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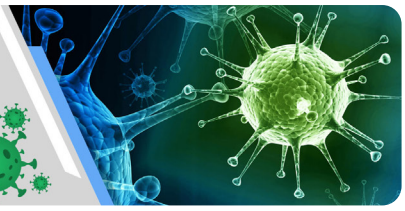

Novel anti-inflammatory Artemisia Naphta oil Extract Efficacious in *In Vivo* Mouse Models of Atopic Dermatitis and Psoriasis

Hu Huang[1,2], Kan Tao[1,2], Ziyan Qin[1,2], Lili Guo[1,2], Corey Fitzgerald[3], José R. Fernández[3], Eduardo Pérez[*,3]

Abstract

Background: *Artemisia annua* has been used in traditional Chinese medicine and has recently emerged in contemporary medicine as an anti-malaria treatment due to the presence of artemisinin, and topically for cosmetics. Since Chinese regulations prohibit the use of artemisinin in consumer products, we previously developed a novel, topical, artemisinin-free *A. annua* extract byproduct called artemisia naphta (AN) oil. We demonstrated that AN oil extract was effective *in vitro* and clinically in subjects with sensitive and/or acne prone skin. Given these findings, we sought to determine the therapeutic potential of AN oil extract for atopic dermatitis (AD) and psoriasis.

Results: Utilizing human peripheral blood mononuclear cells, we screened for AN oil extract's ability to inhibit T-cell mediated inflammation, a hallmark of AD and psoriasis. Results showed that AN oil extract significantly reduced T-cell Receptor induced IL-4 and IL-17A pro-inflammatory cytokine release. Given these promising *in vitro* results, we then tested AN oil extract's activity in topical *in vivo* models for both AD and psoriasis. In the calcipotriol or MC903-AD-induced model, AN oil extract demonstrated reduction in mouse ear thickness (edema) and several serum cytokines IL-1β, IL-6, and IgE. Furthermore, AN oil extract was also effectively ameliorated lesions, significantly reduced psoriasis area and severity index score down to 5.4 and inhibited serum inflammatory mediators (IL-6, TNF-α, IL-1β) in the imiquimod-induced psoriasis mouse model.

Conclusions: The results presented here make AN oil extract an attractive candidate for further development to treat AD and psoriasis as well as continued usage as a cosmetic ingredient

Keywords: Atopic dermatitis, Psoriasis, *Artemisia annua*, *Artemisia naphta*, Anti-inflammatory, Calcipotriol, Imiquimod.

Background

Artemisia annua (A. annua) is an annual herb used in traditional Chinese and contemporary medicine as a remedy for malaria and fever (1). The anti-malarial activity is derived from artemisinin, which was discovered in 1972 (2), and has since been shown to also possess anti-viral, anti-cancer, anti-inflammatory, and anti-bacterial properties (3). More recently, A. annua extracts have been identified as attractive for use as a raw material in cosmetics (4), possessing antioxidant activity (5), and potential for treating topical inflammatory disorders (6). Due to Chinese regulations prohibiting the use of artemisinin in cosmetics, we developed a novel, topical, artemisinin-free A. annua extract byproduct called artemisia naphta (AN) oil. Characterization of this AN oil extract found that its most abundant bioactive molecules were

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camphor, camphene, p- cymene, eucolyptol and D-limonene (7-9), previously reported to provide benefits to the skin. Subsequent in vitro studies for AN oil extract utilizing normal human epidermal keratinocytes (NHEKs) and mouse macrophages demonstrated anti-inflammatory activity (reduction of Interleukin (IL)-6, IL-8 and thymic stromal lymphopoietin (TSLP)), upregulation of filaggrin, and antibacterial activity inhibiting the growth of C. acnes and S. aureus (10). Lastly, a 1% AN oil extract gel formulation was applied topically to human subjects and was well tolerated and effective for sensitive and acne prone skin (10), further suggesting it's potential for treating inflammatory skin disease.

Downregulation of filaggrin (11), TSLP production (12) and Staphylococcus aureus overgrowth (13) have all been previously reported to be major players in the pathogenesis of atopic dermatitis (AD) and itch. Moreover, elevated TSLP levels have also been implicated in the pathogenesis of psoriasis (14), as have IL-6 (15) and IL-8 (16). Psoriasis is a common chronic inflammatory skin disorder affecting ~2% of the worldwide population (17). It is characterized by keratinocyte hyper-proliferation and inflammatory cells infiltrating the dermis and epidermis (18). AD is another common chronic inflammatory skin disease afflicting ~5% of adults and ~20% of children, and is characterized by intensive itch, dry skin, and a defective skin barrier possessing keratinocytes with the inability to combat secondary skin infections leading to further complications (19). In addition to keratinocytes, T-cells play a critical role in inducing and maintaining inflammatory cutaneous conditions such as psoriasis and AD (20). A skewed T-helper (Th)-1 and Th-17 response has emerged to play a central role in the pathogenesis of psoriasis (21). While the predominant phenotype in chronic AD lesions is primarily Th1, the initial stages of AD adopt a predominantly Th2 phenotype (22). The cytokines produced in the skewed immune responses have received great attention as potential targets for therapeutic intervention. Imiquimod (IMQ) is an agonist of Toll-like receptors 7/8 and is widely used to induce psoriasis-like skin lesions in mice (23). Moreover, calcipotriol (CPT or MC903), a vitamin D3 analog, induces changes in skin morphology and inflammation resembling immune perturbations observed in lesions of patients with AD (24). Given the previously reported activity profile for AN oil extract in skin, we sought to determine its therapeutic potential against psoriasis and AD by utilizing these two well established in vivo disease models.

Methods

Reagents

All reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Organic solvents were purchased from Fisher Scientific (Hampton, NH, USA).

Artemisia Naphta Oil Extraction

Extraction method was performed as previously described (10). *A. annua* leaves were heated with petroleum ether and resulting AN oil was filtered. Leaching solution was heated and evaporated to remove the petroleum ether. AN oil was distilled at 100-110°C and then drained to obtain crude AN oil. Crude product of AN oil was placed in a refining tank, heated to 110°C for 30 min and then cooled down. Silica gel was added to the oil, stirred for 30 minutes, and then filtered to decolorize the AN oil extract.

Cell Treatments

Human Peripheral Blood Mononuclear cells (hPBMCs) were obtained from Zen-Bio Inc. (Durham, NC), grown in suspension at normal conditions (5% CO2; 37°C) and later pre- incubated for 2 hours with test materials (0.1% v/v ethanol vehicle) in growth factor-depleted fresh media in triplicate. Cells were induced with Dynabeads T-Cell activator anti-CD3/CD28 (Life Technologies, Carlsbad, CA, USA). Cells were subjected to viability tests by MTS assay (Promega; Madison, WI, USA). Media supernatants were harvested after 24 hours induction for cytokines (IL-4, IL-17A) measured by ELISA kits (R&D Systems; Minneapolis, MN).

Animals

Six-week-old female BALB/c mice were purchased from Hunan Silaikejingda Experimental Animal Co., Ltd. (Hunan, China). All the mice were maintained with a 12-h light/dark cycle at 22–24°C. Animal experimental procedures were ethically reviewed and approved by The Animal Ethics Committee of the Second Affiliated Hospital of Hunan University of Chinese Medicine and in accordance with ARRIVE guidelines.

Atopic Dermatitis *In vivo* Model

AD-like dermatitis was produced by topical application with of 50 nmol/mL of calcipotriol in ethanol on the left ear (20µL) of mice every 48 hours for 14 days. AN oil (0.5-5%) suspended in Tween-20 solution (4%) vehicle was administered once daily (12 mice per group). At the end of treatment, mice were euthanized using CO2 and ear thickness was measured using a micrometer. Ear skin tissue was fixed in formalin for histopathological analysis and serum stored at -80°C for inflammatory markers (IL-1β, IL-6, IgE) protein expression by ELISA.

Psoriasis *In vivo* Model

Imiquimod-induced Psoriasis-like inflammatory model was produced by topical application with of 42mg of 5% imiquimod (IMQ) cream (Sichuan MED-SHINE Pharmaceutical Co., Ltd.; Sichuan, China) on the shaved dorsal skin of mice for 7 consecutive days. AN oil (0.5-

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5%) suspended in Tween-20 solution (4%) vehicle was administered once daily for 14 days. At the end of treatment, mice were euthanized using CO2 and skin lesions were observed. The severity of inflammation of the dorsal skin, an objective scoring system was developed based on the clinical Psoriasis Area and Severity Index (PASI). The score served as a measure of the severity of inflammation (scale 0–21.6). Dorsal skin tissue was fixed in formalin for histopathological analysis and serum stored at -80°C for inflammatory markers (IL-6, TNFα, IL- 1β) protein expression by ELISA.

Histopathological Analysis

Ear skin samples were fixed in 4% formalin, and six micrometer sections were prepared and stained with hematoxylin and eosin (H&E) for evaluation of the epidermal thickness and inflammation.

Enzyme-linked Immunosorbent Assays (ELISA)

The levels of human cytokines were measured from tissue culture media supernatants by sandwich ELISA using appropriate standards and following the manufacturers protocols. IL-4 and IL-17A kits were purchased from R&D Systems Inc. (Minneapolis, MN, USA). The levels of mouse cytokines were measured from blood serum by sandwich ELISA using appropriate standards and following the manufacturer's protocols. IL-6, TNFα, IL-1β, and IgE kits were purchased from Shanghai Xinle Biotechnology Co., Ltd. (Shanghai, China).

Statistical Analysis

Statistical significance was determined by ANOVA followed by a Dunnett multiple comparisons test using p-values less than 0.05 as a significant difference. For all ELISA measurements, samples were assayed in triplicate. Analyte dose–response curves were generated by fitting data with the Hill, three-parameter equation using the Sigma Plot software (Systat Software Inc.; Chicago, IL, USA), from which the maximum inhibition was determined.

Results

AN oil extract inhibits key pro-inflammatory cytokines for atopic dermatitis and psoriasis in PMBCs

AD and psoriasis are T-cell mediated cutaneous inflammatory diseases, making the use of human peripheral blood mononuclear cells (hPBMCs) an attractive cell-based model to screen for activity against these inflammatory disorders. Cells from three donors were treated separately with T-cell receptor (TCR) inducer anti-CD3/CD28 to induce pro-inflammatory cytokines IL-4, a key target for treating AD, and IL-17A, an important contributor to the pathogenesis of psoriasis (Figure 1). After 24-hour treatments, both IL-4 and IL-17A were significantly induced by anti-CD3/CD28 in every donor batch of cells. AN oil tested at 0.0001-0.01µg/mL significantly inhibited IL-4 production by >100% for all donors. For IL-17A, AN oil significantly inhibited cytokine production by an average of 83% (0.0001µg/mL), 54% (0.001µg/mL), and 66% (0.01µg/mL). Clobetasol (1µg/mL), a glucocorticoid used as a positive control, significantly inhibited IL-4 production by >100% for all donors, and had significant IL- 17A inhibition ranging from 56% to >100%.

Beneficial effects of AN oil extract application in atopic dermatitis mouse model

Topical application of calcipotriol (CPT or MC903) has been previously established as an *in vivo* AD model (25). After two weeks of daily CPT application to the left ear, mice of the CPT + vehicle group demonstrated an increase in redness, swelling, telangiectasia (small, widened blood vessels), as well as epidermal and stratum corneum thickening compared to the untreated group. Gross imaging of mouse ears illustrates the increase in redness and telangiectasia from CPT application, as well as an improvement when co-applied with AN oil (Figure 2A). As demonstrated by ear thickness measurements, application of CPT increased ear thickness by 71% compared to untreated (Table 1). Co-application of AN oil at 0.5-5% resulted in a significant dose-dependent reduction (39-66%) in ear thickness compared to CPT + vehicle group. Histology of ear sections further demonstrates the increase in ear thickness caused by CPT application, and the significant reduction in thickness of the epidermis and stratum corneum of mice treated with AN oil (Figure 2B).

In addition to the physical and visual hallmarks of AD, calcipotriol application also induces several AD-related pro-inflammatory cytokines. Two weeks of CPT application to mouse ears significantly increased the production of serum cytokines IL-1□, IL-6, and immunoglobulin E (IgE) by ~56%, ~69%, and ~52%, respectively (Table 2). While AN oil tested at 0.5% did not produce a significant inhibition for all cytokine endpoints in this model, all other higher concentrations (1-5%) produced statistically significant decreases in all cytokines compared to the vehicle control. Peak inhibitory activity was achieved with 1.5% AN oil reducing IL-1□, IL-6, and IgE, by 92%, 70%, and 99% respectively. All other AN oil doses produced inhibition responses between 38-60% for IL-1□, 33-55% for IL-6, and 68-85% for IgE (Table 2).

Beneficial effects of AN oil extract application in psoriasis mouse model

Topical application of imiquimod (IMQ), a Toll-Like Receptor 7/8 (TLR7/8) ligand, is utilized to induce psoriasis-like skin inflammation in mice closely resembling human disease symptoms including skin flakiness or scaling and induction of pro-inflammatory cytokines, making it an attractive *in vivo* psoriasis model (23). After two weeks of daily IMQ application to dorsal skin, mice of the IMQ +

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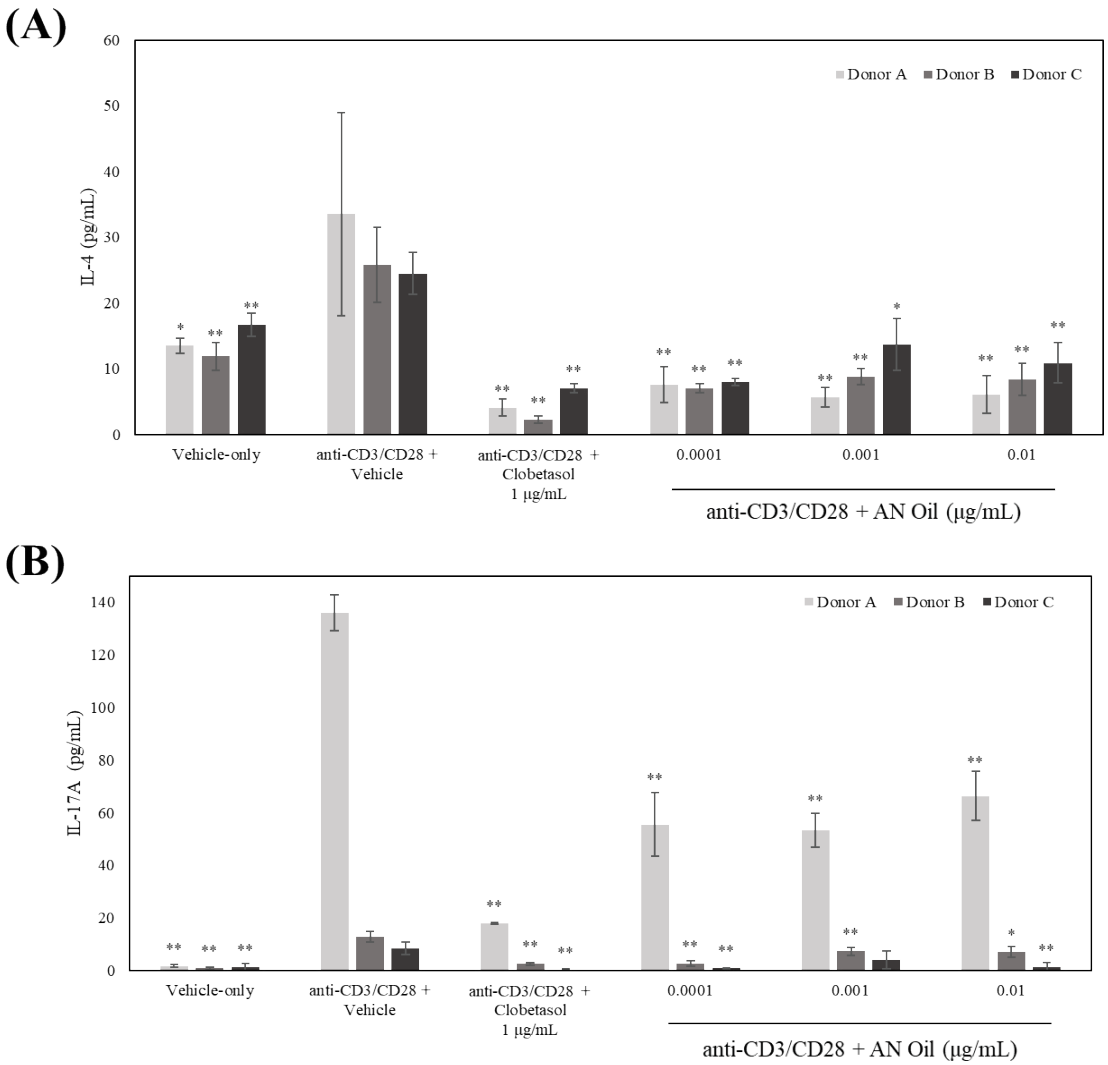

Figure 1: AN oil reduces cytokines in T-cell receptor activated hPBMCs. Cells were activated with TCR inducer anti-CD3/CD28 and measured for IL-4 (A) and IL-17A (B) by ELISA. Data shown represents the cumulative data (Avg ± StDev) from 3 independent experiments (3 donors run in triplicate). *p< 0.05 and **p≤ 0.01 indicate a statistically significant difference relative to each donor's anti-CD3/CD28 + Vehicle group.

vehicle treatment group demonstrated a significant increase in psoriasis area and severity index (PASI) score, and histological changes demonstrative of psoriatic pathology. As expected, IMQ application led to an increase in psoriatic-like scaling, and interestingly, co-application of AN oil ameliorated this condition (Figure 3). For quantitative assessment of dorsal skin inflammation, PASI scores were evaluated. Treatment with IMQ + vehicle produced a significant 10.65-point increase in PASI score compared to untreated (Table 3). Treatments with 3% and 5% AN oil produced significant improvements in score compared to IMQ + vehicle group, with PASI score decreases of 45% and 49%, respectively. To further visualize the pathological changes incurred with IMQ application, hematoxylin and eosin (H&E) staining was performed. The untreated group demonstrated no abnormalities, while tissues treated with IMQ + vehicle showed an increase in epidermal thickening, spinous process growth, and inflammatory cell infiltration (Figure 4). Treatment with AN oil demonstrated a dose- dependent improvement in these conditions, with 5% AN providing the best visual improvement.

Two weeks of IMQ application to mouse dorsal skin also caused a significant induction of serum cytokine production of IL-6, tumor necrosis factor alpha (TNF-α), and IL-1β (Table 4). IMQ application increased IL-6 and TNF-α production by 42% and 70%, respectively, and co- application with both 1% and 5% AN oil significantly inhibited both cytokines by >100%. Interestingly, for TNF-α, even the lowest concentration of AN oil (0.5%) produced a significant 52% inhibition. IL-1β was significantly increased by 76% from IMQ exposure. All AN oil concentration except 1.5% significantly inhibited IL-1β, with inhibitions reaching 58%, 89%, 80%, and 82%, for 0.5%, 1%, 3%, and 5% AN oil, respectively (Table 4).

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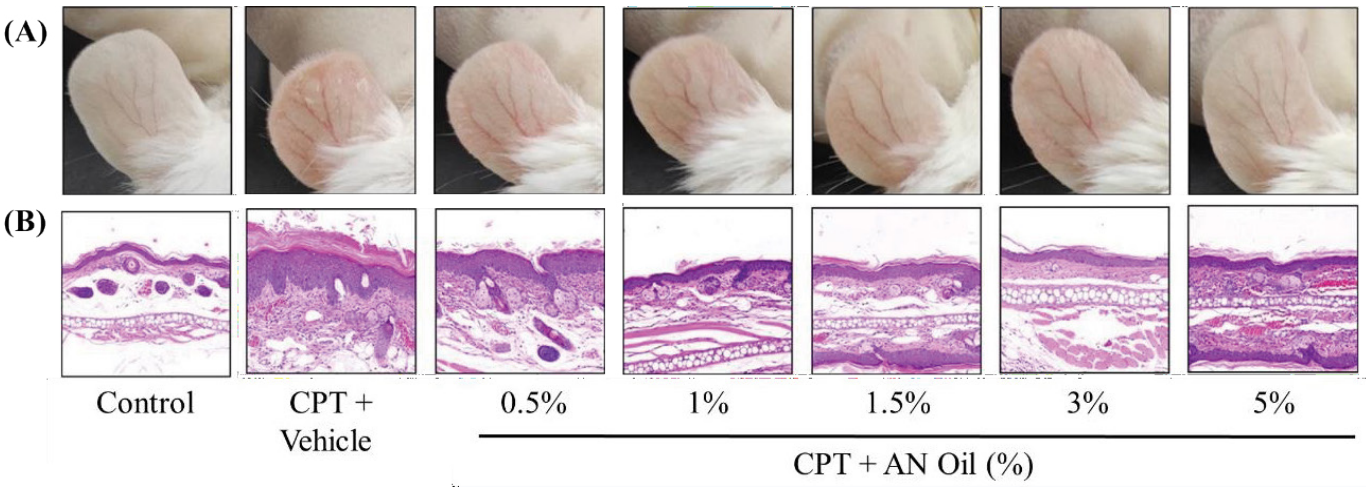

Figure 2: Gross appearance of ears (A) and H&E staining of skin sections at day 14 of calcipotriol (CPT) atopic dermatitis model. Original magnification x 200.

Table 1: AN oil reduces ear thickness in AD model.

Treatment Group	Left Ear Thickness (cm ± SD)#
Untreated	0.21 ± 0.01 **
CPT + Vehicle	0.36 ± 0.04
CPT + 0.5% AN oil	0.30 ± 0.06 **
CPT + 1% AN oil	0.31 ± 0.06 *
CPT + 1.5% AN oil	0.28 ± 0.04 **
CPT + 3% AN oil	0.28 ± 0.04 **
CPT + 5% AN oil	0.26 ± 0.04 **

#Ear thickness measured by micrometer

*p< 0.05 and **p≤ 0.01 indicate a statistically significant difference relative to Calcipotriol (CPT) + Vehicle group n=12 mice per treatment group

Table 2: AN oil reduces pro-inflammatory cytokines in AD model.

Treatment Group	Serum Cytokines (pg/mL ± SD)#		
	IL-1β	IL-6	IgE
Untreated	71.51 ± 19.52 **	57.93 ± 6.02 **	6.29 ± 1.81 **
CPT + Vehicle	111.51 ± 15.65	97.73 ± 13.62	9.54 ± 2.03
CPT + 0.5% AN oil	104.94 ± 13.05	93.25 ± 12.77	8.34 ± 1.16
CPT + 1% AN oil	87.36 ± 9.11 **	84.51 ± 12.01 *	7.15 ± 1.55 **
CPT + 1.5% AN oil	74.82 ± 8.60 **	70.00 ± 9.84 **	6.31 ± 1.06 **
CPT + 3% AN oil	96.24 ± 15.57 *	76.02 ± 10.62 **	7.32 ± 1.47 **
CPT + 5% AN oil	88.98 ± 6.23 **	80.89 ± 10.15 **	6.77 ± 1.43 **

#Serum cytokines measured by enzyme-linked immunosorbent assay (ELISA)
*p< 0.05 and **p≤ 0.01 indicate a statistically significant difference relative to Calcipotriol (CPT) + Vehicle group

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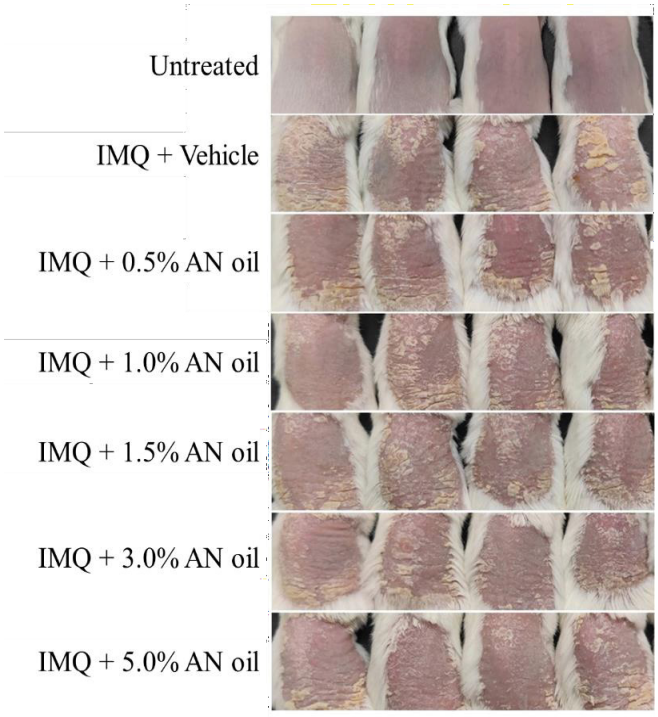

Figure 3: Gross appearance of mouse dorsal skin at day 14 of imiquimod (IMQ) psoriasis model. Original magnification x 200.

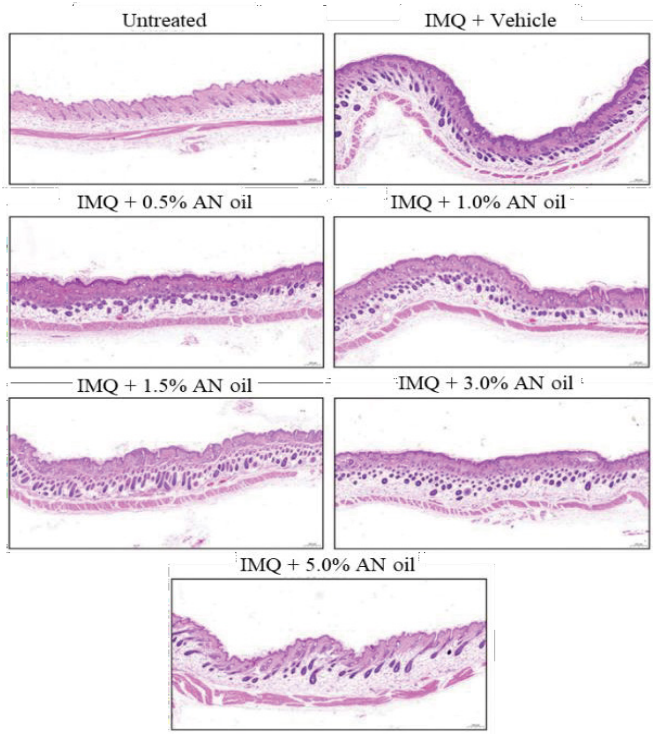

Figure 4: H&E staining of dorsal skin sections at day 14 of imiquimod (IMQ) psoriasis model. Original magnification x 200.

Table 3: AN oil reduces PASI score in psoriasis model.

Treatment Group	PASI Score ± SD#
Untreated	0.00 ± 0.00 **
Vehicle	10.65 ± 2.70
0.5%AN	7.05 ± 1.24
1%AN	7.05 ± 1.24
1.5%AN	6.75 ± 1.72
3%AN	5.85 ± 1.72 *
5%AN	5.40 ± 2.55 *

Table 4: AN oil reduces pro-inflammatory cytokines in psoriasis model.

Treatment Group	Serum Cytokines (pg/mL ± SD)#		
	IL-6	TNF-α	IL-1β
Untreated	72.99 ± 16.11 *	320.52 ± 58.83 **	56.23 ± 10.10 **
IMQ + Vehicle	103.51 ± 14.94	544.74 ± 30.78	98.83 ± 6.40
IMQ + 0.5% AN oil	87.52 ± 13.40	428.47 ± 46.61 **	74.23 ± 14.48 *
IMQ + 1.0% AN oil	65.63 ± 10.53 **	299.45 ± 113.96 *	61.03 ± 14.71 **
IMQ + 1.5% AN oil	98.01 ± 12.80	485.58 ± 73.95	85.08 ± 13.56
IMQ + 3.0% AN oil	86.82 ± 11.19	455.06 ± 90.18	64.56 ± 15.80 *
IMQ + 5.0% AN oil	71.22 ± 8.46 *	319.89 ± 57.68 **	63.76 ± 10.79 **

#Serum cytokines measured by enzyme-linked immunosorbent assay (ELISA)
*p< 0.05 and **p≤ 0.01 indicate a statistically significant difference relative to Imiquimod (IMQ) + Vehicle group

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Discussion

Artemisia species have been utilized for centuries as Traditional Chinese Medicine and have recently emerged as topical skin care actives in cosmetics. There are more than 300 *Artemisia* species and thus far *Artemisia annua*, *Artemisia abrotanum*, *Artemisia absinthium*, *Artemisia capillaries*, *Artemisia dracunculus*, and *Artemisia vulgaris* appear to be the most used as raw materials (26). Interest in *Artemisia* peaked with a Nobel Prize discovery that several species, such as *A. annua*, *abrotanum,* and *A. vulgaris*, contained artemisinin, a sesquiterpenoid lactone proven to be effective for the treatment of malaria (27). This led to phytochemical and biological activity characterization of many of these species and results showed that while there are some shared classes of compounds, the *artemisia* species slightly differ from each other in their chemical composition (26). Our initial *in vitro* biological characterization of AN oil extract (from *A. annua*, artemisinin-free) yielded intriguing results for its potential use to treat inflammatory skin disorders. Specifically, for AD, AN oil extract previously demonstrated the ability to inhibit *S. aureus* growth as well as *S. aureus*-induced TSLP, which is linked to AD skin pruritus (10). Moreover, AN oil extract was shown to increase filaggrin, an essential gene in the formation of the epidermal barrier, and reduce Th2 cytokine-induced IL-8 production in normal human epidermal keratinocytes. All the above have been shown to be key contributors to the pathogenesis of AD, providing the rationale to continue investigation into AN oil's therapeutic potential for AD.

For chronic inflammatory skin disorders like AD and psoriasis, innate and adaptive immunity both play a crucial role. Interestingly, previously published results studying the anti-inflammatory activities of *Artemisia* predominantly focus on reducing the innate immune response in keratinocytes (28, 29), but not the adaptive response. Having previously studied the activity of AN oil extract in keratinocytes and demonstrating its activity in reducing the innate immune response, we next utilized an hPBMC cell-based assay to confirm for the first time that AN oil extract also acts on lymphocytes, the key mediators of the adaptive immune response. Utilizing T- cell receptor inducer anti-CD3/CD28, AN oil extract not only inhibited IL-4 (a key AD cytokine) and IL-17A (an important psoriasis cytokine) release from hPBMCs, but did so at concentrations lower than the potent topical glucocorticoid clobetasol (Figure 1A + B). Furthermore, given we utilized primary hPBMCs to better mimic what happens in human skin instead of human leukemia monocytic cell line (THP-1) cells, we observed expected donor to donor variability (30), but nevertheless, AN oil extract effectively reduced cytokine production for all donors.

Having now demonstrated strong cell-based anti-inflammatory activity for cytokines linked to AD and psoriasis, as well as previously showing clinical improvement reducing redness, swelling and itch when applying AN oil extract topically on human subjects with sensitive and acne prone skin (10), we sought to determine AN oil extract's efficacy in *in vivo* topical models for skin disease. Previously, *Artemisia* species have been shown to be effective in several different mouse models including the 2, 4-Dinitrocholrlbenzene (DNCB)-induced AD model, which is the most commonly utilized model (31-34), NC/Nga mice (35, 36), and the oxazolone-induced AD model (37). We instead selected the CPT-induced AD mouse model for four reasons: 1) This model mimics most of the clinical, skin barrier-related, histological, and immunological characteristics observed in AD patients (38); 2) It does not involve hazardous agents like oxazolone or DNCB; 3) It does not affect systemic calcium metabolism in mice, allowing for the CPT to be applied to the ear and dorsal skin at higher concentrations and for a longer time to induce AD inflammatory phenotypes (24); and 4) This would be the first time an *Artemisia* species would be tested in this specific AD model.

As shown in the results, CPT increased ear thickness (via micrometer measurements and H&E staining), which is a marker for skin inflammation (39), and AN oil extract dose-dependently reduced ear thickness. This reduction in inflammation was also observed visually as mouse ear erythema was greatly reduced as AN oil extract concentration reached 5% (Figure 2). Moreover, a key protagonist for CPT-induced AD-like skin inflammation is the increase of key pro-inflammatory cytokines, and we demonstrated that AN oil extract significantly inhibited serum levels of IL-1β, IgE, and IL-6 (Table 2), all which have been shown to play a potential role in AD pathogenesis. For example, patients with AD often display elevated levels of total serum IgE, and autoreactive IgE antibodies may elicit an allergic-autoimmune process and contribute to perpetuation of inflammation (40). Also, peripheral blood T cells derived from patients with AD spontaneously produce increased amounts of IL-6 compared to T cells from normal subjects, which reflects the increased activation state of T cells in atopic dermatitis (41). Lastly, IL-1β has been shown to be an early key mediator for the acquisition of an AD phenotype through induction of TSLP and alteration of epidermal homeostasis (42). Altogether, this clearly demonstrates that AN oil extract is efficacious when applied topically to reduce the inflammatory pathways and biomarkers associated with AD in this preclinical model. Given that AN oil extract inhibited Th-17A response *in vitro,* which plays an important role in psoriasis (Figure 1), and previously demonstrating downregulation of TSLP in macrophages (10), also linked to psoriasis and AD, we sought to determine if AN oil extract would be effective when applied topically *in vivo*. Several animal models mimicking human psoriasis have been developed and

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utilized. We selected the IMQ-induced psoriasis preclinical model because it allows for the elucidation of underlying mechanisms and the evaluation of new therapies against psoriasis in a rapid and convenient manner (43). Moreover, unlike AD where several different *Artemisia* species were tested in different models, as of this report only one species, *A. capillaris* formulated in a topical cream, has been tested for anti-psoriatic activity and it was performed in the IMQ-induced psoriasis model (44). Thus, despite *A. capillaris* possessing a very different phytochemical profile compared to AN oil extract, we selected the *in vivo* IMQ model to assess its efficacy. As expected, and previously shown utilizing this model, application of IMQ to the dorsal skin of mice led to a visual increase in psoriatic scaling (Figure 3), thickening of the epidermis, and infiltration of inflammatory cells (Figure 4) which were all decreased when AN oil extract was co-applied. To verify these observations quantitatively, we measured PASI, a common psoriasis scoring tool where generally, PASI >12 is severe, PASI 7 to 12 is moderate, and PASI less than 7 is mild (45). IMQ + vehicle application resulted in a PASI score in the 10-11 range, representing the induction of moderate psoriasis. Topical application of AN oil extract demonstrated a dose-dependent and statistically significant reduction in PASI score with AN oil tested at 3% and 5% producing scores in the 5-6 range, improving the psoriatic condition from moderate to mild (Table 3). In addition to being characterized by red, scaly lesions formed by the hyperproliferation of epidermal keratinocytes, several inflammatory cytokines have been shown to be elevated in psoriasis lesions, and the serum concentrations of a subset of these also correlate with psoriasis disease severity (46). Two such cytokines are IL-6 and TNF-α, which have both been reported to be potential biomarkers for psoriasis and targets for treatment response (47) with the development of anti-TNF (48) and IL-6 inhibitor therapeutics (49). In skin lesions of psoriasis patients, IL-1β levels have been found to be increased, and effective treatment leads to a significant decrease in epidermal IL-1β expression, suggesting that IL-1β and its family members play a role in the pathogenesis of this disease (50, 51). As shown in Table 4, AN oil extract significantly reduced the levels of all three of these cytokines. Successful development of AN oil extract for treating psoriasis would offer several benefits over the current injectable biologics being utilized, including being more affordable and being a topical therapeutic option. Moreover, being able to downregulate multiple key pro-inflammatory cytokines contributing to the pathogenesis of the disease may be a better long-term approach than targeting a single one.

Conclusion

Here we demonstrate for the first time that artemisinin-free AN oil extract *in vitro* effectively reduces T-cell mediated production of IL-4 and IL-17A cytokines, which are both associated in the pathogenesis of inflammatory skin diseases including psoriasis and atopic dermatitis (AD). To further characterize the potential activity for AN oil extract for skin, we extended our studies to *in vivo* disease models. We found that topical application of AN oil extract had significant therapeutic effects in the CPT induced-AD mouse model, including reduction in mouse ear thickness and pro-inflammatory cytokine production. Moreover, AN oil extract was also effective in ameliorating lesions and inhibiting inflammatory mediators in the IMQ-induced psoriasis mouse model. Altogether, these results make AN oil extract an attractive candidate for further development to treat AD and psoriasis.

Abbreviations

AN: artemisia naphta; AD: atopic dermatitis; hPBMCs: human peripheral blood mononuclear cells; CPT: calcipotriol; PASI: psoriasis area and severity index; IMQ: imiquimod; NHEKs: normal human epidermal keratinocytes; TSLP: thymic stromal lymphopoietin; Th: T-helper; TCR: T-cell receptor; IgE: immunoglobulin E; H&E: hematoxylin and eosin; TNF-α: tumor necrosis factor alpha; THP-1: human leukemia monocytic cell line; DCNB: 2, 4- Dinitrocholrlbenzene

Declarations

Ethics approval and consent to participate

Not applicable

Consent for Publication

Not applicable

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article

Competing interests

All authors are employees of Shanghai Chicmax Cosmetic Co., Ltd. and/or Signum Biosciences. The funders had no role in experimental design, running experiments, data analysis, data interpretation, or writing of the manuscript.

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Author Contributions

HH, KT conceived and designed the study. CF, KF, ZQ, LG performed experiments. EP drafted the manuscript. HH, KT, CF, ZQ, LG, analyzed and reviewed the manuscript. All authors read and approved the final manuscript.

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