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Fermented rice milk ameliorates atopic dermatitis in mice *via* MAPK and NF-κB signaling pathway

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A B S T R A C T

Atopic dermatitis (AD), which is associated with inflammatory response and skin damage, is increasing worldwide in recent years. Interest is increasing in the protective effects of natural and fermented products on the prevention and treatment of AD. This study aimed to investigate the ameliorative effects of a new rice milk product fermented with *Saccharomyces cerevisiae* on AD mice. Fermented rice milk could effectively attenuated the symptoms of AD mice, such as skin lesions, scaliness and lichenification. Furthermore, fermented rice milk decreased the levels of inflammatory cytokines interleukin (IL)-4, IL-6 and tumor necrosis factor (TNF)-α in 2,4-dinitrochlorobenzene (DNCB) induced mice. It was also observed that fermented rice milk treatment downregulated the levels of p-IKBα, p-NF-κB, p-ERK, p-JNK and p-p38 proteins expression in skin of AD mice, which showed the anti-inflammatory function of fermented rice milk, mainly *via* the NF-κB and MAPK signaling pathways. In addition, *S. cerevisiae* fermentation reduced original flavor of rice milk, and increased the acid and alcohol compounds. Overall, the findings indicated that *S. cerevisiae* fermentation improved the flavor characteristics of rice milk, and fermented rice milk exhibited protective effects against DNCB-induced atopic dermatitis by downregulating NF-κB and MAPK signaling pathways and protecting the skin barrier.

1. Introduction

Atopic dermatitis (AD) is a chronic and relapsing disease, which is characterized by eczematous skin eruption, epidermal hyperplasia, severe pruritus and erythematous plaque[1]. The epidermis is the place of initiation of AD development, and skin barrier disruptions play an important role in the promoting AD[2]. In AD models, the application of 2,4-dinitrochlorobenzene (DNCB) stimulates the disruption of skin barrier and results in production of pro-inflammatory factors, which initiates an immune response[3]. NF-κB and MAPK activation is known to mediate skin inflammation. Activation of the NF-κB and MAPK cascade leaded to pro-inflammatory gene expression and inflammatory cytokine production[3-4]. Therefore, an effective way to alleviate AD is to decrease the inflammatory response in the skin.

Generally, AD is treated with chemical agents such as dexamethasone and antihistamines[2,5-6]. However, the side effects (skin atrophy, bruising tendency, and rebound phenomenon) of drugs led to the necessity of developing safer anti-AD products[7]. Recent data suggested that the effects of probiotics and some functional products (prebiotics and dietary substrates) exhibited the protective effects on AD and showed beneficial effects on experimental animal models[8]. Several strains of *Lactobacillus*, isolated from fermented foods, were shown to inhibit AD-like ear thickness and epidermal thickening, as well as reducing serum immunoglobulin E (IgE) levels[9-11]. Therefore, these results suggested that the application of probiotics and fermented products could exhibit therapy effects on AD.

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In addition, according to meta-analysis of clinical trials in infants and adults, long-term intake of probiotic products reduced the prevalence of AD, incidence of skin lesions and eosinophil counts[12].

Natural active compounds displayed therapeutic effects on AD disorders. Some natural active compounds could effectively curb inflammation and immune responses, and exhibited anti-inflammatory and antioxidant properties[13-14]. Rice is one of the most important food crops all over the world. Rice-based fermented products have been reported for enhancing various biological activities, including antioxidant, anti-cancer, anti-diabetes, anti-wrinkle and anti-melanogenesis activities[15-16]. Recent studies have found that rice products intake could inhibit inflammatory response and exhibit antioxidant property in AD[17-18]. However, the precise effects of rice on AD remains unclear. Thus, considering the health effects of rice may be candidates for potential treatment of AD. In this study, *Saccharomyces cerevisiae* was selected for the fermentation of rice milk, and the chemical properties were evaluated. Moreover, the fermented rice milk showed attenuated effect on DNCB-induced AD mice model.

2. Materials and methods

2.1 Yeast culture

S. cerevisiae CGMCC 20352 was screened from Shaoxing rice wine and grown on yeast peptone dextrose (YPD) medium (2% glucose, 1% yeast extract, 2% peptone, 1.5% agar) at 28 ℃ for 48 h, and maintained at 4 °C.

2.2 Production of fermented rice milk

Rice milk was made by japonica rice (Jinlongyu, Heilongjang, China). To prevent the adverse effects of long soaking times, the dry milling method was selected to break the rice granules. Briefly, rice was ground with a mixer for 1 min, then flour was soaked in water at a flour to water ratio (*m*/*V*) of 1:20 (20%), 1:40 (40%) and 1:60 (60%), respectively. The fermented rice milk was produced according to the method described by previous studies with slight modifications[19-20]. Fermentation was carried out in 500 mL flasks in a shaker incubator at a speed of 200 r/min for 3 days at 28 °C. Samples were collected on 0 h (0D), 24 h (1D), 48 h (2D) and 72 h (3D), and stored at –80 ℃ for further analysis.

2.3 Determination of chemical properties of rice milk

Total starch, amylopectin and amylose content of rice milk were measured using kits (Solarbio Technology Co., Ltd., Beijing, China). The pH value of the sample was determined with a digital pH meter. (FiveEasy plus, Mettler Toledo, Shanghai, China) Total nitrogen was measured by an automatic Kjeldahl apparatus (Kjeltec 8400, FOSS Co., Hilleroed, Denmark). Protein content was estimated by multiplying the total nitrogen percentage by 5.95. All determinations were carried out in triplicate.

2.4 Microbial count

Samples were diluted up to 10^3 times with PBS, 200 μL of dilutions were plated for counts using YPD plates, then the plates were incubated at 28 °C for 48 h. Yeast colony was counted by using Model ZR-1101 Automatic Colony Counter (Junray, Qingdao, China). The results are presented as log colony-forming unit (CFU)/mL.

2.5 Gas chromatography-mass spectrometry (GC-MS) analysis

Volatile compounds from three types of fermented rice milk were extracted using solid-phase microextraction (SPME) coupled with GC-MS following Yang et al.[21]. A total of 3 mL sample and 10 μL 2-octanol (200 μg/mL) were transferred into a 20 mL headspace vial and covered with a silicone septum. The fiber (50 μm DVB/CAR/PDMS) headed into a solid phase microextraction (SPME) device (Superlo Inc. Bellefonte, PA, USA) was inserted into the vials, then volatile compounds were extracted at 50 °C for 30 min. The volatile compounds were then desorbed at 250 °C in 12 min. A GC-MS system (SCIONSQ-456, Bruker Daltonics Inc., USA) equipped with a DB-WAX column (60 m × 0.25 mm × 0.25 μm, Agilent Technologies, USA) and programmed from 40 °C (holding for 3 min) to 210 °C at 6 °C/min, then 210 to 230 °C at 8 °C/min (holding for 15 min). Helium was delivered at a flow rate of 1 mL/min as the carrier gas. The mass detector was operated in electron impact mode at an ionizing voltage of 70 eV. The ion source temperature was set at 220 °C. The extracted volatile compounds were identified by comparing the spectra with a mass spectrum library search (NIST 1.6 and Wiley 6.0). The Kovats index (KI) of unknown compounds was determined by sample injection with a homologous series of alkanes (C7–C30). The quality of volatile compounds was obtained by calculating the relative peak area in relation with internal standard 2-octanol (CAS: 123-96-6, > 99.5%, Anpel, Shanghai, China).

2.6 Sensory analysis

The sensory analysis consisted of 16 panelists evaluating the fermented rice milk samples according to the method described by Feng et al.[22]. The acceptability of fermented rice milk was based on their appearance, fruit aroma, alcohol aroma, viscosity and overall acceptability. For the quantification of the results, a 9-point scale was used (0: none; 1–3: very weak; 4–6: moderate; 7–9: strong)[23-24].

2.7 Animal experimental design

The animal study was approved and performed accordance with the guidelines of the Institutional Animal Care and use Committee of Shanghai Jiao Tong University (201801130). Seven-week-old female BALB/c mice (20–22 g, Shanghai JieSiJie Laboratory Animal Co., Ltd.) were housed under autoregulated temperature ((22 ± 2) °C) and humidity ((50 ± 5)%) in a 12 h light/dark cycle room.

During the experiment, the mice were randomly assigned to 5 groups of 8 mice as follow: N group (received water, 200 μL), M group (received water, 200 μL), Dex group (received dexamethasone, 3 mg/kg), RM group (received rice milk, 200 μL), FRM group (received fermented rice milk, 200 μL). On day 1, the dorsal skin hairs of mice were shaved off using Commercial Hair Removal Cream (Veet, Shanghai, China). According to Zhao et al.[25], expect for N group, all groups received 7% DNCB (acetone/olive, 3:1) applied to the dorsal skin at 2-day intervals for 7 days[25]. From days 8 to 21, each group of mice received orally and applied 3D_20% FRM sample. On the last day, the mice were sacrificed and blood and skin tissues were collected.

2.8 Dermatitis score

The dermatitis score was consisted of skin lesions and scratching behavior[11,26]. The skin lesion was assessed every week according to the following 3 symptoms: erythema (mild), scaling (moderate), and erosion (severe). Scores of 0 (none), 1 (mild), 2–4 (moderate), and 5–7 (severe) were assigned for each symptom. The measurement of scratching behavior was recorded as described in the previous study[3]. Mice were recorded the scratching movements of dorsal skin over an hour. To evaluate convenience, scratching was scored as 0 (< 20 times), 1 (mild, 20–50 times), 2–4 (moderate, 50–100 times), and 5–7 (severe, > 100 times). The sum of the individual scores was defined as the final dermatitis scores.

2.9 Enzyme-linked immunosorbent assay (ELISA) analysis

Serum was collected by centrifugation (4 000 r/min, 10 min) from blood and stored at –80 ℃ until analysis. As previously described[27], mice serum levels of IgE, interleukin (IL)-4, IL-6 and tumor necrosis factor (TNF)-α were analyzed by using a commercial ELISA kits (Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China) according to the manufacturer's instructions. The dorsal skin tissue was cut off and mechanically disrupted. After centrifugation, the supernatant superoxide dismutase (SOD) and malondialdehyde (MDA) were analyzed with kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.10 RNA isolation and quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from dorsal skin using Trizol according to manufacturer's instructions. Then 1 mg of total RNA was reversely transcribed using a PrimeScript RT reagent kit following the manufacturer's protocol and amplified by qRT-PCR using SYBR Premix Ex *Taq*II (TaKaRa Bio) and the appropriate primers. Data were analyzed according to the $2^{-\Delta\Delta Ct}$ method and normalized to an endogenous reference (*β-actin* gene). The primers used in this experiment are listed in Table 1.

2.11 Histological analysis

The dorsal skin was fixed with 4% paraformaldehyde and embedded in paraffin. The skin was cut into 5 μm thick sections and stained with hematoxylin and eosin (H&E) to evaluate epidermal thickness. To evaluate the infiltration of mast cells, skin was stained with toluidine blue (TB) and quantified by microscope (Leica DFC 7000 T, Wetzlar, Germany).

2.12 Western blot analysis

Skin tissues were washed with pre-cooled phosphate buffer saline (PBS) and then homogenized using tissue lysis buffer containing proteinase inhibitor. The lysates were then subjected to centrifugation at 12 000 × *g* for 5 min. Protein concentrations were measured using the BCA protein assay kit (Beyotime, Nanjing, China). Protein samples (20 μg) were separated on 12% SDS-PAGE gels and then transferred to polyvinylidene fluoride (PVDF, IPVH00010, Merck, Germany). Next, the membranes were blocked with a solution containing 5% non-fat milk in Tris-buffered saline with Tween-20 (TBST) for 1 h at room temperature and then incubated overnight with a primary antibody at 4 °C. The membranes were then washed 3 times with TBST and incubated with secondary antibodies for 1 h at room temperature. BeyoECL Plus (Beyotime, Shanghai, China) was used to visualize membranes. Then, the protein gray value was detected by ImageJ software. The following primary antibodies were used at a 1:1 000 dilution and purchased from Santa Cruz Biotechnology (Dallas, TX, USA): p38 (Cat: sc-7972), p-p38 (Cat: sc-166182), C-Jun *N*-terminal kinase (JNK, Cat: sc-7345), p-JNK (Cat: sc-6254), extracellular regulated protein kinases (ERK, Cat: sc-65981) (Cat: sc-65981), p-ERK (Cat: sc-7383), nuclear factor kappa-B (NF-κB, Cat: sc-8008) (Cat: sc-8008) and p-NF-κB (Cat: sc-135769). GAPDH (Santa Cruz Biotechnology, Cat: sc-32233) was used as a control. Inhibitor of kappa B kinase α (IKKα, AF0198) and p-IKKα (AF5839) were used at a 1:1 000 dilution and purchased from Beyotime Biotechnology (Shanghai, China).

2.13 Statistical analysis

All data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) with Duncan's multiple range test was used to compare the data among different groups. All experiments were carried out in triplicate.

3. Results

3.1 Yeast viable count and chemical properties of fermented rice milk

During heating process, the gelatinization could increase the viscosity of rice milk, then directly influence the fermentation. Three

Table 1
RT-PCR primers used in this study.

Gene	Forward primer sequences (3'–5')	Reverse primer sequences (3'–5')
β-actin	AGCCATGTACGTAGCCATCC	CTCTCAGCTGTGGTGGTGAA
TNF-α	TCCTTGATGGTGGTGCATGA	CACAAGATGCTGGGACAGTGA
IL-6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
IL-4	TCAACCCCCAGCTAGTTGTC	AAATATGCGAAGCACCTTGG

different ratios of rice milk were selected to assess the effect of viscosity on fermentation. The fermentation was monitored over 72 h by measuring pH value, starch content, viable count, total nitrogen content. Except for viable count, all results exhibited obvious fold relationships. As shown in Fig. 1A, compared with 0D, starch content of 3D_20%, 3D_40% and 3D_60% decreased by 21.53%, 8.82% and 36.36%, respectively. Besides, it was observed that amylopectin content was decreased with the increasing fermentation time (Fig. 1C). Therefore, the degradation of amylopectin resulted in the decrease of starch content in fermented rice milk. As shown in Fig. 1D, among the different fermented rice milk, only 20% sample showed an increased trend in viable counts at 24 h. The pH was increased in all samples during the fermentation (Fig. 1F). Li et al.[28] also reported that rice milk fermented by yeast could increase the pH value. The change of starch-related compounds and pH suggested that viscosity influenced fermentation of yeast, then affected the chemical properties of fermented rice milk.

3.2 *Apparent viscosity of fermented rice milk*

In general, viscosity affects the quality attributes of food. The viscosity of RM was associated with protein concentration, pH, and starch content[29]. As shown in Fig. S1, it was not observed a significant change in the apparent viscosity between 0D_20% and 3D_20% samples. In 40% samples, apparent viscosity decreased from 39 to 20 mPa·s. The apparent viscosity decreased from 29 to 11 mPa·s in 60% samples.

3.3 *Volatile compounds of fermented rice milk*

In order to investigate the effect of yeast fermentation on RM, the content of volatile compounds was analyzed using GC-MS method. The volatile compounds were shown in Table S1. In this study, total 25 volatile compounds were detected, and the composition of volatile compounds between RM and FRM groups were significantly different. In Fig. 2A, γ-octanoic lactone and octanoic acid were only detected in 3D_20% and 3D_60% samples, respectively. Besides, 7 volatile compounds consisted of 2-octanone, 1-heptanol, 2-ethylhexanol, citronellol, benzothiazole, 4-hydroxy-3-methoxystyrene and palmitic acid ethyl ester were common to all samples (Fig. 2B). After fermentation, 9 volatile flavors (acetic acid, isobutyric acid, phenylethyl alcohol, γ-octanoic lactone, 1-dodecanol, octanoic acid, cedrol, γ-decalactone and geranic acid) occurred, and all of those were produced by metabolic activity of yeast which were reported by previous studies (Table S1)[30]. Compared with 0D_40% and 0D_60% samples, contents of ketones, alcohols and acids were significantly increased by 2 to 5 fold in 3D_40% and 3D_60% samples (Fig. 2C). However, contents of aldehydes and phenol were decreased. In details, aldehyde was decreased to 0 μg/mL in 3D_20% and 3D_40% samples, and phenol was reduced by 3.5-fold in 3D_40% and 3D_60% samples compared to 0D_40% and 0D_60% samples, respectively.

In Fig. 2D, 4 acids were produced by yeast after fermentation. Among different samples, there was a significant difference in the acid content, which indicated that viscosity could affect the production of acids. It was observed that low viscosity was beneficial to produce acetic acid (0.5 μg/mL), octanoic acid (0.2 μg/mL) and geranic acid (1.62 μg/mL) in 3D_20% sample, and high viscosity was beneficial to produce isobutyric acid (range from 0 to 0.29 μg/mL) in 3D_60% sample. The alcohol compounds were the most volatile compounds that were detected in all sample. The highest content of phenylethanol was found in high viscosity rice milk (37.73 μg/mL) (Fig. 2E). There were 5 compounds (1-heptanol, 2-ethylhexanol, phenylethyl alcohol, 2-octanone and

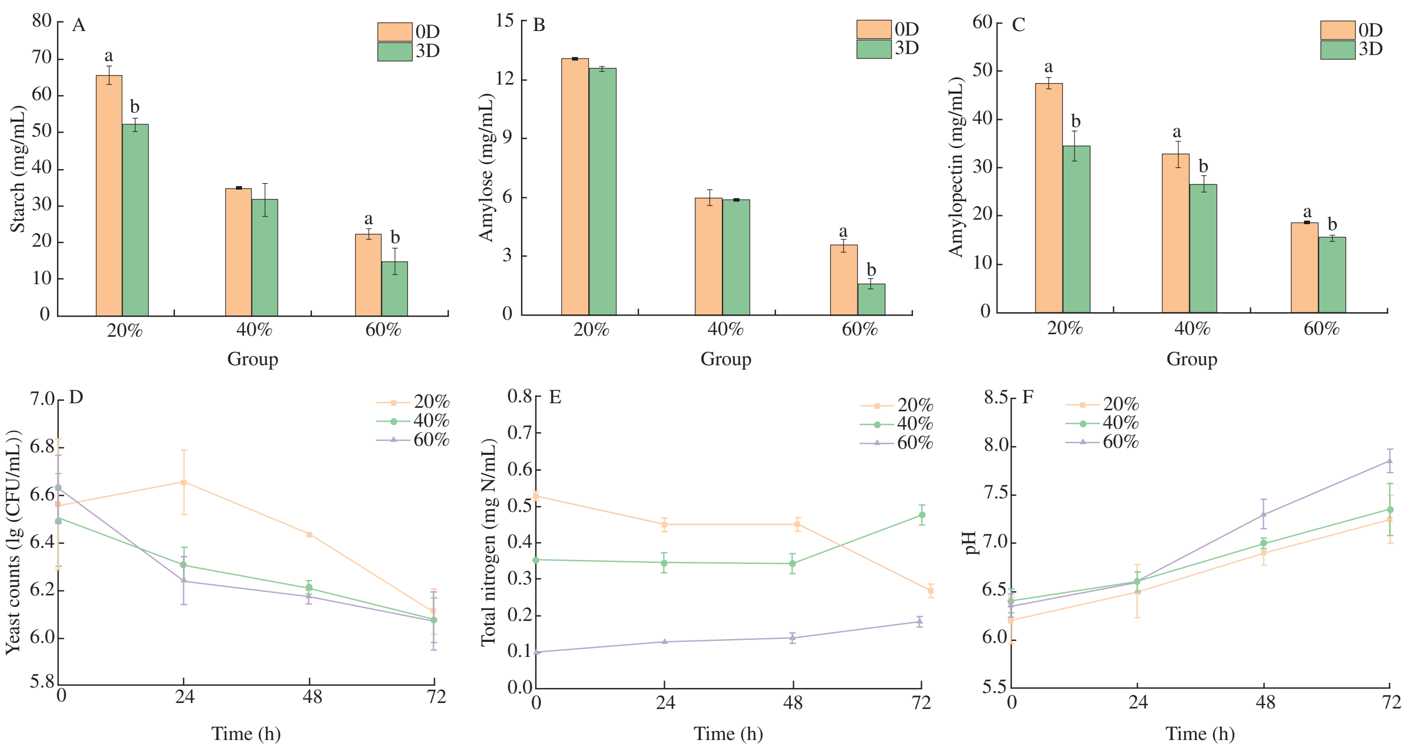

Fig. 1 The content of (A) starch, (B) amylose, (C) amylopectin and (D) change in yeast counts, (E) total nitrogen and (F) change in pH. The data represent the mean ± SD. Different lowercase letters represent significantly different ($P < 0.05$).

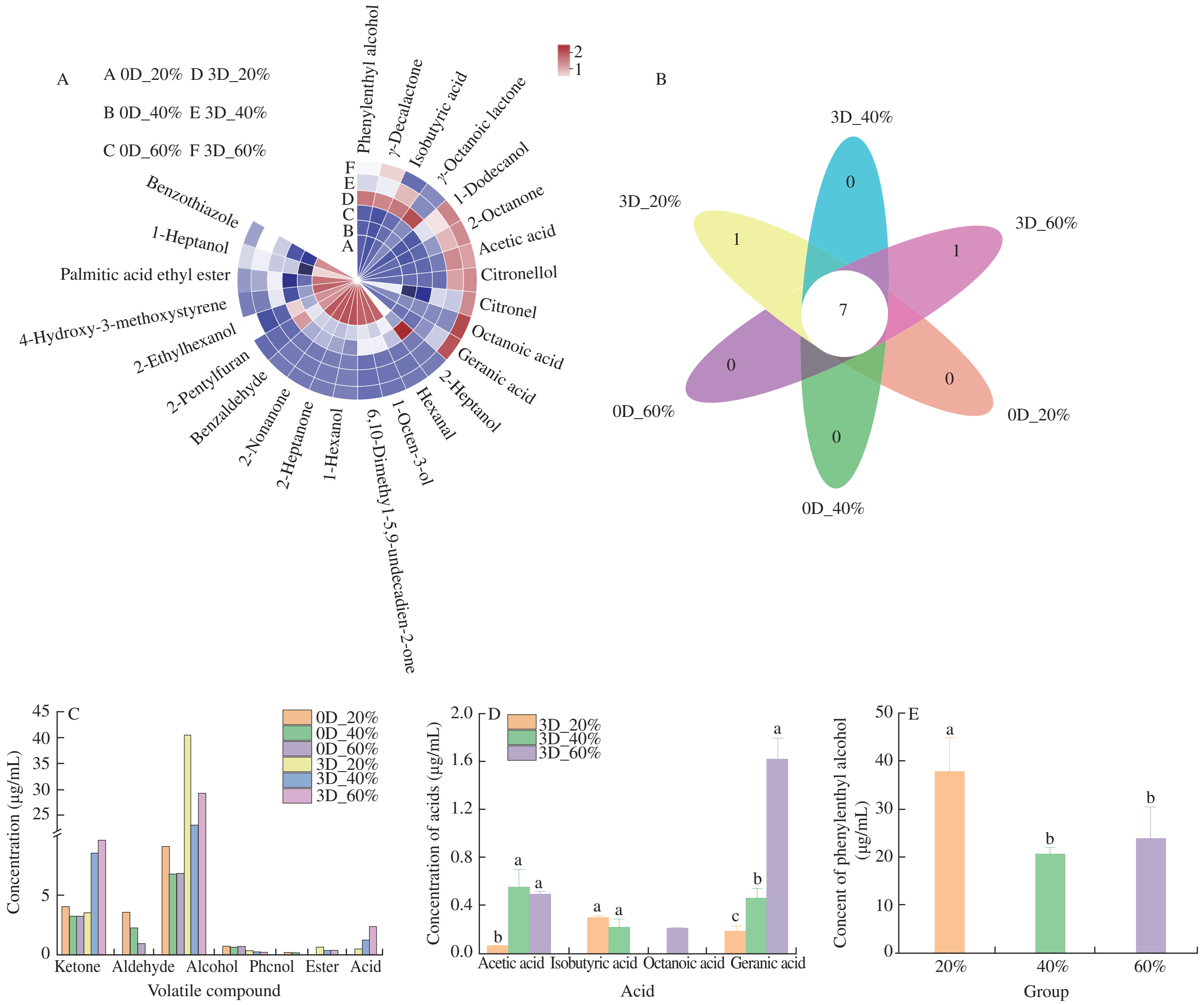

Fig. 2 Concentration of volatile compounds in RM and FRM groups. (A) Circle heatmap of volatile compounds of FRM. (B) Kinds and number of volatile flavor compounds among different volume ratios rice milk. (C) Total content of different volatile compounds. Content of (D) acids and (E) phenylethyl alcohol. The data represent the mean ± SD. Different lowercase letters represent significantly different (P < 0.05).

4-vinylguaiacol) exhibited an odor activity value (OAV) higher than 1 (Table S1). Expect for 2-ethylhexanol, the other 4 compounds offered sweet and fruity odor (cherry and orange aroma). These results showed that yeast fermentation increased the acid and alcohol compounds, and improved flavor profile of fermented rice milk.

3.4 Sensory analysis results

The result of sensory analysis of fermented rice milk was shown in Fig. S2. Among the samples, 3D_20% had the highest overall acceptability (7 scores) and appearance values (6.1 scores). In addition, 3D_20% sample obtained high fruit aroma scores (6.9 scores), and this result was consistent with high concentration of phenylethyl alcohol. In this study, 3D_60% sample also had the high acceptability (6.9 scores), this might be related to personal mouthfeel preference. The sensory results were coincided well with the study by chemical properties, viscosity property, and volatile compound content, demonstrating that the feasibility of fermentation of RM by *S. cerevisiae* especially for high viscosity rice milk. Therefore, 3D_20% sample was selected for the subsequent animal experiments.

3.5 Fermented rice milk ameliorated skin lesions and dermatitis symptoms in AD mice

To assess the protective effects of fermented rice milk against the development of AD-like skin lesions, mice were treated with fermented rice milk (200 μL/day) for 2 weeks after sensitization with 7% DNCB (Fig. 3A). As shown in Figs. 3B and C, repeated application of DNCB resulted in AD symptoms, including weight loss, scaliness and lichenification, and the dermatitis score were markedly increased until the end. However, dexamethasone and fermented rice milk treatment alleviated these changes. Rice milk treatment did not alleviate atopic dermatitis symptoms, but inhibited weight loss. As described in the previous study, dermatitis score was consisted of skin lesions and scratching behavior[3]. The frequently scratching leads to further skin barrier damage and inflammation development. It was observed that the skin lesion was improved in FRM group (Fig. 3B) and scratching count (data not shown) was significantly lower than those in the M group. Therefore, the recovered skin symptom and low scratching count reduced the dermatitis score in FRM group.

Many studies have shown that AD was associated with oxidative stress, and rice products exhibited excellent antioxidant activity[2,31]. Therefore, the levels of MDA and SOD were measured to assess the *in vivo* antioxidant effect of fermented rice milk. As shown in Figs. 3E and F, levels of MDA activity in M group was higher than N group, and SOD activity showed opposite trend. However, these markers were improved after fermented rice milk treatment. It indicated that fermented rice milk could increase the antioxidant ability in AD mice. AD is associated with Th2 cells and it would increase immunoglobulin E (IgE) level. High level of IgE could activate mast cells and lead to itching symptom. As shown in Fig. 3G, fermented rice milk treatment significantly decreased the level of IgE. These findings suggested that fermented rice milk improved the symptoms of AD induced by DNCB.

3.6 Fermented rice milk attenuated skin pathological features in AD mice

In this study, H&E and TB staining were used to assess the protective effects of fermented rice milk on skin barrier of mice after DNCB-induced. Results showed that the mice in N group exhibited thin epidermises and no inflammatory cell infiltration. Inflammatory cell infiltration was significantly increased and skin thickness was significantly higher in the M and RM group than those in the N group. However, dexamethasone and fermented rice milk treatment decreased the thickness of dermis and inflammatory cell infiltration (Figs. 4A and C). Mast cell infiltration was one of the distinguished features of AD[32]. Mast cells could release inflammatory cytokines and aggravate AD disease progression. TB straining results showed that fermented rice milk treatment decreased infiltration and counts

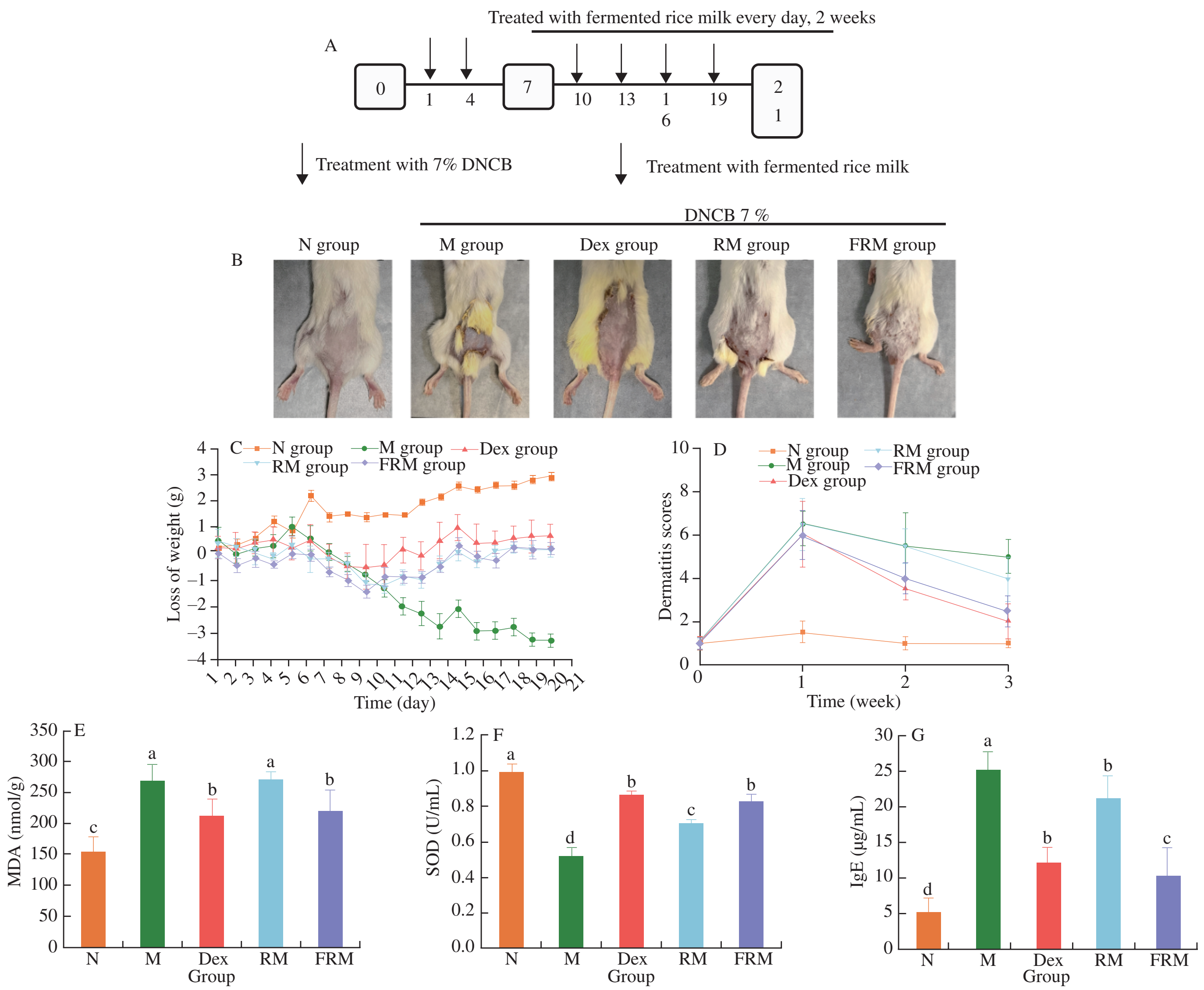

Fig. 3 Effect of FRM on the development of atopic dermatitis in DNCB induced mice. (A) Animal experimental protocol. (B) Representative image of the extent of skin lesions. (C) Body weight change in mice. (D) Atopic dermatitis score. Content of (E) MDA, (F) SOD and (G) IgE. Each value represents the mean ± SD of 8 mice in each group. Different lowercase letters represent significantly different ($P < 0.05$).

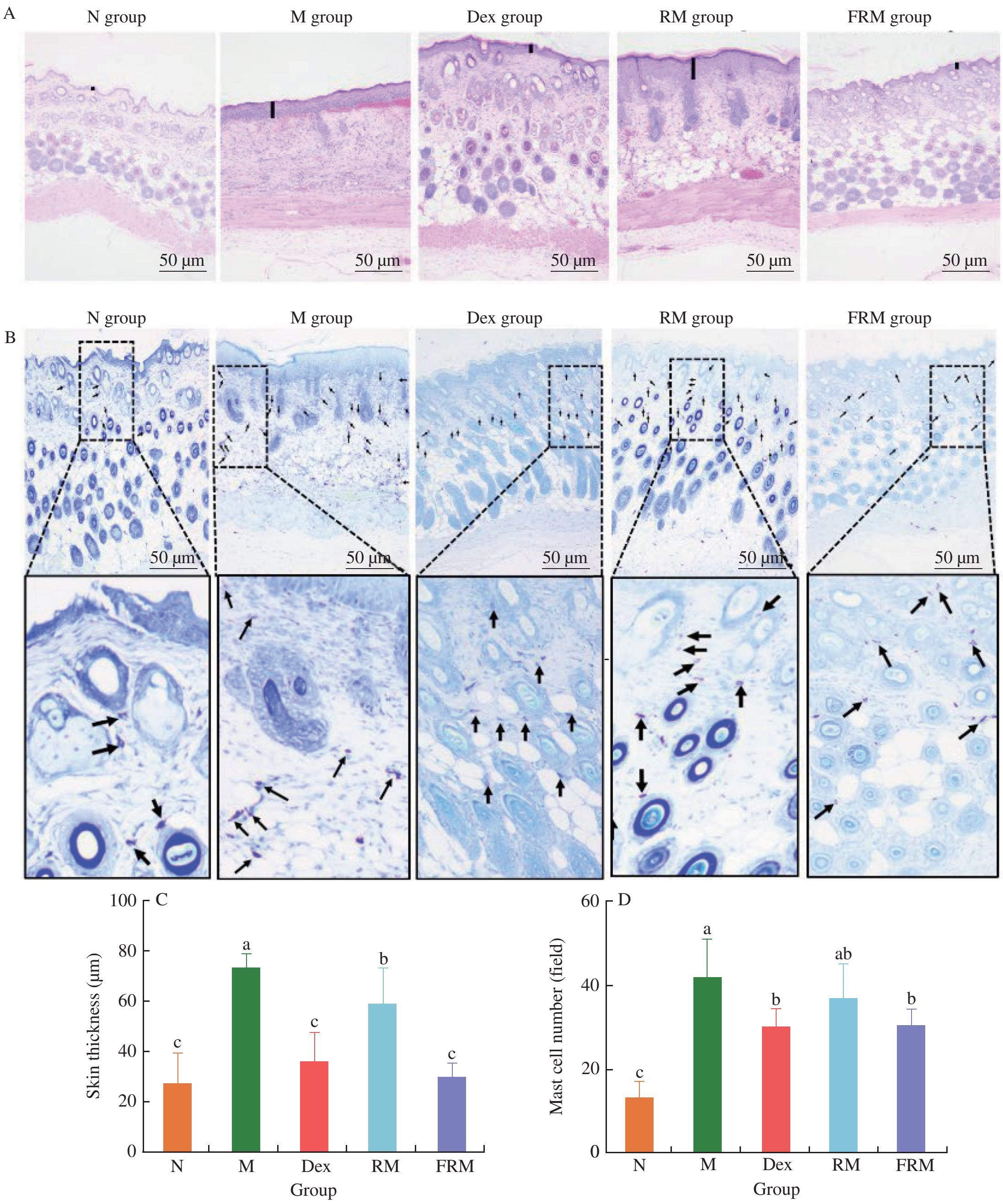

Fig. 4 Effect of fermented rice milk on skin pathological features in AD mice. (A) H&E staining (scale bar = 50 μm). (B) TB staining (scale bar = 50 μm). (C) Statistical analysis of skin thickness in mice based on H&E staining results. (D) Counting of mast cells in TB staining results. Black line mean skin thickness and black arrows mean mast cells. The data represent the mean ± SD. Different lowercase letters represent significantly different ($P < 0.05$).

of mast cells in skin tissue (Figs. 4B and D). Therefore, these results suggested that fermented rice milk improved the skin barrier of AD-like mice by suppressing skin thickening and inflammatory cells infiltration.

3.7 Fermented rice milk inhibited the expression of inflammatory cytokines in AD mice

AD was one of the skin immune disease and caused by excessive skin inflammatory. Levels of pro-inflammatory cytokines and expression of mRNA were measured to investigate the inhibitory effect of fermented rice milk on inflammation. The results showed that fermented rice milk significantly reduced the concentration of TNF-α, IL-6 and IL-4 in serum (Figs. 5A-C). Meanwhile, the expression of AD-associated pro-inflammatory gene, including *TNF-α*, *IL-6* and *IL-4* were significantly inhibited in mice treated with fermented rice milk (Figs. 5D-F). In summary, fermented rice milk down-regulated inflammatory cytokine gene expression and reduced the immune response in AD mice.

3.8 Fermented rice milk inhibited the activation of MAPK and NF-κB pathways in AD mice

Inflammatory response has an important role in the development of AD disease. In order to unravel the mechanisms of fermented

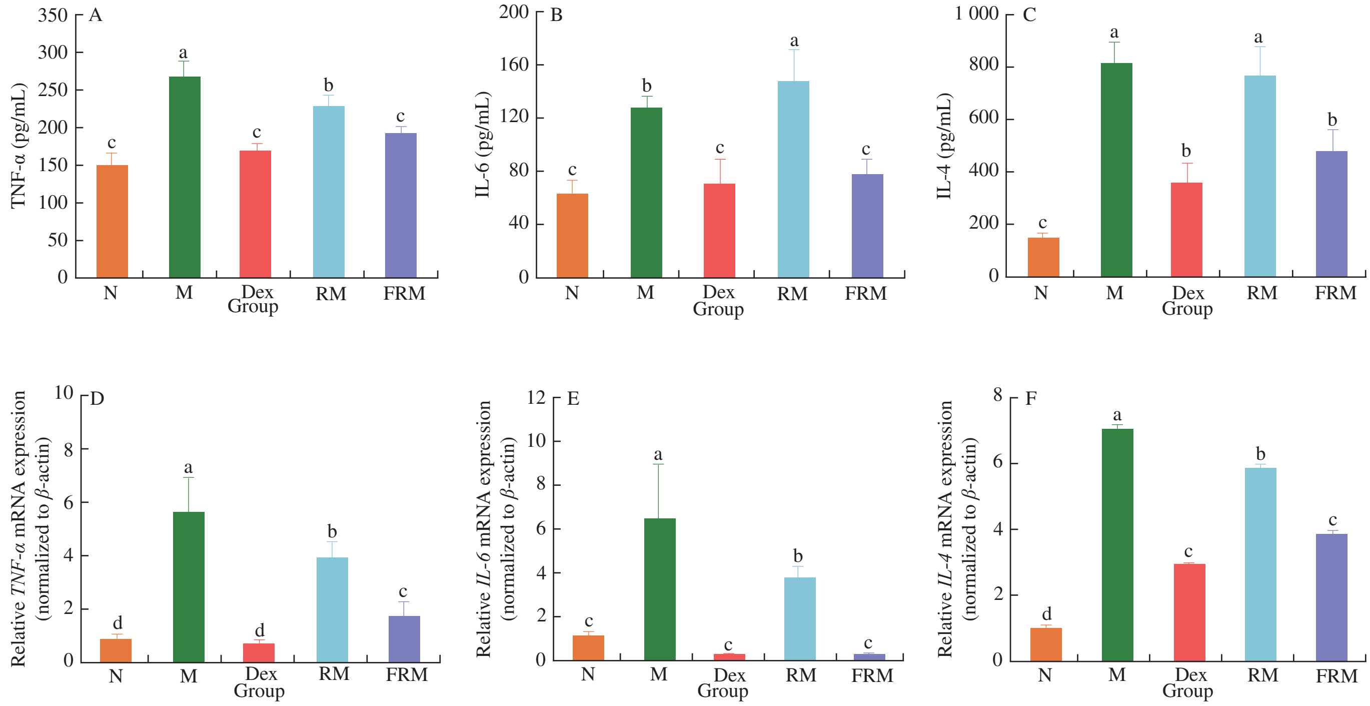

Fig. 5 Fermented rice milk suppressed the pro-inflammatory cytokines content and gene expression. The levels of (A) TNF-α, (B) IL-6 and (C) IL-4. mRNA expression of (D) *TNF-α*, (E) *IL-6*, (F) *IL-4*. The data represent the mean ± SD. Different lowercase letters represent significantly different ($P < 0.05$).

rice milk effects on inflammation and immune dysregulation in AD mice, we focused on the changes in the relevant signaling pathways. Alterations in MAPK and NF-κB pathways were assessed by Western blot analysis (Fig. 6A). As shown in Figs. 6B-D, p-p38, p-JNK and p-ERK in the M group were significantly increased compared with N group. Fermented rice milk treatment suppressed the phosphorylation levels of MAPKs. DNCB treatment increased the expression of p-IKBα and p-NF-κB, which suggested that AD disease was associated with inflammatory response (Figs. 6E and F). In the FRM group, the levels of p-IKBα and p-NF-κB were significantly reduced. These data indicated that fermented rice milk suppressed inflammatory response by regulating the NF-κB and MAPK signaling pathways.

4. Discussion

Atopic dermatitis, which is closely associated with inflammation and immune dysfunction, is a common skin disease and its prevalence has increased steadily in recent decades[33]. Recently, an increasing number of reports on the application of probiotics and its fermented

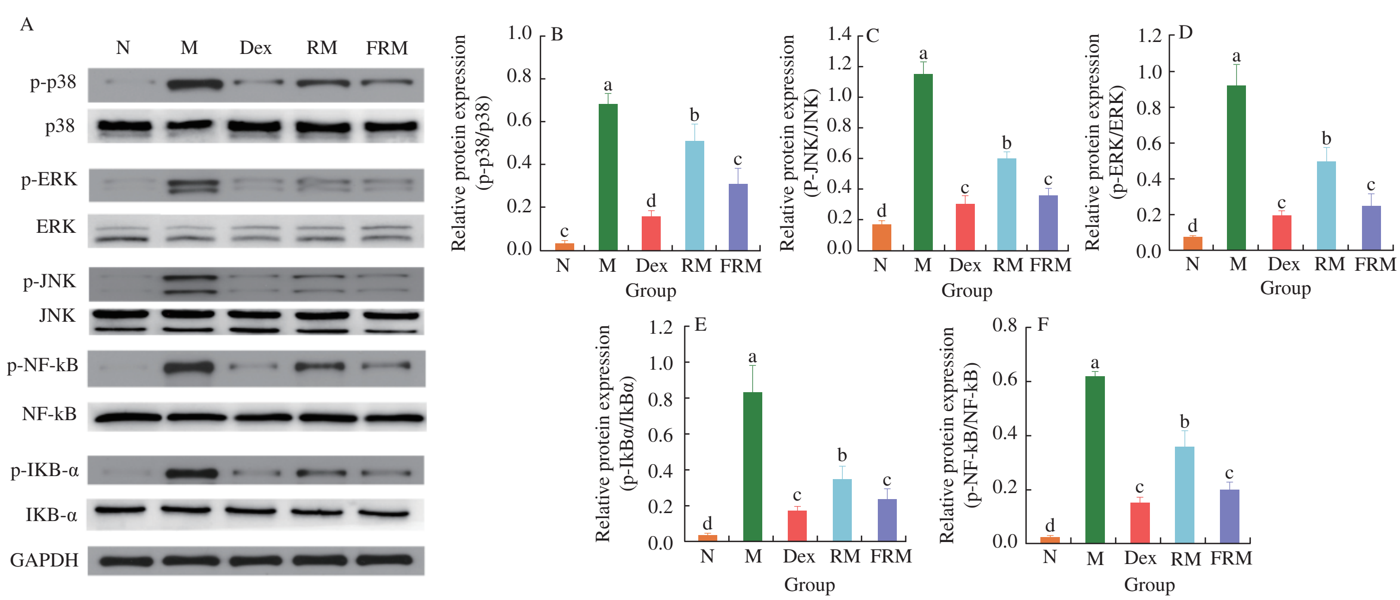

Fig. 6 Effects of fermented rice milk on MAPKs and NF-κB pathway in the dorsal skin of DNCB-induced mice. (A) Representative Western blot bands for MAPKs and NF-κB pathway. The relative folds of (B) p-p38/p38, (C) p-JNK/JNK, (D) p-ERK/ERK, (E) p- IκBα/IκBα and (F) p-NFκB/NFκB were measured. The data represent the mean ± SD. Different lowercase letters represent significantly different ($P < 0.05$).

products as therapeutics for AD have shown beneficial effects[34]. A survey on fermented food intake and AD in Koreans showed that the dietary pattern of high fermented food consumption was negatively related to the prevalence of AD[35]. Fermented foods may be a potential product for AD treatment. In this study, the protective effects of rice milk fermented with yeast on AD treatment was determined *in vivo*, and its action mechanism was also explored. After 2 weeks of oral administration and painted with fermented rice milk, clinical symptoms of DNCB-induced dermatitis lesions were inhibited, such as redness, bleeding and skin thickening. AD was frequently accompanied with oxidative stress and chronic inflammation[31,36]. Fermented rice milk treatment reduced the MDA level by 17.9%, and increased SOD level by 37.3%. Previous study suggested that flavonoids and phenolic compounds in wheat exhibited excellent antioxidant and anti-inflammatory properties and showed potential protective effect on AD mice[37]. Furthermore, H&E and TB results clearly suggested administration of fermented rice milk reduced the infiltration of immune cells.

DNCB administration causes itching and scratching behaviors, contributing to the overactivation of immune response and increased serum IgE concentrations[38]. Serum IgE concentration is a biomarker for AD in experimental animals[39]. Compared with N group, mice in DNCB treatment exhibited increased serum IgE levels with scratching behaviors and high dermatitis scores, but fermented rice milk suppressed their increase (Figs. 3D and G). In addition, serum IgE could induce the production of pro-inflammatory cytokines[40]. In the present study, fermented rice milk treatment greatly reduced pro-inflammatory cytokines (IL-4, IL-6 and TNF-α) levels in serum, and suppressed the expression of mRNA of these pro-inflammatory cytokines. IL-4 is known to induce pruritus by inducing the synthesis of IgE and activating mast cells, which in turn aggravated development of AD[41]. Decreased number of mast cells in FRM group mice also exhibited the inhibitory effects of fermented rice milk on activation of inflammation.

The increased inflammation of AD mice was reported to be associated with the activation of NF-κB and MAPK pathways[8,42]. TNF-α was produced by activated NF-κB pathway and regulated to the inflammation. Fermented rice milk treatment also reduced the concentration of TNF-α. Several studies have already reported that fermentation could improve the biological activity of rice products. Various fermented rice samples prepared from colored rice cultivar and fermented with Loogpang, were demonstrated to inhibit melanogenesis in B16F10 melanoma cells[43]. Yeast extract-treated rice significantly increased anti-skin aging activity[44]. There is evidence that fermented rice will allow the development of nutraceutical and cosmeceutical treatments for skin inflammation[15,45]. Therefore, we focused on the role of fermented rice milk on skin inflammatory response, which was induced by DNCB. From our results, fermented rice milk inhibited the DNCB-induced activation of p38, JNK and ERK. IκBα is a protein which binds to NF-κB protein and inhibits NF-κB. And these 2 proteins were also suppressed by fermented rice milk. Thus, the anti-inflammatory effect of fermented rice milk on DNCB-induced skin inflammation could mainly be attributed to the regulation of NF-κB and MAPK signaling. These evidences suggested that fermented rice milk could be potential products for the treatment of AD.

Most of previous studies are biased towards the alcoholic beverage production by using rice, and the research on rice milk is relatively few[46,47]. In this study, the effect of yeast fermentation on the physicochemical properties and flavor of rice milk were evaluated. The viscosity of all samples was decreased after fermentation (ranged from 36.36% to 8.82%). Results suggested that reduction of amylopectin leaded to a decrease in viscosity. And the results were in accordance with previous study that viscosity of rice milk was decreased after fermentation[48]. The metabolic activity of the yeast imparts a new flavor profile to fermented rice milk. Rice milk contained a large amount of hexanal, 4-vinylguaiacol and 2-pentylfuran, and they were considered as major odor compounds in cooked rice[49-51]. After fermentation, hexanal, 2-pentylfuran, hexanol and 4-vinylguaiacol were not detected or reduced compared with initial value. Hexanal, considered to be undesirable odor (leaf-like and green) in rice milk, was reduced by 93%–100% at the end of fermentation[49]. In addition, alcohols (1-octen-3-ol, hexanol, phenylethyl alcohol, etc.) obtained a higher odor threshold and they were the most abundant compounds except for aldehydes[52]. Phenylethanol could improve the flavor of fermented products as they could import honey rose-like flavor, contributed the most to the aroma of fermented rice milk[53-54]. 1-Octen-3-ol was thought to be produced in lipid oxidation, with a mushroom odor, contributed the most to the aroma of rice milk[54]. Odor analysis revealed that fermentation with yeast could reduce the undesirable odor and increase the fruity and floral flavors, which changed the flavor profile of rice milk.

5. Conclusion

The protective effect of fermented rice milk on AD model mice was investigated. The dermatitis symptoms of AD mice were alleviated after treatment by oral administration and application. Fermented rice milk reduced skin damage and lichenification, serum levels of IL-4, IL-6 and TNF-α and the number of mast cells in AD mice. Further experiments showed that the alleviating effect of fermented rice milk was related to anti-inflammatory ability, and reduced the inflammatory response in AD mice through NF-κB and MAPK signal pathways. Fermentation by *S. cerevisiae* reduced contents of total starch and amylopectin, increased phenylethanol content and improved the flavor profile of fermented rice milk. This study demonstrated that the advantages of fermented rice milk are that, in addition to alleviating the symptoms of AD, it also could reduce inflammatory responses and improve antioxidant capacity in body. Further studies are required to identify the anti-inflammatory component in fermented rice milk. These results increase our understanding of the mechanism underlying the anti-inflammatory effect of fermented rice milk.

Conflict of interest

The authors have no conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://doi.org/10.26599/FSHW.2024.9250353.

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