Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: https://www.sciopen.com/journal/2097-0765

China Food Publishing Co.

Aluminum exposure-exacerbated experimental colitis in mice was antagonized by *Pueraria lobata* extract

Beibei Peng[a,b], Hui Zhao[a,*], Fan Yang[a], Jie Pan[a], Liang Bai[a], Zhibo Han[c,d], Wenqiang Guan[a], Jianfu Liu[a], Yongqing Tao[a,*], Zheng Wang[b,*]

[a] *Tianjin Key Laboratory of Food and Biotechnology, State Experimental and Training Centre of Food and Drug, School of Biotechnology and Food Science, Tianjin University of Commerce, Tianjin 300134, China*

[b] *Department of Pi-Wei Disease, Xuanwu Traditional Chinese Medical Hospital, Beijing 100050, China*

[c] *State Key Lab of Experimental Hematology, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences, Tianjin 300020, China*

[d] *Jiangxi Engineering Research Center for Stem Cell, Shangrao 334113, China*

ARTICLE INFO

Article history:
Received 17 April 2024
Received in revised form 4 May 2024
Accepted 27 November 2024

Keywords:
Aluminum exposure
Pueraria lobata extract
Tight junction
Intestinal barrier

ABSTRACT

Aluminum is the most abundant environmental pollutant. Recent studies suggest that aluminum exposure increases the risk of multiple diseases, including intestinal barrier dysfunction. We investigated whether *Pueraria lobata* extract (PLE) is effective in safeguarding against aluminum chloride exposure-exacerbated intestinal barrier dysfunction. Using an experimental colitis model of aluminum-exacerbated dextran sulphate sodium (DSS)-treated mice, clinical and pathological evidence suggested that the administration of PLE counteracted aluminum exposure-induced intestinal barrier damage. In addition, we found that aluminum toxicities, including loss of tight junction molecules (TJs), upregulated pro-inflammatory cytokines, and enhanced myeloperoxidase (MPO) activity, were significantly suppressed by PLE administration. Furthermore, PLE administration was identified to inhibit activation of MAPKs and NF-κB signal pathways, which contribute to upregulation of myosin light-chain kinase (MLCK) in inflamed intestine. Taken together, these results suggest that PLE might be a potential candidate for aluminum exposure-related intestinal barrier dysfunction.

1. Introduction

The intestinal barrier is mainly composed of tight junction proteins (TJs), intestinal epithelial cell membranes, antimicrobial peptides, mucosal layers, and the immune system[1]. As one of the barriers, the intestinal epithelial cells form a single-cell layer that acts as a selective osmotic barrier, allowing for the stable absorption of nutrients and preventing the entry of harmful molecules (e.g., pathogens, toxins, and antigens) into the mucosal tissues and circulatory system from the luminal environment[2-3]. Given the leaky gut loosening the precise entry and exit of risk factors, the current knowledge suggests that dysfunction of intestinal barrier function not only disrupts the intestine itself but also causes systemic diseases such as obesity, diabetes, and even cognitive disorders[4-5]. Of note, TJs are crucial for intestinal integrity through forming a physical barrier to regulate the paracellular permeability and prevent inflammatory molecules[6]. Therefore, it is important to maintain the intestinal barrier homeostasis for prevention against diseases.

Aluminum has become the most abundant metal pollutant in the global nowadays and is frequently ingested with food and water[7].

* Corresponding authors.
E-mail address: zhaohui@tjcu.edu.cn (H. Zhao); tyqing@tjcu.edu.cn (Y.Q. Tao); 185545572@qq.com (Z. Wang)

Peer review under responsibility of Beijing Academy of Food Sciences.

SciOpen Publishing services by Tsinghua University Press

http://doi.org/10.26599/FSHW.2024.9250423

Long-term exposure to aluminum causes cumulative toxic harm to humans[8]. The reported toxic effects of aluminum include neurotoxicity, immunotoxicity, reproductive toxicity, and skeletal effects[9-12]. In addition, aluminum has been epidemiologically identified to promote intestinal barrier dysfunction[13-14]. The toxicity of aluminum on intestinal damage includes excessively generated pro-inflammatory cytokines, increased reactive oxygen species (ROS), and downregulated TJs[15-16].

Pueraria lobata is the dried root of kudzu, which is widely utilized in East Asia and is even listed in the 50 fundamental dietary herbs of traditional Chinese medicine[17-18]. Various health benefits of *P. lobata* extract (PLE) and its compounds to diseases, such as alcoholic liver injury, cardiovascular diseases, and diabetes, have been well summarized by systemic reviews[19-22]. Interestingly, accumulating reports suggested that PLE lowers the dysfunction of the intestinal barrier. Gegen Qinlian decoction, in which *P. lobata* serves as a key member, had been recommended to alleviate dextran sulphate sodium (DSS)-induced experimental colitis *via* inhibiting NF-κB activation and excessive myeloperoxidase (MPO) activity[23]. In another report, fermented PLE was able to recover the intestinal barrier by reducing pro-inflammatory cytokines[24]. Moreover, Ga et al. [25] performed an *in vivo* research using DSS-induced colitis model and confirmed the protective role of PLE on intestinal barrier function. In addition, products based on PLE have been well developed to prevent against excessive alcoholic intake-induced liver toxicity through restoring the intestinal barrier hemostasis[26].

On the basis of the protective potential of PLE on intestinal barrier hemostasis and the intestinal toxicity of aluminum exposure, we therefore hypothesized that PLE antagonizes intestinal barrier dysfunction induced by aluminum chloride ($AlCl_3$) exposure using DSS-induced colitis. The clinical and pathological evidence suggested that the administration of PLE antagonized the excessive intestinal permeability and intestinal TJs loss induced by aluminum exposure. Furthermore, investigation of mechanisms reflected that alteration of proinflammatory cytokine profile, MPO activity, and inactivation of MAPK/NF-κB-MLCK signaling pathway contribute to PLE antagonizing aluminum exposure-induced intestinal barrier dysfunction.

2. Materials and methods

2.1 Regents, kits and antibodies

For detailed materials including regents, kits, and antibodies unless indicated are listed in Table S1.

2.2 Extraction and composition analysis of PLE

The *P. lobata* was gifted by Shangrao Ecological Agricultural Products Development Co., Ltd. (Shangrao, China). Briefly, the *P. lobata* was peeled, dried, and crushed with a pulverizer and passed through a 40-mesh sieve. After collection, the samples were extracted by ultrasonic-assisted extraction and the PLE was freeze-dried with a vacuum dryer. Appropriate HPLC detection methods were established and 6 flavonoids (puerarin, daidzin, rutin, daidzein, quercetin, genistein) which were suggested to be rich in PLE were assessed with reference to pioneering literature.

2.3 Animals and experimental designs

Male C57BL/6 mice of 7–8 weeks were purchased from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). Mice were acclimatized to the environment in an animal room for 1 week (temperature 23–26 ℃, humidity 45%–60%, maintain a 12 h light/dark cycle) prior to treatments. A total of 40 mice were randomly divided into 4 experimental groups (Fig. 1). DSS and $AlCl_3$ were dissolved in distilled water, and the mice received the same ordinary diet unless indicated. The $AlCl_3$-exacerbated DSS colitis model (DSS + $AlCl_3$) was established according to the method of Pineton de Chambrun et al.[16] with modifications. Groups are as follows: 1) Control (CON) group in which mice received distilled water to drink; 2) DSS group in which mice received distilled water containing 2% DSS; 3) DSS + $AlCl_3$ group in which mice were oral intake of distilled water containing 2% DSS and gavaged with 25 mg/kg of $AlCl_3$ referring to the previously published method regarding mouse model of aluminum-exacerbated colitis[16]; 4) PLE group in which mice were gavaged with PLE (200 mg/kg) in addition to DSS + $AlCl_3$. The dose of PLE available here had been validated by our preliminary experiments according to previous reports[23-24,27].

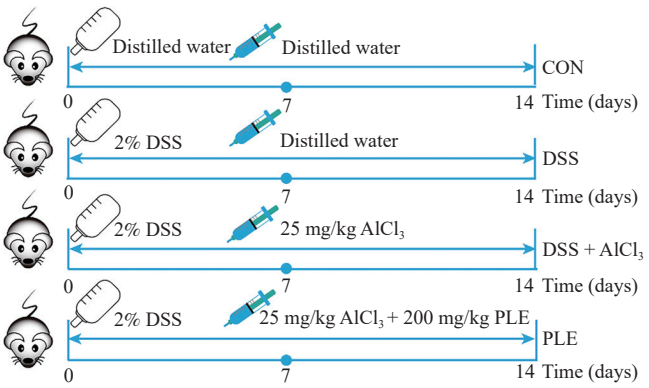

Fig. 1 Schematic diagram of oral administration of PLE to mice.

The body weight, fecal status, and anal bleeding were recorded daily, and all mice were sacrificed on day 15. Animal experimental procedures were approved by the Experimental Animals Ethics Committee of Tianjin University of Commerce (TKLFB-2023YJS-6) according to the Guide for the Care and Use of Laboratory Animals (order No. 2006-398, Ministry of Science and Technology, China).

2.4 Assessment of fluorescein isothiocyanate (FITC)-dextran levels in serum

The serum concentrations of FITC-dextran were tested according to the kit manufacturers. Briefly, mice were gavaged with 40 mg/kg FITC-dextran 4 h before sacrifice and serum samples were extracted from ocular venous blood, protected from light for 2 h, and centrifuged at 4 ℃ for 10 min at 3 000 r/min. The absorbance of samples was measured at 528 nm emission and 485 nm excitation.

2.5 Tissue sampling analysis

On day 15, blood collected from the eye vein was centrifuged at 4 ℃ for 10 min at 3 000 r/min, and the supernatant was collected. The

weight and the length of the colon were recorded, and part of the colon tissue was fixed in 4% paraformaldehyde solution, and the remaining tissue samples were stored in a –80 ℃ refrigerator.

2.6 Histological analysis

Colon tissue was immobilized with 4% paraformaldehyde solution. After 48 h, the fixed tissues were dehydrated, embedded, sliced, and stained with hematoxylin and eosin (H&E). The assessment of intestinal histopathological changes was regarding a previously established scoring system[28].

For immunohistochemistry assessment, the slices were sequentially soaked in xylene, gradient ethanol and water, and then repaired with EDTA under high pressure. Endogenous peroxidase blockers were added according to the manufacturer's instructions, and the sections were washed three times for 5 min with PBS, followed by overnight incubation at 4 ℃ with primary antibodies for claudin-4, occludin, and MLCK, and at 37 ℃ with the corresponding secondary antibodies. Under the condition of avoiding light, the tissues were reacted with DAB chromogenic agent, and hematoxylin staining was carried out according to standard procedure. Photographs of different fields of view were taken using a light microscope (Olympus, Tokyo, Japan). Finally, slices were from 3 different mice per treatment group in which 4 fields (400×) were selected for each slice to be photographed, and the percentage of positive area was calculated by ImageJ software.

All the histopathological features were kept consistently with original magnification 40×, scale bar 500 μm; original magnification 100×, scale bar 200 μm; and original magnification 200×, scale bar 100 μm.

2.7 Western blot analysis

Briefly, colon tissue was taken into RIPA solution with protease and phosphatase inhibitors (Thermo Scientific, North Carolina, USA), ground at low temperature, centrifuged at 4 ℃, and supernatant was collected. Quantitative determination of proteins according to manufacturer's instructions. Equal amounts of protein extracts were separated on sodium dodecyl sulfate polyacrylamide gel and then wet-transferred to a polyvinylidene fluoride (PVDF) membrane. PVDF membrane was blocked with 2% bovine serum albumin (BSA) for 1 h. Subsequently, membranes were incubated with primary antibodies against claudin-4, occludin, ZO-1, p-ERK1/2, ERK1/2, p-JNK, JNK, p-p38, p-38, p-p65, p-65, Lamin B1, GAPDH, and *β*-actin overnight at 4 ℃. Then they were washed with TBST buffer and the correspondingly secondary anti-mouse or anti-rabbit antibodies were incubated for 1 h at room temperature. Finally, western blotting strips were reacted with an enhanced chemiluminescence solution and detected using an imaging system (Monad, Suzhou, China). ImageJ software (Bethesda, MD, USA) was utilized to quantify the protein bands compared with the internal references including GAPDH, *β*-actin.

2.8 Isolation of total RNA and the quantitative real-time polymerase chain reaction (PCR)

Trizol was used to homogenize colon tissue to obtain total RNA, and then the total RNA was reverse transcribed into cDNA with cDNA reversal kit, and finally real-time PCR amplification was performed by MonAmp™ SYBR Green qPCR Mix Kit, and the mRNA expression of tumor necrosis factor-α (*TNF-α*), interleukin-1β (*IL-1β*), and interleukin-6 (*IL-6*) was detected by *GAPDH* as an internal control. Information of primer sequences is listed in Table S2.

2.9 MPO activity assay

The colonic tissues were homogenized with the buffer solution corresponding to the kit. The MPO activity was measured according to the manufacturer's instructions.

2.10 Statistical analysis

The results were analyzed by GraphPad Prism software 8.0, expressed as mean ± standard deviation, and one-way ANOVA was performed by Turkey method. A statistical probability of $P < 0.05$ was considered significant.

3. Results

3.1 PLE is rich with dietary flavonoids puerarin, rutin, and daidzin

On the basis of the previous methods, 6 suggested flavonoids of PLE were tested using HPLC chromatograms (Figs. 2A-C)[29-31]. Our data manifested that puerarin was the most abundant compound with the concentration of 31.69 mg/g, followed by rutin (14.19 mg/g), daidzin (7.75 mg/g), daidzein (1.43 mg/g), quercetin (0.63 mg/g) and genistein (0.57 mg/g) (Table 1).

Table 1
Content of 6 compounds in PLE.

Compound number	Compound name	Retention time (min)	Content (mg/g)
1	Puerarin	15.00	31.69
2	Daidzin	17.04	7.75
3	Rutin	21.43	14.19
4	Daidzein	24.60	1.43
5	Quercetin	25.70	0.63
6	Genistein	26.96	0.57

3.2 PLE ameliorates $AlCl_3$ exposure-exacerbated colitis

Before assessing the effects and mechanisms of PLE prevention against exacerbated DSS-induced colitis by aluminum, it is vital to ensure that the established mouse model well reflects the pathogenesis. As shown in Fig. 3, aluminum administration (25 mg/kg) definitely exacerbated colitis, reflected by broader performances, including weight loss, barrier permeability, the length of colon, and pathological scores, compared with mice that received DSS alone. These results indicated that the current model is competently available in the following investigation.

On the basis of the established aluminum-exacerbated colitis model, we asked whether PLE protects against it. Administration with PLE (200 mg/kg) was available in the investigation, and parameters

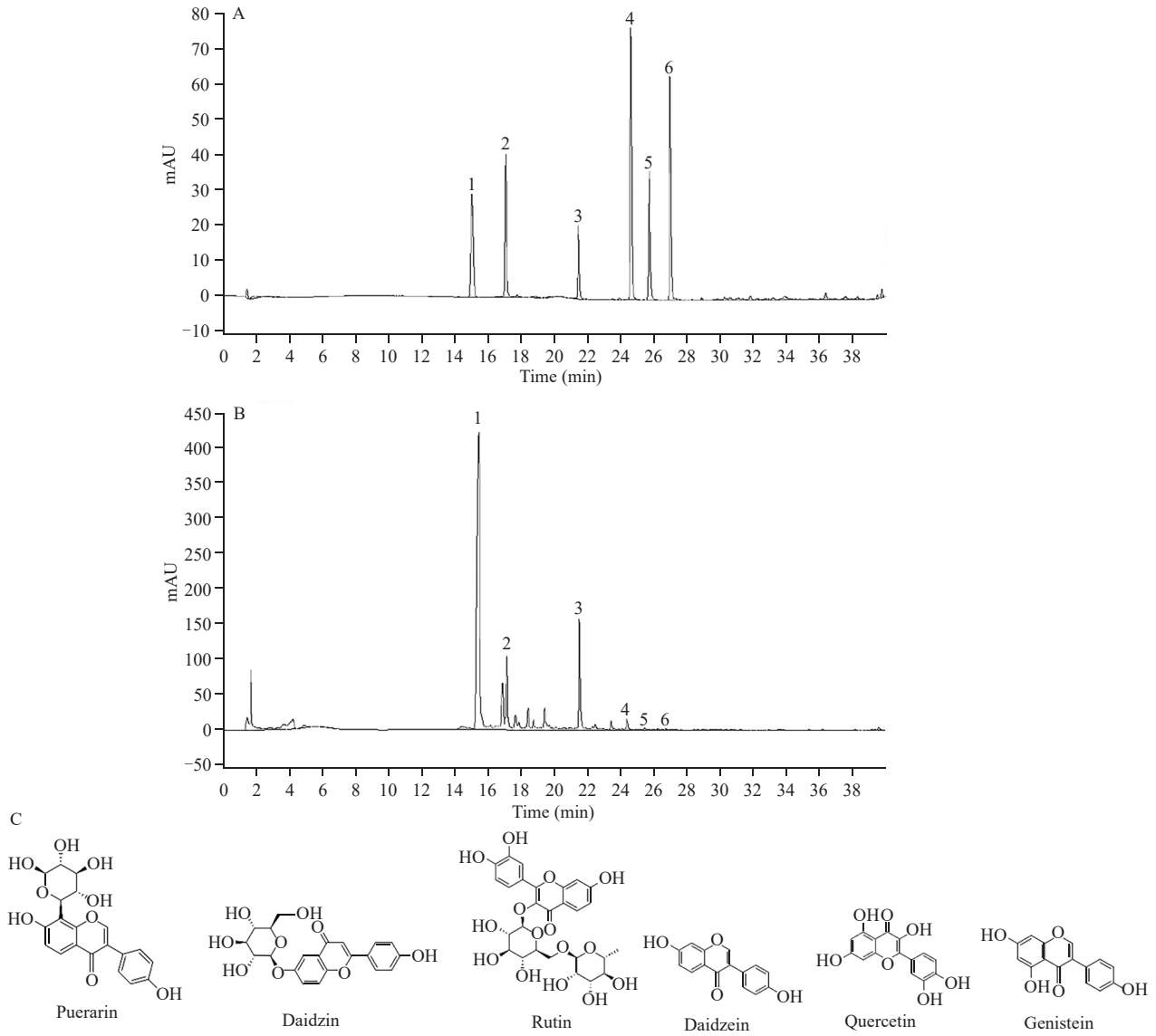

Fig. 2 HPLC chromatograms of 6 representative compounds identified from PLE. (A) Mixed standard of 6 representative compounds. (B) PLE sample profile. 1. Puerarin; 2. daidzein; 3. rutin; 4. daidzein; 5. quercetin; 6. genistein. (C) Chemical structures of the 6 representative compounds from PLE.

for assessment of colitis were recorded according to previous method[23-24,32]. Mice of DSS + $AlCl_3$ group showed enhanced weight loss since day 5 compared with mice of PLE group (Fig. 3A). Furthermore, as shown in Fig. 3B, the mice of DSS + $AlCl_3$ group had the much higher serum FITC-dextran content compared with mice of DSS group, indicating that $AlCl_3$ exposure resulted in more worsened damage of intestinal epithelial permeability. Oppositely, mice of PLE group displayed significantly deceased FITC-dextran content compared with mice exposed to $AlCl_3$, indicating that PLE counteracted $AlCl_3$-indueced intestinal barrier dysfunction. In addition, PLE administration also restored the shortened colon length and weight loss resulting from $AlCl_3$ exposure (Figs. 3C-E). Supportively, the histopathological score of colon samples was characterized by the worst destruction of epithelial structures, infiltration of inflammatory cells, and loss of goblet cells in the DSS + $AlCl_3$ group (Fig. 3F). On the opposite, mice of the PLE group possessed the better intestinal epithelial integrity and the less infiltration of inflammatory cells. Taken together, these findings suggested that exposure to aluminum definitely exacerbated colitis and as was counteracted by PLE administration.

3.3 PLE suppresses loss of tight junctions induced by exposure to $AlCl_3$

The above findings indicated that PLE protected the integrity of the intestinal barrier. Given that TJs are critical for constructing the epithelial barrier, we then investigated whether PLE protected against $AlCl_3$-exacerbated intestinal barrier injury involving in TJs. As expected, intestinal TJs, such as occludin, claudin-4 and ZO-1, were significantly upregulated in mice administrated by PLE as compared with DSS group and DSS + $AlCl_3$ group (Figs. 4A-D), These findings were further confirmed by immunohistochemical tests on colon samples (Figs. 4E and F).

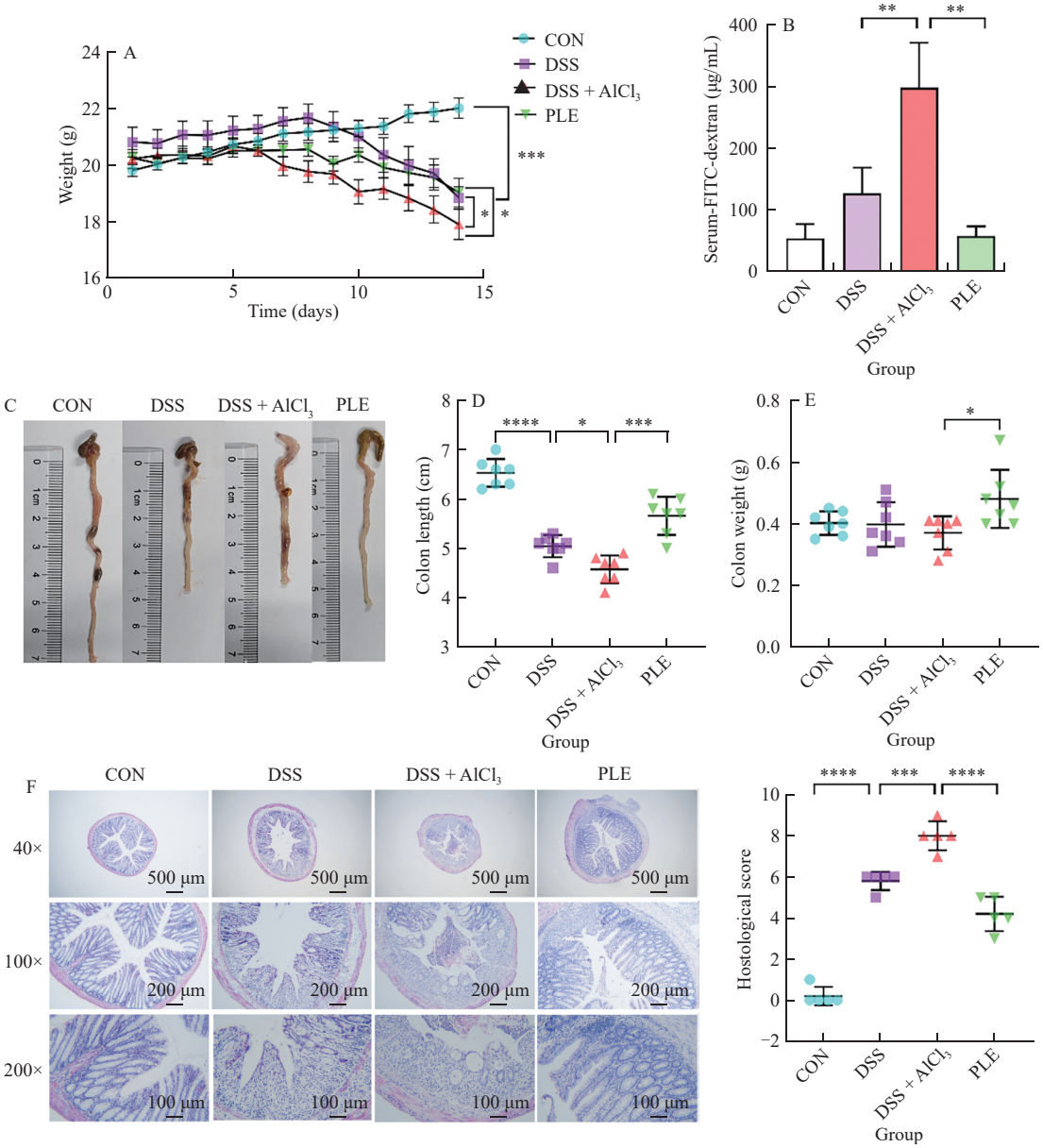

Fig. 3 Exposure to $AlCl_3$ worsens DSS-induced colitis and PLE safeguards the structural integrity of the intestine in mice exposed to DSS and $AlCl_3$. (A) Changes of body weight; (B) concentration of FITC-dextran in mice; (C) photographs of the colon; (D) length and (E) weight of colon samples; (F) H&E staining images and histopathological scores. All values are presented as the mean ± SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.000\ 1$.

3.4 PLE ameliorates inflammation and oxidative stress in flamed intestine

Inflammatory processes and oxidative stress disrupt the epithelial barrier, impairing intestinal paracellular permeability, we then evaluated whether PLE exerted protective effects associated with the suppression of inflammation and oxidative stress. A pioneer report by Jeong et al.[15] presented definite evidence that oral administration of $AlCl_3$ to DSS-treated mice worsened intestinal inflammatory cytokine profile and MPO activation. As shown in Fig. 5, mice with aluminum exposure led to a worsening of production of pro-inflammatory cytokines, including *TNF-α*, *IL-1β*, and *IL-6*, and MPO activity in intestinal samples. Interestingly, these effects induced by aluminum exposure were markedly reversed when mice received PLE addition. These data indicated that PLE effectively suppressed exposure to aluminum-induced enhancement of inflammation and oxidative stress in the colitis model.

3.5 PLE inhibits activation of intestinal MAPK and NF-κB pathways and expression of MLCK

Over the past decades, it has been well established that inflammation and/or oxidative stress independently or collaboratively disrupts the intestinal barrier by activating signal transduction pathways such as NF-κB and MAPK to regulate cytoskeletal proteins leading to loss of TJs and subsequent barrier dysfunction[33]. Indeed, aluminum stimulation leading to intestinal oxidative stress and inflammation activates MAPK and NF-κB pathways, destroying the intestinal barrier[34]. Interestingly, although there is

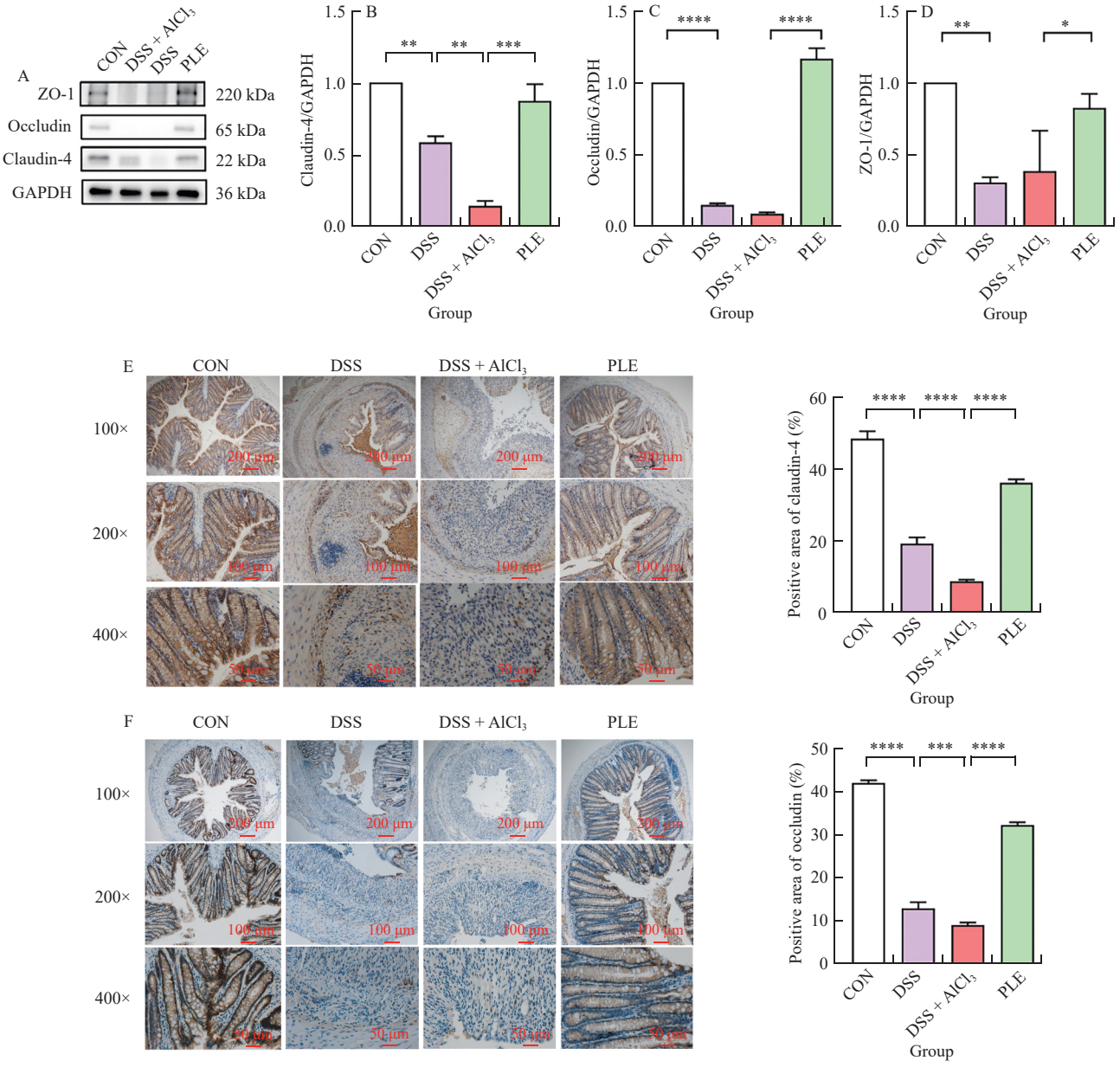

Fig. 4 PLE suppresses loss of tight junctions induced by exposure to $AlCl_3$. (A) TJs were analyzed by Western blot; (B-D) Levels of intestinal proteins TJs, including claudin-4, occludin and ZO-1; (E and F) Immunohistochemical images and evaluation of intestinal TJ molecules claudin-4 and occludin. All values are presented as the mean ± SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.000\ 1$.

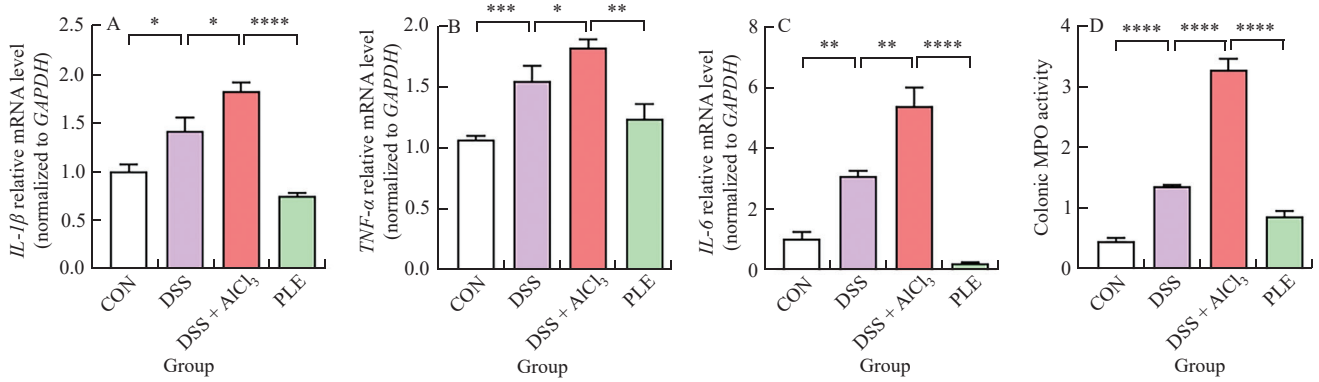

Fig. 5 PLE ameliorates inflammation and oxidative stress in flamed intestine. Intestinal mRNA levels of pro-inflammatory cytokines. (A) *TNF-α*, (B) *IL-1β*, and (C) *IL-6*. (D) Colonic MPO activity. All values are presented as the mean ± SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.000\ 1$.

no direct evidence regarding PLE resistance to aluminum stimulation, PLE is recommended to be protective on intestinal hemostasis[35-36]. Our above findings indicate that PLE addition reversed aluminum exposure-exacerbated dysfunction of intestinal barrier, and we next evaluated that the protective effect of PLE was mediated by suppressing MAPK and NF-κB activation.

As shown in Figs. 6A-C, assessment of intestinal samples indicated that administration of PLE effectively reduced the phosphorylated proteins of ERK, JNK, and p-38 as compared with aluminum stimulation. Likewise, aluminum stimulation-induced phosphorylation of NF-κB p-65 in the nucleus was significantly reduced by PLE administration (Fig. 6D).

Studies have shown that activation of NF-κB and MAPK pathways results in upregulated MLCK expression in epithelial barrier[6,37]. Subsequently, MLCK-dependent MLC phosphorylation disrupts TJs, leading to intestinal barrier dysfunction[38-39]. In subsequent studies, we evaluated whether PLE administration reduced MLCK expression in gut. Immunohistochemistry assessment of the colons manifested that mice exposed to aluminum stimulation had a significantly higher MLCK expression as compared with mice administrated by PLE (Fig. 6E).

4. Discussion

The intestinal barrier function is crucial to keeping tissue homeostasis. Given that the intake of food is the fundamental action of the intestine, the barrier is constantly being challenged by various substances[40]. Intestinal tissue homeostasis is safeguarded by junctions between epithelial cells. In particular, lateral sides of neighboring cells are sealed by TJs, which form the restrictive cellular pores to maintain the permeability of intestinal epithelial layers to substances. TJs loss resulted from risk factors such as toxins, inflammation, and oxidative stress, enabling the intestine to become leaky and lose of barrier integrity[41].

Intake of aluminum is inevitable due to its properties as the most abundant metal element on earth and food containers. The principal route of intake of aluminum is the breakthrough of the intestinal barrier, contributing to diseases due to the disruption of intestinal epithelial integrity[42-45]. Aluminum exposure induces loss of intestinal barrier TJs through activating ERK1/2 and NF-κB signal pathways, which result in upregulated matrix metalloproteinase-9, MLCK, inflammatory cytokines, and MPO activity[15]. Furthermore, aluminum exposure worsened the severity and duration of inflamed intestine

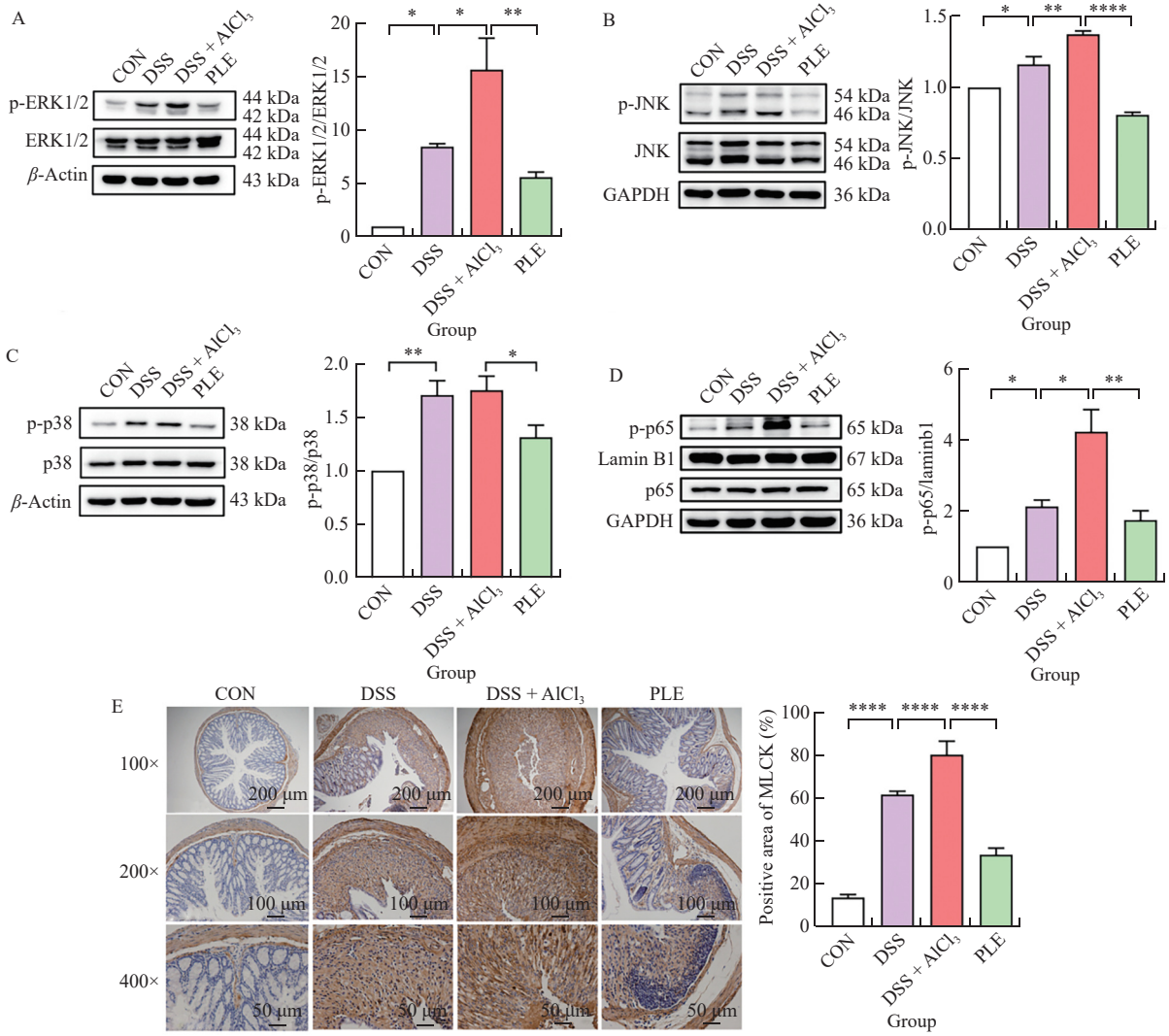

Fig. 6 PLE inhibits activation of intestinal MAPK and NF-κB pathways and expression of MLCK. (A-D) Western blot analysis of p-ERK1/2, p-JNK, p-p38, p-p65; (E) Immunohistochemical assessment of MLCK. All values are presented as the mean ± SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.000\ 1$.

identified with several mouse models of experimental colitis[16]. These researches suggest that aluminum exposure serves as an environmental risk factor for intestinal injury. However, it is far short of effective tactics against intestinal barrier dysfunction associated with aluminum exposure.

Growing reports have suggested that *P. lobata* might provide a probable solution to intestinal barrier dysfunction. In this study, we provide clear evidence that PLE administration mitigates aluminum exposure-exacerbated intestinal barrier dysfunction. Furthermore, we present the mechanisms behind it involving targeting the pro-inflammatory cytokines, oxidative stress, and MAPK/NF-κB-MLCK signaling pathway (Fig. 7).

The current consensus is that the bioactive compounds from PLE are indispensable for the health effects of *P. lobata*[46-47]. There are more than 100 chemical constituents, including isoflavones, triterpenoids, coumarins, alkaloids, and others, currently documented by literature reports[48-49]. Flavonoids and saponins are considered as the main bioactive substances due to the highest population accounting for various PLEs[50]. Of these compounds, isoflavones puerarin and daidzein are the representative compounds reflecting the vast majority of bioactive features of PLE[51-53]. As a unique component of the *Pueraria* genus, puerarin has been chosen to be a quality control marker for *P. lobata* in China[54]. Indeed, the most abundant flavonoid of PLE available in our experiments is puerarin (31.69 mg/g) followed by rutin (14.19 mg/g) and daidzin (7.75 mg/g). In agreement, a recent research reported that there are around 180 chemical ingredients identified in PLE for ameliorating ulcerative colitis in mice and the contents of puerarin, daidzin and daidzein are with the highest relative levels[25]. Interestingly, these flavonoids are commonly abundant regardless of PLEs isolated from various methods and have been frequently documented in maintenance of intestinal barrier hemostasis[55-56]. As the most abundant substance of PLE, puerarin was reported to downregulate NF-κB and NF-E2 p45-related factor 2 (Nrf2) signaling pathways to alleviated mice colitis[27]. In addition, puerarin administration alters gut microbiota to regulate short-chain fatty acids, pyroptosis and macrophage polarization contributing to the enhanced intestinal barrier[25]. Akin to puerarin, rutin and daidzein were also available in treatment of intestinal damage through targeting MPO activity and NF-κB and MAPK activation[57-58]. Therefore, our current clinical evidence and ingredient analysis suggest that PLE has the potential as a protective agent to aluminum exposure-exacerbated intestinal barrier injury.

In conclusion, our experimental findings propose PLE as a potential solution of aluminum toxicity for intestinal barrier dysfunction. Furthermore, we delineate the mechanism by which aluminum-mediated intestinal pathogenesis can be suppressed by PLE involving inhibition of inflammation and oxidative stress through counteraction of intestinal NF-κB and MAPK signal pathways. The demonstration also proposes a potential therapeutic development based on PLE or main compounds of PLE on aluminum toxicity to intestinal dysfunction.

Conflict of interest

Hui Zhao is an associate editor for *Food Science and Human Wellness* and was not involved in the editorial review or the decision to publish this article. The authors declare no conflict of interest.

Acknowledgements

This project was supported by the Shangrao Key Research and Development Project (19A005).

Appendix A. Supplementary information

Supplementary data associated with this article can be found, in the online version, at http://doi.org/10.26599/FSHW.2024.9250423.

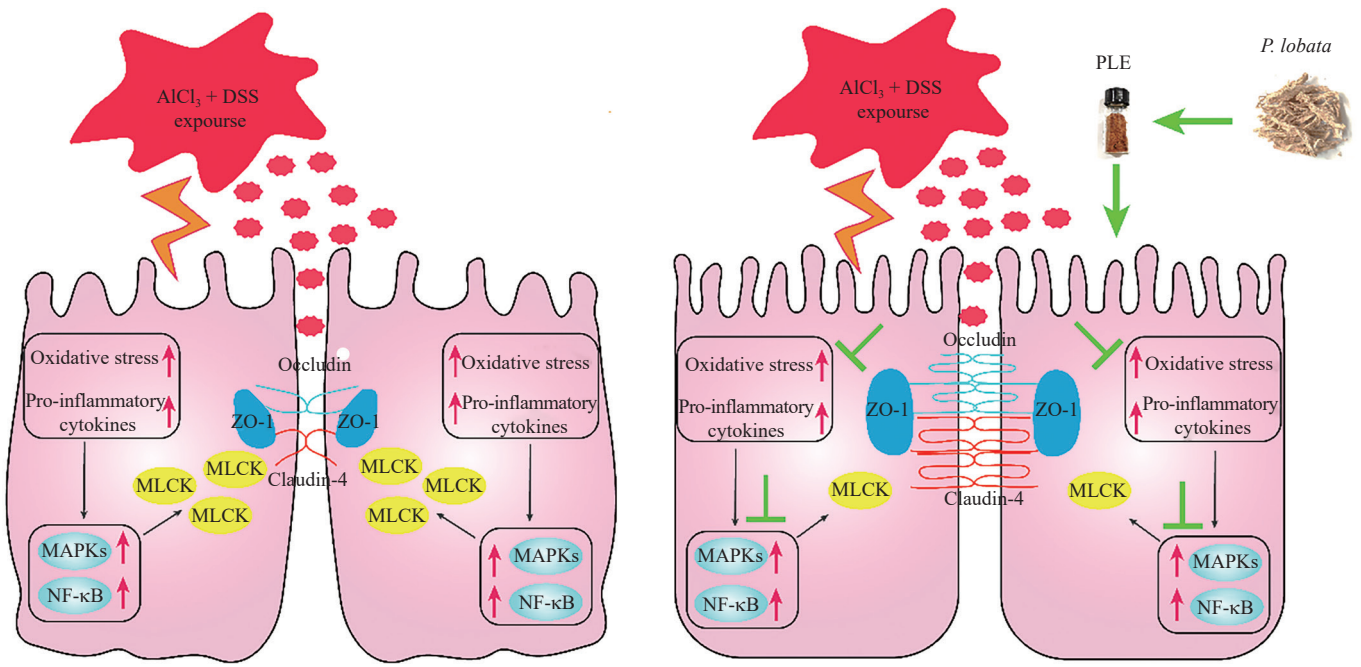

Fig. 7 Schematic representation of the mechanisms by which PLE protects the intestinal barrier.

References

[1] Y. Zhuang, H. Wu, X. Wang, et al., Resveratrol attenuates oxidative stress-induced intestinal barrier injury through PI3K/Akt-Mediated Nrf2 signaling pathway, Oxid. Med. Cell Longev. 2019 (2019) 7591840. http://dx.doi.org/10.1155/2019/7591840.

[2] R. Al-Sadi, J. Engers, M. Haque, et al., Matrix metalloproteinase-9 (MMP-9) induced disruption of intestinal epithelial tight junction barrier is mediated by NF-κB activation, PLoS One 16 (2021) e0249544. http://dx. doi. org/10.1371/journal.pone.0249544.

[3] S.H. Lee, Intestinal permeability regulation by tight junction: implication on inflammatory bowel diseases, Intest Res. 13 (2015) 11-18. http://dx.doi.org/10.5217/ir.2015.13.1.11.

[4] C. Chelakkot, J. Ghim, S.H. Ryu, Mechanisms regulating intestinal barrier integrity and its pathological implications, Exp. Mol. Med. 50 (2018) 1-9. http://dx.doi.org/10.1038/s12276-018-0126-x.

[5] Y. Wang, Y. An, W. Ma, et al., 27-Hydroxycholesterol contributes to cognitive deficits in APP/PS1 transgenic mice through microbiota dysbiosis and intestinal barrier dysfunction, J. Neuroinflammation 17 (2020) 199. http://dx.doi.org/10.1186/s12974-020-01873-7.

[6] T. Suzuki, Regulation of the intestinal barrier by nutrients: the role of tight junctions, Anim. Sci. J. 91 (2020) e13357. http://dx.doi.org/10.1111/asj.13357.

[7] K. Klotz, W. Weistenhöfer, F. Neff, et al., The health effects of aluminum exposure, Dtsch Arztebl Int. 114 (2017) 653-659. http://dx.doi.org/10.3238/arztebl.2017.0653.

[8] I.O. Igbokwe, E. Igwenagu, N.A. Igbokwe, Aluminium toxicosis: a review of toxic actions and effects, Interdiscip Toxicol. 12 (2019) 45-70. http://dx.doi.org/10.2478/intox-2019-0007.

[9] I. ELBini-Dhouib, R. Doghri, A. Ellefi, et al., Curcumin attenuated neurotoxicity in sporadic nnimal model of Alzheimer's disease, Molecules 26 (2021) 3011. http://dx.doi.org/10.3390/molecules26103011.

[10] H. Yu, J. Zhang, Q. Ji, et al., Melatonin alleviates aluminium chloride-induced immunotoxicity by inhibiting oxidative stress and apoptosis associated with the activation of Nrf2 signaling pathway, Ecotox. Environ. Saf. 173 (2019) 131-141. http://dx.doi.org/10.1016/j.ecoenv.2019.01.095.

[11] M.I. Yousef and A.F. Salama, Propolis protection from reproductive toxicity caused by aluminium chloride in male rats, Food Chem. Toxicol. 47 (2009) 1168-1175. http://dx.doi.org/10.1016/j.fct.2009.02.006.

[12] Y. Zhu, F. Xu, X. Yan, et al., The suppressive effects of aluminum chloride on the osteoblasts function, Environ. Toxicol. Pharmacol. 48 (2016) 125-129. http://dx.doi.org/10.1016/j.etap.2016.10.009.

[13] A. Lerner, Aluminum as an adjuvant in Crohn's disease induction, Lupus. 21 (2012) 231-238. http://dx.doi.org/10.1177/0961203311430090.

[14] M. Djouina, C. Waxin, F. Leprêtre, et al., Gene/environment interaction in the susceptibility of Crohn's disease patients to aluminum, Sci. Total Environ. 850 (2022) 158017. http://dx.doi.org/10.1016/j.scitotenv.2022.158017.

[15] C.H. Jeong, H.C. Kwon, D.H. Kim, et al., Effects of aluminum on the integrity of the intestinal epithelium: an *in vitro* and *in vivo* study, Environ. Health Perspect. 128 (2020) 17013. http://dx.doi.org/10.1289/ehp5701.

[16] G. Pineton de Chambrun, M. Body-Malapel, I. Frey-Wagner, et al., Aluminum enhances inflammation and decreases mucosal healing in experimental colitis in mice, Mucosal Immunol. 7 (2014) 589-601. http://dx.doi.org/10.1038/mi.2013.78.

[17] X. Xu, Y. Guo, S. Chen, et al., The positive influence of polyphenols extracted from *Pueraria lobata* root on the gut microbiota and its antioxidant capability, Front. Nutr. 9 (2022) 868188. http://dx.doi.org/10.3389/fnut.2022.868188.

[18] S. Wang, S. Zhang, S. Wang, et al., A comprehensive review on *Pueraria*: insights on its chemistry and medicinal value, Biomed. Pharmacother. 131 (2020) 110734. http://dx.doi.org/10.1016/j.biopha.2020.110734.

[19] X. Chen, J. Yu, J. Shi, Management of diabetes mellitus with puerarin, a natural ssoflavone from *Pueraria lobata*, Am. J. Chin Med. 46 (2018) 1771-1789. http://dx.doi.org/10.1142/s0192415x18500891.

[20] X. Liu, R. Huang, J. Wan, Puerarin: a potential natural neuroprotective agent for neurological disorders, Biomed. Pharmacother. 162 (2023) 114581. http://dx.doi.org/10.1016/j.biopha.2023.114581.

[21] C. Ulbricht, D. Costa, C. Dam, et al., An evidence-based systematic review of kudzu (*Pueraria lobata*) by the Natural Standard Research Collaboration, J. Diet Suppl. 12 (2015) 36-104. http://dx.doi.org/10.3109/19390211.2014.904123.

[22] T. Xuan, Y. Liu, R. Liu, et al., Advances in extraction, purification, and analysis techniques of the main components of Kudzu root: a comprehensive review, Molecules 28 (2023) 6577. http://dx.doi.org/10.3390/molecules28186577.

[23] R. Li, Y. Chen, M. Shi, et al., Gegen Qinlian decoction alleviates experimental colitis *via* suppressing TLR4/NF-κB signaling and enhancing antioxidant effect, Phytomedicine 23 (2016) 1012-1020. http://dx.doi.org/10.1016/j.phymed.2016.06.010.

[24] S. Choi, J.K. Woo, Y.S. Jang, et al., Fermented *Pueraria lobata* extract ameliorates dextran sulfate sodium-induced colitis by reducing pro-inflammatory cytokines and recovering intestinal barrier function, Lab Anim. Res. 32 (2016) 151-159. http://dx.doi.org/10.5625/lar.2016.32.3.151.

[25] Y. Ga, Y. Wei, Q. Zhao, et al., *Puerariae Radix* protects against ulcerative colitis in mice by inhibiting NLRP3 inflammasome activation, Food Sci. Hum. Wellness 13 (2024) 2266-2276. http://dx.doi.org/10.26599/FSHW.2022.9250189.

[26] Q. Wu, P. Li, X. Li, et al., Pueraria extract ameliorates alcoholic liver disease via the liver-gut-brain axis: focus on restoring the intestinal barrier and inhibiting alcohol metabolism, J. Agric. Food Chem. 72 (2024) 24449-24462. http://dx.doi.org/10.1021/acs.jafc.4c05365.

[27] Y.D. Jeon, J.H. Lee, Y.M. Lee, et al., Puerarin inhibits inflammation and oxidative stress in dextran sulfate sodium-induced colitis mice model, Biomed. Pharmacother.124 (2020) 109847. http://dx.doi.org/10.1016/j.biopha.2020.109847.

[28] X. Wu, Y. Yang, Y. Dou, et al., Arctigenin but not arctiin acts as the major effective constituent of *Arctium lappa* L. fruit for attenuating colonic inflammatory response induced by dextran sulfate sodium in mice, Int. Immunopharmacol. 23 (2014) 505-515. http://dx.doi.org/10.1016/j.intimp.2014.09.026.

[29] R.W. Jiang, K.M. Lau, H.M. Lam, et al., A comparative study on aqueous root extracts of *Pueraria thomsonii* and *Pueraria lobata* by antioxidant assay and HPLC fingerprint analysis, J. Ethnopharmacol. 96 (2005) 133-138. http://dx.doi.org/10.1016/j.jep.2004.08.029.

[30] L. Qu, K. Song, Q. Zhang, et al., Simultaneous determination of six isoflavones from *Puerariae Lobatae* Radix by CPE-HPLC and effect of puerarin on tyrosinase activity, Molecules 25 (2020) 344. http://dx.doi.org/10.3390/molecules25020344.

[31] G. Du, H.Y. Zhao, Q.W. Zhang, et al., A rapid method for simultaneous determination of 14 phenolic compounds in *Radix Puerariae* using microwave-assisted extraction and ultra high performance liquid chromatography coupled with diode array detection and time-of-flight mass spectrometry, J. Chromatogr. A. 1217 (2010) 705-714. http://dx.doi.org/10.1016/j.chroma.2009.12.017.

[32] M. Perše, A. Cerar, Dextran sodium sulphate colitis mouse model: traps and tricks, J. Biomed Biotechnol. 2012 (2012) 718617. http://dx.doi.org/10.1155/2012/718617.

[33] P. Liu, Y. Wang, G. Yang, et al., The role of short-chain fatty acids in intestinal barrier function, inflammation, oxidative stress, and colonic carcinogenesis, Pharmacol. Res. 165 (2021) 105420. http://dx.doi.org/10.1016/j.phrs.2021.105420.

[34] L. Yu, Q. Zhai, F. Tian, et al., Potential of *Lactobacillus plantarum* CCFM639 in protecting against aluminum toxicity mediated by intestinal barrier function and oxidative stress, Nutrients 8 (2016) 1891-1900. http://dx.doi.org/10.3390/nu8120783.

[35] S.Y. Che, J.W. Yuan, L. Zhang, et al., Puerarin prevents epithelial tight junction dysfunction induced by ethanol in Caco-2 cell model, J. Funct. Foods. 73 (2020) 104079. http://dx.doi.org/10.1016/j.jff.2020.104079.

[36] S.H. Eom, S.J. Jin, H.Y. Jeong, et al., Kudzu leaf extract suppresses the production of inducible nitric oxide synthase, cyclooxygenase-2, tumor necrosis factor-alpha, and interleukin-6 *via* inhibition of JNK, TBK1 and STAT1 in inflammatory macrophages, Int. J. Mol. Sci. 19 (2018) 1536. http://dx.doi.org/10.3390/ijms19051536.

[37] J. Wang, H. Zhao, K. Lv, et al., Pterostilbene mmeliorates DSS-induced intestinal epithelial barrier loss in mice via suppression of the NF-κB-mediated MLCK-MLC signaling pathway, J. Agric. Food Chem. 69 (2021) 3871-3878. http://dx.doi.org/10.1021/acs.jafc.1c00274.

[38] Y. Xiong, C. Wang, L. Shi, et al., Myosin light chain kinase: a potential target for treatment of inflammatory diseases, Front. Pharmacol. 8 (2017) 292. http://dx.doi.org/10.3389/fphar.2017.00292.

[39] K.E. Cunningham, J.R. Turner, Myosin light chain kinase: pulling the strings of epithelial tight junction function, Ann. N. Y. Acad. Sci. 1258 (2012) 34-42. http://dx.doi.org/10.1111/j.1749-6632.2012.06526.x.

[40] S. Citi, Intestinal barriers protect against disease, Science 359 (2018) 1097-1098. http://dx.doi.org/10.1126/science.aat0835.

[41] A. Buckley, J.R. Turner, Cell biology of tight junction barrier regulation and mucosal disease, CSH Perspect. Biol. 10 (2018) 029314. http://dx.doi.org/10.1101/cshperspect.a029314.

[42] W. Hao, X. Zhu, Z. Liu, et al., Resveratrol alleviates aluminum-induced intestinal barrier dysfunction in mice, Environ. Toxicol. 37 (2022) 1373-1381. http://dx.doi.org/10.1002/tox.23490.

[43] W. Hao, C. Hao, C. Wu, et al., Aluminum induced intestinal dysfunction *via* mechanical, immune, chemical and biological barriers, Chemosphere 288 (2022) 132556. http://dx.doi.org/10.1016/j.chemosphere.2021.132556.

[44] R. Bonfiglio, M. Scimeca, A. Mauriello, The impact of aluminum exposure on human health, Arch. Toxicol. 97 (2023) 2997-2998. http://dx.doi.org/10.1007/s00204-023-03581-6.

[45] P. Nayak, Aluminum: impacts and disease, Environ. Res. 89 (2002) 101-115. http://dx.doi.org/10.1006/enrs.2002.4352.

[46] S.S. Zhang, N.N. Zhang, S. Guo, et al., Glycosides and flavonoids from the extract of *Pueraria thomsonii* Benth leaf alleviate type 2 diabetes in high-fat diet plus streptozotocin-induced mice by modulating the gut microbiota, Food Funct. 13 (2022) 3931-3945. http://dx.doi.org/10.1039/d1fo04170c.

[47] K.H. Wong, V. Razmovski-Naumovski, K.M. Li, et al., Comparing morphological, chemical and anti-diabetic characteristics of *Puerariae Lobatae Radix* and *Puerariae Thomsonii Radix*, J. Ethnopharmacol. 164 (2015) 53-63. http://dx.doi.org/10.1016/j.jep.2014.12.050.

[48] W. Wang, Y. Liu, D. Liu, et al., Profiling of antidiabetic bioactive flavonoid compounds from an edible plant Kudzu (*Pueraria lobata*), J. Agric. Food Chem. 72 (2024) 15704-15714. http://dx.doi.org/10.1021/acs.jafc.4c02564.

[49] W. Dai, M. Lei, Q. Yin, et al., Isolation and characterization of novel pueroside B isomers and other bioactive compounds from *Pueraria lobata* roots: structure elucidation, α-glucosidase, and α-amylase inhibition studies, Int. J. Mol. Sci. 25 (2024) 9602. http://dx.doi.org/10.3390/ijms25179602.

[50] C. Chen, X. Li, Y. Kano, et al., Oriental traditional herbal medicine-*Puerariae Flos*: a systematic review, J. Ethnopharmacol. 306 (2023) 116089. http://dx.doi.org/10.1016/j.jep.2022.116089.

[51] K.H. Wong, G.Q. Li, K.M. Li, et al., Kudzu root: traditional uses and potential medicinal benefits in diabetes and cardiovascular diseases, J. Ethnopharmacol. 134 (2011) 584-607. http://dx.doi.org/10.1016/j.jep.2011.02.001.

[52] Z. Zhang, T.N. Lam, Z. Zuo, *Radix Puerariae*: an overview of its chemistry, pharmacology, pharmacokinetics, and clinical use, J. Clin. Pharmacol. 53 (2013)787-811. http://dx.doi.org/10.1002/jcph.96.

[53] J. Wang, F. Yang, Y. Tao, et al., Phytochemicals and biological activities of *Pueraria* flower: a review, J. Food Bioact. 13 (2021) 40-51. http://dx.doi.org/10.31665/JFB.2021.13258.

[54] S. Yue, Y. Liu, R. Zhou, et al., A highly sensitive and rapid colloidal gold immunoassay for puerarin detection, J. Agric. Food Chem. 72 (2024) 8817-8822. http://dx.doi.org/10.1021/acs.jafc.4c00644.

[55] M.S. Zeng, W.D. Yu, H.X. Wang, et al., Puerarin reduces impairment of intestinal and adipose immune responses to influenza virus infection in mice, Arch. Virol. 166 (2021) 2387-2397. http://dx.doi.org/10.1007/s00705-021-05112-z.

[56] M. Tsuchiya, G. Ito, M. Hama, et al., Functional analysis of isoflavones using patient-derived human colonic organoids, Biochem. Biophys. Res. Commun. 542 (2021) 40-47. http://dx.doi.org/10.1016/j.bbrc.2021.01.021.

[57] A. Sharma, N.V. Tirpude, M. Kumari, et al., Rutin prevents inflammation-associated colon damage via inhibiting the p38/MAPKAPK2 and PI3K/Akt/GSK3β/NF-κB signalling axes and enhancing splenic Tregs in DSS-induced murine chronic colitis, Food Funct. 12 (2021) 8492-8506. http://dx.doi.org/10.1039/d1fo01557e.

[58] J. Shen, N. Li, X. Zhang, Daidzein ameliorates dextran sulfate sodium-induced experimental colitis in mice by regulating NF-κB signaling, J. Environ. Pathol. Toxicol. Oncol. 38 (2019) 29-39. http://dx.doi.org/10.1615/JEnvironPatholToxicolOncol.2018027531.