





## Article

# Assessing Antipsoriatic Effects of Bitter Pu'er Tea and Its Three Major Compounds, Strictinin, Theacrine and Epigallocatechin Gallate, in Imiquimod-Treated Mice

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**Abstract:** Psoriasis is a chronic inflammatory skin disease with hyperproliferation and aberrant differentiation of keratinocytes in association with the elevation of interleukin-17A (IL-17A) and IL-23 levels. In an animal model, psoriasis-like dermatitis was induced on the shaved dorsal skin of BALB/c mice by topical application of imiquimod (IMQ), a synthetic ligand of Toll-like receptor 7. Administration of bitter Pu'er tea significantly reduced psoriasis-like dermatitis in IMQ-treated mice, including a reduction in dorsal skin lesions, splenomegaly and the mRNA expression levels of IL-17A and IL-23. To examine putative antipsoriatic constituents, three major compounds in bitter Pu'er tea, strictinin, theacrine and epigallocatechin gallate (EGCG), were separately given as supplements to IMQ-treated mice. The results showed that all the three compounds attenuated the severity of psoriasis by reducing epidermal thickness. Only theacrine significantly attenuated splenomegaly. All the three compounds inhibited the expression of IL-23 mRNA in the skin as well as reduced the content of IL-17A<sup>+</sup>CD4<sup>+</sup> T cells in the spleen, and strictinin was found to be relatively effective. It seemed that the antipsoriatic activity of bitter Pu'er tea was attributed to the additive effects of its multiple active compounds.

**Keywords:** bitter Pu'er tea; epigallocatechin gallate; imiquimod; psoriasis; strictinin; theacrine



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## 1. Introduction

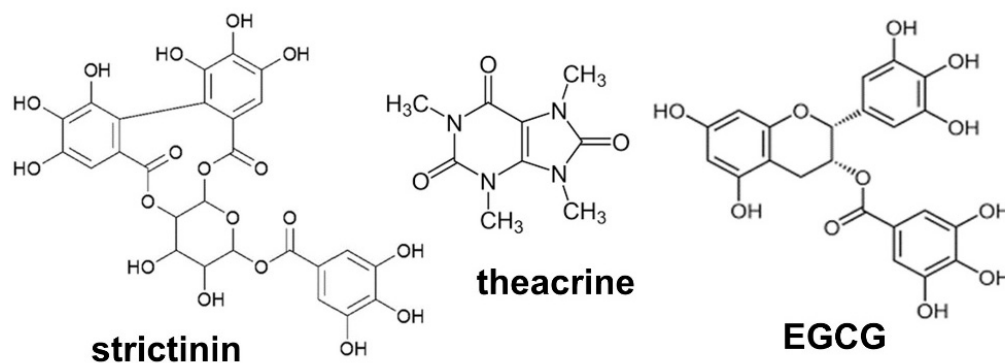
Psoriasis is a noncontagious skin disease driven by the hyperproliferation of abnormal keratinocytes in the epidermis [1]. It causes a rash with scaly patches frequently found on the elbows and knees, and is assumed to result from environmental factors as well as personal bad behaviors [2]. Although psoriasis is a chronic disease with no immediate threat to life, it can be painful and troublesome as the patients suffer physical discomfort as well as psychological pressure and social stigma, which in turn, can lead to a decline in emotional control, productivity and self-esteem in their work, affecting their quality of life [3]. Moreover, it has been shown that psoriasis is a risk factor for the development of many other diseases, such as psoriatic arthritis, postinflammatory hypopigmentation, conjunctivitis, type 2 diabetes, high blood pressure, cardiovascular disease, autoimmune dysfunction and mental problems, such as low self-esteem and depression [4]. Medicinal treatments with steroid creams, vitamin D3 cream, ultraviolet light and immunosuppressive drugs are currently used to control or alleviate the symptoms since there is no known cure for psoriasis thus far [5]. Searching for novel oral medicines or developing new topical agents for the patients suffering from psoriasis is strongly needed [6].

Psoriasis is not only a skin problem, but also a systemic inflammatory disease. The level of interleukin-17A (IL-17A) in the skin and blood of psoriatic lesions was found to

be higher than that in healthy skin, and was positively correlated with the severity of psoriasis [7]. IL-17A, a proinflammatory cytokine that regulates the expression of other inflammatory factors, is generated from T-helper 17 cells after being stimulated by IL-23 produced from dermal dendritic cells [8,9]. It has been proposed that the IL-23/IL-17 axis is the primary signaling pathway leading to the pathological changes in psoriatic skin [10,11]. Scientific evidence shows that IL-17A-producing CD4<sup>+</sup> T cells may be potential targets for the therapeutic treatment of psoriasis [12].

Aldara<sup>TM</sup>, used to treat genital warts, superficial basal cell carcinoma and actinic keratosis, is a commercial topical ointment containing 5% imiquimod (IMQ), a positive immune response modifier [13]. IMQ stimulates Toll-like receptor 7 on dendritic cells in the skin, and then regulates the immune response via the inflammatory signaling pathway of IL-23/IL-17 to produce lesions similar to plaque psoriasis in the topical area [14]. It has been shown that psoriasis-like dermatitis was induced on the shaved dorsal skin of rodents by topical application of Aldara<sup>TM</sup> [15]. Therefore, the IMQ-treated mice serve as a suitable animal model for the screening of potential drugs for psoriasis.

Pu'er tea, prepared from *Camellia assamica* grown in certain areas of Yunnan, China, has become popular in Chinese society in recent decades due to its special flavor. Besides caffeine and catechins, strictinin is also identified as a relatively abundant compound in Pu'er tea [16]. Bitter Pu'er tea, also named Pu'er Kucha tea, is prepared from a mutant variety of the wild Pu'er tea plant [17]. In addition to strictinin, theacrine is also identified as a relatively abundant compound in bitter Pu'er tea. Theacrine is a purine alkaloid with a chemical structure similar to caffeine, and is responsible for the bitterness of this tea [13]. Pu'er tea and bitter Pu'er tea, as well as their major compounds such as strictinin, theacrine and epigallocatechin gallate (EGCG; a major catechin) (Figure 1), have been demonstrated to possess several health-promoting properties, such as antioxidant, antiobesity, antiviral, anti-inflammatory, antitumor and antipsoriatic activities [18–23].



**Figure 1.** Chemical structures of strictinin, theacrine and epigallocatechin gallate (EGCG).

In this study, we aimed to assess the antipsoriatic effects of bitter Pu'er tea and identify its putative active compounds. Psoriasis-like dermatitis was, firstly, induced in mice by treatment with IMQ. The effects of bitter Pu'er tea as well as its three major compounds, strictinin, theacrine and EGCG, on the alleviation of psoriasis-like dermatitis in IMQ-treated mice were evaluated, and the improvement in dorsal skin induration, splenomegaly and the expression levels of IL-17A and IL-23 were also analyzed.

## 2. Materials and Methods

### 2.1. Chemicals and Materials

Bitter Pu'er tea was purchased from Yunnan Puer Yifeng Tea Co., Ltd. (Yunnan, China). The contents of strictinin, theacrine and EGCG in 1 g of the bitter Pu'er tea used in this study were estimated to be 20, 12 and 13 mg, respectively; strictinin was purified as described previously [17]. Theacrine was purchased from Bolise (Shanghai, China). EGCG was purchased from Sigma-Aldrich (St Louis, MO, USA). Aldara<sup>TM</sup>, 5% imiquimod (IMQ) cream, was obtained from MEDA Pharmaceuticals (Solna, Sweden).

## 2.2. Animals and Experiment Design

Male BALB/c mice were purchased from BioLasco, Taiwan Co., Ltd. (Taipei, Taiwan). They were housed in an environment controlled for temperature and humidity, and kept in 12 h light/dark cycles with free access to water and a standard chow diet (5001 Rodent LabDiet, PMI Nutrition International Inc., St. Louis, MO, USA). Mice were randomly divided into the following groups (n = 8 per group): control group, 5% IMQ-induced group (topical dose with 62.5 mg of IMQ cream), IMQ + Pu'er tea group (500 mg/kg/day), IMQ + strictinin group (150 mg/kg/day), IMQ + theacrine group (150 mg/kg/day), IMQ + EGCG group (150 mg/kg/day). The mice were intragastrically administered with saline (control group), bitter Pu'er tea infusion, strictinin, theacrine or EGCG daily for two weeks [22,24]. To establish a psoriasis-like animal model, hair on the dorsal of mice was shaved using an electric shaver and a depilatory cream (Nair™, Church & Dwight Canada Corp., Mississauga, ON, Canada) was applied to remove the remaining hairs prior to Vaseline (Jonesboro, AR, USA) (control group) or IMQ treatment. The psoriasis-like symptoms were induced by topical administration of the 5% IMQ cream for 7 consecutive days [25]. The body weight of different groups was individually recorded during the experiment and presented as a fold-change in the body weight growth rate compared to the first day. In the end, the dorsal skin and spleen were dissected, and the spleen was weighed. The animal experiment was approved by the Institutional Animal Care and Use Committee of National Chung-Hsing University, with an approval number of IACUC 109-078.

## 2.3. Histological Analysis

The dorsal skin samples dissected at the end of the experiment were fixed with 10% formalin and embedded in paraffin (Sigma-Aldrich, St Louis, MO, USA). Briefly, the skin sections were dewaxed and stained with the hematoxylin and eosin (H&E) solution (Sigma-Aldrich) according to the standard protocol developed previously [26]. To capture the images, a light microscope (Olympus, BX43, Tokyo, Japan) equipped with a digital camera (Canon EOS 600 D, Tokyo, Japan) was used, and the skin thickness of the epidermis was measured by using the ImageJ software (NIH, Bethesda, MD, USA).

## 2.4. Reverse Transcription-Polymerase Chain Reaction (RT-PCR) Analysis

Total RNA was extracted from the dorsal skin using the Quick-RNA™ Miniprep Kit (Zymo Research, Irvine, CA, USA), and the purity and quantity of RNA were determined with a NanoDrop spectrophotometer (Quawell Technology Inc, San Jose, CA, USA). Synthesis of cDNA was performed by using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA), and amplified using a PCR machine (Thermo Fisher Scientific, Waltham, MA, USA) at 95 °C for 3 min, followed by 39 cycles of 95 °C for 3 min, 95 °C for 3 s and 60 °C for 20 s. The primer sequences used in the study are shown in Table 1. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control for normalization. Finally, PCR products were separated by electrophoresis on a 1.2% agarose gel and visualized by staining with ethidium bromide (Sigma-Aldrich). The signal density of each band was captured by a gel imaging system (Syngene, Cambridge, UK), and analyzed using the ImageJ software (NIH, Bethesda, MD, USA).

**Table 1.** Primers used in RT-PCR.

Name		Primer Sequence	bp	Reference
IL-17A	F	5'-GACAACCACGGCCTTCCCTACTTC-3'	165	[27]
	R	5'-CTTCCCTCCGCATTGACAC-3'		
IL-23	F	5'-ATGCTGGATTGCAGAGCAGTA-3'	213	[28]
	R	5'-ACGGGGCACATTATTTTAGTCT-3'		
GAPDH	F	5'-AGGTCGGTGTGAACGGATTG-3'	195	[29]
	R	5'-GGGGTCGTTGATGGCAACA-3'		

### 2.5. Flow Cytometric Analysis

Mice were sacrificed via cardiac puncture under deep anesthesia at the end of the experiments, and their spleens were collected for the following assay. The single cells were separated from the spleens after being gently pressed across a 70  $\mu\text{m}$  cell strainer (Jet Biofil, Guangzhou, China) and placed in precooled PBS. The cell suspensions were fixed and permeabilized with the Intracellular Fixation & Permeabilization Buffer Set (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's procedure. The cells were stained with a PE-conjugated anti-IL17A antibody and FITC-conjugated CD4 antibody (Biolegend, San Diego, California, USA) for 30 min at 4 °C prior to the performance on the flow cytometer. Finally, 10,000 events of the population were acquired and analyzed on a BD Accuri™ C6 Plus Flow Cytometer (BD Biosciences, San Jose, CA, USA).

### 2.6. Statistical Analyses

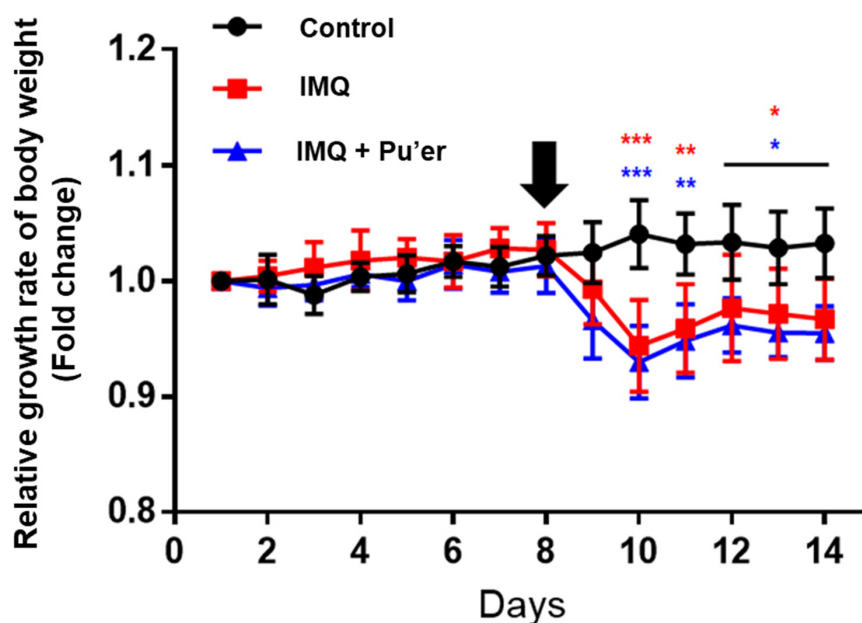
All data were presented as the mean  $\pm$  standard deviation. The statistically significant differences were compared by a one-way analysis of variance, followed by Tukey's post hoc test using GraphPad Software Prism 7.0 (GraphPad Software, San Diego, CA, USA), with  $p < 0.05$  (\*),  $p < 0.01$  (\*\*) and  $p < 0.001$  (\*\*\*) considered to be statistically significant.

## 3. Results

### 3.1. Effects of Bitter Pu'er Tea on Psoriasis-Like Dermatitis in IMQ-Treated Mice

#### 3.1.1. Effects on Body Weight of Mice after IMQ Treatment

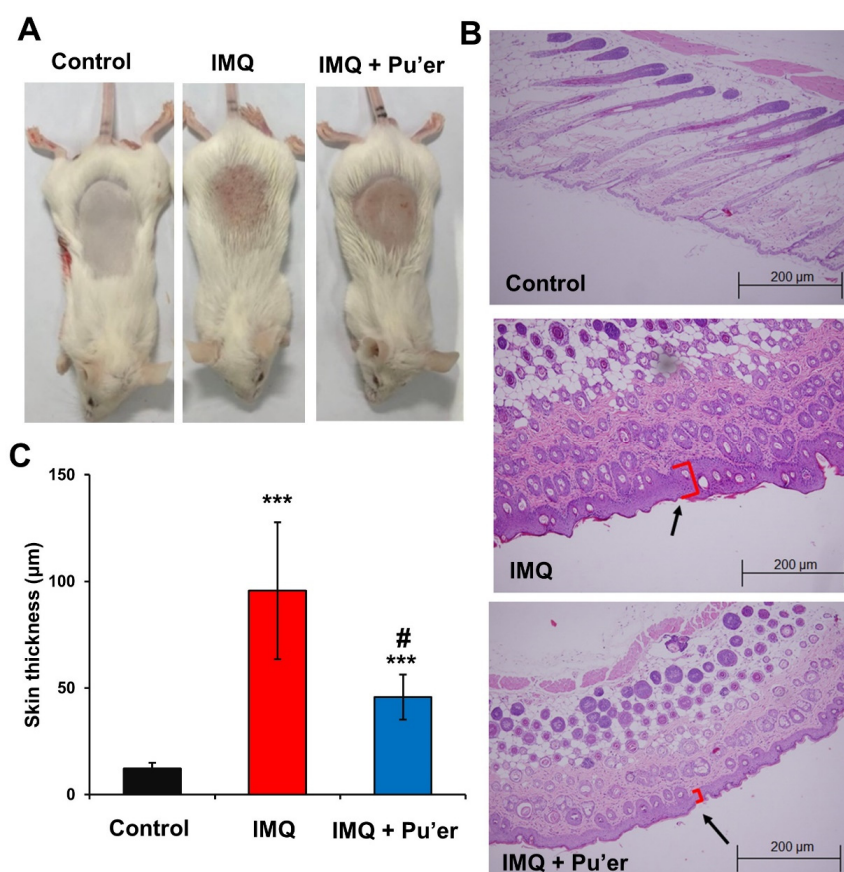
Mice were adapted for a week with or without (control) supplementation of the bitter Pu'er tea infusion daily, and then treated with IMQ daily with or without supplementation of the bitter Pu'er tea infusion in the following week. The body weight of mice was found to drop significantly after IMQ treatment for two days (Figure 2). The loss of body weight was slightly recovered in mice after IMQ treatment for three days. The supplementation of the bitter Pu'er tea infusion to mice did not affect the fluctuation of body weight caused by the IMQ treatment.



**Figure 2.** Body weight growth rate of IMQ-treated mice with or without supplementation of bitter Pu'er tea infusion. Body weight of mice was individually recorded for two weeks. Mice were divided into three groups: control group, IMQ group and IMQ + Pu'er group. Bitter Pu'er tea was supplemented daily for two weeks, and IMQ treatment was provided daily in the second week. Statistical significance was shown at \*  $p < 0.05$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$  as compared with the control group.

### 3.1.2. Reduction in Dorsal Skin Lesions in IMQ-Treated Mice by Bitter Pu'er Tea

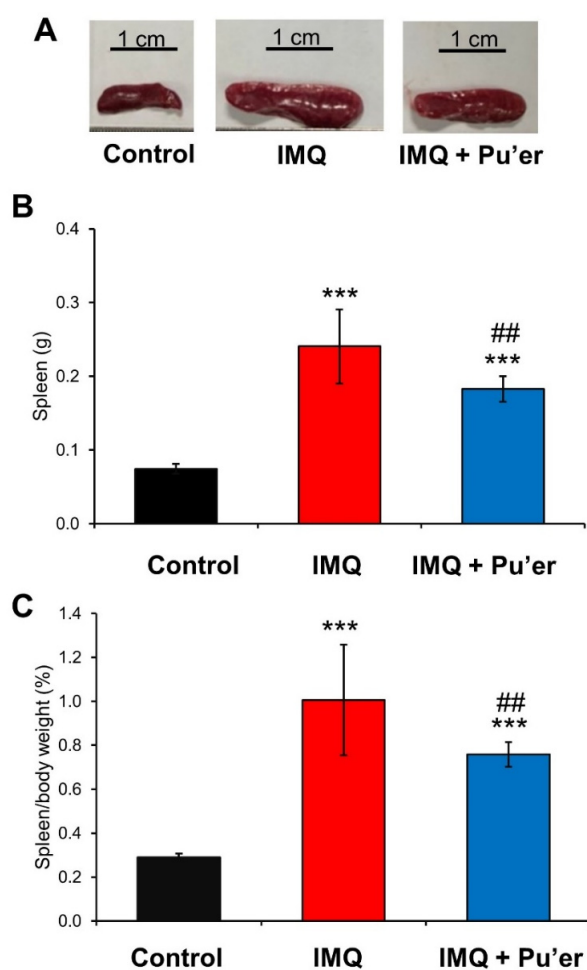
Dorsal skin lesions were observed in mice after being treated with IMQ, and the lesions were evidently reduced when the mice were supplemented with the bitter Pu'er tea infusion (Figure 3A). As observed through light microscopy, the skin thickness of the epidermal layer was substantially expanded in the lesions of IMQ-treated mice, and the expansion of skin thickness was significantly reduced when the IMQ-treated mice were supplemented with the bitter Pu'er tea infusion (Figure 3B). Quantitatively, the skin thickness in the lesions of IMQ-treated mice was expanded, being 6.5 times higher in comparison with the normal skin thickness in untreated mice (control), and the expansion was reduced, being 4 times lower, after the supplementation of the bitter Pu'er tea infusion (Figure 3C).



**Figure 3.** Effects of bitter Pu'er tea on dorsal skin lesions of IMQ-treated mice. Lesions were examined on dorsal skin of mice in the three groups: control group, IMQ group and IMQ + Pu'er group (A). Thickness of dorsal skin from mice in the three groups was observed through light microscopy (B). Dorsal skin thickness of mice in the three groups was measured and compared (C). Statistical significance was shown at \*\*\*  $p < 0.001$  as compared with the control group, and #  $p < 0.05$  as compared with the IMQ group.

### 3.1.3. Attenuation of Splenomegaly in IMQ-Treated Mice by Bitter Pu'er Tea

Splenomegaly was observed in mice after being treated with IMQ, and the enlargement of the spleen was diminished when the mice were supplemented with the bitter Pu'er tea infusion (Figure 4A). Quantitatively, the spleens of IMQ-treated mice were enlarged, being 2.25 times higher in comparison with the spleens of untreated mice (control), and the enlargement was reduced, being 1.4 times lower, after the supplementation of the bitter Pu'er tea infusion (Figure 4B). Similar results were detected when the ratio of the spleen over body weight was measured for the mice (Figure 4C).



**Figure 4.** Effect of bitter Pu'er tea on splenomegaly of IMQ-treated mice. Spleens were dissected from mice in the three groups: control group, IMQ group and IMQ + Pu'er group (A). Weight of spleens from mice in the three groups was measured and compared (B). The ratio of spleen over body weight from mice in the three groups was measured and compared (C). Statistical significance was shown at \*\*\*  $p < 0.001$  as compared with the control group, and ##  $p < 0.01$  as compared with the IMQ group.

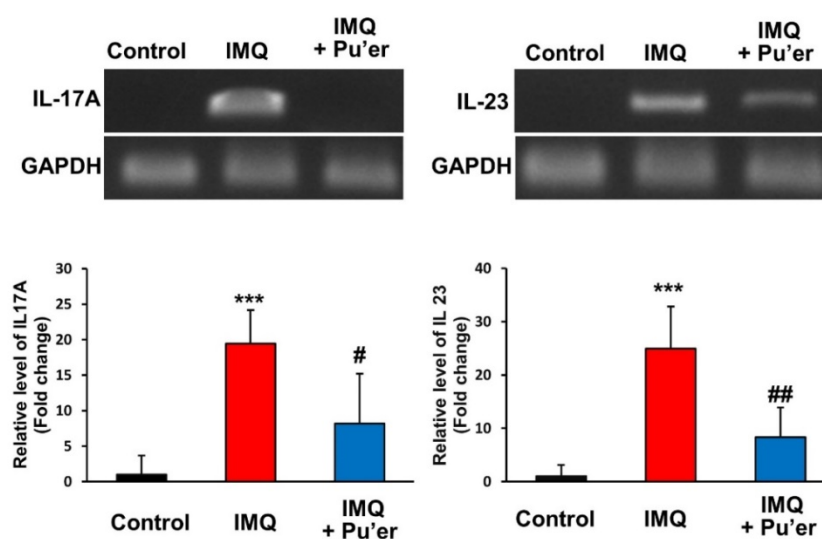
#### 3.1.4. Effects of Bitter Pu'er Tea Supplementation on the mRNA Expression Levels of IL-17A and IL-23 in IMQ-Treated Mice

Relatively low mRNA expression levels of IL-17A and IL-23 were detected in the dorsal skin of mice without IMQ treatment, and both levels were apparently elevated when mice were treated with IMQ (Figure 5). The elevated expression levels of IL-17A and IL-23 in IMQ-treated mice were significantly reduced when they were supplemented with the bitter Pu'er tea infusion. Approximately, two thirds of the mRNA expression levels of IL-17A and IL-23 induced by IMQ treatment were abolished when the mice were supplemented with the bitter Pu'er tea infusion.

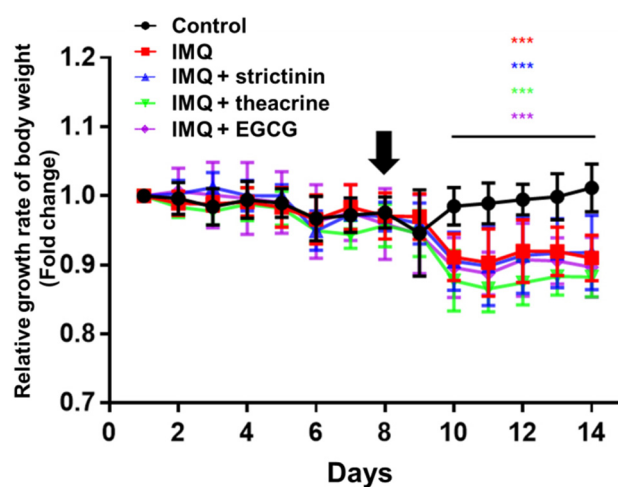
### 3.2. Effects of Strictinin, Theacrine and EGCG on Psoriasis-Like Dermatitis in IMQ-Treated Mice

#### 3.2.1. Effects of Strictinin, Theacrine and EGCG on Body Weight of IMQ-Treated Mice

Mice were adapted for a week with or without (control) supplementation of strictinin, theacrine or EGCG daily, and then treated with IMQ daily with or without supplementation of strictinin, theacrine or EGCG in the following week. Similarly, the body weight of mice was found to drop significantly after IMQ treatment for two days (Figure 6). As expected, the supplementation of strictinin, theacrine or EGCG to mice did not affect the fluctuation of body weight caused by the IMQ treatment in the second week.



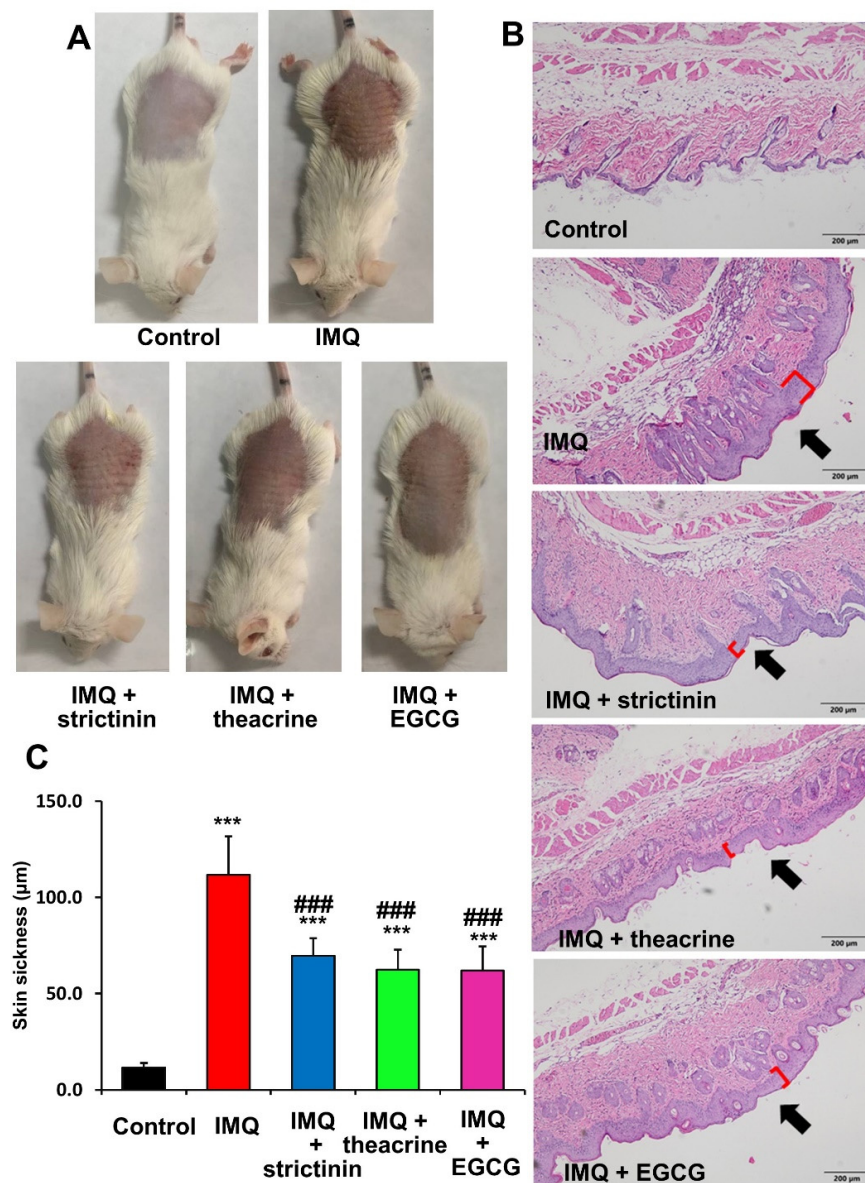
**Figure 5.** Effects of bitter Pu'er tea on the expressions of IL-17A and IL-23 in the dorsal skin of IMQ-treated mice. The mRNA expression levels of IL-17A and IL-23 were detected in the three groups: control group, IMQ group and IMQ + Pu'er group. Statistical significance was shown at <sup>\*\*\*</sup>  $p < 0.001$  as compared with the control group, and #  $p < 0.05$  or ##  $p < 0.01$  as compared with the IMQ group.



**Figure 6.** Effects of strictinin, theacrine and EGCG on body weight of IMQ-treated mice. Body weight of mice was recorded for two weeks. Mice were divided into five groups: control group, IMQ group, IMQ + strictinin group, IMQ + theacrine group and IMQ + EGCG group. Strictinin, theacrine or EGCG was supplemented daily for two weeks, and IMQ treatment was provided daily in the second week. Data were statistically significant at <sup>\*\*\*</sup>  $p < 0.001$  as compared with the control group.

### 3.2.2. Effects of Strictinin, Theacrine and EGCG on Dorsal Skin Lesions of IMQ-Treated Mice

Dorsal skin lesions were observed in mice after being treated with IMQ, and the lesions were evidently reduced when the mice were supplemented with strictinin, theacrine or EGCG (Figure 7A). As observed through light microscopy, the skin thickness of the epidermal layer was substantially expanded in the lesions of IMQ-treated mice, and the expansion of skin thickness was significantly reduced when the IMQ-treated mice were supplemented with strictinin, theacrine or EGCG (Figure 7B). Quantitatively, skin thickness in the lesions of IMQ-treated mice was expanded, being 8.5 times higher in comparison with the normal skin thickness in untreated mice (control), and the expansion was reduced, being 5.8, 4.5 and 4.5 times lower, after the supplementation of strictinin, theacrine and EGCG, respectively (Figure 7C).

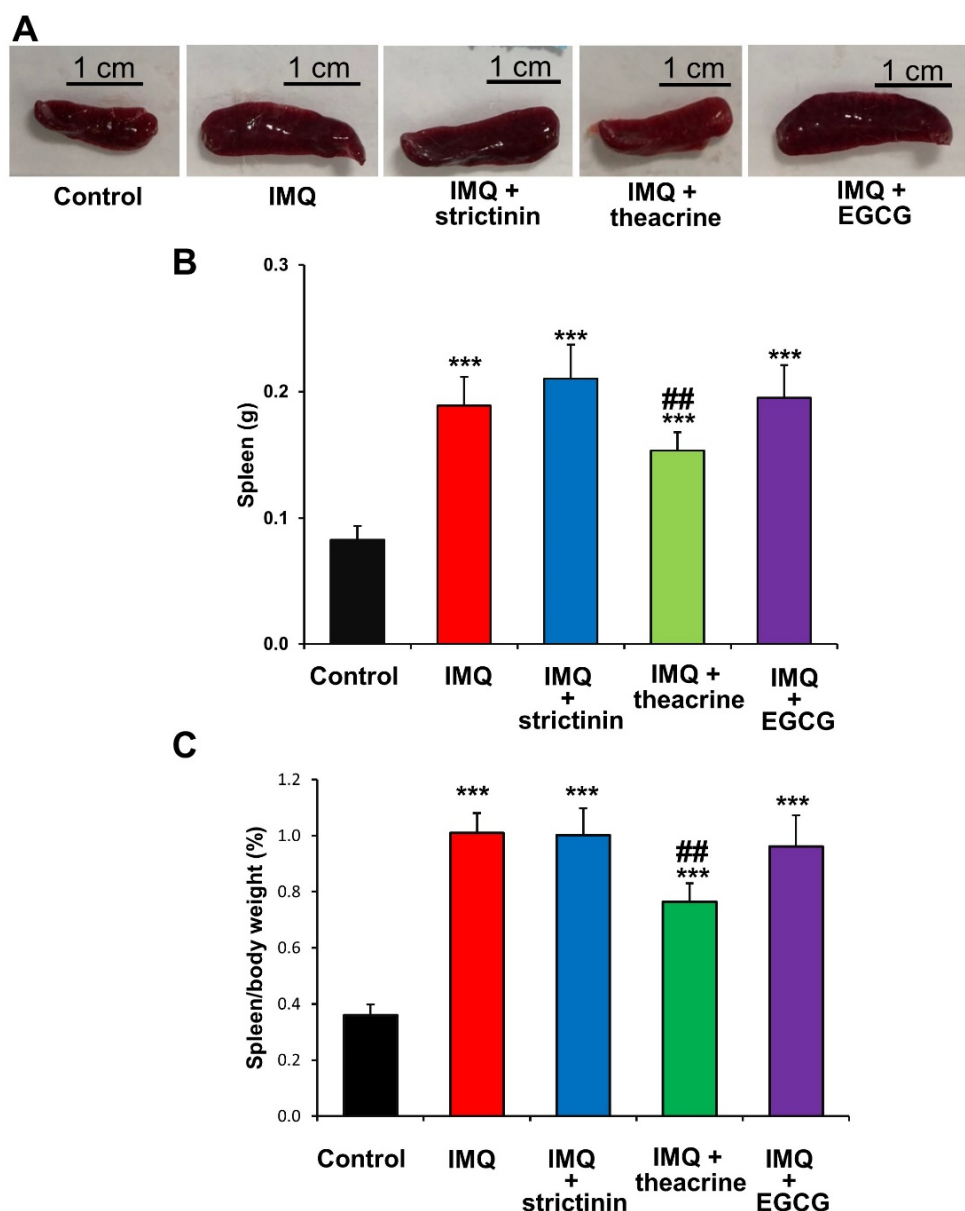


**Figure 7.** Effects of strictinin, theacrine and EGCG on dorsal skin lesions of IMQ-treated mice. Lesions were examined on dorsal skin of mice in the five groups: control group, IMQ group, IMQ + strictinin group, IMQ + theacrine group and IMQ + EGCG group (A). Thickness of dorsal skin from mice in the five groups was observed through light microscopy (B). Dorsal skin thickness of mice in the five groups was measured and compared (C). Statistical significance was shown at \*\*\*  $p < 0.001$  as compared with the control group, and ###  $p < 0.001$  as compared with the IMQ group.

### 3.2.3. Effects of Strictinin, Theacrine and EGCG on Splenomegaly of IMQ-Treated Mice

Splenomegaly was observed in mice after being treated with IMQ, and the enlargement of the spleen was diminished when the mice were supplemented with theacrine, but not strictinin or EGCG (Figure 8A). Quantitatively, the spleens of IMQ-treated mice were enlarged, being 1.3 times higher in comparison with the spleens of untreated mice (control), and the enlargement was reduced, being 0.85 times lower, after the supplementation of theacrine (Figure 8B). Similar results were observed when the ratio of the spleen over body weight was measured for the mice (Figure 8C).

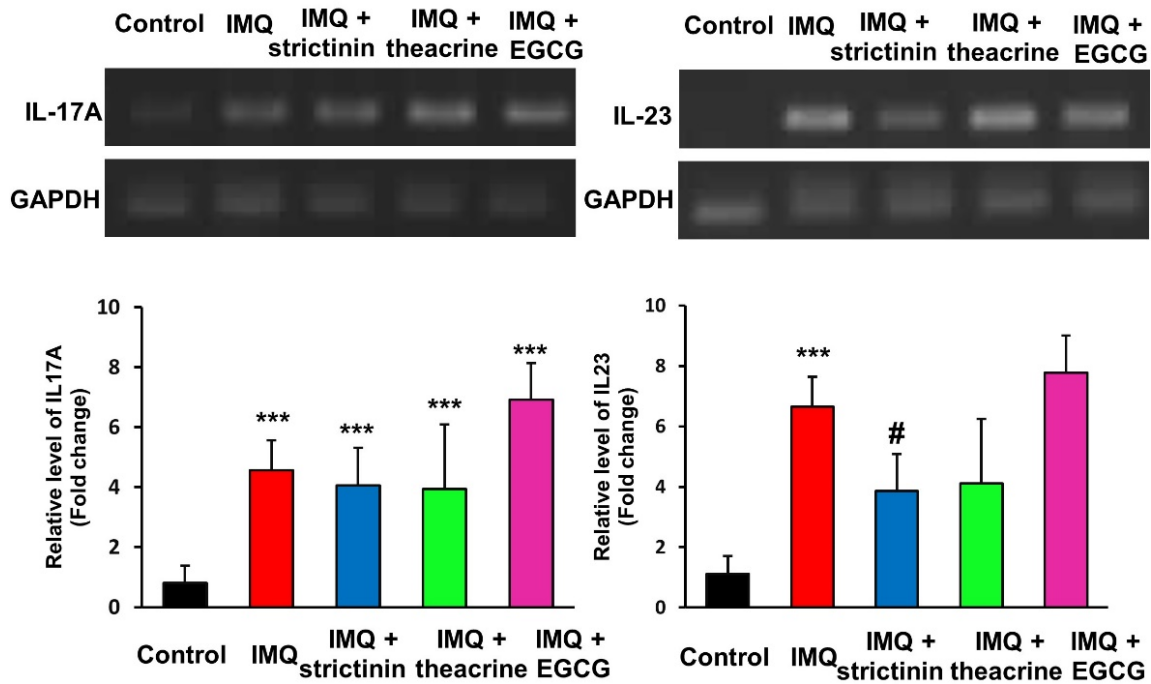




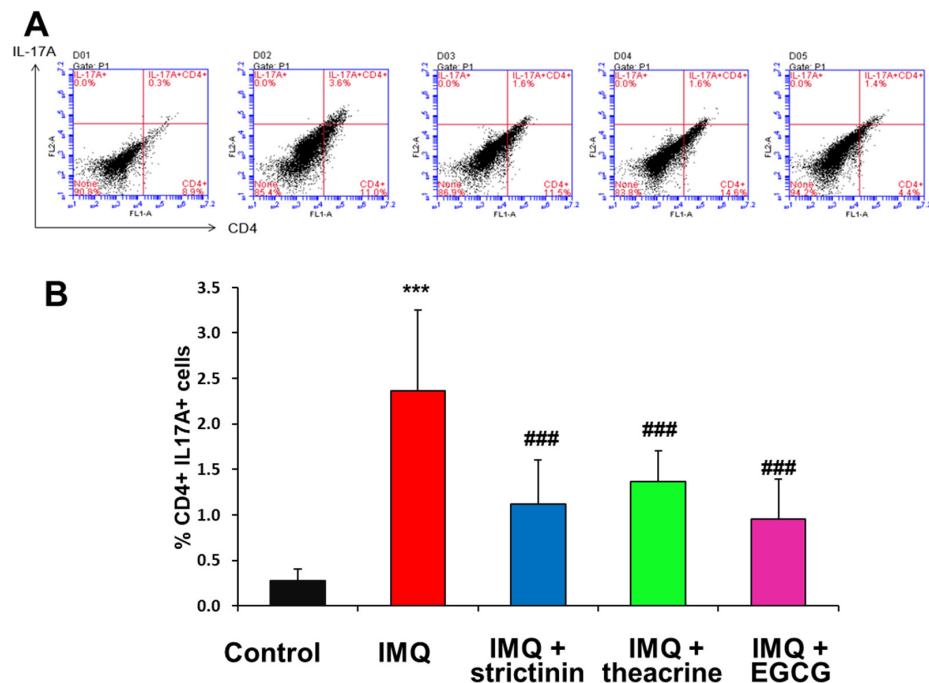
**Figure 8.** Effects of strictinin, theacrine and EGCG on splenomegaly of IMQ-treated mice. Spleens were dissected from mice in the five groups: control group, IMQ group, IMQ + strictinin group, IMQ + theacrine group and IMQ + EGCG group (A). Weights of spleens from mice in the five groups were measured and compared (B). The ratio of spleen over body weight from mice in the five groups was measured and compared (C). Statistical significance was shown at \*\*\*  $p < 0.001$  as compared with the control group, and ##  $p < 0.01$  as compared with the IMQ group.

### 3.2.4. Effects of Strictinin, Theacrine and EGCG on the mRNA Expression Levels of IL-17A and IL-23 in IMQ-Treated Mice

Relatively low mRNA expression levels of IL-17A and IL-23 were detected in the dorsal skin of mice without IMQ treatment, and both levels were apparently elevated when mice were treated with IMQ (Figure 9). The elevated expression levels of IL-23 in IMQ-treated mice were reduced when they were supplemented with strictinin, theacrine or EGCG, and strictinin seemed to be relatively effective for the reduction. In contrast, no significant reduction was observed on the elevated expression levels of IL-17A in the skin of IMQ-treated mice when they were supplemented with strictinin, theacrine or EGCG. However, strictinin, theacrine and EGCG significantly reduced the content of IL-17A<sup>+</sup>CD4<sup>+</sup> T cells in the spleens of IMQ-treated mice (Figure 10).



**Figure 9.** Effects of strictinin, theacrine and EGCG on the expressions of IL-17A and IL-23 in the dorsal skin of IMQ-treated mice. The mRNA expression levels of IL-17A and IL-23 were detected in the five groups: control group, IMQ group, IMQ + strictinin group, IMQ + theacrine group and IMQ + EGCG group. Statistical significance was shown at \*\*\*  $p < 0.001$  as compared with the control group, and #  $p < 0.05$  as compared with the IMQ group.



**Figure 10.** Effects of strictinin, theacrine and EGCG on the content of IL-17A<sup>+</sup>CD4<sup>+</sup> T cells in the spleens of IMQ-treated mice. Flow cytometric analysis was performed for single cells isolated from the spleens of mice in the five groups: control group, IMQ group, IMQ + strictinin group, IMQ + theacrine group and IMQ + EGCG group (A). The content of IL-17A<sup>+</sup>CD4<sup>+</sup> T cells from each group was measured and compared (B). Statistical significance was shown at \*\*\*  $p < 0.001$  as compared with the control group, and ###  $p < 0.001$  as compared with the IMQ group.

#### 4. Discussion

In this study, psoriasis-like dermatitis was successfully induced on the shaved dorsal skin of BALB/c mice by topical application of IMQ, and was used to evaluate the antipsoriatic effects of bitter Pu'er tea. The results indicated that bitter Pu'er tea possessed antipsoriatic activity, including a significant reduction in dorsal skin lesions, alleviation of splenomegaly and a decrease in the mRNA expression levels of IL-17A and IL-23 in the skin lesions of IMQ-treated mice. Furthermore, three potential active compounds, strictinin, theacrine and EGCG, which are abundantly found in bitter Pu'er tea, were evaluated for their antipsoriatic activity using the same animal model. The results showed that all three compounds displayed antipsoriatic activity in terms of a significant reduction in dorsal skin lesions. However, none of the three compounds displayed antipsoriatic activity equivalent to bitter Pu'er tea. Presumably, the antipsoriatic activity of bitter Pu'er tea was attributed to the additive effects of its multiple active compounds. Of course, it should not be ruled out that some minor compounds in bitter Pu'er tea might also play important roles in its antipsoriatic activity.

The contents in tea infusions prepared from different Pu'er tree leaves varied drastically, particularly those leaves harvested from wild tea plants [16,30]. Bitter Pu'er tea, a natural mutant with an additional bitter compound (theacrine), is not commercially cultivated thus far due to its bitterness, and thus, the contents of bitter Pu'er teas vary significantly among tea leaves collected from different sources. The content of theacrine in 1 g of the bitter Pu'er tea used in this study was approximately 12 mg [17]. The three compounds (strictinin, theacrine and EGCG), in association with caffeine, represented the major compounds in the infusion of the bitter Pu'er tea. Relatively, the content of 20 mg in 1 g of dry leaves in the bitter Pu'er tea is approximately 3–5 times higher than that in the commercially cultivated Pu'er teas [16,17]. Moreover, theacrine is basically undetectable in the commercially cultivated Pu'er teas. According to this study, theacrine, but not strictinin and EGCG, significantly attenuated splenomegaly (Figure 8), and strictinin seemed to possess better anti-inflammatory activity than theacrine and EGCG (Figure 9). Therefore, it is reasonable that the antipsoriatic activity of bitter Pu'er teas is empirically perceived to be higher than that of commercially cultivated Pu'er teas.

Structurally similar to caffeine, theacrine is also an antagonist for the adenosine receptor, and thus, it can increase the cAMP content and inhibit the inflammatory response in animals [31]. The cAMP levels in patients with psoriasis were found to be relatively low [32]. Topical application of 10% caffeine was shown to effectively reduce the severity of psoriasis in patients [33]. Accordingly, oral supplementation of theacrine was also found to reduce the dorsal skin lesions of IMQ-treated mice (Figure 7). In addition, theacrine significantly attenuated splenomegaly (Figure 8), although the detailed mechanism is still unknown. Whether the attenuation of splenomegaly by theacrine is related to the increase in the cAMP level remains to be clarified. It has been reported that the spleen, an important organ for the regulation of immune cells, was found to be enlarged (both in weight and size) and positively correlated with the increased composition of immune cells in the IMQ-treated mice [34]. It is also possible that theacrine might attenuate splenomegaly by regulating the immune system.

EGCG and other tea catechins ingested orally are mostly distributed on the surface of intestinal mucosa, and are finally excreted into the feces [35]. Certain portions of catechins are conjugated with glucuronide or sulfate in the intestinal mucosa, liver and kidney, though a certain portion of catechins are found in nonconjugated forms [36]. Therefore, the active compounds displaying antipsoriatic activity after oral consumption of EGCG were proposed to be its metabolic derivatives. Similarly, metabolic derivatives might also serve as the active compounds displaying antipsoriatic activity after oral consumption of strictinin. Strictinin is classified as a hydrolyzable tannin of the ellagitannin family [37]. Dietary ellagitannins are hydrolyzed to release ellagic acid in the human gastrointestinal tract [38]. Both ellagitannins and ellagic acid are metabolized to dibenzopyranones, known as urolithin A and its monohydroxylated derivative, urolithin B, by the colon microbiota

of different mammals [39]. Ellagic acid was demonstrated to possess anti-inflammatory effects on acute lung injury induced by acid in mice, and the effects were also attributed to its metabolites, urolithins (A and B), since urolithins have much better bioavailability than ellagic acid [40].

It has been demonstrated that the topical application of EGCG for six consecutive days alleviated psoriasiform dermatitis and improved the skin pathological structure, whereas oral supplementation of EGCG (150 or 300 mg/kg/day) attenuated IMQ-induced psoriasis-like inflammation in BALB/c mice [22]. In this study, oral supplementation of the bitter Pu'er tea infusion containing strictinin, theacrine and EGCG was also shown to attenuate psoriasis in the IMQ-induced BALB/c mice. Similarly, it is predictable that topical application of the bitter Pu'er tea infusion will also be therapeutically effective for the treatment of psoriasis. As described above, the active compounds in topical application and oral supplementation of the bitter Pu'er tea infusion might be drastically different (natural compounds vs. their derivatives). Therefore, it is feasible that the topical application of powder or cream containing bitter Pu'er tea can be applied to the skin lesions of patients in tandem with oral supplementation of the bitter Pu'er tea infusion for an additive efficacy in the treatment of psoriasis.

## 5. Conclusions

This study demonstrated that bitter Pu'er tea possessed antipsoriatic activity, including a significant reduction in dorsal skin lesions, alleviation of splenomegaly and a decrease in the mRNA expression levels of IL-17A and IL-23 in the skin lesions of imiquimod-treated mice. The antipsoriatic activity of bitter Pu'er tea seemed to be additively contributed to by several compounds, including, at least, three active compounds, strictinin, theacrine and EGCG. These three compounds are considered to be functional ingredients and possess great potential in the utilization of antipsoriatic treatment.

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