

Anti-inflammatory effects of oridonin on an *in vitro* model using LPS-treated human intestinal Caco-2 cells

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Abstract. Oridonin (Ori) attenuates dextran sulfate sodium-induced ulcerative colitis (UC) in mice, and its underlying mechanism involves the sirtuin 1 (SIRT1)/NF- κ B/p53 pathway. In the present study, lipopolysaccharide (LPS)-stimulated Caco-2 cells were used as an *in vitro* model to mimic human intestinal epithelial inflammation, to ascertain the anti-inflammatory effects of Ori on a cell model of UC. The concentrations of TNF- α and IL-1 β were quantified using ELISA, the expression levels of SIRT1, NF- κ B and p53 were assessed through western blot analysis, and cell viability was determined using the Cell Counting Kit-8. The results showed that cell viability was affected by treatment with Ori at different doses. Ori significantly inhibited the optical density values in a dose-dependent manner at the doses of 5.0–80.0 μ M whereas it did not affect viability at the doses of 0.625–2.50 μ M, compared with in the control group. These findings suggested that the inhibitory effect of Ori on intestinal epithelial cells did not result from cytotoxic action at the doses of 0.625–2.50 μ M. Furthermore, Ori suppressed the inflammatory response in intestinal epithelial cells by significantly reducing LPS-induced secretion of the pro-inflammatory cytokines TNF- α and IL-1 β with a ~2-fold reduction. Additionally, Ori significantly increased the protein expression levels of SIRT1, and decreased the protein expression levels of NF- κ B and p53. In conclusion, the present results indicated that the inhibition of NF- κ B-mediated inflammation may be a key mechanism by which Ori exerts its therapeutic effects on UC.

Introduction

Ulcerative colitis (UC) refers to prevalent chronic, non-specific localized inflammation of the intestine, marked

by the presence of local ulcers (1). This condition can lead to damaged intestinal mucosa, causing symptoms such as abdominal pain, diarrhea, and bloody or pus-filled stools, which markedly affect patient health (2). The exact etiology of UC remains unclear, but involves immune, genetic, environmental and dietary factors, and treatment strategies typically encompass a combination of pharmacological interventions, dietary modifications and psychological support to alleviate symptoms, control disease progression and enhance the quality of life of patients. Notably, the incidence of colitis and colitis-related colon cancer has been increasing in China; despite extensive research into the influencing factors of UC, the precise pathogenesis remains unclear (3,4). Advancements in understanding UC pathogenesis have emphasized the role of dysregulated inflammatory responses, particularly the extensive tissue damage caused by the release of pro-inflammatory mediators (5,6).

Sirtuin 1 (SIRT1), a histone deacetylase III widely present in human cells, has been implicated in regulating various biological processes, including modulation of the tumor suppressor p53 and NF- κ B through deacetylation, thereby influencing multiple biological activities (7). Acetylation of p53 affects its transcriptional activity, and SIRT1 specifically deacetylates the lysine 382 of p53. Beyond acetylation, histone glycooxidation can induce aggregation and conformational changes in nuclei that perturb chromatin architecture and gene regulation, intersecting with SIRT1/NF- κ B/p53 crosstalk in inflamed epithelia (8). Moreover, p53 forms a negative feedback loop by inhibiting SIRT1 transcription through binding to the p53 response element in the SIRT1 promoter. Increasing evidence has reported that pro-inflammatory mediators, including TNF- α and IL-1 β , contribute to intestinal inflammation in UC primarily via NF- κ B-mediated pro-inflammatory effects (7,9). Use of natural products and their derivatives in treating UC is gaining traction; Donglingcao (*Rabdosia rubescens*) is commonly used in traditional Chinese medicine to treat inflammatory conditions, and is often administered for chronic tonsillitis, pharyngitis, laryngitis and stomatitis in China (6). Notably, spontaneous recovery from UC has been reported following Donglingcao administration (10). These phenomena have demonstrated that Donglingcao may exert anti-UC effects; however, Donglingcao has not been formally approved for the treatment of UC in China. Oridonin (Ori), a

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bioactive tetracyclic diterpenoid compound, is derived from Donglingcao (6,11), and our previous study indicated that SIRT1 is a primary target of Ori, which may mitigate dextran sulfate sodium-induced UC in mice by activating the SIRT1 signaling pathway (12). Therefore, Ori shows promise as a future therapeutic agent for UC; however, the molecular mechanisms underlying its efficacy require further investigation.

The present study aimed to evaluate the SIRT1 signaling pathway in an *in vitro* model of lipopolysaccharide (LPS)-treated human intestinal Caco-2 cells, and to evaluate the safe concentrations for the potential treatment of UC. LPS-stimulated Caco-2 cells were used to establish a cell model representing the inflamed human intestinal epithelium; subsequently, the impact of Ori on the SIRT1/NF- κ B/p53 signaling pathway within the inflamed Caco-2 cells was assessed. Using this approach, the current study aimed to provide insights into the potential therapeutic mechanisms of Ori in mitigating inflammation and preserving intestinal barrier function *in vitro*.

Materials and methods

Reagents. LPS (batch no. L2630) was purchased from MilliporeSigma and Ori (batch no. 17073013) was obtained from Baoji Chenguang Biotechnology Co., Ltd.; the purity of Ori (as determined by high-performance liquid chromatography) was 98%. Both reagents were dissolved in sterile distilled water to a concentration of 10 mM and stored at -20°C for later use. The following antibodies were purchased from Cell Signaling Technology, Inc. and were used for western blotting: SIRT1 (1:1,000; cat. no. 8469), NF- κ B (1:1,000; cat. no. 6956), p53 (1:1,000; cat. no. 18032) and GAPDH (1:1,000; cat. no. 51332); the horseradish peroxidase (HRP)-conjugated secondary antibody (cat. no. 7074) was purchased from Cell Signaling Technology, Inc. (1:5,000).

Cell culture and cell viability assay. The Caco-2 human colon adenocarcinoma cell line was used in the present study. The cells were cultured in 24-well plates according to a previously published method (9). Briefly, the fully differentiated Caco-2 cells were seeded into 96-well plates at a density of 10,000 cells/well and were cultured in DMEM (cat. no. C11995500BT; Gibco; Thermo Fisher Scientific, Inc.) containing 10% FBS (cat. no. 10091148; Gibco; Thermo Fisher Scientific, Inc.) in an incubator containing 5% CO_2 at 37°C . After adhering to the plate for 24 h, the cell culture medium was discarded and fresh DMEM was supplemented with Ori at distinct concentrations (0.625, 1.25, 2.5, 5.0, 10.0, 20.0, 40.0 and $80.0\ \mu\text{M}$). After treatment for 24 h at 37°C , $10\ \mu\text{l}$ Cell Counting Kit (CCK)-8 solution (Sigma-Aldrich; Merck KGaA) was added to each well and the plate was incubated for 1.5 h at 37°C . The optical density (OD) was determined at 450 nm using a microplate reader (model 550; Bio-Rad Laboratories, Inc.). A total of 6 parallel wells were established at each concentration. Cell viability calculations were repeated three times.

ELISA. Differentiated, well-grown Caco-2 cells (20 days post-confluence) in 24-well plates at a density of 10,000 cells/well were randomly divided into four groups: Normal control (NC), $20\ \mu\text{g/ml}$ LPS, and LPS ($20\ \mu\text{g/ml}$) +

high-dose Ori ($2.5\ \mu\text{M}$, Ori-H) and LPS ($20\ \mu\text{g/ml}$) + low-dose Ori ($1.25\ \mu\text{M}$, Ori-L) groups. Briefly, both Ori-H and Ori-L groups were incubated with Ori for 1 h prior to stimulation with LPS ($20\ \mu\text{g/ml}$) for 12 h at 37°C . The NC group was incubated with $10\ \mu\text{l}$ 0.75% saline, whereas the LPS group was incubated with LPS ($20\ \mu\text{g/ml}$) for 12 h at 37°C . At the end of the experiment, the supernatants were collected by centrifugation of the cell culture medium at $3,000 \times g$ for 10 min at 4°C . The levels of TNF- α and IL-1 β in cells were measured by ELISA using commercially available ELISA kits according to the manufacturer's instructions. The human TNF- α (cat. no. 430204) and IL-1 β (cat. no. 437007) ELISA kits were obtained from BioLegend, Inc.

Western blot analysis. Western blot analysis was performed as previously described (7). Briefly, differentiated cells (20 days post-confluence) in 6-well plates were incubated, grouped and treated with various concentrations of Ori and LPS as aforementioned; however, they were only stimulated with LPS for 30 min, not 12 h. Cells were harvested and lysed with lysis buffer [50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 0.5% NP-40, 5 mM EDTA, 50 mM NaF and 1 mM PMSF; Gibco, Thermo Fisher Scientific, Inc.]. After complete homogenization, the supernatant was centrifuged at $10,000 \times g$ for 10 min at 4°C and collected, and the protein concentration in the supernatant was determined using the BCA quantitative method. Subsequently, the supernatant was placed in boiling water for 10 min to denature the protein and $10\ \mu\text{g}$ proteins/lane underwent SDS-PAGE on 10% gels and were transferred to a PVDF membrane. The membrane was blocked with 5% skimmed milk (Procell Life Science & Technology Co., Ltd.) for 1 h at room temperature and was then incubated overnight at 4°C with SIRT1, NF- κ B and p53 antibodies (1:1,000); after which, an appropriate HRP-conjugated secondary antibody was added (1:5,000) and incubated at room temperature for 2 h. The blots were visualized by enhanced chemiluminescence imaging (Amersham; Cytiva), and proteins levels were semi-quantified using ImageJ software (version 1.46; National Institutes of Health) to analyze the grey value of each protein band, with GAPDH used as an internal control.

Statistical analysis. All data were statistically analyzed and plotted using SPSS 20.0 (IBM, Corp.) and GraphPad Prism software 8.0 (Dotmatics). The data are presented as the mean \pm standard deviation. One-way analysis of variance followed by Bonferroni post hoc analysis was used for data analysis between the groups. All measurements were performed in triplicate. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Effects of Ori on the viability of Caco-2 cells. To investigate the effect of Ori on the viability of Caco-2 cells, cell viability was assessed using the CCK-8 method after treatment with different concentrations of Ori for 24 h. The results demonstrated that cell viability was affected by treatment with Ori at different doses; 5.0 – $80.0\ \mu\text{M}$ Ori significantly inhibited the OD values in a dose-dependent manner ($P < 0.01$), whereas 0.625 – $2.50\ \mu\text{M}$ Ori did not inhibit viability ($P > 0.01$), compared with in the

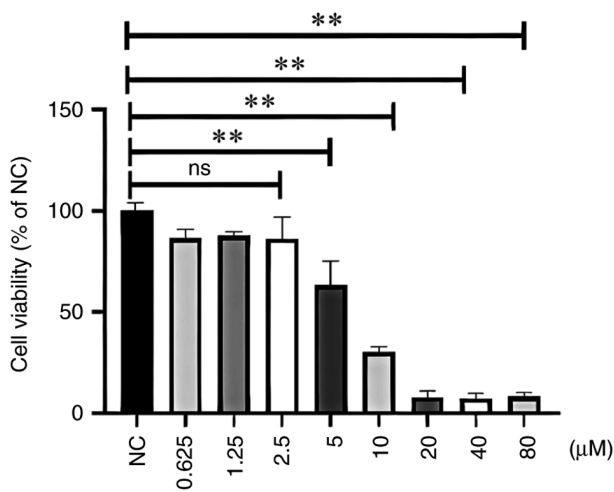


Figure 1. Effect of oridonin on the viability of Caco-2 cells. **P<0.01. NC, normal control; ns, not significant.

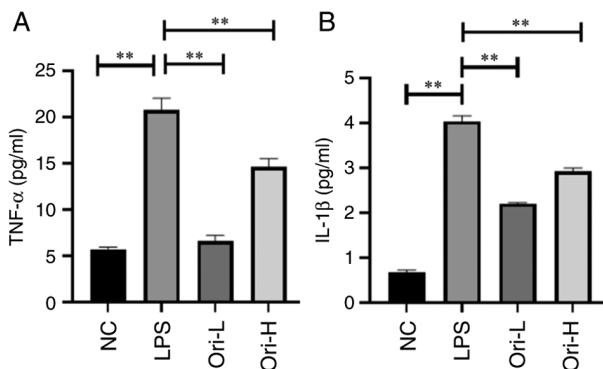


Figure 2. Ori inhibits the secretion of pro-inflammatory mediators TNF- α and IL-1 β in LPS-exposed, differentiated Caco-2 cells. (A) Densitometric analysis of TNF- α levels. (B) Densitometric analysis of IL-1 β levels. **P<0.01. H, high-dose; L, low-dose; LPS, lipopolysaccharide; NC, normal control; Ori, oridonin.

control group (Fig. 1). These findings suggested that the inhibitory effect of Ori on intestinal epithelial cell viability did not result from a cytotoxic action at doses of 1.25 or 2.50 μ M. Therefore, two concentrations of Ori, 1.25 and 2.50 μ M, were selected for subsequent functional and mechanistic experiments due to their lack of toxicity. In addition, the IC₅₀ value of Ori on Caco-2 cells was calculated to be 7.51 μ M.

Effects of Ori on LPS-induced secretion of inflammatory factors in Caco-2 cells. After pre-treatment of Caco-2 cells with Ori for 0.5 h and stimulation with 20 μ g/ml LPS for 24 h, it was observed that LPS stimulation significantly induced the release of TNF- α and IL-1 β in Caco-2 cells, compared with that in the NC group (P<0.01; Fig. 2). However, Ori treatment significantly reduced the LPS-induced secretion of TNF- α and IL-1 β in a concentration-dependent manner (P<0.01), indicating that Ori may suppress the inflammatory response in intestinal epithelial cells at doses of 1.25 and 2.50 μ M.

Effect of Ori on LPS-induced protein expression in Caco-2 cells. Compared with in the NC group, LPS significantly

decreased the protein expression levels of SIRT1, and increased those of NF- κ B and p53 (P<0.01; Fig. 3). Conversely, Ori significantly increased the protein expression levels of SIRT1, and decreased those of NF- κ B and p53, compared with in the LPS group (P<0.01; Fig. 3). These findings indicated that Ori may reduce the LPS-induced expression of members of the NF- κ B signaling pathway and increase SIRT1 expression, which might inactivate NF- κ B/p53 acetylation and promote SIRT1 transcription through inhibiting nuclear translocation and binding to the p53 response element in the SIRT1 promoter (9), thereby reducing intestinal inflammatory response and improving UC.

Discussion

At present, the cause and onset of UC is not yet fully understood; however, it is known that it is related to immunity and is associated with recurrent attacks (4). UC is mainly treated with symptomatic drugs, such as aminosalicylic acids, corticosteroids, immunosuppressants and biologics (5); however, while these drugs can alleviate disease symptoms, they have toxic side-effects (6). Therefore, research into the pathogenesis and treatment of UC is increasingly important. Studies have reported that the SIRT1/NF- κ B/p53 signaling pathway has marked effects on inflammation and inflammation related to cancer development (13,14), and is therefore of interest as a target to treat UC.

LPS is a notable pathological stimuli of intestinal epithelial cells, which stimulates macrophages to increase the secretion of IL-1 β and TNF- α . Pro-inflammatory cytokines produced in the intestine cause damage to intestinal epithelial cells through various pathways, thus adversely affecting their function (14). Furthermore, IL-1 increases intestinal tight junction permeability by activating NF- κ B and myosin light chain kinase, thus decreasing barrier function in experimental colitis (15). TNF induces pro-inflammatory processes in the pathogenesis of UC, including neoangiogenesis and activation of myofibroblasts, macrophages and T cells; consequently, the release of cytokines is regarded as a marker of the inflammatory response. Inhibition of IL-1 and TNF- α function has been shown to ameliorate the inflammatory activity of experimental colitis in animal models and reduce inflammation-associated carcinogenesis (16). Therefore, in the current study, LPS was used to stimulate Caco-2 cells to simulate the inflammatory environment, with the aim of assessing the effects of Ori on inflammatory factors in this environment. The results demonstrated that LPS significantly activated the inflammatory markers TNF- α and IL-1 β in Caco-2 cells compared with that in the NC group. After treatment with Ori, the levels of these markers were significantly decreased, indicating that Ori may inhibit the secretion of pro-inflammatory cytokines induced by LPS stimulation. In addition, after treatment with Ori, the expression levels of SIRT1 were increased, whereas those of NF- κ B and p53 were decreased, compared with those in the LPS-treated group. Notably, Ori has garnered considerable attention from researchers due to its pharmacological properties, including antibacterial, anti-inflammatory, pro-apoptotic and antitumor effects (12). Accumulating evidence has demonstrated that the mechanisms underlying the pharmacological activities of Ori are primarily mediated through signaling

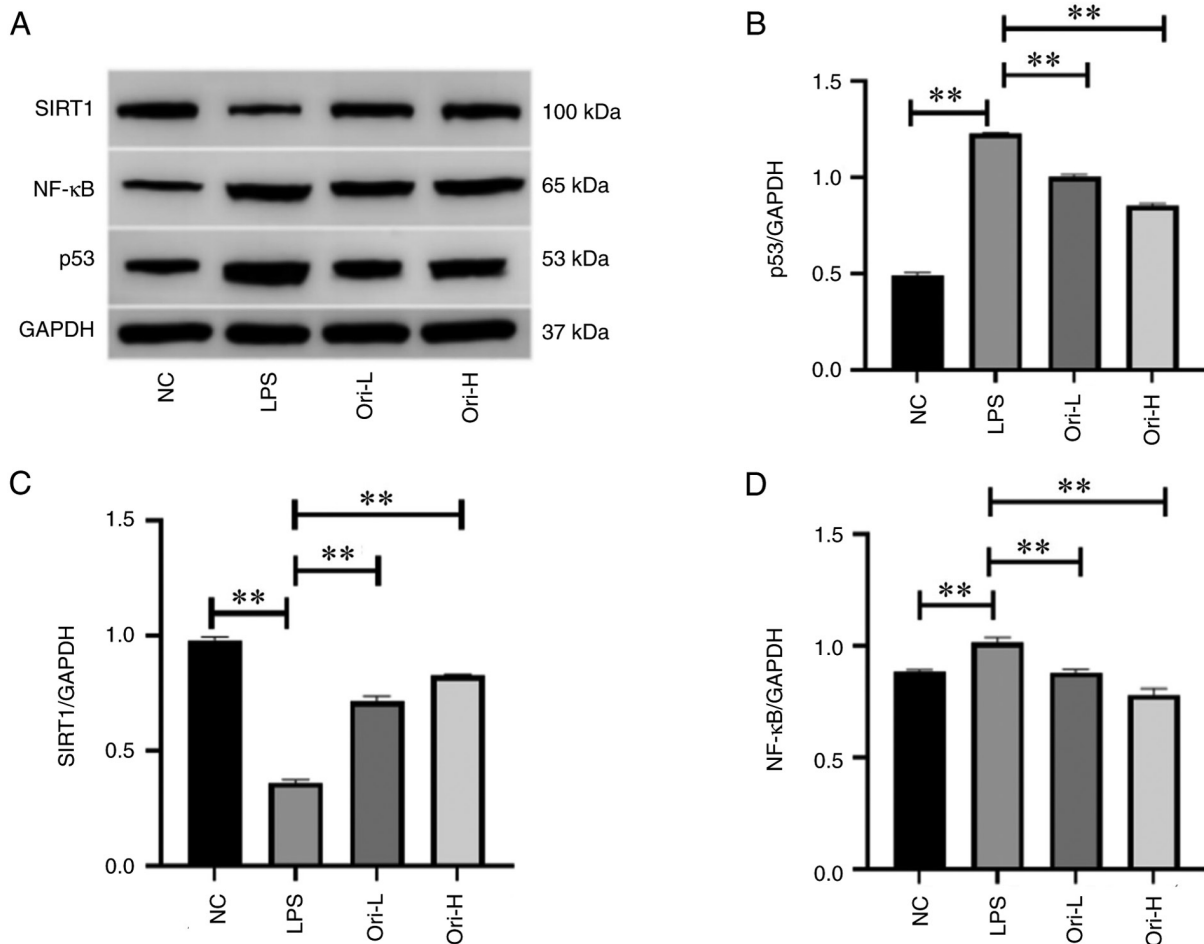


Figure 3. Effect of Ori on the protein expression levels of SIRT1, NF- κ B and p53 in LPS-exposed, differentiated Caco2 cells. (A) SIRT1, NF- κ B and p53 were detected by western blot analysis, with GAPDH as the internal control. Densitometric analysis of (B) p53/GAPDH, (C) SIRT1/GAPDH, and (D) NF- κ B/GAPDH. ** $P < 0.01$. H, high-dose; L, low-dose; LPS, lipopolysaccharide; NC, normal control; Ori, oridonin.

pathways such as c-Met, Notch and VEGF (15,16). Consistent with previous findings, studies have reported that Ori reduces the inflammatory response and inhibits activation of the key SIRT1 signaling axis, which is associated with inflammation in LPS-induced cells (13,17).

In the present study, the findings demonstrated that Ori inhibited LPS-induced inflammatory cytokine response in intestinal epithelial cells to alleviate the inflammatory response. In a previous study, Ori has been reported to inhibit the phosphorylation of SIRT1 and prevent the nuclear translocation of NF- κ B in a mouse model of colitis (13). Similarly, this finding shows that Ori suppresses the activation of the NF- κ B signaling pathway. Mechanistically, SIRT1-mediated deacetylation notably impacts multiple biological processes, including cellular senescence, apoptosis, sugar and lipid metabolism, oxidative stress and inflammation (18). A recent study has revealed marked links between SIRT1 and inflammation, in which alterations to SIRT1 expression and activity have been linked to inflammatory diseases (19). Specifically, the present study determined that Ori notably increased the protein expression levels of SIRT1, and decreased those of NF- κ B and p53 through the NF- κ B signaling pathway, which may reduce the intestinal inflammatory response and promote UC recovery. Another study showed that Ori can inhibit the

phosphorylation of SIRT1 and prevent the nuclear translocation of NF- κ B in a mouse model of UC (13). As such, SIRT1-targeted anti-inflammatory therapies are attracting increasing attention for their clinical applications in treating inflammatory diseases (7). The present study investigated the effect of Ori on UC-related inflammation in LPS-induced Caco-2 cells, an *in vitro* model of inflamed human intestinal epithelium. The Caco-2 cell line, derived from a human colon carcinoma, has been proven to be a good alternative to an intestinal epithelial cell model, and is the most commonly used cell line for studies of the structural and functional characteristics of human differentiated intestinal epithelial cells (20-22). Taken together, the present study showed that Ori may be considered a promising drug for treating UC depending on its suitable concentration (14,19,20).

However, the present study has some limitations; the study mainly focused on the *in vitro* Caco-2 cell model and therefore does not fully represent the intestinal epithelium in UC. In addition, another limitation of the present study is that it contains preliminary research, which did not systematically reveal if Ori directly modulates SIRT1 activity or whether changes are secondary to reduced inflammation. Furthermore, the effect of Ori on cytokine levels in cells that were not treated with LPS was not explored. In future studies, *in vitro*

and *in vivo* experiments will be supplemented using pharmacological inhibitors or small interfering RNA to confirm the dependence of the effects of Ori on SIRT1.

In conclusion, to the best of our knowledge, the present study is the first to provide evidence of the suppressive effects of Ori on LPS-induced inflammatory responses in intestinal epithelial cells through inhibition of the SIRT1/NF- κ B/p53 signaling pathway, and the increased expression of SIRT1.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

MNW and MCL contributed to the study design. BX, LYL and YW conducted the investigation. MNW and LYL wrote the original draft, and MCL reviewed and edited the manuscript. LYL and YW confirm the authenticity of all the raw data. All authors discussed the results and agreed to be accountable for all aspects of the work. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Cauchi S, Van Venetien F, Sciberras M and Ellul P: Colitis trouble up high: a case of gastroduodenal ulcerative colitis and literature review. *J Gastrointest Liver Dis* 34: 128-132, 2025.
- Ruan G, Sun Y, Yu Z, Bai X, Yang H and Qian J: Global, regional, and national burden of inflammatory bowel disease from 1990 to 2021: Findings from the global burden of disease 2021. *Gastroenterol Rep (Oxf)* 13: goaf082, 2025.
- Wu Y, He S, Cao M, Teng Y, Li Q, Tan N, Wang J, Zuo T, Li T, Zheng Y, *et al*: Comparative analysis of cancer statistics in China and the United States in 2024. *Chin Med J (Engl)* 137: 3093-3100, 2024.
- Harpaz N and Polydorides AD: Upper gastrointestinal manifestations of inflammatory bowel disease. *Surg Pathol Clin* 13: 413-430, 2020.
- Katsandegwaza B, Horsnell W and Smith K: Inflammatory bowel disease: A review of pre-clinical murine models of human disease. *Int J Mol Sci* 23: 9344, 2022.
- Chen B, Dong X, Zhang JL, Sun X, Zhou L, Zhao K, Deng H and Sun Z: Natural compounds target programmed cell death (PCD) signaling mechanism to treat ulcerative colitis: A review. *Front Pharmacol* 15: 1333657, 2024.
- Shen P, Deng X, Chen Z, Ba X, Qin K, Huang Y, Huang Y, Li T, Yan J and Tu S: SIRT1: A potential therapeutic target in autoimmune diseases. *Front Immunol* 12: 779177, 2021.
- Mir AR, Moinuddin and Islam S: Circulating autoantibodies in cancer patients have high specificity for glycooxidation modified histone H2A. *Clin Chim Acta* 453: 48-55, 2016.
- Jia X, Liu H, Ren X, Li P, Song R, Li X, Guo Y and Li X: Nucleolar protein NOC4L inhibits tumorigenesis and progression by attenuating SIRT1-mediated p53 deacetylation. *Oncogene* 41: 4474-4484, 2022.
- Barjasteh AH, Al-Asady AM, Latifi H, Al Okla S, Al-Nazwani N, Avan A, Khazaei M, Ryzhikov M, Nadi-Yazdi H and Hassanian SM: Maximizing treatment options for IBD through drug repurposing. *Curr Pharm Des* 30: 2538-2549, 2024.
- Zhang Y, Wang S, Dai M, Nai J, Zhu L and Sheng H: Solubility and bioavailability enhancement of oridonin: A review. *Molecules* 25: 332, 2020.
- Li X, Zhang C, Ma W, Xie X and Huang Q: Oridonin: A review of its pharmacology, pharmacokinetics and toxicity. *Front Pharmacol* 12: 645824, 2021.
- Wang M, Xu B, Liu L and Wang D: Oridonin attenuates dextran sulfate sodium-induced ulcerative colitis in mice via the Sirt1/NF- κ B/p53 pathway. *Mol Med Rep* 26: 312, 2022.
- Tu J, Xu Y, Xu J, Ling Y and Cai Y: Chitosan nanoparticles reduce LPS-induced inflammatory reaction via inhibition of NF- κ B pathway in Caco-2 cells. *Int J Biol Macromol* 86: 848-856, 2016.
- Chen M, Chen C, Gao Y, Li D, Huang D, Chen Z, Zhao X, Huang Q, Wu D, Lai T, *et al*: Bergenin-activated SIRT1 inhibits TNF- α -induced proinflammatory response by blocking the NF- κ B signaling pathway. *Pulm Pharmacol Ther* 62: 101921, 2020.
- Ban J, Peng X, Zhang Y, Liu Y, Wei Y, Ao L, Tian H, He X, Zhao H and Li J: Oridonin alleviates SiNPs-induced pulmonary fibrosis by inhibiting pyroptosis via IRE1 α -XBP1s-NLRP3 pathway. *Int Immunopharmacol* 164: 115388, 2025.
- Shao YY, Guo Y, Feng XJ, Liu JJ, Chang ZP, Deng GF, Xu D, Gao JP and Hou RG: Oridonin attenuates TNBS-induced post-inflammatory irritable bowel syndrome via PXR/NF- κ B signaling. *Inflammation* 44: 645-658, 2021.
- Yang Y, Liu Y, Wang Y, Chao Y, Zhang J, Jia Y, Tie J and Hu D: Regulation of SIRT1 and its roles in inflammation. *Front Immunol* 13: 831168, 2022.
- Jia XM, Hao H, Zhang Q, Yang MX, Wang N, Sun SL, Yang ZN, Jin YR, Wang J and Du YF: The bioavailability enhancement and insight into the action mechanism of poorly soluble natural compounds from co-crystals preparation: Oridonin as an example. *Phytomedicine* 122: 155179, 2024.
- Zhao G, Zhang T, Ma X, Jiang K, Wu H, Qiu C, Guo M and Deng G: Oridonin attenuates the release of pro-inflammatory cytokines in lipopolysaccharide-induced RAW264.7 cells and acute lung injury. *Oncotarget* 8: 68153-68164, 2017.
- Artursson P, Palm K and Luthman K: Caco-2 monolayers in experimental and theoretical predictions of drug transport. *Adv Drug Deliv Rev* 46: 27-43, 2001.
- Sambuy Y, De Angelis I, Ranaldi G, Scarino ML, Stamatii A, Zucco F: The Caco-2 cell line as a model of the intestinal barrier: Influence of cell and culture related factors on Caco-2 cell functional characteristics. *Cell Biol Toxicol* 21: 1-26, 2005.