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Picrasma quassioides (D.DON) Benn. Ethanolic Extract Improves Atopic Dermatitis and Hyperactivity Disorder in DNCB-Treated BALB/c Mice

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Abstract: Atopic dermatitis (AD) is a chronic inflammatory skin disorder that can be associated with psychiatric disorders. *Picrasma quassioides* (D.Don) Benn (Gomokpi, GMP), a traditional medicinal herb, has been used to treat skin diseases, including AD. The current study examined the effects of an ethanolic extract of GMP on 2,4-dinitrochlorobenzene (DNCB)-induced AD mice. The severity of skin symptoms and behavioral changes in AD mice were evaluated. GMP alleviated the AD-like skin inflammation and hyperlocomotion activity in DNCB-treated BALB/c mice. The effects of GMP behavioral abnormalities might occur by inhibiting TNF- α production in the PFC. GMP suppressed the production of TARC (Th2 chemokine) in TI-stimulated HaCaT keratinocytes. Moreover, GMP also exerted immunosuppressive effects by reducing TNF- α production in LPS-stimulated Raw264.7 macrophages, IL-17 expression in PI-stimulated EL4 cells, and VEGF secretion in SP-stimulated HMC-1 cells. These findings suggest that GMP could be useful for treating AD by modulating inflammatory responses and comorbid behavioral changes.

Keywords: *Picrasma quassioides* (D.Don) Benn.; atopic dermatitis; skin inflammation; ADHD; neuroinflammation

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1. Introduction

Atopic dermatitis (AD) is a common inflammatory skin disease that requires long-term treatment because of its frequent recurrence [1]. Typical AD manifestations include dryness, redness, swelling, and severe itching. The pathogenesis of AD is unclear, but genetic factors, environmental factors, and immune system imbalance are associated with the cause of AD [2–4]. Upon the invasion of external allergens into the body, the balance of T helper (Th) 1 and Th2 cytokines is disrupted, resulting in the overproduction of Th2 cytokines and stimulation of B cells to produce IgE [2]. IgE can bind to Fc epsilon receptor 1 (Fc ϵ RI) on the mast cell surface, leading to mast cell degranulation and the secretion of inflammatory mediators, such as histamine, cytokines, chemokines, and growth factors [5]. These degranulation substances cause and worsen AD symptoms, such as swelling and itching [6]. The interaction between immune cells and keratinocytes plays a crucial role in skin inflammatory responses in AD. Keratinocytes are the major component of the epidermis that can produce various cytokines and chemokines to recruit immune cells, such as T cells, macrophages, eosinophils, and mast cells, into lesional skins [7]. In turn, the inflammatory mediators produced by these immune cells can promote cytokine production and decrease the expression of skin barrier peptides in keratinocytes, leading to skin inflammation and barrier disruption in AD lesions [8].

Several psychiatric comorbidities have been indicated in patients with AD [9]. A previous study reported that AD is associated with an increased risk of attention deficit hyperactivity disorder (ADHD) in both children and adults, with a higher risk in those

with more severe symptoms of AD and frequent sleep disturbances [10]. The possible underlying mechanisms for the higher prevalence of psychiatric disorders in AD patients have been suggested [11]. The persistent excessive release of inflammatory cytokines in AD affects the development of brain areas, such as the prefrontal cortex (PFC) and neurotransmitter system, which play crucial roles in ADHD [12]. Hyperactivity patterns, decision-making, and attention deficits in ADHD are associated with the PFC region, and clinical studies have shown changes in neuronal activity in the PFC region after chronic AD [13,14]. Elevated levels of pro-inflammatory molecules, such as TNF- α , IFN- γ , and IL-10 in AD lead to the dysregulation of the hypothalamus-pituitary-adrenal (HPA) axis and the secretion of corticosteroids, which might contribute to the development of ADHD [15]. This suggests a close relationship in the pathophysiology of AD and psychiatric disorders. Thus, therapeutic approaches that manage AD symptoms and psychological disorders should be investigated to improve the patients' satisfaction with AD treatment.

Picrasma quassioides (D.Don) Benn. bark, also known as Gomokpi (GMP) in traditional Korean, can strengthen the stomach, eliminate dampness, remove intestinal parasites, and promote pus discharge. GMP has been used to treat several symptoms, such as indigestion, gastroenteritis, pulmonary tuberculosis, and eczema [16,17]. The main components of GMP are quassinoids, triterpenoids, and alkaloids, which have a range of pharmacological effects, including anti-inflammatory, anti-cancer, and neuroprotective effects [18]. Previous studies reported that the GMP extract inhibited the allergic response in an ovalbumin-induced mouse model of asthma and suppressed the production of inflammatory mediators in RAW264.7 macrophages stimulated with lipopolysaccharide (LPS) [19,20]. Hence, this study investigated the beneficial effects of GMP on skin symptoms and behaviors in a 2,4-dinitrochlorobenzene (DNCB)-induced mouse model of AD.

2. Materials and Methods

2.1. Chemicals and Reagents

Iscove's Modified Dulbecco's Medium (IMDM) was purchased from (Merck Millipore, Darmstadt, Germany), and high glucose Dulbecco's modified Eagle's medium (DMEM) was obtained from (Welgene Inc., Gyeongsangbuk, Korea). Fetal bovine serum (FBS) and penicillin-streptomycin were acquired from (Invitrogen, Carlsbad, CA, USA). Substance P (SP), 2,4-dinitrochlorobenzene (DNCB), lipopolysaccharide (LPS), and dexamethasone (DEX) were purchased from (Sigma-Aldrich, St. Louis, MO, USA). The mouse VEGF, TNF- α , IFN- γ , IL-1 β , IL-6, IL-17, and human VEGF ELISA kits were purchased from (Koma Biotech, Seoul, Korea). Human MDC and TARC ELISA kits were supplied by (R&D Systems, Minneapolis, MN, USA). Tissue extraction reagent I was obtained from (ThermoFisher Scientific, Carlsbad, CA, USA).

2.2. Preparation of Herbal Ethanolic Extracts of *Picrasma quassioides* (D.Don) Benn. Bark

Picrasma quassioides (D.Don) Benn. bark (GMP) was provided and verified by Professor Heung-Mook Shin (Department of Physiology, Dongguk University). The dried GMP (204 g) was extracted in 70% ethanol at 80 °C for 3 h. The fluid extract was passed through 0.2 mm filter paper (Whatman, Maidstone, UK), evaporated with a rotary vacuum evaporator, and freeze-dried for three days. The yield of GMP was 4.47%. The GMP extract was stored at −20 °C for further experiments.

2.3. High-Performance Liquid Chromatography (HPLC) Analysis

Picrasin B and quassin were obtained from (ChemFaces, Wuhan, China). The chemical constituents were analyzed by an HPLC system (1290 series, Agilent, Santa Clara, CA, USA). The GMP extract and standard samples were separated on a Kinetex C18 column (4.6 × 250 mm, 5 μ m, Phenomenex) with two mobile phases: (A) 0.1% phosphoric acid and (B) acetonitrile. The solvent gradient was 10–90% (B) for 25 min, followed by 5 min equilibration. The column was kept at 35 °C with a flow rate of 0.9 mL/min. Picrasin B

and quassin were detected at 210 nm and 254 nm, respectively. The concentrations of the compounds in GMP were calculated using the calibration lines.

2.4. Animal Experiments

BALB/c (three-week-old, male) mice were obtained from Koatech (Seoul, Korea). Male mice were used in this study because the individual estrous cycle in the female mice might result in more variable data than male mice. Moreover, female hormones (estrogen, progesterone) have been reported to promote Th2 activity that affects clinical outcomes in the AD model [21]. Animal studies were conducted according to the protocols approved by the Institutional Animal Care and Use Committee of Dongguk University (IACUC-2020-05). The animals were stabilized for two weeks before the experiments. The mice were divided randomly to five groups ($n = 7$ –8 per group): normal control group (NC), DNCB treatment group (DNCB), DNCB plus 0.1% of GMP (0.1% GMP), DNCB plus 1% of GMP (1% GMP), DNCB plus 0.1% DEX (DEX). Figure 1 shows a schematic diagram of the experimental schedule. BALB/c mice were treated topically with 4% SDS and 1% DNCB three times per week for one week (sensitization period). The AD-like skin lesions were maintained by topical treatment with 0.3% DNCB three times per week for five weeks. DNCB, GMP, and DEX were dissolved in a 3:1 mixture of acetone and olive oil. The mice were treated topically with vehicles or drugs (200 μ L) five times per week for five weeks. The severity of skin lesions was assessed for symptoms of erythema, edema, scale/dryness, and abrasions in the epidermis. The scale for each symptom includes the following: 0 (asymptomatic), 1 (mild), 2 (moderate), and 3 (severe). At the end of the experiment, all mice were sacrificed using isoflurane, and the body weight and spleen weight of each mouse was recorded. The spleen index was defined as the ratio of spleen weight to body weight (mg/g). For protein expression analysis, skin lesions on the back and prefrontal cortex (PFC) were excised and homogenized using a tissue extraction reagent.

Figure 1. Experimental design for induction of AD and GMP treatment in BALB/c mice. AD, atopic dermatitis; GMP, Gomokpi; DNCB, 2,4-dinitrochlorobenzene; OFT, open field test; EPM, elevated plus maze.

2.5. Open Field Test (OFT)

On day 36 of the experiment, an OFT was conducted to evaluate the effects of GMP on locomotor activity in DNCB-treated BALB/c mice. All mice were moved to the test room 1 h before the experiment for habituation. The mice were placed in the center of an OFT box (40 × 40 × 40 cm) and were allowed to explore freely for 15 min. At the end of each session, the box was wiped with 70% alcohol to remove the odor. Smart V3.0 software (Panlab Harvard Apparatus, MA, USA) was used to assess the total distances (cm), distances in the periphery and the center, and mean speeds (cm/s).

2.6. Elevated Plus Maze (EPM)

On day 38 of the experiment, the EPM was conducted to measure the anxiety-like behaviors in mice. All mice were moved to the test room 1 h before the experiment for habituation. Each mouse was placed in the center of a plus maze (two opposite open arms and two opposite closed arms) elevated 50 cm above the floor. The EPM test was conducted for 5 min. At the end of each session, the box was wiped with 70% alcohol to remove the odor. Smart V3.0 software (Panlab Harvard Apparatus, MA, USA) was used to analyze the number of entries and time spent in the closed and open arms in a 5 min session.

2.7. Histological Analysis

The collected dorsal skin samples were fixed in 4% paraformaldehyde and paraffin-embedded. The sections were cut at 5 μ m and stained with hematoxylin and eosin (H&E), toluidine blue, and Congo red. The histological observation was conducted using a Lionheart FX Microscope and Gen5 Imager software (Biotek Instruments Inc., Winooski, VT, USA). The epidermal thickness and the number of eosinophils and mast cells were determined in three randomly selected areas per sample.

2.8. Cell Culture and Treatments

HaCaT cells (a human keratinocyte cell line) were obtained from KIOM (Daegu, Korea). Raw264.7 cells (a mouse macrophage cell line), EL4 cells (a mouse T lymphoblast cell line), and HMC-1 cells (a human mast cell line) were obtained from the Korean Cell Line Bank (Seoul, Korea). The HaCaT, Raw264.7, and EL4 cells were cultured in DMEM supplemented with 1% penicillin-streptomycin and 10% FBS in a humidified environment of 5% CO₂ at 37 °C. The HMC-1 cells were cultured in IMDM supplemented with 10% FBS, 1% penicillin-streptomycin, 1.2 mM 1-thioglycerol, in a 5% CO₂ humidified incubator at 37 °C. All cell types were pre-treated with selected concentrations of GMP for 1 h before stimulation. HaCaT, Raw264.7, and EL4 cells were stimulated with TI (TNF- α and IFN- γ , 10 ng/mL each), LPS (1 μ g/mL), and PI (PMA 10 ng/mL, ionomycin 100 ng/mL), respectively, for 24 h. The HMC-1 cells were stimulated with SP (10 μ M) for 48 h.

2.9. Cell Viability Assay

The cytotoxic effects of GMP on HaCaT, Raw264.7, EL4, and HMC-1 cells were investigated using a Cell Proliferation Kit II (XTT) (Sigma-Aldrich, Mannheim, Germany). The cells were seeded at a density of 5×10^4 cells/well (100 μ L) into 96-well plates and cultured for 24 h, and treated with GMP (50, 100, 300, and 500 μ g/mL) for another 24 h. Subsequently, XTT solution (50 μ L/well) was added and the plates were incubated for 4 h. The optical densities were assessed at 450 nm (reference wavelength: 650 nm) using a microplate reader (Tecan Sunrise, Männedorf, Switzerland).

2.10. Enzyme-Linked Immunosorbent Assay (ELISA)

Commercial ELISA kits were used to determine the levels of chemokines and cytokines in cell-cultured media and skin tissue lysates, including TARC and MDC in HaCaT cells, TNF- α in Raw264.7 cells, IL-17 in EL4 cells, VEGF in HMC-1 cells, and IFN- γ , VEGF, and IL-10 in skin lysates. All procedures were conducted following the manufacturer's instructions. The absorbances were evaluated at 450–540 nm using a microplate reader (Tecan Sunrise, Männedorf, Switzerland).

2.11. Statistical Analysis

All experiments were performed at least three times independently. The data are presented as the means $\pm$ standard errors followed by the statistical significance (Student's t-test for unpaired experiments) with a *p*-value < 0.05. The Pearson correlation was used to evaluate the correlation between the two different parameters.

3. Results

3.1. Effect of GMP on Skin Lesions in DNCB-Treated BALB/c Mice

As shown in Figure 2A,B, after applying DNCB to the back skin for six weeks, the clinical skin symptoms of the DNCB group was significantly more severe than the NC group. In contrast, the skin scores were reduced significantly in the 0.1% GMP and 1% GMP treated groups compared to the DNCB group (Figure 2A,B). In addition, the spleen index was reduced significantly in the DNCB group compared to the NC group, which was significantly lower in the GMP-treated groups (Figure 2C). The topical application of GMP decreased DNCB-induced epidermal hyperplasia significantly in BALB/c mice (Figure 2D). Toluidine blue and Congo red staining results showed that eosinophil and mast cell infiltrations in the skin lesions were less severe in the 0.1% GMP and 1% GMP groups than in the DNCB group (Figure 2D).

Figure 2. Effect of GMP on AD-like symptoms in DNCB-treated BALB/c mice. (A) Images of dorsal skin lesions from each group were taken at the end of the experiment. (B) The total skin score of dorsal skin lesions (C) Spleen index was measured. (D) Histological and immunohistochemical staining of dorsal skin samples. Scale bar: 100 μm. Data represent the means ± SDs. * $p < 0.05$ compared to the NC group, # $p < 0.05$ compared to the DNCB group. AD, atopic dermatitis; GMP, Gomokpi; DEX, dexamethasone; DNCB, 2,4-dinitrochlorobenzene.

3.2. Effect of GMP on Serum IgE Levels and Inflammatory Cytokine Levels in Skin Lesions in DNCB-Treated BALB/c Mice

The effects of GMP on the levels of serum IgE and inflammatory cytokines in the skin tissues were evaluated using an enzyme-linked immunosorbent assay (ELISA). The results show that the serum IgE level was significantly increased in the DNCB group, compared with the NC group (Figure 3A). In contrast, treatment with 1% GMP reduced the IgE level in the serum significantly. The levels of IFN- γ , VEGF, and IL-10 in the skin lysates were significantly higher in the DNCB group than in the NC group. On the other hand, these increases were lowered by treatment with GMP (Figure 3B–D).

Figure 3. Effect of GMP on serum IgE and inflammatory cytokines. Serum IgE and cytokine levels were evaluated by ELISA. (A) Serum IgE level. (B–D) Levels of inflammatory cytokine IFN- γ , VEGF, and IL-10 in the skin lesions. Data represent the means $\pm$ SDs. * $p < 0.05$ vs. the NC group, # $p < 0.05$ vs. the DNCB group.

3.3. Effect of GMP on the Behavioral Changes in DNCB-Treated BALB/c Mice

The effects of GMP on locomotor activity and anxiety-like behavior were evaluated using OFT and EPM tests. In the OFT test, the DNCB group showed a significant increase in the total distance and distance traveled in the center compared to the NC group. The 0.1% GMP and 1% GMP groups showed a significant decrease compared to the DNCB group. The mean speed was also higher in the DNCB group than the NC group and lower in the GMP treatment groups (Figure 4A,B). The DNCB group showed a moderate increase in the distance traveled in open arms of EPM, but there was no significant difference between the NC and DNCB groups (Figure 4C,D). In contrast, compared to the DNCB group, the distance in the open arms was significantly reduced in the 0.1% GMP and 1% GMP groups. The distance traveled in closed arms and the mean speed of the DNCB group were not significantly different from the NC group, and the GMP treatment did not have any effects (Figure 4C,D).

Figure 4. Effect of GMP on behavioral changes in DNCB-treated BALB/c mice. **(A)** Representative tracking images of each group in the OFT. **(B)** Total distance, distance in center, and mean speed in the OFT were assessed on day 36. **(C)** Representative tracking images of each group in the EPM. **(D)** Distance in open arms, closed arms, and mean speed in the EPM were assessed on day 38. Data represent the means $\pm$ SDs. * $p < 0.05$ vs. the NC group, # $p < 0.05$ vs. the DNCB group.

3.4. Effect of GMP on Neuroinflammation in DNCB-Treated BALB/c Mice

ELISA showed that the levels of TNF- α and IL-1 β in the PFC were increased in the DNCB group compared to the NC group. The GMP treatment reduced TNF- α levels significantly but did not affect the IL-1 β levels (Figure 5A). IL-6 expression was similar in the NC group and the DNCB group, and the 1% GMP group decreased significantly compared to the DNCB group (Figure 5A). The relationship between the total distance in the OFT and the expression levels of the inflammatory cytokines in the PFC were analyzed further to determine if the hyperactivity behavior correlates with neuroinflammation. The total distance in the OFT showed a positive correlation with TNF- α levels ($R^2 = 0.3594$, $p < 0.0001$) (Figure 5B).

Figure 5. Effect of GMP on the inflammatory cytokine levels in the PFC in DNCB-treated BALB/c mice. (A) Inflammatory cytokines TNF- α , IL-6, and IL-1 β levels in the PFC area of the brain. (B) Correlation between inflammation cytokine and total distance in OFT. Data represent the means $\pm$ SDs. * p < 0.05 vs. the NC group, # p < 0.05 vs. the DNCB group.

3.5. Effect of GMP on Inflammatory Responses In Vitro

HaCaT, HMC-1, EL4, and Raw264.7 cells were used to investigate the anti-inflammatory effects of GMP in vitro. The XTT assays showed that GMP had cytotoxic effects at 300–500 μ g/mL (Figure 6A,C,E,G). Therefore, the doses of 50 and 100 μ g/mL of GMP were selected for further experiments. The production levels of TARC and MDC after TI stimulation in HaCaT cells were measured to examine the effect of GMP on Th2 chemokines. The production of TARC was increased significantly in the TI group compared to the Con group, and GMP reduced TARC production in a dose-dependent manner (Figure 6B). On the other hand, the MDC levels were similar in groups (Figure 6B). In Raw264.7 cells, LPS stimulation increased the production of TNF- α , which was decreased by the GMP pretreatment (Figure 6D). Similarly, preincubation with GMP also lowered the levels of IL-17 in PI-stimulated EL4 cells and decreased the levels of VEGF in the SP-stimulated HMC-1 cells (Figure 6F,H).

Figure 6. Cont.

Figure 6. Effect of GMP on the inflammatory responses *in vitro*. **(A)** Effect of GMP on cell viability of HaCaT cells. **(B)** Effect of GMP on TARC and MDC production in TI-stimulated HaCaT cells. **(C)** Effect of GMP on cell viability of Raw264.7 cells. **(D)** Effect of GMP on TNF-α level in LPS-stimulated Raw264.7 cells. **(E)** Effect of GMP on cell viability of EL4 cells. **(F)** Effect of GMP on IL-17 level in PI-stimulated EL4 cells. **(G)** Effect of GMP on cell viability of HMC-1 cells. **(H)** Effect of GMP on VEGF production in SP-stimulated HMC-1 cells. Data represent the means ± SDs. * $p < 0.05$ vs. the Con, # $p < 0.05$ vs. the TI-treated cells, # $p < 0.05$ vs. the LPS-treated cells, # $p < 0.05$ vs. the PI-treated cells. # $p < 0.05$ vs. the SP-treated cells.

3.6. Quantification of Chemical Constituents in GMP

The bioactive compounds in GMP were quassin and picrasin B (Figure 7A) [18]. Figure 7B presents chromatograms of two standard compounds. Quassin, but not picrasin B, was detected in the GMP extract (Figure 7C). The retention time of quassin was 12.733 min, and the concentration of quassin in GMP was 2.963 mg/g.

Figure 7. Analysis of chemical constituents of GMP by HPLC. (A) Structures of picrasin B and quassin. HPLC chromatograms of the standard compounds (B) and GMP (C).

4. Discussion

AD is a common chronic inflammatory skin disease with an incidence from 3–20% in children and 2–5% in adults worldwide [22,23]. Accumulating evidence suggests that AD is associated with various neuropsychiatric comorbidities, such as ADHD, anxiety, and depression, which reduce the quality of life of AD patients significantly [24]. Recent studies indicated that AD exhibits a transient association with the later onset of ADHD and approximately 7% of total AD patients suffer from ADHD symptoms [25,26]. Therefore, managing both skin symptoms and psychiatric disorders might be a more effective approach for AD treatment. This study revealed the inhibitory effects of GMP on AD-skin inflammation and AD-induced hyperactivity behavior in DNCB-treated mice, suggesting its potential in the drug development for AD.

DNCB-induced cutaneous inflammation in mice was reported as an appropriate model for AD studies [27]. In this study, the topical application of DNCB to the back skin of BALB/c mice induced AD-like symptoms, such as redness, dryness, and excoriation. On the other hand, the GMP treatment could ameliorate these symptoms in AD mice significantly. Epidermal hyperplasia and infiltration of inflammatory cells in skin lesions are typical characteristics of AD [28]. These results indicated that the topical treatment of GMP alleviated DNCB-induced epidermal thickening and infiltration of mast cells and eosinophils in skin lesions. The roles of mast cells in allergic disease, including AD,

were well characterized in previous studies [29,30]. Mast cells are generally activated by binding IgE to FcεRI receptors on the cell surface, and activated mast cells can secrete a range of inflammatory molecules, such as histamine, TNF-α, and VEGF [31]. Interestingly, GMP could decrease both IgE and VEGF levels in DNCB-treated mice. Pretreatment with GMP also reduced SP-induced production of VEGF in HMC-1 cells, suggesting the inhibitory effects of GMP on the mast cell response in AD. In addition to mast cells, macrophages, another innate immune cell type, play a pivotal role in developing AD. The accumulation of macrophages is observed in acute and chronic AD inflamed skin. In the early stages of inflammation, macrophages have pro-inflammatory functions, such as antigen presentation and the production of inflammatory cytokines [32]. These results suggest that GMP inhibited the LPS-induced TNF-α production in Raw264.7 macrophages. These results suggest the suppressive effects of GMP on the innate immune system for the treatment of AD symptoms.

T lymphocytes, particularly T helper cells, play crucial roles in the pathogenesis of AD [33]. The infiltration of CD4+ T cells into the dermis was observed in AD skin lesions [34]. In the early phase of AD, Th2 cells are prominent infiltrates in lesional skin. The recruitment of Th2 lymphocytes into AD lesions might be triggered by several chemokines, such as MDC and TARC, mainly produced by keratinocytes [35]. A previous study reported that the levels of these Th2 chemokines were elevated in AD patients compared to healthy subjects [36]. Infiltrated Th2 cells secrete many inflammatory cytokines, including IL-4, IL-5, and IL-10, to promote skin inflammation and skin barrier disruption in AD [37]. In this study, GMP reduced the expression of IL-10 in skin lesions of AD mice and suppressed the production of TARC in TI-stimulated HaCaT keratinocytes, suggesting the effects of GMP on the Th2 response in AD. In addition to Th2 cells, Th1 and Th17 cells, as well as their effector cytokines are involved in the progression of AD. Th1 cells can produce various cytokines, such as IFN-γ, TNF-α, and IL-2, while Th17 cells are well known for the production of IL-17 [38]. IL-17 triggers the Th2 response and B cell differentiation to promote an inflammatory response in AD [39,40]. In the current study, the GMP treatment decreased the levels of IFN-γ in AD mice and reduced the production of IL-17 in PI-stimulated EL4 cells, suggesting that GMP can also modulate the activities of Th1/Th17 cells to improve the AD symptoms.

ADHD is one of the most prevalent neuropsychiatric comorbidities in AD patients. The severity of AD may result in emotional and behavioral problems, contributing to an increased incidence of ADHD [41,42]. In this study, AD mice exhibited ADHD-like symptoms, as inferred from the increased total distance travel and speed in the OFT. The hyperactivity in AD mice was also indicated by a moderate increase in the distance in open arms of the EPM. In contrast, treatment with GMP could attenuate the hyperlocomotion behavior in AD mice, indicating the beneficial effects of GMP on psychiatric comorbidities of AD. Neuroinflammation plays an important role in the development of ADHD [43]. Several immune cells, such as microglia, astrocytes, and mast cells are involved in the development of neuroinflammation by secreting inflammatory cytokines, such as TNF-α, IL-6, and IL-1β [44,45]. In the present study, the level of TNF-α in PFC was increased in the AD mice, and the TNF-α level positively correlated with hyperactivity behavior. This was reduced by the GMP treatment, suggesting that GMP improved behavioral changes in AD mice by inhibiting neuroinflammation.

Topical steroids are commonly used to treat AD because they can reduce inflammation and itching symptoms in dermatitis [46]. On the other hand, long-term skin treatment of these steroids might result in various adverse effects such as skin atrophy, impaired skin barrier function, and drug resistance [47]. The results showed that some biomarkers were improved by the DEX treatment, but the overall clinical severity was worsened in the DEX group compared to the GMP group. The GMP group did not have any side effects, even after long-term treatment, highlighting the safety of GMP for AD treatment.

HPLC showed that quassin is a major component of GMP. A previous study indicated the anti-inflammatory activity of quassin by suppressing the expression of inflammatory

molecules, including iNOS2, TNF- α , IL-10, and IL-12 in murine macrophages [48]. In addition, quassin exerted neuroprotective effects by increasing the viability of SH-SY5Y neuroblastoma cells under H₂O₂ stimulation [49]. These findings suggest that quassin might improve the therapeutic effects of GMP against DNCB-induced skin inflammation and behavioral changes in mice.

This study still had some limitations that can be improved in future studies. Several other behavioral tests for evaluation of other psychiatric symptoms in AD mice, such as depression (sucrose preference test, tail suspension test) should be conducted for a more detailed conclusion about AD-associated psychiatric disorders in the mouse model. The effects of GMP on signaling pathways related to AD can also be examined to investigate the underlying mechanisms. Therefore, further studies should be performed to provide more scientific evidence for the efficacy of GMP for AD treatment.

5. Conclusions

GMP alleviated the AD-like skin inflammation and hyperlocomotion activity in DNCB-treated BALB/c mice. The effects of GMP behavioral abnormalities might be through inhibiting TNF- α production in the PFC. GMP also showed the anti-inflammatory effects in vitro by suppressing the production of inflammatory cytokines and chemokines in Ha-CaT keratinocytes, Raw264.7 macrophages, EL4 T cells, and HMC-1 mast cells. These findings suggest that GMP could be useful for the treatment of AD by modulating inflammatory responses and comorbid behavioral changes.

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