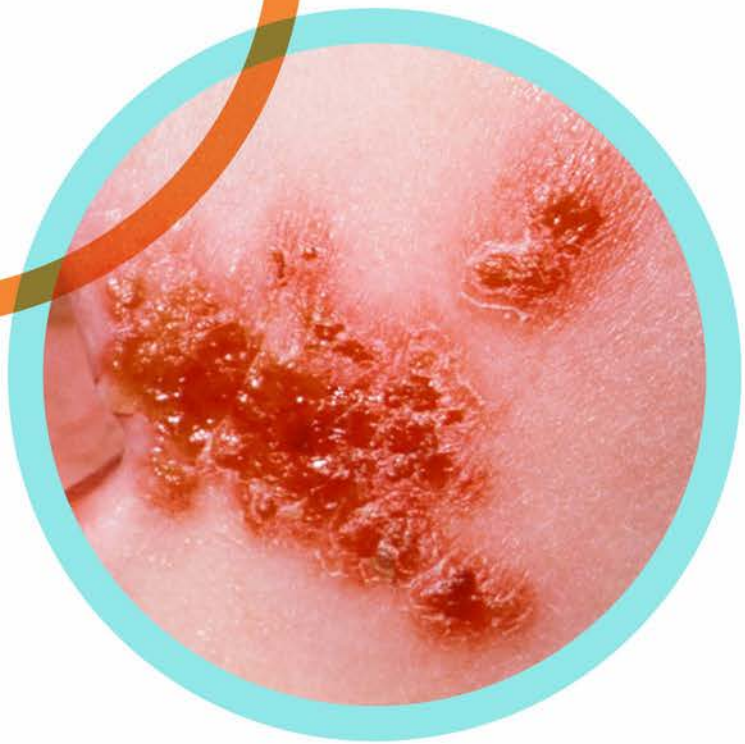

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Research Article
Biochemical Mechanisms Mediating the Immunomodulatory and Anti–Atopic Dermatitis Effects of GammaQ in a DNCB-Induced BALB/c Mouse Model

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Abstract

Background and Objective: Individual components, such as gamma-linolenic acid, quercetin and bromelain, yield positive effects on immune regulation and atopic dermatitis (AD). Despite this, the collective influence and clinical effectiveness of GammaQ, a composite containing gamma-linolenic acid, quercetin and bromelain, in modulating immunity and preventing AD is still unclear. Thus, this study aimed to assess the clinical efficacy and immunomodulatory mechanisms of GammaQ using a DNCB-induced atopic dermatitis mouse model. **Materials and Methods:** A total of 30-day dietary interventions with 0, 2.0, 3.5 and 5.0% GammaQ were administered, after which biochemical markers, skin lesion scores, histopathological changes and gene expression profiles were assessed in DNCB-induced atopic dermatitis model mice. Data were analyzed using SAS (2023), with results expressed as mean±SE; group differences were assessed by ANOVA followed by Duncan's multiple range test at $p<0.05$ (95% confidence level). **Results:** The group receiving 5.0% GammaQ (providing daily: Gamma-linolenic acid 3.15 mg/day, quercetin 0.43 mg/day and bromelain 0.72 mg/day) exhibited significant reductions in both histamine and IgE concentrations, with improved clinical and histopathological features of the skin, as well as substantial downregulation of genes associated with atopic dermatitis compared to the other groups ($p<0.05$). **Conclusion:** These results indicate that GammaQ may serve as a natural bioactive agent for enhancing immune function and mitigating atopic dermatitis symptoms in both humans and companion animals.

Key words: GammaQ, gamma-linolenic acid, quercetin, bromelain, immune, atopic dermatitis

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Eczema and atopic dermatitis (AD) are prominent inflammatory skin disorders that are strongly linked to dysregulation of immune homeostasis, significantly reducing the quality of life in both humans and companion animals[1]. The AD is driven by an interplay of genetic predisposition, environmental triggers, skin barrier dysfunction and immune dysregulation. Key clinical features encompass xerosis, severe pruritus and repetitive scratching behavior, each of which exacerbates structural damage and aggravation of inflammation in the skin. Allergen exposure leads to hyperactivation of T helper type 2 (Th2) cells, which in turn results in elevated secretion of cytokines, including IL-4, IL-5 and IL-13. This Th2-skewed immune response facilitates IgE-mediated allergic reactions and induces activation of mast cells, prompting the release of inflammatory mediators such as histamine and prostaglandins[2]. The resulting mediators sensitize cutaneous nerve endings and intensify pruritic sensations; persistent scratching damages the skin barrier further, predisposing it to secondary bacterial infections and progressive inflammation.

Moreover, reduced immune competence worsens these pathophysiological mechanisms. Chronic stress, aging, environmental toxins and nutritional imbalance are established contributors to weakening both innate and adaptive immunity, subsequently lowering the skin's capacity for defense and regeneration. This dysregulated cytokine profile, marked by heightened production of TNF-α, IL-6 and IL-31 and a concurrent reduction in IL-10-mediated anti-inflammatory responses, plays a central role in the persistence of immune imbalance. Thus, restoring Th1/Th2 equilibrium via immune modulation, fortifying the skin barrier and upregulating anti-inflammatory and antioxidant mechanisms are regarded as essential strategies for the prevention and therapeutic management of eczema and AD[3].

In recent years, researchers have concentrated on clarifying the clinical efficacy and underlying biochemical mechanisms of natural plant-derived bioactive materials, including gamma-linolenic acid (GLA), quercetin and bromelain, with respect to immune enhancement and the prevention of atopic dermatitis (AD) and other skin disorders in both humans and companion animals. As a result, interventions using these bioactive compounds have been proposed as viable methods to restore skin immunity by inhibiting mast cell degranulation, decreasing pro-inflammatory cytokines and promoting activation of antioxidant pathways. Thus, immune-modulating strategies are increasingly recognized as a progressive paradigm for the management of AD and other skin disorders, underlining the significance of advancing immune-based technologies for skin health management[4-6].

Meanwhile, there has been growing acknowledgment of the need for preventive management of atopic dermatitis (AD; affecting approximately 10-20% of all dogs) and eczema, in addition to major diseases associated with immunosenescence, such as cancer in elderly pets. In companion animals, immune dysregulation may result in immune-mediated disorders, while autoimmune responses, in which the immune system targets normal cells, may compromise host defenses and elevate the risk of cancer. Importantly, cancer remains a primary cause of mortality in companion animals, accounting for roughly 47% of deaths in dogs and 33% in cats. These trends indicate that consistent screening and preventive interventions are particularly important in aging pets[7,8].

Gamma fatty acids (gamma-linolenic acid, GLA, 18:3n-6 and dihomo-gamma-linolenic acid, DGLA, 20:3n-6) have been documented to suppress the gene expression of inflammatory cytokines such as IL-4 and IL-13, thereby mitigating inflammatory responses and aiding in the management of AD and other skin diseases, in addition to supporting systemic immune homeostasis and contributing to cancer prevention. In clinical trials involving adults with AD, the recommended daily intake of GLA ranges from 80 to 640 mg, while in companion animals with AD, a therapeutic high-dose intake of approximately 45-50 mg/kg/day has been recommended[9,10]. As a biologically active polyphenol, quercetin counteracts oxidative stress induced by excess reactive oxygen species (ROS), thereby promoting cellular resilience and facilitating immune regulatory and anticancer functions. Although an established daily intake for AD prevention in humans has not been determined, typical supplementation ranges from 500 to 1,000 mg/day. For companion animals, the recommended quercetin dosage is 5-15 mg/kg/day for dogs and approximately 3 mg/day for cats[5,11]. Bromelain, an immunomodulatory enzyme, has been shown to inhibit the gene expression of TNF-α and IL-31, while reducing IgE production, which helps attenuate allergic responses and supports immune enhancement and cancer prevention. In humans, the recommended intake for anti-inflammatory effects is between 800 and 1,500 mg/day, but no standardized dosage has been specified for AD in companion animals; nevertheless, an empirical dosage of 10-30 mg/kg/day for dogs has been proposed. These bioactive substances each function via unique metabolic routes *in vivo*, but together they foster immune homeostasis, regulate inflammation and contribute to the prevention of cancer and AD[7].

This study analyzed the biochemical mechanisms responsible for the protective effects of GammaQ in a DNCB-induced atopic dermatitis model using BALB/c mice. The immunomodulatory effects of GammaQ were assessed by measuring serum IgE concentrations, evaluating mast cell degranulation and examining changes in the skin barrier structure.

MATERIALS AND METHODS

Study area: This study was conducted at the Institute of Animal Resources, Kangwon National University, Chuncheon City, Gangwon Province, Republic of Korea, from November 1, 2020, to March 31, 2021. This work was supported by the Basic Science Research Program of the National Research Foundation of Korea (NRF), funded by the Ministry of Education (Project No. 2019R1D1A3B07047548).

Preparation of GammaQ: GammaQ, a novel composite formulation originally devised by the authors, comprises GLA, quercetin and bromelain; its biochemical mechanisms (Fig. 1) pertinent to immune enhancement and AD prevention have been identified[9]. The well-balanced formulation yields synergistic effects on immune regulation and suppression of inflammation, thereby offering potential benefits for AD prevention. Additionally, GammaQ shows antioxidant properties and aids both skin and systemic immune equilibrium, with possible implications for lowering cancer risk through comprehensive physiological pathways. Importantly, early investigations indicate potential anti-tumor activities via modulation of the p53 signaling cascade and alteration of the Bax/Bcl-2 ratio, which suggest involvement in apoptosis-related molecular processes[9,12,13]. However, evidence regarding these combined mechanisms is still limited.

GammaQ was formulated from natural plant-derived extracts intended for human consumption. The main active components included gamma-linolenic acid (GLA), quercetin and bromelain. The GLA was derived from borage oil (GLA 20%, Nordic Natures, Mfg, US) and evening primrose oil (GLA 7%, Chuncheon, Gangwon, Republic of Korea). Bromelain was obtained from pineapple extract powder (2,400 GDU/g) and quercetin was sourced from Sophora japonica extract powder (purity 95%). The preparation of GammaQ per kilogram of diet consisted of 91.11 g of GLA-enriched mixed oil (70% borage oil and 30% evening primrose oil), 5.00 g of α-tocopherol, 2.11 g of quercetin, 3.33 g of bromelain and 898 g of filler (corn sugar 50: Granular sugar 50). The computed nutrient profile of the final GammaQ diet was as follows: GLA 14,667 mg/kg, quercetin 2,000 mg/kg, bromelain 3,333 mg/kg (4,800,000 GDU/kg) and α-tocopherol 5,000 mg/kg diet (calculated values).

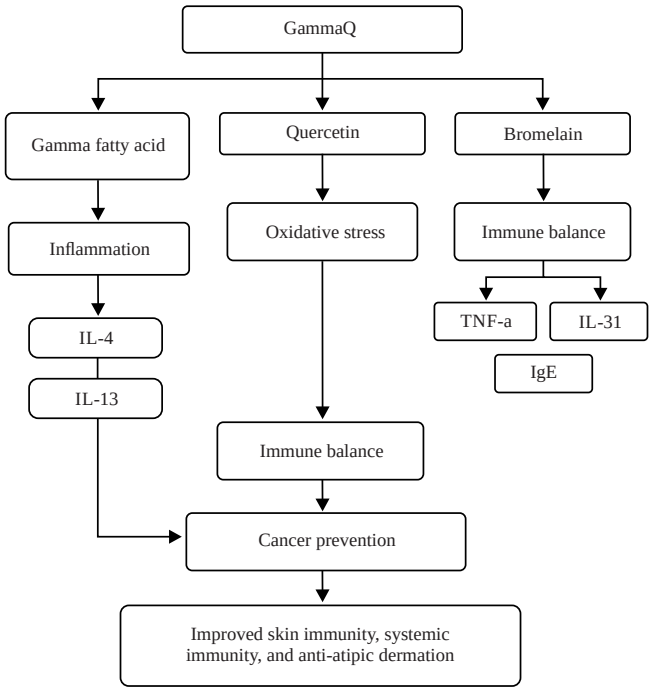

Fig. 1: Biochemical mechanism of GammaQ in immune enhancement and prevention of atopic dermatitis[9,10]

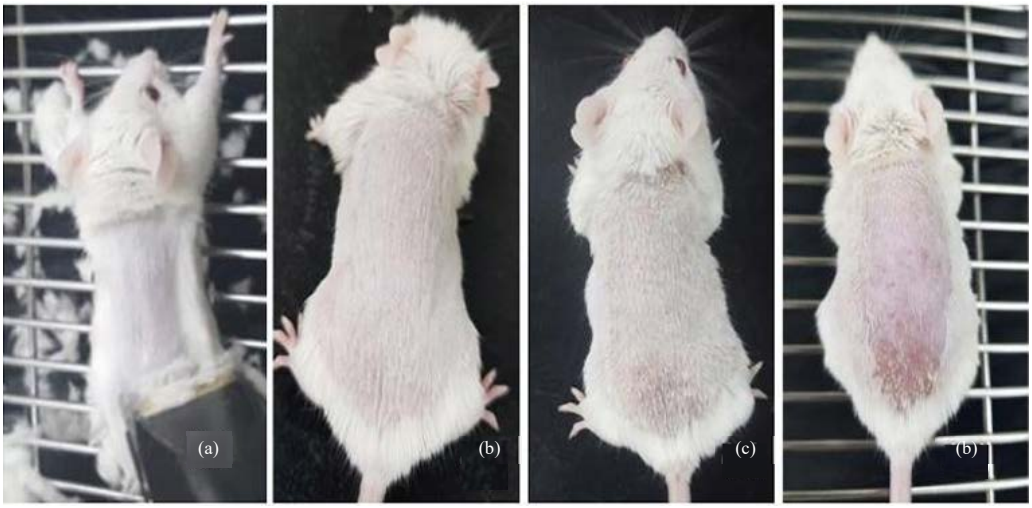

Fig. 2: Establishment of a DNCB-induced atopic dermatitis animal model, (a) Shaved dorsal skin on day 1, (b) 200 µL of 2.5% DNCB applied on day 2, (c) 150 µL of 1.0% DNCB applied on days 5-8 and (d) Atopic dermatitis lesions appearing after day 8

Experimental design: Twenty specific pathogen-free (SPF) male BALB/c mice (7 weeks old, 22±1.2 g) were purchased from Daehan BioLink Co. (Eumseong, Korea). Animals underwent a one-week acclimation period before the experiment. Mice were randomly distributed into four groups: a control group (CON; diet without GammaQ) and three groups receiving GammaQ supplementation at 2.0, 3.5 and 5.0%. Each experimental group had five replicates, with one mouse per cage and the study was conducted using a Completely Randomized Design (CRD). The experimental diets, manufactured as purified pellet diets according to the AIN-93 formula, were administered for 30 consecutive days before AD induction with DNCB. The purified pellet diets (%) consisted of casein-vitamin free 20, corn starch 39.75, maltodextrin 13.20, sucrose 10.00, soybean meal 7.00, cellulose 5.00 and AIN 93G mineral mix. 3.50, AIN 93G vitamin mix. 1.00, L-cystine 0.30, choline bitartrate 0.25 and t-butylhydroquinone 0.0014 cats[13]. The specific dietary concentrations of GammaQ components were as follows:

- **GammaQ 2.0% group:** GLA 293 mg/kg, quercetin 40 mg/kg, bromelain 67 mg/kg, α-tocopherol 100 mg/kg
- **GammaQ 3.5% group:** GLA 513 mg/kg, quercetin 70 mg/kg, bromelain 117 mg/kg, α-tocopherol 175 mg/kg
- **GammaQ 5.0% group:** GLA 733 mg/kg, quercetin 100 mg/kg, bromelain 167 mg/kg, α-tocopherol 250 mg/kg

All experimental diets were formulated to provide an identical gross energy of 3,868 kcal/kg and a crude protein content of 18.0%. Both feed and water were available *ad libitum* for the entire duration of the experiment. Procedures for animal care and handling adhered to the guidelines approved by the Institute of Animal Resources, Kangwon National University. In the DNCB-induced AD mouse model, we assessed biochemical markers, skin lesion characteristics, histopathological changes and gene expression profiles.

Induction of atopic dermatitis model in mouse: To establish an atopic dermatitis (AD) model, the dorsal hair of BALB/c mice was completely shaved and the animals were allowed a 24 hrs rest period for stabilization. Between days 2 and 4, 200 µL of a 2.5% 2,4-dinitrochlorobenzene (DNCB; Sigma-Aldrich, St. Louis, MO, USA) solution in acetone and olive oil (3:1, v/v) was applied topically to the shaved dorsal skin once daily. From days 5 to 8, 150 µL of a 1.0% DNCB solution was similarly applied daily to the same dorsal region. After day 8, development of skin crusts, increased scratching and subsequent crust detachment were observed, which verified the successful induction of atopic dermatitis lesions cats[14] (Fig. 2a-d).

Analysis of blood biomarkers: After anesthesia with ethyl ether, blood samples (1.0 mL) were drawn via cardiac puncture and collected in serum separation tubes (SST; Ikinbin Technology Co., Ltd, Guangzhou, China). The samples were centrifuged to obtain serum for biochemical assays. Serum triacylglycerol (TAG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and phospholipids (PL) concentrations were measured using commercial diagnostic kits (Sigma Chemical Co., St. Louis, MO, USA) and analyzed on a Roche Hitachi 917 automatic analyzer (Japan).

Table 1: Primer sequences used for RT-PCR analysis of cytokine gene expression in mouse spleen

Gene	NCBI accession No.	Forward primer (5'-3')	Reverse primer (5'-3')
Interferon-γ (IFN-γ)	NM_008337.3/NM_138880.2	GCCTAGAAAGTCTGAAGAAC	GAGATAATCTGGCTCTCAAG
IL-4	NM_021283.3/NM _201270.1	CACCTTGCTGTCACCCTGTT	ACATCTCGGTGCATGGAGTC
IL-5	NM_021834.1	CAGTGGTGAAAGAGACCTTG	GTATGTCTAGCCCCTGAAAG
IL-13	NM 053828.1	ATCGAGGAGCTGAGCAACAT	ATCCGAGGCCTTTTGGTTAC
β-actin	NM 031144.3	GTCGTACCACTGGCATTGTG	GCTGTGGTGGTGAAGCTGTA

Primer sequences are shown in the 5→3 orientation, NCBI accession numbers indicate the reference mRNA sequences used for primer design, β-actin was used as the housekeeping gene for normalization of gene expression in RT-PCR analysis, IFN-γ: Interferon-gamma, IL-4: Interleukin-4, IL-5: Interleukin-5, IL-13: Interleukin-13 and RT-PCR: Reverse transcription–polymerase chain reaction

Serum IgE analysis: Serum IgE levels were quantified using a Mouse IgE ELISA kit (Shibayagi Co., Ltd, Gunma, Japan). The absorbance of the reaction mixture was read with a Precision Microplate Reader (Molecular Devices Inc., New York, USA) and serum IgE concentrations were determined by reference to a standard curve.

Serum histamine analysis: Serum histamine concentrations were assayed using a Mouse Histamine ELISA kit (IBL-America, Inc., Minneapolis, MN, USA). Absorbance measurements were collected with a Precision Microplate Reader (Molecular Devices Inc., New York, USA) and used to compute serum histamine concentrations based on standard curves.

Skin clinical observation and histopathological analysis: In the AD mouse model, the overall skin condition, mast cell infiltration and degranulation, as well as eosinophil infiltration, were assessed. Dorsal skin lesions were excised as 1.5×1.5 cm^2 biopsy samples and fixed in 10% formaldehyde solution at 4°C before further histological staining. Samples underwent additional fixation in 4% glutaraldehyde (0.1 M cacodylate buffer, pH 7.4), then dehydration, substitution, infiltration, embedding, polymerization and semithin sectioning. The ultrastructure was analyzed using an energy-filtering transmission electron microscope (EFTEM, Leo 912AB, Carl Zeiss Inc., Germany)[14].

Gene expression analysis: Spleen tissues were used to evaluate atopic dermatitis (AD)-related cytokine gene expression. The β-Actin served as the housekeeping gene for normalization of relative expression levels of interleukin (IL) and interferon-γ (IFN-γ) genes using real-time polymerase chain reaction (RT-PCR). Total RNA was isolated with an RNA Mini Kit (Philekorea Technology, PKT, Korea) and RNA concentration and purity were determined using a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, USA). Synthesized cDNA from extracted RNA functioned as the template for RT-PCR and relative mRNA expression of target genes was quantified using GAPDH as the reference gene. RT-PCR was carried out employing the Quantimix SYBR Kit (PKT, Korea). The cDNA was diluted 1:5 and combined with gene-specific forward and reverse primers. Gene expression analysis followed the manufacturer's instructions for the Eco Real-Time PCR system (Illumina Inc., USA). Primers for each target gene were designed with the NCBI database and commercially synthesized. β-Actin was used as the housekeeping gene (Table 1).

Statistical analysis: All collected data were statistically analyzed using the SAS program 2023. The mean and standard error for each treatment group were calculated, followed by variance analysis. Statistical significance ($p<0.05$) was determined at the 95% confidence level using Duncan's multiple range test.

RESULTS

Blood biomarkers: Dietary supplementation with GammaQ significantly altered blood biomarker profiles in DNCB-induced atopic dermatitis mice. Serum TAG levels decreased progressively from 122.5 mg/100 mL in the control group to 109.0 mg/100 mL at 3.5% GammaQ, followed by a slight increase at 5.0% (112.5 mg/100 mL; $p = 0.012$). Total cholesterol showed a similar reduction, declining from 206.7 mg/100 mL (control) to 195.3 and 192.8 mg/100 mL at 3.5 and 5.0% GammaQ, respectively ($p = 0.015$). In contrast, HDL-C increased significantly with GammaQ supplementation, reaching the highest values at 3.5% (60.58 mg/100 mL) and 5.0% (59.85 mg/100 mL) compared with the control (50.15 mg/100 mL; $p = 0.010$). LDL-C levels decreased dose-dependently from 56.31 mg/100 mL in controls to 47.55 mg/100 mL at 3.5% GammaQ ($p = 0.022$). Blood glucose was significantly reduced at higher GammaQ levels, decreasing from 148.6 mg/100 mL (control) to ~140 mg/100 mL at 3.5-5.0% ($p = 0.012$). Phospholipid concentrations increased significantly with GammaQ, peaking at 78.77 mg/100 mL at 3.5% ($p = 0.011$). Liver enzymes ALT and AST were markedly reduced in GammaQ-fed groups, with ALT decreasing from 65.61 U/L (control) to ~36 U/L at 3.5-5.0% ($p = 0.015$) and AST from 48.63 U/L to ~39-40 U/L ($p = 0.020$), indicating improved hepatic function (Table 2).

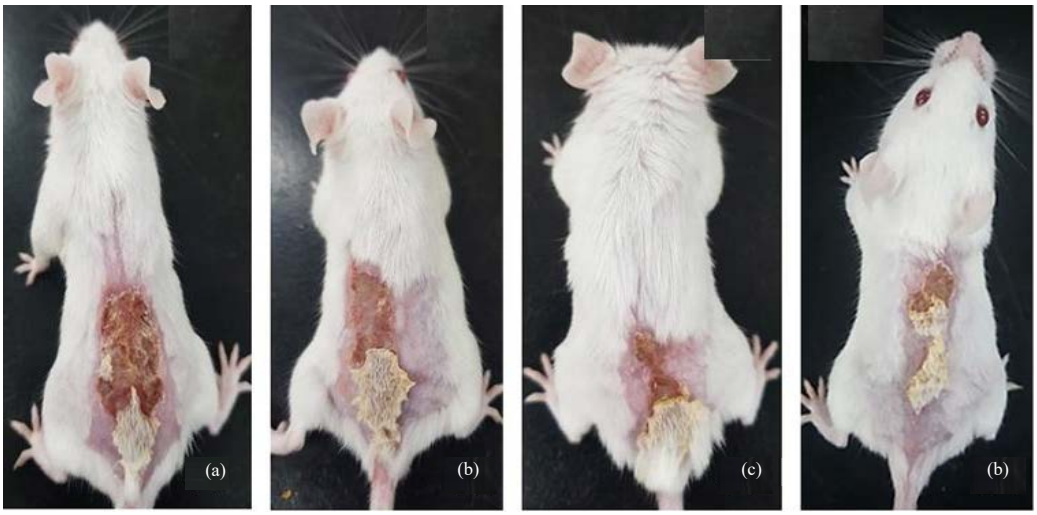

Fig. 3: Clinical presentation of skin lesions in DNCB-induced atopic dermatitis mice following administration of a purified diet containing GammaQ, (a) Control group without GammaQ, (b) Group fed a diet containing 2% GammaQ, (c) Group fed a diet containing 3.5% GammaQ and (d) Group fed a diet containing 5.0% GammaQ

Table 2: Blood biomarkers (mg/100 mL) in DNCB-induced atopic dermatitis mice after consuming a purified diet containing GammaQ

		Diet with GammaQ				
	CON	2.0%	3.5%	5.0%	SEM	p-value
TAG	122.5^{a}	120.3^{a}	109.0^{c}	112.5^{b}	1.197	0.012
TC	206.7^{a}	205.1^{a}	195.3^{b}	192.8^{b}	1.593	0.015
HDL-C	50.15^{c}	58.03^{b}	60.58^{a}	59.85^{a}	0.818	0.010
LDL-C	56.31^{a}	53.05^{b}	47.55^{c}	48.08^{c}	0.337	0.022
Glucose	148.6^{a}	146.9^{a}	140.7^{b}	140.2^{b}	1.701	0.012
PL	68.57^{d}	73.04^{c}	78.77^{a}	75.84^{b}	1.028	0.011
ALT (U/L)	65.61^{a}	62.72^{b}	35.33^{c}	36.02^{c}	1.093	0.015
AST (U/L)	48.63^{a}	43.39^{b}	38.58^{c}	40.07^{c}	1.035	0.020

CON: Control group without gamma G, GammaQ: Complex of gamma linolenic acid, quercetin and bromelain, SEM: standard error of mean values (n = 5), ^{a,b,c,d}Values within the same row with different superscript are significantly different (p<0.05), TAG: Triacylglycerol, TC: Total cholesterol, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, PL: Phospholipids, ALT: Alanine aminotransferase and AST, Aspartate aminotransferase

Table 3: Serum IgE and histamine levels (mg/100 mL) in DNCB-induced atopic dermatitis mouse after consuming a purified diet containing GammaQ

		Diet with GammaQ				
	CON	2.0%	3.5%	5.0%	SEM	p-value
IgE	75.7^{a}	66.1^{b}	33.7^{c}	31.5^{d}	1.802	0.012
Histamine	52.5^{a}	31.6^{b}	21.3^{c}	18.5^{d}	1.302	0.020

CON: Control group without gamma G, GammaQ: Complex of gamma linolenic acid, quercetin and bromelain, SEM: Standard error of mean values (n = 5), ^{a,b,c}Values within the same row with different superscript are significantly different (p<0.05)

Blood IgE and histamine: Table 3 provides the values of serum IgE and histamine in DNCB-induced AD model mice administered purified diets containing GammaQ. When compared to the CON group, which received a purified diet lacking GammaQ, all GammaQ-treated groups demonstrated significantly reduced serum IgE and histamine levels (p<0.05). Moreover, a dose-dependent reduction was noted, with the GammaQ 5.0, 3.5 and 2.0% groups exhibiting a progressive decline in both biomarkers (p<0.05).

Clinical observation of skin lesions: Figure 3a-d displays the clinical presentation of skin lesions in DNCB-induced AD model mice administered purified diets containing GammaQ. In comparison to the CON group on a diet lacking GammaQ, the GammaQ-treated groups demonstrated a notable improvement in skin condition and the GammaQ 5.0% group exhibited the most substantial recovery. Collectively, these results indicate that dietary GammaQ supplementation may aid in reducing DNCB-induced inflammatory skin responses and facilitating skin barrier repair.

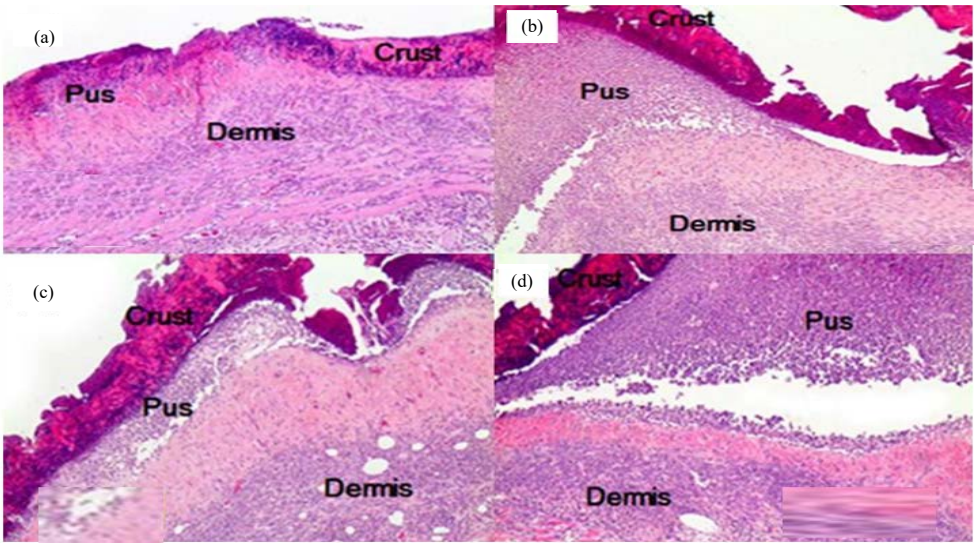

Fig. 4: Histopathological characteristics of skin tissue in DNCB-induced atopic dermatitis mice following administration of a purified diet containing GammaQ, (a) Control group without GammaQ, (b) Group fed a diet containing 2% GammaQ, (c) Group fed a diet containing 3.5% GammaQ and (d) Group fed a diet containing 5.0% GammaQ

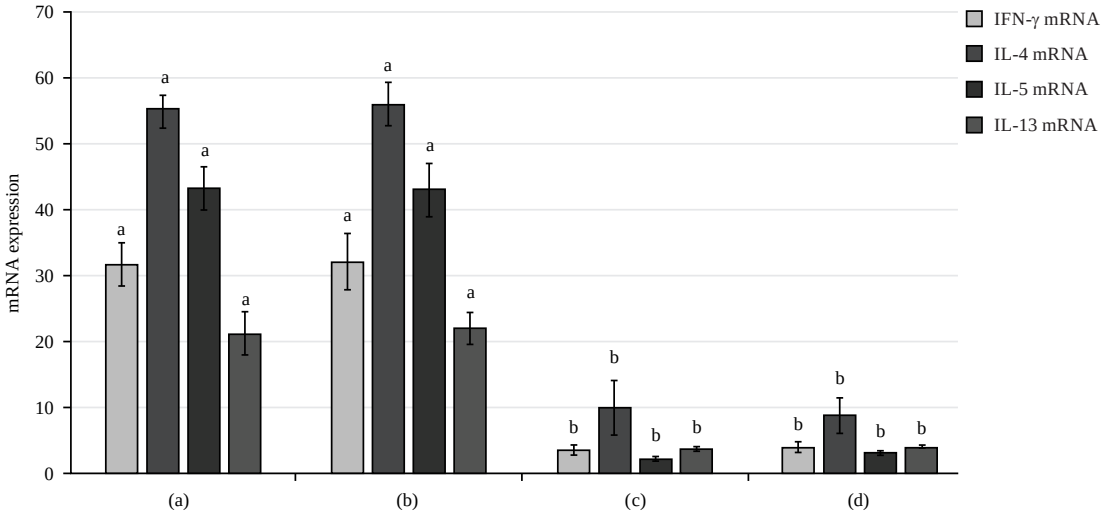

Fig. 5: Gene expression of cytokines in DNCB-induced atopic dermatitis mouse after consuming a purified diet containing GammaQ, (a) Control group without GammaQ, (b) Group fed a diet containing 2% GammaQ, (c) Group fed a diet containing 3.5% GammaQ and (d) Group fed a diet containing 5.0% GammaQ

Histopathological findings: Figure 4a-d shows the histopathological characteristics of skin tissue in DNCB-induced AD model mice administered purified diets containing GammaQ. Within the CON group pronounced epidermal hyperplasia, infiltration of dermal inflammatory cells, mast cell degranulation and edema were evident. Conversely, these pathological manifestations were considerably alleviated in all GammaQ-treated groups and the GammaQ 5.0% group exhibited the most significant improvement. These findings propose that supplementation with GammaQ may assist in reducing inflammatory cell infiltration and restoring physiological skin tissue structure under AD conditions.

Gene expression: Gene expression profiles of immune-related cytokines in the spleen of DNCB-induced AD model mice fed purified diets containing GammaQ are shown in Fig. 5. The mRNA expression levels of IFN-γ, IL-4, IL-5 and IL-13, which are associated with atopic inflammation, did not differ significantly between the CON group (without GammaQ) and

the GammaQ 2.0% group. In contrast, compared with the CON group, the mRNA expression levels of these cytokines were significantly downregulated in both the GammaQ 3.5 and 5.0% groups ($p<0.05$), as evidenced by reduced relative mRNA expression levels shown in Fig. 5. Collectively, these results indicate that GammaQ supplementation may effectively attenuate Th2-mediated cytokine responses and contribute to the restoration of immune homeostasis under atopic dermatitis conditions.

DISCUSSION

In DNCB-induced atopic dermatitis (AD) model mouse fed with a purified diet containing GammaQ, the GammaQ 3.5% (= 5.0%) group demonstrated increased concentrations of HDL-C, glucose and phospholipids (PL), while the levels of triglycerides (TAG), total cholesterol (TC), LDL-C and hepatic enzymes (ALT and AST) were significantly decreased (Table 2, $p<0.05$). Blood biomarkers in AD serve as auxiliary indicators for monitoring disease progression and changes in lipid composition may function as potential predictive markers for atopic manifestations. Previous studies have established that elevated circulating lipotoxic components correlate with systemic biochemical and inflammatory dysregulation in AD patients cats[15]. Moreover, compared to the CON group, the significant reductions in ALT and AST observed within the GammaQ-supplemented groups imply that GammaQ may provide a hepatoprotective effect by mitigating hepatic stress induced by AD (Table 2). Aspartate Aminotransferase (AST) is an important mitochondrial enzyme and is recognized as a well-established biomarker of hepatocellular injury. Elevated AST concentrations indicate mitochondrial impairment and injury to hepatocytes commonly results in higher serum levels of AST and ALT[16,17]. In the CON group of DNCB-induced AD model mice, serum concentrations of lipotoxic substances (TC, TAG, LDL-C) and liver enzymes (AST and ALT) were significantly higher than those in the GammaQ-supplemented groups, suggesting that these circulating biomarkers are closely related to AD pathogenesis[18]. The active components of GammaQ including gamma-linolenic acid, quercetin and bromelain have each been shown to improve serum lipid indices by decreasing TC and LDL-C and elevating HDL-C and phospholipid levels. The current findings support the premise that these bioactive agents may serve as biochemical markers for predicting or ameliorating AD-related symptom worsening through lipid profile modulation, in line with existing literature[19-21]. In our prior work, the clinical benefits and biochemical mechanisms of the GammaQ complex were found to include immunomodulatory and dermal protective actions in AD model animals[10].

In the GammaQ-supplemented groups of DNCB-induced AD model mouse, a marked suppression of both serum IgE elevation and histamine release was observed when compared with the control group (Table 3). Elevated serum IgE concentration is widely acknowledged as a critical immunological marker for atopic dermatitis, as excessive IgE stimulates mast cell and basophil degranulation, thereby intensifying inflammatory responses. Histamine, which is a principal mediator involved in the pathogenesis of pruritus in atopic dermatitis, significantly contributes to the itch-scratch cycle and drives allergic inflammation. The activation of immune cells by skin irritation and inflammation leads to IgE-dependent histamine release, which further amplifies allergic reactions. An overabundance of histamine secretion facilitates eosinophil infiltration, provokes acute hypersensitivity reactions and exacerbates severe pruritus, thereby worsening AD symptoms[22,23].

Clinical skin observations in DNCB-induced AD model mouse demonstrated substantial improvement in skin integrity in the GammaQ-treated groups versus the control group, with the most pronounced and rapid recovery occurring in the GammaQ 5.0% group (Fig. 3). These results indicate that dietary supplementation with 5.0% GammaQ alleviated AD symptoms through a biochemical action involving the reduction of serum IgE levels, inhibition of histamine release and regulation of related gene expression (Fig. 1, Table 3, Fig. 5). Following DNCB administration, AD symptoms presented on day 8, as seen by scab formation, pronounced scratching due to pruritus, erythema, dryness and focal minor bleeding (Fig. 2). By day 10 after AD induction, the GammaQ-treated groups showed significant recovery of skin lesions compared with the control group and notably, the GammaQ 5.0% group exhibited almost complete normalization of the epidermal surface, with evident exfoliation of the stratum corneum (Fig. 3).

In the GammaQ-fed AD model mouse, the epidermal architecture showed greater uniformity in thickness and a more organized alignment of connective tissue compared to the severely affected control (CON) group. Remarkably, increased regeneration of hair follicles and sebaceous glands was evident within the dermis, alongside more pronounced re-epithelialization and marked proliferation of the stratum corneum, basal layer and reticular layer (Fig. 4). In allergic dermatitis, infiltration and subsequent degranulation of mast cells and eosinophils induce epidermal hyperkeratosis. These inflammatory reactions lead to trans-epidermal water loss (TEWL), which contributes to dryness and roughness of the skin, thus permitting antigen penetration and worsening hypersensitivity. This process results in

impaired skin barrier function and greater vulnerability to infection and inflammation[24]. Existing studies on the regeneration of hair follicles and cutaneous appendages, activation of follicular stem cells and histopathological changes in DNCB-induced AD models corroborate the current observations regarding dermal follicular and sebaceous gland remodeling and improved epidermal regeneration in the GammaQ-treated groups[25-27].

Unlike the hemorrhagic and ulcerated skin surfaces present in the AD control mice, the GammaQ-treated mice demonstrated a smooth and fully intact epidermal morphology. The control group also exhibited severe necrosis of basal keratinocytes, accompanied by dense infiltration of inflammatory cells throughout both the epidermal and dermal layers. These pathological distinctions align with prior literature documenting hemorrhagic and ulcerative lesions in DNCB-treated mice[28] and support the report by Kim *et al.*[29], which highlights reduced hemorrhage and tissue injury in groups treated for AD[29]. Additionally, the GammaQ-treated mice presented with a markedly reduced presence of inflammatory cells and the epidermal thickness nearly returned to nor-mal levels (Fig. 4).

From an immunological standpoint, the GammaQ-treated groups showed a significant reduction in Th2-associated proinflammatory cytokines, including IFN-γ, IL-2, IL-4 and IL-13 mRNA expression, compared with the severely affected control (CON) group in the DNCB-induced AD model (Fig. 5). Th2-mediated immune responses are primarily orchestrated by type 2 helper T cells (Th2), which release cytokines such as IL-4, IL-5 and IL-13 to promote IgE-mediated humoral immunity via activation of B cells. This pathway is a central mechanism contributing to the development of atopic and allergic inflammation. Thus, the observed inhibition of Th2 cytokine expression in the GammaQ-treated mice reinforces its anti-inflammatory and immunomodulatory properties at the molecular level[30,31]. Although the exact mechanisms by which GammaQ prevents or mitigates atopic dermatitis remain to be fully determined[10], the present results demonstrate that GammaQ supplementation regulates T-cell-driven im n the AD model. These findings indicate that GammaQ may serve as a novel immunoregulatory mune gene expression following antigen stimulation, thereby supporting immune homeostasis compound, aiding the management of IgE-centered humoral responses and facilitating protection against allergic and atopic dermatitis (Fig. 1, Fig. 5).

CONCLUSION

This study demonstrated the immunomodulatory and anti-atopic effects of GammaQ, a formulation consisting of gamma-linolenic acid, quercetin and bromelain, in a DNCB-induced atopic dermatitis (AD) model using BALB/c mouse. In this model, GammaQ supplementation led to an improved blood lipid profile, significantly lowered hepatic enzyme levels (ALT and AST) and profoundly inhibited the increase in serum IgE and histamine release. Clinical observations showed marked improvement in skin lesions, with the GammaQ 5.0% group experiencing the fastest recovery. At the molecular level, GammaQ effectively reduced the mRNA expression of Th2-related cytokines (IL-4, IL-5, IL-13) as well as IFN-γ, indicating suppression of inflammatory processes. The daily administration corresponding to the 5.0% GammaQ group 3.15 mg of gamma-linolenic acid, 0.43 mg of quercetin and 0.72 mg of bromelain proved efficacious in suppressing IgE production and histamine release, thereby preventing and alleviating AD symptoms by modulating immune-related gene expression. In summary, these findings imply that GammaQ holds promise as a functional biogenic agent for immune enhancement and atopic dermatitis prevention. Nonetheless, further molecular and mechanistic investigations are needed to elucidate its underlying mechanisms. Moreover, comprehensive long-term studies across various ages, body weights, breeds and immune conditions in both human and companion animal populations are required to fully evaluate the safety profile and therapeutic efficacy of GammaQ.

SIGNIFICANCE STATEMENT

This study presents biochemical data that substantiate the clinical nutritional effectiveness of GammaQ a formulation containing gamma-linolenic acid, quercetin and bromelain in supporting immune function, maintaining skin barrier integrity and modulating gene expression for the prevention and alleviation of atopic dermatitis (AD) in DNCB-induced murine models. The results indicate that dietary supplementation with GammaQ may represent a viable natural strategy to enhance immune function and skin health in both humans and animals, with benefits including reduced pruritus and decreased epidermal barrier disruption, potentially leading to improved quality of life.

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