Agarwood Extract Mitigates Intestinal Injury in Fluorouracil-Induced Mice

Canhong Wang, a Shuai Wang, b Deqian Peng, c Zhangxin Yu, a Peng Guo, a, b and Jianhe Wei a, b

a Hainan Provincial Key Laboratory of Resources Conservation and Development of Southern Medicine, Key Laboratory of State Administration of Traditional Chinese Medicine for Agarwood Sustainable Utilization, Hainan Branch Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College; Haikou 570311, China; b National Engineering Laboratory for Breeding of Endangered Medicinal Materials, Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College; Beijing 100193, China; c School of Pharmacy, Hainan Medical College; Haikou 571199, China.

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Agarwood is used to treat gastrointestinal diseases. Although our previous studies demonstrated that agarwood ethanol extract produced by the whole-tree agarwood-inducing technique (WTAAE) improves intestinal peristalsis, the intestinal protective effect of WTAAE remains unclear. This study aimed to evaluate the protective effect of WTAAE on the intestinal injury induced by fluorouracil (5-FU) and explore its potential mechanism. Institute of Cancer Research (ICR) mice were given agarwood ethanol extracts (AAEs) (details in materials part), including WTAAE (0.71, 1.42 and 2.84 g/kg), wild agarwood ethanol extract (WAAE) and burning-chisel-drilling agarwood ethanol extract (FBAAE) (2.84 g/kg). A colon injury model was induced by 5-FU. After 14 d of treatment, the histopathology and biochemical and molecular parameters were measured. Our results indicated that WTAAE enhanced the intestinal advancing rate and alleviated the severity of colon injury similar the WAAE and better than FBAAE. Simultaneously, WTAAE reduced the nitric oxide (NO) concentration and increased the glutathione (GSH) and superoxide dismutase (SOD) levels. WTAAE also reduced the levels of interleukin-17 (IL-17) and IL-33 and elevated the level of IL-10. Furthermore, WTAAE upregulated the mRNA expression of the nuclear factor-E2-related factor 2–antioxidant response element (Nrf2–ARE) pathway and downregulated the mRNA levels of the nuclear factor-kappaB (NF-κB) pathway. WTAAE had a mitigating effect on intestinal damage, suggesting that it could be used as an intestinal protective and adjuvant therapy drug for intestinal injury induced by chemical drugs.

Key words fluorouracil (5-FU); intestinal injury; anti-inflammation; agarwood ethanol extract produced by the whole-tree agarwood-inducing technique (WTAAE); antioxidation

INTRODUCTION

Agarwood (Chinese name: ChenXiang), is a valuable non-timber fragrant product of Aquilaria spp. (Thymelaeaceae). Artificial agarwood-inducing methods provide ways to supply agarwood and conserve wild Aquilaria stock. Agarwood is a precious herb medicine that has been used for centuries in Asian countries including China to treat many diseases, especially gastrointestinal diseases. Previous investigations have revealed that sesquiterpenes and 2-(2-phenylethyl)-chromone derivatives are two predominant constituents of agarwood. Via HPLC-GC-MS, we previously revealed that the components of agarwood ethanol extract produced by the whole-tree agarwood-inducing technique (WTAAE) include sesquiterpenes (10.615%), chromosome (31.678%), aromatics (31.831%) and other known compounds (25.760%). Extracts from different parts of the plant were found to possess anti-inflammatory, pain-alleviating, antioxidant and other biological activities, supporting its folkloric and clinical use for diseases related to painful and inflammatory conditions, such as intestinal injury, gastric ulcer, gastritis, angina, trauma, and cough. Meanwhile, extracts, essential oils and primary compounds from agarwood exhibited a wide array of pharmacological properties, such as laxative, anti-inflammatory, anti-neuroinflammatory, neuroprotective, antioxidant and antitumor activities. Moreover, our earlier study revealed that WTAAE has an obvious effect on intestinal peristalsis. According to its traditional efficacy and modern application, we boldly hypothesize that agarwood has a treatment effect on inflammatory bowel diseases. However, the mechanism of action of WTAAE for anti-intestinal mucosal injury has not been verified by modern pharmacological studies.

Fluorouracil (5-FU), a common therapeutic and effective chemotherapy drug, is used to treat gastrointestinal malignancies. However, in the clinic, 50–80% of patients who receive 5-FU report considerable side effects, such as diarrhea symptoms and gastrointestinal discomfort. Intestinal injury is a severe limiting factor in clinical applications of 5-FU. Previous studies in animal models have demonstrated that intestinal injury is induced by 5-FU, and the pathogenetic mechanisms of the drug has been investigated in experimental models.

Intestinal mucosa damage, a common side effect of chemotherapeutic drugs in clinical application, is characterized by a shorter length of intestinal villi, disruption of crypt cells and infiltration of inflammatory cells in intestinal tissues. The mechanisms of the intestinal inflammatory response include an increase in reactive oxygen species, inflammatory mediators, necrosis and apoptosis. Unfortunately, there is no curative intervention for intestinal mucosa damage induced
by chemotherapy drugs. Therefore, much work is needed to search for and develop natural drugs that can prevent and treat this intestinal side effect.

In this study, our goal was to investigate the protective effect of WTAAE on colon mucosal injuries induced by 5-FU and explore its potential mechanism of action. Moreover, we compared the protective effect of the different agarwood ethanol extracts (AAEs) against the damage. This paper provides experimental evidence for further research into agarwood’s protective mechanisms of action against gastrointestinal inflammatory diseases.

MATERIALS AND METHODS

Reagents  Fluorouracil injection was provided by Shanghai Xu Dong Hai Pu Pharmaceutical Co., Ltd. (Shanghai, China). All of the biochemical indicator kits, including for nitric oxide (NO), glutathione (GSH), superoxide dismutase (SOD) and protein quantification, were obtained from Jian Cheng Biotech Co. (Nanjing, China). Enzyme-linked immunosorbent assay (ELISA) kits for the determination of the cytokines interleukin-17 (IL-17), interleukin-33 (IL-33) and interleukin-6 (IL-6) were provided by TransGen Biotech (Beijing, China). The primers for the nuclear factor-kappaB (NF-κB) and nuclear factor-E2-related factor 2–antioxidant response element (Nrf2–ARE) signaling pathways were synthesized by Sangon Biotech (Beijing, China).

Materials  The whole-tree agarwood-inducing technique (Patent Number: ZL201010104419.5) induced agarwood, wild agarwood and burning-chisel-drilling agarwood of *Aquilaria* spp. (Thymelaeaceae) purchased from Guangdong, China, were all authenticated by Jian-He Wei from the Institute of Medicinal Plant Development, Chinese Academy Sciences & Peking Union Medical College (Beijing, China). Dried and powered agarwood (1000×g) induced by the whole-tree agarwood-inducing technique were soaked with 95% alcohol (5L) for 2h, refluxed for 1h three times and added to the solution. The resulting solution was evaporated in vacuo to obtain a dark brown alcohol extract (140 g, 14%) and stored at −20°C. Alcohol extracts of wild agarwood and burning-chisel-drilling agarwood were produced by the same method (10.5% for wild agarwood; 14% for fire burned agarwood).

Ethics Statement  All the animal experiments were performed in accordance with the National Institutes of Health regulations for the care and use of animals. All mouse protocols were approved by the Animal Ethics Committee at the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences (No. SLXD-15-10-15) and were in compliance with the Chinese Association for Laboratory Animal Sciences guidelines.

Animal  Male ICR mice weighing 18–20g were purchased from Vital River Laboratory Animal Technology Co., Ltd. (certificate no. SCXK-2016-0011, Beijing, China). Animals were kept on a 12h light/dark cycle with a controlled temperature (20–24°C) and humidity (50–70%). Mice were housed with free access to food and water for 3d prior to the experiments.

Animal Experiments  Mice were randomly divided into seven groups, with ten mice in each group: normal group, model 5-FU (25mg/kg) group, wild agarwood alcohol extract (WAAE) (2.84g/kg) + 5-FU (25mg/kg) group, burning-chisel-drilling agarwood alcohol extract (FBAAE) (2.84g/kg) + 5-FU (25mg/kg) group, and WTAAE (0.71, 1.42, 2.84g/kg) + 5-FU (25mg/kg). Mice in the normal and model groups were orally administered distilled water (20mL/kg, once/d). Mice in model 5-FU group were intraperitoneally (ip) injected with 5-FU (25mg/kg, 10mL/kg) once every 2d. Mice in the combined therapy groups were orally administered the AAEs once per d and ip injected with 5-FU once/2d. Each group was administered continuously for 14d. Animals received no food 12h prior to sacrifice.

Measurement of the Intestinal Propulsion Rate  Two hours after the last treatment, mice were administered a mixed solution containing 5% charcoal powder and 10% arabic gum powder. After 20min, blood samples were collected, and then, mice were sacrificed. The intestines were removed, and the charcoal powder pushing length of intestine and intestinal total length were measured. The intestinal propulsion rate was calculated as follows: intestinal propulsion rate (%) = carbon pushing length/intestinal total length × 100.

Hematoxylin–Eosin (HE) Staining for Colon Tissue and Histopathological Evaluation  Colon tissues were fixed in a 10% formalin solution and embedded in paraffin. Then, the paraffin-embedded samples were cut into 5μm thick sections, which were deparaffinized in xylene and rehydrated in a graded series of ethanol. For histological analysis, sections were stained with HE using the standard techniques and photographed under a microscope at a magnification of 200×.

During observation, the semi-quantitative histopathological examination was performed by microscopic observation. The severity of colon mucositis damage was assessed according to ten different parameters: the reduction of the intestinal crypts and villi, disruptions and abscess formation in the crypts, thickening of the outer muscle layer, integrity of the epithelium and muscular layers, inflammatory cell infiltration, vacuolization and edema. The scores for each parameter ranged from 0 to 3. Histological damage was calculated using the following score system: 0, absent (no destruction); 0.5, few (0–20% destruction); 1, mild (20–50% destruction); 2, moderate (50–80% destruction); and 3, severe (80–100% destruction).

Measurement of Lipid Peroxidation in the Supernatant of Homogenates of the Colon  Colons were clipped and homogenized with ice-saline at a ration of 1:9 (w/v) using a homogenizer. The homogenate was centrifuged at 3000rpm at 4°C for 15min. The supernatant was collected and stored at −80°C. NO, GSH and SOD were detected using special kits according to the manufacturer's instructions.

Detection of the Serum IL-17, IL-33 and IL-10 Levels  The serum levels of cytokines involved in inflammatory response, including IL-17, IL-33 and IL-10, were measured using mouse-specific ELISA kits according to the manufacturer’s instructions.

Analysis of the mRNA Levels of Anti-oxidation and Inflammation  The mRNA levels of the genes involved in anti-oxidation and inflammation were determined by RT-PCR. Total RNA in colon tissues from intestinal injury model mice and the AAE (2.84g/kg) group were extracted and reverse-transcribed into cDNA for 30min at 42°C and 5min at 85°C.
using an RT kit. cDNA was amplified with a RT-PCR system via a PCR Super-mix kit using the Power SYBR Green PCR Mix. Samples were cycled 40 times using CFX96 Touch TM PCR System (BioRad, Hercules, CA, U.S.A.). The conditions were as follows: 5 min at 95°C followed by 40 cycles of 15 s at 95°C, 34 s at 60°C, and 40 s at 72°C. The cycle threshold (CT) was calculated under the default setting for real-time sequence detection software.24 At least three independent biological replicates were performed to check the reproducibility of the data. The following gene-specific primer sequences, as defined in Table 1, were used: reactive oxygen species (ROS), Kelch-like ECH associating protein 1 (Keap1), Nrf2, nicotinamide adenine dinucleotide phosphate (NADPH), heme oxygenase-1 (HO-1), glutathione-S-transferase (GST), tumor necrosis factor-α (TNF-α), TNF receptor associated factor 6 (TRAF6), myeloid differentiation factor 88 (MyD88), IκB kinase (IKKβ), inhibitory factor kappaB-β (IκB-β), NF-κB and β-actin.

Table 1. Primer Sequences Used in RT-PCR

| Genes          | Primer sequences                          |
|----------------|-------------------------------------------|
| ROS            | Forward 5’-CTGCCCTAAGCTCTCGTGTAAC-3’       |
|                | Reverse 5’-CAGAGTTCGAAACACTGACATCCA-3’     |
| Keap1          | Forward 5’-TGCCCCCTGTGGTCAAGTGC-3’         |
|                | Reverse 5’-GGTTCGGTACCCTCCTGC-3’           |
| Nrf2           | Forward 5’-CTTGAAAGATCTGCAATAGTGC-3’       |
|                | Reverse 5’-GTGAAAACCTGAAGCCAAAGGC-3’       |
| NADPH          | Forward 5’-ATGGGAGGTGTGCAACTCGA-3’         |
|                | Reverse 5’-GCCCTCTCTCAGCGGATAGTGA-3’       |
| HO-1           | Forward 5’-AACGGCAAGATCTGATCTGCA-3’        |
|                | Reverse 5’-GCCGCTGATAGATGTCAGAAAGA-3’      |
| GST            | Forward 5’-TGCCAGTCCAGCGAAATACC-3’         |
|                | Reverse 5’-CATTCTATCTCGTCCGCTCC-3’         |
| TNF-R          | Forward 5’-CCGGGAGAGGAGGGATAGCTT-3’        |
|                | Reverse 5’-TCGGAGACTCATTCAACAGT-3’         |
| TRAF6          | Forward 5’-AAAGCGAGAGGATCTCTTCCTCG-3’      |
|                | Reverse 5’-ACTGCGAGCCTCAGCTAAGCAG-3’       |
| MyD88          | Forward 5’-CTCATGGTCTCCTACCATCCCGT-3’      |
|                | Reverse 5’-AAACTCGGAGTGCGGTCAAG-3’         |
| IKKβ           | Forward 5’-CAACCGCAAGGAGATGCTAGC-3’        |
|                | Reverse 5’-CAACGGTGTACGACTGATGAC-3’        |
| IκB-β          | Forward 5’-CAACCGCAAGGAGATGCTAGC-3’        |
|                | Reverse 5’-CAACGGTGTACGACTGATGAC-3’        |
| NF-κB          | Forward 5’-GGAGCGATCTGCGTGAAGG-3’          |
|                | Reverse 5’-CCCTCGGTGGATTTCTGCTG-3’         |
| β-Actin        | Forward 5’-GGCTGTATTCCTCCCTCAGTC-3’        |
|                | Reverse 5’-CCAGTTGGTAAACATGCCATGT-3’       |

**Statistical Analysis** Data are presented as the means ± standard deviation (S.D.) for ten animals in each group and were statistically evaluated using SPSS17 software (IBM Corporation, New York, U.S.A.). Differences between groups were analyzed by one-way ANOVA and Tukey’s *post hoc* test (TTTEST), and *p*<0.05 was considered to denote statistical significance.

RESULTS

**Effect of WTAAE on the Body Weight** The changes of body weight are shown in Table 2. Compared with the normal group, the body weight was obviously loss in model group (*p*<0.01). However, the body weights were increased in the AAEs treatments. Especially, treatment of WTAAE obviously increased the body weight loss in the thirteenth day (*p*<0.05 or *p*<0.01).

**Effect of WTAAE on the Intestine Propulsion Rate** As shown in Fig. 1, the intestine propulsion rate of the 5-FU group (88.52 ± 7.23) was obviously lower than that of the normal group (66.83 ± 5.99). However, the intestine propulsion rates were significantly increased in the WAAE (64.57 ± 7.31) group and the 1.42 kg/kg (63.53 ± 6.02) and 2.84 kg/kg (65.84 ± 6.90) WTAAE (*p*<0.05) groups, all of which were better than that of the FBAAE (62.21 ± 5.81) group.

Table 2. Effects of Orally Administered AAEs on the Body Weight of the 5-FU-Induced Intestinal Injury Mice

| Group/days | First day | Fifth day | Ninth day | Thirteenth day |
|------------|-----------|-----------|-----------|---------------|
| Normal     | 24.69 ± 1.31 | 28.35 ± 1.02 | 30.70 ± 1.23 | 32.10 ± 1.45 |
| Model      | 22.08 ± 2.40** | 25.19 ± 2.06** | 26.89 ± 2.08** | 27.45 ± 2.28** |
| WAAE       | 23.06 ± 1.76 | 26.37 ± 1.50 | 28.06 ± 1.85 | 30.36 ± 1.75** |
| FBAEE      | 23.11 ± 1.88 | 25.40 ± 2.00 | 26.46 ± 2.71 | 27.86 ± 2.31 |
| WTAAEL     | 23.09 ± 1.45 | 26.19 ± 1.46 | 27.89 ± 1.57 | 28.78 ± 1.97** |
| WTAAEM     | 22.91 ± 2.20 | 26.22 ± 2.04 | 28.15 ± 2.00 | 30.47 ± 2.54** |
| WTAAEH     | 23.33 ± 1.41 | 26.41 ± 0.97** | 28.10 ± 1.68 | 30.45 ± 1.23** |

The data are presented as means ± S.D. (n = 10). **p<0.01 vs. normal group; *p<0.05 vs. model group indicate a significant difference.**
result suggested that WTAAE had a beneficial effect on intestine propulsion, supporting the earlier results of intestinal peristalsis.

Colon Tissue Histopathological Examination To observe the degree of damage of colon tissues, biopsies were performed, the results of which are shown in Figs. 2A–G. Colon tissues were significantly damaged in the 5-FU-induced group, characterized by villi shortening and crypt disruption, inflammatory cell infiltration, goblet cell reduction, and mucosa and muscle layer thinning (Fig. 2B); moreover, there were more colon histopathological damage index (HDI) lesion sores than those in other groups \( (p < 0.001) \) (Fig. 2H). However, AAE treatment obviously protected against this histopathological damage. Furthermore, the degree of colon tissue damage was lower in WAAE (Fig. 2C), FBAAE (Fig. 2D) and WTAAE (Figs. 2E–G); this effect of WTAAE at a 2.84 g/kg dose was similar to that of WAAE and better than that of FBAAE. These results support the idea that WTAAE can significantly relieve the colon histopathological injury caused by 5-FU.

Effect of WTAAE on NO, GSH and SOD in the Supernatant of the Colon As shown in Figs. 3A–C, the NO level \( (10.35 \pm 12.96) \) was obviously higher \( (p < 0.01) \) in the 5-FU group than that in the normal group \( (4.60 \pm 1.58) \), suggesting that the colon caused oxidative and inflammatory injury. However, treatment with AAEs significantly reduced the level of NO in the supernatant of colon tissues. By contrast, the content of GSH and activity of SOD were decreased by 56.27 and 37.63% in the model group, respectively, and then significantly increased by the treatment with AAEs \( (p < 0.05 \) or \( p < 0.01) \). Meanwhile, the effect of WTAAE was similar to that of WAAE and better than that of FBAAE at the same dose. That is, WTAAE obviously reduced the colon injury induced by 5-FU via an antioxidant effect.

Effect of WTAAE on the Serum Cytokine Level of IL-17, IL-33 and IL-10 Various inflammatory cytokines, such as IL-1\( \beta \), IL-6, IL-17 and IL-33, have been shown to be produced during drug-induced colon injury and are involved in promoting further tissue damage. Therefore, we evaluated the serum levels of IL-17 and IL-33 of intestinal injury mice induced by 5-FU. As shown in Figs. 4A and B, the levels of IL-17 \( (47.56 \pm 5.27) \) and IL-33 \( (5.26 \pm 1.16) \) were significantly increased \( (p < 0.01) \) in mice with an intestinal injury compared to those of normal mice \( (21.75 \pm 7.12 \) and \( 1.21 \pm 0.66) \), confirming that the inflammatory response occurred after 5-FU treatment. However, AAEs markedly decreased the levels of IL-17 and IL-33 \( (p < 0.05 \) or \( p < 0.01) \) compared with those in the model group. By contrast, the anti-inflammatory cytokine level of IL-10 was significantly increased in the WAAE \( (p < 0.05) \) group and 2.84 g/kg WTAAE group \( (p < 0.01) \) (Fig. 4C) compared to that of the model group, showing the potential anti-inflammatory effect of WTAAE.

Effect of WTAAE on of Gene Expression of the Nrf2–ARE Signaling Pathway To explore the protective mechanisms of WTAAE anti-oxidation in colon damage induced by 5-FU, we further detected the mRNA expression of the Nrf2–ARE pathway in colon tissue by RT-PCR. As expected, treatment with WAAE and WTAAE markedly decreased the mRNA level of ROS (Fig. 5A) \( (p < 0.01 \) or \( p < 0.05) \) compared to that of the model group. Moreover, AAEs significantly increased the mRNA expression levels of Keap1, Nrf2, NADPH,
HO-1 and GST (Fig. 5B; \( p < 0.05, p < 0.01 \) or \( p < 0.001 \)) in colon tissue of 5-FU-induced injury mice, showing a significant antioxidant effect by activating and upregulating the Nrf2–ARE pathway.

**Effect of WTAAE on of Gene Expression of the NF-κB Signaling Pathway** Next, to explore the protective mechanisms of WTAAE anti-inflammation in colon injury induced by 5-FU, we also detected the mRNA expression of the NF-κB pathway by RT-PCR in colon tissue. As shown in Figs. 6A and B, WTAAE markedly decreased the mRNA expression levels of TNF-R, TRAF6, MyD88, IKKβ, 1xβ-β and NF-κB \( (p < 0.05 \) or \( p < 0.01 \)) in the colon tissues of injured mice, showing a sharp anti-inflammatory effect by inhibiting and downregulating the NF-κB pathway.

**DISCUSSION**

Recently, many studies have emerged on natural products with few side effects for treating chronic diseases, including inflammatory and damage bowel disease.\(^\text{25–27}\) Agarwood, a precious traditional Chinese medicine, has been used for centuries to treat gastrointestinal diseases.\(^\text{7}\) Our earlier study revealed that WTAAE significantly improved intestinal peristalsis.\(^\text{15}\) Intestinal injury is common in chronic and inflammatory diseases. Previous studies in animal models
have demonstrated that colon injury is induced by 5-FU and investigated the pathogenetic mechanisms of this damage.

Therefore, in the present study, we verified that intestinal injury is induced by 5-FU in mice. First, we found that WTAAE protected against 5-FU-induced colon damage using biochemical parameters and histological assessments. Second, WTAAE markedly inhibited oxidative stress, inflammatory factor secretion and the inflammatory response, alleviating the 5-FU-induced colon injury, in correlation with the regulation of the Nrf2–ARE pathways. Third, the protective effect of WTAAE was similar to that of WAAE and better than that of FBAAE.

The research team’s previous study revealed that 99 components of WTAAE include sesquiterpenes (10.615%), chromone (31.678%), aromatics (31.831%) and other known compounds (25.760%). Chromone and aromatics are the major components of WTAAE. Several studies showed that 2-(2-phenethyl)-chromones, methanol extract and ethyl acetate extract of agarwood had anti-inflammatory effect. Beside, our review paper also revealed that nearly thirty 2-(2-phenylethyl) chromones of agarwood showed the anti-inflammatory effect. Therefore, we guess that the 2-(2-phenylethyl)chromones of agarwood probably play an inflammatory protective action, which is an important event that we will continue study and clarify. Intestinal damage has been shown to be induced by drugs with severe oxidative stress, including increased levels of ROS, NO and malondialdehyde (MDA) and decreased levels of GSH and SOD. However, a major mechanism of defense against oxidative stress is the activation of Nrf2–ARE signaling. Nrf2–ARE, a central controller, is responsible for diverse cyto-protective processes. Under normal conditions, Nrf2 is localized in the cytoplasm and interacts with Kelch-like ECH associating protein 1 (Keap1), inhibiting Nrf2 ubiquitin-mediated degradation. Upon exposure to ROS or other stimuli, Keap1 degrades and is activated, translocates into the nucleus and binds to ARE, enhancing the production of many antioxidant enzyme genes, such as glutathione peroxidase.

![Fig. 5. Effects of Orally Administered AAEs on the mRNA Factors Expression Levels of Nrf2–ARE Signaling Pathway in Colon Tissues of the 5-FU-Induced Intestinal Injury Mice](image)

The data are presented as means ± S.D. (n = 3) of triplicate experiments. *p < 0.05, **p < 0.01 vs. model group indicate a significant difference.

![Fig. 6. Effects of Orally Administered AAEs on the mRNA Factors Expression Levels of NF-κB Signaling Pathway in Colon Tissues of the 5-FU-Induced Intestinal Injury Mice](image)

The data are presented as means ± S.D. (n = 3) of triplicate experiments. *p < 0.05, **p < 0.01 vs. model group indicate a significant difference.
(GPx), glutathione-S-transferase (GST), heme oxygenase (HO), superoxide dismutase (SOD), glutathione (GSH) and NADPH, which protect cells from oxidative damage.\(^{27,30}\) In the present study, our results showed that AAEs obviously enhanced the mRNA expression of Nrf2, Keap1, NADPH, HO-1, and GST as well as GSH content and SOD activity and decreased the mRNA level of ROS and the NO content compared with those of the model group. AAEs had a significant effect on free radical scavenging and anti-oxidative damage; the effects of WTAAE were similar to those of WAAE and higher than those of FBAAE, indicating the AAE-mediated colon protective effect by upregulation of the Nrf2–ARE pathway.

Inflammation is another crucial factor involved in the pathogenesis of intestinal damage. NF-κB is a vital transcription factor that regulates many immune and acute inflammatory processes. Activation of NF-κB upregulated pro-inflammatory cytokines, including IL-17, IL-33 and IL-6.\(^{40,41}\) The present study suggested that AAEs, especially WTAAEs, significantly decrease the level of inflammatory cytokines, including IL-17 and IL-33, and markedly enhanced the level of IL-10. These results were especially true for the 2.84 g/kg WTAAE group, which showed a more beneficial effect than the other groups and indicated that AAEs were effective at inhibiting and alleviating symptoms and protecting the intestines from inflammatory damage.

It is well known that NF-κB controls the regulated effect of inflammatory processes in drug-induced tissues injury and activates genes that are involved in the inflammatory response.\(^{26}\) Normally, NF-κB is located in the cytoplasm in its inactive form by binding to IκB-α/β. IκB-α/β is controlled upstream by the IκB kinase (IKK) complex.\(^{27}\) However, in response to a variety of stimuli, NF-κB dissociates from IκB-α/β and translocates into the nucleus to modulate the transcription of downstream genes and induce the inflammatory response.\(^{39,42}\) The present study suggested that AAEs, especially WTAAEs, significantly decrease the mRNA expression of TNF-α, TRAF6, MyD88, IκBα, IκBβ, and NF-κB by down-regulating and inhibiting the NF-κB pathway and contribute to the anti-inflammatory effect by controlling the inflammatory response in colon tissues.

CONCLUSION

Our results demonstrated that WTAAE mitigated the symptoms of colon mucosa oxidative damage and inflammation in 5-FU-induced mice. The effect of WTAAE was similar to that of WAAE and better than that of FBAAE. From these results, we conclude that the potential mechanisms of this effect are related to anti-inflammatory and anti-oxidative effects. However, the specific mechanism of this action needs to be further studied and confirmed. Generally, our study suggests that agarwood may be a potential protection drug in the prevention and treatment of inflammatory and oxidative injury bowel diseases.

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Conflicts of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

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