

Anti-Psoriasis Inflammation And Anti-Oxidation of The Extract From The Flower of *Datura Metel* L.

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Research

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Abstract

Background At present, Flos Datura L. has been reported to have obvious anti-psoriasis effect, but its active ingredient is still unknown.

Objectives: The chemical characteristics and anti-psoriasis inflammatory and antioxidant activities of partially purified flavonoids components of Flos Daturae (FC-FD) were investigated.

Methods: FC-FD were analyzed by ultra-performance liquid chromatography (UPLC)-electrospray ionization (ESI)-quadrupole time-of-flight (Q-TOF)-mass spectrometry (MS^E) combined, while antioxidant potency was monitored using a drosophila model. And the immune inflammatory response was measured in guinea pig animal model.

Results: After comparison, 14 products were identified. In vitro antioxidant activity assays showed that FC-FD could effectively scavenge hydroxyl radicals. Furthermore, the longest survival time of the male and female *Drosophila melanogaster* was extended by 100.50% and 96.82%, respectively. In addition, in the psoriasiform guinea pigs model, FC-FD could decrease the histopathological score and the expression of PCNA protein, and block the production of TNF- α . Notably, the partial purified flavonoids extracted from Flos Datura was and identified for the first time.

Conclusions: The results indicated that FC-FD are potential natural antioxidants in extending lifespan and anti-psoriatic inflammatory drugs.

Introduction

Datura metel L. (Solanaceae) flowers are one of the traditional Chinese herbal medicine commonly used to cure and prevent pain, asthma, rheumatism, begma, and convulsions etc. [1–2]. Withanolides, alkaloids, flavonoids, sesquiterpenoids, lignans, and phenolic acids are generally viewed as the major bioactive compounds of *Datura metel* L. [3]. Other plants of the genus *Datura* have been extensively studied, mainly due to the presence of alkaloids and withanolides [4]. Withanolides possess multiple bioactivities, such as immunomodulation, anti-cancer, neuroprotection, antihyperglycemic, and reversal of Alzheimer's disease [5]. These papers reported the extraction of that withanolides extracted from *Datura metel* L. made a difference to inhibitory on immune responses [6]. In recent years, accumulating studies have reported that *Datura metel* L. has obvious anti-psoriasis effects, and these have been clinically validated at the First Affiliated Hospital of Heilongjiang University of Chinese Medicine [7]. The effective part for psoriasis has been demonstrated that it mainly included withanolides and flavonoids.

In previous studies, a variety of flavonoids have been isolated from the flower, mainly kaempferol and quercetin as parent nuclei. *David w. ate* and *John e.* found that there were eight types of flavonoids in the flower of *Datura metel* L. [8–9], including Quercetin-3-O-rutinoside; 7-O-glucoside; 3,7-O-diglucoside; 3-O-rutinoside-7-O-glucoside; Kaempferol-3-O-rutinoside; 7-O-glucoside; 3,7-O-diglucoside; 3-O-rutinoside-7-O-glucoside. In recent years, Kaempferol-3-O- β -D-glucopyranosyl-(1 \rightarrow 2)- β -D-glucopyranoside; Kaempferol-3-

O-β-D-glucopyranosyl (1→2)-β-D-glucopyranosyl,7-O-α-L-rhamnopyranoside; Kaempferol-7-O-α-L-rhamnopyranoside; Kaempferol-7-O-β-D-glucopyranoside; Kaempferol-3-O-β-D-glucopyranosyl, 7-O-α-L-rhamnopyranoside; and Kaempferol-3-O-α-L-rhamnopyranosyl(1→6)-β-D-glucopyranosyl, 7-O-β-D-glucopyranoside ect. has been isolated in our research group [10]. Although flavonoids have a variety of biological activities, such as antioxidant, antitumor, antibacterial, anti-inflammatory, cardiovascular resistance, etc., but the research on the role of less reported on the effect of flavonoids from *Datura metel* L.. Tang [10] quantified the content of total flavonoids in AF-FD (the anti-psoriatic active fraction of Flos Daturae) by using compound Kaempferol-3-O-β-D-glucopyranosyl (1→2)-β-D-glucopyranosyl,7-O-α-L-rhamnopyranoside as standard, and found that more than 53% of the AF-FD was flavonoids. However, the responsibility of flavonoids extracted from *Datura metel* L. has not been clarified [11].

In this study, we aim to analyze the flavonoids using UPLC-Q-TOF/MS technology, and the therapeutic effects of flavonoids were then evaluated using guinea pig psoriatic model induced by propranolol, drosophila biological model and human keratinocytes. We hope to further understand the role of these drugs in the treatment of psoriasis - like inflammation and antioxidant. We expect to gain further knowledge of these drugs in treating psoriasis-like inflammation and anti-oxidation.

Materials And Methods

1 Plant Materials

The dried flower of *Datura metel* L. (30 kg) was purchased from the First Hospital of Heilongjiang University of Traditional Chinese Medicine and was appraised by Professor Zhenyue Wang of the Pharmaceutical Department of Heilongjiang University of Traditional Chinese Medicine.

2 Chemicals and reagents

Acetonitrile and methanol were purchased from fisher (Fisher, USA); leucine enkephalin was purchased from sigma-Aldrich (Hongkong, China); 2,2-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from TCI Development Company (Shanghai, China); Proliferating Cell Nuclear Antigen (PCNA) antibody was purchased from Boshide Company (Wuhan, China); Propranolol was purchased from Changzhou Pharmaceutical Factory (Shanghai, China); H₂O₂ solution was obtained from Libo Commerce Company (Guangdong, China); MTT kit, SABC kit and IL-6 immunoassay kit were obtained from Boster Biological Technology Company (California, USA).

3 Extraction and isolation

The extraction and preparation process has been previously described, and 52 g of the active fraction of Flos Daturae (AF-FD) had been extracted from Yang by some adjustments [5]. To get the purity, we separated AF-FD by Glucose gel (Sephadex LH-20) column chromatography with water and methanol for a week at 20 °C. Methanol eluent components were flavonoids components of Flos Daturae (FC-FD).

4 Ultra-high Performance Liquid Chromatography mass spectrometry analysis

Adding the appropriate FC-FD that was accurately determined to methanol, we adjusted the concentration to $1 \text{ mg}\cdot\text{ml}^{-1}$. Concentrated solution using a $0.22 \mu\text{M}$ micropore filter, sampling $10 \mu\text{L}$ injection UPLC-Q-TOF / MS analysis [12–14].

Chromatographic separation was performed on a Waters Acquity UPLC consisting of a quatpump, autosampler and oven (Waters, USA). The samples were eluted using an ACQUITY UPLCTM HSS T_3 column ($100\times 2.1 \text{ mm}$, $1.8 \mu\text{m}$ Waters Corp Milford, USA) with setting column temperature at $40 \text{ }^\circ\text{C}$. The solvent flow rate was $0.40 \text{ mL} / \text{min}$, and $4 \mu\text{L}$ of the sample solution was injected each time. The mobile phase was consisted of 0.1% acetonitrile of formic acid (solvent A) and 0.1% water of formic acid (solvent B). The gradient elution steps are as follows: A; 0–1 min, 2.0–3.6% A; 1–34 min, 3.6–26% A; 34–36 min, 26–100%.

UPLC was coupled to a Q-TOF-MS system (Waters, USA) equipped with an electrospray ionization source (ESI) for scanning samples in positive ion mode. Mass spectrometry was performed by electrospray ionization, MS^E centroid testing was performed in positive and negative modes, and screening was performed over a m/z scan range of 50-2000 D. The scan condition was set to scan every 0.6s. Collision energy was set for two functions. The first function does not use collision energy at low energy, and the second function used collision energy slope of $30 \sim 60 \text{ eV}$ at high energy. In positive mode, the following MS program conditions settings were used: desolvation gas flow: $600 \text{ L}\cdot\text{hr}^{-1}$; drying gas temperature: $400 \text{ }^\circ\text{C}$; cone gas flow: $50 \text{ L}\cdot\text{h}^{-1}$; source temperature $110 \text{ }^\circ\text{C}$; sampling cone 40V ; capillary voltage: 3.0 kV ; collision energy: 6 eV ; Leu-enkephalin ions at m/z 556.2771. In addition, leucine enkephalin (Leu-enk) lockmass solution ($50 \text{ fmol}\cdot\mu\text{l}^{-1}$) at $60 \mu\text{l}$ injection flows parallel to the flow, scans and automatically corrects submitted sequences to verify accurate quality assurance with high quality accuracy throughout the scan range. Masslynx v4.1 software (Waters, USA) was used to control the instrument and also analyzed the data.

5 DPPH free radical scavenging assay

The DPPH assay followed the protocol of Wang with some adjustments [15]. A reaction consisted of 2 ml $0.08 \text{ mg}\cdot\text{ml}^{-1}$ DPPH of absolute alcohol solution and samples depended by each experiment [15]. In the experiment examining the antioxidant capacity of FC-FD, FC-FD added to the reaction at final concentrations of 0.0156 , 0.0313 , 0.0625 , 0.125 , 0.25 , and $0.5 \text{ mg}\cdot\text{ml}^{-1}$. Each sample also had no DPPH blank and negative control. The reaction was carried out at $30 \text{ }^\circ\text{C}$ under light resistance. After the reaction, residual DPPH was evaluated by measuring the absorbance (Abs) at 517 nm . The half maximal inhibitory concentration (IC_{50}) was calculated based on the graph.

The antioxidant activity (AA) at each concentration was calculated by the formula:

$$\text{AA}\% = 1 - \frac{\text{Abs sample} - \text{Abs blank sample}}{\text{Abs negative control}} * 100\%$$

Ascorbic acid was often used as a standard antioxidant. We calculated the DPPH free radical removal rate, and fitted the removal rate to the concentration of nonlinear regression, obtaining the quantitative effect relationship between the two, and getting the corresponding concentration when the clearance rate was 50%, so as to obtain the respective IC_{50} values.

6 Climbing assay, Subacute damage assay and lifespan assay of drosophila melanogaster

The wild-type strain of *Drosophila* was taken from The *Drosophila* Technology Platform of The Shanghai Academy of Life Sciences of The Chinese Academy of Sciences and used it in all the experiments. *Drosophila* were brought up in bottles at 25°C, 35–40% humidity, and 12 hours dark/light cycle. The basic medium contained 16 g of sugar, 26.8 g of corn flour, 10 g of yeast, 4 g of agar, and 400 ml of distilled water with adding propionic acid prevented mold growth [16–17]. FC-FD powder was dissolved in the basic medium, and the final concentration of FC-FD supplementary medium was 0.025%, 0.0125% and 0.00625% (w/v), respectively. Then pour 5 ml of the prepared medium into each bottle. All experiments using the DAMS system in the present study were performed at 25°C.

During the climbing test, the unhatched *drosophila* within 12 hours were divided into four groups, male and female, according to their sex ($n = 10$). The four groups were control group, low dose group, medium dose group and high dose FC-FD group. The control group was given basic medium, and the low, medium and high dose FC-FD groups were given medium supplemented with FC-FD for 7 days at doses of 0.025%, 0.0125% and 0.00625% (w/v), respectively. *Drosophila* were maintained under standard conditions. On days 10, 20 and 30, the *drosophila* was transferred into the empty bottle (bottle length was 10 cm; 0.7 cm in diameter). With a tap from the bottom, the *drosophila* moved from the bottom to the top. The number of *Drosophila* that climbed more than 8 cm from the bottom in 10 seconds was counted after the DAMS system flooded for 1 min. In each experiment, the climbing index was calculated as the climbing length. Each trial was repeated five times.

In subacute damage assay, we took 30-day-old *drosophila* and separated by sex. Then, they were divided into four groups ($n = 100$ each) after being hungry for 2 h, which four groups were given 20% H_2O_2 with 6% glucose solution and the same dose of FC-FD as the climbing assay, respectively. We calculated the longest survival time and the extension rate until all were dead.

In lifespan assay, unhatched *drosophila* during 12 h separated by sex and were divided into four groups ($n = 100$ each). The four groups were given the same dose of FC-FD for 7 days as the climbing assay and the environmental conditions also were same. From these cultures, 100 *Drosophila* were collected from 0–7 day and maintained in a new vial. Survivors were transferred to a new vial within every 4-day interval and the maximal lifespan was counted until all were dead. There were at least three replicate bottles for each experiment [18].

7 Hematoxylin-eosin staining (HE) assay, PCNA assay and elisa assay

40 adult inbred guinea pigs (5 weeks old, 320–350 g) were obtained from the Animal Experimental Center of Heilongjiang University of Traditional Chinese Medicine. And the study was approved by national

legislation of China and local guidelines. All animals were placed in a SPF grade laboratory under normal temperature ($25 \pm 1^\circ\text{C}$) and normal humidity (40–50%). Food and running water were provided at random. All animal studies were approved by the Animal Experimental Ethical Committee of Heilongjiang University of Chinese Medicine. The surface of the ears of 32 guinea pigs was applied daily with 5% propranolol emulsion for 4 times a day for 6 to 8 weeks.

The therapeutic drug was administered orally once a day for 24 hours after the last application of propranolol at 9 to 10 weeks of age. Guinea pigs were randomly divided into four groups ($n = 8$). The three treatment groups were: (1) acitretin-group, with acitretin treatments (2.86 mg/kg i.g.). (2) FC-FD high-dose-treated group, with FC-FD treatment (12.8 mg/kg i.g.). (3) FC-FD low-dose-treated group, with FC-FD treatment (3.2 mg/kg i.g.). Dosages of FC-FD were determined on the basis of preliminary experiments. Untreated animals were used as blank controls. Animals were sacrificed 24 hours after the last dose and ear samples were collected. Then, we observed the pathologic status by HE assay and PCNA assay. The secretion of Tumor Necrosis Factor (TNF- α) was detected by elisa assay [19].

The clinical condition assessment grade of psoriasis-like skin was defined as erythema, edema, erosion, dry skin/swarf and scratches according to the clinical dermatological condition, with grades of 0 to 3, respectively. "Skin Score" was defined as the sum of the individual scores (Table 1) [20].

Table 1
Evaluation of the clinical skin conditions of psoriasiform model animals

	Pathological change	Score
Corneous layer	Munro abscess	1.5
	Hyperkeratosis	0.5
	Parakeratosis	1.0
Epidermis	Lengthening of rete ridges	0.5–1.5
	Lack of granular layer	1.0
	Acanthosis	1.0
Dermis	Lymphocytic infiltrate	0.5–1.5
	Papillary papillae congestion	1.0
	Thinning above papillae	0.5

Detection of PCNA protein expression in guinea pig ear tissue according to the guidance of SABC immunohistochemistry kit.

8 Statistic analysis

We processed statistical analysis of data applying SPSS 19.0 software. The data were expressed as means \pm standard deviation (SD). One-way ANOVA and a post hoc LSD test were used for statistical analysis. $P < 0.05$ was considered statistically significant.

Results

1 UPLC-MS detected the FC-FD

The total flavonoids content was 51.5% determined by UV-1601 spectrophotometer at 268 nm. Meanwhile, the content of total flavonoids in FC-FD was 67.2% [10]. The fact was that the compound parent nuclear structure of the FC-FD was dominated by the structure of kaempferol. We used UPLC-Q-TOF/MS technology, and detected the characteristics of FC-FD under ultraviolet conditions and mass spectrometry conditions, and used the MSE and Metabolynx methods for data processing. A Metabolynx analysis of $m/z287$, which was shared with the $m/z287$ of the mountain phenol, was obtained. Comparing this Metabolynx analysis chart (Fig. 1, B) with the UV feature absorption map (*figure S1, A*) detected by UPLC, the types and quantity of flavonoids could be clearly and intuitively distinguished. After MSE and Metabolynx treatment, the FC-FD UV absorption map corresponds to the ion peaks of the mother core $m/z287$ in mass spectroscopy, and the results showed that the magenta FC-FD component mainly contains a series of chemical components of the kaempferol parent nucleus.

2 UPLC-Q/TOF-MS result

After the data of UPLC-Q/TOF-MS was treated standardly, we used Markerlynks to obtain the manages of ion peaks and fragment ions. According to the analysis of the molecular ion peaks and major fragment peaks of each chromatography peak, and compared with the literature and the controls, 14 chemical components of the flavonoid component were initially determined (*figure S2, Table 2*).

3 DPPH Analysis for FC-FD of Antioxidants

In Table 3, results for antioxidant activity (DPPH) for ascorbic acid and FC-FD were given. The semi-inhibitory concentration (IC₅₀) of FC-FD was 0.0520 mg·ml⁻¹, while the IC₅₀ of ascorbic acid was 0.0401 mg·ml⁻¹. So It was important to emphasize that FC-FD had a strong clearance ability to DPPH free radicals. Moreover, the antioxidant capacity of FC-FD was gradually enhanced with the increase of concentration. In the concentration scope of 0.0156 to 0.500 mg·mL⁻¹ of FC-FD, the free radical clearance rate was 22.0–97.1% while in the concentration scope of 0.0625 to 1.00 mg·mL⁻¹ of Ascorbic acid, the free radical clearance rate of 54.8–97.4%, which was showing a high free radical removal ability.

Table 3
Antioxidant effect of flavonoids from *Datura.metel*/L. (n = 5)

Group	FC-FD		Ascorbic acid	
	concentration /mg·mL-1	free radical clearance rate /%	concentration /mg·mL-1	clearance rate /%
1	0.0156	22.0 ± 1.22	0.0625	54.8 ± 1.33
2	0.0313	39.7 ± 0.78	0.125	76.2 ± 1.60
3	0.0625	51.5 ± 0.69	0.250	96.0 ± 0.63
4	0.125	70.3 ± 0.51	0.500	96.8 ± 1.60
5	0.250	94.4 ± 1.30	0.750	97.2 ± 1.33
6	0.500	97.1 ± 0.75	1.000	97.4 ± 1.50

Note: 0.0156, 0.0313, 0.0625, 0.125, 0.25, and 0.500 mg·ml-1 FC-FD were added to 96 well to examine the antioxidant capacity of FC-FD which was showing a high free radical removal ability. Ascorbic acid was used as a standard antioxidant to obtain the respective IC50 values.

4 Effect of FC-FD on the climbing ability of drosophila

In this research, the results of climbing assays revealed that the climbing capacity of male and female drosophila on 10 d at the concentration of 0.025% and male drosophila on 10 d and 20 d at the concentration of 0.0125% could improve the climbing ability of drosophila, compared to control group (Fig. 1). We also found that compared with control, male and female drosophila on 30 d at the any concentration had no significant difference. These results indicated that FC-FD at feasible concentrations could increase the antioxidant ability which maybe bring about the improvement in locomotive ability of drosophila.

5 Effect of FC-FD on the subacute damage of drosophila

In the subacute oxidative injury experiment, the maximum survival time of male and female drosophila showed significant differences at a drug concentration of 0.025% and 0.0125%, compared to control group (Table 4). However, the extension rate of 0.025% group in male and female drosophila reached 92.86% and 100.50%, respectively. While the extension rate of 0.0125% group in male and female drosophila reached 23.65% and 18.54%, respectively. The extension rate of 0.025% group in male and female drosophila had significant difference, compared to 0.0125% group (Fig. 2). The result showed that FC-FD had the strong antioxidant ability. Meanwhile, it can reduce the degree of drosophila damage.

Table 4
Survival time parameters in drosophila exposed to H₂O₂ following FC-FD (n = 100, $\bar{x} \pm$ SD)

Group	Sexuality/number	The Longest survival time(d)	Extension rate(%)
Control	♂n = 100	73.33 ± 6.11	-
	♀n = 100	68.33 ± 1.53	-
0.025%	♂n = 100	144.33 ± 8.74**	96.82
	♀n = 100	137.00 ± 9.54**	100.50
0.0125%	♂n = 100	90.67 ± 13.58*	23.65
	♀n = 100	81.00 ± 6.00*	18.54
0.00625%	♂n = 100	77.67 ± 11.02	5.92
	♀n = 100	73.67 ± 5.13	7.82
Note: *p < 0.05, **p < 0.01 VS. control group			

6 Effect of FC-FD on the lifespan of drosophila

The effects of three different doses of FC-FD on the lifespan of male and female drosophila were studied. The survival time of drosophila treated with FC-FD was longer than that of control group, seeing Fig. 3. In the 0.025% group, male and female drosophila had the longest survival time, and the maximum lifespan was significantly higher than that of the control group ($P < 0.01$). The average lifespan of male drosophila (79.00 days) and female drosophila (72.67 days) were lengthened than that of the control group (56.33 days / 57.33 days). Compared to control, the mean lifespan of male and female drosophila reached 71.30% and 78.89%, respectively. While in the 0.0125% group, the female drosophila only had the longest survival time and significantly longer life expectancy compared with the control group ($p < 0.05$). Compared with the control group, the average lifespan of female drosophila (66.67 days) reached 84.72%. The maximum and average life expectancy of the male drosophila in the 0.025% group was higher than that in the female drosophila, but the difference between the two groups was not statistically significant. Therefore, we speculate that 0.025% may be the most appropriate concentration of FC-FD, which was of interest to future research.

7 Effect of FC-FD in the psoriasisiform model

As shown in Fig. 4(A) and Fig. 5, in the guinea pig control group, the stratum corneum was very thin, and there were 3–5 layers of spinous cells, and the dermal matrix was lined with single-layer columnar cells, and no inflammatory cells were found. Stimulation of propranolol caused psoriatic lesions, including hyperkeratosis, hypokeratosis, acanthosis, cristae extension, and lymphocyte infiltration (Fig. 4A.2). Significant differences in histopathological scores between the control group and the propranolol group presented ($P < 0.001$). Compared with the propranolol group, the histopathological scores were

significantly decreased after FC-FD treatment (10.61 mg/kg and 2.65 mg/kg) (Fig. 4A.5, 6) ($P < 0.01$). Although the application of acitretin and TWP could decrease the HaCaT cells growth, the histopathological score of psoriasiform changed inapparent in guinea pigs.

The expression of PCNA protein in ear specimens was shown in Fig. 4(B). Immunohistochemistry showed that PCNA-expressing cells aggregated in basal and spinous keratinocytes. PCNA protein expression was significantly enhanced by propranolol stimulation (Fig. 4B.9), while the expression was decreased by FC-FD (Fig. 4B.11, 12). In contrast, acitretin showed relatively weaker influence on the expression (Fig. 4B.10). These results indicated that FC-FD might inhibit the hyper-proliferation of the epidermal keratinocytes.

8 Detection the change of TNF- α

The inflammation of the back of the guinea pigs' ear, which caused the increase of TNF- α expression, was nonspecific dermatitis. As shown in Fig. 10, the secretion of TNF- α in the model group was increased, compared to control group. Both the high dose group of FC-FD and Propranolol-treated group significantly blocked the production of TNF- α from the psoriasiform models. That meant that FC-FD may control pro-inflammatory cytokine production at the translational levels. No statistical differences between the positive groups and control group. However, the high and low dose group of FC-FD even the TWP group had significant difference, compared to model group, which fully proved that FC-FD inhibited the occurrence of inflammatory reactions.

Discussion

During our research, the anti-psoriatic active fraction were found, the partial purified flavonoids(FC-FD) and withanolides were separated, simultaneously [21–24]. The UPLC-ESI-Q-TOF-MS^E method was successfully employed for identification of FC-FD. This rapid, efficient, and accurate identification method was efficient for screening 14 compounds. The results provided a basis for the study of pharmacology and pharmacokinetics of Flos Datura, overcome the shortcomings of traditional Chinese medicine chemical analysis to some extent, and promote the development of Chinese medicine research [25].

In order to obtain more information about the functions of FC-FD, we studied their chemical properties and antioxidant and anti-aging activities in vitro and in vivo. FC-FD had a strong ability to remove hydroxyl groups, DPPH and superoxide radicals, and had the strongest scavenging effect on hydroxyl radicals. Furthermore, the maximum lifespan and survival time of drosophila can be extended by FC-FD medium ($P < 0.01$). The results showed that some purified flavonoids extracted from Flos Datura could enhance the antioxidant activity in vivo and in vitro and prolong the lifespan of drosophila.

Flos Datura has been extensively used in TCM for centuries. It has been reported that clinical use of Flos Datura showed a significant effect on the treatment of psoriasis [26–27]. Psoriasis is one of the most common immune-mediated chronic inflammatory skin diseases. This condition is characterized by hyperproliferation of keratinocytes and massive infiltration of white blood cells [28]. Although the

pathogenesis of psoriasis has not been fully understood, there is evidence that IL-10, IL-6, and IFN- α produce many immuno-derived cytokines, including IL-23, IL-17A, IL-20, and IL-22, TNF- γ and TNF- α are involved in the pathogenesis and interaction network of psoriasis [29–36].

In the psoriasis-like guinea pig model, oral FC-FD treatment for 14 days resulted in a significant reduction in histopathological scores and PCNA protein expression. These results provided further evidence that FC-FD ameliorates Propranolol-induced the psoriasiform pathological changes, directly illustrating its effects within the target tissue. Furthermore, after propranolol challenge, FC-FD significantly blocked the production of TNF- α . Therefore, it was suggested that AC-FD may control pro-inflammatory cytokine production at some level. These results demonstrate that FC-FD could be anti-psoriasis inflammatory infiltration.

Conclusion

To sum up, we used UPLC-ESI-Q-TOF-MS^E method to screen 14 compounds from FC-FD, and evaluated the effects on anti-inflammatory and antioxidative in vivo and in vitro. Overall, our findings are beneficial for *Datura metel* L. being developed as a potential natural antioxidants in extending lifespan and anti-psoriatic inflammatory drugs. Further studies should clarify the mechanism of above-mentioned effects of *Datura metel* L.

Abbreviations

FC-FD	Flavonoids components of Flos Daturae
UPLC	Ultra-performance Liquid Chromatography
ESI	Electrospray Ionization
Q-TOF	Quadrupole Time-of-Flight
MS ^E	Mass Spectrometry
AF-FD	The active fraction of Flos Daturae
DPPH	2,2-Diphenyl-2-picrylhydrazyl
PCNA	Proliferating Cell Nuclear Antigen
TNF- α	Tumor necrosis factor
UV	Ultra Violet
HE	Hematoxylin-eosin staining
PCNA	Proliferating Cell Nuclear Antigen
TCM	Traditional Chinese Medicine

Declarations

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Authors' contributions

WYY designed, organized, and supervised the study. ZH, WZ, MYH, YBY and LTL contributed to literature review and data analyses. KHX contributed to the project design and paper writing. All authors read and approved the final manuscript.

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Availability of data and materials

All the data used to support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

All animal studies were approved by the Animal Experimental Ethical Committee of Heilongjiang University of Chinese Medicine.

Consent for publication

We declare that the Publisher has the Author's permission to publish the relevant Contribution.

Competing interests

The authors declare that they have no competing interests.

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Tables

Due to technical limitations, table 2 is only available as a download in the Supplemental Files section.

Figures

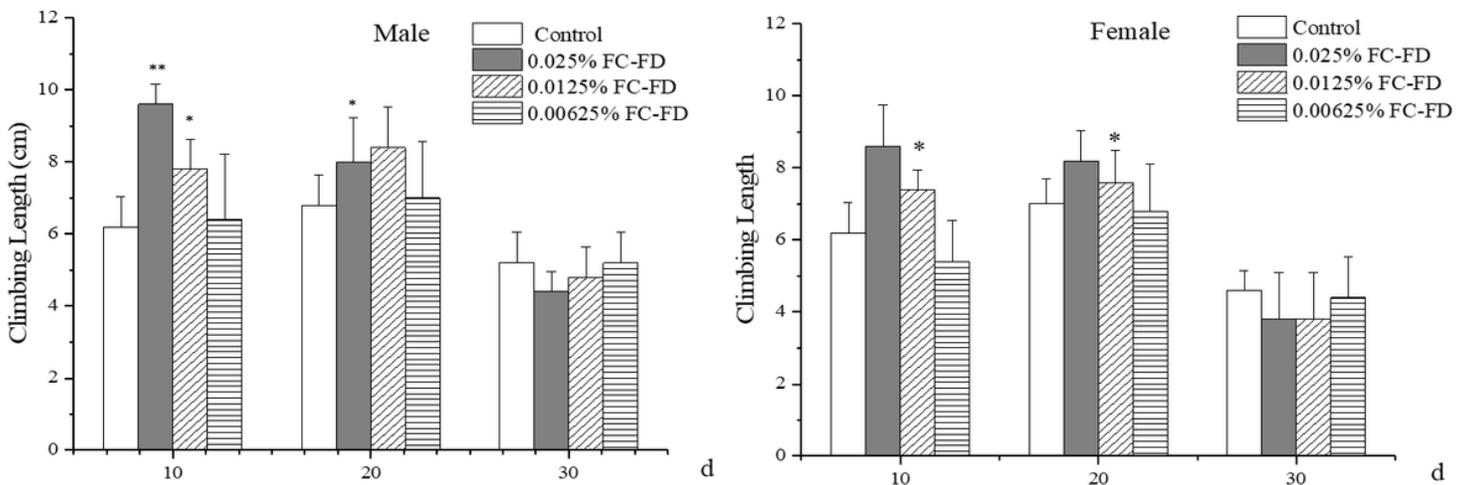


Figure 1

Climbing ability in drosophila following FC-FD. The unhatched drosophila within 12 hours were fed 0.025%, 0.0125%, and 0.00625% FC-FD for 7 days, respectively. On days 10, 20 and 30, the drosophila were transferred into the empty bottle (bottle length was 10 cm; 0.7 cm in diameter). The number of drosophila that climbed more than 8 cm from the bottom in 10 seconds was counted after the DAMS system flooded for 1 min. * $p < 0.05$ vs. control group; ** $p < 0.01$ vs. control group ($n = 10$, $\bar{x} \pm SD$).

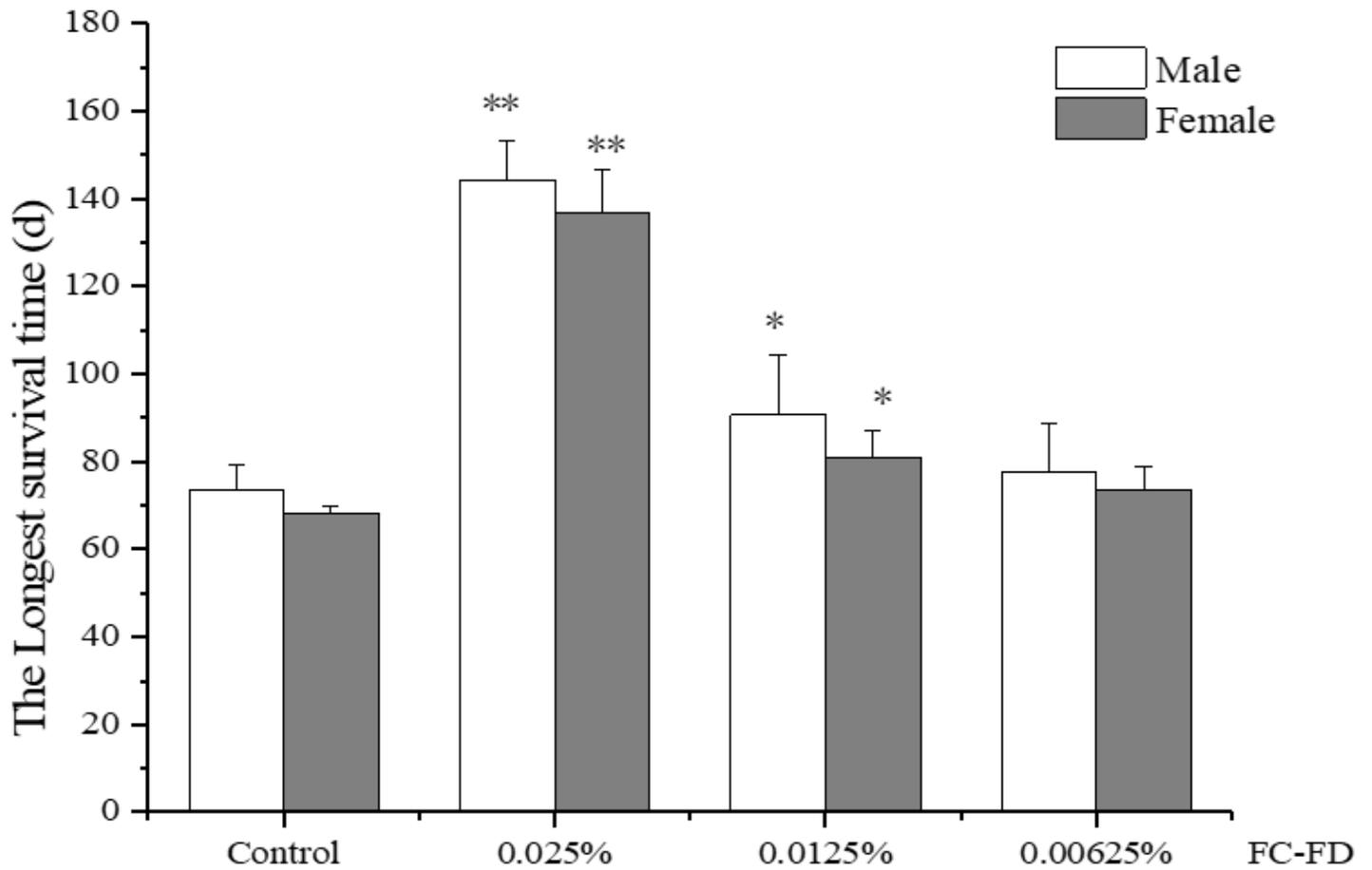


Figure 2

Survival time parameters in drosophila exposed to H₂O₂ following FC-FD. Drosophila at 30 days old were selected and fed in medium containing 0.025%, 0.0125% and 0.00625% FC-FD with the same dose respectively. Meanwhile, 20% H₂O₂ with 6% glucose solution was given, and the life condition was tested until all the drosophila died. * p<0.05 vs. control group; ** p<0.01 vs. control group (n=100, $\bar{x} \pm SD$).

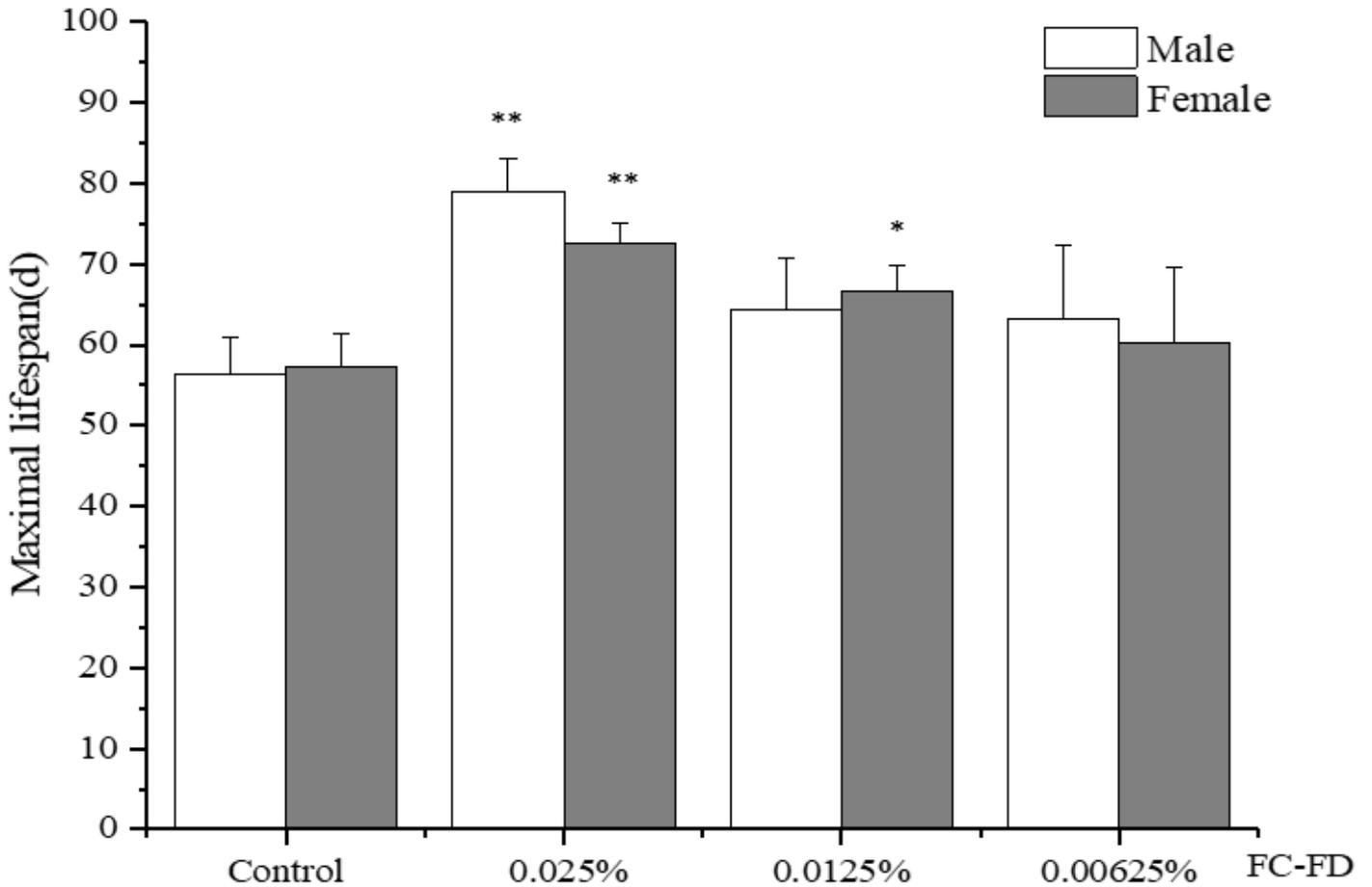


Figure 3

Lifespan parameters in drosophila following FC-FD. 100 unhatched drosophila during 12 h were collected for 7 days and separated by sex. They were fed FC-FD at different concentrations of 0.025%, 0.0125% and 0.00625%, respectively. Then they were transferred to a new vial within every 4-day interval until all the drosophila died. * $p < 0.05$ vs. control group; ** $p < 0.01$ vs. control group ($n = 100$, $\bar{x} \pm SD$).

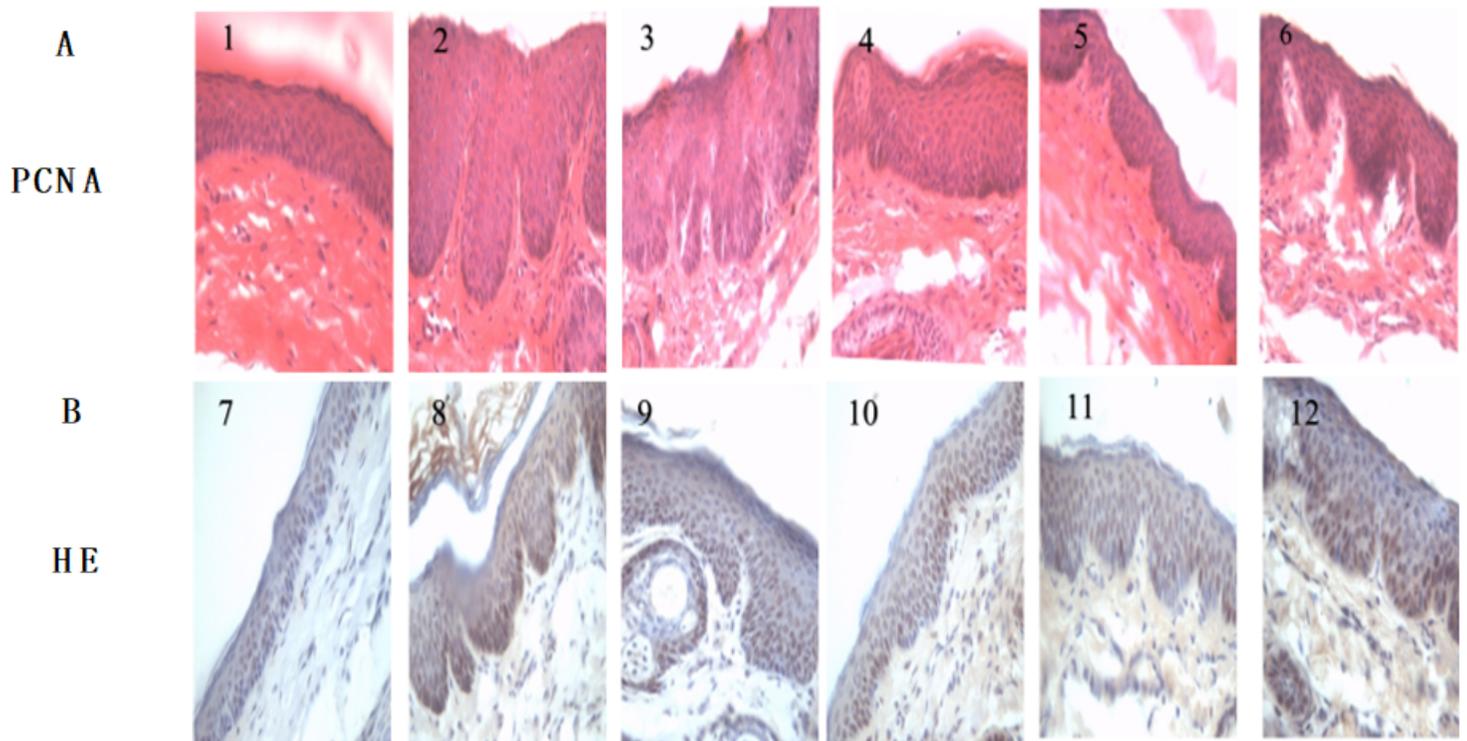


Figure 4

(A) Effect of FC-FD on psoriasiform changes in the psoriasiform model. 1: Control group; 2: propranolol-treated group; 3: 2.86 mg/mL acitretin group; 4: 7.34 mg/mL TWP group; 5: 10.61 mg/mL FC-FD group; 6: 2.65 mg/mL FC-FD group. A significant difference in histopathological score was detected between the control group and the propranolol group ($P < 0.001$). Histopathological score decreased significantly after FC-FD (12.8 mg/kg and 3.20 mg/kg) treatment (figure A.5, A.6) compared with that of the propranolol treatment group ($P < 0.01$). Although the application of acitretin could decrease the HaCaT cells growth, the histopathological score of psoriasiform changed inapparent in guinea pigs. (B) Effect of FC-FD on PCNA protein changes in the ear sections. 7: Control group; 8: propranolol-treated group; 9: 2.86 mg/mL acitretin group; 10: 7.34 mg/mL TWP group; 11: 10.61 mg/mL FC-FD group; 12: 2.65 mg/mL FC-FD group. Propranolol challenge significantly enhanced the expression of PCNA protein, compared with the control group. Furthermore, after propranolol challenge, FC-FD treatment decreased the expression of PCNA protein. The application of acitretin after propranolol challenge had no influence on the expression of PCNA protein, which is different from the propranolol group.

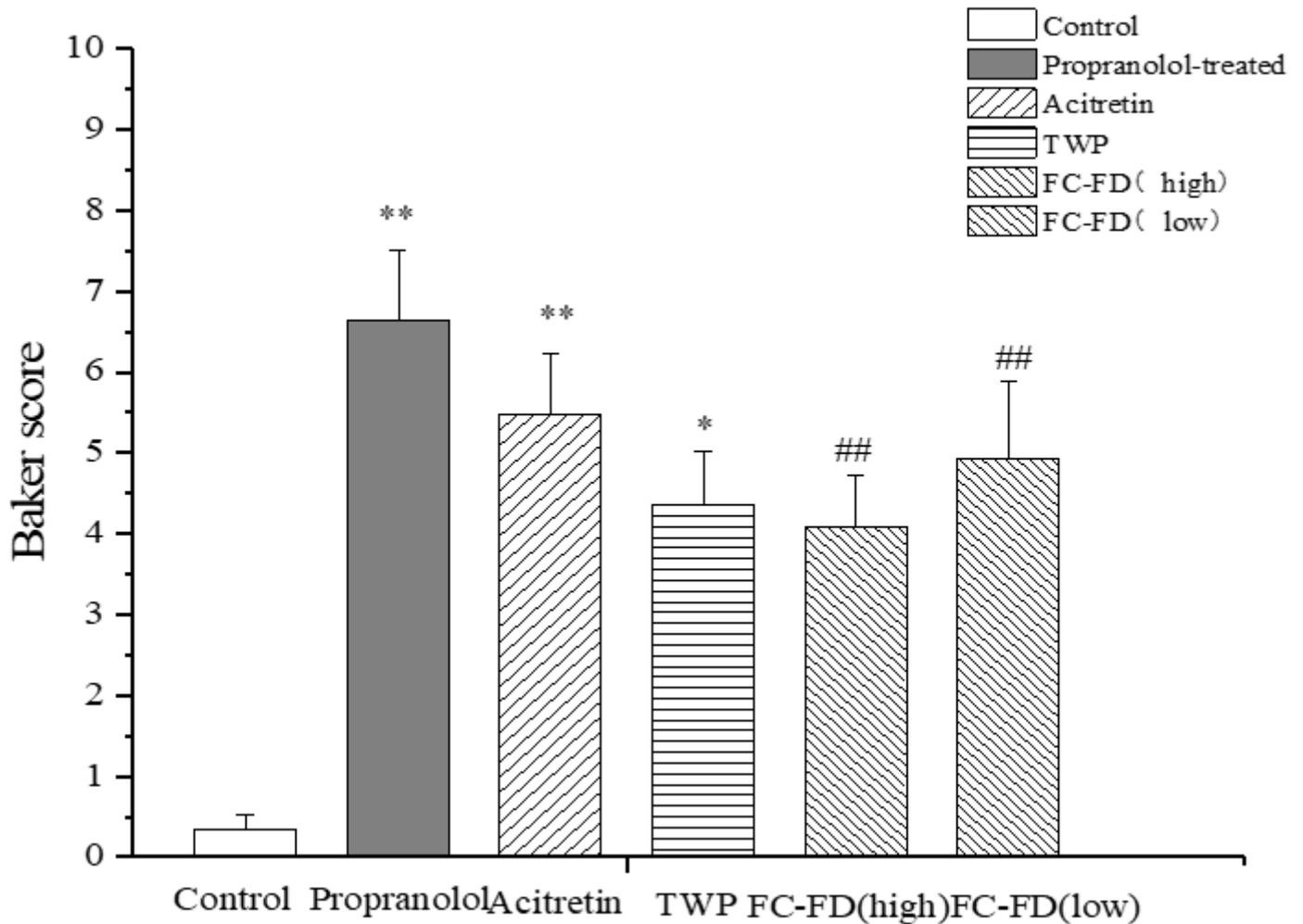


Figure 5

Effect of FC-FD in the psoriasiform model. The clinical condition assessment grade of psoriasis-like skin was defined as erythema, edema, erosion, dry skin/swarf and scratches according to the clinical dermatological condition, with grades of 0 to 3, respectively. "Skin Score" was defined as the sum of the individual scores. * $p < 0.05$ vs. control group; ** $p < 0.01$ vs. control group; ## $p < 0.01$ vs. propranolol-treated group ($n=8$, $\bar{x} \pm SD$).

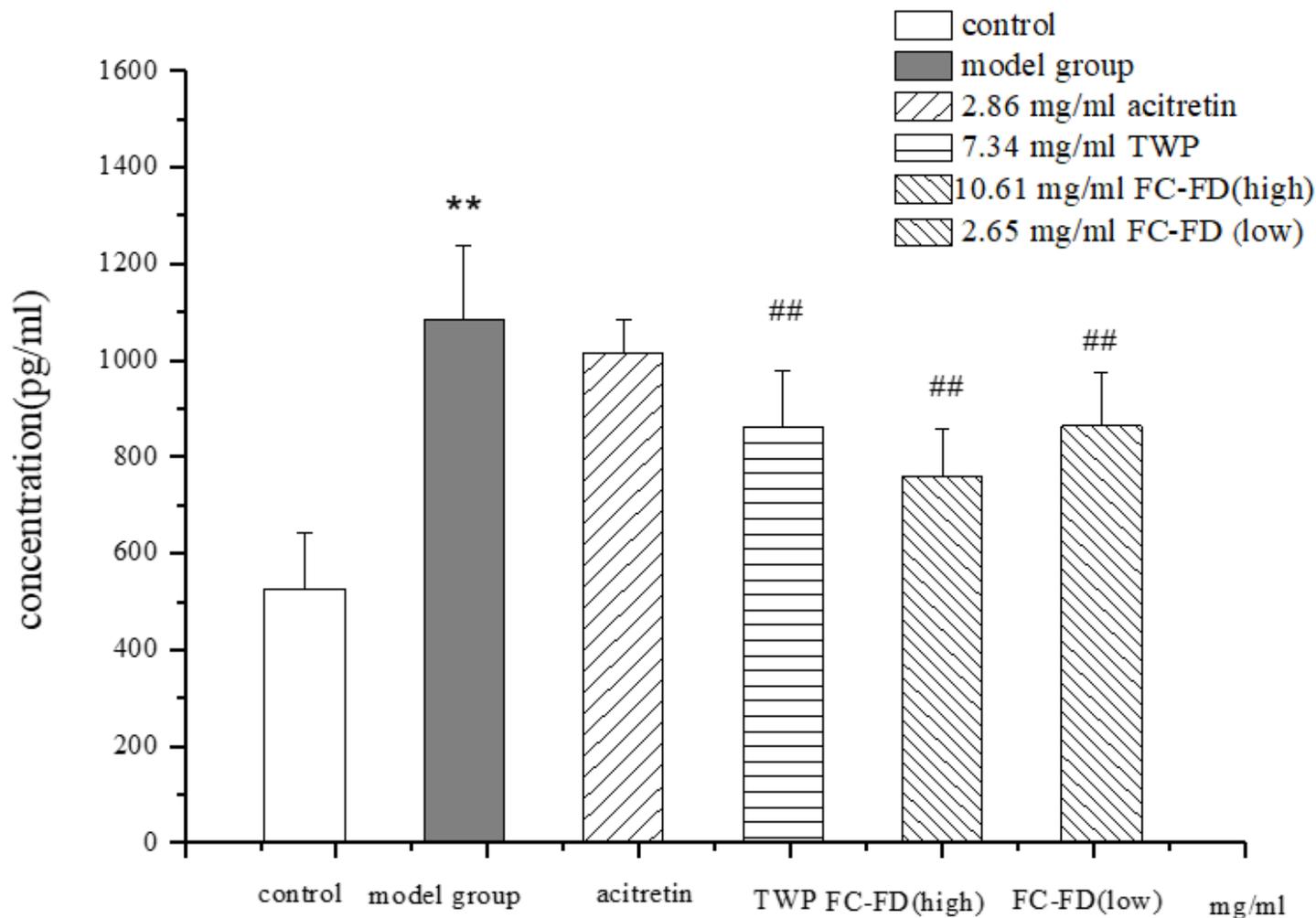


Figure 6

Effect of psoriasis-like skin damage on the secretion of TNF- α in the back of guinea pigs' ear. The guinea pig ear samples were ground in a grinder, centrifuged and centrifuged to extract the supernatant, then 50 μ l of samples were collected. The absorbance value was determined at 470 nm according to the instructions of TNF- α Elisa. ** $p < 0.01$ vs. control group; ## $P < 0.01$ vs. model group ($n = 8, \bar{x} \pm SD$)

Supplementary Files

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