RESEARCH ARTICLE

Effect of anthocyanin consumption on ulcerative colitis: a systematic review and meta-analysis

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Anthocyanin could reduce intestinal inflammatory symptoms or prevent the reactivation of disease initiation and progression. The effect of anthocyanin consumption on ulcerative colitis (UC) has not been fully elucidated to date. This study attempted to determine the effect of anthocyanins on the expression of inflammatory cytokines and the enrichment of signaling pathways. A literature search was performed by using PubMed, Embase, and the Cochrane Library databases up to July 2021. The impact of anthocyanins on human immunity including inflammatory cytokines of MCP-1, TNF-α, IL-2, IL-8, and IL-10 were meta-analyzed. Subsequently, bioinformatic analysis was performed to determine the enrichment pathways. A total of 5 studies with 208 participants were included in the literature search. From the meta-analysis, the results showed that anthocyanin consumption could decrease the expression of inflammatory cytokines, especially the expression of TNF-α. From the bioinformatic analysis, the inflammatory and Jak/STAT signaling pathways were identified as the enriched pathways, which was also in keeping with the mechanism of action of anthocyanins. This study suggested that anthocyanin consumption may be safe and may have positive effects on patients with UC which enriched in inflammatory and Jak/STAT signaling pathways. Therefore, the widespread use of anthocyanins in adjuvant therapy for UC was encouraged. Further high-quality studies are warranted to characterize the effects of anthocyanins more thoroughly.

Keywords: anthocyanin; ulcerative colitis; inflammatory cytokines; meta-analysis; bioinformatic analysis.

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Introduction

Ulcerative colitis (UC) is a type of chronic idiopathic inflammatory bowel disease (IBD) that starts with superficial mucosal inflammation in the rectum and extends proximally in a continuous fashion as shown by endoscopic evidence, which may involve any section of the colon. Typical symptoms of UC are bloody diarrhea, abdominal pain, fecal urgency, and/or tenesmus, which are characterized by a relapsing and remitting course [1-3]. Pharmacotherapy for UC includes aminosalicylic acid, glucocorticoids, and immunosuppressants. However, still up to 15% of UC patients need surgery or have complications of dysplasia. Currently, alternative natural therapies including dietary changes are investigated as means of managing or treating UC. A high-fat diet has been associated with an increased risk of UC, therefore, the dietary intervention of adopting a low-fat, high-fiber diet may benefit patients with UC in remission [4].

Anthocyanins have been widely detected as

natural plant molecules all over the world. These compounds are members of the subclass dietary flavonoids, which are water-soluble vascular pigments responsible for red-orange to blue-violet colors in many fruits, vegetables, and flowers. At present, people are primarily concerning about the health benefits of anthocyanins that have shown considerable promise as a means of reducing intestinal inflammatory symptoms or preventing the reactivation of disease initiation/progression [5]. Anthocyanins in blueberry was observed to suppress JAK/STAT-3 phosphorylation and nuclear translocation, thereby, inducing cell cycle arrest and mitochondrial-mediated apoptosis, which may attenuate clinical symptoms and immune cell infiltration in animal models [6-7]. Several publications demonstrated that anthocyanins have anti-inflammatory efficacy, which suggest the feasibility of dietary strategies employing anthocyanins for UC mitigation [8-9]. However, no previous systematic reviews have undertaken quantitative research with meta-analyses.

The aim of this systematic review study was to determine the anti-inflammatory effects of anthocyanins in human body and to determine whether anthocyanins can be utilized in the treatment of UC by using meta-analysis. In addition, the bioinformatic study to identify if anthocyanins can inhibit the occurrence and aggravation of UC based on characterizing signaling pathways of UC was also performed.

Materials and methods

Search strategy and selection criteria

The synthesis and meta-analysis were followed by the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement [10-11] for determining the anthocyanin consumption efficacy in UC with a predesigned structure. The protocol was registered with the PROSPERO online system (No. CRD42021232676) (https://www.crd.york.ac.uk/PROSPERO), which is the international prospective register of systematic reviews [12]. Three online databases, PubMed (https://pubmed.gov), Embase (https://www.embase.com), and Cochrane Library (https://www.cochranelibrary.com), were searched with the keywords of “anthocyanins”, “ulcerative colitis”, and “inflammatory”, and their Medical Subject Headings (MeSH) terms were searched from their inception to July 2021. Controlled studies investigating the effect of anthocyanins on immunity indexes were included.

Data extraction and outcomes

Baseline and data extraction from each originally included study was independently performed by two researchers (MY and YJY) using preset forms. For the included studies, the basic characteristics of the first author, publication year, anthocyanin source, subject type, duration of treatment, type of study, outcome, and quantifiable results in the meta-analysis were all extracted and presented in the table. Subsequently, to quantitatively investigate the impact of anthocyanins on human immunity, the inflammatory cytokines such as MCP-1, TNF-α, IL-2, IL-8, IL-10 and the indicators that evaluate the degree of UC were used for the quantitative meta-analysis. The pooled standardized mean differences (SMDs) with 95% confidence intervals (CIs) were applied to obtain a summary of significant differences. Regardless of heterogeneous results, random effects models were applied [13], and statistical heterogeneity I^2 > 50% indicated high heterogeneity among the included studies [14]. All the statistical analysis was done by using STATASE (version 15.1) (StataCorp, College Station, Texas, USA).

Downloading online data and identifying differentially expressed genes

Data were acquired from the Gene Expression Omnibus (GEO), a free public functional genomics website (www.ncbi.nlm.nih.gov/geo). The standard for including online data in this bioinformatic research was that the sample must include UC and normal tissue mRNA data. R4.0.2 software (www.r-project.org) was utilized to

Table 1. Baseline characteristics.

First author (year)	Anthocyanins source	Subjects type	Sample Size	Duration of treatment	Type of study	Outcomes	Quantifiable results in meta-analysis
Roth S (2016) [15]	Bilberry extract	UC patients	8	7 weeks	Open label clinical trial	IFN-γ, STAT1, IFGR2, TNFα, MCP-1	MCP-1 serum levels
Kamali M (2015) [16]	Punica granatum peel extract	UC patients	62	4 weeks, 10 weeks (follow-up)	A randomized, placebo controlled, clinical trial	LCAI score, Nocturnal diarrhea, *etc.*	LCAI score
Kuntz S (2014) [17]	Fruit juices	Healthy young female volunteers	90	2 weeks	Randomized, double-blind, placebo-controlled, cross-over study	TNF-α, MCP-1, hs-CRP, sCD40, sICAM-1, sVCAM-1, IL-2, IL-6, IL-8, IL-10	TNF-α, MCP-1, hs-CRP, sCD40, sICAM-1, sVCAM-1, IL-2, IL-6, IL-8, IL-10
Biedermann L (2013) [18]	Bilberry extract	Mild to moderate UC patients	24	6 weeks, 9 weeks (follow-up)	Open pilot study	Mayo Score, calprotectin levels	Mayo Score, calprotectin levels
Kaspar KL (2010) [19]	Pigmented potato	Healthy men	24	6 weeks	Open pilot study	IL-1α, IL-1β, IL-2, IL-4, IL-8, IL-10, IFNγ, TNFα	IL-1α, IL-1β, IL-2, IL-4, IL-8, IL-10, IFNγ, TNFα

collate and calculate the downloaded data with the purpose of identifying differentially expressed genes (DEGs) on the basis of |log2 FC| > 2.0, which was performed by using the edgeR package. Since the role of high mRNA levels in UC were explored, the first 1,000 DEGs in UC were selected for follow-up studies.

Gene ontology (GO) annotation terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis

To identify the functions of DEGs, enriched gene ontology (GO) functions from three aspects including biological process (BP), cellular component (CC), and molecular function (MF) were applied. The Kyoto encyclopedia of genes and genomes (KEGG) enrichment analysis was also applied to determine which pathway DEGs might be enriched in. GO terms and KEGG pathway enrichment analysis for identification of DEGs were performed by using the DAVID database (the Database for Annotation, Visualization, and Integrated Discovery) (version 6.8) (https://david.ncifcrf.gov). The p adjust (FDR) value less than 0.05 was considered to be significant.

Results

Study characteristics of meta-analysis

From the initial search of electronic databases, 45 potential publications were identified. After removing duplicates and inspecting title and abstract, 14 full-text articles were evaluated for eligibility. Through thoroughly examination and removal of inappropriate research, 5 studies were determined to be eligible for quantitative meta-analysis with 208 participants [15-19]. Three of these studies investigated UC patients [15, 16, 18], while the other 2 studies examined healthy people [17, 19]. For the included studies published from 2010 to 2016, anthocyanin sources were bilberry extract, Punica granatum peel extract, fruit juices, and pigmented potato. All included publications were well-designed and suitable for meta-analysis (Table 1).

Meta-analysis results

Three of included studies described outcomes regarding inflammatory cytokines [15, 17, 19]. For MCP-1, no significant results were obtained with substantial heterogeneity (SMD = 0.02, 95% CI: -0.91 to 0.94; p = 0.011, I^2 = 77.7%). The results showed that anthocyanin consumption could decrease the expression of TNF-α in participants with significant results (SMD = 0.50, 95% CI: 0.06 to 0.95) and low heterogeneity (p = 0.193, I^2 =

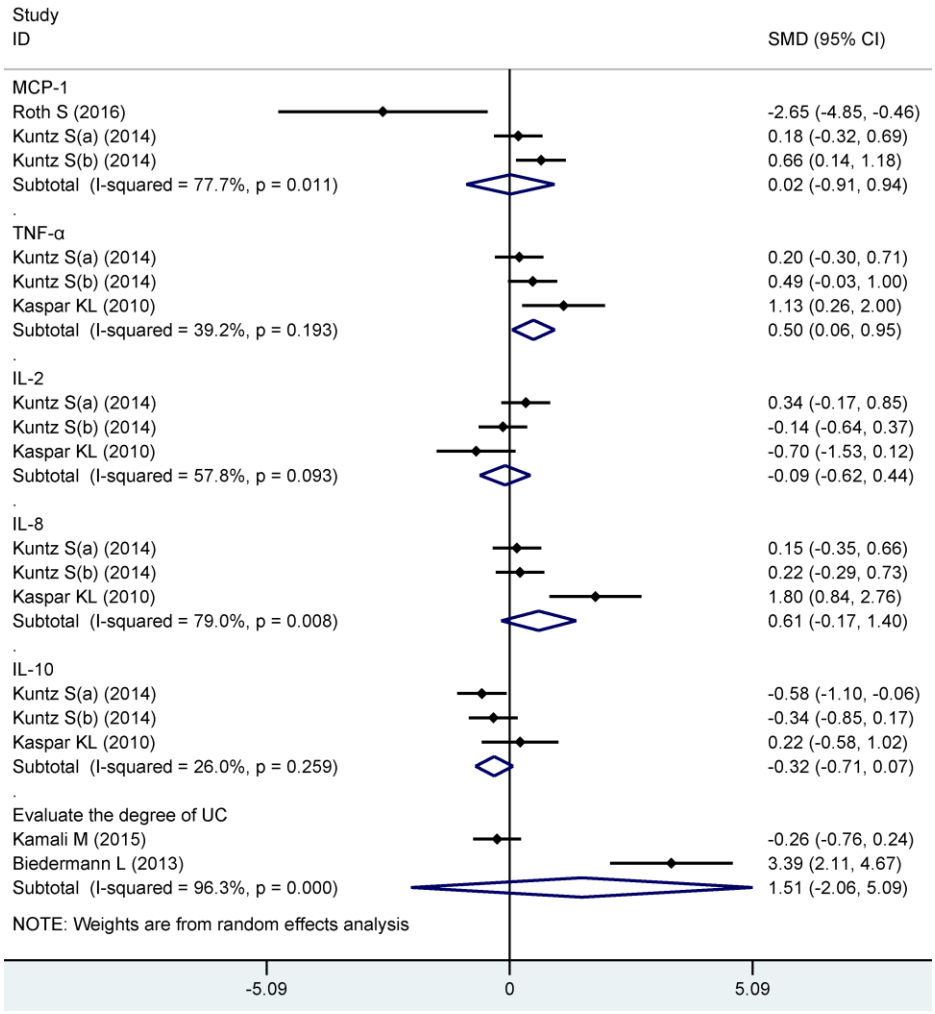

Figure 1. Forest plot for anthocyanin consumption versus control for MCP-1, TNF-α, IL-2, IL-8, and IL-10 and for evaluating the degree of UC in meta-analysis.

39.2%). In addition, for IL-2, IL-8, and IL-10, no significant differences were observed with moderate to substantial heterogeneity. Moreover, when evaluating the degree of UC from 2 included studies [16, 18], anthocyanin consumption exhibited a tendency to reduce the severity of UC disease with substantial heterogeneity (SMD = 1.51, 95% CI: -2.06 to 5.09; p = 0.000, I^2 = 96.3%) (Figure 1).

GO and KEGG pathway analysis from bioinformatic analysis

Bioinformatic analysis was performed by using data of 5 normal tissues and 21 UC samples from GEO database. The 6,493 DEGs that were differentially expressed between UC and normal tissues and the abovementioned outcome were presented in the form of a volcano plot (Figure 2). The top 1,000 DEGs was used for subsequent analysis. GO functional and KEGG pathway enrichment analysis were performed by using the DAVID website. 24 GO functional analysis results and the 24 most enriched KEGG pathways were demonstrated in Figure 3 and 4, respectively. The outcomes of the analysis indicated that, in terms of BP, UC tissue was enriched in the immune response (GO:0006955) and inflammatory response (GO:0006954), while, in terms of CC and MF, cytosol (GO:0005829) and protein binding (GO:0005515) were enriched (Figure 3).

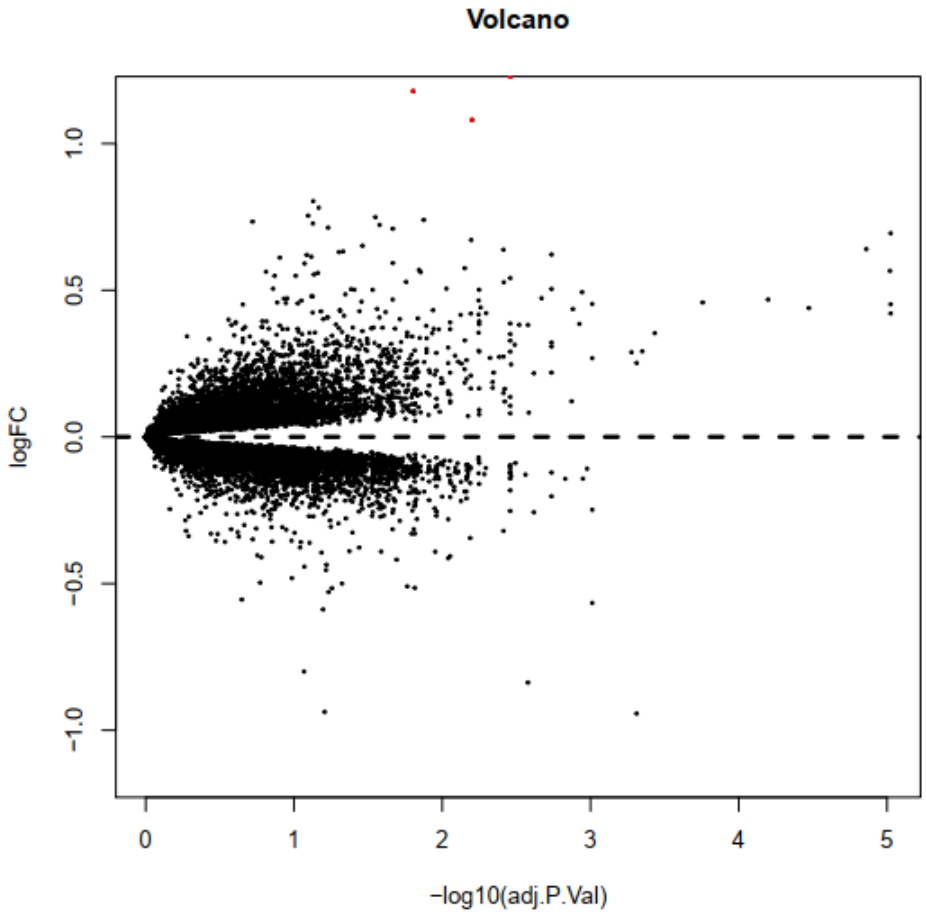

Figure 2. The volcano plot of DEGs.

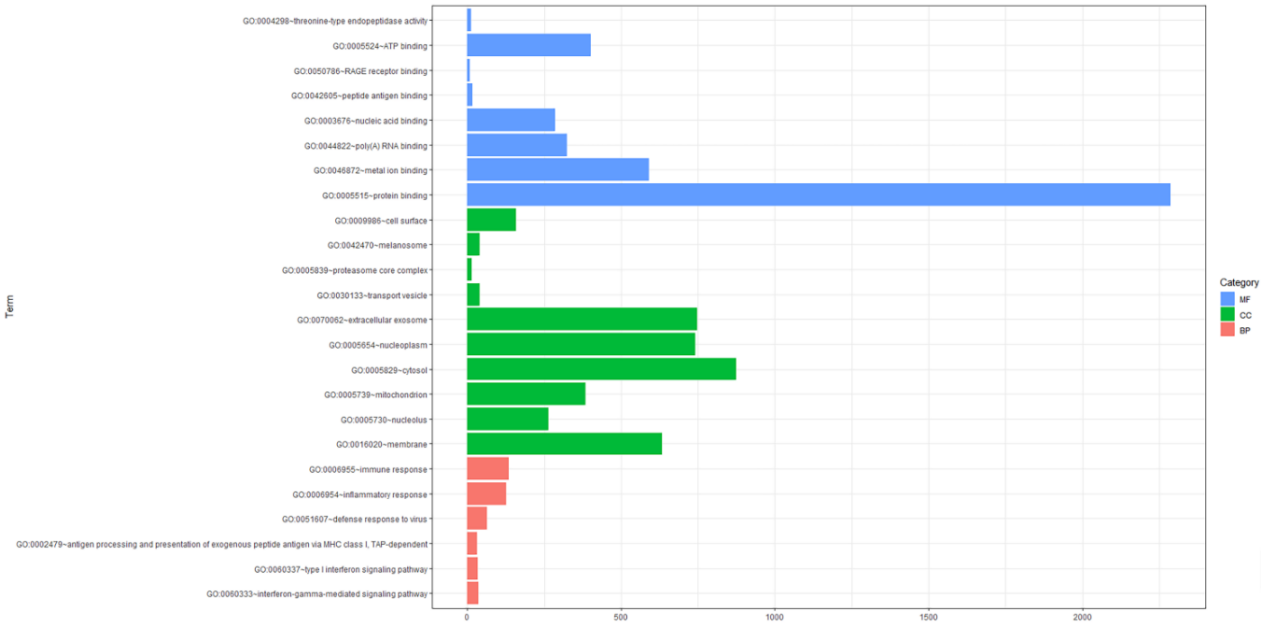

Figure 3. Gene ontology (GO) annotation analysis of DEGs in UC.

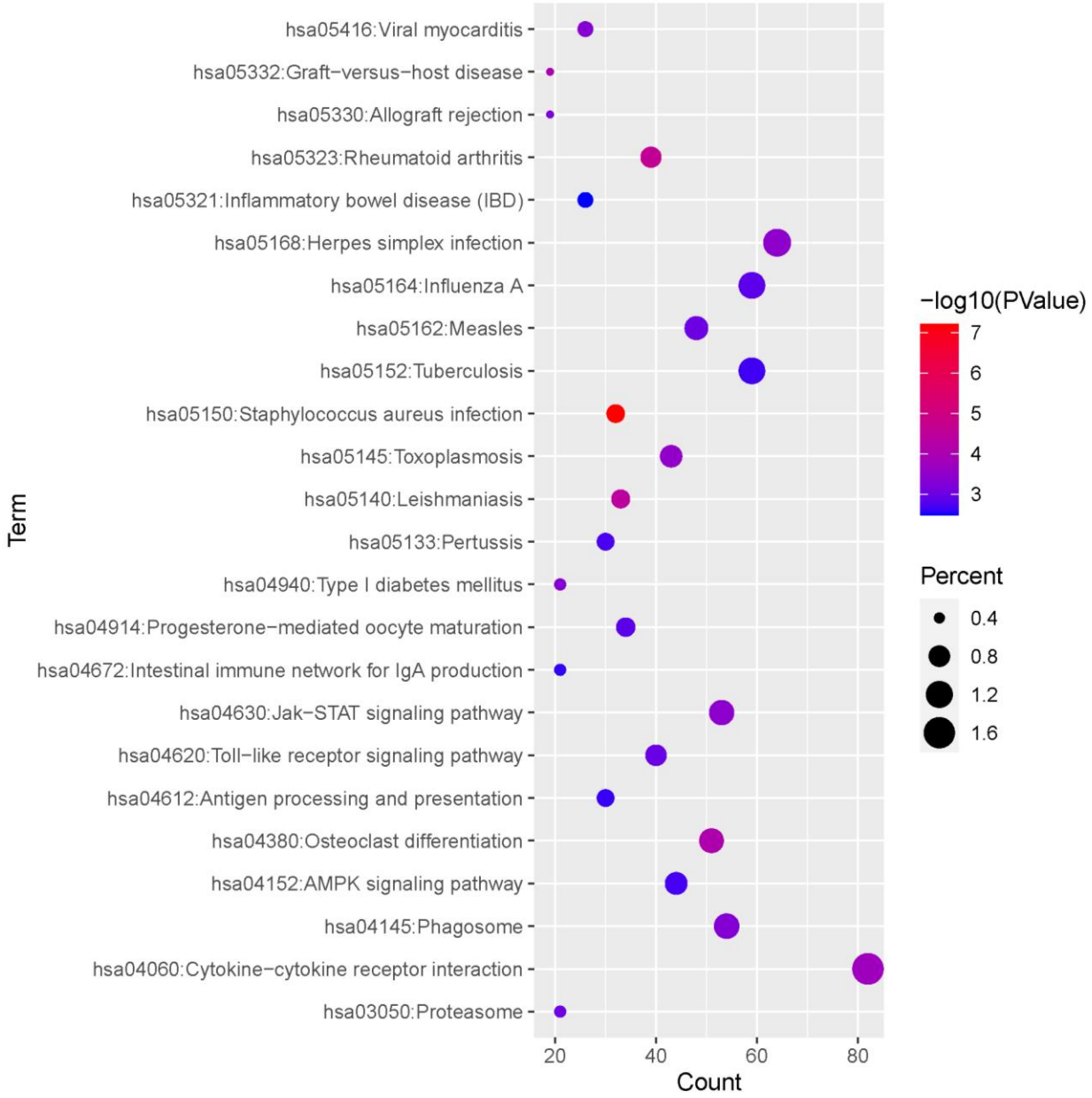

Figure 4. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of DEGs in UC.

KEGG pathway analysis indicated that cytokine-cytokine receptor interactions (hsa04060) and the Jak-STAT signaling pathway (hsa04630) were enriched in UC tissues (Figure 4).

Discussion

The meta-analysis and bioinformatic analysis were applied to investigate the effects of anthocyanin consumption on UC. Five publications that studied a total of 208 participants were included in this study, and inflammatory cytokines could be utilized for quantitative analysis (Table 1). In addition, the inflammatory cytokines MCP-1, TNF-α, IL-2, IL-8, and IL-10 and biomarkers to evaluate the degree of UC were also employed for quantitative meta-analysis, and a significant decrease of TNF-α in the anthocyanin consumption group was

observed (Figure 1). Through GO enrichment and KEGG pathway analysis, the results confirmed that the expression of inflammatory factors in UC patients was indeed increased and might be enriched in the Jak-STAT signaling pathway, which was consistent with the mechanism of action of anthocyanins (Figure 3 and 4).

This study described the first time of systematic review, which provided quantitative data from a meta-analysis and indicated that anthocyanins could reduce TNF-α. The research performed by Del Bo, *et al*. demonstrated that anthocyanins from blueberry could inhibit TNF-α-mediated production of E-selectin and adhesion of monocytes to endothelial cells [20]. In addition, Sari, *et al*. identified anthocyanins from black rice that might function as anti-inflammatory factors related to TNF-α signaling [21]. All those previous research results support the findings of this study that anthocyanins may reduce the expression of TNF-α. Moreover, Nizamutdinova, *et al*. demonstrated that anthocyanins differentially regulated TNF-α-mediated expression of VCAM-1 and ICAM-1 through modulation of GATA and IRF-1 binding activity *via* Jak/STAT-3 activation [22]. In keeping with this finding, Baba, *et al*. also determined that blueberry abrogated the Jak/STAT-3 pathway and modulated downstream targets that affected cell proliferation and apoptosis in a hamster model of oral oncogenesis [7]. In addition, anthocyanins also inhibit inflammation of the allergic inflammation and the effect of protection against intestinal barrier damage [23-24]. This study determined that anthocyanin supplementation could reduce the expression of TNF-α, and therefore, indicated that anthocyanins affected both the inflammatory signaling pathway and the Jak/STAT signaling pathway.

There were several limitations to this study. First, regarding the meta-analysis, only 5 studies were included, which means the sample size was relatively small for a meta-analysis. However, based on the current body of research on this subject, no more suitable studies were identified for inclusion. Future studies should include more human, controlled, and quantifiable studies investigating anthocyanin consumption. Second, the studied population of two of the included studies was healthy people instead of patients with UC. This population was included because these studies presented quantitative immunoinflammatory factor results, which might increase baseline heterogeneity. Third, because the studies that we included could only be counted as case-control studies and there was no reasonable control scheme design, the risk of bias of the included studies was not evaluated. For future study, the quality of publications needs to be evaluated when there are better-designed studies available. Finally, through bioinformatic analysis, only inflammation and the Jak/STAT signaling pathways were enriched. It was hard to conclusively determine the relationship of these pathways, and a large number of experimental studies are required in future.

To ensure the safety of anthocyanin consumption, anthocyanins are primarily derived from food such as blueberries and black rice. The safety of these compounds is relatively good, and there is no adverse reaction mentioned in the included publications. Moreover, in safety research on anthocyanins, published studies have mentioned that anthocyanins are beneficial to human health and have beneficial effects [23, 25]. Therefore, although the safety of anthocyanins was not evaluated in this study, the safety of anthocyanins on human is not in doubt.

In conclusion, from the meta-analysis, the results indicated that anthocyanin consumption could decrease the expression of inflammatory cytokines, especially in the expression of TNF-α. From the bioinformatic analysis, the results demonstrated that inflammatory and Jak/STAT signaling pathways were enriched, which was also in keeping with the mechanism of action of anthocyanins. In general, anthocyanins are recommended for patients with UC, as these compounds exhibit good efficacy and safety. The widespread use of anthocyanins in adjuvant therapy for UC was encouraged.

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