International Immunopharmacology 69 (2019) 79–87

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Preventive effect of Diallyl Trisulfide on cutaneous toxicities induced by EGFR inhibitor

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ARTICLE INFO

Keywords:
DATS
EGFRIs
Cutaneous toxicities
Inflammatory

ABSTRACT

Cutaneous toxicities are the commonest side effects in patients with cancer treated using epidermal growth factor receptor inhibitors such as erlotinib. For patients with such toxicities, there is a lack of safe, effective pharmacological agents. Here we established a skin toxicity model and investigated the preventive and therapeutic effect of Diallyl Trisulfide (DATS) in vivo. The mouse skin toxicities model was established through continuous administration of erlotinib for 49 days. Meanwhile, the mice in the experimental group underwent DATS treatment for 49 days. Hematoxylin and eosin (HE) staining and oil red O staining of back and limb skin was performed to determine whether DATS aqueous extract can reverse the skin toxicities caused by erlotinib. Compared with the erlotinib group, the incidence of rash in the DATS group was lower. In addition, in the DATS group, the degree of skin redness and herpes was mild, the body weight was stable, and the activity was favorable. By comparing the HE and oil red O staining results for the mouse skin, the degree of keratin hyperplasia was determined to be lower in the experimental group than in the erlotinib group, and the number of purulent neutrophils decreased. The number of follicles was relatively less. The release of TNF-α, IL-6 and other inflammatory factors was reduced by DATS. Erlotinib hydrochloride can cause severe skin toxicities, and DATS prevents skin toxicities, its mechanism may be related to DATS reduced erlotinib-induced inflammatory injury.

1. Introduction

EGFR tyrosine kinase inhibitors are a standard first-line treatment for many malignant tumors [1]. EGFR's distribution in the body causes these tyrosine kinase inhibitors to have adverse reactions on the skin and limbs. Among them, the commonest and most difficult to tolerate are dermal toxicities with an incidence of 79%–88%. Many patients treated using EGFRI develop dermatological side effects, most frequently papulopustular (acneiform) eruption, but also xerosis, eczema, telangiectasia, hyperpigmentation, hair changes or paronychia [2].The mechanism of these cutaneous toxicities has not yet been fully elucidated, and is generally believed to be related to the skin follicles and interstitial cell follicle signaling pathway of interference [3].

In normal skin tissue, phosphorylated EGFR is mainly expressed in the basal lamina and basal layer, and mitogen-activated protein kinase (MAPK) is expressed in the basal layer. The inhibition of EGFR expression in basal keratinocytes may cause proliferation inhibition and advanced differentiation accompanied by inflammatory chemokines release and leukocyte recruitment. These leukocytes release enzymes that lead to keratinocyte apoptosis. The dead cells accumulate beneath the dermis, causing further skin damage [4,5]. Such cutaneous toxicities may result in reduction or even cessation of anti-EGFR therapy and were proved to compromise patients' quality of life [6]. Studies on preventing skin toxicities caused by EGFRIs remain in the exploratory stage. The commonest methods used in Western medicine are topical or oral antibiotics, steroids, or immunomodulators [7]. However, long-term use of these drugs results in other side effects and leads to substandard patient quality of life.

Garlic (*Allium sativum* L. fam. Alliaceae) is a medicinal, edible plant with numerous biological properties and a sizeable effect on skin disease [8]. Evidence suggests that oral administration of garlic has an effect on the immune system, cutaneous microcirculation, protection against UVB [9], and cancer treatment. The possible mechanisms might be its anti-oxidative [10], anti-inflammatory [11], and immunomodulatory properties [12,13]. DATS (Diallyl Trisulfide) is the most effective fat-soluble component of garlic. Recently, several independent studies have demonstrated that DATS may prevent carcinogenic and mutagenic processes of the skin in vitro and in vivo [14,15]. However, the effect of DATS consumption on EGFRI induced skin toxicities remains largely unknown.

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https://doi.org/10.1016/j.intimp.2019.01.023
Received 6 December 2018; Received in revised form 9 January 2019; Accepted 15 January 2019
Available online 23 January 2019
1567-5769/ © 2019 Published by Elsevier B.V.

On the basis of the afore-mentioned findings, we investigated whether DATS has skin toxicities prevention effect on erlotinib hydrochloride induced mouse cutaneous toxicities and attempted to elucidate the potential involved mechanisms. The erlotinib hydrochloride induced mouse skin toxicity model was used to test the skin protection potential of DATS. In the present study, we demonstrated the effects of oral application of DATS on erlotinib hydrochloride-induced mouse cutaneous toxicity biomarkers, such as slow hair regeneration, abnormal keratinocyte hyperplasia, inflammatory factor release. Furthermore, MPO, p-p65, TLR4, JAK1, STAT3 and ERK expression was investigated in erlotinib hydrochloride induced mouse cutaneous toxicities to elucidate the possible mechanisms.

2. Materials and methods

2.1. Animals

Six weeks old female BALB/c mice (17–23 g), free from infection, were obtained from Nantong University (Nantong, China) and raised at the Animal Center of Jiangsu Province Hospital of Integrated Traditional Chinese and Western Medicine. Throughout the experiments, the mice were maintained in plastic cages at 21 ± 2 °C in a 12 h light/dark cycle with free access to food and water. Animal welfare and experimental procedures were performed in strict accordance with the care standards for laboratory animals.

2.2. Chemicals and regents

DATS (purity > 98%) was purchased from LKT Laboratories, Inc., (USA), Erlotinib hydrochloride (purity: 99.3%) was purchased from Aladdin (Shanghai Aladdin Bio-Chem Technology Co., Ltd., CAS: 183319-69-9).

2.3. In vivo skin toxicity experiment

The female BALB/c mice underwent adaptive feeding for 1 week and were randomly divided into four groups (blank; 150 mg/kg erlotinib hydrochloride; 150 mg/kg erlotinib hydrochloride/12.5 mg/kg DATS; 150 mg/kg erlotinib hydrochloride/25 mg/kg DATS), each comprising of 10 mice. Administration was through oral gavage. Erlotinib hydrochloride was administered at 9 a.m., and DATS was administered at 3 p.m. The blank group was given the same dose of solvent. All animals underwent back-hair removal before the experiment. Mouse body weight was recorded at the beginning of the experiment, thereafter at 1 week intervals, and at the time of death. Daily activities and changes to the fur, including hair regeneration, rashes, and papules, were recorded from the beginning of the experiment. Skin from each mouse's limbs, back, neck, and eyelids was removed for subsequent experimental studies after the mice were sacrificed. Skin samples were used for hematoxylin and eosin (HE) staining and oil red O staining; hair follicle expansion, blockage, and neutrophil pus clusters were observed. Experiments were approved by the Animal Ethics Committees of Nanjing University of Chinese Medicine and performed in strict accordance with the U.S. National Institutes of Health's guide for the Care and Use of Laboratory Animals.

2.4. Oil red O staining

Tissue sections were fixed with methanol (4% neutral formazan fixative) for 10 min; rinsed with distilled water; 60% isopropanol dipped; oil red O stained for 10 min (dye can be recycled and reused); 60% isopropyl Alcohol color separation to background colorless; distilled water rinse; Mayer hematoxylin counterstain; tap water wash (blue) 1–3 min; distilled water rinse; glycerin gelatin seal. The lipid droplets in the tissue cells are orange-red and the nucleus is blue.

2.5. Immunohistochemistry

Tissue processing: animals in all groups were sacrificed through cervical dislocation 1 h after the last application, the dorsal skin tissue were processed for sub-cellular fractionation. Mouse skins were preserved in 10% neutral buffered formalin for histological observation. Subsequently, 10% homogenates were prepared in chilled phosphate buffer (0.1 M, pH 7.4) using a Polytron homogenizer (Kinematica, Inc., Switzerland). The homogenized tissue was centrifuged at 12,000*g* for 15 min at 4 °C to obtain the supernatant. The dorsal skins ($2 \times 2\ cm^2$) of mice were prepared for immune histochemical analysis of EGFR and p-EGFR expression. These skins were fixed in 10% formalin and then embedded in paraben. Five-micrometer-thick sections were cut and mounted on silanized glass slides. The sections of dorsal skin were deparaffinized in xylene, and dehydrated in graded ethanol. The deparaffinized sections were heated and boiled twice for 6 min in 10 mM citrate buffer (pH 6.0) for antigen retrieval. To block the non-specific binding, each section was treated using 3% H_2O_2 for 20 min and then using blocking solution (1% fat-free skimmed milk) for 30 min. Each section was incubated using monoclonal anti-EGFR and anti-p-EGFR antibody at room temperature for 2 h and subsequently incubated using secondary antibody at room temperature for 1 h. The color was developed using DAB as a substrate for 3–5 min. The sections were subsequently rinsed using distilled water and counterstained using hematoxylin. The DAB-peroxidase reaction resulted in a brown reaction product. Finally, the sections were analyzed using a microscope (Motic Digital Microscope) in at least six different regions.

2.6. Cytokine determination

Blood samples of the mice treated using various formulations were collected for inflammatory cytokines analysis. Various types of cytokine reflecting the influence of treatment on inflammation functions, such as interferon-γ (IFN-γ), interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-12A (IL-12A), interleukin-10 (IL-10), and tumor necrosis factor-a (TNF-a) were quantified in accordance with the manufacturer's protocol for enzyme-linked immunosorbent assay kits (Keygen Co. Ltd.)

2.7. Statistical analysis

The significant between the different groups were ascertained using the Student-Newman-Keuls-test after a one-way analysis of variance (ANOVA) was conducted by GraphPad 7.0. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Preventive effect of DATS on erlotinib-induced mouse skin dermal toxicity

First, we established a mouse model of skin toxicity induced by erlotinib hydrochloride in BALB/c mice and investigated the effect of DATS using the mouse model. As shown in Fig. 1, mice that were administered 150 mg/kg erlotinib hydrochloride exhibited significant skin toxicities, as indicated by redness and swelling of the extremities (Fig. 1A), hair regeneration obstruction, whisker loss, abnormal eyelids, herpes, and rashes around the mouth and limbs (Fig. 2). Mice that were administered DATS could exhibited a significant reduction in skin toxicities induced by erlotinib hydrochloride. Promotion of hair growth and mitigation of rash incidence were observed demonstrated by the apparent observation. We calculated the rate of redness, swelling, herpes, and rash for each group of mice. The results indicated that DATS administration could reduce the incidence of skin toxicities induced by EGFRI (Table 1).

To observe the pathological changes of the skin tissue after the establishment of the model and administration of different doses of DATS,

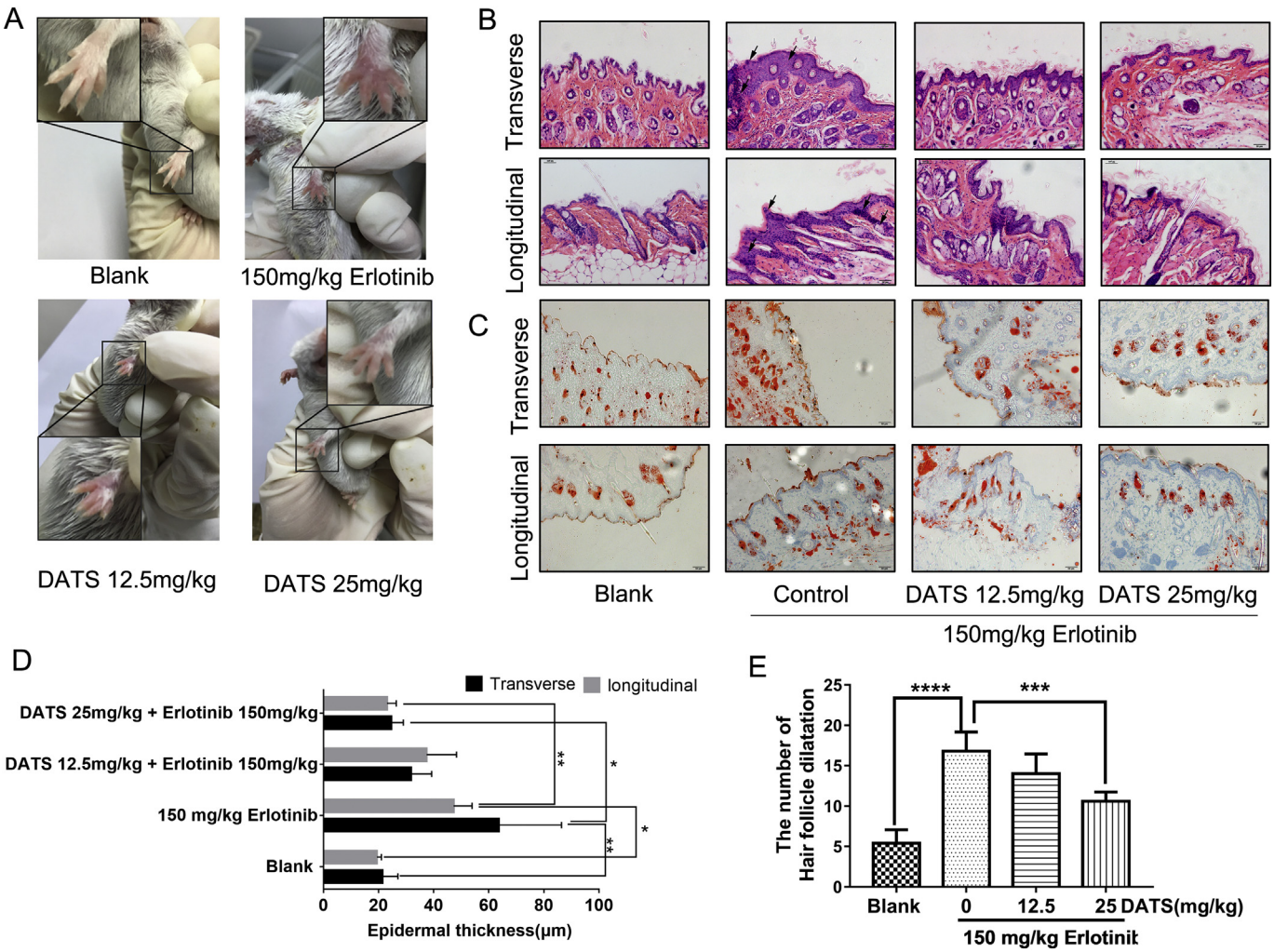

Fig. 1. Preventive effect of DATS on Erlotinib-induced mouse skin dermal toxicity. Group of 10 female BALB/c mice, 6 weeks of age, were treated with 150 mg/kg Erlotinib Hydrochloride and different doses of 12.5 and 25 mg/kg DATS. (A) Observation of the appearance of red and swollen limbs. (B) H&E staining of the transverse section (Up) and the longitudinal section (Bottom) of the skin tissue (200×). (C) Oil red staining of skin tissues of the transverse section (Up) and the longitudinal section (Bottom) of the skin tissue (200×). (D) Quantitative analysis of Epidermal thickness (n = 3). (E) Statistics of the number of hair follicle expansion (n = 3). The data are presented as the mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 (versus 150 mg/kg Erlotinib). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

HE staining of skin tissue of the mice in each group was monitored after treatment [16]. Fig. 1B shows that the hallmarks of skin inflammation i.e., hair follicle plugging, pus-like neutrophil clusters surrounding the hair follicle canal, parakeratosis (retention of the nuclei in stratum corneum cells), and ectatic (dilated) follicles were observed in erlotinib hydrochloride treated mice but not in the mouse groups treated with different does of DATS. The thickness of the skin epidermis was calculated in the present study: compared with the model group, neutrophil-rich inflammation with abnormal epidermal thickening was lower in the DATS treatment group (Fig. 1D). We next found that the increased the number of hair follicle expansions in erlotinib treated group while reduced in 25 mg/kg DATS treated group (Fig. 1E).

In addition, EGFR inhibition may result in sebaceous gland over activation, sebocyte maturation, and oil production. We subsequently evaluated skin samples through oil red O staining. As Fig. 1C shows, enlarged sebaceous glands were observed to feed into oil-filled follicular distensions near the sebaceous ducts on erlotinib hydrochloride -treated skin but not on 12.5 mg/kg DATS treated skin.

The body weight of the mice and supplemental statistics were recorded weekly during the experimental period. The key immune system organs of mice such as the liver, spleen, and thymus were stripped on the last day of the experiment and weighed. As Fig. 3A shows, body weight increased gradually in the blank group, whereas mice in the model group underwent significant weight loss after erlotinib administration. Mice in the different DATS treated groups underwent significant weight loss during the first 3 weeks, but a exhibited significant weight gain from week 4. The results showed that the liver index and spleen index of the mice were significantly increased, whereas the thymus index was significantly reduced, indicating that the mice's immune systems were severely damaged by erlotinib hydrochloride administration. The organ index of the mice in the DATS group recovered to a certain extent, close to the level of normal healthy mice, indicating that DATS can reverse the immune damage caused by erlotinib hydrochloride and protect against it (Fig. 3B–D).

3.2. Effect of DATS on erlotinib-induced skin inflammation

Evidence suggests that IL-12A, IL-2, TNF-α, IL-6, IFN-γ, and other factors are positively correlated with the occurrence of inflammation in skin toxicities [16,17]. Therefore, we collected sera from various groups of mice in animal experiments and examined these cytokines. The results showed that compared with those in the blank group, the expression levels of IL-10, IL-12A, IL-2, TNF-α and IL-6 in the erlotinib group were significantly elevated, but the expression of the afore

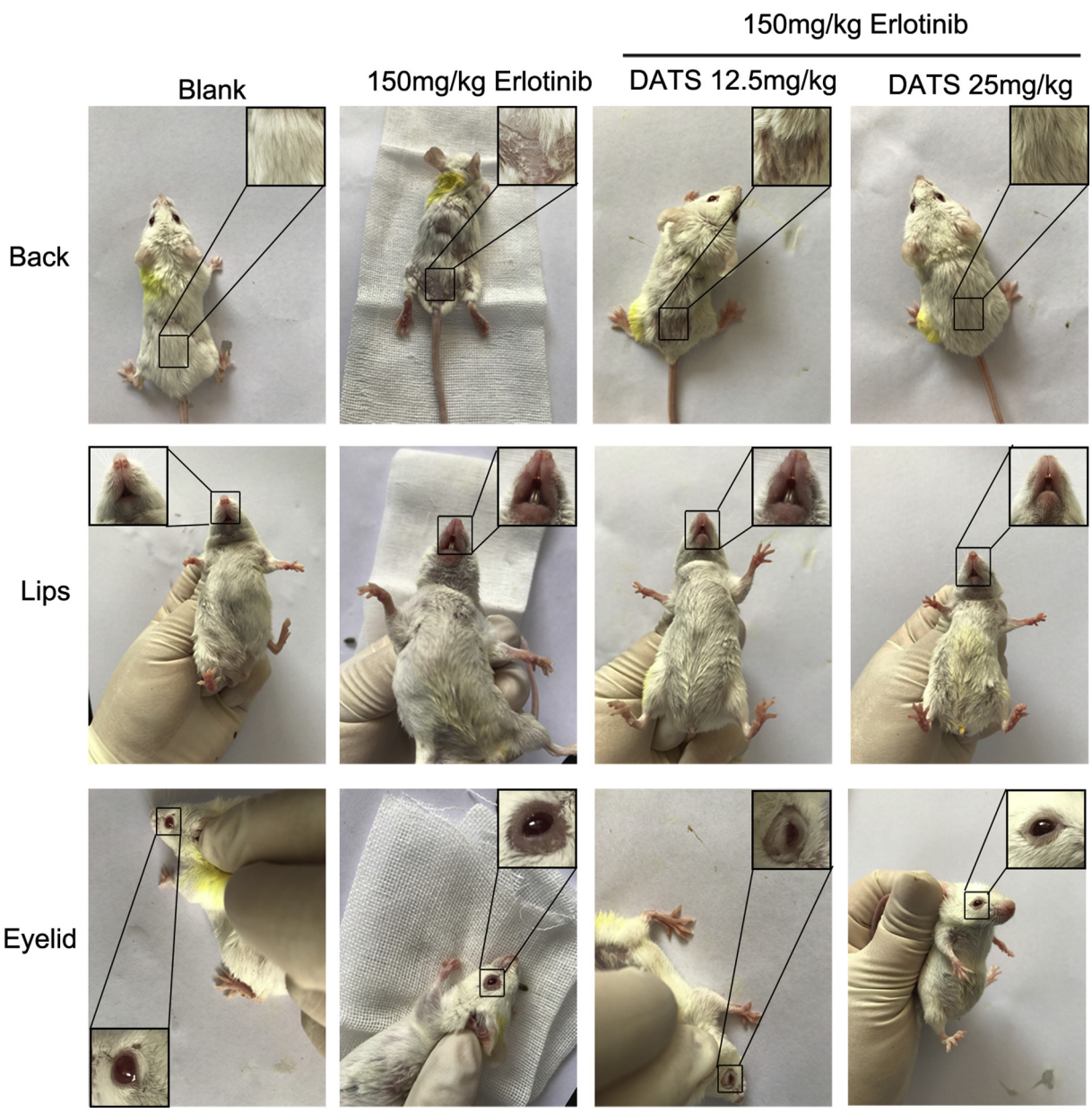

Fig. 2. Representative graphs of change in hairs and skins in different treatment groups on 7 weeks are shown. Hair regeneration (Up), Changes in the skin around the lips (Mid) and Eyelid abnormalities (Bottom) of different groups are observed with an unaided eye.

mentioned inflammation-associated cytokines was significantly reduced after DATS administration and tended to be normal, as shown in Fig. 4. The prevention and treatment of the erlotinib-induced dermatological toxicity of DATS may be related to its intervention in the down-regulation of inflammatory factors.

3.3. Effect of DATS on EGFR inflammatory proteins in EGFRI-induced skin toxicities

Neutrophils are thought to be important inflammatory cells in the stages of skin toxicities induced by EGFRI and play an important role in initiating inflammatory responses. When the body is in an

Table 1
The incidence rate of red and swollen, herpes and rash after the interventions with 150 mg/kg Erlotinib Hydrochloride or/and different concentration of DATS in BALB/c mouse.

Incidence rate (100%)	Red and swollen				Herpes				Rash			
Groups	Non	Mild	Moderate	Severe	Non	Mild	Moderate	Severe	Non	Mild	Moderate	Severe
Blank	100%	–	–	–	100%	–	–	–	100%	–	–	–
150 mg/kg Erlotinib Hydrochloride	–	–	80%	20%	20%	40%	40%	–	20%	80%	–	–
150 mg/kg Erlotinib Hydrochloride + 12.5 mg/kg DATS	–	20%	40%	40%	20%	80%	–	–	40%	60%	–	–
150 mg/kg Erlotinib Hydrochloride + 25 mg/kg DATS	20%	60%	20%	–	80%	20%	–	–	100%	–	–	–

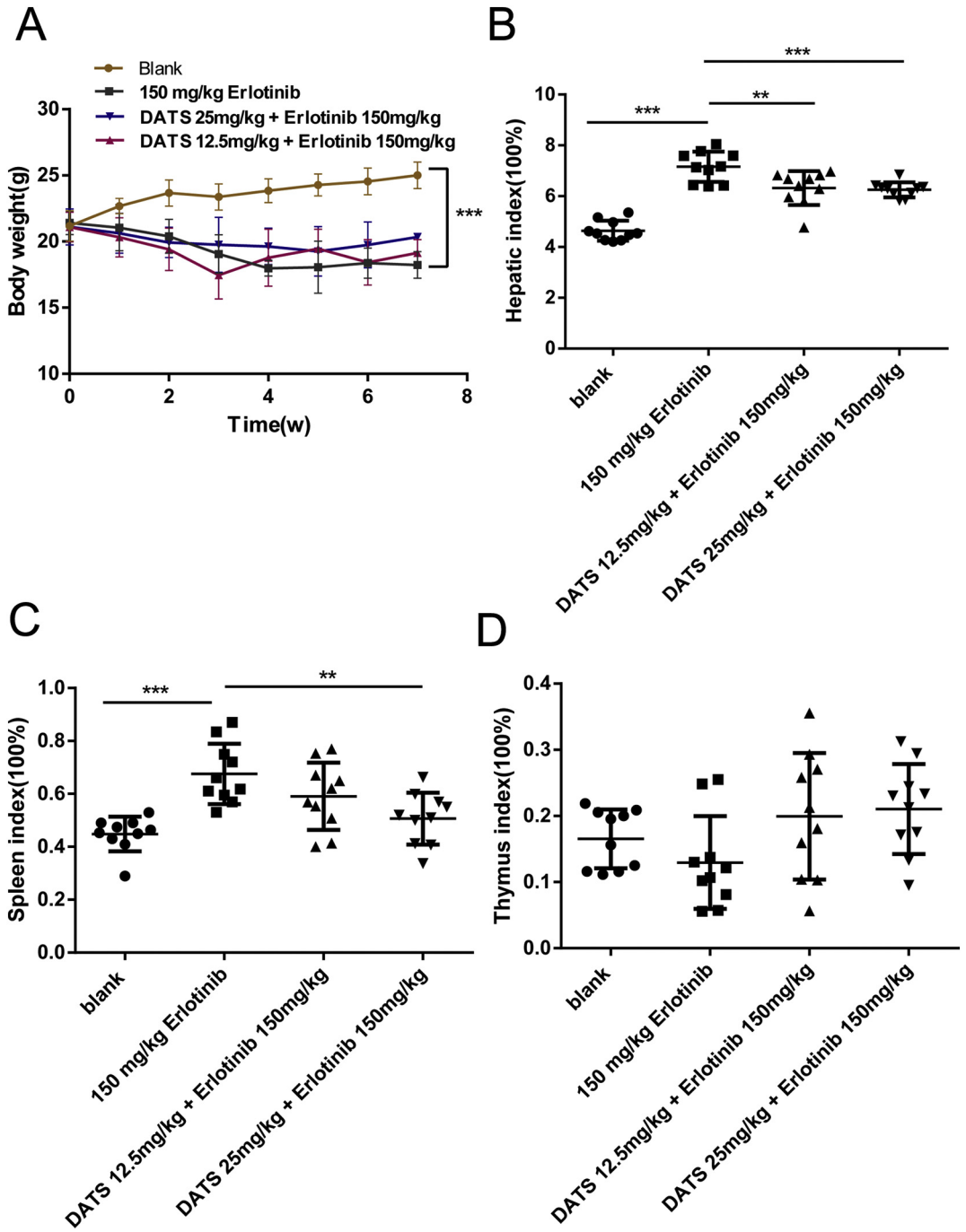

Fig. 3. The effect of DATS on body weights and viscera index of mice in each group. (A) Body weights were recorded weekly. Body weights are plotted, showing changes observed every second week until the killing of the animals. Data are expressed as means ± SD of 10 animals in each group. (B–D) Hepatic index, spleen index and thymus index are shown. Data are expressed as means ± SD of 10 animals in each group.

inflammatory or stress state, neutrophil activation releases MPO, which produces a large amount of oxygen free radicals and oxidants, further activating the inflammatory signaling pathway of the cells. Therefore, MPO can sensitively reflect the degree of tissue neutrophil infiltration. We first detected the effect of DATS on the expression of MPO. The results showed that application of Erlotinib hydrochloride could up-regulate the expression of MPO compared with normal control group. Treatment with DATS showed significant decrease on the expression of MPO in a dose-dependent manner as compared with the model group in mouse skin.

We have previously confirmed that the massive release of inflammatory factors induced by EGFRI. Studies have demonstrated that EGFRIs induce arrest of basal keratinocyte growth, premature maturation, neutrophil differentiation and inflammatory factors release by increasing the expression of the TLR4/NF-κB, JAK/STAT3 and ERK. We next investigated the expression of TLR4, p-p65, JAK1, STAT3 and ERK using immunohistochemistry assay in erlotinib and/or DATS treated groups. The results indicated that erlotinib significantly increased the expression of TLR4, p-p65, STAT3 and ERK in the erlotinib group compared with the control group; the administration of DATS reduced these inflammatory proteins (Fig. 5A and B). Thus, these findings indicated that DATS could extenuate the skin inflammation induced by EGFRI.

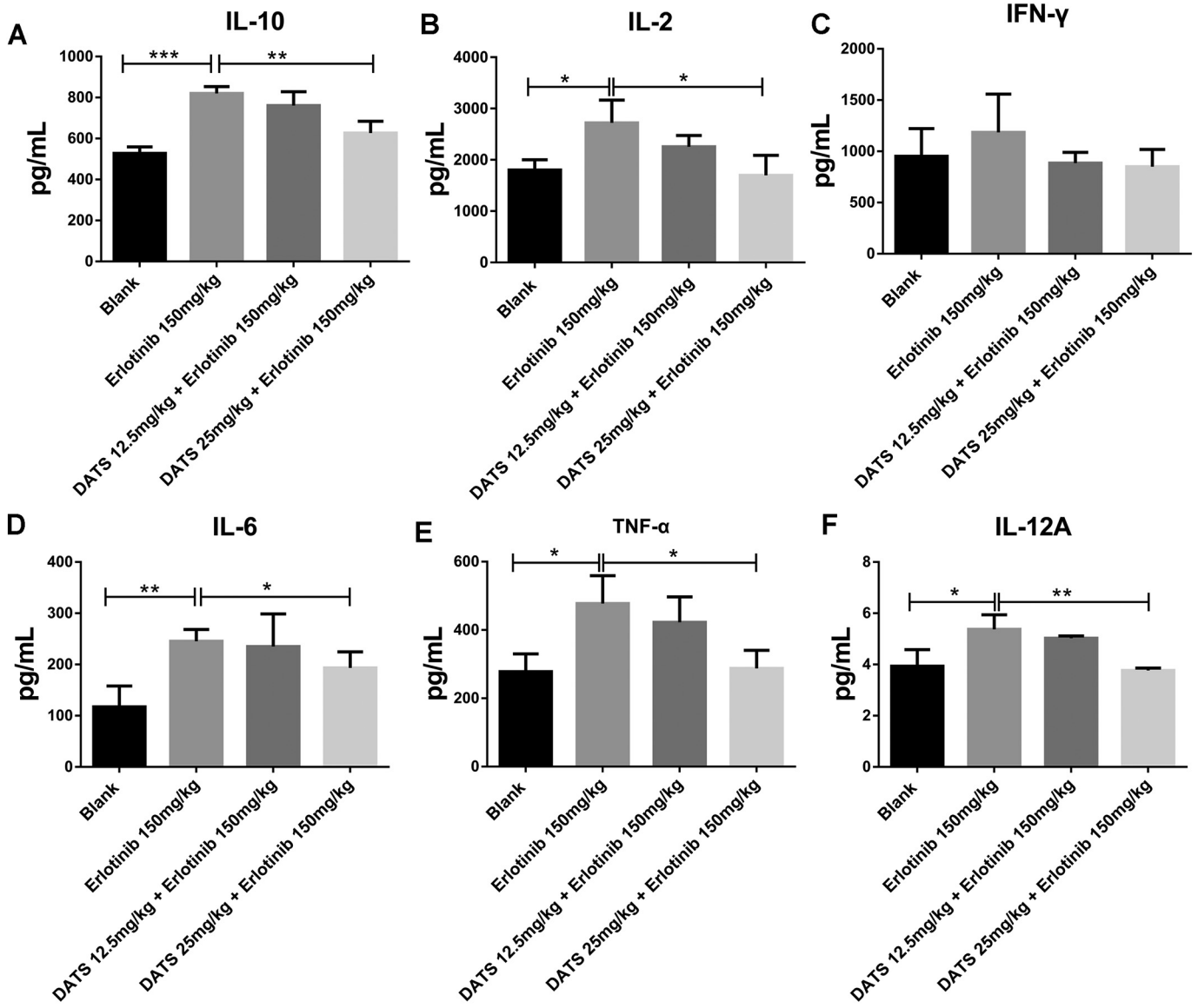

Fig. 4. The effect of DATS on Erlotinib-induced skin inflammation. (A–F) Cytokinedetections. The content of (A) IL-10, (B) IL-2, (C) IFN-γ, (D) IL-6, (E)TNF-α, and (F) IL-12A in the serum of different group mice.

4. Discussion

The emergence of molecular-targeted anti-tumor drugs is promising development for patients with cancer. An increasing number of molecular targeted anti-cancer drugs have been clinically applied and have achieved favorable efficacy. Of these, EGFRIs have led to considerable progress in the treatment of various solid tumors since its introduction. However, clinical results have increasingly found severe adverse reactions accompanying EGFRI treatment. Among these, skin toxicities are the commonest adverse reaction and have a severe effect on the patients' physical and mental health [18]. Skin toxicities affect patients' daily lives even anti-tumor therapy without intervention treatment although it is considered a hallmark event in the emergence of molecular targeted drugs [19].

DATS (Diallyl Trisulfide), the major organic sulfur compound found in garlic (*A. sativum* L. fam. Alliaceae), has long been used as a medicinal and edible food with cancer chemoprevention properties worldwide [20,21]. Interestingly, there are two viewpoints on the use of garlic in dermatological diseases: Exposure of the skin to high concentrations of garlic oil may cause contact dermatitis because of irritation caused by garlic [22]. However, reports have increasingly concluded that garlic and garlic extracts can be used to treat skin-related diseases, such as psoriasis, alopecia areata, and infectious wounds [23–25]. Its mechanism may be related to its anti-oxidative, anti-inflammatory properties and its other activities [26,27]. However, little is known about its effect and underlying the mechanisms of the effect of DATS on EGFRI induced skin toxicities.

In this study, we established an EGFRI-induced mouse skin toxicity model to verify the efficacy of DATS. We chose erlotinib as a skin-toxicity animal model inducer because it is a molecular-target anti-tumor drug that specifically targets tumor cells in the anticancer process and inhibits tumor cells by inhibiting the activity of a specific enzyme in epidermal growth factor in human cells. The formation, growth, or promotion of tumor cell apoptosis to achieve anti-tumor effect was confirmed to have a reliable curative effect on advanced non-small-cell lung cancer and can effectively improve tumor survival rate. It has clinically significant efficacy, however, in clinical use during the process, many patients with tumors have different degrees of skin-related adverse reactions, especially on the face [28]. By using the described animal model, we assessed the effects of DATS on skin toxicity

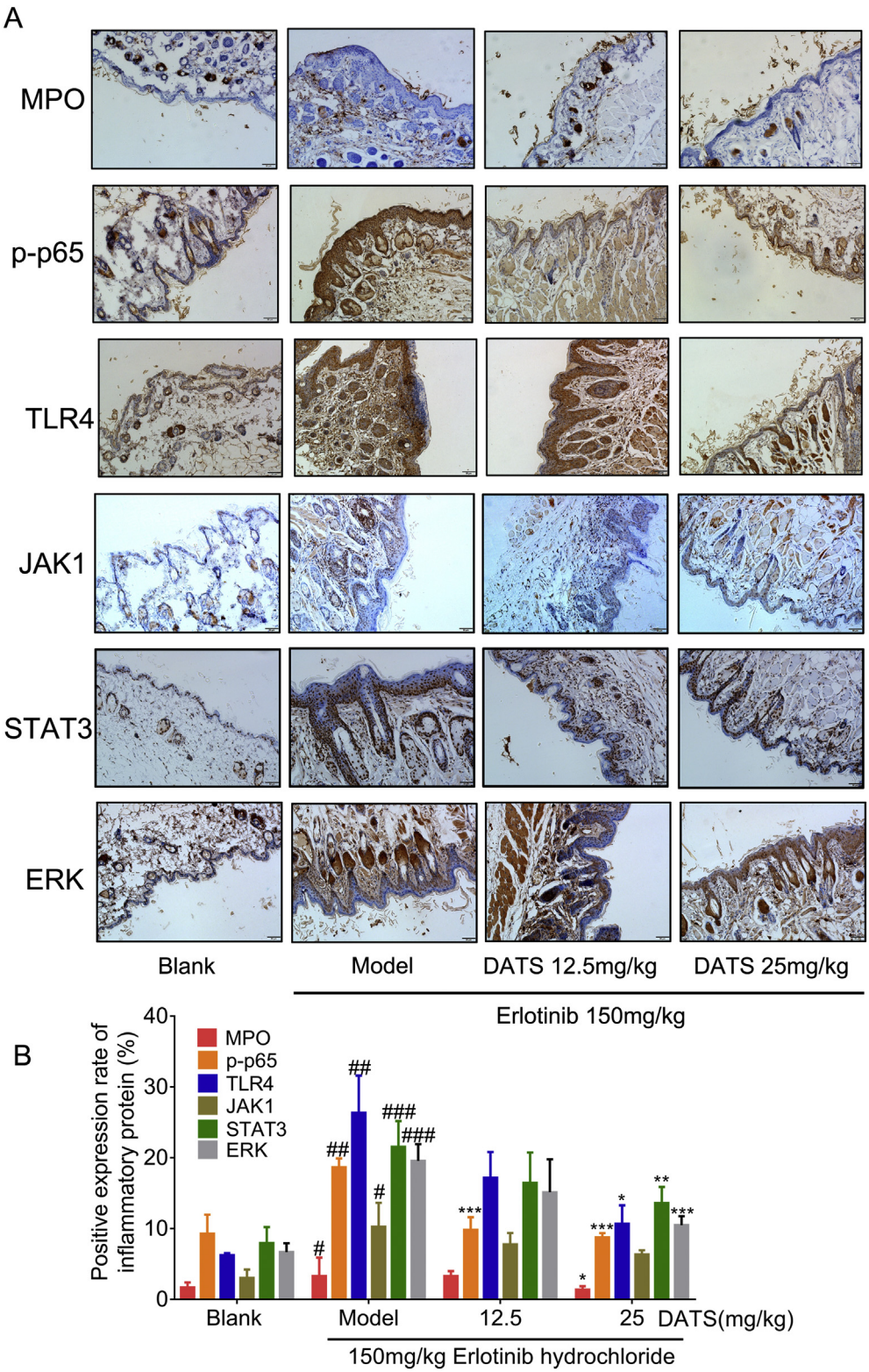

Fig. 5. Immunohistochemistry analysis of protein expression of (A) MPO, p-p65, TLR4, JAK1, STAT3 and ERK, *Scale bars*:50 μm. (B) Quantitation of the result of positive expression rate of MPO, p-p65, TLR4, JAK1, STAT3 and ERK. Significance: $^{\#\#\#}P < 0.001$, $^{\#\#}P < 0.01$, $^{\#}P < 0.05$, Erlotinib-treated mice versus Blank group; $^{***}P < 0.001$, $^{**}P < 0.001$, $^{*}P < 0.05$, Erlotinib/DATS-treated mice versus DATS treated mice group.

models induced by 7 weeks of erlotinib hydrochloride administration. During continuous administration, mouse body weight and status were recorded every other week. Skin toxicity effects, such as desquamation, shedding and slow hair regeneration in the epidermis, were observed following continuous intragastric administration of 150 mg/kg erlotinib hydrochloride. The incidence of limb swelling in the erlotinib hydrochloride group was 100%, and the degree was moderate to severe. DATS reduces the incidence and severity of skin toxicity according to the macro observation statistics. DATS reversed abnormal skin changes, such as abnormal epidermis thickening, inflammatory cell infiltration,

pus-like neutrophil accumulation, and clogging according to HE and oil red O staining (Fig. 1).

Researchers have identified the following four stages of the skin toxicity process induced by EGFRIs:1) skin irritation and increased skin sensitivity; 2) inflammatory cytokine secretion and local infiltration; 3) T-lymphocyte-rich papules and pustules; and 4) scleroderma [2,29]. Inflammation plays a key role in the skin toxicity process with the release of numerous cytokines. TNF-α and IL-6 were the commonest pro-inflammatory factors in multiple inflammatory diseases including skin problem [30,31]. The main role is to cause tissue damage, and release of secondary inflammatory mediators. TNF-α is mainly produced by macrophages and monocytes, it can activate inflammatory cells, up-regulate adhesion factors, NO, and free radicals to damage tissues, and cause sepsis. In addition, elevated levels of TNF-α can stimulate the release and increase the quantity of cytokines such as IL-2, thereby disrupting the network balance between cytokines and causing multiple inflammations. We examined the changes in some inflammatory cytokines after the administration of erlotinib hydrochloride, DATS, or both. Our results confirmed that EGFRIs could induce the release of most pro-inflammatory factors such as IL-10, IL-2, IL-6, TNF-α, and IL-12A, whereas DATS could normalize them to normal levels. The cytokine IL-10 acts as both an inducer of Th2 type responses and a regulator of sputum cell activation, which regulates skin inflammation and inhibits the production of cytokines such as IFN-γ, TNF-α, and IL-12 [32]. The up-regulation of IL-10 that was observed in this experiment may be a negative feedback regulation mechanism of the body.

Innate immune response was considered to drive the rash related skin inflammation by inhibition of EGFR. Large amount of immune cells such as B cell, T cell, neutrophils, et al. infiltration was observed in Egfr lacking mouse but local deletion of neutrophils of mast cell activation reduced skin inflammation [33]. We first detected the expression of the neutrophil marker protein MPO. The results showed that DATS could reduce the neutrophil infiltration induced by EGFRI.

Although the phosphorylation of STAT3 in human skin is not directly affected by EGFR activity, it might result from the activation of alternative pathways that are triggered by cytokines or other growth factors, because EGFRIs lead to increased STAT3 staining in the basal layer of the epidermis. Our experimental results confirmed that the number of STAT3-positive cells in the skin of mice administered with erlotinib hydrochloride was significantly increased. Treatment with DATS could significantly suppress the erlotinib hydrochloride -induced increase of STAT3 positive cells in a dose-dependently manner, suggesting that STAT3 is a promising target in treating inflammation-related skin toxicity. In addition, daily administration of erlotinib or its metabolites may cause reactive oxygen production or oxidative stress in patients, leading to the activation of the ERK and STATS signaling pathways. Both pathways may contribute to inflammatory reaction and rash development [34]. Thus, to elucidate the mechanisms underlying the effect of DATS on skin protection, the expression of EEK and JAK1 in erlotinib hydrochloride -mediated skin toxicities was determined. Our results indicated that DATS could inhibit the expression of these proteins. Collectively, the results suggest that the intervention of STAT3 and MAPK-mediated pro-inflammation signaling plays a pivotal role in the skin-protection effect of DATS on erlotinib hydrochloride-induced skin toxicity.

TLRs (Toll like receptors, TLRs) play an important role in skin diseases, especially infectious skin diseases, allergic skin diseases and autoimmune skin diseases. Activation of NF-κB by TLR4 signaling pathway induces the production of cytokines such as TNF-α and IL-6, which is characterized by increased inflammatory cell infiltration and thickening of the epidermis [35,36]. Our results showed that DATS could strongly inhibit the expression of p-p65. This was the same as the protection mechanism of DATS for other normal tissues such as cardiomyocytes [37]. Therefore, we hypothesized that inhibition of inflammation was an important mechanism of DATS on protecting normal tissue damage.

This study has several limitations. The dermal toxicity caused by EGFRIs is clinically produced by patients with tumors who took EGFRIs. A tumor-bearing mouse skin toxicity model should be established for further assessment of the effect of EGFRIs on skin toxicities and on the anti-tumor effect of DATS. In addition, we postulated that DATS reduced STAT3 and EGFR-MAPK signaling, which, requires further validation. The effect of DATS on the expression of these proteins remains unknown. Whether the anti-oxidation, anti-free radical generation and antibacterial effects of DATS are related to the reversal of the skin toxicities induced by EGFRIs requires further research [38].

In conclusion, our findings revealed the significant role of a potent organosulfur compound, DATS, from the homology of medicine and food, in protecting skin from EGFRIs. DATS inhibited inflammatory cells infiltration, pro-inflammation cytokines release, TLR4-NF-κB, JAK1-STAT3, ERK expression. The findings suggest that consuming garlic that contains organic sulfide DATS may be beneficial to the prevention of skin toxicities in patients treated using EGFRIs. This paper provides a new strategy for the skin toxicity prevention in patients undergoing EGFRIs therapy.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This work was supported in part by Natural Science Foundation of Jiangsu Province (BK20171097), Science and Technology Project of Jiangsu Provincial Bureau of traditional Chinese Medicine (FY201505), (YB2017033).

Disclosure statement

None.

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