Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2025.338

RESEARCH ARTICLE

Vaccarin Ameliorates Colitis by Enhancing Autophagy and Suppressing Inflammation Mediated by FSTL3 in Mice

Yuexiao Li[1#], Haonan Kong[2#], Yilin Wang[3#], Shinong Dong[4], Pengfei Zhao[5], Jianan Li[4], Hong Yu[6*] and Yichao Liang[2*]

[1]Department of Oral and Maxillofacial Surgery, School and Hospital of Stomatology, China Medical University, Shenyang 110002, China; [2]Department of General Surgery, Shengjing Hospital of China Medical University, Shenyang 110004, China; [3]Fourth Clinical College, China Medical University, Shenyang 110032, China; [4]College of Animal Science and Veterinary Medicine, Shenyang Agricultural University, Shenyang 110866, China; [5]Department of Preventive Veterinary Medicine, College of Veterinary Medicine, Shandong Agricultural University, Taian 271017, China; [6]Department of Oncology, Shengjing Hospital of China Medical University, Shenyang 110022, China.
[#] These authors contributed equally to this work.
*Corresponding author: liangyc@sj-hospital.org (YL); 18940253638@163.com (HY)

ARTICLE HISTORY (25-1048)

Received: October 28, 2025
Revised: December 13, 2025
Accepted: December 15, 2025
Published online: December 19, 2025

Key words:
Vaccarin
Ulcerative colitis
FSTL3
Autophagy
Inflammation
Intestinal barrier

ABSTRACT

Colitis is an intestinal disorder characterized by uncontrolled inflammation and epithelial barrier dysfunction, with limited effective pharmacological options available for veterinary clinical application. Our work evaluated the protective effects and mechanisms of Vaccarin (a flavonoid glycoside from *Vaccaria segetalis*) in a C57BL/6 murine model of dextran sulfate sodium (DSS)-induced colitis. The mice were randomized into 5 groups: Control, DSS, DSS+Vaccarin-L (1mg/kg), DSS+Vaccarin-H (4mg/kg), and DSS+mesalazine (100mg/kg, a reference drug used off-label in veterinary practice). Vaccarin improved clinical presentation, including reduced DAI, weight loss, and colon shortening. Besides, the results indicate that Vaccaria preserved epithelial structure, attenuated apoptosis, restored tight junction proteins, suppressed IL-1β and IL-18 production, and reversed autophagy impairment. Transcriptomics and protein validation identified FSTL3 as a novel target, with DSS-induced FSTL3 upregulation suppressed by Vaccarin in a dose-dependent manner. Autophagy inhibitor 3-MA abrogated Vaccarin's protective effects, confirming the FSTL3-autophagy axis. In conclusion, Vaccarin ameliorates colitis by maintaining intestinal epithelial integrity, suppressing inflammation, and restoring autophagy flux via FSTL3 regulation, highlighting its potential as a veterinary therapeutic for colitis to alleviate gut-related morbidity and improve animal welfare.

To Cite This Article: Li Y, Kong H, Wang Y, Dong S, Pengfei Z, Li J, Yu H and Liang Y, 2025. Vaccarin ameliorates colitis by enhancing autophagy and suppressing inflammation mediated by FSTL3 in mice. Pak Vet J, 45(4): 1964-1971. http://dx.doi.org/10.29261/pakvetj/2025.338

INTRODUCTION

Colitis is a chronic intestinal inflammatory disorder that poses a significant threat to the health and welfare of animals worldwide (Hussein & Sakuma, 2005; Hussein, 2007; Zhang & Li, 2014). In veterinary settings, colitis often manifests as diarrhea, weight loss, and reduced productivity in livestock, or chronic gastrointestinal distress in companion animals, severely impacting animal quality of life and imposing economic losses in the livestock industry (Fluke *et al*., 1989; Marks *et al*., 2011; King *et al*., 2024; Salvarani *et al*., 2025). Companion animals such as dogs and cats frequently suffer from chronic enteropathies that share pathological similarities with human colitis (Marks *et al*., 2011; Jergens, 2012; Suchodolski, 2022; Remmel *et al*., 2023). Likewise, livestock species, particularly swine and poultry, are vulnerable to intestinal inflammatory disorders that compromise nutrient absorption, growth performance, and overall animal welfare, leading to substantial economic losses in animal husbandry (Mohsin *et al*., 2024; Finatto *et al*., 2025; Wei *et al*., 2025). Although current therapies, including corticosteroids, immunosuppressants, and biologics targeting cytokines or adhesion molecules, have improved patient outcomes, their long-term use is often limited by high costs, adverse side effects, and the risk of secondary infections (Baumgart & Le Berre, 2021; Altieri *et al*., 2025). Thus, novel therapeutic approaches that

restore intestinal homeostasis and limit inflammation are highly relevant to veterinary clinical practice and the livestock industry (Lin et al., 2020).

Dysregulated autophagy plays a pivotal role in the pathogenesis of colitis (Li & Law, 2022). Autophagy maintains intestinal homeostasis by regulating epithelial barrier integrity, immune cell activation, and microbial clearance (Li & Law, 2022). Given that impaired autophagy aggravates mucosal injury, while enhanced autophagy strengthens tight junctions, preserves barrier integrity, eliminates intracellular pathogens, and suppresses excessive inflammation, thereby maintaining intestinal homeostasis (Lassen & Xavier, 2018; Pott *et al*., 2018; Zhang *et al*., 2021). Therefore, targeting autophagic function has emerged as a promising therapeutic strategy for ulcerative colitis. In this context, attention has increasingly focused on upstream regulatory molecules that may affect autophagic processes. Among them, follistatin-like 3 (FSTL3), a secreted glycoprotein of the follistatin family, has been implicated in immune regulation, tissue remodeling, and metabolic homeostasis (Li *et al*., 2024), suggesting it may also play an important but as yet undefined role in the modulation of autophagy during colitis both in humans and animals.

Natural products provide a valuable reservoir for drug discovery in inflammatory diseases (Liu *et al*., 2022; Wang *et al*., 2024a). Vaccarin, a bioactive flavonoid glycoside isolated from Vaccaria segetalis (Caryophyllaceae), has been reported to modulate signaling pathways related to oxidative stress and immune responses (Gong *et al*., 2019). Preliminary studies suggest that Vaccarin modulates signaling pathways involved in oxidative stress and immune responses, yet its therapeutic potential in colitis and the underlying mechanisms remain unclear (Xie *et al*., 2015; Fan *et al*., 2025).

Based on these findings, we hypothesized that Vaccarin might ameliorate colitis by regulating autophagic flux through FSTL3 and suppressing excessive inflammatory responses, thereby holding relevance for both human and veterinary colitis management. To test this hypothesis, we established a murine model of DSS-induced colitis, a well-validated preclinical model widely used to mimic colitis pathologies observed in veterinary patients, such as dogs, cats, and pigs, and evaluated the therapeutic efficacy of Vaccarin (Chassaing *et al*., 2014). The aims of this study were to evaluate the therapeutic efficacy of Vaccarin in DSS-induced colitis and investigate whether its effects are mediated by the FSTL3-autophagy axis, focusing on the regulation of epithelial barrier function, inflammation, and autophagic flux. This study confirmed Vaccarin's veterinary translational value, including easing reliance on antibiotics and mitigating side effects of conventional anti-inflammatory drugs, while highlighting the cross-species FSTL3-autophagy axis for animal colitis therapy.

MATERIALS AND METHODS

Animal Model: Male C57BL/6 mice (Beijing Huafukang Biotechnology Co., Ltd) were maintained under standard environmental conditions (temperature: 22±2°C, humidity 50±10%, 12-hour light/12-hour dark cycle, free access to standard mouse food and water).

Male C57BL/6 mice were randomized into 5 groups: Control, DSS, DSS+Vaccarin-L, DSS+Vaccarin-H, and DSS+mesalazine. Experimental ulcerative colitis was induced by administration of 3% (wt/vol) dextran sulfate sodium (DSS, MP Biomedicals, USA) in drinking water for 10 consecutive days (Cui *et al*., 2021). Mice in the DSS + Vaccarin-L and DSS + Vaccarin-H groups received intraperitoneal injections of 1 mg/kg and 4 mg/kg Vaccarin (Sun *et al*., 2021b), respectively, while mice in the positive control group were administered 100 mg/kg Mesalazine intragastrically (Sun *et al*., 2021b). On day 11, all animals were sacrificed and colon tissues were harvested, colon length, and representative colon imaging were recorded. The disease activity index (DAI) and body weight of the mice were monitored by the research team throughout the experimental period.

To further evaluate the role of autophagy, an additional cohort of mice (n = 8 per group) was treated with DSS + Vaccarin-H or DSS + Vaccarin-H combined with 10 mg/kg 3-methyladenine (3-MA, intraperitoneal, every other day).

H&E staining: Colon sections (4 μm) were prepared according to the standard procedure and stained with hematoxylin and eosin (Sun *et al*., 2021b).

TUNEL staining: The fixed colon sections exposed to 50 μL TUNEL reaction mixture (KeyGEN BioTECH, China), followed by DAPI counterstaining and imaging under a fluorescence microscope (Olympus BX53, Japan) (Bai *et al*., 2020).

Immunohistochemistry (IHC): Colon sections (4 μm) were with primary antibodies against FSTL3 (1:2000, AB232761, Abcam, USA) or p62 (1:500, AB91526, Abcam, USA) incubated overnight at 4°C, followed by 1 h of incubation at room temperature with HRP-conjugated secondary antibodies (1:4000, RGAR011, Proteintech, China), visualized using DAB chromogen, and finally imaged under a light microscope (Ren *et al*., 2020).

Immunofluorescence (IF): Colon sections were incubated with the corresponding primary antibodies overnight at 4°C and Alexa Fluor-conjugated secondary antibodies (1:50, 128-545-003, Jackson ImmunoResearch, USA) for 1h at 25°C in the dark, counterstained with DAPI, and imaged by fluorescence microscopy (Zhang *et al*., 2024).

The primary antibodies information: Occludin (1:200, AB216327, Abcam, USA) and ZO-1 (1:500, AB307799, Abcam, USA).

ELISA assay: Serum and colon tissue homogenates were collected, and IL-1β and IL-18 concentrations were determined by commercial ELISA kits (eBioscience, USA) with experimental procedures strictly following the manufacturers' instructions. Serial dilutions of homologous recombinant standard substances were used to generate standard curves. In short, after completing the antibody incubation and color development steps with tetramethylbenzidine (TMB) substrate, the OD values were measured at a wavelength of 450nm using a microplate reader (Yao *et al*., 2017).

RNA Sequencing: Total RNA was extracted from colon tissues of DSS and DSS + Vaccarin-H groups and high-throughput mRNA sequencing was implemented on an Illumina NovaSeq 6000 platform.

Quantitative Real-Time PCR: Total RNA from colon tissues was reverse transcribed into cDNA using a commercial kit (Takara, Japan). qRT-PCR was performed using SYBR Green Master Mix (Applied Biosystems, USA). Primer sequences are provided in Table 1.

Table 1: Primer sequences used for qRT-PCR

Gene	Primer sequences(5′→3′)
GAPDH-F	TCACCATCTTCCAGGAGCGAGAC
GAPDH-R	TGAGCCCTTCCACAATGCCAAAG
FSTL3-F	CTACATCTCCTCGTGCCACA
FSTL3-R	TCTTCTGCAGACTCACCACCT

Western blotting: Proteins from colon tissues were extracted, quantified, and subjected to SDS-PAGE, followed by transfer to PVDF membranes. In turn, the membranes were incubated with primary antibodies and HRP-conjugated secondary antibodies, bands were visualized by ECL and quantified with ImageJ (Li *et al*., 2023).

The primary antibodies information: LC3 (1:5000, AB48394, Abcam, USA), p62 (1:1000, AB109012, Abcam, USA), Beclin-1 (1:2000, AB207612, Abcam, USA), (Occludin (1:2000, AB216327, Abcam, USA), ZO-1 (1:2000, AB307799, Abcam, USA), and FSTL3 (1:2000, AB232761, Abcam, USA)

Statistical analysis: All data are expressed as mean ± SD. For comparisons between two groups, a student's t-test was employed; for groups of three or more, one-way analysis of variance (ANOVA) followed by Tukey's post-test was used. A *P*-value less than 0.05 was considered statistically significant.

RESULTS

Vaccarin alleviates clinical and histological features of DSS-induced colitis: Mice exhibited typical symptoms, including colon shortening, elevated DAI, and body weight loss after modeling (P<0.01; Fig. 1A-C). Vaccarin treatment alleviated the reductions in colon length, increases in DAI scores, and decreases in body weight. Histologically, DSS induced extensive epithelial damage and crypt destruction, while Vaccarin markedly reduced mucosal injury (Fig. 1D). TUNEL staining confirmed increased apoptosis in DSS colons, which was strongly attenuated by Vaccarin, particularly at high dose, similar to mesalazine (Fig. 1E). Collectively, Vaccarin improved clinical manifestations and inhibited epithelial apoptosis in mice.

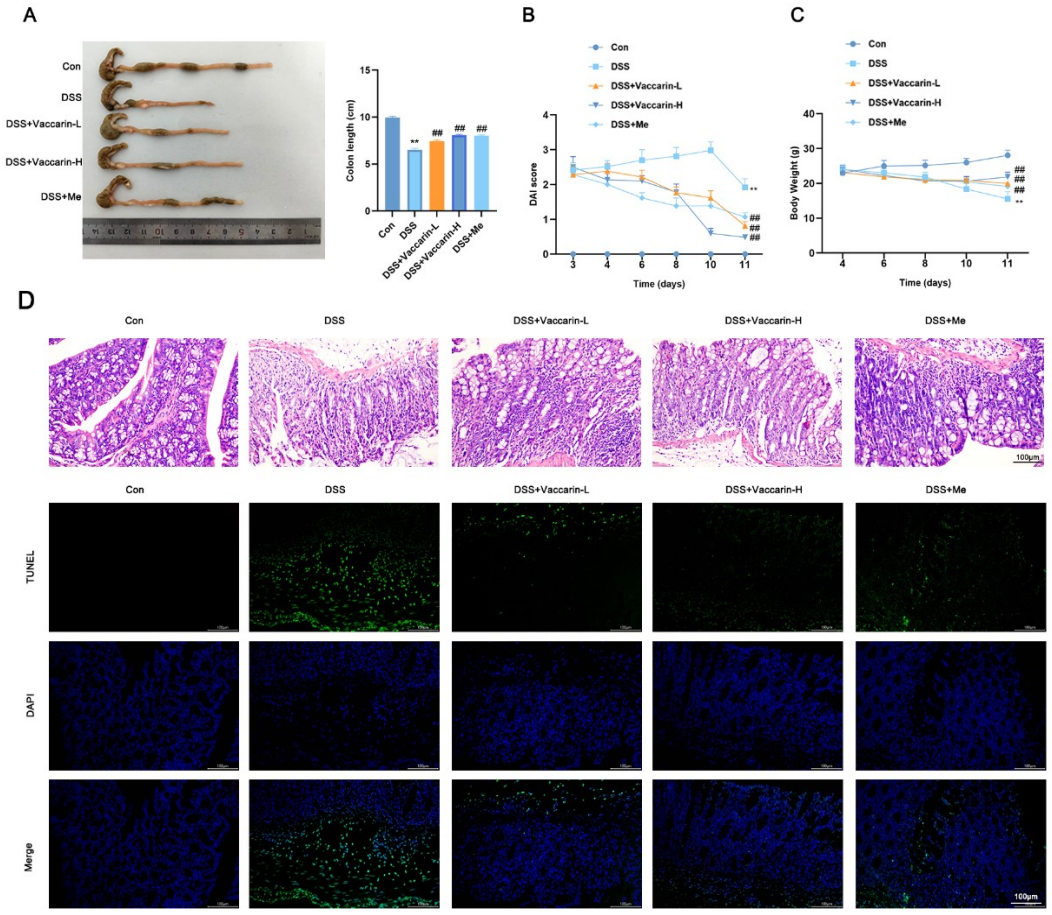

Fig. 1: Vaccarin ameliorates DSS-induced colitis in mice. (A) Colon length. (B) DAI scores during disease course. (C) Body weight changes. (D) Representative H&E staining of colon tissues. (E) TUNEL staining showing epithelial apoptosis. Data are expressed as mean ± SD (n = 8–10). **P< .01 vs. Con; ##P<0.01 vs. DSS (one-way ANOVA).

Vaccarin restores intestinal barrier integrity: DSS challenge markedly disrupted the intestinal barrier, as evidenced by reduced Occludin and ZO-1 staining in colonic tissues compared with controls (Fig. 2A and B). High dose Vaccarin treatment restored barrier protein expression almost to the control group, which was in consistent with the results of Western blotting ($P<0.01$; Fig. 2C). Together, these findings indicate that Vaccarin effectively preserves intestinal barrier integrity in mice.

Vaccarin attenuates inflammatory responses in DSS-induced colitis: Then, the pro-inflammatory cytokine IL-1β and IL-18 in both serum and colon tissues were detected. DSS markedly increased IL-1β (Fig. 3A and C) and IL-18 (Fig. 3B and D) levels in both serum and colon tissues compared with controls. At the same time, Vaccarin treatment significantly reduced these elevations ($P<0.01$), indicating that Vaccarin effectively suppresses DSS-induced inflammatory cytokine production in both systemic circulation and local intestinal tissues.

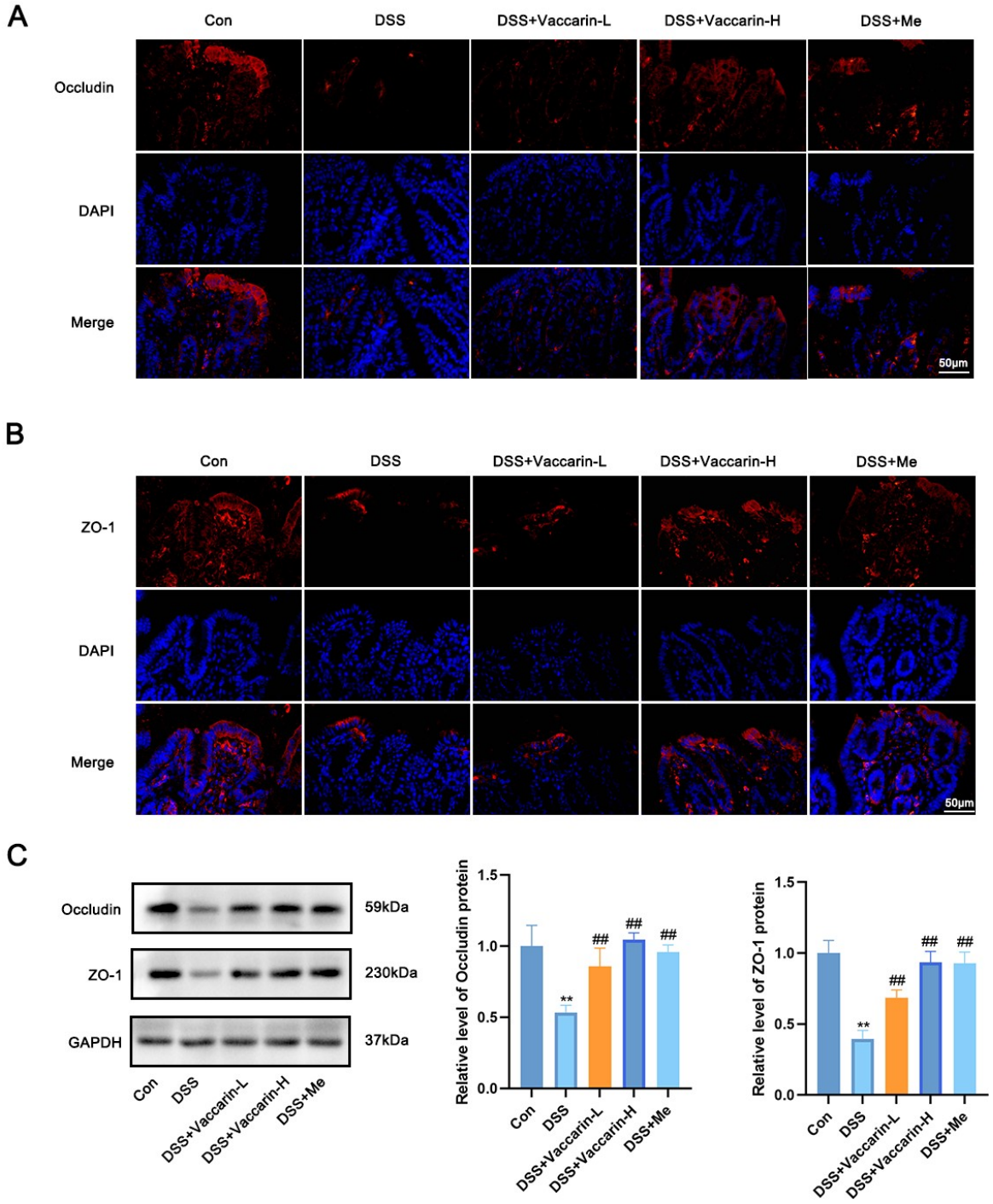

Fig. 2: Vaccarin restores intestinal barrier integrity in DSS-induced colitis. (A) Immunofluorescence of Occludin and ZO-1 in colon tissues. (B) Western blot analysis of Occludin and ZO-1 expression, with GAPDH as a loading control. Data are expressed as mean ± SD (n = 6–8). **P < 0.01 vs. Con; ##P<0.01 vs. DSS (one-way ANOVA).

Vaccarin regulates FSTL3 expression in DSS-induced colitis: To explore the molecular mechanism of Vaccarin, transcriptomic analysis was performed on colon tissues from DSS and DSS+Vaccarin-H mice. Volcano plots revealed significantly differentially expressed mRNAs between the two groups (Fig. 4A), while GO and KEGG enrichment analyses indicated enrichment of pathways related to autophagy and inflammation (Fig. 4B-C). Further validation of FSTL3 expression was conducted. DSS markedly upregulated FSTL3 expression compared with controls, while Vaccarin treatment reduced FSTL3 expression ($P<0.01$; Fig. 4D-E). This phenomenon was subsequently confirmed by immunohistochemical testing targeting FSTL3 (Fig. 4F), indicating that Vaccarin ameliorates DSS-induced colitis at least in part by downregulating intestinal FSTL3 expression.

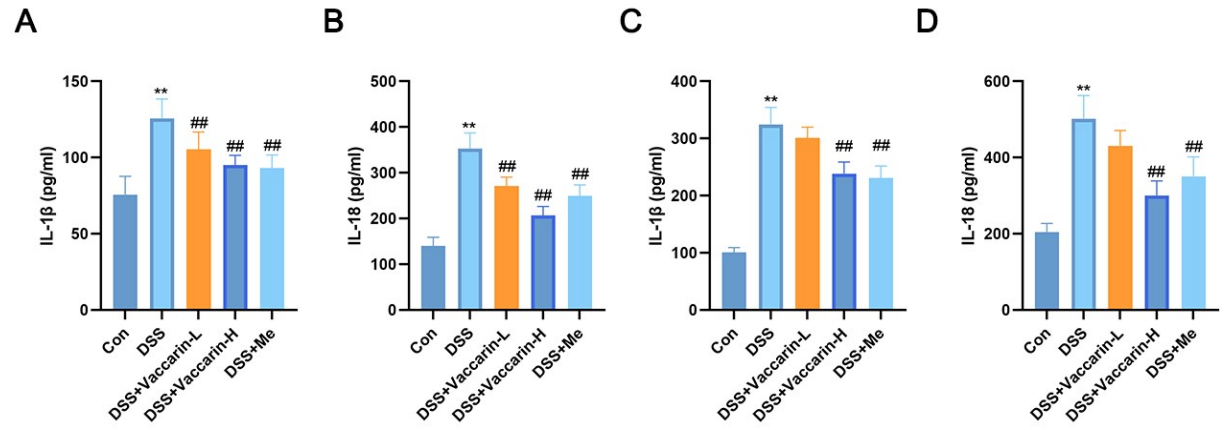

Fig. 3: Vaccarin suppresses inflammatory cytokine production in DSS-induced colitis. (A) Serum IL-1β levels measured by ELISA. (B) Serum IL-18 levels measured by ELISA. (C) Colonic IL-1β levels measured by ELISA. (D) Colonic IL-18 levels measured by ELISA. Data are expressed as mean ± SD (n = 6–8). **P < 0.01 vs. Con; ##P < 0.01 vs. DSS (one-way ANOVA).

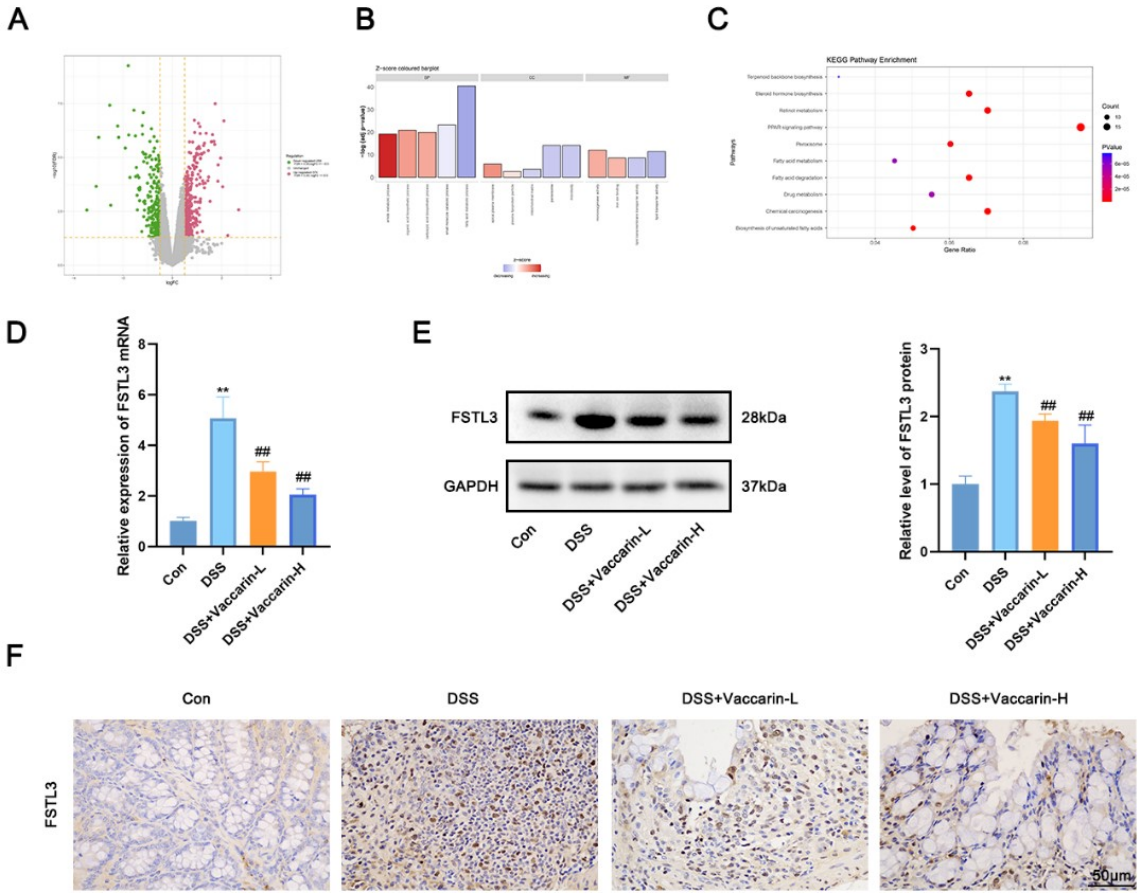

Fig. 4: Vaccarin regulates FSTL3 expression in DSS-induced colitis. (A) Volcano plot showing differentially expressed mRNAs between DSS and DSS + Vaccarin-H groups. (B) GO enrichment analysis of differentially expressed genes. (C) KEGG pathway analysis. (D) Real time-PCR detection of FSTL3 expression in colon tissues. (E) Western blot analysis of FSTL3 expression, with GAPDH as a loading control. (F) Immunohistochemistry of FSTL3 expression in colon tissues. Data are expressed as mean ± SD (n = 6–8). **P<0.01 vs. Con; ##P<0.01 vs. DSS (one-way ANOVA).

Vaccarin regulates autophagy in DSS-induced colitis: DSS modeling markedly suppressed autophagy, as indicated by decreased LC3II/I and Beclin-1 levels and increased p62 accumulation compared with controls, and Vaccarin treatment reversed these changes ($P<0.01$; Fig. 5A). Immunohistochemistry further confirmed enhanced p62 staining in DSS colons, which was attenuated by Vaccarin treatment (Fig. 5B). To investigate whether autophagy mediates the protective effects of Vaccarin, a well-established autophagy inhibitor 3-MA was co-administered with high-dose Vaccarin. In comparison to the DSS+Vaccarin-H group, combined treatment with 3-MA significantly shortened colon length and abrogated the beneficial effects of Vaccarin on DAI scores as well as body weight recovery. (Fig. 5C, E, and F). Histological examination by H&E also revealed more severe mucosal damage in the DSS + Vaccarin-H + 3-MA group compared with Vaccarin alone (Fig. 5D), indicating that Vaccarin restores autophagic flux, and that inhibition of autophagy abolishes its protective effects.

DISCUSSION

In animals, colitis-like intestinal inflammation is often presented as chronic diarrhea, weight loss, and reduced productivity in livestock or impaired welfare in companions. Its pathogenesis is conserved across species (Le Berre *et al*., 2023; Peng *et al*., 2023). Current interventions targeting colitis have notable limitations, though they overlap, and there is an overlap between human and veterinary practice. In animals, conventional options, including corticosteroids and antibiotics for secondary infections and supportive care, offer symptomatic relief but are constrained by side effects such as gut dysbiosis and immunosuppression (Wangchuk *et al*., 2024). Natural bioactive compounds with pleiotropic effects are therefore receiving increasing attention as alternative or adjunctive therapies. For example, sedanolide, a natural phthalide from celery seed oil, alleviated DSS-induced colitis by reducing inflammation and fortifying the intestinal barrier (Li *et al*., 2025). Vaccarin, a flavonoid glycoside derived from *Vaccaria segetalis*, significantly ameliorates DSS-induced colitis by improving clinical and histological outcomes, preserving intestinal barrier integrity, attenuating inflammatory cytokine release, downregulating FSTL3, and restoring autophagic flux. These findings not only highlight Vaccarin as a promising therapeutic candidate for human colitis but also underscore its considerable translational value for veterinary medicine, offering a potential novel option for managing colitis-like disorders in companion animals and livestock.

Our results confirmed that DSS administration induces classical colitis symptoms, such as colon shortening, body weight loss, increased DAI, epithelial destruction, and apoptosis, consistent with previous studies (Oh *et al*., 2014; Cui *et al*., 2021). Vaccarin dose-dependently ameliorated these manifestations, with efficacy comparable to mesalazine, a standard drug for colitis. Similar protective effects have been described for

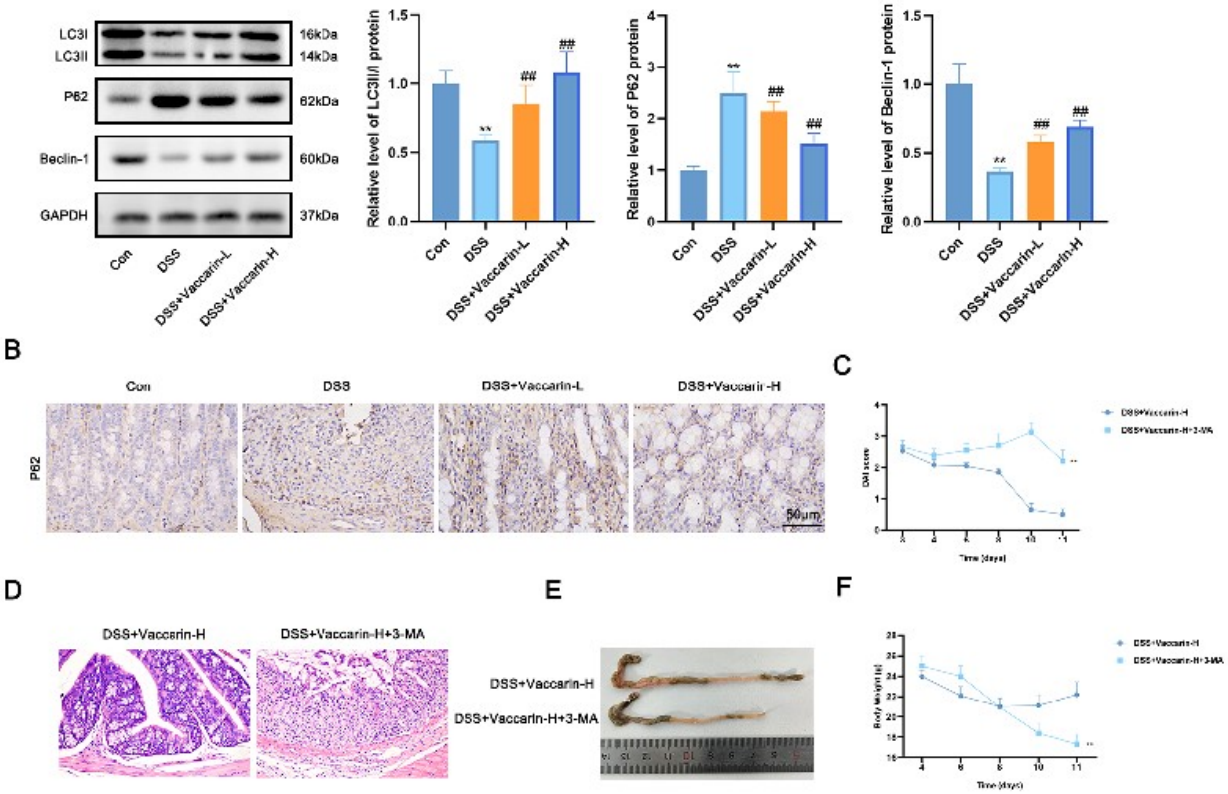

Fig. 5: Vaccarin regulates autophagy in DSS-induced colitis. (A) Western blot analysis of LC3II/I, Beclin-1, and p62 expression in colon tissues, with GAPDH as a loading control. (B) Immunohistochemistry of p62 in colon tissues. (C) Representative colon images and DAI scores in DSS + Vaccarin-H and DSS + Vaccarin-H + 3-MA groups. (D) H&E staining of colon tissues. (E) Body weight changes during treatment. Data are expressed as mean ± SD (n = 6–8). **P<0.01 vs. Con; ##P<0.01 vs. DSS (one-way ANOVA).

phytochemicals such as curcumin, resveratrol, and quercetin, which also reduce histological injury and improve survival in DSS colitis models (Gao *et al*., 2024; Wang *et al*., 2024b; Yu *et al*., 2024). By extending this evidence, our study identifies Vaccarin as an additional bioactive compound mitigating colitis progression, reinforcing the therapeutic value of plant-derived agents in intestinal inflammation.

Epithelial barrier dysfunction is widely recognized as a key driver of colitis pathogenesis (Moeser *et al*., 2017; Fang *et al*., 2021). Tight junction proteins, including occludin and ZO-1, are critical for maintaining epithelial integrity, and their disruption facilitates bacterial translocation and mucosal immune activation (Kuo *et al*., 2021; Pham *et al*., 2021). Vaccarin was found to reverse the impaired expression of Occludin and ZO-1 following DSS challenge, thereby strengthening the epithelial barrier. Similarly, to the established action of berberine, this highlights the potential of Vaccarin and other natural compounds to target and fortify epithelial junctions (Yan *et al*., 2022). Thus, our findings underscore barrier preservation as a fundamental mechanism by which Vaccarin confers mucosal protection.

Inflammation plays a central role in the perpetuation of colitis (Li *et al*., 2021). Our data revealed pronounced DSS-induced increases in IL-1β and IL-18 levels in serum and colon tissue, which were significantly suppressed by Vaccarin. These cytokines are canonical products of NLRP3 inflammasome activation, a pathway strongly implicated in colitis pathogenesis (Lv *et al*., 2021). Comparable anti-inflammatory effects have been reported for natural flavonoids that attenuate NF-κB and MAPK signaling cascades (Ren *et al*., 2020; Chu *et al*. 2025). Collectively, these findings suggest that Vaccarin acts as a potent immunomodulator, disrupting pro-inflammatory signaling at multiple levels.

Transcriptomic and experimental validation identified FSTL3 as a novel target of Vaccarin. DSS markedly upregulated FSTL3 expression, whereas Vaccarin suppressed it in a dose-dependent manner. Although FSTL3, a member of the follistatin family, is known to regulate TGF-β signaling, metabolism, and tissue remodeling (Liu *et al*., 2021; Sun *et al*., 2021a), its role in colitis remains unclear. Our results suggest that aberrant FSTL3 expression may exacerbate inflammation and impair autophagy, whereas Vaccarin-induced downregulation of FSTL3 promotes mucosal recovery.

The mechanistic analysis confirmed that autophagy is indispensable for Vaccarin's protective effects. DSS impaired autophagic flux, as reflected by decreased LC3II/I and Beclin-1 and accumulation of p62, in agreement with earlier findings that defective autophagy promotes uncontrolled inflammation and tissue injury in colitis (Larabi *et al*., 2020). Vaccarin restored autophagy, while co-treatment with 3-MA abolished its beneficial effects, underscoring the pivotal role of autophagy in mediating its actions. These findings are consistent with studies showing that pharmacological agents or genetic interventions that restore autophagic flux ameliorate experimental colitis (Duan *et al*., 2021; Kim *et al*., 2021). Together, our data establish the FSTL3–autophagy axis as a novel mechanism through which Vaccarin protects against colitis.

Beyond its implications for human medicine, our findings may also bear translational significance for veterinary practice. Chronic enteropathies are highly prevalent in companion animals such as dogs and cats, as well as in livestock, where intestinal inflammation compromises growth performance and welfare (Makielski *et al*., 2019). The ability of Vaccarin to restore barrier integrity, suppress inflammation, and modulate autophagy suggests potential applications across species, bridging preclinical and veterinary medicine.

In conclusion, this study demonstrates that Vaccarin effectively ameliorates DSS-induced colitis in mice by targeting a FSTL3-mediated regulatory axis. Our findings advance knowledge of Vaccarin's pharmacological effects and provide robust support for its development as an IBD therapeutic significant veterinary value for addressing key unmet needs in treating colitis-like disorders in animals, alongside its use in humans. Collectively, these future endeavors will not only refine our understanding of the FSTL3-mediated pathogenesis of colitis but also accelerate the translational potential of Vaccarin as a targeted therapy for both human and veterinary IBD.

Ethical approval of the study: All animal experiments were approved by the Institutional Animal Care and Use Committee of the China Medical University (No. CMUKT2025210).

Authors contribution: Yuexiao Li performed conceptualization, formal analysis and original draft writing; Haonan Kong designed the methodology and conducted validation; Yilin Wang provided resources and prepared data visualization. Shinong Dong, Zhao Pengfei, and Jianan Li carried out the key experiments and data curation. Corresponding authors Hong Yu and Yichao Liang led conceptualization, supervision and critical manuscript revision.

REFERENCES

Altieri G, Zilli A, Parigi TL, *et al*., 2025. Dual therapy in inflammatory bowel disease. Biomolecules 15: 222.

Bai Y, Wang W, Yin P, *et al*., 2020. Ruxolitinib alleviates renal interstitial fibrosis in UUO mice. Int J Biol Sci 16**:** 194-203.

Baumgart DC, and Le Berre C, 2021. Newer biologic and small-molecule therapies for inflammatory bowel disease. N Engl J Med 385**:** 1302-1315.

Chassaing B, Aitken JD, Malleshappa M, *et al*., 2014. Dextran sulfate sodium (DSS)-induced colitis in mice. Curr Protoc Immunol 104:15.25.1-15.25.14.

Chu T, Wang Y, Wang S, *et al*., 2025. Kaempferol regulating macrophage foaming and atherosclerosis through Piezo1-mediated MAPK/NF-κB and Nrf2/HO-1 signaling pathway. J Adv Res 75**:** 635-650.

Cui L, Guan X, Ding W, *et al*., 2021. Scutellaria baicalensis Georgi polysaccharide ameliorates DSS-induced ulcerative colitis by improving intestinal barrier function and modulating gut microbiota. Int J Biol Macromol 166**:** 1035-1045.

Duan C, Tang X, Wang W, *et al*., 2021. Lactobacillus rhamnosus attenuates intestinal inflammation induced by Fusobacterium nucleatum infection by restoring the autophagic flux. Int J Mol Med 47:125-136.

Fan Q, Liu D, Chu C, *et al*., 2025. Vaccarin alleviates renal ischemia-reperfusion injury by inhibiting inflammation and ferroptosis. Int Immunopharmacol 153**:** 114463.

Fang J, Wang H, Zhou Y, *et al*., 2021. Slimy partners: the mucus barrier and gut microbiome in ulcerative colitis. Exp Mol Med 53**:** 772-787.

Finatto AN, Yang C, and de Oliveira Costa M, 2025. Porcine β-defensin 5 (pBD-5) modulates the inflammatory and metabolic host intestinal

response to infection. Sci Rep 15: 7568.
Fluke MH, Hawkins EC, Elliott GS, *et al*., 1989. Short colon in two cats and a dog. J Am Vet Med Assoc 195: 87-90.
Gao F, Zhu F, Shuai B, *et al*., 2024. Quercetin ameliorates ulcerative colitis by restoring the balance of M2/M1 and repairing the intestinal barrier via downregulating cGAS–STING pathway. Front Pharmacol 15: 1351538.
Gong L, Lei Y, Liu Y, *et al*., 2019. Vaccarin prevents ox-LDL-induced HUVEC EndMT, inflammation and apoptosis by suppressing ROS/p38 MAPK signaling. Am J Transl Res 11: 2140-2154.
Hussein HS, 2007. Prevalence and pathogenicity of Shiga toxin-producing Escherichia coli in beef cattle and their products. Journal of animal science 85: E63-72.
Hussein HS and Sakuma T, 2005. Prevalence of shiga toxin-producing Escherichia coli in dairy cattle and their products. Journal of dairy science 88: 450-465.
Jergens AE, 2012. Feline idiopathic inflammatory bowel disease: what we know and what remains to be unraveled. J Feline Med Surg 14: 445-458.
Kim S, Lee JY, Shin SG, *et al*., 2021. ESRRA (estrogen related receptor alpha) is a critical regulator of intestinal homeostasis through activation of autophagic flux via gut microbiota. Autophagy 17: 2856-2875.
King AP, Donovan TA, Cohen E, *et al*., 2024. Short colon syndrome in cats. J Vet Intern Med 38: 2138-2150.
Kuo WT, Zuo L, Odenwald MA, *et al*., 2021. The tight junction protein ZO-1 is dispensable for barrier function but critical for effective mucosal repair. Gastroenterology 161: 1924-1939.
Larabi A, Barnich N and Nguyen HTT, 2020. New insights into the interplay between autophagy, gut microbiota and inflammatory responses in IBD. Autophagy 16: 38-51.
Lassen KG, and Xavier RJ, 2018. Mechanisms and function of autophagy in intestinal disease. Autophagy 14: 216-220.
Le Berre C, Honap S and Peyrin-Biroulet L, 2023. Ulcerative colitis. Lancet 402: 571-584.
Li B, Du P, Du Y, *et al*., 2021. Luteolin alleviates inflammation and modulates gut microbiota in ulcerative colitis rats. Life Sci 269: 119008.
Li H, Zheng N, Guo A, *et al*., 2024. FSTL3 promotes tumor immune evasion and attenuates response to anti-PD1 therapy by stabilizing c-Myc in colorectal cancer. Cell Death Dis 15: 107.
Li S, Zhuge A, Chen H, *et al*., 2025. Sedanolide alleviates DSS-induced colitis by modulating the intestinal FXR-SMPD3 pathway in mice. J Adv Res 69: 413-426.
Li Y, and Law HKW, 2022. Deciphering the role of autophagy in the immunopathogenesis of inflammatory bowel disease. Front Pharmacol 13: 1070184.
Li ZY, Lin LH, Liang HJ, *et al*., 2023. Lycium barbarum polysaccharide alleviates DSS-induced chronic ulcerative colitis by restoring intestinal barrier function and modulating gut microbiota. Ann Med 55: 2290213.
Lin X, Mohsin M, Abbas RZ, *et al*., 2020. Evaluation of Immunogenicity and Protective Efficacy of Eimeria maxima Immune Mapped Protein 1 with EDA Adjuvant in Chicken. Pak Vet J 40: 209-213.
Liu Y, Li BG, Su YH, *et al*., 2022. Potential activity of Traditional Chinese Medicine against Ulcerative colitis: A review. J Ethnopharmacol 289: 115084.
Liu YJ, Li JP, Zhang Y, *et al*., 2021. FSTL3 is a Prognostic Biomarker in Gastric Cancer and is Correlated with M2 Macrophage Infiltration. Onco Targets Ther 14: 4099-4117.
Lv Q, Xing Y, Liu J, *et al*., 2021. Lonicerin targets EZH2 to alleviate ulcerative colitis by autophagy-mediated NLRP3 inflammasome inactivation. Acta Pharm Sin B 11: 2880-2899.
Makielski K, Cullen J, O'Connor A, *et al*., 2019. Narrative review of therapies for chronic enteropathies in dogs and cats. J Vet Intern Med 33: 11-22.
Marks SL, Rankin SC, Byrne BA, *et al*., 2011. Enteropathogenic bacteria in dogs and cats: diagnosis, epidemiology, treatment, and control. J Vet Intern Med 25: 1195-1208.
Moeser AJ, Pohl CS, Rajput M, 2017. Weaning stress and gastrointestinal barrier development: Implications for lifelong gut health in pigs. Anim Nutr 3: 313-321.
Mohsin M, Aleem MT, Goraya MU, *et al*., 2024. Editorial: Natural products and pseudo-natural products against veterinary disease-causing microorganisms. Front Vet Sci 11: 1429587.
Oh SY, Cho KA, Kang JL, *et al*., 2014. Comparison of experimental mouse models of inflammatory bowel disease. Int J Mol Med 33: 333-340.
Peng S, Shen L, Yu X, *et al*., 2023. The role of Nrf2 in the pathogenesis and treatment of ulcerative colitis. Front Immunol 14: 1200111.
Pham HHS, Matsubayashi M, Tsuji N, *et al*., 2021. Relationship between Eimeria tenella associated-early clinical signs and molecular changes in the intestinal barrier function. Vet Immunol Immunopathol 240: 110321.
Pott J, Kabat AM, Maloy KJ, 2018. Intestinal epithelial cell autophagy is required to protect against TNF-induced apoptosis during chronic colitis in mice. Cell Host Microbe 23: 191-202.e4
Remmel P, Gros L, Mortier J, *et al*., 2023. Congenital partial colonic agenesis in dogs and cats: clinical, biological, diagnostic imaging, endoscopic and histopathologic characterization, a retrospective study. Vet Sci 10:577..
Ren Q, Guo F, Tao S, *et al*., 2020. Flavonoid fisetin alleviates kidney inflammation and apoptosis via inhibiting Src-mediated NF-κB p65 and MAPK signaling pathways in septic AKI mice. Biomed Pharmacother 122: 109772.
Salvarani FM, Oliveira H and Uzal FA, 2025. Clostridioides difficile in Animal Inflammatory Bowel Disease: A One Health Perspective on Emerging Zoonotic Threats. Microorganisms 13:1233.
Suchodolski JS, 2022. Analysis of the gut microbiome in dogs and cats. Vet Clin Pathol 50 Suppl 1: 6-17.
Sun F, Sun P, Yang X, *et al*., 2021a. Inhibition of FSTL3 abates the proliferation and metastasis of renal cell carcinoma via the GSK-3β/β-catenin signaling pathway. Aging (Albany NY) 13: 22528-22543.
Sun JN, Yu XY, Hou B, *et al*., 2021b. Vaccarin enhances intestinal barrier function in type 2 diabetic mice. Eur J Pharmacol 908: 174375.
Wang K, Yin J, Chen J, *et al*., 2024a. Inhibition of inflammation by berberine: Molecular mechanism and network pharmacology analysis. Phytomedicine 128: 155258.
Wang L, Zheng W, Men Q, *et al*., 2024b. Curcumin-loaded polysaccharide microparticles alleviated DSS-induced ulcerative colitis by improving intestinal microecology and regulating MAPK/NF-κB/Nrf2/NLRP3 pathways. Int J Biol Macromol 281: 136687.
Wangchuk P, Yeshi K and Loukas A, 2024. Ulcerative colitis: clinical biomarkers, therapeutic targets, and emerging treatments. Trends Pharmacol Sci 45: 892-903.
Wei L, Liu X, Tan Z, *et al*., 2025. Chlorogenic acid mitigates avian pathogenic Escherichia coli-induced intestinal barrier damage in broiler chickens via anti-inflammatory and antioxidant effects. Poult Sci 104: 105005.
Xie F, Cai W, Liu Y, *et al*., 2015. Vaccarin attenuates the human EA.hy926 endothelial cell oxidative stress injury through inhibition of Notch signaling. Int J Mol Med 35: 135-142.
Yan S, Chang J, Hao X, *et al*., 2022. Berberine regulates short-chain fatty acid metabolism and alleviates the colitis-associated colorectal tumorigenesis through remodeling intestinal flora. Phytomedicine 102: 154217.
Yao F, Zhang L, Yin Z, *et al*., 2017. The renal level of a novel cytokine IL-35 is related to sepsis-associated acute kidney injury in mice. Int J Clin Exp Pathol 10: 10998-11005.
Yu B, Wang Y, Tan Z, *et al*., 2024. Resveratrol ameliorates DSS-induced ulcerative colitis by acting on mouse gut microbiota. Inflammopharmacology 32: 2023-2033.
Zhang H, Cui Z, Cheng D, *et al*., 2021. RNF186 regulates EFNB1 (ephrin B1)-EPHB2-induced autophagy in the colonic epithelial cells for the maintenance of intestinal homeostasis. Autophagy 17: 3030-3047.
Zhang H, Pan Y, Jiang Y, *et al*., 2024. Akkermansia muciniphila ONE effectively ameliorates dextran sulfate sodium (DSS)-induced ulcerative colitis in mice. NPJ Sci Food 8: 97.
Zhang YZ, Li YY, 2014. Inflammatory bowel disease: pathogenesis. World J Gastroenterol 20: 91-99.