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Pharmacodynamics of frigid zone plant *Taxus cuspidata S. et Z.* against skin melanin deposition, oxidation, inflammation and allergy

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Abstract

Background: *Taxus cuspidata S. et Z.* is a precious species of frigid zone plant belonging to the Taxaceae family, which possesses anticancer, anti-inflammatory, hypoglycemic, and antibacterial pharmacological properties. While taxane extracted from Taxus chinensis has been reported to elicit antioxidant activities, whether *Taxus cuspidata S. et Z.* has skin-protective actions against injuries remained unknown. This study aims to explore the pharmacological effects of three Taxus extracts on skin melanin deposition, oxidation, inflammation, and allergy so as to provide new ideas for the prevention and treatment of various diseases related to skin damage. **Methods:** Skin melanin deposition was evaluated by measuring melanin content in the skin of guinea pigs by alkali lysis method. Antioxidant capacity was evaluated by measuring superoxide dismutase (SOD) concentration and glutathione (GSH) content in skin tissue homogenates of Kunming mice by SOD assay kit and micro reduced GSH assay kit. The quantitative real-time polymerase chain reaction (qRT-PCR) and western blotting were used to examine the levels of both SOD and recombinant glutathione peroxidase 4 (GPX4). Skin inflammation was evaluated by xylene-induced ear swelling test and egg-white-induced paw swelling test in mice. In a mouse model of skin allergy induced by 4-aminopyridine (4-AP) , allergy was determined by licking body counts and histamine concentrations in tissue homogenates using enzyme-linked immunosorbent assay (ELISA) kits. Two proinflammatory factors tumor necrosis factor (TNF)-α and interleukin (IL)-1β were measured by qRT-PCR. Hematoxylin and eosin (HE) staining was conducted to assess the degree of skin lesion. **Results:** All three Taxus extracts including Taxus chinensis essential oil, Taxus chinensis extract and Taxus chinensis extract compound reduced the melanin deposits in the back skin relative to the non-treated control animals, of which Taxus chinensis essential oil produced the greatest effect. In contrast, the three Taxus extracts elevated SOD and GSH levels in the skin tissues, and the highest increase was seen with Taxus chinensis essential oil. Three Taxus extracts, especially Taxus chinensis essential oil, effectively reduce the rate of ear and paw swelling. All three Taxus extracts reduced the number of body licks, the levels of TNF-α and IL-1β, and the histamine content in tissue homogenates of mice and alleviated skin damage. Consistently, Taxus chinensis essential oil yielded the greatest magnitude of decreases. **Conclusion:** While all three Taxus extracts possessed the anti-skin melanin deposition, oxidation, and allergy properties, Taxus chinensis essential oil produced the superior effects.

Keywords

Taxus cuspidata S. et Z.; skin melanin deposition; oxidation; inflammation; allergy

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1 Introduction

The role of skin in the human body is indispensable, serving as a barrier to moderate homeostasis[1]. Damaged skin epidermis and dermis lead to a variety of pathological phenotypes, including hyperpigmentation, skin oxidation, skin inflammation and allergies. Chronic exposure of mammalian skin to solar ultraviolet (UV) radiation can induce multiple serious biological reactions, including hyperpigmentation, skin cancer development, skin oxidation, photoaging, erythema, edema, and sunburn cell formation[2]. It has been reported that dark melanin protects against the deleterious effects of UV radiation[3]. Melanin is a key

component of the pigmentary systems of human skin, eyes and hair, which is produced by melanocytes through a process of melanogenesis[4]. However, hyperpigmentation can be induced by constant solar UV radiation. The total number of melanocytes in human skin decreases with age[5], and aged skin often exhibits irregular pigmentation that is associated with hyperpigmentation[6]. Therefore, regulating melanogenesis is a crucial strategy for the treatment of hyperpigmentary disorders[7].

Skin oxidation is affected by both intrinsic and extrinsic factors. Extrinsic factors include solar radiation and cigarette smoke, while intrinsic factors may be the degree of pigmentation[8]. Skin oxidation is associated with skin aging, a highly complex and not yet fully understood process in which reactive oxygen species (ROS) plays an important role in both over time and radiation ageing of the skin[9]. More precisely, oxidative stress induced by the accumulation of ROS can damage lipid, protein, nucleic acid, and organelle, thus leading to the occurrence of cellular senescence, which is one of the core mechanisms mediating skin aging[10-11]. Hence, it is essential to maintain the balance of oxidation and antioxidant reactions in human body.

The epithelial barrier constitutes the first line of physical, chemical, and immune defenses and provides a protective wall against environmental factors[12]. Skin inflammation occurs once the barrier is invaded[13]. Allergic dermatitis is the most common chronic inflammatory skin disease with a lifetime prevalence of up to 20% and substantial impacts on quality of life, characterized by intense pruritus, recurrent eczematous lesions and fluctuations in the course of the disease driven by the differentiation of two terminal keratinocytes[14-16]. Moreover, allergic diseases have in common a dysfunctional epithelial barrier, which allows the penetration of allergens and microbes, leading to the release of type 2 cytokines that drive allergic inflammation[17]. Furthermore, effective management of atopic dermatitis requires a multipronged approach, such as restoring cutaneous barrier function, modulating microbial flora and immune homeostasis, and enhancing skin epithelial differentiation[17]. Current treatments for allergic dermatitis include topical moisturizers, anti-inflammatory agents, phototherapy and systemic immunosuppressants.

Taxus cuspidata S. et Z. is a kind of precious frigid plant belonging to the Taxaceae family[18], which mainly grows in Chinese Northeast and distributes in Laoye Ridge, Zhangguangcai Ridge and Changbai Mountains, Jilin, China. Since the discovery of paclitaxel isolated from the bark of Pacific Taxus (Taxusbrevifolia) in the 1960s, the genus Taxus has been the main source of novel compound discovery by medicinal chemistry/pharmacy[19]. Meanwhile, it is meaningful to investigate whether Taxus extracts have other pharmaceutical effects. Considerable traditional Chinese medicine (TCM) extracts have been reported for their efficacies in the treatment of diverse skin-related diseases. For instance, Aoki and his colleagues showed that Oolong tea extract could inhibit the progression of melanogenesis[20], and Shi *et al.* found that Gingko extract has inhibitory effects on oxidative damage[21]. Cao *et al.* revealed that kaempferol extracted form Kaempferia galanga L has an inhibitory effect on anaphylaxis[22]. However, the potential pharmacological effects of Taxus on skin-related diseases remained unexplored.

In the present study, we aim to reveal the pharmacological effects of Taxus on skin damage, focusing on whitening, anti-oxidative, anti-inflammatory and anti-allergic efficacies. The results demonstrate that three kinds of Taxus extracts (Taxus chinensis essential oil, Taxus chinensis extract and Taxus chinensis extract compound) have beneficial effects on skin disorders, among which, the essential oil extract possesses the prominent whitening, anti-oxidative, anti-inflammatory, and anti-allergic effects. These newly identified pharmacological effects of Taxus in addition to its anti-tumor property suggest the possibility of expanding the horizon of its clinical applications.

2 Materials and methods

2.1 Drug preparation

2.1.1 Preparation of Taxus chinensis extract compound solution

Olive oil of 50 mL was mixed with 50 mL of saline, followed by addition of 2 mL of Tween-80 and mix by vortex shaking. A total of 5 g Taxus chinensis extract powder was added into the mixed solution in several batches with each in a small quantity, which was then subject to vortex shaking to obtain Taxus chinensis extract compound solution (50 mg/mL).

2.1.2 Preparation of Taxus chinensis extract solution

After 50 mL of olive oil had been mixed with 50 mL of saline, 2 mL of Tween-80 was added and mixed well by vortex shaking. A total of 5 g Taxus chinensis extract powder was added into the mixed solution in several batches with each in a small quantity, and the solution was mixed well by vortex shaking to obtain Taxus chinensis extract solution (50 mg/mL).

2.2 Evaluation of anti-skin melanin deposition

A total of healthy adult black guinea pigs were randomly divided into 5 groups (N = 10 for each): control group, Taxus chinensis essential oil group, Taxus chinensis extract compound group, Taxus chinensis extract group, and positive control group (6.5%

arbutin). The hairs on both sides of the guinea pigs' backs were shaved (2 cm × 3 cm), and these three extracts of Taxus chinensis and arbutin were applied to the skin surface of the respective experimental groups. After 28 days of administration, the guinea pigs were executed by cervical dislocation and photographed. The skin tissue was cut off at the site of drug administration and trimmed to 10 mg pieces. The skin specimen was grinded thoroughly with a grinding rod in a grinder containing 1 mL of NaOH (1N, containing 5% DMSO). The preparation was then subject to measurement of optical density (OD) values at 405 nm (the OD value indicates the melanin content).

2.3 Evaluation of anti-oxidation

Sixty healthy Kunming mice (20 g ± 5 g) of equal numbers of males and females were randomly divided into 6 groups (*N* = 10 for each group): control, allergic dermatitis, Taxus chinensis essential oil, Taxus chinensis extract compound, Taxus chinensis extract, and positive control (acetone) groups. Allergic dermatitis was established by removing the hair on the back of mice (2 cm × 3 cm) with remover lotion (Nair, USA) to expose the back skin surface, which was immediately smeared with 1% 2, 4-dinitrochlorobenzene (DNCB). These three extracts and acetone were applied separately to the skin of the depilated area once daily for 7 days. The control group received an equal volume of distilled water.

2.3.1 Determination of skin SOD concentration and GSH content

After 7 days, the mice were immediately sacrificed by cervical dislocation. An equal area (1.5 cm × 2.0 cm) of allergic dermatitis skin was cut off from the same area on the back of mice, and tissue homogenates were prepared. The superoxide dismutase (SOD) concentration and glutathione (GSH) content were determined by SOD assay kit (GC-NJ-A001-3-2, Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and micro reduced glutathione (GC-NJ-A006-2-1, Nanjing Jiancheng Bioengineering Institute, Nanjing, China) assay kit, respectively.

2.3.2 RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA samples were extracted from dorsal skin of mice by using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. The quality of the RNA samples was measured by NanoDrop ND-8000 to ensure the RNA/DNA ratio of 1.8-2.0. Integrity of RNAs was assessed by standard denaturing agarose gel electrophoresis. RNA was reversely transcribed to cDNA using Reverse Transcription Kit (No.4368814, Applied Biosystems, Carlsbad, USA). Real-time PCR was then performed with SYBR Green (04913914001, Roche, Basel, Swiss). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. Each sample was analyzed in triplicate. The sequences of primer pairs used in the present study are as follows:

SOD: forward 5'-AACCAGTTGTGTTGTCAGGAC-3' and reverse 5'-CCACCATGTTTCTTAGAGTGAGG-3';
Recombinant Glutathione Peroxidase 4 (GPX4): forward 5'-TGTGCATCCCGCGATGATT-3' and reverse 5'-CCCTGTACTTATCCAGGCAGA-3';
GAPDH: forward 5'-AAGAAGGTGGTGAAGCAGGC-3' and reverse 5'-TCCACCACCCAGTTGCTGTA-3'.

2.3.3 Western blot analysis

Total protein was extracted from the skin of Kunming mice using the same procedures as described in detail elsewhere[23]. The protein concentrations were determined by BCA Protein Assay (P0009, Beyotime Co., Ltd, Shanghai, China) kit. Equal amounts of protein lysates were separated by SDS-PAGE and transferred onto nitrocellulose membranes followed by blocking with 5% skimmed milk at room temperature for 1 hour. Subsequently, the membranes were incubated with the primary antibodies against GPX4 (1:1 000, ab252833, Abcam Co., Ltd, Cambridge, UK) and GAPDH (1:2 000, 60004-1-Ig, Proteintech Co., Ltd, Chicago, USA) at 4°C overnight. After washing with PBST three times, the membranes were incubated with the fluorescence-conjugated anti-rabbit or anti-mouse IgG secondary antibody (1:10 000) for 1 hour. Western blot bands were examined and analyzed by using the Odyssey Imaging System (LI-COR, Inc., Lincoln, USA).

2.4 Evaluation of anti-inflammatory

Fifty healthy Kunming mice (20 g ± 5 g) of equal numbers of males and females were randomly divided into 5 groups (*N* = 10 for each): control group, Taxus chinensis essential oil group, Taxus chinensis extract compound group, Taxus chinensis extract group, and positive control group (Dexamethasone ointment).

2.4.1 Xylene-induced ear swelling

The mice in each group were evenly coated with the three extracts or hexadecadrol on both sides of the left ear, once daily for 5 d at a dose of 0.02 mL. After 30 minutes of the last administration, the left ear was inflamed by applying 0.02 mL of xylene and the right ear was used as control. After 30 minutes of inflammation, the mice were executed by cervical dislocation, and the left and right round-shaped ear tissues were dissected from the same areas of both ears with an ear punch. The ear specimens were weighed,

and the ear swelling degree and swelling rate were calculated according to the following formula:

Swelling degree = left ear-piece weight – right ear-piece weight
Swelling rate = Swelling degree value / right ear-piece weight × 100%

2.4.2 Egg white-induced paw swelling

The mice in each group were smeared with three extracts or hexadecadrol on the left rear foot twice a day for 5 consecutive days. After 30 minutes of the last administration, the toes of mice were inflamed with 10% egg white solution. The left rear foot volume was measured with a vernier caliper before inflammation and 0.5, 1.0, 3.5, 5.0 and 7.0 hours after inflammation. The data were recorded, and the paw swelling rate was calculated according to the following formula:

(Post-inflammatory foot volume – pre-inflammatory foot volume) / pre-inflammatory foot volume × 100 %

2.5 Evaluation of anti-allergy

Sixty healthy Kunming mice (20 g ± 5 g), half males and half females, were randomly divided into 6 groups (N = 10 for each): control group, skin allergy model group (4-Aminopyridine [4-AP]), Taxus chinensis essential oil group, Taxus chinensis extract compound group, Taxus chinensis extract group, and positive control group (hexadecadrol ointment).

2.5.1 Antipruritic test

The back skin was depilated (2 cm × 3 cm) using 8% sodium sulfide alcohol solution, three extracts or hexadecadrol was applied to the skin of the depilated area once a day for 7 days. The control group and skin allergy model group were coated with equal volumes of distilled water. After 40 minutes of the last administration, 0.01 mL/g of 4-AP (0.02% with saline) solution was injected subcutaneously at the application site on the back of the neck, and the control group was injected with an equal volume of saline. The licking times of mice in each group within 10 minutes were recorded (one licking time was calculated from continuous licking to a short pause).

2.5.2 Determination of skin histamine concentration

After the antipruritic test, the mice were immediately sacrificed by cervical dislocation. An equal area (1.5 cm × 2 cm) of allergic skin was cut off from the same area on the back of mice, and tissue homogenates were prepared. The skin histamine concentration was measured by an enzyme-linked immunosorbent assay (E-EL-0032c-96T, Elabscience Biotechnology Co., Ltd, Wuhan, China) kit.

2.5.3 HE staining

The dorsal skin specimen of Kunming mice was fixed in 4% paraformaldehyde, then embedded with paraffin, and cut cross-sectionally into 5-μm thick sections. Tissue sections were deparaffinized and stained with hematoxylin and eosin (HE) reagent. Images of the preparations were captured with a light microscope (Carl Zeiss Microscopy, Germany) and were analyzed using Image J software.

2.5.4 RNA Extraction and qRT-PCR

Total RNA samples were extracted from the dorsal skin of Kunming mice by using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. And the subsequent procedure is the same as described in section of Evaluation of anti-oxidation. The sequences of primer pairs are as follows:

Tumor Necrosis Factor (TNF)-α: forward 5'-CAGGCGGTGCCTATGTCTC-3' and reverse 5'-CGATCACCCCGAAGTTCAGTAG-3';
Interleukin (IL)-1β: forward 5'-GAAATGCCACCTTTTGACAGTG-3' and reverse 5'-TGGATGCTCTCATCAGGACAG-3'.

2.6 Statistical analysis

Statistical analyses were performed using GraphPad Prism 8 (GraphPad Software, San Diego, USA). All data are presented as the standard error of the mean (mean ± SE). Statistical comparisons were performed by Student's *t*-test between two groups. Differences among groups were analyzed by one-way ANOVA by Dunnett's test. $P < 0.05$ was considered statistically significant.

All procedures were approved by the Institutional Animal Care and Use Committee of Harbin Medical University (Protocol [2009]-11) (No. IRB3017621). The use of animals was compliant with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). All sacrifices were performed under anesthesia, and every effort was made to minimize animal suffering.

3 Results

3.1 Anti skin melanin deposition effect of three Taxus chinensis extracts

Previous studies have shown that melanin is the main cause

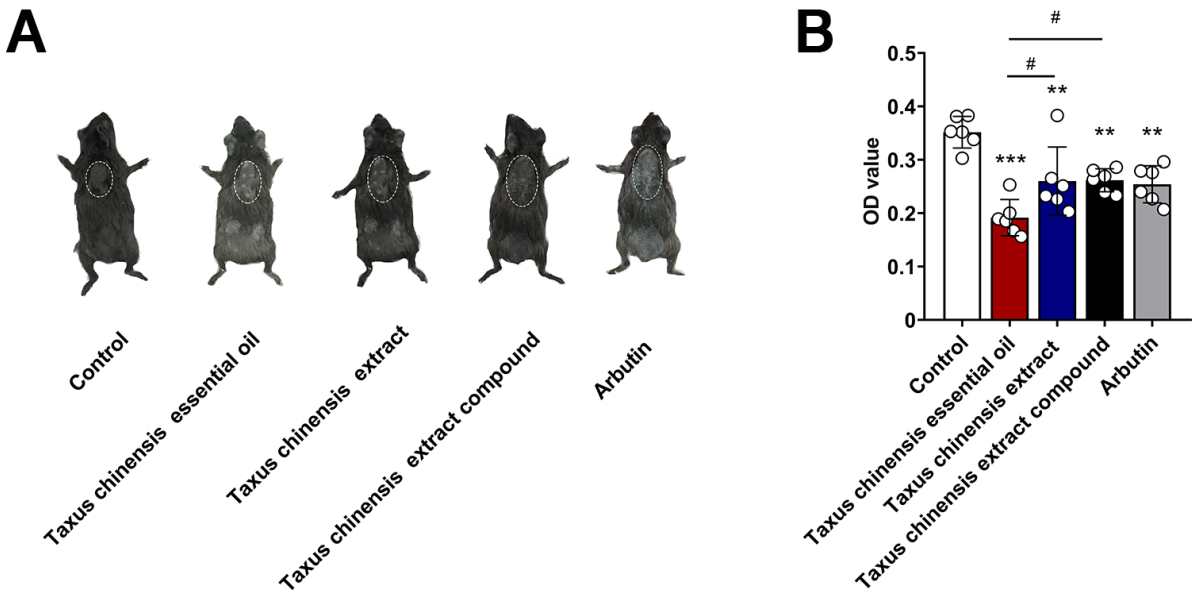


Fig. 1 Anti skin melanin deposition effect of three Taxus chinensis extracts ($N = 6$)

(A) Representative images of guinea pigs in each group after 28 days of administration; (B) Melanin content of back skin of guinea pigs in each group after 28 days of administration; Data represent the mean ± SE; $^{**}P < 0.01$; $^{***}P < 0.001$ *vs.* Control group; $^{\#}P < 0.05$ *vs.* Taxus chinensis essential oil group.

of black and brown plaque deposition. In order to explore the potential inhibitory effect of Taxus chinensis on skin melanin deposition, we conducted experiments with black adult guinea pigs. After 28 days of administration of Taxus chinensis extracts on skin once a day, the skin color on the back of guinea pigs in each group altered. Among the three extracts of Taxus chinensis, the skin of guinea pigs in the essential oil group was significantly whiter than that in the control group compare with administration of Taxus chinensis extract and Taxus chinensis extract compound (Fig. 1A). The content of melanin in guinea pig skin tissues was detected by alkali lysis method. Compared with the control group, all three extracts of Taxus chinensis reduced the melanin content in guinea pig skin tissues, with the most significant reduction being observed in the Taxus chinensis essential oil group (Fig. 1B). Overall, all three extracts of Taxus chinensis can reduce skin melanin deposition, with Taxus chinensis essential oil performing the best whitening effect.

3.2 Anti skin oxidation effect of three Taxus chinensis extracts

At the same time, we explored the anti-oxidative effect of Taxus chinensis. Allergic dermatitis was established by removing the hair on the back of mice, exposing the back skin, and smearing with 1% DNCB. The three Taxus chinensis extracts or positive drug acetone was applied to the skin of the depilated area once daily for 7 days. The skin damage induced by DNCB was reversed by three extracts of Taxus chinensis and acetone as well, yet Taxus chinensis essential oil and acetone produced stronger anti-oxidative efficacies than the other two extracts (Fig. 2A). The allergic dermatitis skin tissues of the same size were cut off from the same area on the back after cervical dislocation execution of mice, and tissue homogenates were prepared to measure the SOD concentration and GSH content. The results showed that only Taxus chinensis essential oil increased the skin SOD concentration in mice treated with 1% DNCB, and none of the three Taxus chinensis extracts affected GSH content (Fig. 2B and C). To provide further evidence for the anti-oxidative effect of Taxus chinensis extracts, qRT-PCR and western blotting were used to detect the mRNA and protein level of SOD and GPX4. The results demonstrated that the decrease in mRNA level of SOD induced by DNCB was reversed by all three Taxus chinensis extracts and acetone. Consistently, among all three Taxus chinensis extracts, Taxus chinensis essential oil gave rise to the greatest increase in the mRNA level of SOD (Fig. 2D). Both mRNA and protein levels of GPX4 were diminished by DNCB, effects mitigated by either Taxus chinensis essential oil or acetone, but not by Taxus chinensis extract compound or Taxus chinensis extract (Fig. 2E). The data suggest that Taxus chinensis essential oil elicit anti-oxidative effect essentially *via* increasing the expression of anti-oxidant enzymes SOD and GPX4.

3.3 Anti skin inflammatory effects of three Taxus chinensis extracts

In order to investigate the effect of Taxus chinensis against

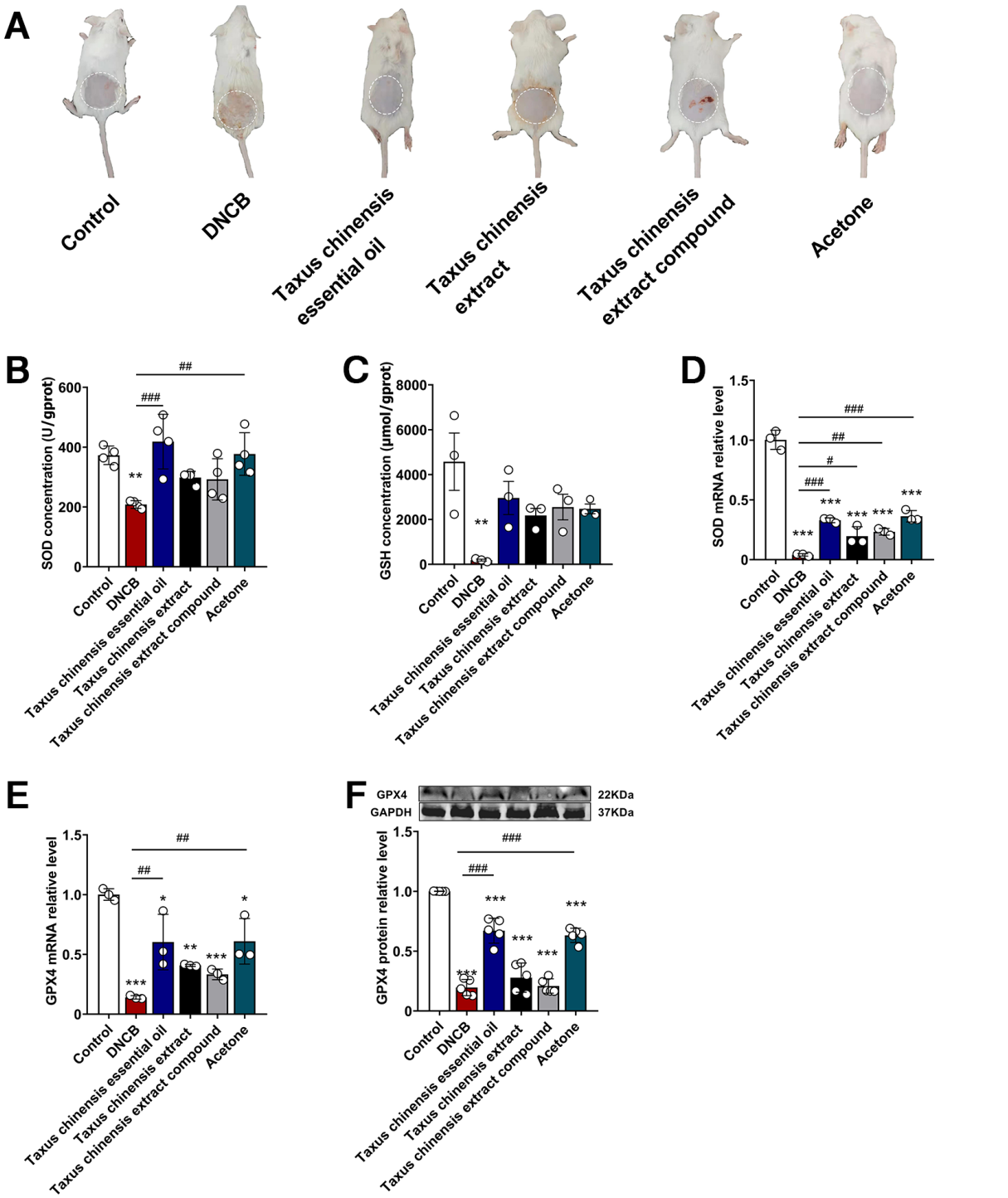


Fig. 2 Anti skin oxidation effect of three Taxus chinensis extracts ($N = 3$-5)

(A) Representative images of Kunming mice in each group after 7 days of drug administration; (B) SOD content in the skin tissue homogenates of mice in each group; (C) GSH content of in the skin tissue homogenates of mice in each group; (D) (E) mRNA level of SOD and GPX4 in the skin tissue of each group were tested; (F) Protein relative level of GPX4 in the skin tissue of each group were tested; Data represent the mean ± SE; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ *vs.* Control group; $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$ *vs.* DNCB group; DNCB, dinitrochlorobenzene; SOD, superoxide dismutase; GPX4, Glutathione Peroxidase 4; GSH, glutathione.

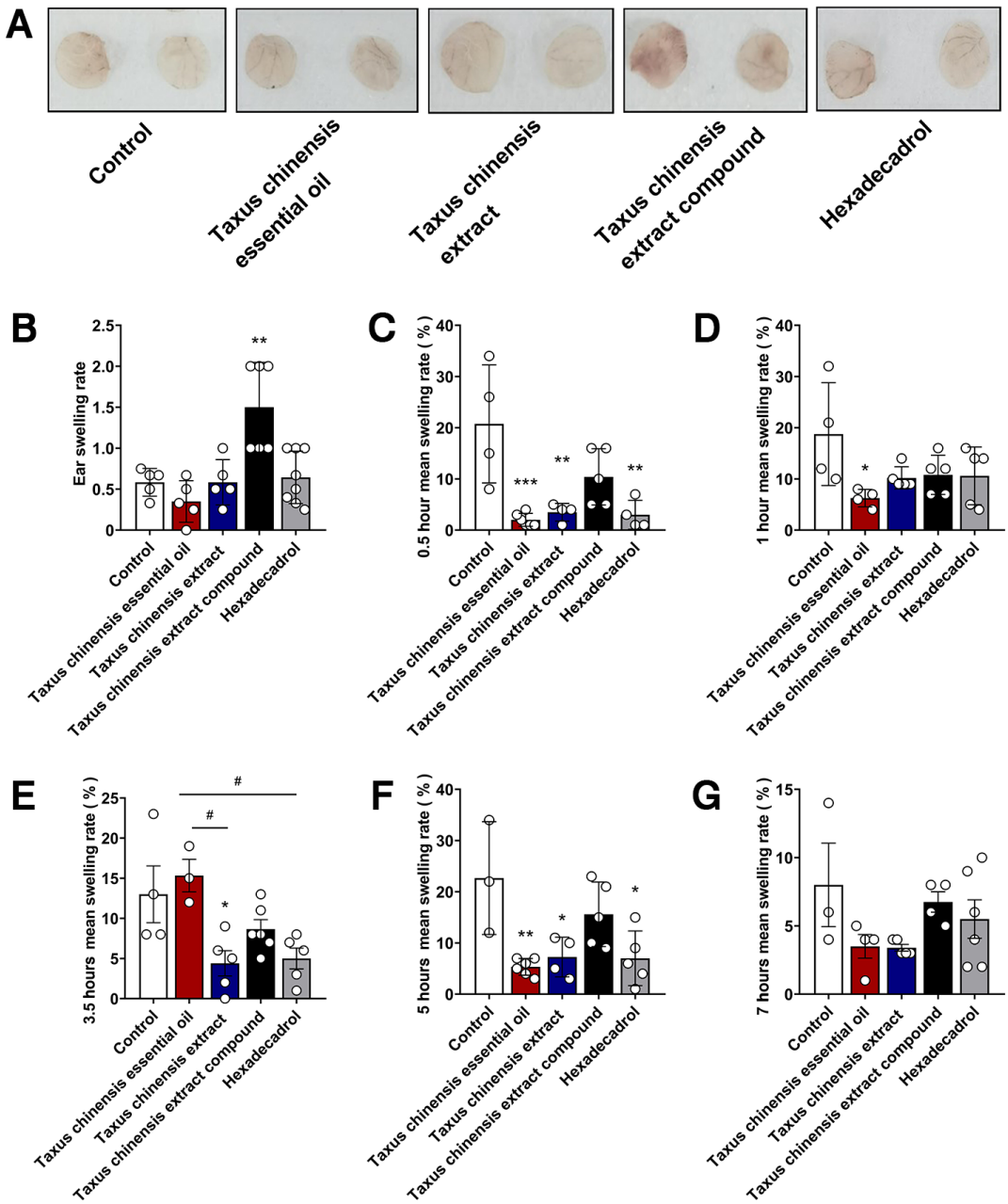
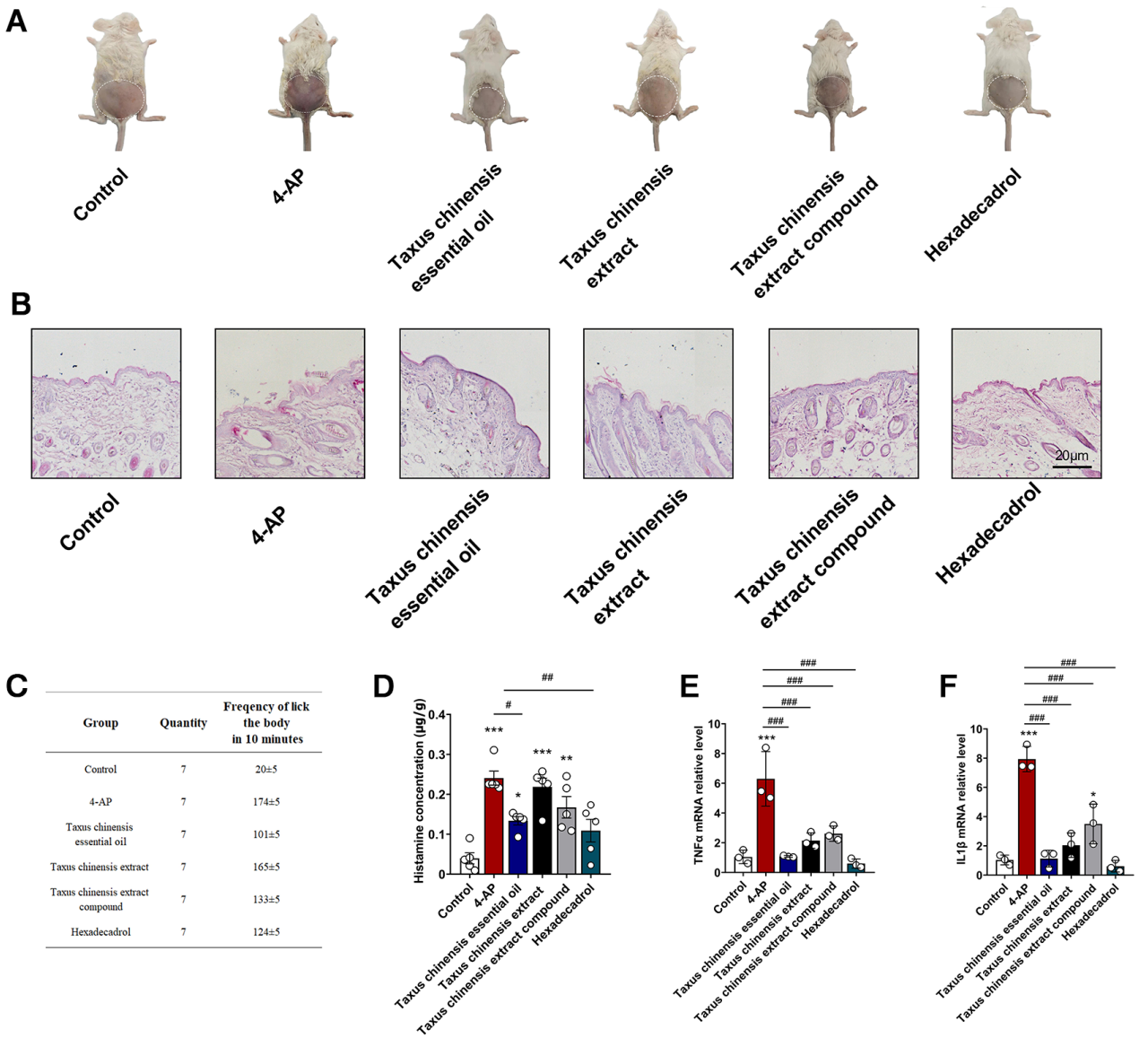


Fig. 3 Anti skin inflammation effect of three Taxus chinensis extracts ($N \geq 3$)

(A) Representative ears photos of mice in each group after 30 minutes of inflammation induction; (B) Ear swelling rate of mice in each group after 30 minutes of inflammation induction; (C-G) Swelling rate of mice toes at different time points post inflammation induction; Data represent the mean ± SE; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ *vs.* Control group; $^{\#}P < 0.05$ *vs.* Taxus chinensis essential oil group.

skin inflammation, we first performed the xylene-induced ear swelling experiment in mice. As shown in Fig. 3A and B, compared with the control group, Taxus chinensis essential oil produced apparent but non-significant reduction of the inflammation-induced ear swelling in mice, Taxus chinensis extract did not alter the swelling, and Taxus chinensis extract compound unexpectedly aggravated the swelling. We then went on to investigate the effects of Taxus chinensis on inflammation



Group	Quantity	Freqency of lick the body in 10 minutes
Control	7	20±5
4-AP	7	174±5
Taxus chinensis essential oil	7	101±5
Taxus chinensis extract	7	165±5
Taxus chinensis extract compound	7	133±5
Hexadecadrol	7	124±5

Fig. 4 Anti skin allergy effect of three Taxus chinensis extracts ($N = 5$-7)

(A) Representative skin photos of mice in each group after 4-AP injected to induce allergy; (B) Pathological changes of skin tissue were observed by HE staining in each group (bar = 20 μm); (C) Body licking times of mice in each group within 10 minutes after allergic stimulation; (D) Histamine content in the skin tissue homogenates of mice in each group; (E) (F) mRNA level of SOD and GPX4 in the skin tissue of each group were tested; Data represent the mean ± SE; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ *vs.* Control group; $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$ *vs.* 4-AP group; 4-AP, 4-aminopyridine; DNCB, dinitrochlorobenzene; HE, hematoxylin and eosin; SOD, superoxide dismutase; GPX4, Glutathione Peroxidase 4; TNF, tumor necrosis factor; IL, Interleukin.

using another inflammatory model: the egg white-induced paw swelling model. As shown in Fig. 3C-G, all three Taxus chinensis extracts ameliorated inflammation-induced swelling of left rear foot, and the effect of Taxus chinensis essential oil was consistently superior to the other two extracts. The above results indicate the potential effect of Taxus chinensis against skin inflammation, especially the essential oil extract.

3.4 Anti skin allergy effect of three Taxus chinensis extracts

Finally, we evaluated the effects of Taxus chinensis on skin

allergy. 4-AP (0.01 mL/g) was injected subcutaneously into the neck to establish the skin allergy model. The skin allergy induced by 4-AP was reversed by all three extracts of Taxus chinensis and hexadecadrol as well. In agreement with the above-described data, Taxus chinensis essential oil afforded stronger anti-allergic effect relative to the other two extracts (Fig. 4A). HE staining of the dorsal skin exhibited that anomalous keratinization induced by 4-AP was effectively blunted by Taxus extracts (Fig. 4B). In addition, the licking times within 10 minutes were counted after injected 4-AP and the results showed that the mice of the 4-AP group had the highest number of licking times compared to the control group, while the Taxus chinensis essential oil group had the lowest number of licking times among the model groups (Fig. 4C). This result suggests that the anti-allergic effect of Taxus chinensis essential oil extract is greater than that of Taxus chinensis extracts and Taxus chinensis extract compound.

Since the production of histamine is a marker of allergic reaction, we then measured the histamine content in skin tissues by ELISA. We found that the content of histamine in the 4-AP group was significantly higher than that in the control group. And compared with the 4-AP model group, the content of histamine in the Taxus chinensis essential oil group decreased obviously, but not in the Taxus chinensis extracts and Taxus chinensis extract compound groups (Fig. 4D).

Since allergy is usually accompanied by inflammation, we also determined the expression level of TNF-α and IL-1β transcripts using qRT-PCR. The results revealed that the mRNA levels of both TNF-α and IL-1β were significantly up-regulated by 4-AP, and Taxus chinensis extracts mitigated the increases (Fig. 4E and F). The above results further confirmed the anti-allergic effect of Taxus chinensis, and the greater efficacy of the essential oil extract than the other extracts against skin allergy.

4 Discussion

Taxus is a precious woody species with important medicinal value. Natural Taxus has been previously demonstrated to possess anti-cancer, anti-diabetes, anti-fibrosis, anti-bacteria, anti-Alzheimer's Disease and anti-high blood pressure properties[24-26]. Taxus L. is not refer to a single plants but refer to a general term for genus taxus. Up to now, it is discovered that 15 species genus taxus worldwide, significantly, there are 10 species which occur in Asia. *Taxus cuspidata S. et Z.* is a kind of genus taxus belonging to the Taxaceae family. However, amount of researches have suggested that Taxol, clinically applied against tumor, is the principle bioactive ingredient of *Taxus cuspidata S. et Z.*, considering the exorbitant cost of Taxol extraction, it is meaningful and imperative to exploit other Taxus-derived compounds with significant pharmaceutical values. This study for the first time reveals that three extracts (Taxus chinensis essential oil, Taxus chinensis extract and Taxus chinensis extract compound) from *Taxus cuspidata S. et Z.* performed excellent effects on skin damages like melatonin deposition, oxidation, inflammation and allergy.

Firstly, considering the involvement of hyperpigmentation in numerous pathological processes such as skin aging, oxidation, and skin cancer, we focus on investigating the whitening effect of Taxus in guinea pigs. Surprisingly, we found that Taxus extracts have an imperative whitening effect. Particularly, Taxus chinensis essential oil is the most prominent among the three extracts. The melanosomes of melanocytes synthesize melaninin in mammals, which is catalyzed by diverse melanogenic enzymes including tyrosinase, tyrosinase-related protein (TRP) -1 and TRP-2[27]. In the process of melanogenesis, tyrosinase is the pivotal rate-limiting enzyme that catalyzes the hydroxylation of L-tyrosine to 3, 4-dihydrox-yphenylalanine (DOPA) and the oxidation of DOPA to dopaquinone[28]. It is obvious that these biochemical reactions are crucial for melanogenesis. Moreover, tyrosinase has an important biochemistry function in melanogenesis. Interestingly, many agents, such as arbutin, ellagic acid and kojic acid, restrain melanin synthesis *via* inhibiting tyrosinase activity[29-30]. Hence, tyrosinase was proposed as a potential target for the action of Taxus given its anti-pigmentation property. Yet, better understanding and thorough exploration of molecular mechanism underlying Taxus extracts for their anti-pigmentation effects are highly desirable.

Secondly, the researchers identified the anti-glycative component from the leaf of Taxus. and found that component cleared methyl glyoxal (MGO) in chemical and cell models to exert its ability which prompted us to guess that *Taxus cuspidata S. et Z.* also exerts similar function on skin oxidation. Redox balance plays a central role in cellular metabolism[31]. Several mechanisms exist to control ROS generation and elimination, ensuring physiological ROS levels in the cells. Consequently, the balance between ROS-generating and ROS-eliminating enzymes defines the proper redox state thereby the cellular function and cell fate[32]. In view of the key role of SOD and GSH in balancing redox homeostasis[33-34], we measured DNBC-induced SOD and GSH levels in the skin of Kunming mice. The data showed that all three Taxus extracts could reverse the downregulation of SOD and GSH induced by DNBC. Prominently, GPx (glutathione peroxidase) play a central role in deoxidation[35-36]. GPX4, one of GPx, is also a pivotal enzyme that participates in regulation of ferroptosis. Published papers showed that ferroptosis associated with oxidative stress. Therefore, to dissect the molecular mechanisms of Taxus extracts for their antioxidative effects, we examined both the mRNA and protein levels of GPX4 in the skin and our data strongly indicated that Taxus chinensis essential oil acts by promoting and maintaining the endogenous antioxidative capacity.

Recently, a research have studied the anti-inflammation effect of *Taxus cuspidata S. et Z.* water decoction[37]. Even though, our data further verified the anti-inflammation effect of the extracts from *Taxus cuspidata S. et Z. via* assaying both xylene-induced ear swelling and egg white-induced paw swelling. The results showed that both Taxus chinensis essential oil and Taxus chinensis extract could suppress skin inflammation; on the contrary, Taxus chinensis extract compound produced pro-inflammatory effect in this model. However, comparison with *Taxus cuspidata S. et Z.* water decoction, the extracts from *Taxus cuspidata S. et Z.* used in this study might perform better bioavailability.

Moreover, skin allergic reactions are also associated with inflammation, therefore, we hypothesized that Taxus extracts also have pharmacological effect on skin allergy. It is known that histamine sharply increases in allergic diseases, hence, we tested the level of histamine in the dorsal skin of mice induced by 4-AP. The data indicated that Taxus chinensis essential oil slightly suppress the level of histamine. HE staining displayed that Taxus extracts effectively reversed anomalous keratinization induced by 4-AP. It is well accepted that mastocyte plays central role in the course of allergy[38], therefore, we speculated that the anti-allergy property of Taxus extracts partially results from retarding the release of inflammation factors, and partially due to inhibition of the mastocyte discharge histamine. When skin inflammation occurs, the cell membrane metabolism of keratinocytes, fibroblasts, mastocyte and endothelial cells is activated, and the phospholipid of membrane synthesizes various proinflammatory factors through different pathways like TNF-α and IL-1β[39]. Meanwhile, histamine released by mast cell degranulation during acute inflammatory response. To provide further evidence for the mechanism of Taxus extracts in skin inflammation, the mRNA level of TNF-α and IL-1β were determined by qRT-PCR, and the results showed that all three Taxus extracts downregulated the expression of TNF-α and IL-1β, indicating that Taxus extracts mitigated the inflammatory response of the irritated skin *via* suppressing pro-inflammation signaling.

The findings of the present study prompted us to propose that Taxus chinensis essential oil, Taxus chinensis extract and Taxus chinensis extract compound could be used for the treatment of skin damages in terms of whitening, anti-oxidation, anti-inflammation, and anti-allergy *via* directly applied to skin. Especially, Taxus chinensis essential oil appears to be the most promising drug candidate for the management of skin diseases. Our investigation provides a new insight in the pharmacological value of *Taxus cuspidata S. et Z.* extracts and widens the understanding of their potential molecular mechanisms on skin damages.

Although the present study has revealed the novel pharmacological effects of Taxus extracts against skin damage, it unavoidably contains some limitations and future in-depth investigations are required to improve our better understanding of their actions and mechanisms. It is necessary to further analyze and identify the active ingredients against skin diseases of Taxus extracts, especially the essential oil extract, and it is also indispensable to demonstrate the safety of Taxus extracts using the pathological models of the human skin as well as exploring which medium could help them maximize the effectiveness. In addition, the specific molecular mechanism of Taxus extracts against skin damage remains unclear, which merits more rigorous investigations to decipher.

5 Conclusion

In summary, the present study reveals that three Taxus extracts have the effects of anti-skin melanin deposition, oxidation, and allergies, among which the Taxus chinensis essential oil extract has the superior effects. Taxus extracts, mainly essential oil extract, also have a certain effect on skin inflammation.

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Authorship contributions

Zhang Y proposed the conception for the study. Wang Q designed the experiments. Shi Y and Liu L wrote the manuscript. Shi Y, Liu L, Sun H Y, Chen C, Feng J, Chen Y C performed most of the experiments shown in the manuscript. Wang Q, Lin Y and Kopylov P gave critical discussions and revisions on manuscript.

Ethical approval

All procedures were approved by the Institutional Animal Care and Use Committee of Harbin Medical University (Protocol [2009]-11) (No. IRB3017621). The use of animals was compliant with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). All sacrifices were performed under anesthesia, and every effort was made to minimize animal suffering.

Conflicts of interests

Zhang Y is an Editorial Board Member of the journal. The article was subject to the journal's standard procedures, with peer review handled independently of this editor and his research groups.

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