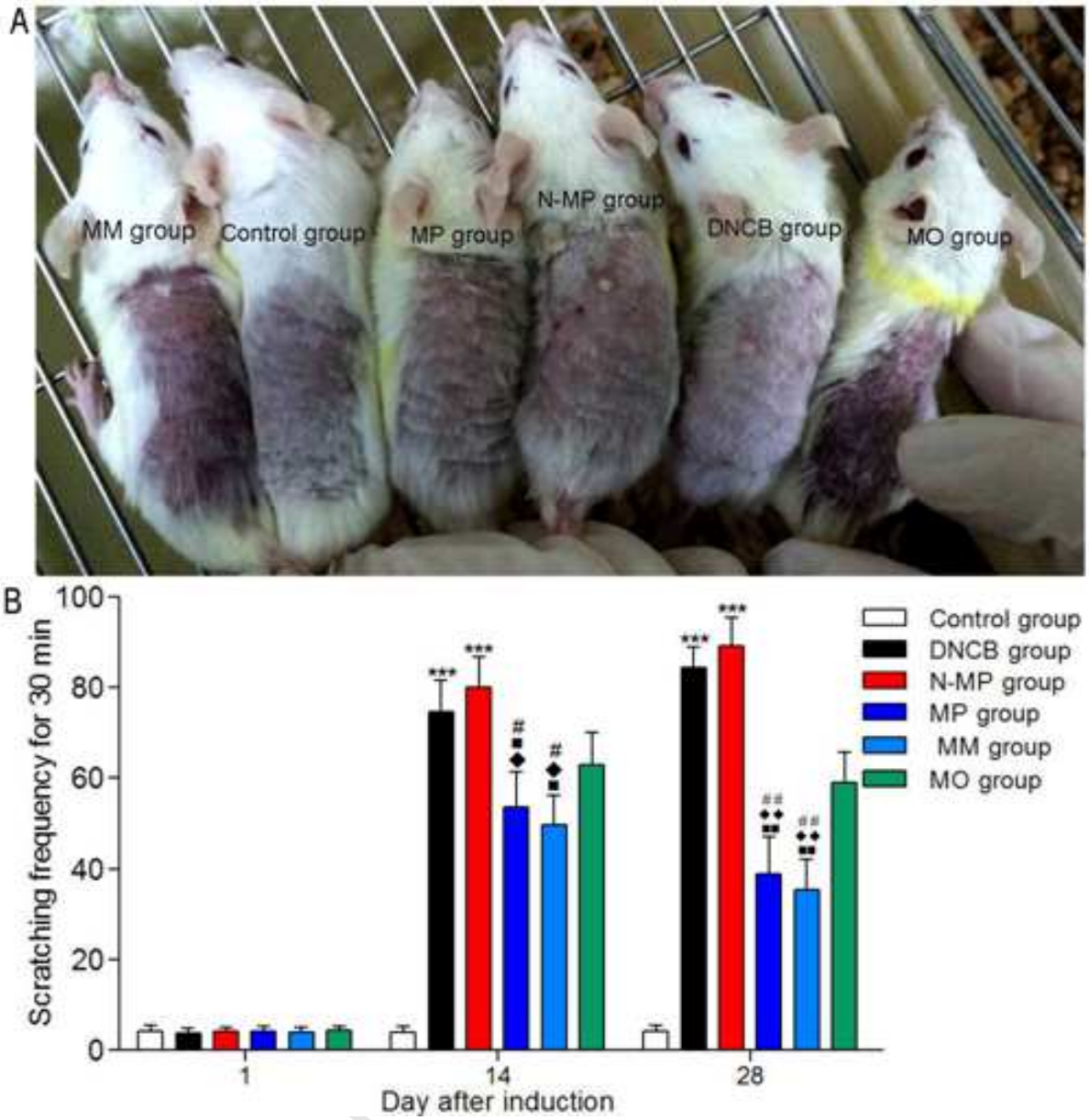
A
MM group
Control group
MP group
N-MP group
DNCB group
MO group
B
Scratching frequency for 30 min
100
80
60
40
20
0
1
14
28
Day after induction
Control group
DNCB group
N-MP group
MP group
MM group
MO group

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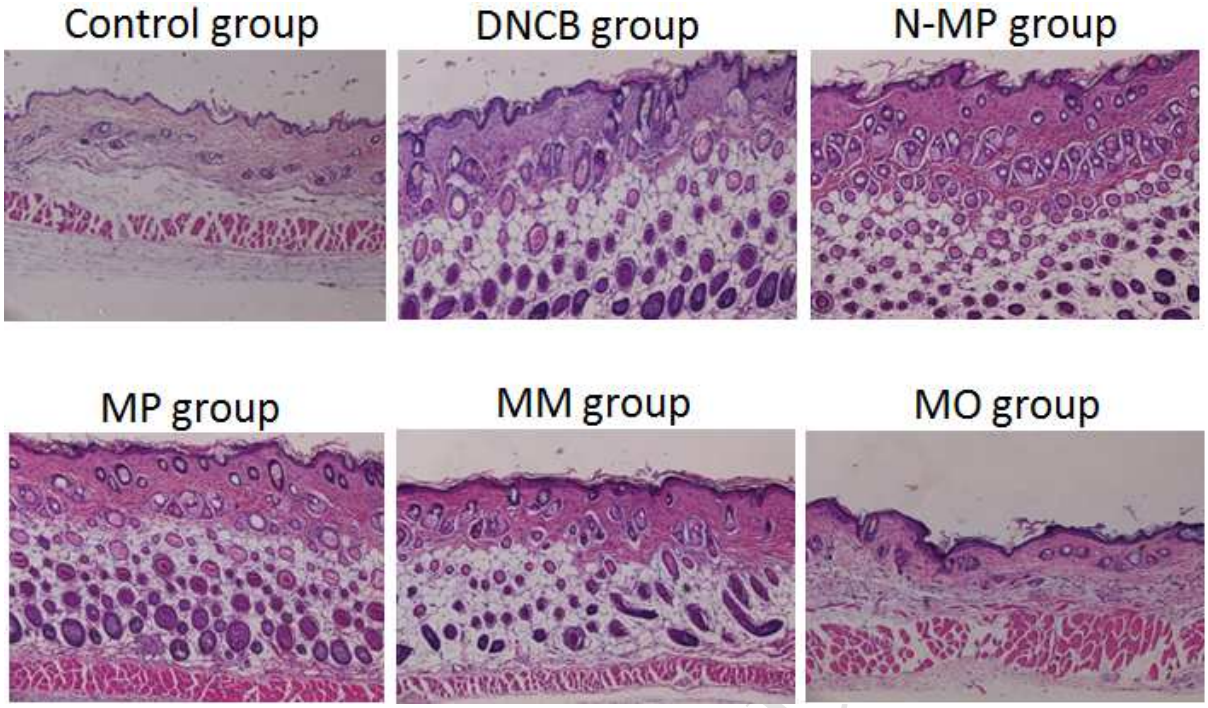

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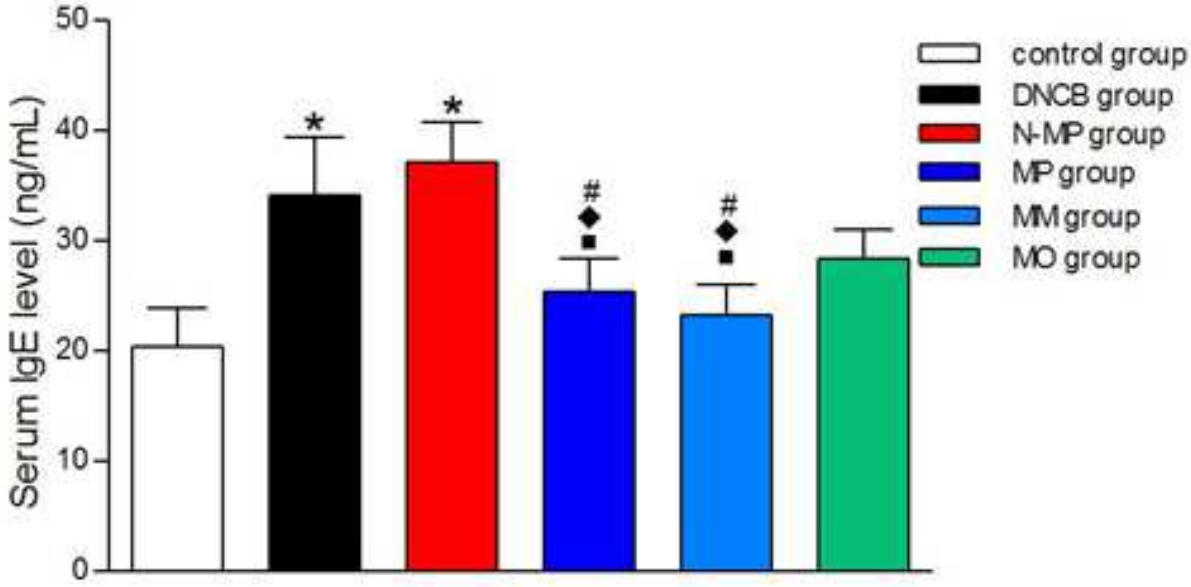

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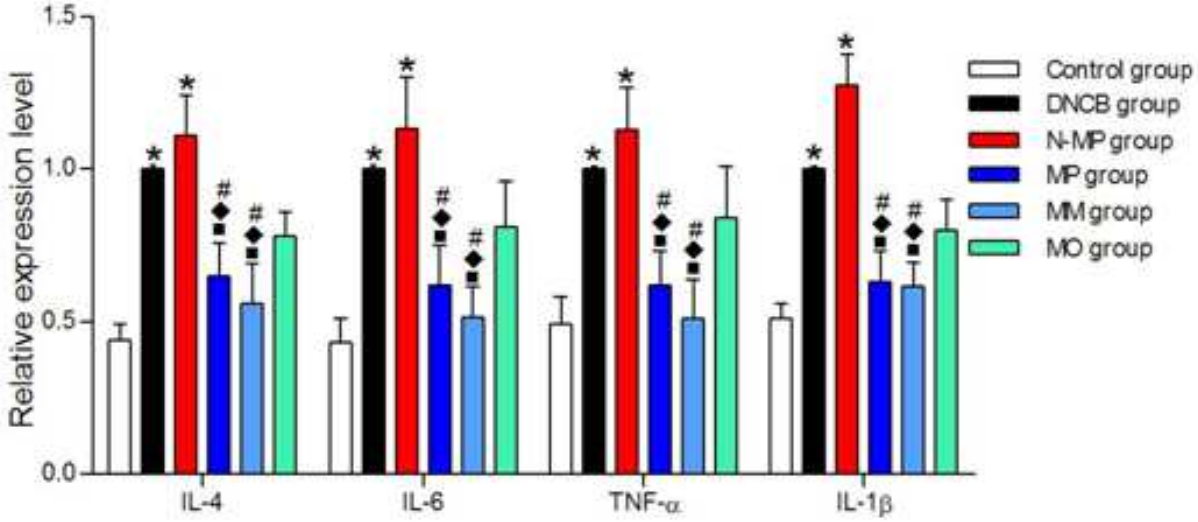

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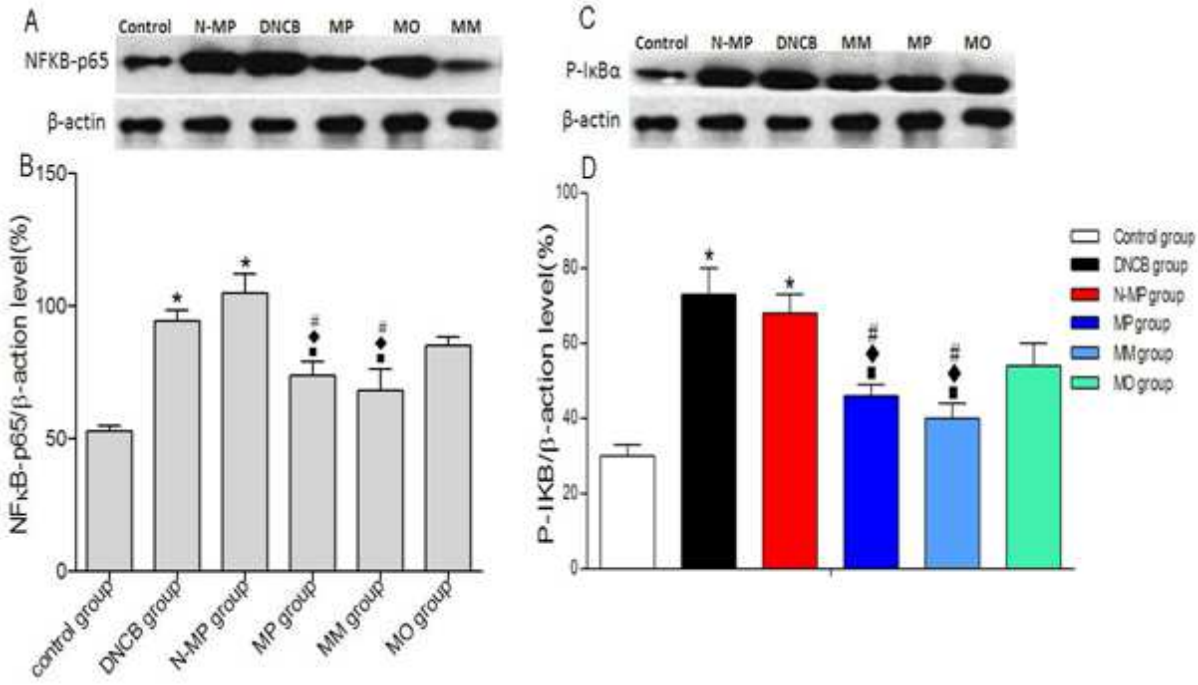

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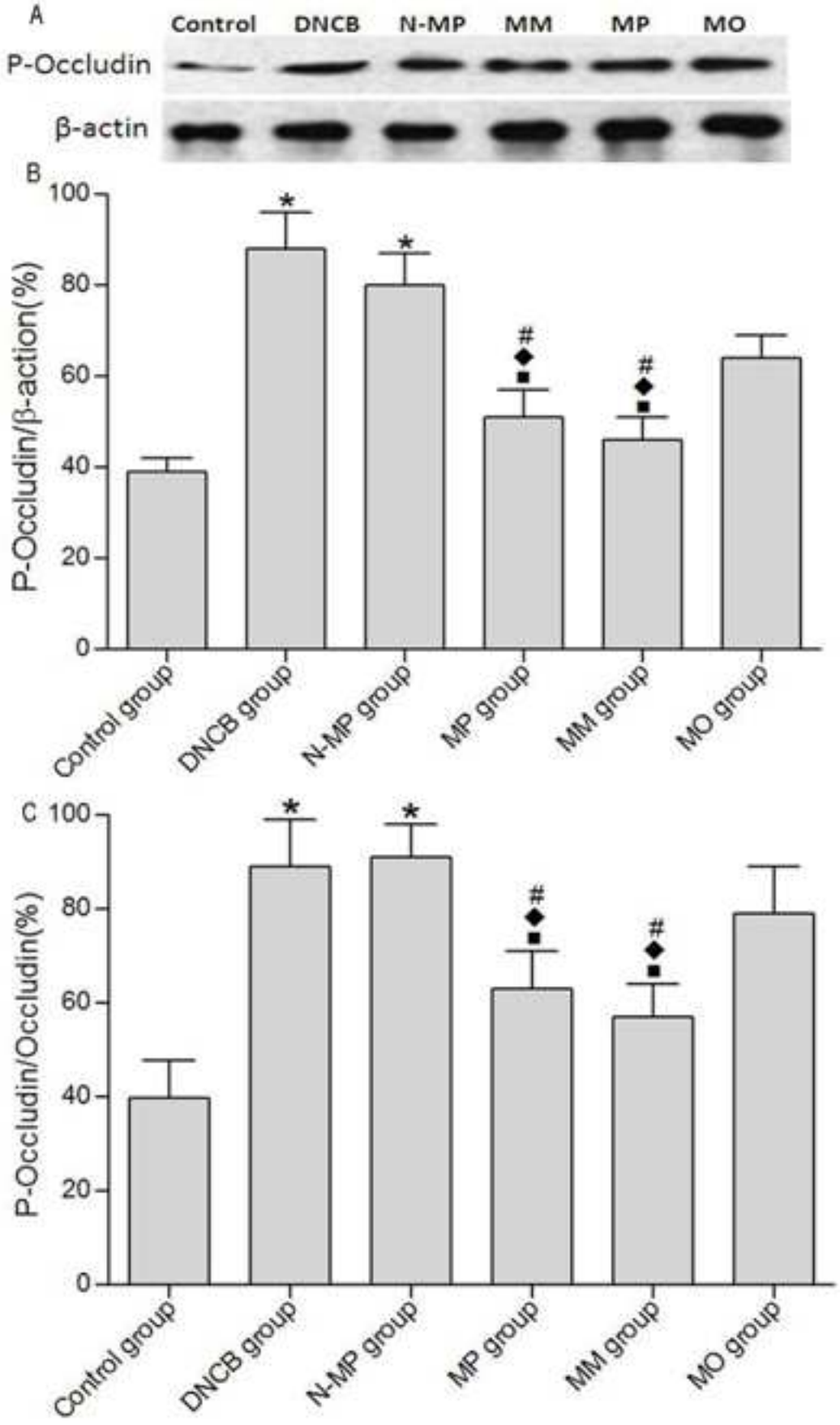
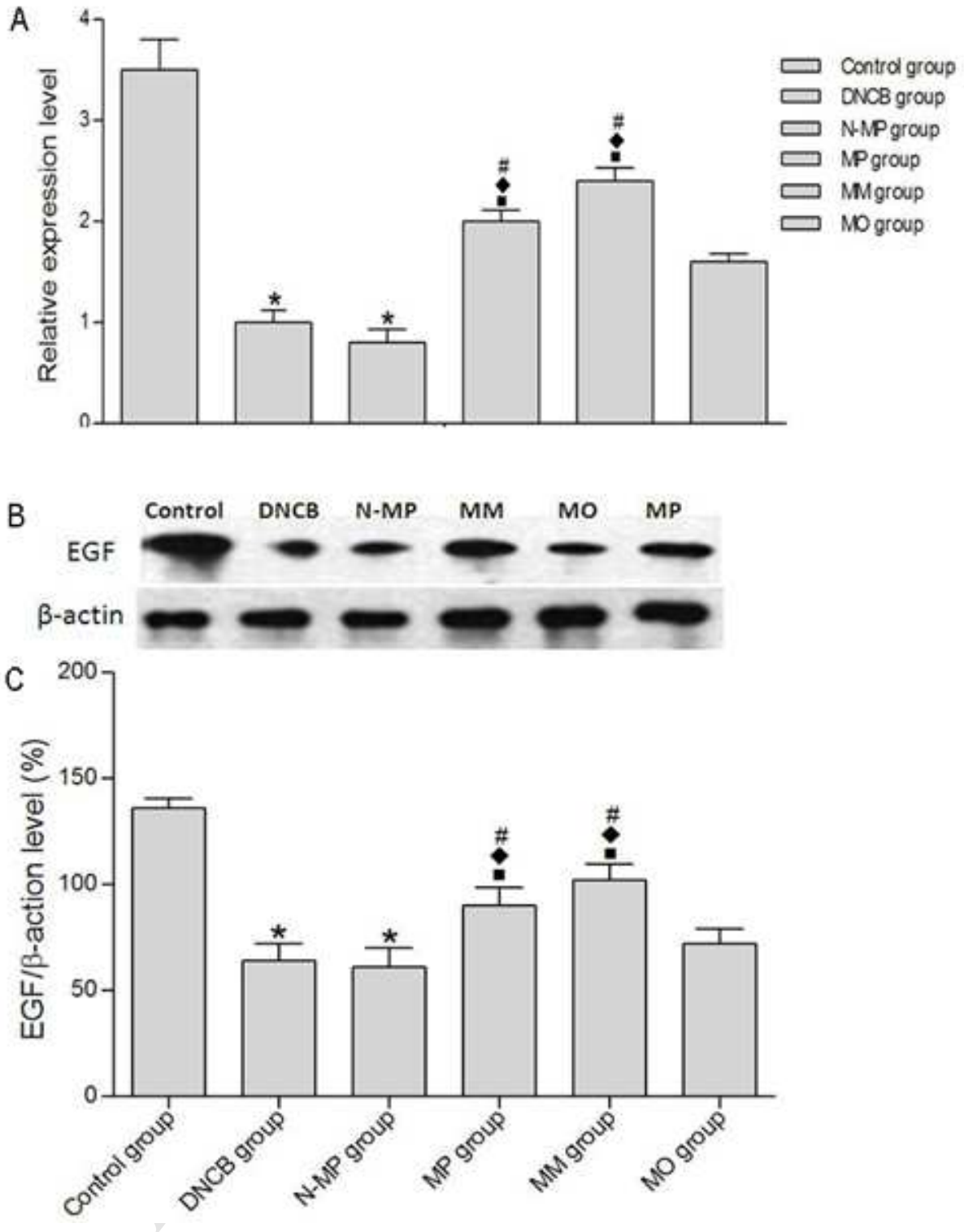

A
Control
DNCB
N-MP
MM
MP
MO
P-Occludin
β-actin
B
P-Occludin/β-action(%)
100
80
60
40
20
0
Control group
DNCB group
N-MP group
MP group
MM group
MO group
C
P-Occludin/Occludin(%)
100
80
60
40
20
0
Control group
DNCB group
N-MP group
MP group
MM group
MO group


A
Relative expression level
0
1
2
3
4
Control group
DNCB group
N-MP group
MP group
MM group
MO group
B
Control
DNCB
N-MP
MM
MO
MP
EGF
β-actin
C
EGF/β-action level (%)
0
50
100
150
200
Control group
DNCB group
N-MP group
MP group
MM group
MO group