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Coix seed as a microbiota-targeted functional food: Postbiotic inosine mediates protection against chemotherapy-induced intestinal mucositis

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ABSTRACT: 5-Fluorouracil (5-FU)-induced intestinal mucositis (IM) is a dose-limiting complication of chemotherapy that severely compromises patient quality of life and treatment adherence. Coix seed (CS), a traditional cereal grain with established “medicine food homology” status in East Asia, has demonstrated potential benefits for intestinal health. However, the protective mechanisms of this functional food against chemotherapy-induced gastrointestinal toxicity remain unexplored. Here, we demonstrate for the first time that dietary CS supplementation effectively alleviates 5-FU-induced IM without compromising antitumor efficacy. Integrated 16S rDNA sequencing revealed that CS ameliorated 5-FU-induced gut microbiota dysbiosis by suppressing pathogenic bacteria while enriching beneficial commensals. Transcriptomic analysis identified the PI3K/Akt-NF- κ B pathway as the key inflammatory cascade inhibited by CS. Notably, untargeted metabolomics demonstrated that CS promoted the accumulation of gut microbiota-derived inosine, a postbiotic purine metabolite. In vitro anaerobic fermentation confirmed that CS serves as a fermentable substrate for beneficial bacteria to produce inosine. Importantly, exogenous inosine administration recapitulated the gastroprotective effects of CS by suppressing PI3K/Akt-NF- κ B-mediated inflammation through adenosine A₁ receptor activation. These findings establish a novel “diet-microbiota-metabolite-health” axis linking dietary CS intake to intestinal protection, providing scientific evidence for developing microbiota-targeted functional foods for the nutritional management of chemotherapy-induced IM.

Keywords: 5-fluorouracil; Intestinal mucositis; Coix Seed; Functional food; Gut microbiota; Inosine

1. Introduction

Chemotherapy-induced intestinal mucositis (IM) represents a critical clinical challenge that frequently necessitates dose reduction or discontinuation of cancer treatment. 5-Fluorouracil (5-FU), a cornerstone chemotherapeutic agent for breast and colorectal carcinomas, induces IM in 50%–80% of treated patients, manifesting as intractable diarrhea, malabsorption, gastrointestinal hemorrhage, and emesis ^[1]. These debilitating complications severely compromise treatment adherence and patient quality of life, often compelling therapy abandonment. Although the precise pathophysiology of 5-FU-induced IM remains to be fully elucidated, accumulating evidence implicates the Nuclear Factor- κ B (NF- κ B)-mediated inflammatory cascade as a central driver of mucosal damage ^[2–4]. Current management strategies rely predominantly on

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symptomatic interventions, such as loperamide and octreotide, which provide neither substantial nor sustained therapeutic benefit [3, 5]. Thus, there is an urgent unmet need for effective preventive and therapeutic approaches against 5-FU-induced IM.

Emerging evidence has established the gut microbiota—comprising trillions of metabolically active microorganisms—as a pivotal environmental determinant of host physiology and disease susceptibility [6–8]. Notably, the composition and metabolic activity of gut microbiota are profoundly influenced by dietary factors, positioning nutrition as a key modulator of intestinal health. However, 5-FU administration severely disrupts this delicate microbial ecosystem through multiple interconnected mechanisms. As a thymidylate synthase inhibitor, 5-FU disrupts DNA synthesis in rapidly dividing intestinal epithelial cells, leading to villus atrophy, crypt hypoplasia, and compromised barrier integrity [9]. This epithelial damage fundamentally alters the intestinal microenvironment, including oxygen tension, nutrient availability, bile acid composition, and antimicrobial peptide secretion, thereby creating ecological niches that preferentially support pathogenic bacteria [10]. Furthermore, 5-FU-induced intestinal inflammation generates reactive oxygen species (ROS) and pro-inflammatory cytokines (IL-6, TNF- α , IL-1 β), establishing a pro-oxidative environment that selectively favors the proliferation of facultative anaerobic pathogens while suppressing obligate anaerobes [9]. Even a single intraperitoneal injection of 5-FU substantially depletes beneficial commensals, including *Bifidobacterium* and *Lactobacillus*, while concurrently enriching potentially pathogenic bacteria such as *Escherichia*, *Clostridium*, and *Enterococcus*. This microecological perturbation exhibits a robust association with IM pathogenesis [11], as dysbiosis can exacerbate mucosal injury and amplify inflammatory responses [9, 12]. Compelling mechanistic support derives from observations that antibiotic-mediated microbial depletion attenuates IM severity and reduces pro-inflammatory cytokine levels in 5-FU-treated mice [12]. These findings collectively suggest that dietary interventions targeting gut microbiota homeostasis may represent a promising nutritional strategy for ameliorating 5-FU-induced IM.

The emerging concept of “food as medicine” has garnered increasing attention in the management of chemotherapy-related complications. For example, dietary polysaccharides represent a major class of nutrients that escape digestion by host enzymes in the upper gastrointestinal tract and reach the colon largely intact [13]. There, they serve as primary fermentable substrates for gut microbiota, particularly beneficial anaerobic bacteria such as *Bifidobacterium*, *Lactobacillus*, *Faecalibacterium*, and *Akkermansia*. These commensal bacteria possess specialized enzymatic repertoires that enable the degradation of complex polysaccharides into oligosaccharides and monosaccharides [14]. The subsequent microbial fermentation of these carbohydrates generates a diverse array of bioactive metabolites that exert profound effects on intestinal epithelial barrier function, immune homeostasis, and inflammatory signaling, including short-chain fatty acids, amino acid derivatives, phenolic compounds, and purine nucleosides [15]. Importantly, dietary nutrients serve as essential substrates for microbial metabolite production, establishing a critical “diet-microbiota-metabolite-health” axis [16]. This paradigm opens new avenues for preventing chemotherapy-induced IM through functional food interventions that modulate gut microbial metabolism.

Coix seed (CS), the mature kernel of *Coix lacryma-jobi* L. (Poaceae), represents a paradigmatic example of “medicine food homology—a traditional concept in Asian cultures where certain foods possess both nutritional and therapeutic value. As a cereal grain consumed for over 2,000 years in East Asia, CS is rich in bioactive constituents including coixenolide, coixol, polysaccharides, dietary fiber, essential amino acids, and unsaturated fatty acids ^[17]. Modern pharmacological studies have substantiated its traditional applications, revealing pronounced anti-inflammatory, immunomodulatory, and gut microbiota-regulating properties ^[18]. Notably, microbial fermentation of CS polysaccharides has been shown to biotransform these compounds into highly bioavailable metabolites, including bioactive peptides, phenolic compounds, short-chain fatty acids, and purine nucleosides, thereby significantly amplifying their therapeutic potential ^[19]. The Chinese National Health Commission has officially classified CS as both a food and a traditional Chinese medicine, underscoring its safety profile for long-term dietary consumption. However, whether this traditional functional food confers protection against 5-FU-induced IM—and if so, whether this effect is mediated through the gut microbiota-metabolite axis—remains unexplored.

In this study, we demonstrate for the first time that dietary supplementation with CS effectively alleviates 5-FU-induced IM without compromising antitumor efficacy. Integrating 16S rDNA sequencing, transcriptomics, and metabolomics approaches, we reveal that CS restores gut microbiota homeostasis, suppresses PI3K/Akt-NF- κ B-mediated inflammation, and promotes the accumulation of the microbial metabolite inosine. Importantly, exogenous inosine administration recapitulates the protective effects of CS. These findings establish a mechanistic framework for CS-mediated IM protection via the diet-gut microbiota-metabolite axis, providing scientific evidence for the development of microbiota-targeted functional food interventions in the nutritional management of chemotherapy-induced gastrointestinal toxicity.

2. Materials and methods

2.1 Chemicals and reagents

The CS used in this study was acquired from Guoda drug store Co., Ltd. (Shenyang, China) and Dr. Dong Wang of Shenyang Pharmaceutical University verified its identity as the seeds of *Coix lacryma-jobi* L. A water extraction method was employed to obtain CS extract preparation. Briefly, 100 g of CS powder (passed through 60-mesh sieve) was extracted with water (1:10 w/v ratio) under reflux conditions at 100 °C and atmospheric pressure (101.3 kPa), twice, each time for 1 hour. The combined water extracts were concentrated under reduced pressure and then lyophilized to obtain the dried CS extract powder.

5-FU, inosine (Ino) and loperamide (Lop) were acquired from Chengdu Chroma Biotechnology Ltd. in Sichuan, China. Their purity standards were at least 98.0%. Thermo Fisher Scientific of New Jersey, USA, supplied the acetonitrile and formic acid that were up to HPLC standards. All of the water utilized in the study came from Wahaha Co., Ltd. in Hangzhou, China.

2.2. Animal experiments

A group of male BALB/c mice aged 6 to 8 weeks were obtained from Shenyang Pharmaceutical University (Quality Certification No. SCXK2021-0009) and housed in a specific pathogen-free environment. All experimental procedures were formally approved by the Institutional Animal Care and Use Committee of Shenyang Pharmaceutical University (Approval No. SYPUIACUC-20240311021). The experimental design of the animal experiments was shown in the Supplementary Figure. S1.

To establish a syngeneic tumor model, 4T1 cells (2×10^6) were subcutaneously inoculated into the right flank of the mice. Once the tumor volume reached approximately 200 mm³, the animals were randomly allocated into four experimental groups (n = 6 per group). Group I served as the control and received daily oral administration of normal saline. Groups II (5-FU), III (5-FU+CS_L), and IV (5-FU+CS_H) were intraperitoneally administered 5-fluorouracil (5-FU, 50 mg/kg) on days 1–5. Additionally, from days 1–7, groups III and IV were orally administered CS at doses of 0.6 g/kg and 1.2 g/kg, respectively. All animals were euthanized on day 8 for further analysis.

For the non-tumor-bearing model, Group I (control) received daily oral administration of normal saline. Groups II (5-FU), III (5-FU+CS_L), IV (5-FU+CS_H), and V (5-FU+Lop) were treated with 5-fluorouracil (5-FU, 50 mg/kg, intraperitoneal injection) from days 1 to 5. Additionally, from days 1 to 7, Groups III and IV were administered CS via oral gavage at doses of 0.6 g/kg and 1.2 g/kg, respectively. In Group V, mice received an additional anti-diarrheal treatment consisting of loperamide (2 mg/kg, oral gavage, twice daily) on days 4–7. All animals were euthanized on day 8 for subsequent analysis.

In the inosine treatment study, Group I (control) received daily oral gavage of normal saline. Groups II (5-FU), III (5-FU+Ino_L), and IV (5-FU+Ino_H) were administered 5-fluorouracil (5-FU, 50 mg/kg, intraperitoneal injection) daily from days 1 to 5. Concurrently, from days 1 to 7, Groups III and IV were supplemented with inosine via oral gavage at doses of 200 mg/kg and 400 mg/kg, respectively. All mice were humanely euthanized on day 8 for endpoint analysis.

2.3. Histological evaluation and inflammatory cytokine analysis

The pathological alterations in the colon were distinguished by means of hematoxylin and eosin (HE) staining via a slightly modified methodology from a prior study^[20]. Commercial kits from the Jiancheng Bio-engineering Institute (Nanjing, China) were used to quantify interleukin (IL)-6, IL-1 β , and TNF- α concentrations in the colon, following the instructions given by the manufacturer. All analyses were performed using samples from all six mice per group (n = 6).

2.4. Gut microbiota analysis

16S rDNA gene sequencing was performed by Metware Biotechnology Co., Ltd. (Wuhan, China). Briefly, total genomic DNA was extracted from colon contents, and the V3-V4 hypervariable region of 16S rDNA was amplified and sequenced on an Illumina NovaSeq platform. Detailed procedures are provided in the Supplementary Materials. Samples from all six mice per group were used for sequencing (n = 6).

2.5. Transcriptomic analysis

Transcriptomic analysis of colon tissues was conducted by Metware Biotechnology Co., Ltd. (Wuhan, China). Briefly, RNA was extracted using Trizol reagent, with library preparation and sequencing conducted on the Illumina HiSeq platform. Differentially expressed genes (DEGs) were identified using DESeq2 (adjusted P -value < 0.05 , $|\log_2 \text{Fold Change}| \geq 1$). Complete methodological details are provided in the Supplementary Materials. Four mice per group were randomly selected for transcriptomic analysis ($n = 4$).

2.6. Non-targeted metabolomics

The method described in our previous report was used for the non-targeted metabolomics analysis with slight modifications [21]. Supplementary materials provide detailed descriptions of the sample preparation protocols, chromatographic separation conditions, mass spectrometric acquisition parameters, and computational data processing workflows. Samples from all six mice per group were used for metabolomic analysis ($n = 6$).

2.7. Quantitative analysis of purine metabolites

In order to quantify inosine, deoxyinosine, guanosine, and deoxyguanosine, the LC-MS/MS system was used. Supplementary materials offer comprehensive details regarding the sample preparation, chromatographic settings, and mass spectrometry parameters.

2.8. Anaerobic fermentation conditions

Under anaerobic circumstances, 0.5 g of fresh feces from male Balb/c mice were mixed with Gifu anaerobic medium. The mixture was then cocultured with CS extract powder at concentrations of 10 and 20 mg/mL. After 24 hours of post-incubation, quantitative analysis of purine metabolites and bacterial composition was performed on the cultured samples.

2.9. Cell culture and treatment

The NCM460 cell line, a non-transformed human colonic mucosal epithelial cell model, was obtained from EallBio Biomedical Technology Co., Ltd. in Beijing, China. Cells were maintained in Dulbecco's Modified Eagle Medium containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin antibiotic mixture. Following 80%–90% cell confluence, the cells were exposed to 5-FU (5.0 $\mu\text{mol/L}$) alone or in combination with inosine, deoxyinosine, guanosine, or deoxyguanosine (0.1, 0.25, 0.5, 1.0, or 2.0 mmol/L) for 48 h. A methyl thiazolyl tetrazolium (MTT; Solarbio, Beijing, China) test kit was used to evaluate cell viability, following the method previously reported [22].

2.10. Quantitative real-time PCR

Total RNA was extracted from colon tissue using Trizol reagent (Transgen, China) and quantified spectrophotometrically. cDNA synthesis and real-time PCR were performed using a SYBR kit (Cwbio

Century Biotechnology Co., China) on a CFX-96 system (Bio-RAD, Singapore). Thermal cycling conditions were: 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Relative expression was calculated using the $2^{-\Delta\Delta C_t}$ method with β -actin as reference. Primer sequences are listed in Supplementary Table S1.

2.11. Western blot analysis

Protein lysates from colon tissue were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then electro-transferred onto a polyvinylidene difluoride membrane (Merck, Darmstadt, Germany). The incubation of membrane was conducted with primary antibodies that targeted p-AKT (phospho T308, 1:1000, ab278565, Abcam), NF- κ B pp65 (phosphorylation site Ser536, 1:1000, 3033T, CST), COX2 (1:1000, ab179800, Abcam), A₁R (1:1000, 20332-1-AP, Proteintech) and β -Actin (1:1000, 4970T, CST) after being blocked using a Western Quick Block Kit (Genefist, Nanjing, China). The next step was to incubate the membrane for 2-3 h with secondary antibodies coupled to horseradish peroxidase. An imaging system for digital gels (Tanon-5200Multi, Tanon, Shanghai, China) was employed for protein signal visualization and detection.

2.12. Statistical analysis

Statistical analyses were performed using SPSS v19.0. Data are presented as mean $\pm$ SD. Comparisons between multiple groups were analyzed by one-way ANOVA, while two-group comparisons used unpaired Student's t-test. Statistical significance was defined as: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ versus control group; # $P < 0.05$, ## $P < 0.01$, and ### $P < 0.001$ versus 5-FU treatment group.

3. Results

3.1 Dietary CS supplementation preserved the antitumor efficacy of 5-FU in a breast cancer model

A primary consideration for any dietary adjuvant therapy during chemotherapy is whether it compromises anticancer efficacy. To address this critical question, we evaluated the potential modulatory effects of CS on 5-FU chemotherapeutic activity using a syngeneic 4T1 breast cancer model in mice. As illustrated in Figure 1A, 5-FU monotherapy significantly suppressed tumor growth compared to vehicle-treated controls ($P < 0.001$). Importantly, concomitant dietary supplementation with CS at either low (0.6 g/kg) or high (1.2 g/kg) doses did not significantly alter tumor growth kinetics compared to 5-FU monotherapy, indicating that CS neither potentiates nor attenuates the antitumor activity of 5-FU under these experimental conditions. Consistent with tumor volume measurements, endpoint analysis of excised tumors revealed that 5-FU treatment induced a marked reduction in tumor weight relative to controls ($P < 0.001$; Figure 1B–C). Notably, no statistically significant differences in tumor weight were observed between the combination therapy groups (5-FU+CS_L and 5-FU+CS_H) and the 5-FU monotherapy group ($P > 0.05$). These findings collectively demonstrate that dietary CS supplementation does not interfere with the tumor-suppressive efficacy of 5-FU,

thereby establishing a foundation for investigating its potential protective effects against chemotherapy-induced gastrointestinal toxicity.

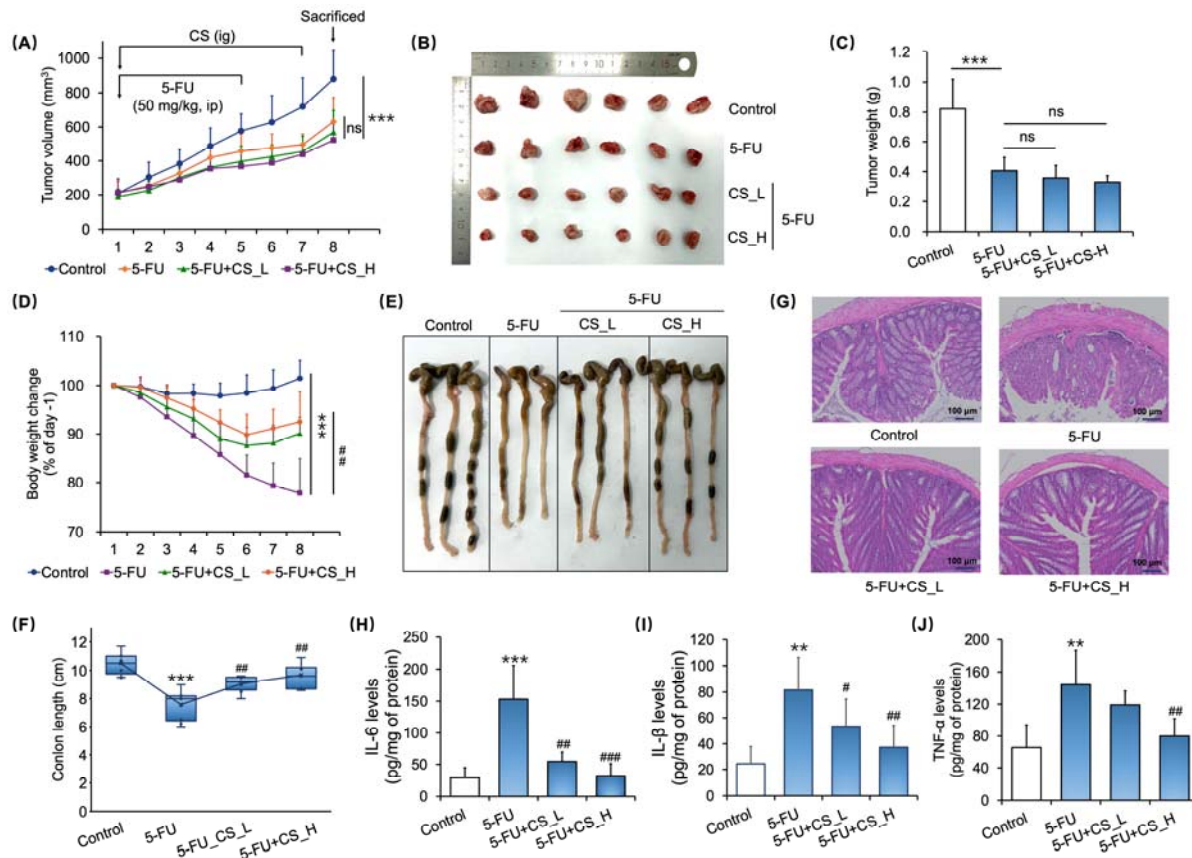


Fig. 1 CS mitigated 5-FU-induced IM without compromising 5-FU anti-tumor efficiency. (A) Tumor growth curves. (B) Macroscopic appearance of tumors. (C) Tumor weights recorded at the experimental endpoint. (D) Daily body weight changes during the treatment. (E) Macroscopic appearance of colon tissues. (F) Colon length measured. (G) Histological assessment of colon tissues via HE staining. (H–J) Concentrations of pro-inflammatory cytokines in colon tissues. Significance: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ versus control group; # $P < 0.05$, ## $P < 0.01$, and ### $P < 0.001$ versus 5-FU treatment group. ns, non-significant variations.

3.2. Dietary CS intervention effectively alleviated 5-FU-induced intestinal mucositis in both tumor-bearing and non-tumor-bearing mice

Having established that dietary CS supplementation does not compromise 5-FU antitumor efficacy, we next investigated whether CS confers protective effects against chemotherapy-induced gastrointestinal toxicity. Strikingly, while 5-FU monotherapy induced pronounced body weight loss—a hallmark of systemic toxicity and reduced food intake—concomitant CS supplementation significantly attenuated this adverse effect (Figure 1D). Upon necropsy on day 8 post-treatment, macroscopic and morphometric analyses revealed that the 5-FU-treated group exhibited marked colon shortening compared to vehicle-treated controls, a characteristic manifestation of severe intestinal mucositis. Notably, dietary CS co-administration dose-dependently attenuated this 5-FU-induced colon shortening, providing initial evidence for its gastrointestinal protective effects (Figure 1E–F). Histopathological examination using hematoxylin and eosin (HE) staining further corroborated these macroscopic observations. Mice receiving 5-FU monotherapy displayed severe mucosal damage characterized by villus blunting, crypt destruction, and epithelial denudation. In contrast, dietary CS supplementation substantially preserved intestinal mucosal architecture, with notably improved

villus height and crypt integrity (Figure 1G). These histological findings provide direct morphological evidence that CS protects against 5-FU-induced intestinal barrier disruption. Given the central role of inflammatory cascades in IM pathogenesis, we further quantified pro-inflammatory cytokine levels in colonic tissues. As illustrated in Figure 1H–J, 5-FU treatment dramatically elevated colonic concentrations of IL-6, IL-1 β , and TNF- α compared to controls. Remarkably, dietary CS supplementation significantly suppressed these pro-inflammatory cytokines in a dose-dependent manner compared to 5-FU monotherapy. These results demonstrate that CS exerts potent anti-inflammatory activity in the context of chemotherapy-induced intestinal inflammation.

To exclude potential confounding effects from tumor burden, we validated these findings in non-tumor-bearing mice. Consistent with observations in the tumor model, dietary CS intervention comparably prevented 5-FU-induced body weight loss, colon shortening, histopathological damage, and pro-inflammatory cytokine elevation (Figure 2). Collectively, these comprehensive analyses demonstrate that dietary CS supplementation effectively mitigates 5-FU-induced intestinal mucositis through attenuation of mucosal inflammation, independent of tumor status. These findings position CS as a promising functional food candidate for the nutritional management of chemotherapy-associated gastrointestinal complications.

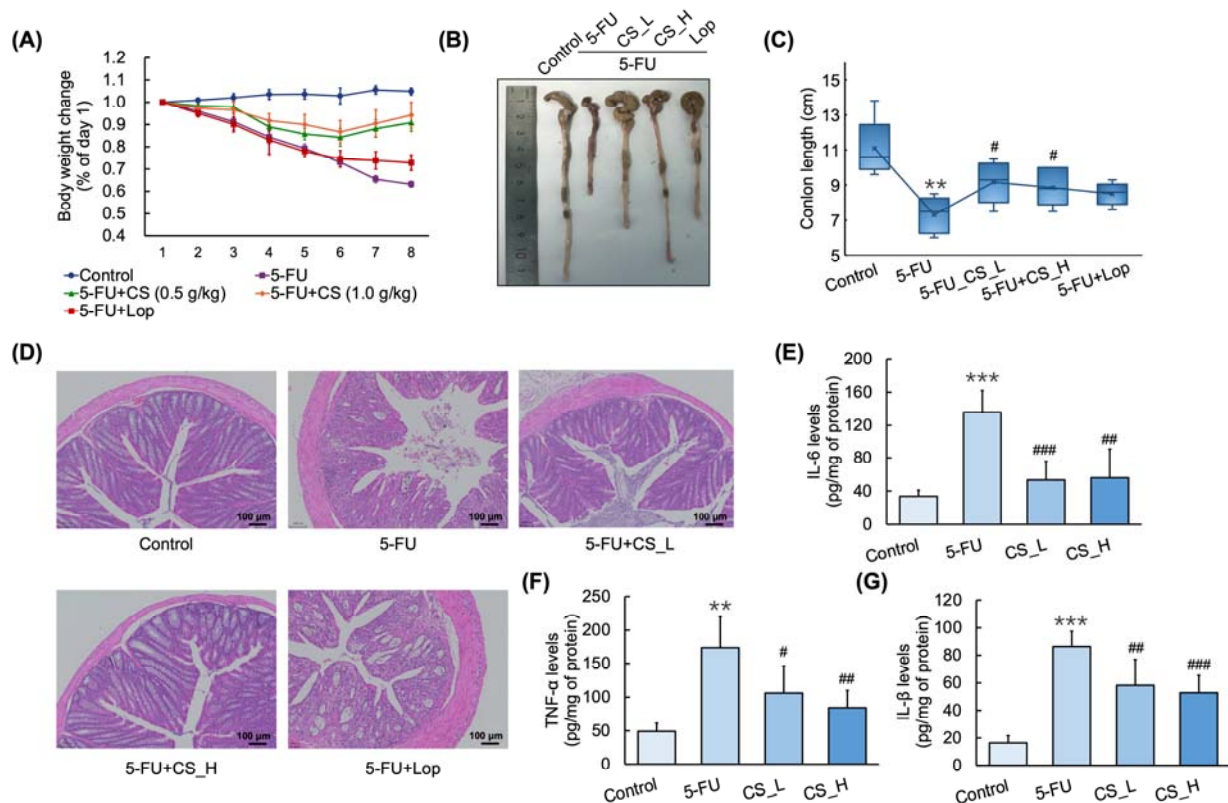


Fig. 2 CS mitigated 5-FU-induced IM in a non-tumor animal model. (A) Daily body weight changes during the treatment. (B) Macroscopic appearance of colon tissues. (C) Colon length measured. (D) HE staining of colon tissues. (E–G) Concentrations of pro-inflammatory cytokines in colon tissues. Significance: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ versus control group; # $P < 0.05$, ## $P < 0.01$, and ### $P < 0.001$ versus 5-FU treatment group.

3.3. Dietary CS supplementation restored gut microbiota homeostasis disrupted by 5-FU treatment

Gut microbiota dysbiosis has emerged as a critical mediator of chemotherapy-induced intestinal mucositis, and dietary components are increasingly recognized as key modulators of microbial community

structure and function [23]. To investigate whether the gastroprotective effects of CS are associated with modulation of the gut microbiome, we performed 16S rDNA sequencing analysis of colonic contents. Venn diagram analysis revealed distinct patterns of shared and unique operational taxonomic units (OTUs) across experimental groups (Figure 3A), while rarefaction and rank-abundance curves confirmed adequate sequencing depth and coverage for reliable downstream analysis (Figure 3B–C). UniFrac-based principal coordinates analysis (PCoA) revealed pronounced shifts in gut microbial community structure following 5-FU treatment, with clear separation between the 5-FU group and healthy controls along the primary coordinate axes (Figure 3D). Notably, dietary CS supplementation substantially restored the microbial community profile toward that of untreated controls, suggesting that CS attenuated 5-FU-induced disruptions in microbial community structure. This observation aligns with the established role of dietary factors in shaping gut microbiota composition and resilience. Consistent with β -diversity findings, α -diversity metrics further substantiated the microbiota-protective effects of CS. The Chao1 index, reflecting microbial richness, and the Shannon index, indicating community diversity, were both significantly diminished following 5-FU treatment compared to controls. Dietary CS supplementation significantly attenuated the reduction in species richness (Chao1 index), though no significant improvement was observed in the Shannon diversity index (Figure 3E), suggesting that CS may partially preserve microbial community complexity during chemotherapeutic stress.

Taxonomic profiling at both phylum and genus levels revealed specific microbial signatures associated with 5-FU-induced dysbiosis and CS-mediated protection. At the phylum level, 5-FU treatment markedly elevated the relative abundance of Proteobacteria and Campylobacterota—phyla consistently associated with intestinal inflammation and epithelial barrier dysfunction (Figure 3F). Dietary CS supplementation effectively suppressed the bloom of these potentially pathogenic taxa. At the genus level, 5-FU induced a pronounced expansion of *Enterobacteriaceae*, a well-established biomarker of gut dysbiosis and inflammatory states [24]. Remarkably, CS co-administration significantly inhibited this *Enterobacteriaceae* bloom, consistent with its anti-inflammatory effects observed at the tissue level. LEfSe analysis further identified discriminative microbial taxa across treatment groups, corroborating the distinct microbiota signatures observed in compositional analyses (Figure 3G). Collectively, these microbiome analyses demonstrate that dietary CS supplementation effectively counteracts 5-FU-induced gut dysbiosis by preserving microbial diversity, maintaining community stability, and suppressing the expansion of pro-inflammatory bacterial taxa. These findings support the concept that CS functions as a prebiotic-like functional food that maintains gut microbial homeostasis during chemotherapy, thereby contributing to its protective effects against intestinal mucositis.

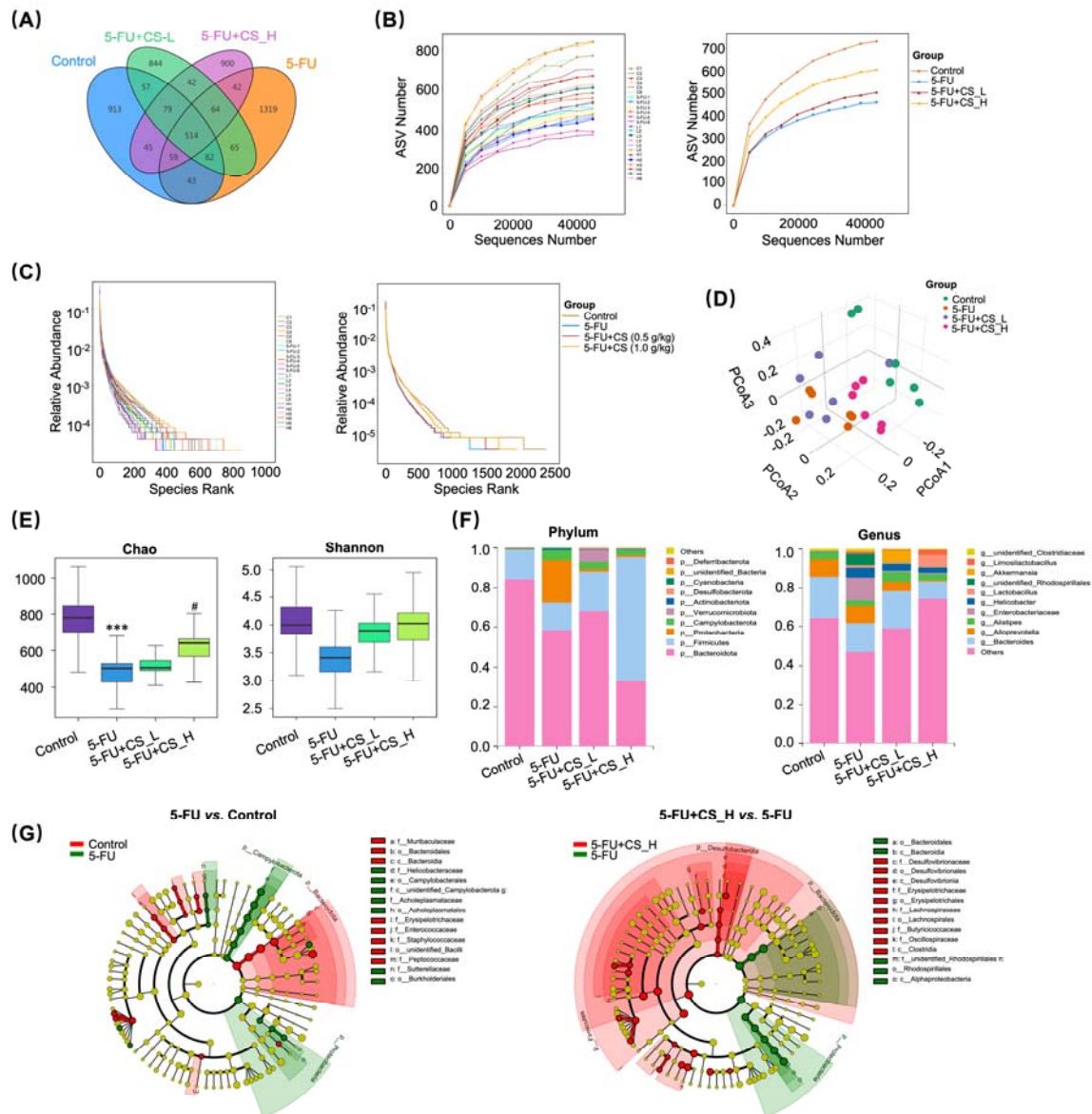


Fig. 3 CS mitigated 5-FU-induced gut microbiota dysbiosis. (A) Venn diagram illustrating shared and unique operational taxonomic units among treatment groups. (B) Rarefaction curves demonstrating sequencing depth adequacy and sample coverage. (C) Rank-abundance curves reflecting species evenness and richness within microbial communities. (D) PCoA plot of gut microbiota composition. (E) Alpha diversity analysis, such as the Chao1 index (richness) and Shannon index (diversity). (F) The phylum and genus levels of the taxonomy of the gut microbiota. (G) Cladogram of significant taxonomic changes at all levels based on LefSe analysis. Significance: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ versus control group; # $P < 0.05$, ## $P < 0.01$, and ### $P < 0.001$ versus 5-FU treatment group. $n = 6$ per group.

3.4. Transcriptomic profiling revealed that dietary CS supplementation suppressed the PI3K/Akt-NF- κ B inflammatory signaling cascade

To elucidate the molecular mechanisms underlying the protective effects of dietary CS against 5-FU-induced intestinal mucositis, we performed comprehensive transcriptomic profiling of murine colonic tissues using RNA sequencing. Principal component analysis (PCA) revealed distinct gene expression signatures across experimental groups (Figure 4A). Notably, while 5-FU treatment induced substantial transcriptional reprogramming with clear separation from controls, dietary CS supplementation markedly shifted the

expression profile toward that of untreated animals, suggesting that CS counteracts 5-FU-induced transcriptional perturbations at the global level.

Differential expression analysis identified 919 significantly altered genes between control and 5-FU groups, while 824 and 1,464 differentially expressed genes (DEGs) were detected between 5-FU versus 5-FU + CS_L and 5-FU versus 5-FU + CS_H comparisons, respectively ($P < 0.05$; $|\log_2FC| > 1$; Figure 4B). Volcano plot and heatmap visualizations further delineated these transcriptional changes: 5-FU treatment upregulated 554 genes and downregulated 365 genes compared to controls, whereas high-dose CS supplementation reversed a substantial proportion of these alterations, with 1,088 genes suppressed and 376 genes elevated relative to 5-FU monotherapy (Figure 4C–D). This dose-dependent transcriptional modulation suggests that dietary CS exerts broad regulatory effects on the intestinal gene expression program disrupted by chemotherapy.

To identify the signaling networks mediating CS-conferred protection, we performed pathway enrichment analysis on DEGs between 5-FU and 5-FU + CS_H groups. Strikingly, the PI3K/Akt signaling pathway emerged as one of the most significantly enriched pathways (Figure 4E). This pathway serves as a central hub integrating cellular metabolism, survival, and inflammatory responses, and has been increasingly implicated in diet-mediated modulation of intestinal homeostasis (Zhou et al., 2022). Heatmap analysis of PI3K/Akt-related DEGs and gene set enrichment analysis (GSEA) consistently demonstrated that genes within this pathway were predominantly downregulated following dietary CS supplementation (Figure 4F–G), indicating that CS suppresses PI3K/Akt signaling activation. The NF- κ B signaling cascade, a critical downstream effector of PI3K/Akt, orchestrates the expression of pro-inflammatory mediators and has been established as a key driver of 5-FU-induced intestinal mucositis pathogenesis (Dahlgren et al., 2021; Guo et al., 2024). GSEA revealed significant downregulation of the NF- κ B pathway following dietary CS administration (Figure 4H). To validate these transcriptomic findings, we quantified the mRNA expression of key pathway components using qRT-PCR. Consistent with sequencing data, 5-FU treatment dramatically upregulated PI3K, Akt, NF- κ B, and COX-2 transcript levels, while dietary CS supplementation markedly attenuated the expression of all four genes in a dose-dependent manner (Figure 4I).

Collectively, these transcriptomic analyses reveal that dietary CS supplementation exerts its anti-inflammatory effects by suppressing the PI3K/Akt-NF- κ B signaling axis, thereby attenuating pro-inflammatory mediator production in the intestinal mucosa. These findings provide mechanistic insight into how this traditional functional food ameliorates chemotherapy-induced gastrointestinal toxicity at the molecular level, and support the concept that bioactive food components can modulate host inflammatory signaling pathways to confer health benefits.

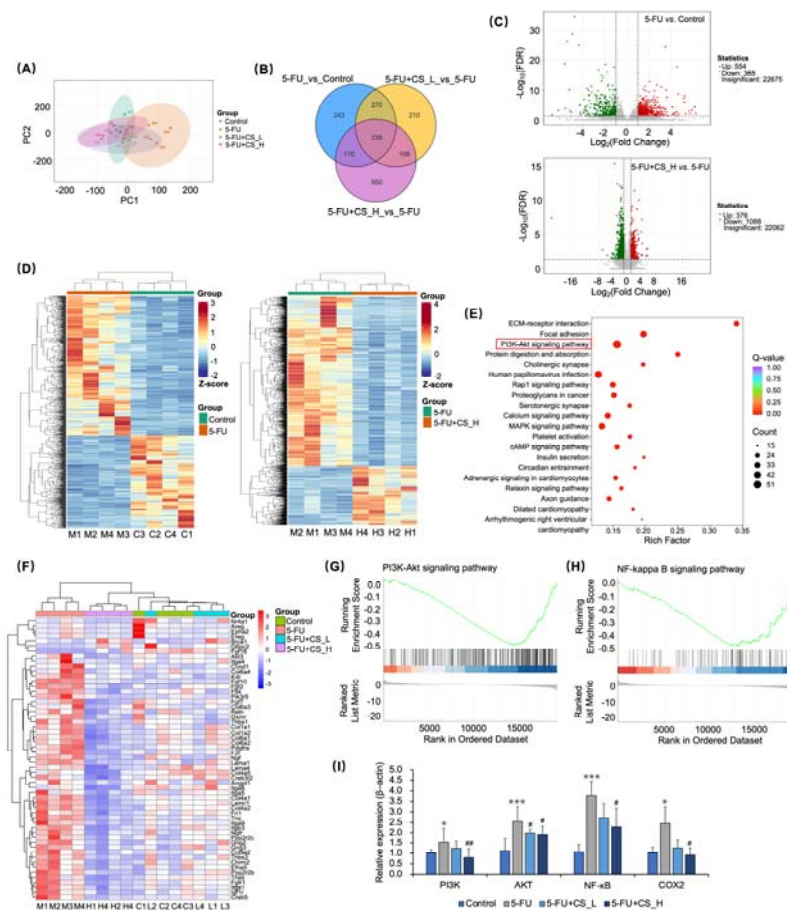


Fig. 4 CS modulated gut gene expression and inhibited the PI3K/Akt-NF-κB pathway. (A) Principal component analysis of transcriptional profiles across groups. (B) Venn diagram illustrating overlapping differentially expressed genes (DEGs). (C) Volcano plot highlighting significant DEGs. (D) Heatmap visualization of DEGs. (E) KEGG pathway analysis of enriched DEGs. (F) Heatmap comparing PI3K/Akt-related DEGs in 5-FU vs. 5-FU+CS_H groups. (G) Gene Set Enrichment Analysis (GSEA) of the PI3K/Akt pathway. (H) GSEA of the NF-κB pathway. (I) qPCR analysis of PI3K, Akt, NF-κB, and COX-2 mRNA levels in colon tissue. $n = 6$ per group for RNA sequencing analysis.

3.5. Untargeted metabolomics revealed that dietary CS supplementation enriched gut microbiota-derived purine metabolites

Gut microbiota-derived metabolites serve as critical molecular mediators of host-microbiome crosstalk, and their production is profoundly influenced by dietary substrate [25, 26]. Given the established role of CS as a fermentable food substrate, we hypothesized that dietary CS supplementation may exert its protective effects, at least in part, by modulating the gut metabolome through microbial biotransformation. To test this hypothesis, we performed untargeted metabolomic profiling of murine colonic contents using LC-MS/MS analysis.

Representative extracted ion chromatograms in both positive and negative ionization modes demonstrated excellent chromatographic resolution (Supplementary Figure S2). Rigorous quality control assessment revealed tight clustering of QC samples with low coefficients of variation for peak distributions, confirming analytical stability and reproducibility (Supplementary Figure S3). PCA score plots further validated data quality, with QC samples tightly grouped at the center of the plot (Figure 5A–B). Orthogonal partial least squares discriminant analysis (OPLS-DA) revealed clear metabolic separation among experimental groups (Figure 5C–D), indicating that both 5-FU treatment and dietary CS supplementation

induced distinct metabolic reprogramming in the gut environment. Differential metabolite analysis identified 58 significantly altered metabolites between 5-FU and 5-FU+CS_H groups, comprising 47 upregulated and 11 downregulated species (Figure 5E–F; Supplementary Table S2). This substantial metabolic remodeling underscores the capacity of dietary CS to reshape the intestinal metabolome during chemotherapy challenge. KEGG pathway enrichment analysis of differentially abundant metabolites revealed significant enrichment in multiple metabolic pathways associated with energy homeostasis and nutrient metabolism, including primary bile acid biosynthesis, cholesterol metabolism, and amino acid metabolism (Figure 5G). Notably, the purine metabolism pathway emerged as one of the most prominently enriched pathways following dietary CS supplementation, suggesting that CS may selectively modulate nucleoside metabolism in the gut microenvironment. Focused analysis of purine metabolites revealed that four key intermediates—inosine, deoxyinosine, guanosine, and deoxyguanosine—were markedly elevated in the colonic contents of CS-supplemented mice compared to 5-FU monotherapy controls. Targeted quantitative LC-MS/MS analysis validated these findings, confirming significant accumulation of all four purine nucleosides following dietary CS supplementation (Figure 5H).

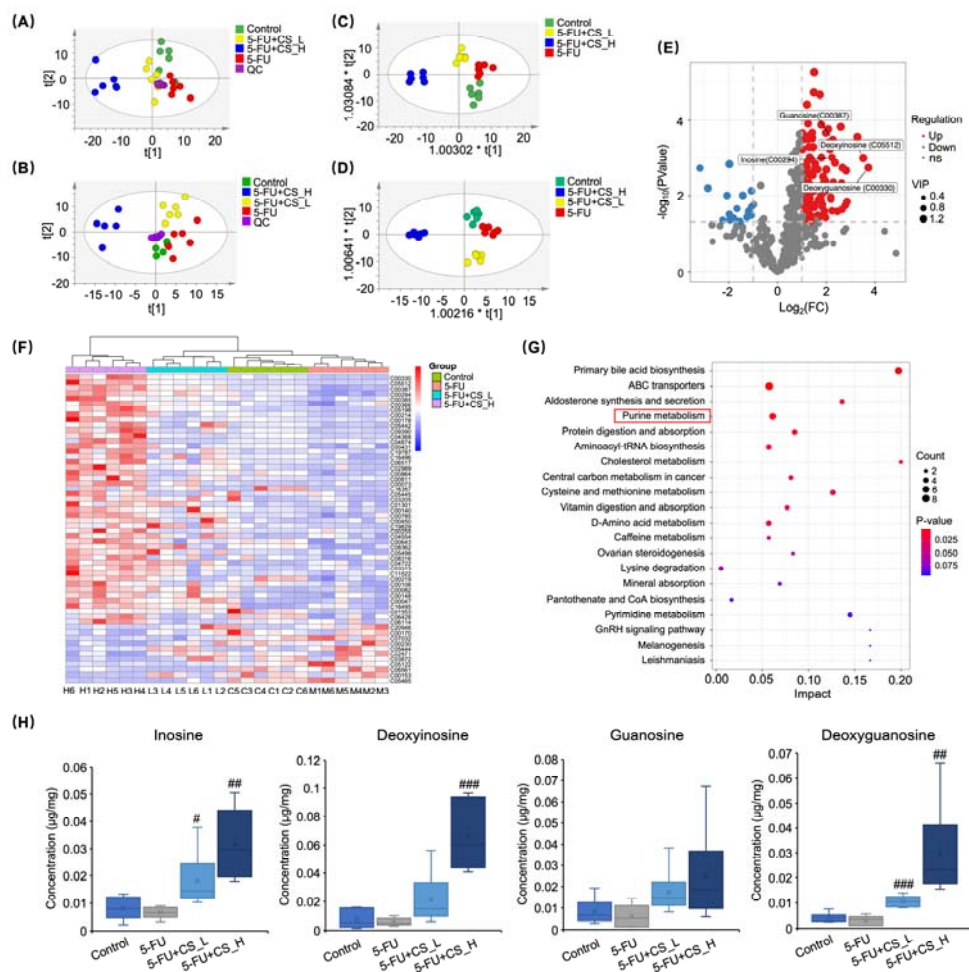


Fig. 5 The effects of CS on metabolic profile and the enrichment of purine metabolites produced by gut microbiota. (A–B) PCA score plots of metabolomic profiles. (C–D) OPLS-DA score plots of metabolomic profiles. (E) Volcano plot illustrating differentially expressed metabolites. (F) Heatmap depicting significantly altered metabolites. (G) Pathway enrichment analysis of metabolites with significant alteration. (H) Inosine, deoxyinosine, guanosine, and deoxyguanosine concentrations in colonic contents. Significance: Compared to the 5-FU group, $P < 0.05$ (#), $P < 0.01$ (##), or $P < 0.001$ (###). $n = 6$ per group.

Collectively, these metabolomic analyses demonstrate that dietary CS supplementation substantially remodels the gut metabolome, with particular enrichment of microbiota-derived purine metabolites. These findings support the emerging paradigm of the “diet-microbiota-metabolite” axis, wherein dietary components serve as substrates for microbial metabolism to generate bioactive metabolites that influence host physiology. The identification of purine nucleosides as CS-enriched metabolites provides a mechanistic link between this traditional functional food and its protective effects against chemotherapy-induced intestinal mucositis.

3.6. *In vitro* fermentation confirmed that gut microbiota mediates CS-induced purine metabolite production through selective enrichment of beneficial bacteria

To establish a causal relationship between gut microbiota and CS-induced purine metabolite enrichment, we performed *in vitro* anaerobic fermentation experiments using fresh murine fecal microbiota as the inoculum and CS as the fermentable substrate (Figure 6A). Notably, CS fermentation by gut microbiota dramatically elevated the concentrations of all four purine nucleosides—inosine, deoxyinosine, guanosine, and deoxyguanosine—compared to vehicle controls (Figure 6B). These results provide direct evidence that gut microbiota mediates CS-induced purine metabolite production through microbial biotransformation pathways, supporting the concept that dietary CS serves as a fermentable substrate for gut bacteria to generate bioactive metabolites.

To identify the specific bacterial taxa responsible for CS-mediated purine production, we characterized the microbial community composition following 24 h of *in vitro* fermentation using 16S rDNA sequencing. Principal coordinates analysis (PCoA) revealed distinct clustering between CS-fermented and control groups (Figure 6C), indicating that CS fermentation induces substantial restructuring of the microbial community. At the phylum level, Verrucomicrobiota exhibited a marked increase in relative abundance following CS fermentation (Figure 6D). Genus-level taxonomic analysis revealed striking enrichment of two well-established beneficial bacterial genera following CS fermentation. Specifically, *Akkermansia*—a next-generation probiotic increasingly recognized for its gut barrier-protective and anti-inflammatory properties—increased dramatically from $3.28\% \pm 1.42\%$ to $26.72\% \pm 7.06\%$. Similarly, *Bifidobacterium*, a classical probiotic genus with well-documented immunomodulatory and intestinal health-promoting effects, expanded from $0.22\% \pm 0.20\%$ to $15.09\% \pm 6.14\%$ (Figure 6E). These findings demonstrate that CS exhibits potent prebiotic-like activity, selectively promoting the growth of beneficial gut bacteria.

To establish a mechanistic link between bacterial enrichment and purine metabolite production, we performed Pearson correlation analysis. Both *Akkermansia* and *Bifidobacterium* abundances exhibited significant positive correlations with the concentrations of all four purine nucleosides ($P < 0.01$; Figure 6F). Notably, extensive genomic analyses have established that *Akkermansia* possesses complete *de novo* purine biosynthesis pathways with experimentally validated metabolic activity, while *Bifidobacterium* species have been directly shown to produce inosine through purine salvage pathways [27, 28]. These results implicate that *Akkermansia* and *Bifidobacterium* may as key contributors to CS-mediated inosine production.

Collectively, these *in vitro* fermentation studies establish a clear mechanistic link between dietary CS, gut microbiota composition, and purine metabolite production. The selective enrichment of *Akkermansia* and *Bifidobacterium* by CS fermentation reveals a prebiotic mechanism whereby this traditional functional food promotes beneficial bacteria that, in turn, generate bioactive purine metabolites with anti-inflammatory potential. These findings provide compelling evidence for the “food-microbiota-metabolite-health” paradigm and position CS as a promising microbiota-targeted functional food for gastrointestinal health promotion.

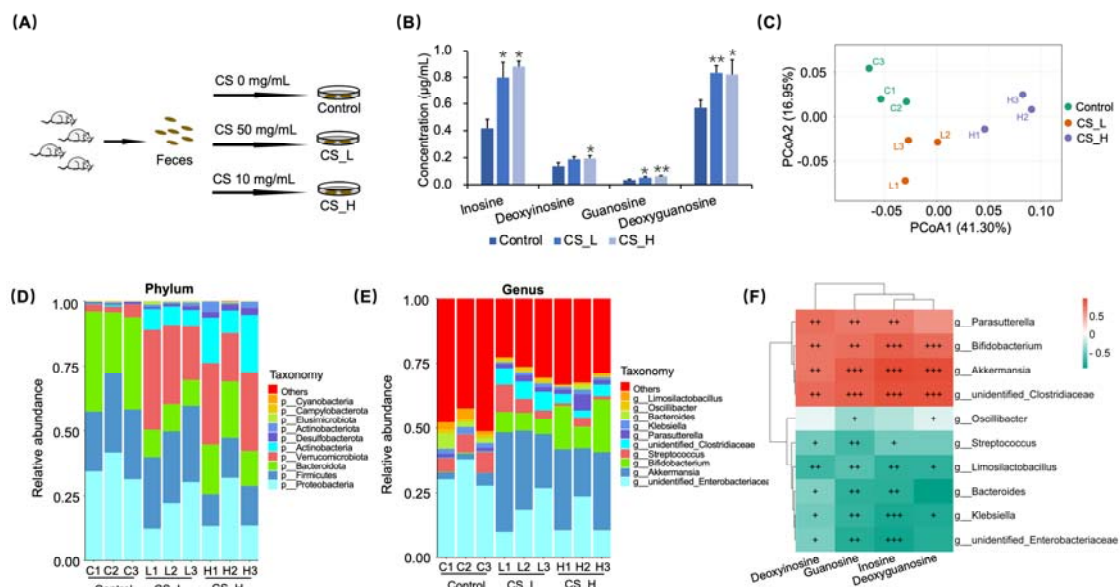


Fig. 6 Fermentation of CS modulated gut microbiota composition and facilitated the accumulation of purine metabolites. (A) Experimental design of the *in vitro* anaerobic fermentation. (B) Inosine concentrations in anaerobic cultures following 24 h of fermentation. (C) PCoA plot illustrating bacterial community composition. (D) Taxonomic distribution of bacterial composition at the phylum level. (E) Taxonomic distribution of bacterial composition at the genus level. (F) Pearson correlation analysis of the relative abundance of microbiota and concentrations of inosine, deoxyinosine, guanosine, and deoxyguanosine in anaerobic cultures.

3.7. The gut microbiota-derived metabolite inosine recapitulated the protective effects of dietary CS against 5-FU-induced intestinal mucositis via PI3K/Akt-NF-κB signaling suppression

Given the intimate proximity of gut microbiota-derived metabolites to the intestinal epithelium, we hypothesized that CS-enriched purine nucleosides may directly exert cytoprotective effects on intestinal epithelial cells. To test this hypothesis, we first evaluated the effects of inosine, deoxyinosine, guanosine, and deoxyguanosine on human colonic epithelial NCM460 cells *in vitro*. Cytotoxicity assays demonstrated that none of these purine metabolites exhibited significant toxicity at concentrations ranging from 0.1 to 2.0 mmol/L (Supplementary Figure S4). Notably, co-treatment with inosine or deoxyinosine significantly enhanced cell viability in 5-FU-challenged NCM460 cells, suggesting direct cytoprotective effects of these gut microbiota-derived purine nucleosides against chemotherapy-induced epithelial damage.

Among these metabolites, inosine has garnered particular attention as a bioactive purine nucleoside with documented anti-inflammatory and immunomodulatory properties [29]. Inosine, a naturally occurring purine nucleoside, has recently emerged as a bioactive metabolite with potent anti-inflammatory and immunomodulatory properties, showing therapeutic promise in various inflammatory conditions including

pneumonia and hepatitis [29–31]. Based on these findings and our observation that dietary CS supplementation substantially enriched intestinal inosine levels, we investigated whether exogenous inosine administration could recapitulate the protective effects of CS against 5-FU-induced intestinal mucositis. Notably, oral inosine supplementation effectively ameliorated multiple hallmarks of 5-FU-induced intestinal mucositis in mice. Inosine treatment significantly attenuated 5-FU-induced body weight loss and colon shortening in a dose-dependent manner (Figure 7A–C). Histopathological examination revealed that inosine supplementation substantially preserved intestinal mucosal architecture, with marked improvements in villus height and crypt integrity compared to 5-FU monotherapy controls (Figure 7D). These phenotypic improvements closely paralleled those observed with dietary CS supplementation, supporting the hypothesis that inosine serves as a key bioactive metabolite mediating the gastroprotective effects of this functional food. Consistent with its anti-inflammatory properties, inosine administration significantly suppressed the elevation of pro-inflammatory cytokines in colonic tissues. Specifically, the concentrations of IL-6, IL-1 β , and TNF- α were markedly reduced in inosine-treated mice compared to 5-FU monotherapy controls (Figure 7E). The magnitude of cytokine suppression achieved by inosine supplementation was comparable to that observed with dietary CS intervention, further supporting the role of this microbiota-derived metabolite as a functional effector of CS-mediated intestinal protection.

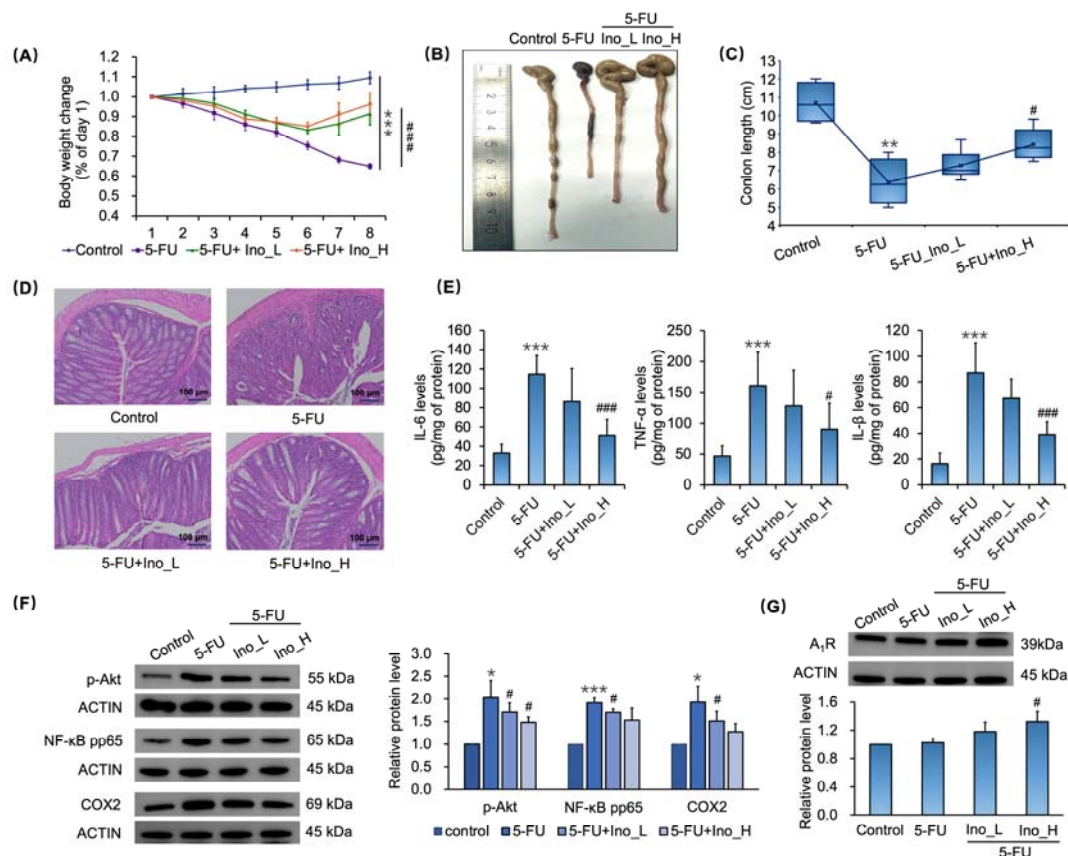


Fig. 7 Inosine relieved 5-FU-induced IM by means of the A₁R-PI3K/Akt-NF- κ B pathway. (A) Daily body weight fluctuations throughout the treatment regimen. (B) Macroscopic appearance of colon tissues. (C) Measurement of colon length at the experimental endpoint. (D) HE staining of colonic tissue. (E) Quantification of pro-inflammatory cytokine expression in colonic tissues. (F) Western blot analysis of p-Akt, NF- κ B pp65, and COX-2 expression in colonic tissues. (G) Western blot analysis of A₁R expression in colonic tissues. Significance: * P < 0.05, ** P < 0.01, and *** P < 0.001 versus control group; # P < 0.05, ## P < 0.01, and ### P < 0.001 versus 5-FU treatment group.

To elucidate the molecular mechanisms underlying inosine-mediated protection, we examined the activation status of the PI3K/Akt-NF- κ B signaling cascade—the key inflammatory pathway suppressed by dietary CS supplementation. Western blot analysis revealed that inosine treatment markedly attenuated the phosphorylation of Akt (p-Akt) and NF- κ B p65 (pp65), along with reduced expression of the downstream inflammatory mediator COX-2 (Figure 7F, Supplementary Figure S5). These molecular findings demonstrate that inosine suppresses the same inflammatory signaling axis as dietary CS, providing mechanistic evidence that this gut microbiota-derived purine nucleoside functions as a bioactive effector molecule in the CS-mediated attenuation of chemotherapy-induced intestinal inflammation. The demonstration also provided compelling evidence for the “diet-microbiota-metabolite-host” paradigm in gastrointestinal health.

3.8. Inosine suppresses PI3K/Akt-NF- κ B inflammatory signaling through adenosine A₁R activation and cAMP pathway modulation

Having established that inosine recapitulates the anti-inflammatory effects of dietary CS through PI3K/Akt-NF- κ B suppression, we next sought to elucidate the upstream molecular mechanism by which this gut microbiota-derived metabolite modulates this signaling cascade. Notably, our transcriptomic analysis revealed significant enrichment of the cAMP signaling pathway among CS-regulated genes (Figure. 4E). The cAMP pathway functions as a critical upstream regulator of PI3K/Akt signaling: adenylate cyclase (AC) catalyzes cAMP production, which subsequently activates Exchange protein directly activated by cAMP (Epac), thereby initiating the PI3K/Akt-NF- κ B inflammatory cascade [32–34]. Consistent with this mechanistic framework, transcriptomics analysis demonstrated that dietary CS supplementation significantly downregulated the expression of both AC and Epac genes in colonic tissues (Supplementary Figure S6). This observation suggests that CS-mediated suppression of the PI3K/Akt-NF- κ B pathway may involve upstream inhibition of the cAMP signaling cascade, providing a mechanistic link between dietary intervention and anti-inflammatory signaling. Notably, Inosine is a well-characterized endogenous agonist of the adenosine A₁ receptor (A₁R), a G_{i/o}-coupled receptor that inhibits AC activity upon activation, thereby suppressing cAMP production and downstream signaling [35]. Western blot analysis revealed that inosine treatment markedly upregulated A₁R protein expression in mouse colonic tissues compared to 5-FU monotherapy controls (Figure 7G, Supplementary Figure S5). This upregulation of A₁R expression, combined with the known agonistic activity of inosine at this receptor, provides mechanistic evidence that inosine activates A₁R signaling to suppress the cAMP-PI3K/Akt-NF- κ B inflammatory cascade.

Collectively, these findings delineate a complete mechanistic pathway linking dietary CS intake to anti-inflammatory protection against chemotherapy-induced intestinal mucositis (Figure 8). Specifically, dietary CS supplementation promotes the growth of beneficial gut bacteria (*Akkermansia* and *Bifidobacterium*), which metabolize CS components to generate inosine. This gut microbiota-derived purine nucleoside activates intestinal A₁R, inhibiting AC activity and suppressing cAMP production. The resultant downregulation of the cAMP-Epac-PI3K/Akt-NF- κ B signaling cascade attenuates pro-inflammatory cytokine expression, thereby protecting the intestinal mucosa from chemotherapy-induced damage.

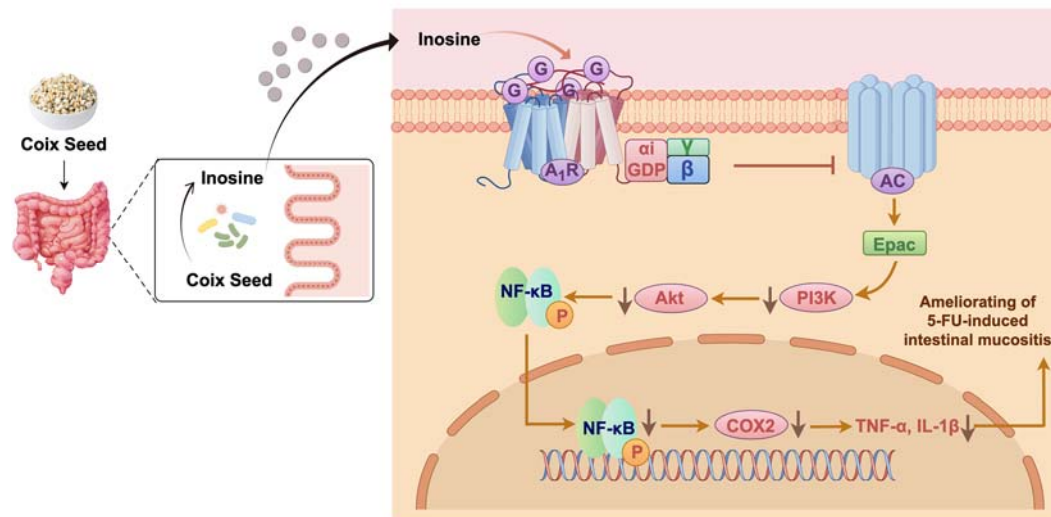


Fig. 8 Schematic illustration of the proposed mechanism by which dietary coix seed (CS) supplementation alleviates 5-FU-induced intestinal mucositis via the gut microbiota-inosine-A₁R-PI3K/Akt-NF-κB axis.

4. Discussion

Chemotherapy-induced IM represents one of the most prevalent and debilitating complications of 5-FU treatment, severely compromising patient quality of life and often necessitating dose reduction or treatment discontinuation [36]. Despite its clinical significance, current pharmacological interventions remain largely palliative, providing neither substantial nor sustained therapeutic benefits [5]. This critical unmet medical need has prompted the exploration of dietary and nutritional strategies, particularly those leveraging the emerging “food as medicine” paradigm and the “diet-gut microbiota-metabolite” axis. In the present study, we provide the first comprehensive evidence that dietary supplementation with CS—a traditional cereal grain with a long history of safe consumption in East Asia—effectively alleviates 5-FU-induced IM through a novel mechanism involving gut microbiota-derived inosine and subsequent suppression of the PI3K/Akt-NF-κB inflammatory signaling cascade. These findings establish CS as a promising functional food for managing chemotherapy-induced gastrointestinal toxicity and exemplify the therapeutic potential of traditional food resources in modern nutritional interventions.

The concept of “medicine food homology”, deeply rooted in traditional Asian dietary culture, posits that certain foods possess both nutritional and therapeutic value. CS epitomizes this concept, having been consumed as both a staple grain and a traditional remedy for over two millennia in China, Japan, and Korea. Unlike pharmaceutical agents that may introduce additional toxicity burdens, CS offers the distinct advantages of established safety profiles, cultural acceptability, ease of dietary incorporation, and cost-effectiveness—attributes particularly valuable for cancer patients requiring long-term supportive care. The Chinese National Health Commission’s official classification of CS as both food and medicine further underscores its suitability for nutritional interventions. Our demonstration that CS effectively ameliorates 5-FU-induced IM without compromising anticancer efficacy addresses a fundamental concern in developing supportive dietary interventions for chemotherapy patients, as previous studies have reported that certain herbal supplements and

functional foods may adversely affect chemotherapy outcomes through alterations in drug metabolism or modulation of drug transporter expression [37, 38]. The preservation of 5-FU's therapeutic efficacy alongside the gastroprotective effects positions CS as an ideal functional food candidate for adjunctive nutritional support during chemotherapy.

From a food science perspective, the bioactive potential of CS derives from its rich nutritional composition, including polysaccharides, dietary fiber, essential amino acids, unsaturated fatty acids, and bioactive compounds such as coixenolide and coixol [18]. Importantly, many of these components serve as fermentable substrates for gut microbiota, positioning CS as a prebiotic-like functional food ingredient. Our comprehensive microbiome analysis revealed that CS supplementation effectively reversed 5-FU-induced gut dysbiosis through two complementary mechanisms. First, CS treatment substantially reduced the relative abundance of potentially pathogenic bacteria, particularly members of the *Enterobacteriaceae* family, which are known to proliferate during gut dysbiosis and contribute to intestinal inflammation [24, 39, 40]. Second, CS supplementation selectively enriched beneficial bacterial genera, including *Bifidobacterium* and *Lactobacillus*, which play essential roles in maintaining gut barrier integrity, immune homeostasis, and inflammatory regulation [41, 42]. These findings highlight the prebiotic functionality of CS and align with the growing recognition that dietary fibers and complex carbohydrates from whole grains can modulate gut microbiota composition to promote intestinal health.

To elucidate the molecular mechanisms underlying CS-mediated protection, we performed comprehensive transcriptomic profiling of intestinal tissues. Pathway enrichment analysis revealed that CS supplementation significantly modulated the expression of genes associated with inflammatory signaling, with the PI3K/Akt pathway emerging as a prominently affected cascade. The PI3K/Akt signaling axis plays a central role in regulating diverse cellular processes including metabolism, survival, and inflammation [43]. Upon activation, this pathway promotes phosphorylation and nuclear translocation of NF- κ B, a master transcription factor that orchestrates the expression of pro-inflammatory cytokines such as IL-6, IL-1 β , and TNF- α , which are key drivers of intestinal injury during chemotherapy [44]. Our data demonstrate that dietary CS intake effectively attenuates the inflammatory response through coordinated downregulation of critical genes within the PI3K/Akt-NF- κ B signaling cascade, providing mechanistic insight into how this traditional food exerts its anti-inflammatory properties at the molecular level.

The emerging field of nutritional metabolomics has revealed that the health benefits of functional foods are often mediated through gut microbiota-derived metabolites rather than direct effects of parent food compounds [45]. Within this “diet-microbiota-metabolite-health” paradigm, the gut microbiota functions as a metabolic organ that biotransforms dietary substrates into bioactive metabolites with profound effects on host physiology [25]. Our untargeted metabolomic analysis revealed that CS supplementation induced significant remodeling of the intestinal metabolome, with purine metabolites—particularly inosine—emerging as prominently enriched species. This finding is consistent with the understanding that CS polysaccharides can be fermented by gut bacteria to produce various bioactive metabolites [19]. Complementary *in vitro* anaerobic

fermentation experiments, simulating the gut fermentation environment, established a causal relationship between gut microbiota activity and inosine production, demonstrating that CS serves as a fermentable food substrate for beneficial bacteria to generate this bioactive metabolite. Notably, *Akkermansia* and *Bifidobacterium*, two genera with well-documented probiotic properties, were significantly enriched during CS fermentation and exhibited strong positive correlations with inosine production. This microbial biotransformation of dietary CS into bioactive inosine exemplifies the concept of “postbiotics” and provides a mechanistic framework for understanding how traditional food resources can influence host health through the gut microbiome.

Inosine, a purine nucleoside naturally present in various foods including meat broths and fermented products, has garnered considerable interest in both food science and pharmacology due to its well-documented anti-inflammatory and immunomodulatory properties^[29]. To validate the functional significance of microbiota-derived inosine as the key effector molecule mediating CS's protective effects, we examined whether exogenous inosine administration could recapitulate the gastroprotective effects of dietary CS. Strikingly, oral inosine supplementation effectively ameliorated multiple hallmarks of 5-FU-induced IM, including body weight loss, colon shortening, mucosal architectural disruption, and pro-inflammatory cytokine elevation, to an extent comparable to that achieved by dietary CS intervention. Mechanistically, inosine exerted its anti-inflammatory effects through suppression of the same PI3K/Akt-NF- κ B signaling axis modulated by CS, as evidenced by reduced phosphorylation of Akt and NF- κ B p65, along with decreased expression of the downstream inflammatory mediator COX-2. Further investigation revealed that inosine upregulated the expression of adenosine A₁ receptor (A₁R) in colonic tissues. Given that A₁R is a G_{i/o}-coupled receptor whose activation inhibits adenylate cyclase activity and suppresses PI3K/Akt-NF- κ B signaling axis^[32-34], our findings suggest that inosine mediates its anti-inflammatory effects through A₁R-dependent inhibition of the PI3K/Akt-NF- κ B signaling cascade. These results collectively establish inosine as the key postbiotic metabolite responsible for CS-mediated intestinal protection.

It should be noted that while our study demonstrates the critical role of the gut microbiota-inosine axis in CS-mediated protection, CS contains multiple bioactive components that may exert direct protective effects independent of microbiota modulation. Recent studies have shown that CS polysaccharides can directly inhibit intestinal mucositis by enhancing intestinal barrier function, reducing inflammatory cytokine production, and modulating immune responses^[46]. Similarly, CS proteins, lipids (coix seed oil), and phenolic compounds have been reported to possess anti-inflammatory and antioxidant properties that may directly benefit intestinal health^[17]. Therefore, the overall protective effects of CS likely result from synergistic actions of both direct biochemical effects and indirect microbiota-mediated mechanisms. Future studies employing polysaccharide-depleted CS extracts or purified CS components could help dissect the relative contributions of these complementary pathways and optimize CS-based nutritional interventions for chemotherapy-induced intestinal mucositis.

From a translational perspective, our findings have several important implications for the development of functional food products and nutritional strategies for cancer patients. First, CS-based food products, including whole grain preparations, CS beverages, and CS-fortified formulations, may serve as practical dietary interventions for patients undergoing chemotherapy [47]. The traditional preparation methods of CS, such as porridge and soup, are well-suited for patients experiencing gastrointestinal discomfort, as these soft-textured preparations are gentle on the compromised intestinal mucosa. Second, the identification of inosine as the key effector metabolite opens possibilities for developing targeted postbiotic supplements or CS-derived functional food ingredients with standardized inosine-producing capacity. Third, the prebiotic-like effects of CS suggest potential synergistic benefits when combined with probiotic strains capable of inosine production, pointing toward the development of synbiotic formulations for enhanced gastrointestinal protection.

While our findings establish CS as a promising microbiota-targeted functional food, several challenges must be addressed before clinical implementation. First, substantial inter-individual variability in baseline gut microbiota composition may influence treatment response, as CS's protective effects depend partly on enriching *Akkermansia* and *Bifidobacterium*. Patients with low baseline abundance of these bacteria may exhibit reduced responsiveness, suggesting that microbiota profiling could help identify individuals most likely to benefit. Second, standardization of CS preparation is critical, as different processing methods (decoction, fermentation, extraction) may yield products with variable bioactive profiles and prebiotic capacities. Clinical-grade CS products would require quality control assays quantifying key constituents, batch-to-batch consistency testing, and good manufacturing practice certification.

Moreover, several limitations warrant consideration. First, while the murine 5-FU-induced mucositis model is well-established for mechanistic investigations, species-specific differences in gut microbiota composition, immune responses, and drug metabolism necessitate cautious extrapolation to clinical settings. Future clinical trials are essential to validate the translational potential of CS supplementation in cancer patients. Second, although our multi-omics analyses demonstrate strong associations between CS-modulated microbiota and mucositis amelioration, fecal microbiota transplantation experiments and antibiotic-mediated microbiota depletion studies would provide more definitive causal evidence by directly transferring the protective microbiota phenotype to recipient animals. Third, while our *in vitro* experiments found inosine suppresses A₁R activation, conditional A₁R knockout models or intestine-specific antagonist delivery systems would strengthen mechanistic conclusions. In addition, future studies employing integrated correlation network analyses combining metagenomics, metabolomics, and host transcriptomics data, coupled with metabolic flux modeling, could further dissect the complex interactions within the microbiota-metabolite-host axis. Despite these limitations, our study provides valuable insights into the diet-microbiota-metabolite axis and establishes a robust foundation for future translational research.

5. Conclusion

This study provides the first evidence that dietary CS supplementation effectively alleviates 5-FU-induced intestinal mucositis without compromising antitumor efficacy. Through integrated multi-omics

analyses, we elucidated a novel protective mechanism mediated by the gut microbiota-inosine-PI3K/Akt-NF- κ B signaling axis. CS restored gut microbial homeostasis, enriched beneficial bacteria, and promoted the accumulation of the postbiotic metabolite inosine, which subsequently attenuated intestinal inflammation via A₁R-mediated suppression of the PI3K/Akt-NF- κ B cascade. These findings provide mechanistic support for the “food as medicine” paradigm and highlight CS as a promising microbiota-targeted functional food for the nutritional management of chemotherapy-induced gastrointestinal toxicity.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

References

- [1] S. Vodenkova, T. Buchler, K. Cervena, et al., 5-fluorouracil and other fluoropyrimidines in colorectal cancer: Past, present and future, *Pharmacol Ther.* 206 (2020) 107447. <https://doi.org/10.1016/j.pharmthera.2019.107447>.
- [2] D. Dahlgren, M. Sjoblom, P.M. Hellstrom, et al., Chemotherapeutics-Induced Intestinal Mucositis: Pathophysiology and Potential Treatment Strategies, *Front Pharmacol.* 12 (2021) 681417. <https://doi.org/10.3389/fphar.2021.681417>.
- [3] H.I. Akbarali, K.H. Muchhala, D.K. Jessup, et al., Chemotherapy induced gastrointestinal toxicities, *Adv Cancer Res.* 155 (2022) 131-166. <https://doi.org/10.1016/bs.acr.2022.02.007>.
- [4] A.T. Sougiannis, B.N. VanderVeen, J.M. Davis, et al., Understanding chemotherapy-induced intestinal mucositis and strategies to improve gut resilience, *Am J Physiol Gastrointest Liver Physiol.* 320 (2021) G712-G719. <https://doi.org/10.1152/ajpgi.00380.2020>.
- [5] Y. He, J. Zheng, B. Ye, et al., Chemotherapy-induced gastrointestinal toxicity: Pathogenesis and current management, *Biochem Pharmacol.* 216 (2023) 115787. <https://doi.org/10.1016/j.bcp.2023.115787>.
- [6] A. Srivastava, M.R. Prabhakar, A. Mohanty, et al., Influence of gut microbiome on the human physiology, *Syst Microbiol and Biomanufacturing.* 2 (2021) 217-231. <https://doi.org/10.1007/s43393-021-00052-w>.
- [7] Y. Niu, J. Feng, J. Ma, et al., Targeting the tumor microbiota in cancer therapy basing on nanomaterials, *Exploration.* 5 (2025) e20210185. <https://doi.org/10.1002/EXP.20210185>.
- [8] J. Lu, X. Shen, H. Li, et al., Recent advances in bacteria-based platforms for inflammatory bowel diseases treatment, *Exploration.* 4 (2024) 20230142. <https://doi.org/10.1002/EXP.20230142>.
- [9] A.T. Sougiannis, B.N. VanderVeen, R.T. Enos, et al., Impact of 5-fluorouracil chemotherapy on gut inflammation, functional parameters, and gut microbiota, *Brain Behav Immun.* 80 (2019) 44-55. <https://doi.org/10.1016/j.bbi.2019.02.020>.
- [10] J.M. Pickard, M.Y. Zeng, R. Caruso, et al., Gut microbiota: Role in pathogen colonization, immune responses, and inflammatory disease, *Immunol Rev.* 279 (2017) 70-89. <https://doi.org/10.1111/imr.12567>.

- [11] L. Wei, X.S. Wen, C.J. Xian, Chemotherapy-induced intestinal microbiota dysbiosis impairs mucosal homeostasis by modulating toll-like receptor signaling pathways, *Int J Mol Sci.* 22 (2021) <https://doi.org/10.3390/ijms22179474>.
- [12] N. Hamouda, T. Sano, Y. Oikawa, et al., Apoptosis, dysbiosis and expression of inflammatory cytokines are sequential events in the development of 5-Fluorouracil-induced intestinal mucositis in mice, *Basic Clin Pharmacol Toxicol.* 121 (2017) 159-168. <https://doi.org/10.1111/bcpt.12793>.
- [13] Q. Yang, S. Chang, X. Zhang, et al., The fate of dietary polysaccharides in the digestive tract, *Trends Food Sci Tech.* 150 (2024) <https://doi.org/10.1016/j.tifs.2024.104606>.
- [14] P. Biscarrat, F. Pepke, C. Defois-Fraysse, et al., Interactions between gut commensal bacteria and polysaccharides derived from algae and legumes: identification of metabolites produced and pathways involved, *Curr Res Microb Sci.* 10 (2026) 100567. <https://doi.org/10.1016/j.crmicr.2026.100567>.
- [15] X. Lin, K. He, Z. Gu, et al., Emerging chemophysiological diversity of gut microbiota metabolites, *Trends Pharmacol Sci.* 45 (2024) 824-838. <https://doi.org/10.1016/j.tips.2024.07.006>.
- [16] Z. Liu, M. Liu, J. Meng, et al., A review of the interaction between diet composition and gut microbiota and its impact on associated disease, *J Future Foods.* 4 (2024) 221-232. <https://doi.org/10.1016/j.jfutfo.2023.07.004>.
- [17] R.D. Devaraj, S.P.K. Jeepipalli, B. Xu, Phytochemistry and health promoting effects of Job's tears (*Coix lacryma-jobi*) - A critical review, *Food Biosci.* 34 (2020) <https://doi.org/10.1016/j.fbio.2020.100537>.
- [18] H. Li, L. Peng, F. Yin, et al., Research on Coix seed as a food and medicinal resource, its chemical components and their pharmacological activities: A review, *J Ethnopharmacol.* 319 (2024) 117309. <https://doi.org/10.1016/j.jep.2023.117309>.
- [19] Q. Ge, C.L. Hou, X.H. Rao, et al., In vitro fermentation characteristics of polysaccharides from coix seed and its effects on the gut microbiota, *Int J Biol Macromol.* 262 (2024) 129994. <https://doi.org/10.1016/j.ijbiomac.2024.129994>.
- [20] U. Kolli, R. Jalodia, S. Moidunny, et al., Multi-omics analysis revealing the interplay between gut microbiome and the host following opioid use, *Gut Microbes.* 15 (2023) 2246184. <https://doi.org/10.1080/19490976.2023.2246184>.
- [21] J. Qu, F. Ke, X. Yang, et al., Induction of P-glycoprotein expression by dandelion in tumor and heart tissues: Impact on the anti-tumor activity and cardiotoxicity of doxorubicin, *Phytomedicine.* 104 (2022) 154275. <https://doi.org/10.1016/j.phymed.2022.154275>.
- [22] J. Qu, F. Ke, Z. Liu, et al., Uncovering the mechanisms of dandelion against triple-negative breast cancer using a combined network pharmacology, molecular pharmacology and metabolomics approach, *Phytomedicine.* 99 (2022) 153986. <https://doi.org/10.1016/j.phymed.2022.153986>.
- [23] X. Sun, Z. Pei, H. Wang, et al., Bridging dietary polysaccharides and gut microbiome: How to achieve precision modulation for gut health promotion, *Microbiol Res.* 292 (2025) 128046. <https://doi.org/10.1016/j.micres.2025.128046>.
- [24] M.I. Moreira de Gouveia, A. Bernalier-Donadille, G. Jubelin, Enterobacteriaceae in the Human Gut: Dynamics and Ecological Roles in Health and Disease, *Biology (Basel).* 13 (2024) <https://doi.org/10.3390/biology13030142>.
- [25] L. Fan, Y. Xia, Y. Wang, et al., Gut microbiota bridges dietary nutrients and host immunity, *Sci China Life Sci.* 66 (2023) 2466-2514. <https://doi.org/10.1007/s11427-023-2346-1>.
- [26] Y. Zhang, R. Chen, D. Zhang, et al., Metabolite interactions between host and microbiota during health and disease: Which feeds the other?, *Biomed Pharmacother.* 160 (2023) 114295. <https://doi.org/10.1016/j.biopha.2023.114295>.
- [27] L.F. Mager, R. Burkhard, N. Pett, et al., Microbiome-derived inosine modulates response to checkpoint inhibitor immunotherapy, *Science.* 369 (2020) 1481-1489.
- [28] N. Ottman, M. Davids, M. Suarez-Diez, et al., Genome-scale model and omics analysis of metabolic capacities of *Akkermansia muciniphila* reveal a preferential mucin-degrading lifestyle, *Appl Environ Microbiol.* 83 (2017) <https://doi.org/10.1128/AEM.01014-17>.
- [29] I.S. Kim, E.K. Jo, Inosine: A bioactive metabolite with multimodal actions in human diseases, *Front Pharmacol.* 13 (2022) 1043970. <https://doi.org/10.3389/fphar.2022.1043970>.
- [30] N. Wang, E. Li, H. Deng, et al., Inosine: A broad-spectrum anti-inflammatory against SARS-CoV-2 infection-induced acute lung injury via suppressing TBK1 phosphorylation, *J Pharm Anal.* 13 (2023) 11-23. <https://doi.org/10.1016/j.jpha.2022.10.002>.
- [31] F. Yang, L. Zhou, Y. Shen, et al., Metabolic heterogeneity caused by HLA-DRB1*04:05 and protective effect of inosine on autoimmune hepatitis, *Front Immunol.* 13 (2022) 982186. <https://doi.org/10.3389/fimmu.2022.982186>.

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- [32] D. Schuster, B. Khanppnavar, I. Kantarci, et al., Structural insights into membrane adenylyl cyclases, initiators of cAMP signaling, *Trends Biochem Sci.* 49 (2024) 156-168. <https://doi.org/10.1016/j.tibs.2023.12.002>.
- [33] H. Zhang, Y. Liu, J. Liu, et al., cAMP-PKA/EPAC signaling and cancer: the interplay in tumor microenvironment, *J Hematol Oncol.* 17 (2024) 5. <https://doi.org/10.1186/s13045-024-01524-x>.
- [34] K. Ogiwara, M. Hoyagi, T. Takahashi, A central role for cAMP/EPAC/RAP/PI3K/AKT/CREB signaling in LH-induced follicular Pgr expression at medaka ovulationdagger, *Biol Reprod.* 105 (2021) 413-426. <https://doi.org/10.1093/biolre/ioab077>.
- [35] F.P. Nascimento, S.J. Macedo-Junior, F.A. Pamplona, et al., Adenosine A1 receptor-dependent antinociception induced by inosine in mice: pharmacological, genetic and biochemical aspects, *Mol Neurobiol.* 51 (2015) 1368-1378. <https://doi.org/10.1007/s12035-014-8815-5>.
- [36] A. Alcorta, L. Lopez-Gomez, R. Capasso, et al., Vitamins and fatty acids against chemotherapy-induced intestinal mucositis, *Pharmacol Ther.* 261 (2024) 108689. <https://doi.org/10.1016/j.pharmthera.2024.108689>.
- [37] P.S. Fasinu, G.K. Rapp, Herbal interaction with chemotherapeutic drugs-A focus on clinically significant findings, *Front Oncol.* 9 (2019) 1356. <https://doi.org/10.3389/fonc.2019.01356>.
- [38] P. Gougis, M. Hilmi, A. Geraud, et al., Potential cytochrome P450-mediated pharmacokinetic interactions between herbs, food, and dietary supplements and cancer treatments, *Crit Rev Oncol Hematol.* 166 (2021) 103342. <https://doi.org/10.1016/j.critrevonc.2021.103342>.
- [39] M.Y. Zeng, N. Inohara, G. Nunez, Mechanisms of inflammation-driven bacterial dysbiosis in the gut, *Mucosal Immunol.* 10 (2017) 18-26. <https://doi.org/10.1038/mi.2016.75>.
- [40] N.R. Shin, T.W. Whon, J.W. Bae, Proteobacteria: microbial signature of dysbiosis in gut microbiota, *Trends Biotechnol.* 33 (2015) 496-503. <https://doi.org/10.1016/j.tibtech.2015.06.011>.
- [41] D.H. Jung, C.S. Park, Resistant starch utilization by Bifidobacterium, the beneficial human gut bacteria, *Food Sci Biotechnol.* 32 (2023) 441-452. <https://doi.org/10.1007/s10068-023-01253-w>.
- [42] C. Li, K. Peng, S. Xiao, et al., The role of Lactobacillus in inflammatory bowel disease: from actualities to prospects, *Cell Death Discov.* 9 (2023) 361. <https://doi.org/10.1038/s41420-023-01666-w>.
- [43] Y. He, M.M. Sun, G.G. Zhang, et al., Targeting PI3K/Akt signal transduction for cancer therapy, *Signal Transduct Target Ther.* 6 (2021) 425. <https://doi.org/10.1038/s41392-021-00828-5>.
- [44] Q. Guo, Y. Jin, X. Chen, et al., NF-kappaB in biology and targeted therapy: new insights and translational implications, *Signal Transduct Target Ther.* 9 (2024) 53. <https://doi.org/10.1038/s41392-024-01757-9>.
- [45] N. Zmora, J. Suez, E. Elinav, You are what you eat: diet, health and the gut microbiota, *Nat Rev Gastroenterol Hepatol.* 16 (2019) 35-56. <https://doi.org/10.1038/s41575-018-0061-2>.
- [46] H.-M. Yin, S.-N. Wang, S.-P. Nie, et al., Coix polysaccharides: Gut microbiota regulation and immunomodulatory, *Bioact Carbohydr Diet Fibre.* 16 (2018) 53-61. <https://doi.org/10.1016/j.bcdf.2018.04.002>.
- [47] F.D. Meng, L. Yuan, D.D. Lu, et al., Anti-tumor effect of coix seed based on the theory of medicinal and food homology, *World J Clin Oncol.* 14 (2023) 593-605. <https://doi.org/10.5306/wjco.v14.i12.593>.