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Article Review

Antiinflammatory Effect of Andrographolide in *Sambiloto* Extract (*Andrographis paniculata)* on Ulcerative Colitis

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Abstract—*Ulcerative colitis (UC) is an idiopathic chronic inflammatory disease of the gastrointestinal tract which is one of the inflammatory bowel diseases (IBD). In Indonesia, epidemiological data obtained from hospital reports, generally show that the incidence of UC is higher than Crohn's Disease (CD). Mesalamine as a drug of choice for UC, is related to some side effects. Therefore, herbal plants such as sambiloto (Andrographis paniculata) could be used as a complementary therapy in UC. The purpose of this article is to provide information about the potential mechanisms of andrographolide (AG) as a bioactive compound in sambiloto (Andrographis paniculata) extract as an anti-inflammatory agent. The method used by authors in this article is a narrative review method, by collecting studies about the anti-inflammatory effect of AG on UC through a database search. The results showed that one of the ingredients of sambiloto, diterpenoid labdane compound in the active form of AG, is able to inhibit the expression of pro-inflammatory cytokines so that it has the potential to act as an anti-inflammatory similar to mesalamine in UC therapy. Additionally, sambiloto contains flavonoids and polyphenols which serve as antioxidants. In conclusion, AG has an anti-inflammatory property that might be utilized as a part of UC complementary therapy.*

Keywords: *andrographolide,* andrographis paniculata*, inflammation, ulcerative colitis, sambiloto*

Abstrak—Kolitis ulseratif (KU) adalah penyakit inflamasi kronis idiopatik saluran pencernaan termasuk dalam salah satu inflammatory bowel disease (IBD). Di Indonesia, data epidemiologi diperoleh dari pelaporan rumah sakit, secara umum menunjukkan insidensi KU lebih tinggi daripada Crohn's Disease (CD). Mesalamine sebagai pilihan terapi untuk KU, dapat menimbulkan beberapa efek samping. Oleh karena itu, tanaman herbal seperti sambiloto Andrographis paniculata dapat digunakan sebagai terapi komplementer pada KU. Tujuan artikel ini adalah memberikan informasi mengenai potensi dan mekanisme senyawa bioaktif andrographolide (AG) pada ekstrak sambiloto (*Andrographis paniculata)* sebagai agen antiinflamasi. Metode yang digunakan oleh penulis dalam artikel ini adalah metode narrative review, dengan mengumpulkan beberapa studi tentang efek antiinflamatori dari AG pada KU melalui pencarian database. Hasil dari analisis data menunjukkan bahwa salah satu kandungan sambiloto, senyawa labdane diterpenoid dalam bentuk aktif AG, mampu menghambat ekspresi sitokin proinflamasi sehingga berpotensi sebagai antiinflamasi serupa dengan mesalamin pada terapi KU. Selain itu, sambiloto memiliki kandungan flavonoid dan polifenol yang berfungsi sebagai antioksidan. Sebagai simpulan, AG memiliki properti antiinflammatori yang dapat digunakan sebagai bagian dari terapi komplementer pada KU.

Kata kunci: *andrographolide, andrographis paniculata, inflamasi, kolitis ulseratif, sambiloto*

INTRODUCTION

Ulcerative colitis (UC) is an idiopathic chronic inflammatory disease of the gastrointestinal tract which is one of the inflammatory bowel diseases (IBD) [1]. Patients typically present with bloody diarrhea, with a genetic predisposition after environmental exposures are thought to develop ulcerative colitis, abnormalities in the gut epithelial barrier, the microbiota, and a dysregulated immune response are significantly associated with the development of ulcerative colitis. The diagnosis is made using a combination of clinical, biological, endoscopic, and histological evidence [2].

Worldwide, North America and Northern Europe have the highest incidence and prevalence of inflammatory bowel disorders globally. There is a strong correlation between a Westernized lifestyle and environment and inflammatory bowel disease. The annual incidence of ulcerative colitis is 9–20 cases per 100,000 people. Its annual prevalence ranges from 156 to 291 cases per 100,000 people. Adults are more likely to have ulcerative colitis than Crohn's disease, which is more common in pediatric patients [3]. Epidemiological data shows that UC, which was formerly thought to occur more commonly in industrialized nations, is now really on the rise in underdeveloped and developed nations alike including those in Asia, such as Indonesia, over the last two decades [4].

The pathophysiology of ulcerative colitis involves defects in the immune system, leukocyte recruitment, epithelial barrier, and colon microbiota [3]. Some experts suggest that IBD is an autoimmune disorder in which the immune system of the host attacks the cells and tissues of the gastrointestinal tract (GIT). These inflammatory manifestations include two diseases, namely: Crohn's disease (CD) and UC. UC is characterized by a lifelong condition that causes inflammation of the colonic mucosa, starting in the rectum, and extending proximally. Common symptoms that often appear are bloody diarrhea, abdominal cramping, urgency, tenesmus, and fever. Most people with UC experience periods when they experience symptoms (flare-ups or exacerbation), followed by longer periods of no symptoms (remission) [5,6]. Administering 5-aminosalicylates, antibiotics, steroids and immunomodulators has been used to manage ulcerative colitis and reduce symptoms and their remissions. However, long-term use of these drugs can result in mild to severe toxicity as well as the possibility of UC-related complications. Even with the use of related drugs, about 15% of patients even have to undergo colectomy or proctocolectomy, despite taking related drugs [7].

The aim of medical management is, first, to induce a rapid clinical response and normalize biomarkers and, second, to maintain clinical remission and reach endoscopic normalization to prevent long-term disability. Corticosteroids and medications containing 5-aminosalicylic acid such as mesalamine, are used as remission-inducing therapies [2]. Mesalamine is well known for its main side effect: renal toxicity so that monitoring renal function before and during the use of this drug is required. Other conditions such as: bone marrow suppression should also be monitored with CBC, particularly in older individuals. There is also a chance of liver failure, cholestatic hepatitis, liver insufficiency, and other hepatotoxic signs and symptoms. The liver function needs to be monitored for long-term use of mesalamine [8,9]. With such serious complications, inefficiency for some patients, and high recurrence rates, thus investigating a novel, safe, and cost-effective treatment strategy for UC is needed.

Alternative therapies based on phytopharmaceuticals have been researched to be a part of the strategy for preventing complications as well as medications used over an extended period of time, with minimal side effects. These therapies can be used as adjuvant therapy or first-line therapy in patients who are less responsive or unresponsive to mesalamine [10]. The purpose of this paper is to review the therapeutic effects of andrographolide (AG) from *sambiloto* extract that are potentially beneficial to human health particularly regarding the pathogenesis of UC. Several have been demonstrated by several in vitro and in vivo studies have demonstrated these effects, which relate to the underlying mechanism of UC and provide additional insight about AG in *sambiloto* extract as a newer modality, of natural, and safe complementary medicine for UC treatment in the future.

METHOD

This literature review was carried out using the narrative review method. The literature search used several keywords such as: "*Andrographis paniculata"*, "ulcerative colitis", "andrographolide" "anti-inflammatory", and "inflammatory bowel disease". Literature search was carried out using Google Scholar, PubMed, and Science Direct search engines.

The inclusion criteria consist of: (1) original articles or review articles published in the last 10 years, (2) full text available in English or Indonesian language, (3) The studies discussed the antiinflammatory effect of AG on UC. The exclusion criteria consist of: (1) full text not available, (2) the study is written in other than English or Indonesian language.

After obtaining the studies that matched inclusion criteria, the studies were assessed and compiled according to titles, study designs, methods and doses, results, and conclusions. Mechanisms of AG as anti-inflammatory against oxidative stress in UC are summarized and discussed in this literature review.

RESULT

We found 6 articles that matched our inclusion criteria, and we presented the summary of those studies in Table 1 below.

DISCUSSION

Pathology of Ulcerative Colitis

By microscopic findings, the GIT consists of four layers, each of which reflects its anatomical and physiological functions, namely: mucosa, submucosa, tunica muscularis externa, and tunica adventitia [11]. The inner lining of the digestive tract, or mucosa, is crucial in creating the mucus barrier or mucus between internal organs and the digestive tract lumen. This layer, which is mostly made up of epithelial cells joined by tight junctions, is crucial for the transfer of nutrients as well as for blocking the entry of pathogenic microbes and substances that are harmful to the host [12]. In the mucosal layer, goblet cells and endocrine cells establish the epithelium. Endocrine cells work to secrete specific hormones that assist in digestion, while goblet cells are the primary cell type of the crypts in the left side of the colon, producng mucin that forms water-soluble mucus. This unique structure of the colon with parallel test tube-like arrangement of crypts differentiated by a consistent amount of lamina propria in between, can display chronic injury when there is an expansion of this lamina propria and a loss of crypts [13].

In UC, there is a defect in the synthesis and secretion of mucus in the mucosal layer, creating damage to the integrity of the tight junctions in the epithelial layer and causing increased mucosal permeability making it easier for antigens to enter from both environmental exposures, toxins, and pathogenic microorganisms. The mucosa and submucosa are both affected by the progression of the inflammatory process [6]. There is a sharp border between normal and affected tissue. The mucosal membrane is erythematous, granular, and friable, with loss of the typical vascular pattern and often followed by scattered hemorrhagic areas. Only in severe manifestations which involving the tunica muscularis, distinguished by large mucosal ulcers and abundant purulent discharge [14]. Antigen exposure to the immune system in the mucosa activates macrophages and dendritic cells which will present antigens and triggers a series of inflammatory responses, triggering the production of proinflammatory cytokines and differentiation of T-cells into effector T-helper (Th) cells. Th cells are part of the lymphocytes which mediate the immune response. Th also activates other immune cells in determining the specificity of antibodies secreted by B cells [15]. After proliferating, Th differentiates into: Th1, Th2, and several other derivatives. Th1 initiates the cell-mediated immune response by releasing proinflammatory cytokines like interferon gamma (IFN-), tumor necrosis factor alpha (TNF-), and interleukin-2 (IL-2). Whereas, Th2 stimulates the humoral immune response by producing anti-inflammatory cytokines like: IL-4, IL-5, IL-6, and IL-10. Th1 cytokines stimulate the development of Th1 cells to suppress Th2 cells in a reciprocal interconnection between Th1 and Th2 lymphocytes. Th2 cytokines, on the other hand, cause Th2 cells to suppress Th1 cells. So that under normal conditions there is homeostasis between the number of Th2 and Th1 cells. The increased polarization of Th1 is

believed to be closely related to the pathogenesis and chronic inflammatory response in UC [15,16].

Other immune responses from dendritic cells also trigger Toll-like receptors (TLRs) which initiate the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) which stimulates the production of other pro-inflammatory cytokines such as: IL-12, IL-23, and IL-1β , along with mitogen-activated protein kinase (MAPK) and their upstream signaling pathways, induce the AMP activated protein kinase (AMPK) pathway in LPS-induced macrophages which also contributes to chronic inflammatory responses. These conditions facilitate the recruitment of leukocytes to the GIT mucosa which is mediated by the release of chemoattractants such as CXCL8 which is found in the incidence of UC and amplifies the inflammatory response [17,18].

Mechanism of AG as Anti-inflammatory & Potential Antioxidant Agent for Ulcerative Colitis

AG*[C20H30O5;(3-[2-{decahydro-6-hydroxy-5-(hydroxymethyl)-5,8α-dimethyl-2-methylene-1-napthalenyl} ethylidene]dihydro-4-hydroxy-2(3H)-furanone)]* is a labdane diterpenoid that has been isolated from the stem and leaves of *A. paniculata* or *sambiloto* and has been studied for its therapeutic effects including anti-inflammatory, anti-platelet aggregation, antioxidant, immunomodulation, and potential antineoplastic properties. The extract is presented by colorless bicyclic diterpene lactone crystals and is extremely bitter. AG is contained in all sections of the *sambiloto*, but the highest levels are found in the leaves [19]. *Sambiloto* also has several other active compounds such as: deoxyandrographolide, diterpene glycosides, lactones, flavonoids, flavonoid glycosides, and polyphenols. Some other literature explains that the leaves and roots of the plant as a whole have also been used as anti-inflammation and anti-bacteria herbal medicine since ancient times for various diseases in Asia and Europe [20].

Inflammation is the main pathophysiology underlying the course of UC disease. A study by Fragoulis *et al* (2016) explained that the IL-23/IL-17 axis pathway in CD4+ T lymphocytes also plays important role in the expression of proinflammatory factors such as: TNF-α, IL-1β, and IL-6, which are closely associated with the pathogenesis of UC, and serve as the foundation for the novel development of anti-inflammatory therapeutic for UC. AG inhibits TNF-α expression thereby suppressing TNF-α-induced intercellular adhesion molecule-1 (ICAM-1) and also inhibits TNF-induced monocyte and endothelial adhesion, which are both key factors in the inflammatory responses and progression in the colonic mucosa [21, 22]. Another subsequent study by Zhu *et al* (2018) demonstrated that AG extract inhibited Th17 activity in CD4+ cells found in the colonic mucosa of mice with TNBS-induced colitis (4,6-trinitrobenzene-sulfonic acid) morphology, thereby inhibiting the production of IL-23, IL-17, and ROR-ɣ (Th17 transcription factor) at several concentrations (0, 10, 20 and 30 µg/mL) in combination with mesalamine.

Additionally, peripheral blood mononuclear cells (PBMCs) were isolated from UC patients, AG demonstrated inhibitory effects on Th17 cell percentages, proinflammatory factor levels, and relative protein expression at several concentrations as stated previously, resulting in the relief of UC [23]. These findings were consistent with a study related to the treatment of IL-23/IL-17 axis-mediated diseases such as UC, Crohn's disease, psoriasis and spondyloarthritis, which showed that AG performed similar efficacy to mesalamine. The data suggested that AG inhibits Th1/Th17 response while increasing the concentrations of IL-4 (a Th2 cell produced cytokine) in the culture medium, the percentages of Th2 cells, and the protein levels of GATA-3 (Th2 lineage-specific transcription factor), resulting in the balance of Th1/Th17/Th2 as part of the UC management. However, whether andrographolide affects the T cell responses of UC patients has not been explored [10].

In addition, previous studies by Guo *et al* (2014) showed the anti-inflammatory effect of AG by inhibiting NLRP-3 inflammasome in macrophages. AG reduced in a concentration-

dependent inhibition of pro-inflammatory IL1B secretion from lipopolysaccharide (LPS)-treated human monocytic THP-1 cells and murine bone marrow-derived macrophages (BMDM) by the ELISA assay. Furthermore, the activation of CASP1 (as indicated by the presence of the cleaved form and enzyme activity) was also significantly inhibited by AG, thus protecting the mucosal layer and lamina propria in conjunction with active colitis which correlated with the severity of inflammation, and tumor development [24,25]. Other anti-inflammatory effects were also shown by AG by inhibiting proinflammatory cytokines including NF-κB and MAPK in colon tissue, upstream the signaling pathways, as well as activating the AMPK pathway in LPS-induced macrophages, thus attenuating DSS-induced intestinal barrier dysfunction and inflammation [17]. AG also inhibits the expression of proinflammatory cytokines including cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), which are involved in the synthesis of prostaglandin E2 (PGE2) and free radical molecules nitric oxide (NO), by preventing Nf-kB from binding to host cell DNA [26], while it showed pro-resolving function by accelerating M1 to M2 macrophage conversion and up-regulating resolution-related genes (IL-10, TGF-β, and HO-1) [27].

AG also showed its potential antioxidant activity by preventing the activation and binding of iNOS to lipopolysaccharide (LPS) in the mucosal cell walls, so that the formation of NO molecules can be suppressed. Additionally, the phytochemical analysis of the extracts revealed variability in the peak of the non-standardized constituents, which are part of the flavonoid and phenylcarboxylic acid. It is widely known that flavonoids and phenolic acids work well as antioxidants and free radical scavengers, while oxidative stress is known to contribute to inflammatory tissue damage and plays a role in cytokine signaling including in UC as well [28,29]. Several other *in vitro* studies have also reported that AG has an anti-inflammatory effect by inhibiting macrophage and neutrophil adhesion-transmigration mediated by macrophage adhesion molecule-1 (Mac-1), thus contributing in suppressing the production of reactive oxygen species (ROS) mediated by protein kinase C (PKC). In addition, AG reduced the formation of the proteins caspase 3/8 and Bax as well as the phosphorylation of p38 in a Balb/c mouse model of intestinal mucositis caused by 5-fluorouracil (5-Fu) [26,30].

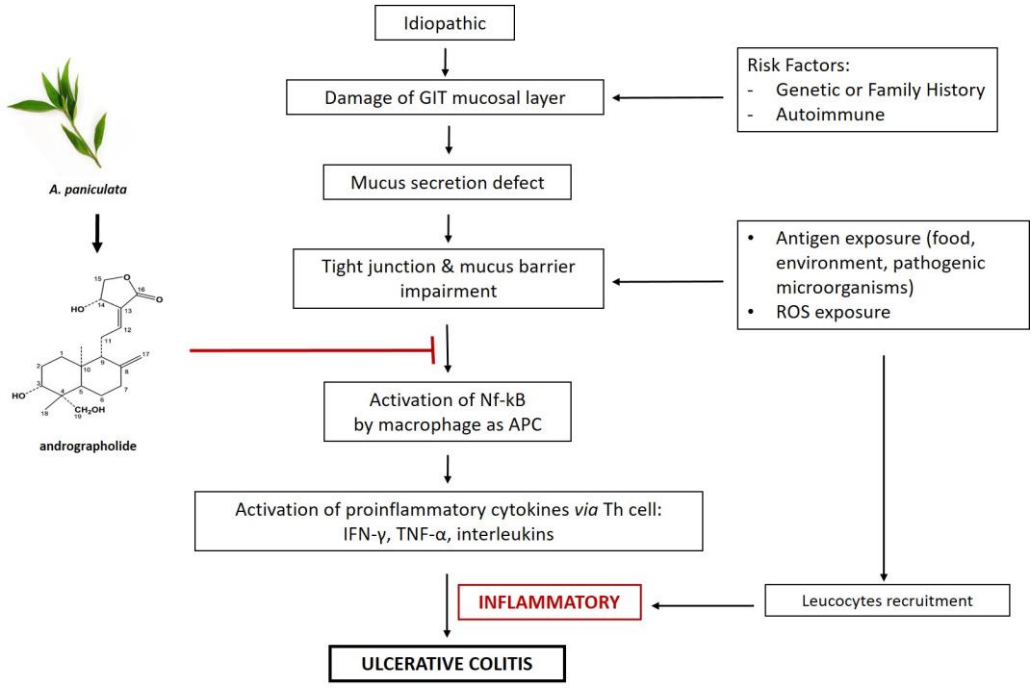

Figure 1. Mechanism effect of andrographolide (AG) extract on the pathogenesis of UC.

CONCLUSION

UC is an idiopathic chronic inflammatory disease of the GIT that occurs due to exposure to antigens originating from food, environmental factors and microorganisms that invade the mucosal lining of the GIT due to tight junction damage of the mucosal barrier. UC is commonly seen with symptoms like bloody diarrhea, abdominal pain, nausea and vomiting. One of the many medicinal plants easily found in Indonesia is *sambiloto (Andrographis paniculata*) which has been extensively researched and developed for both the characteristics and the pharmacological effects of the bioactive compound andrographolide (AG) which has numerous therapeutic benefits. AG is widely used as an anti-inflammatory agent with potential effects as an antioxidant that is believed to help improve the colonic mucosa by reducing and preventing the inflammatory process that occurs in the pathogenesis of UC, thus helping to control and decrease the severity of its symptoms, progression, recurrence, and UC-related complications as well as reducing unwanted side effects from the long-term drug use.

Further and advanced preclinical studies are needed in various colitis model trials followed by clinical studies to explore the efficacy and biological effects of AG and other active compounds contained in *sambiloto* in several range of doses as well as development in various preparations and techniques especially in Indonesia, with newer and modern extraction methods, to obtain optimal efficacy without triggering toxicity.

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Attachment

Table 1
Studiesa Related To Anti-inflammatory Effects Of AG in Sambiloto E

Titles	Study Designs	Methods & Doses	Results	Conclusions	Reference
Andrographolide inhibits TNFα-induced ICAM-1 expression via suppression of NADPH oxidase activation and induction of HO-1 and GCLM expression through the PI3K/Akt/Nrf2 and PI3K/Akt/AP-1 pathways in human endothelial cells	In-vitro experimental study	Using cell-line EA.hy926 in Cell culture medium (RPMI-1640), RPMI-1640 without phenol red, Dulbecco's modified Eagle's medium (DMEM). Analyzed & examinations include: Western Blot, RT-PCR, subcellular fractionation, Reactive oxygen species measurement, Cellular GSH measurement, Monocyte adhesion assay.	- 7,5 µg AG supressed TNFa-induced p65 nuclear translocation, ICAM-1 gene expression - 7.5 µg AG significantly decreased ROS generation. - AG increases GSH expression in time-dependent manner, inducing HO-1 as protection against oxidative stress via the PI3K/Akt pathway. - AG improved cellular GSH content and GCLM gene expression.	AG attenuates TNFα-induced ICAM-1 expression at least partially through suppression of NADPH oxidase activation and induction of HO-1 and GCLM expression, which is PI3K/Akt pathway-dependent.	(Lu et al., 2014)
Small molecule-driven mitophagy-mediated NLRP3 inflammasome inhibition is responsible for the prevention of colitis-associated cancer	Laboratory-experimental study	Using colitis-associated colonic (CAC) in the mouse model of azoxymethane (AOM)-dextran sulfate sodium (DSS) tumorigenesis. Later, divided into two groups of: AOM-DSS group & AOM-DSS with 15mg/kg AG group. Examinations were performed using: - immunofluorescence analysis - ELISA	AG was found to trigger mitophagy leading to a reversed mitochondrial membrane potential collapse, which in turn inactivated the NLRP3 inflammasome & downregulating the PIK3CA-AKT1-MTOR-RPS6KB1 pathway accounted for AG-induced autophagy. Showed by the average number of tumors per mouse in the AOM-DSS group was more than 2 times higher than that in the 15 mg/kg	AG is responsible for the prevention by inhibits CAC mitophagy-mediated NLRP3 inflammasome	(Guo et al., 2014)

			Andro-treated group. The tumor size was reduced by AG in a dose-dependent manner.		
Andrographolide presents therapeutic effect on ulcerative colitis through the inhibition of IL-23/IL-17 axis	In-vivo experimental study	C57BL mice age 5-6 weeks, divided into 4 groups (n=6): control group, UC group (TNBS-induced experimental colitis), experiment group (UC+AG), positive control group (UC+ mesalazine). AG dosing: 0.1 g/kg/day, for 7 days. Examinations were performed using: - Blood test: ELISA, flow cytometry analyses. - Colon tissue sample examination methods: histopathology + HE staining, ELISA, Western Blot -	The UC group showed cell swelling, necrosis and degeneration with massive neutrophil infiltration, elevating proinflammatory factors, followed by increased TH17 via IL-23/IL-17 axis, while tissue repair was seen in the AG and Mesalazine group. This shows the protective effect of AG. AG treatment significantly reduced the concentration of proinflammatory cytokines on the IL-23/IL-17 axis in a dose-dependent manner (p=<0.01). AG treatment decreased Th17 (p=0.01) in CD4+ cells.	AG inhibited the activity of IL-23/IL-17 axis and down-stream pro-inflammatory factors so as to suppress inflammation response, resulting in the relieving of UC.	(Zhu, Zheng, Chen, et al., 2018)
Andrographolide affects Th1/Th2/Th17 responses of peripheral blood mononuclear cells from ulcerative colitis patients	Laboratory-experimental study	Studied the effects of AG on the T cell responses of patients with UC; using peripheral blood mononuclear cells (PBMCs) were received & isolated from patients with UC and treated with several concentrations of AG (0, 10, 20 and 30 µg/ml). Examinations were performed using: - ELISA assay - Flow-cytometry analysis - Western blot	- AG supressed interferon γ (IF- γ), interleukin (IL)-23 and IL-17A, & improved IL-4 in a dose-dependent manner. - AG treatment lowered percentage of Th1 and Th17 cells and an improved proportion of Th2 cells. - T-bet (a Th1-specific transcription factor) and RAR-related orphan receptor γt (key transcription factor of Th17 cells) expression was reduced.	AG demonstrated the inhibitory effects on Th1/Th17 responses and the promoting effects on Th2, similarly on IL-23-treated PBMCs from healthy donors	(Zhu, Zheng, Zhou, et al., 2018)
Andrographolide inhibits inflammatory responses in LPS-stimulated macrophages and murine acute colitis through activating AMPK	In-vitro experimental study	Using murine macrophage cell line RAW264.7 & human promonocytic leukemia cell line U937, treated with AG (1-10 µg).	- AG inhibits the production of NO and proinflammatory cytokines (markedly increased by LPS stimulation) without affecting cell viability, determined by the MTT assay	AG effectively inhibits LPS-induced inflammatory responses via AMPK activation in macrophages, whereby AG	(Kim et al., 2019)

			- The expression and mRNA levels of IL-6, TNF-α and IL-1β expression were decreased by AG in a dose-dependent manner, measured by ELISA and RT-PCR ($P < 0.05$* compared to the controlled group) - AG suppressed the phosphorylation of IKKα/β, IκBα, ERK1/2, JNK, p38 and IRAK4 in the NF-κB and MAPK signaling pathways, in a dose-dependent manner.	also ameliorate DSS-induced acute colitis in mice.	
	In-vivo experimental study	Using BALB/c male mice (19 ± 1 g, 6–8 weeks of age). Acute colitis was induced by the oral administration of 3.5% DSS (dextran sulfate sodium)	- AG inhibited the loss of body weight shown in DAI score of the AG (100 mg/kg) treatment group was notably lower than that of the DSS treatment group, additionally improved colon shortening. - AG (100 mg/kg) attenuate large numbers of inflammatory cells, as shown as IHC analysis that demonstrated CD68 and F4/80 macrophage expression was decreased in AG treatment group compared to DSS treatment group.		
Oral Nanotherapeutics of Andrographolide / Carbon Monoxide Donor for Synergistically Anti-inflammatory and Pro-resolving Treatment of Ulcerative Colitis	In-vitro study	dextran-functionalized PLGA nanocarrier for efficient delivery of AG and a carbon monoxide donor (CORM-2) for synergistically anti-inflammatory/pro-resolving treatment of UC (AG/CORM-2@NP-Dex) in treating Colon-26 and Raw 264.7 cells as UC models in vitro	AG/CORM-2@NP-Dex performed anti-inflammatory effects by eliminating the over-production of pro-inflammatory mediator, nitric oxide (NO), and down-regulating the expression of pro-inflammatory cytokines (TNF-α, IL-1β and IL-6), while it showed pro-	AG performed anti-inflammatory/ pro-resolving effects, therefore relieving UC effectively.	(Zhou et al., 2023)

			resolving function by accelerating M1 to M2 macrophage conversion and up-regulating resolution-related genes (IL-10, TGF-β, and HO-1).		
Andrographolide inhibits TNFα-induced ICAM-1 expression via suppression of NADPH oxidase activation and induction of HO-1 and GCLM expression through the PI3K/Akt/Nrf2 and PI3K/Akt/AP-1 pathways in human endothelial cells	In-vitro experimental study	Using cell-line EA.hy926 in Cell culture medium (RPMI-1640), RPMI-1640 without phenol red, Dulbecco's modified Eagle's medium (DMEM). Analyzed & examinations include: Western Blot, RT-PCR, subcellular fractionation, Reactive oxygen species measurement, Cellular GSH measurement, Monocyte adhesion assay.	- 7,5 µg AG supressed TNFa-induced p65 nuclear translocation, ICAM-1 gene expression - 7.5 µg AG significantly decreased ROS generation. - AG increases GSH expression in time-dependent manner, inducing HO-1 as protection against oxidative stress via the PI3K/Akt pathway. - AG improved cellular GSH content and GCLM gene expression.	AG attenuates TNFα-induced ICAM-1 expression at least partially through suppression of NADPH oxidase activation and induction of HO-1 and GCLM expression, which is PI3K/Akt pathway-dependent.	(Lu *et al.*, 2014)
Small molecule-driven mitophagy-mediated NLRP3 inflammasome inhibition is responsible for the prevention of colitis-associated cancer	Laboratory-experimental study	Using colitis-associated colonic (CAC) in the mouse model of azoxymethane (AOM)-dextran sulfate sodium (DSS) tumorigenesis. Later, divided into two groups of: AOM-DSS group & AOM-DSS with 15mg/kg AG group. Examinations were performed using: - immunofluorescence analysis - ELISA	AG was found to trigger mitophagy leading to a reversed mitochondrial membrane potential collapse, which in turn inactivated the NLRP3 inflammasome & downregulating the PIK3CA-AKT1-MTOR-RPS6KB1 pathway accounted for AG-induced autophagy. Showed by the average number of tumors per mouse in the AOM-DSS group was more than 2 times higher than that in the 15 mg/kg Andro-treated group. The tumor size was reduced by AG in a dose-dependent manner.	AG is responsible for the prevention by inhibits CAC mitophagy-mediated NLRP3 inflammasome	(Guo *et al.*, 2014)

Andrographolide presents therapeutic effect on ulcerative colitis through the inhibition of IL-23/IL-17 axis	In-vivo experimental study	C57BL mice age 5-6 weeks, divided into 4 groups (n=6): control group, UC group (TNBS-induced experimental colitis), experiment group (UC+AG), positive control group (UC+ mesalazine). AG dosing: 0.1 g/kg/day, for 7 days. Examinations were performed using: - Blood test: ELISA, flow cytometry analyses. - Colon tissue sample examination methods: histopathology + HE staining, ELISA, Western Blot	The UC group showed cell swelling, necrosis and degeneration with massive neutrophil infiltration, elevating proinflammatory factors, followed by increased TH17 via IL-23/IL-17 axis, while tissue repair was seen in the AG and Mesalazine group. This shows the protective effect of AG. AG treatment significantly reduced the concentration of proinflammatory cytokines on the IL-23/IL-17 axis in a dose-dependent manner (p=<0.01). AG treatment decreased Th17 (p=0.01) in CD4+ cells.	AG inhibited the activity of IL-23/IL-17 axis and down-stream pro-inflammatory factors so as to suppress inflammation response, resulting in the relieving of UC.	(Zhu, Zheng, Chen, *et al.*, 2018)
Andrographolide affects Th1/Th2/Th17 responses of peripheral blood mononuclear cells from ulcerative colitis patients	Laboratory-experimental study	Studied the effects of AG on the T cell responses of patients with UC; using peripheral blood mononuclear cells (PBMCs) were received & isolated from patients with UC and treated with several concentrations of AG (0, 10, 20 and 30 µg/ml). Examinations were performed using: - ELISA assay - Flow-cytometry analysis - Western blot	- AG supressed interferon γ (IF- γ), interleukin (IL)-23 and IL-17A, & improved IL-4 in a dose-dependent manner. - AG treatment lowered percentage of Th1 and Th17 cells and an improved proportion of Th2 cells. - T-bet (a Th1-specific transcription factor) and RAR-related orphan receptor γt (key transcription factor of Th17 cells) expression was reduced.	AG demonstrated the inhibitory effects on Th1/Th17 responses and the promoting effects on Th2, similarly on IL-23-treated PBMCs from healthy donors	(Zhu, Zheng, Zhou, *et al.*, 2018)
Andrographolide inhibits inflammatory responses in LPS-stimulated macrophages and murine acute colitis through activating AMPK	In-vitro experimental study	Using murine macrophage cell line RAW264.7 & human promonocytic leukemia cell line U937, treated with AG (1-10 µg).	- AG inhibits the production of NO and proinflammatory cytokines (markedly increased by LPS stimulation) without affecting cell viability, determined by the MTT assay - The expression and mRNA levels of IL-6, TNF-α and IL-1β expression were decreased by AG in a	AG effectively inhibits LPS-induced inflammatory responses via AMPK activation in macrophages, whereby AG also ameliorate DSS-induced acute colitis in mice.	(Kim *et al.*, 2019)

			dose-dependent manner, measured by ELISA and RT-PCR ($P < 0.05$* compared to the controlled group) - AG suppressed the phosphorylation of IKKα/β, IκBα, ERK1/2, JNK, p38 and IRAK4 in the NF-κB and MAPK signaling pathways, in a dose-dependent manner. - AG inhibited the loss of body weight shown in DAI score of the AG (100 mg/kg) treatment group was notably lower than that of the DSS treatment group, additionally improved colon shortening. - AG (100 mg/kg) attenuate large numbers of inflammatory cells, as shown as IHC analysis that demonstrated CD68 and F4/80 macrophage expression was decreased in AG treatment group compared to DSS treatment group.		
	In-vivo experimental study	Using BALB/c male mice (19 ± 1 g, 6–8 weeks of age). Acute colitis was induced by the oral administration of 3.5% DSS (dextran sulfate sodium)			
Oral Nanotherapeutics of Andrographolide / Carbon Monoxide Donor for Synergistically Anti-inflammatory and Pro-resolving Treatment of Ulcerative Colitis	In-vitro study	dextran-functionalized PLGA nanocarrier for efficient delivery of AG and a carbon monoxide donor (CORM-2) for synergistically anti-inflammatory/pro-resolving treatment of UC (AG/CORM-2@NP-Dex) in treating Colon-26 and Raw 264.7 cells as UC models *in vitro*	AG/CORM-2@NP-Dex performed anti-inflammatory effects by eliminating the over-production of pro-inflammatory mediator, nitric oxide (NO), and down-regulating the expression of pro-inflammatory cytokines (TNF-α, IL-1β and IL-6), while it showed pro-resolving function by accelerating M1 to M2 macrophage conversion and up-regulating resolution-related genes (IL-10, TGF-β, and HO-1).	AG performed anti-inflammatory/ pro-resolving effects, therefore relieving UC effectively.	(Zhou *et al.*, 2023)