Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Luteolin Suppressed Growth of Colon Tumor via Inflammation, Oxidative Stress, and NLRP3/IL-1β Signal Axis

Qi Yao, Yuan Luo[1], Lingjia Sun, Hongxia Wang, Wenjie Li

Departments of Geriatrics, Ningbo City First Hospital, [1]Department of Gastroenterology, Ningbo City Second Hospital, Zhejiang, Ningbo, China

Submitted: 27-Jun-2021 Revised: 23-Sep-2021 Accepted: 02-Dec-2021 Published: 07-Jul-2022

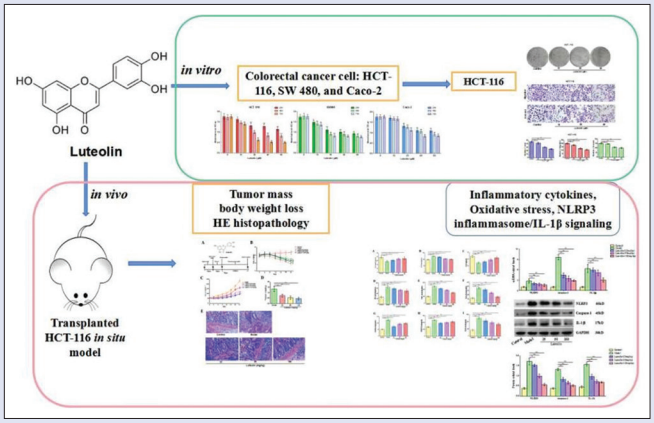
ABSTRACT

Background: Luteolin, a natural flavonoid, exerts anticancer activities. In this study, we evaluated the role of luteolin in colon cancer, and its underlying mechanisms. **Materials and Methods:** The effect of luteolin on the proliferation of human colon tumor (HCT)-116, SW480, and Caco-2 cells was evaluated. Subsequently, the effect of luteolin on the proliferation, migration, and invasion of HCT-116 cells was monitored. Then, we analyzed the level of inflammation and oxidative stress in the mouse model transplanted with HCT-116 under *in situ* conditions. Furthermore, we investigated whether luteolin attenuates growth of colon tumor under *in vivo* conditions. **Results:** Our results demonstrate that luteolin reduced the proliferation of HCT-116, SW480, and Caco-2 cells. In addition, it blocked the migration and invasion of HCT-116 cells. Moreover, luteolin increased the production of interleukin (IL)-2/10 and decreased the production of IL-6, interferon-β, tumor necrosis factor-α, and IL-1 β. Furthermore, it increased the level of superoxidase dismutase and glutathione peroxidase and decreased the levels of malondialdehyde significantly. Luteolin significantly lowered the sensitization of NOD-leucine-rich repeat and pyrin-containing protein 3 (NLRP3)/caspase-1/IL-1 β signal axis. Luteolin exerted the above actions in a dose-dependent manner. **Conclusion:** The results of this study indicate that luteolin mitigates the growth of colon cancer via suppressing the inflammatory processes, oxidative stress, and NLRP3/IL-1 β signal axis. It might serve as a promising candidate for colon cancer.

Key words: Colon cancer, inflammation, luteolin, NOD-leucine-rich repeat and pyrin-containing protein 3/interleukin-1 β signal axis, oxidative stress

SUMMARY

- NOD-leucine-rich repeat and pyrin-containing protein 3 (NLRP3)/interleukin (IL)-1 β signal axis may be a crucial target for anti-colon tumors
- Luteolin, a natural flavonoid, suppresses the growth of colon tumors via suppressing inflammation, oxidative stress, and NLRP3/IL-1 β signal axis.



Abbreviations used: HCT: human colon tumor; NLRP3: NOD-leucine-rich repeat and pyrin-containing protein 3; ASC: apoptosis-associated speck-like protein containing a CARD; caspase-1: cysteine aspartic acid-specific protease; IL: interleukin; IFN: interferon; TNF: tumor necrosis factor; SOD: superoxidase dismutase; GSH-Px: glutathione peroxidase; MDA: Malondialdehyde.

Correspondence:

Dr. Qi Yao,
Department of Geriatrics, Ningbo City First Hospital, Liuting Street, Haishu District, Ningbo 315000, Zhejiang, China.
E-mail: qiyao_qy@163.com
DOI: 10.4103/pm.pm_284_21

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

Globally, colon cancer is one of the leading tumors known to affect human health. The annual survival rate of colon cancer is low, and the rate of recurrence and metastasis is high.[1] It comprises a substantial proportion of global cancer morbidity and mortality. Annually, about 600,000 deaths occur due to colon cancer.[1,2] So far, surgical resection, radiotherapy, and chemotherapy are the primary treatment modalities for colon cancer,[3,4] and the high rate of postoperative metastasis is a serious complication. Although immune checkpoint inhibitors and ancillary agents have been approved in the treatment of colon cancer, the incidence and rate of mortality from colon cancer remain alarming.[5,6] Therefore, it is very important to develop anticancer agents suitable for colon cancer.

Natural products show tumor-suppressive property and are known to be safe. Luteolin, a kind of natural flavonoid, is rich in common herbal plants.[7] Studies show that it exhibits antivirus, antifibrotic, anti-inflammatory, and antioxidant properties.[8,9] A recent study has shown antitumor properties of luteolin against colon cancer cells.[10] Another study reported that luteolin modulates p53-dependent pathway to mediate the apoptosis and autophagy of human colon tumor (HCT)-116 cells.[11] However, to the best of our knowledge, there are no studies conducted to identify the mechanism of action luteolin in colon cancer.

Due to the heterogeneous nature of this cancer, the planned intervention should act via various oncogenic signaling pathways. NOD-leucine-rich repeat and pyrin-containing protein 3 (NLRP3)/caspase-1/interleukin (IL)-1 β signal axis is one of the pathways that can be used as the target to treat colon cancer.[12,13] NLRP3

Cite this article as: Yao Q, Luo Y, Sun L, Wang H, Li W. Luteolin suppressed growth of colon tumor via inflammation, oxidative stress, and NLRP3/IL-1β signal axis. Phcog Mag 2022;18:494-501.

inflammasome (NLRP3-ASC-caspase-1) participates in mediating inflammatory responses induced by the inflammation and tumor stimulation.[14,15] Subsequently, the cytokine IL-1 β is further induced following the NLRP3 inflammasome.[16] Therefore, in the pathogenesis of cancer, NLRP3/caspase-1/IL-1 β signal axis may play a significant role. The activated caspase-1 stimulates the IL-1 β and IL-18 precursors, and cell pyrolytic executive molecules Gasdermin D, which can induce cell pyroptosis, then promote IL-1 β maturity and IL-18 secretion. IL-1 β is known to cause colitis-related illness, such as colon cancer.[17] Although extensive research has been conducted on inflammasome, the specific implications of inflammasome activation in colon cancer have not yet been understood clearly.

Previous studies have revealed the antioxidative and anti-inflammatory activity of luteolin.[18] For example, Kang *et al.* revealed that luteolin regulates apoptotic cell death of HCT cells through its antioxidative activity.[19] Park and Kim indicated that luteolin alleviated the azoxymethane-induced colon oncogene mouse with high-fat diet-fed obese via both colonic inflammation and oxidative stress.[20] In addition, a previous study showed that NLRP3/caspase-1/IL-1 β can exhibit anti-oxidative stress and anti-inflammation as well.[21] Therefore, in this study, we aimed to evaluate the efficacy and action of luteolin in suppressing the growth of colon cancer cells, and further elucidated the possible mechanism via inflammation, oxidative stress, and NLRP3 signal axis.

MATERIALS AND METHODS

Cells and reagents

Colon tumor cell lines HCT-116, SW480, and Caco-2 were obtained from the Chinese Academy of Sciences Shanghai Cell Repository. The cells were cultured (5% CO_2, 37°C) and maintained in RPMI-1640 medium (Gibco™, USA), containing 10% fetal bovine serum (FBS; PeproTech, Inc., USA) and 1% penicillin–streptomycin as the antibiotic (Sigma-Aldrich). Luteolin (Lot no: 111520-201605, HPLC >99.3%) was obtained from China National Institutes for Drug Control, Beijing, China. Luteolin was dissolved ultrasonically in dimethyl sulfoxide (DMSO, Sigma-Aldrich), and then diluted using sterile distilled H_2O.

Cell viability assay

The HCT-116, SW480, and Caco-2 cells were cultured in 96-well plates (1000 cells/100 μL/well) in a humidified CO_2 (5%) incubator at 37°C. The cells were allowed to grow until a monolayer was obtained. Subsequently, the culture medium was replaced with the serum-free medium containing different concentrations of luteolin (0, 10, 20, 40, and 80 μM) and incubated for 24, 48, and 72 h. The cell viability was assessed by performing the tetrazolium-based colorimetric assay (MTT, Shanghai Biyuntian Biotechnology Co., Ltd, China). MTT (5 mg/mL) was added into each well (20 μL/well) and incubated for 4 h. Then, the blue formazan crystals formed were dissolved in DMSO (150 μL/well) and the optical density was read using a microplate reader (BIO-RAD). The control group cells were not incubated with luteolin, and all experiments were conducted with six replicates. Then, the optical density was read at 490 nm, and the survival rate was calculated. Moreover, the optimal concentration range of inhibitory effects was used for the next trials of anti-colon tumor cells activity with the treatment of luteolin.

Cell cloning assays

HCT-116 cells (200 cells/dish/10 mL) were inoculated in 60-mm cell culture dishes and incubated at 37°C with 5% CO_2. The formation of cell clones was visible to the naked eye after 2 weeks.

Cloning observation procedure

The culture medium was carefully drained, discarded, and washed twice with phosphate-buffered saline (PBS). The cell clones were fixed and incubated for 15 min with 4% paraformaldehyde, and then stained with 0.1% crystal violet for 20 min. After staining, the dishes were washed and rinsed with water and air-dried. The cell clones were randomly selected and counted under a fluorescence microscope (Leica, Germany).

Cell transwell migration and invasion assays

In this study, 8-μm transwell chamber (Shanghai Ziqi Biotechnology, Co., Ltd, China) was used. Briefly, 500 μL of medium containing 15% FBS was added to the 24-well plate without any air bubble. After a period of starvation, the cells were detached and counted to reach about 0.8×10^5 cells per well. The cells were suspended in a serum-free medium and seeded in 96-well plates with or without luteolin (200 μL). For the invasion assay, cells were seeded after the upper surfaces of the membranes were coated with 50 μL of Matrigel for 6 h. The HCT-116 cells were cultured at 37°C and were allowed to migrate for 36 h. Cells in the upper chamber were fixed with ice-cold methanol, and stained with 0.1% crystal violet for 20 min. Then, the liquid in the chamber around the cells was wiped with a cotton swab. Finally, the transwell cells were visualized and observed with an optical microscope (Olympus, Japan). Those transwell cells were counted and observed for migration and invasion by Image-Pro Plus 6.0.

Animals and experimental design *in vivo*

Male BALB/c mice, weighing about 18–22 g, were purchased from the Animal Experimental Center, Ningbo University, Zhejiang, China [SPF, Certificate No. SCXK (Zhe) 2018-0021]. The mice were acclimated and housed under standard laboratory conditions (20°C–25°C, 40%–45% humidity, and a 12/12-h light/dark cycle). Animals had access to water *ad libitum* and were fed with granular food. All experiments were authorized by the Institutional Ethical Committee of Ningbo University, and were conducted in the light of local rules for laboratory animal care.

In this study, we established a mouse model with transplanted colon cancer cells under *in situ* conditions.[22] After adaptation for 7 days, mice were anesthetized via intraperitoneal injection of pentobarbital sodium (concentration: 2.5%, dosage: 40 mg/kg, volume: 0.32 mL). Then, about 1.5 cm incision was made in the right flanks of the mice, and cecum was exposed. Then, 50 μL of HCT-116 cells (4×10^7/mL) growing in logarithmic phase were injected into the incision using a sterile microsyringe, between the cecal serous membrane and muscular layer. Then, the injection area was disinfected with 3% iodine, and the abdominal wall was sutured. Control group animals were injected with 50 μL of sterile 0.9% saline solution. The animals were maintained until the xenograft tumor volumes reached about 100 mm^3. Subsequently, the mice were randomly divided into five groups: control group (normal mice), model group, and luteolin group with three different concentrations of 25, 50, and 100 mg/kg, respectively. Each group had 10 mice. Seven days after xenograft, the tumor growth of each mouse was analyzed. Tumor diameter of more than 10 mm indicated successful modeling. Then, luteolin (25, 50, and 100 mg/kg) was administered to the animals via oral gavage at a dose of 0.02 mL/g (*v*/w). The treatment dose was repeated every day for 3 weeks. The control and model groups were administered with an equal volume of PBS by oral. The change in mouse weight was recorded once in every 3 days. The humane endpoints for tumor trials were set as follows: the weight of tumor beyond 10% of the original mouse weight or the average diameter of tumor over 20 mm. On day 21, after first administration, mice were sacrificed via an over dose of pentobarbital (4%, i. p.), and tumors

were harvested and weighed. The formula used to calculate the tumor volume was as follows: $V = 0.5 \times \text{length} \times \text{width}^2$.

H and E Staining

The cancer tissues and the normal tissues from the control group were collected and washed with physiological saline at 4°C. Then, the tissues were fixed in 10% formalin for a week. The tissue sections were dehydrated with ethanol, permeabilized with xylene, embedded in paraffin, and cut using a microtome (4-μm thick). The sections were stained using hematoxylin and eosin, and the lesions and pathological changes of the tumor tissues were evaluated using optical microscopy.

Measurement of inflammatory cytokines and serum antioxidants

The serum was collected through the orbital venous plexus, which was centrifuged at 3000 × g for 10 min under 4°C. Then, the supernatant serum was collected by the fluid transfer gun, and the level of inflammatory cytokines was determined by enzyme-linked immunosorbent assay (ELISA) kits with the appropriate amount of serum. The mouse cytokine antibodies (IL-2, IL-6, IL-10, IL-1 β, interferon [IFN]-β, and tumor necrosis factor [TNF]-α) were purchased from RND Co. (USA). Furthermore, the antioxidants levels of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and malondialdehyde (MDA) were

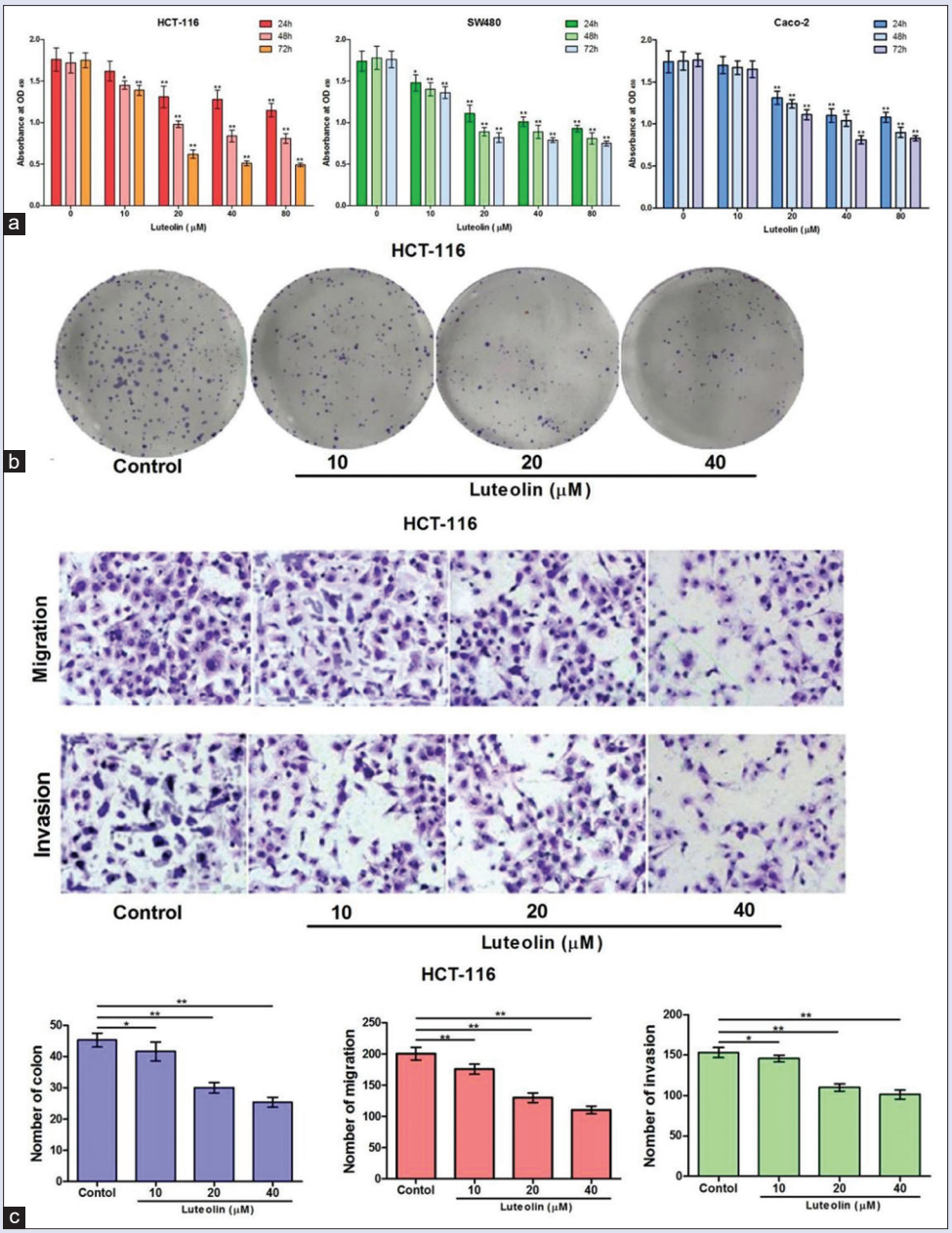


Figure 1: Luteolin inhibited growth of colon tumor cells *in vitro*. (a) The proliferation of human colon tumor-116, SW480, and Caco-2 cells was tested by MTT assay after treatment with 0, 10, 20, 40, d 80 μM dosage of luteolin for 24, 48, or 72 h; (b) human colon tumor -116 cell clone posttreatment with 10, 20, and 40 μM dosage of luteolin; (c) Migration and invasion of human colon tumor-116 posttreatment with 10, 20, and 40 μM dosage of luteolin. Compared with the control, $^{*}P < 0.05$, $^{**}P < 0.01$

Table 1: Real-time fluorescence quantitative polymerase chain reaction primer sequence

Gene		Primer sequences	Primer length (bp)
NLRP3	Forward	5' CTCGCATTGGTTCTGAGCTCA 3'	153
	Reverse	5' AGTAAGGCCGGAATTCACCA 3'	
Caspase-1	Forward	5' ACTCGTACACGTCTTGCCCTC 3'	190
	Reverse	5' CTGGGCAGGCAGCAAATTC 3'	
IL-1β	Forward	5' CCCTGAACTCAACTGTGAAATAGCA 3'	300
	Reverse	5' CCCAAGTCAAGGGCTTGGAA 3'	
GAPDH	Forward	5' GTGACACCCACTCTTCCACC 3'	162
	Reverse	5' GTGGTCCAGGAGGCTCTTAC 3'	

NLRP3: NOD-leucine-rich repeat and pyrin-containing protein 3; IL: interleukin

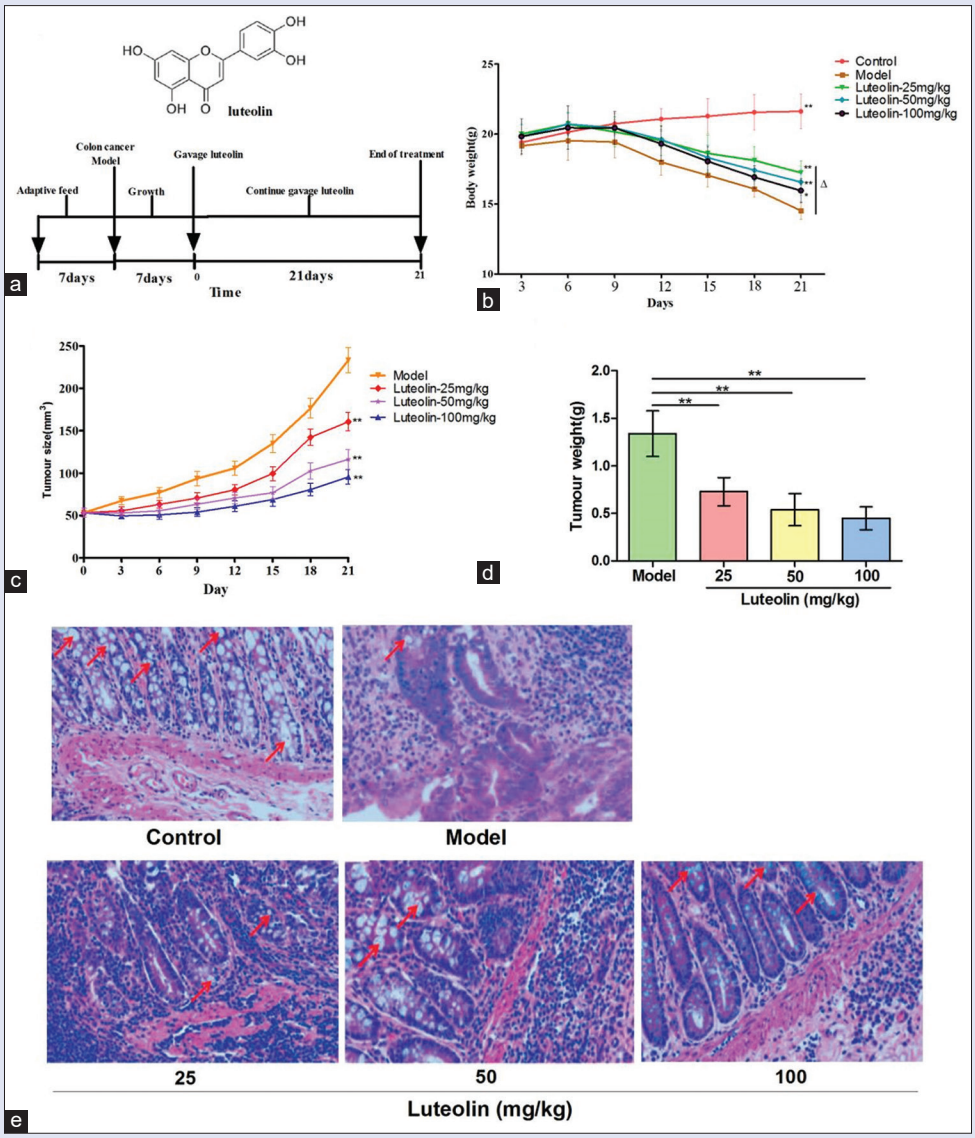


Figure 2: Effect of luteolin on anti-colon tumor *in vivo*. (a) Time-course diagram of treatment *in vivo* experiment; (b) Body weight of mice was observed after treatment with luteolin at a dosage of 25, 50, and 100 mg/g; (c and d) Tumor size recorded every day and tumor weight last day of mice bearing human colon tumor-116 colon tumor; (e) Pathological observation of tumor tissue was measured by HE assay (Magnification × 100). Compared with the model, $^{*}P < 0.05$, $^{**}P < 0.01$; Compared with the control, $^{\triangle}P < 0.05$

determined, and those antioxidant antibodies were purchased from Ricca Newmark Design, USA. The serum levels of inflammatory cytokines and antioxidants were measured by double-antibody-sandwich ELISA assay through type R-4100 Enzyme Marker (Dynatech, USA).

Real-time quantitative polymerase chain reaction assay

In this study, the primers [Table 1] used to amplify the genes of NLRP3, caspase-1, IL-1 β, and GAPDH were designed by Kelton

Biotechnology (Shanghai) Co., Ltd. (China) and synthesized by Generay Biotech Co., Ltd. (China).

The total RNA of each group in tumor tissues was extracted according to the manufacturer's instructions (Trizol, Lot No. 20191108, Invitrogen Inc., USA). The purity and concentrations of RNA were determined by nucleic acid detector and agarose gel electrophoresis. The reverse transcription reactions were conducted using a reverse transcription kit (Thermo Fisher Scientific Inc., USA). A real-time quantitative polymerase chain reaction (RT-qPCR) was conducted to amplify the cDNA via SYBR Green PCR Kit (Lot No. 0016161945, Thermo Fisher Scientific Inc., USA) using an ABI 7500 Fluorescent Quantitative PCR detector (Applied Biosystems Inc., USA). The PCR was programmed to run 40 cycles for amplification. *GAPDH* was chosen as the internal reference gene. The relative levels of mRNA were calculated using the formula $2^{-\triangle\triangle Ct}$.[23]

Western blot analysis

The tumor tissues of mice were ground in liquid nitrogen, and then RIPA lysis buffer was added at a ratio of 1:10 (g/mL), and the total protein was determined by BCA protein quantitative kit (Lot No. 00041305, Beijing ComWin Biotech Co., Ltd., China).

Then, sodium dodecyl sulfate–polyacrylamide gel electrophoresis was performed to separate the proteins, and the separated bands were transferred onto polyvinylidene fluoride membranes. The transferred proteins were blocked with 5% fat-free milk for 2 h. Subsequently, the membranes were washed thrice by Tris-buffered saline with 0.05% Tween (TBST) and were incubated at 4°C with rabbit-anti-mouse primary antibody (Abcam, UK) overnight (~12 h). Then, the membranes were rinsed thrice with TBST for 10 min. The membrane was incubated with horseradish peroxidase-labeled goat-anti-rabbit secondary antibody IgG (Proteintech, USA) for 2 h at room temperature. Subsequently, the membranes were rinsed thrice, and the bands were visualized using enhanced chemiluminescence technique, and analyzed using Image-Pro Plus 6.0 software (Type Omega Lum G, Applegen, USA). *GAPDH* was used as an internal loading vehicle in the western blot analysis.

Statistical analysis

The data (mean ± standard deviation [$\overline{x} \pm s$]) were analyzed by using the SPSS software version 19.0 (International Business Machines Corporation, New York, USA). The significance comparisons among multiple groups were performed via one-way analysis of variance, followed by Tukey's *post hoc* test. The combined charts were plotted by GraphPad Prism 8 software. If $P < 0.05$, the results were considered statistically significant.

RESULTS

Luteolin inhibited growth of colon tumor cells *in vitro*

To analyze the effect of luteolin against colon tumor cells, we performed experiments using HCT-116, SW480, and Caco-2 cells. According to our results, luteolin inhibited the proliferation of HCT-116, SW480, and Caco-2 cells effectively. The inhibitory activity was shown as proportion with the change of the concentration and incubation time [Figure 1a]. According to our results, 10 μM or higher doses of luteolin inhibited the proliferation of colon tumor cells significantly compared with the control. Besides, within the same treatment time of luteolin, the growth of HCT-116 cells was inhibited more sensitively [shown in Figure 1a].

Second, the inhibitory activity of luteolin against HCT-116 cells clone, migration, and invasion was examined. As shown in Figure 1, luteolin-treated HCT-116 cells showed a significant reduction in the cells clone, migration, and invasion ($P < 0.01$), and luteolin exerted this

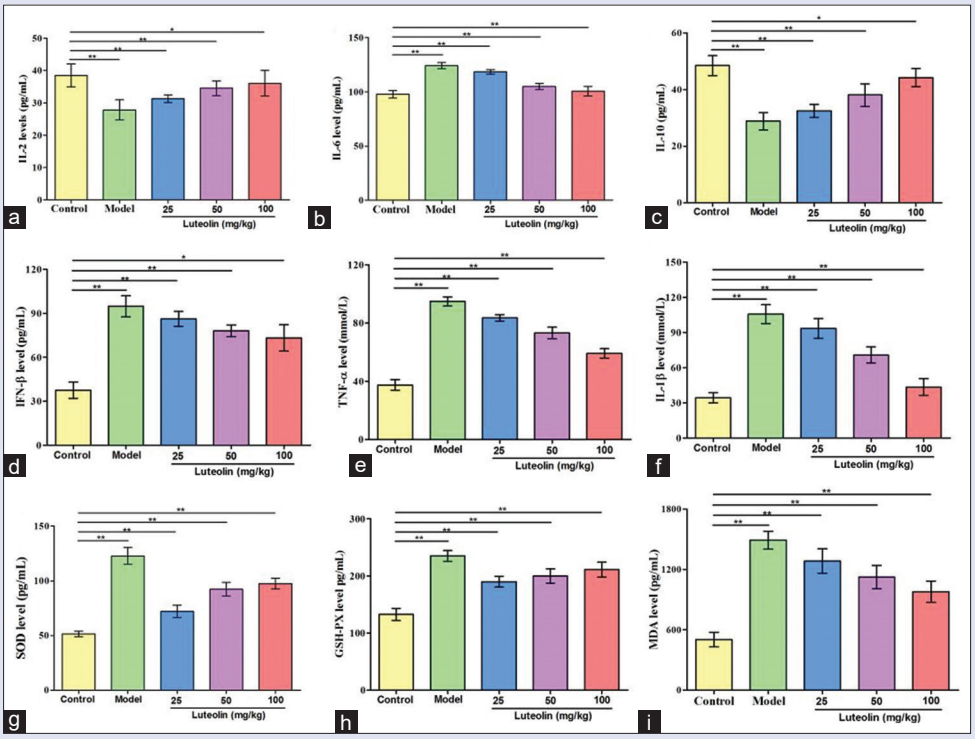


Figure 3: Luteolin regulated the inflammatory cytokines and anti-oxidation factor. (a-f) Serum interleukin-2, interleukin-10, interleukin-6, interferon-β, tumor necrosis factor-α, and interleukin-1 β levels, respectively, after treatment with luteolin at dosage of 25, 50, and 100 mg/g; (g-i) superoxidase dismutase, glutathione peroxidase, and malondialdehyde levels post treatment with luteolin. Compared with the control, *$P < 0.05$, **$P < 0.01$

effect in a dose-dependent manner. Therefore, luteolin exhibited obvious effects on clone, migration, and invasion for HCT-116 cells over a dose of 10 μM [Figure 1b-c], suggesting that luteolin exerted the ability of hindering the colon tumor cells.

Luteolin ameliorated colon tumor *in vivo*

After establishing the antiproliferative effect of luteolin under *in vitro* conditions using HCT-116, SW480, and Caco-2 cells, we sought to address whether luteolin inhibited the growth of HCT-116 cells under *in vivo* conditions. After the HCT-116 cells were injected into BALB/c mice (process shown in Figure 2a), the body weight, tumor size and weight, and pathological observation were analyzed to study the effect of luteolin against colon tumor. As shown in Figure 2b-e, compared with the model group, luteolin (25, 50, and 100 mg/kg) relieved the weight loss of mice, and decreased the tumor size and tumor weight in mice significantly ($P < 0.05$). In the case of pathological morphology of tumor tissue, compared with the model group, the specimens in luteolin groups were more holonomic and smooth, the size of the nucleus decreased, and the nuclear staining was light. These manifestations were observed in a dose-dependent manner. These results show that luteolin suppressed the growth of HCT-116 cells under *in vivo* conditions.

Luteolin attenuated inflammation and oxidative stress

According to the results of this study, luteolin acted via multiple ways to inhibit cancer. The inflammation and oxidative stress-related cytokines were investigated to evaluate the underlying mechanism of action of luteolin. As shown in Figure 3, luteolin significantly decreased the serum levels of IL-6, IFN-β, TNF-α, and IL-1 β compared with the model group. In addition, it increased the levels of IL-2 and IL-10 when compared with

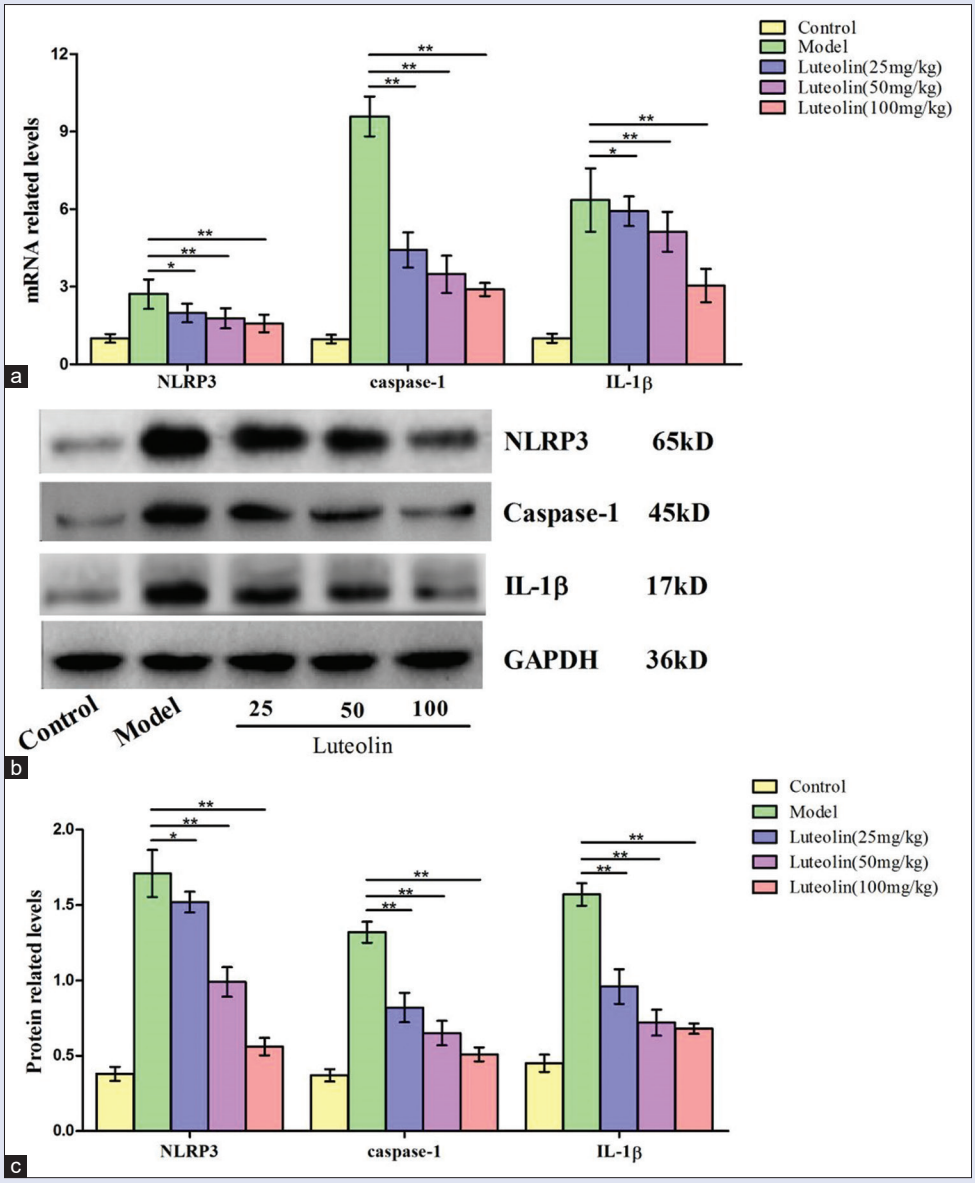


Figure 4: Effect of luteolin on blocking the NLPR3/interleukin-1 β signal axis in tumor tissues. (a) Luteolin downregulated the key targets mRNA in NOD-leucine-rich repeat and pyrin-containing protein 3/interleukin-1 β signal axis; Luteolin decreased NOD-leucine-rich repeat and pyrin-containing protein 3, caspase-1 and interleukin-1 β protein expressions ([b] Western blotting; [c] Quantitative analysis of the protein expressions). Compared with the control, *$P < 0.05$, **$P < 0.01$

the model group ($P < 0.05$). Furthermore, luteolin increased the levels of SODs and GSH-Px, and decreased the levels of MDA ($P < 0.01$) in a dose-dependent manner. These results indicate that luteolin inhibited the growth of cancer cells via attenuation of inflammatory and oxidative stress-related cytokines.

Luteolin blocked the NOD-leucine-rich repeat and pyrin-containing protein 3/interleukin-1 beta signal axis

To elucidate the possible targets and the underlying mechanism of action of luteolin, we investigated the NLRP3/IL-1 β signal axis, which is associated with inflammation and oxidative stress. According to our results, theNLRP3, caspase-1, and IL-1 β protein, and mRNA expression levels in luteolin-treated groups were blocked significantly ($P < 0.05$; Figure 4a-c). In addition, compared with the vehicle group, model group showed significantly increased activation of NLRP3/IL-1 β signal axis, and luteolin reduced the activation in a dose-dependent manner. These results indicate that luteolin inhibited the growth of tumor cells via suppression of NLRP3/IL-1 β signal axis.

DISCUSSION

Nowadays, colorectal cancer is the leading cause of health issue worldwide. Although there is a substantial amount of research conducted on anticancer therapies to treat colon cancer, novel therapeutic avenues and strategies are still deficient.[24] Comprehensive literature and the results of this study support the idea that natural herbal products are novel therapeutic strategies as adjuvant treatment of colon cancer.[25] In this study, we conducted *in vitro* research on luteolin and found that it inhibits the growth and proliferation of colon cancer cells significantly. Therefore, we conducted *in vivo* study, which showed that luteolin alleviated the weight loss, and restrained growth of tumor in mice (inhibit tumor size and weight). Furthermore, luteolin improved the pathological changes in the tumor cells and suppressed the growth of HCT-116 cells under *in vivo* conditions.

Furthermore, we analyzed the anti-inflammatory and antioxidative activity of luteolin and found that luteolin could increase the level of IL-2/10 and decrease the level of IL-6, IFN-β, TNF-α, and IL-1 β. In addition, it increased the level of SOD and GSH-Px and decreased the level of MDA. Previous studies have reported that the level of the aforementioned inflammatory cytokines and antioxidant factors can accurately reflect the level of damage in patients with tumor.[26-28] As reported earlier, CD4+ regulatory cells participate in cytokine secretion and restore immune homeostasis in inflammation-associated cancer by downregulating the inflammatory processes. Reducing inflammation is very important in promoting health and reducing risk of cancer.[29] In terms of oxidative stress, Wang *et al*. reported that luteolin extracted from *Rhus typhina* fruits showed the potent anti-proliferative activity against HT-29 cells via blocking the SOD-2 gene expressions and production of reactive oxygen species.[30]

Furthermore, among the signaling pathways associated with the pathogenesis colon cancer, NLRP3/caspase-1/IL-1 β signal axis has been identified as a promising drug target.[31] This signal axis can regulate acute inflammation induced by inflammatory bowel disease.[32] On the contrary, inflammatory cytokines (e.g., IL-6 and IFN-β) can further modulate NLRP3/caspase-1/IL-1 β[20,33] and regulate antioxidant factors (SOD, GSH-Px, and MDA).[34] NLRP3 refers to a cytoplasmic pattern recognition receptor, and it combined with downstream signal ASC and caspase-1 together to make up the NLRP3/ASC/caspase-1 inflammasome. During the process of signal transduction, stimulated NLRP3 activates the adaptor apoptosis-associated speck-like protein containing CARD, which, in turn, activates to form caspase-1 from procaspase-1. During this time, the signal can significantly mediate the maturity of IL-1 β and secretion of IL-18.[35,36] In this study, our results show that luteolin inhibited the growth of colon cancer cells under *in vitro* and *in vivo* conditions. It reduced inflammation and blocked oxidative stress. Furthermore, luteolin significantly downregulated the expression of the key targets of NLRP3/caspase-1/IL-1 β signal axis. These results suggest that luteolin inhibits colon carcinogenesis by acting as an inhibitor of the signal axis, followed by suppression of inflammation and oxidative stress.

For researches in drug screening, efficacy evaluation, or clinical prediction of cancer, the establishment of a transplanted tumor model has a obviously unique advantage. Therefore, in this study, we evaluated the efficacy and effect of HCT-116 cell transplantation tumor model, instead of the primary colon cancer induced by dimethylhydrazine.[37] Transplantation tumor model is more suitable for evaluating the activity of antitumor agents by investigating tumor mass and volume. Previously, it has been reported that luteolin is effective against colon cancer, but further research needs to be conducted in this regard. Therefore, the results of this study might provide a better reference for the development of luteolin as an anticancer drug for colon cancer.

CONCLUSION

The results of this study show that luteolin inhibits the proliferation of colon cancer cells and mitigates the growth of colon tumors via suppression of inflammatory processes, oxidative stress, and NLRP3/IL-1 β signal axis. Thus, our results indicate that luteolin may serve as a promising candidate for the treatment of colon cancer.

Acknowledgements

This research would like to thank the Animal Experimental Center, Ningbo University, Zhejiang, China, for animal experiments and equipment facility support.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015;65:87-108.
2. Strillacci A, Griffoni C, Lazzarini G, Valerii MC, Di Molfetta S, Rizzello F, *et al*. Selective cyclooxygenase-2 silencing mediated by engineered *E. coli* and RNA interference induces anti-tumour effects in human colon cancer cells. Br J Cancer 2010;103:975-86.
3. Jahanafrooz Z, Mosafer J, Akbari M, Hashemzaei M, Mokhtarzadeh A, Baradaran B. Colon cancer therapy by focusing on colon cancer stem cells and their tumor microenvironment. J Cell Physiol 2020;235:4153-66.
4. Gao P, Huang XZ, Song YX, Sun JX, Chen XW, Sun Y, *et al*. Impact of timing of adjuvant chemotherapy on survival in stage III colon cancer: A population-based study. BMC Cancer 2018;18:234.
5. Pagès F, Mlecnik B, Marliot F, Bindea G, Ou FS, Bifulco C, *et al*. International validation of the consensus Immunoscore for the classification of colon cancer: A prognostic and accuracy study. Lancet 2018;391:2128-39.
6. Goggi JL, Hartimath SV, Xuan TY, Khanapur S, Jieu B, Chin HX, *et al*. Granzyme B PET imaging of combined chemotherapy and immune checkpoint inhibitor therapy in colon cancer. Mol Imaging Biol 2021;23:714-23.
7. Lee HJ, Seo HS, Ryu J, Yoon YP, Park SH, Lee CJ. Luteolin inhibited the gene expression, production and secretion of MUC5AC mucin via regulation of nuclear factor kappa B signaling pathway in human airway epithelial cells. Pulm Pharmacol Ther 2015;31:117-22.

8. Yao YY, Rao CH, Zheng G, Wang SS. Luteolin suppresses colorectal cancer cell metastasis via regulation of the miR384/pleiotrophin axis. Oncol Rep 2019;42:131-41.
9. Yuan J, Che S, Ruan Z, Song L, Tang R, Zhang L. Regulatory effects of flavonoids luteolin on BDE-209-induced intestinal epithelial barrier damage in Caco-2 cell monolayer model. Food Chem Toxicol 2021;150:112098.
10. Potočnjak I, Šimić L, Gobin I, Vukelić I, Domitrović R. Antitumor activity of luteolin in human colon cancer SW620 cells is mediated by the ERK/FOXO3a signaling pathway. Toxicol *In Vitro* 2020;66:104852.
11. Yoo HS, Won SB, Kwon YH. Luteolin induces apoptosis and autophagy in HCT116 coloncancer cells via p53-dependent pathway. Nutr Cancer 2022;74:677-86.
12. Fu Y, Han P, Yan W. Tu1993 high mobility group box 1 activates NLRP3 inflammasome through toll like receptor 4 in colon cancer cells. Gastroenterology 2015;148:S-954.
13. Fan Z, Yang J, Yang C, Zhang J, Cai W, Huang C. MicroRNA24 attenuates diabetic vascular remodeling by suppressing the NLRP3/caspase-1/IL-1-1/Ignaling pathway. Int J Mol Med 2020;45:1534-42.
14. Ma TH, Sheng T, Tian CM, Xing MY, Yan LJ, Xia DZ. Effect of ethanolic extract of *Polygonum cuspidatum* on acute gouty arthritis in mice through NLRP3/ASC/caspase-1 axis. Zhongguo Zhong Yao Za Zhi 2019;44:546-52.
15. Oh NH, Han JW, Shim DW, Sim EJ, Koppula S, Kwak SB, *et al.* Anti-inflammatory properties of *Morus bombycis* Koidzumi via inhibiting IFN-β signaling and NLRP3 inflammasome activation. J Ethnopharmacol 2015;176:424-8.
16. Guan X, Guan Y, Shi C, Zhu XM, He YN, Wei ZC, *et al.* Estrogen deficiency aggravates apical periodontitis by regulating NLRP3/caspase-1/IL-1β axis. Am J Transl Res 2020;12:660-71.
17. He WT, Wan H, Hu L, Chen PD, Wang X, Huang Z, *et al.* Gasdermin D is an executor of pyroptosis and required for interleukin-1β secretion. Cell Res 2015;25:1285-98.
18. Chen Y, Sun XB, Lu HE, Wang F, Fan XH. Effect of luteoin in delaying cataract in STZ-induced diabetic rats. Arch Pharm Res 2017;40:88-95.
19. Kang KA, Piao MJ, Ryu YS, Hyun YJ, Park JE, Shilnikova K, *et al.* Luteolin induces apoptotic cell death via antioxidant activity in human colon cancer cells. Int J Oncol 2017;51:1169-78.
20. Park JE, Kim E. Effects of luteolin on chemical induced colon carcinogenesis in high fat diet-fed obese mouse. J Nutr Health 2018;51:14.
21. Dong Z, Shang H, Chen YQ, Pan LL, Bhatia M, Sun J. Sulforaphane protects pancreatic acinar cell injury by modulating Nrf2-mediated oxidative stress and NLRP3 inflammatory pathway. Oxid Med Cell Longev 2016;2016:7864150.
22. Dupaul-Chicoine J, Arabzadeh A, Dagenais M, Douglas T, Champagne C, Morizot A, *et al.* The Nlrp3 inflammasome suppresses colorectal cancer metastatic growth in the liver by promoting natural killer cell tumoricidal activity. Immunity 2015;43:751-63.
23. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C (T)) method. Methods 2001;25:402-8.
24. Zhao R, Choi BY, Wei L, Fredimoses M, Yin F, Fu X, *et al.* Acetylshikonin suppressed growth of colorectal tumour tissue and cells by inhibiting the intracellular kinase, T-lymphokine-activated killer cell-originated protein kinase. Br J Pharmacol 2020;177:2303-19.
25. Gupta A, Mittal A, Kumar A. Nature's treasurer: Plants acting on colon cancer. J Stress Physiol Biochem 2011;7:217-31.
26. Dabrowska M, Uram L, Zielinski Z, Rode W, Sikora E. Oxidative stress and inhibition of nitric oxide generation underlie methotrexate-induced senescence in human colon cancer cells. Mech Ageing Dev 2018;170:22-9.
27. Park KW, Kundu J, Chae IG, Kim DH, Yu MH, Kundu JK, *et al.* Carnosol induces apoptosis through generation of ROS and inactivation of STAT3 signaling in human colon cancer HCT116 cells. Int J Oncol 2014;44:1309-15.
28. Schmitt M, Greten FR. The inflammatory pathogenesis of colorectal cancer. Nat Rev Immunol 2021;21:653-67.
29. Erdman SE, Poutahidis T. Roles for inflammation and regulatory T cells in colon cancer. Toxicol Pathol 2010;38:76-87.
30. Wang L, Xu ML, Xin L, Ma C, Yu GY, Saravanakumar K, Wang MH. Oxidative stress induced apoptosis mediated anticancer activity of *Rhus typhina* fruits extract in human colon cancer. Med Chem Res 2019;28:917-25.
31. Wang H, Wang Y, Du Q, Lu P, Fan H, Lu J, *et al.* Inflammasome-independent NLRP3 is required for epithelial-mesenchymal transition in colon cancer cells. Exp Cell Res 2016;342:184-92.
32. Rogler G, Vavricka S. Exposome in IBD: Recent insights in environmental factors that influence the onset and course of IBD. Inflamm Bowel Dis 2015;21:400-8.
33. Chen C, Ma XQ, Yang CJ, Nie W, Zhang J, Li HD, *et al.* Hypoxia potentiates LPS-induced inflammatory response and increases cell death by promoting NLRP3 inflammasome activation in pancreatic β cells. Biochem Biophys Res Commun 2018;495:2512-8.
34. Zhao CC, Xu J, Xie QM, Zhang HY, Fei GH, Wu HM. Abscisic acid suppresses the activation of NLRP3 inflammasome and oxidative stress in murine allergic airway inflammation. Phytother Res 2021;35:3298-309.
35. Ning ZW, Luo XY, Wang GZ, Li Y, Pan MX, Yang RQ, *et al.* MicroRNA-21 mediates angiotensin II-induced liver fibrosis by activating NLRP3 Inflammasome/IL-1β axis via targeting Smad7 and Spry1. Antioxid Redox Signal 2017;27:1-20.
36. Lee MN, Lee Y, Wu D, Pae M. Luteolin inhibits NLRP3 inflammasome activation via blocking ASC oligomerization. J Nutr Biochem 2021;92:108614.
37. Fazio V, Robertis M, Massi E, Poeta M, Carotti S, Morini S, *et al.* The AOM/DSS murine model for the study of colon carcinogenesis: From pathwaysto diagnosis and therapy studies. J Carcinog 2011;10:9.