Original Article

Therapeutic Effect of *Syzygium aromaticum* and *Abelmoschus esculentus* Mixture on Induced Ulcerative Colitis in Rats Through Its Effects on Pro- and Anti-Inflammatory Mediators and Oxidative Stress

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Abstract

Background: Ulcerative colitis (UC) is a chronic inflammatory bowel disease, characterized by dysregulated local immune defense with a constant influx of leucocytes. Many factors can cause UC such as environmental factors and genetic factors. **Objectives:** Treat UC with a mixture of plants and assess the levels of interleukin (IL-8), IL-10, reactive oxygen species (ROS), and malondialdehyde (MDA) before and after treatment. **Materials and Methods:** This was done by drawing blood from rat with induced UC, divided into different groups, including a negative group (healthy rat) and a positive control group (rats with induced UC), along with three treatment concentrations (400, 600, and 800 mg/kg/day) from a plant mixture for UC treatment. **Results:** The total leukocyte count, IL-8, ROS, and MDA levels appeared to increase in the positive control group compared with all other groups, while IL-10 showed a significant decrease in the positive control compared with all groups. Histological findings of the positive control group appeared necrosis, thickening of the submucosal layer, hyperplasia of goblet cells, more progressive of fiber connective tissue, enlargement of colon wall, and infiltration of inflammatory cells. Inflammation in the mucosa lead to disruption of the normal crypt architecture, with distortion, branching, and loss of structure, along with the presence of immune cells. Conversely, histological findings in the 400 mg/kg group showed normal colorectal histology with hyperactivity goblet cells, increased mucus production, thickening of the mucosal layer, and myenteric plexus. On the other hand, histological findings in the fourth group (600 and 800 mg/kg) revealed normal colorectal tissue with a chronic inflammatory response. **Conclusions:** The mixture of plants has a therapeutic effect on UC because it contains phenolic compounds that act as anti-inflammatory and antioxidant agents.

Keywords: *Abelmoschus esculentus*, IL-10, IL-8, MDA, ROS, *Syzygium aromaticum*, ulcerative colitis

Introduction

Ulcerative colitis (UC) is a chronic inflammatory disorder of the large intestine. Its etiology is still unknown, although most experts agree that it is a complex illness caused by variables such as environmental exposure, genetic predisposition, aberrant gut flora, and an improper immune response.[1] Additionally, clinical research has revealed that patients have lower antioxidant levels and higher levels of reactive oxygen molecules.[2] In addition to causing tissue inflammation, this oxidative stress may also play an etiological role in the pathophysiology of UC.[3]

The two most typical signs of UC are diarrhea with blood or pus and abdominal discomfort. Additional symptoms may include anemia, exhaustion, fever, nausea, appetite

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loss, weight loss, rectal bleeding, loss of bodily fluids and nutrients, skin lesions, and stunted growth in children.[4]

The inflammatory mucosa produces excessive amounts of reactive oxygen species (ROS), which may be a significant factor in the etiopathogenesis of inflammatory bowel disease.[5]

An imbalance between pro-oxidative molecules and antioxidant defenses results in oxidative stress, compromising cellular integrity and causing membrane malfunction, protein aggregation, and DNA damage. Through positive feedback, oxidative stress induced by ROS creation triggers an initial inflammatory response, leading to more ROS production and tissue damage.[3] Further tissue damage may arise from increased ROS formation during respiration, as well as during prostaglandin and leukotriene metabolism by other immune cells, including monocytes and leukocytes, during inflammation.[6]

Abelmoschus esculentus is a significant vegetable crop indigenous to tropical Africa.[7] *A. esculentus* is the most well-known species in the Malvaceae family and is cultivated globally.[8] *A. esculentus* is also used in traditional medicine for treating diseases of the digestive system, displaying antiulcer activity. Its immature pods and seeds contain a polysaccharide rich in phenolics, including hydroxycinnamic derivatives, quartering derivatives, and catechin oligomers, which possess powerful biological properties.[9]

Syzygium aromaticum (cloves) are dried flower buds native to the Indonesian Maluku islands and belong to the Myrtaceae family. They are considered one of the most adaptable spices.[10] The clove tree consists of leaves and buds, with the commercial production of flowering buds starting approximately 4 years after planting.[11] Cloves have been traditionally used for their antiseptic, antimicrobial, analgesic, and local anesthetic properties.[12] In addition, studies have shown that clove powder may influence growth performances, immunity response, hematological parameters, and lymphoid systems.[13]

Cloves contain a significant amount of active biologically compounds, including eugenol, eugenol acetate, rutin, quercetin, and β-caryophyllene.[14] Eugenol, constituting 70%–90% of cloves, is the most active compound.[14] Quercetin, a natural bioflavonoid, is another essential component of cloves.[13] Studies have shown that quercetin possesses anti-inflammatory, anticarcinogenic, antioxidative, free-radical scavenging, antifibrotic, and antiproliferative properties in various cell types and animal models.[15] Additionally, it has been observed to reduce the synthesis of interleukin (IL)-6, IL-8, and MCP-1 in 4-hydroxynonenal-stimulated ARPE-19 cells human retinal pigment epithelial cell line (ARPE-19).[15] In animal models, rutin has been shown to reduce inflammatory pain. Its analgesic effects are attributed to its ability to stimulate the NO–cGMP–PKG–KATP channel signaling, suppress NFκB activation, and modulate the Nrf2/HO-1 pathway. Furthermore, rutin holds promise as a potential pharmaceutical strategy for managing pain induced by G-CSF without interfering with its main therapeutic function of releasing hematopoietic progenitor cells into the bloodstream.[16]

Materials and Methods

Animals

The present study was conducted using adult male albino rats weighting between 180 and 225 g. They were obtained from the animal house at Babylon University, Iraq. They were placed in lab cages and provided with tap water and food in the same environment for 1 week before the beginning of the experiments.

Experimental protocol

Fifty male rats were randomly divided into five groups, each consisting of 10 rats. (1) The first group served as the negative control (n = 10) and was treated orally with 0.5 mL of distal water for 60 days. (2) The second group served as the positive control (n = 10) and was induced with ulcers without treatment. (3) The third group (n = 10) was induced with ulcers and treated orally with 400 mg/kg/day of a mixture of plants for 60 days. (4) The fourth group (n = 10) was induced with ulcers and treated orally with 600 mg/kg/day of a mixture of plants for 60 days. (5) The fifth group (n = 10) was induced with ulcers and treated orally with 800 mg/kg/day of a mixture of plants for 60 days.

Induced ulcer

Using 0.5 mL of 5% acetic acid injected locally into the colorectal region, after 24 h of the last dose, eight animals from each group were dissected after being anesthetized with diethyl ether. The abdominal cavity was opened with a sharp scalpel, and then the colorectal region was removed and washed in a petri dish containing normal saline. The tissue was then preserved in 4% formalin.[17]

Assessment of IL-8 and IL-10

After blood was collected and serum was isolated, the assessment of IL-8 was conducted using a rat IL-8 enzyme-linked immunosorbent assay (ELISA) kit (Cat. No. E1167Ra) from Bioassay Technology Laboratory, and IL-10 was assessed using a rat IL-10 ELISA kit (Cat. No. E0108 Ra) from the same laboratory-.

ROS determination

The FOX2 technique was used to measure reactive oxygen species (ROS). The test system is based on the oxidantion of the ferrous ion–odianisidine complex to ferric ion by different forms of oxidants present in the plasma samples.

Xylenol orange binds to the Fe(III) ion produced, creating a complex with an absorbance peak at 560 nm.[18]

Estimation of serum malondialdehyde

A 2-thiobarbituric acid reactive substances assay kit was used to measure serum malondialdehyde (MDA) concentration.

Malondialdehyhe was estimated by Thiobarbituric acid (TBA) assay method of Buege Aust, 1978, using a spectrophotometer.[19]

Histopathology in colorectal region

Tissues were cleaned with normal saline solution and then fixed for 24 h in 4% paraformaldehyde. Afterward, paraffin blocks were made by processing the fixed tissues, including dehydration with different concentrations of ethanol, clearing with xylene, impregnating with wax, and cutting the paraffin block into 4-mm sections. Finally, the sections were stained with hematoxylin and eosin (H&E) and tested under a microscope for histopathological changes. This process was performed using full automated tissue processing by a bio-based company.

Ethical approval

The study protocol was approved by the committee on publication ethics at College of of Biotechnolgy, AL-Qasim Green University, Babylon, under the reference No. 109 on May 8, 2023.

Results

Results showed that the total leukocyte count (TLC) was increased in the positive group compared to all other groups.

In addition to a significant increase in IL-8 levels obtained in the positive control compared to the negative control, the treatment in three groups resulted in decreased IL-8 levels. Furthermore, IL-10 levels showed a significant decrease in the positive control compared to the negative as well as other treatments [Table 1]. Additionally, there were significant differences observed between group three (400 mg/day) and the negative control, as well as other treatments [Tables 1 and 2].

Table 1: Immunological assessment by measured IL-8, IL-10, and TLC in experimental rats treated with mixture of plants (*S. aromaticum* L and pods of *A. esculentus* L.) (mean ± SD)

Concentrations	IL-8 (pg/mL)	IL-10 (pg/mL)	WBC (μL)
Negative control	262.8 ± 27 A	407.4 ± 20.5 C	3590 ± 1558 A
Positive control	369.6 ± 66.08 B	304.6 ± 77.6 A	13220 ± 5708 B
400 mg/kg	289.2 ± 47.6 A	380 ± 70 B	8410 ± 451.7 A
600 mg/kg	289 ± 1.15 A	443.6 ± 22.2 C	7546 ±709.4 A
800 mg/kg	257.6 ± 17.09 A	461.2 ± 9 C	5635 ± 1020 A

WBC = white blood cells

Table 2: Change in ROS and MDA in experimental rats treated with mixture of plants (*S. aromaticum* 25% and pods of *A. esculentus* 75%) (mean ± SD)

Groups	ROS (μg/mL)	MDA Con. (mmol/l)
Negative group (DW)	1.866 ± 0.3 A	0.46 ± 0.1 a
Positive group (UC)	2.37 ± 0.45 B	0.61 ± 0.11 b
400 mg/day	1.73 ± 0.08 A	0.52 ± 0.04 A
600 mg/day	1.64 ± 0.023 A	0.44 ± .131 A
800 mg/day	1.6 ± 0.36 A	0.41 ± 0.04 A

DW = distal water

Statistical analysis of the data

Results were analyzed using one-way analysis of variance followed by the Duncan test, and presented as in means ± SEM. Differences with P value < 0.05 were described significant. IBM SPSS Statistics version 26 was used for the analysis.

Histological study

The histological findings in the negative control group showed normal tissue with four layers of the colon (mucosa, submucosa, muscularis layer, myenteric layer, and serosa layer). However, in the positive control group, abnormalities such as crossion, slephing, necrosis, thickening of the submucosal layer, enlargement and hyperplasia of goblet cells, more progressive of fiber connective tissue, enlargement of the colon wall, and infiltration inflammatory cells. Inflammation in the mucosa led to disruption of the normal architecture of the crypts, resulting in distortion, branching, and loss of structure, accompanied by the presence of immune cells (lymphocytes and neutrophils) [Figures 1–4].

The histological findings in the 400 mg/kg group revealed normal colorectal histology, characterized by hyperactivity of goblet cells, hypermucus production thickness of mucosal layer, and myenteric plexus [Figures 5–7]. On the other hand, the histological findings of the fourth and fifth groups (600 and 800 mg/kg) appeared normal tissue in the colorectal region with the presence of chronic inflammatory response [Figures 8–10].

Discussion

In the present study, we treated rats with UC to investigate the therapeutic effects and antioxidant potential of *S. aromaticum* (clove) and *A. esculentus* (okra) pods.

Previous research has indicated that patients with inflammatory bowel disease often exhibit an unbalanced oxidative state in their bowels.[20] Elevated concentrations of ROS are typically observed in the colonic mucosa of individuals with UC compared to healthy individuals. These ROS molecules can inflict damage on DNA and proteins, contributing to the pathophysiology of UC[3]

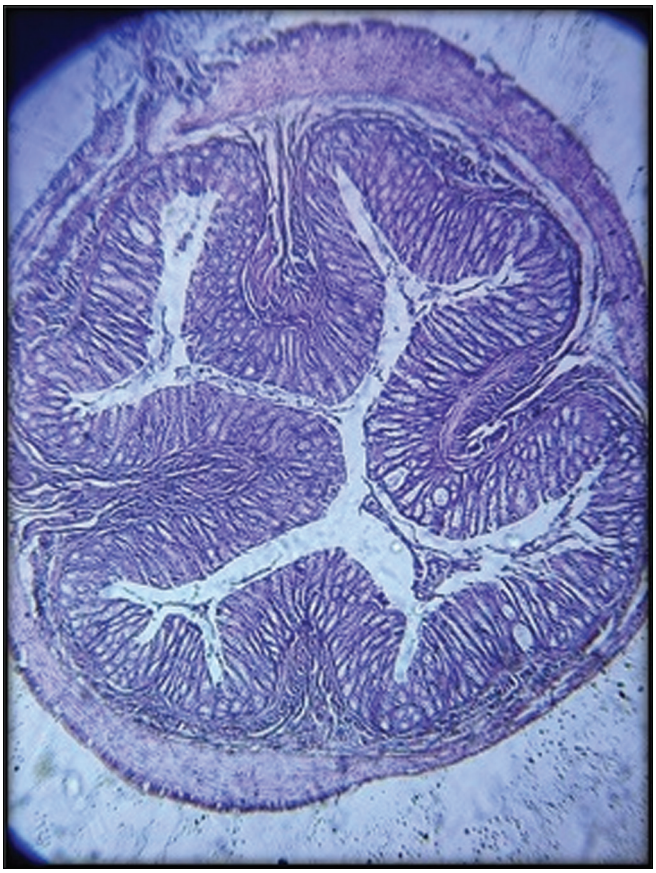

Figure 1: Cross-section of colorectal region in the negative control group, treated with distal water for 60 days, shows normal tissue in four layers of colon (mucosa, submucosa, muscularis layer, myenteric layer, and serosa layer). The histological section was stained with H&E and observed at 40× magnification

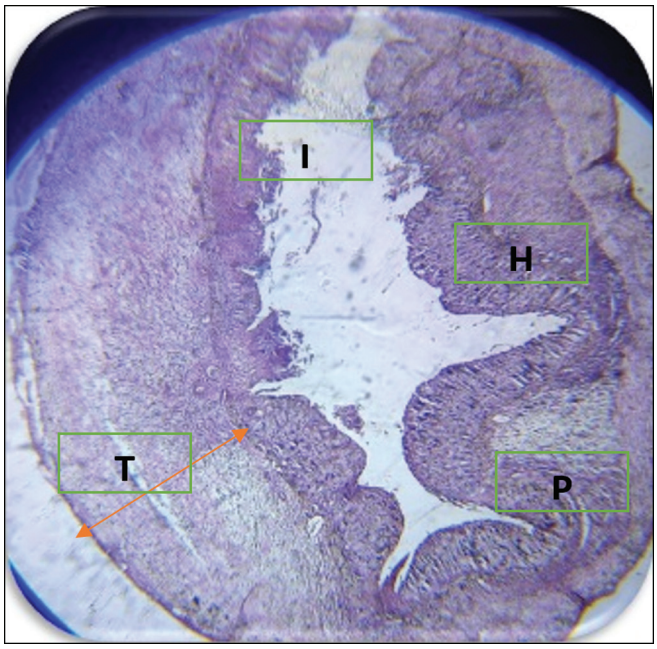

Figure 2: Cross-section of colorectal region in the positive control group, induced with UC without treatment, reveals histological abnormalities. These include thickening of the submucosal layer (T), enlargement of goblet cells, hyperplasia of goblet cells (H), more progressive of fiber connective tissue (P), enlargement of colon wall, infiltration inflammatory cells (I), and inflammation in the mucosa leading to disruption of the normal architecture of the crypts, characterized by distortion, branching, and loss of structure. The histological section was stained with H&E and observed at 40× magnification

and tissue damage. Our results demonstrated a decrease in ROS levels in all groups induced with ulcers and treated with a mixture of plants. This reduction may be attributed to the presence of quercetin in clove, as quercetin is known to act as an antioxidant by targeting ROS, the primary source of oxidative stress, and thereby preventing a decrease in NO production.[21] Additionally, eugenol may also play a role in reducing ROS levels.[22]

The results demonstrated a decrease in MDA in the treated group compared to the positive control. This finding is consistent with Deri and Denny, who reported that inducing nicotine at a dose of 2 mg/kg body weight may increase oxidative stress and inflammation, leading to endothelial dysfunction and elevated serum MDA levels.[3,6,23,24] Additionally, it has been noted that quercetin possesses antioxidant and anti-inflammatory properties, which may prevent nicotine-induced oxidative stress and inflammation.[25]

Our results showed a decrease in IL-8 levels in all treatment groups, which may be attributed to the presence of crude okra polysaccharides. These polysaccharides are known to enhance phagocytic activity-related immune responses and regulate cytokine production-related immune responses.[23] Additionally, the presence of chemical compounds like quercetin in clove may contribute to the inhibition of IL-8, as documented by Kempuraj *et al*.[24] This finding is consistent with previous research indicating that flavonoid, including quercetin, have a role in inhibiting IL-8.

Previous ocular investigations have demonstrated that quercetin reduces both the RNA and protein levels of IL-6 and IL-8, as well as the mRNA expression of these substances in cultured tissue from Graves' orbitopathy.[26]

Mounting evidence suggests that quercetin may shield retinal pigment epithelial cells from harm *in vitro*.[27-29] In IL-1-stimulated ARPE-19 cells, quercetin suppresses the expression of IL-6, IL-8, MCP-1, ICAM-1, and sICAM-1 at both the mRNA and the protein levels.

Cheng *et al*.[30] showed that IL-1 stimulation induces phosphorylation of c-Jun, as well as transcription factors (CREB response element-binding protein and ATF2), along with mitogen-activated protein kinases (extracellular signal-regulated kinases, p38, and JNK1/2) in ARPE-19 cells. Quercetin strongly inhibits this phosphorylation,

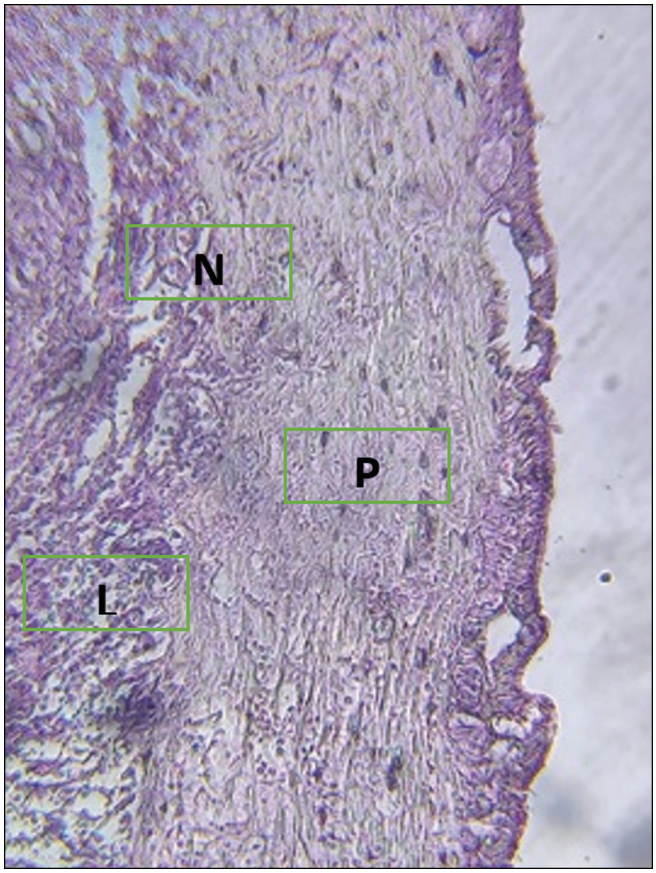

Figure 3: Cross-section of the colorectal region in the positive control group, induced with UC without treatment, exhibits histological abnormalities. These include crossion, slephing, necrosis (N), more progressive of fiber connective tissue (P), increased inflammatory cells (including the presence of immune cells such as lymphocytes (L) and neutrophils), and infiltration of inflammatory cells. The histological section was stained with H&E and observed at 40× magnification

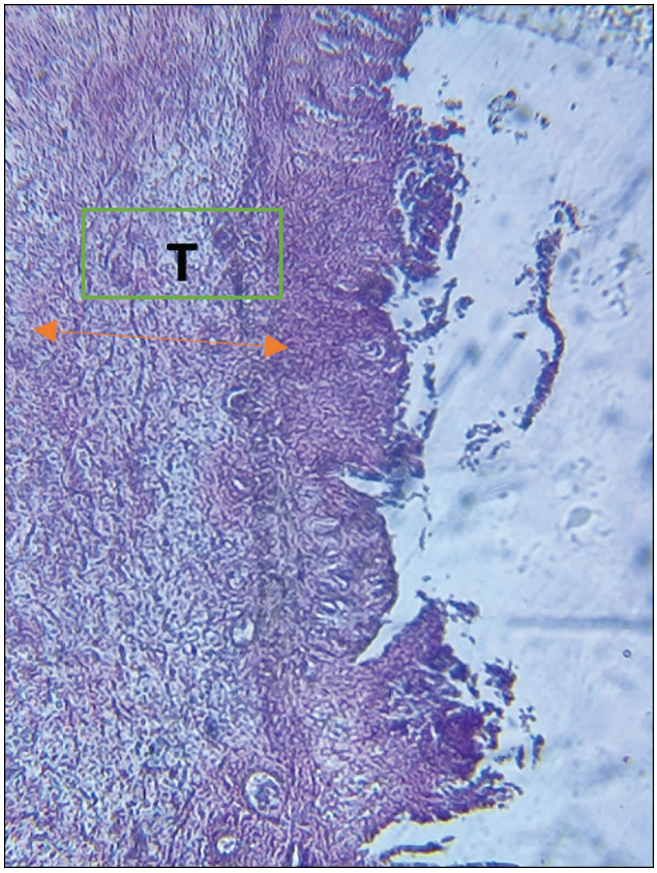

Figure 4: Cross-section of the colorectal region in the positive control group, induced with UC without treatment, exhibits histological abnormalities. These include thickening of the submucosal layer (T), enlargement of goblet cells, hyperplasia of goblet cells, more progressive of fiber connective tissue, enlargement of colon wall, infiltration of inflammatory cells, deformation of the tissue, and sever necrosis of mucosal layer. The histological section was stained with H&E and observed at 40× magnification

resulting in a reduction in the production of MCP-1, sICAM-1, IL-6, IL-8, and ICAM-1.[30]

Our results showed an increase in IL-10 levels in the treatment groups compared with the infected groups of ulcerative colitis. This result is consistent with the study by Mateen *et al*.,[22] which demonstrated the effectiveness of two phytochemicals, in collagen induced arthritis in Wistar rats—cinnamaldehyde and eugenol—against arthritis. They found that treatment with eugenol and cinnamaldehyde and eugenol led to a reduction in arthritis score and swelling of the hind paw. Furthermore, they observed improvements in antioxidant enzymes and biomolecular oxidation indicators. Treatment with these phytochemicals also enhanced the levels of Tumor necrosis factor- α (TNF-*α*), IL-6, and IL-10, while reducing ROS.[22]

Histological study revealed that the mixture of plants induced UC in rats. However, after treatment with different doses of plant mixture, the condition improved and the rats recovered. This may be because of the presence of the phenolic compound eugenol and other components. This result is compatible with Santin *et al*.,[31] who stated that eugenol has gastroprotective properties; pretreatment with this bioactive substance prevented acute gastric lesions induced by ethanol by increasing mucus production. Moreover, Morsy and Fouad[32] reported that eugenol (100 mg/kg, p.o.) protected rats against stomach ulcers caused by indomethacin, and they hypothesized that this protective effect might be connected to the suppression of aggressive forces.

Bleeding per rectum; the most common diagnosis was nonspecific colitis and hyperplastic polyp along with internal hemorrhoid,[33] thickening of the colon, massive tissue necrosis, and ulceration.[34] Longo *et al*.[35] approved the oral treatment or rats with 1 mg/kg of eugenol, showing significant improvement in mucus production and antioxidant imbalance after acetic acid-induced ulceration. Furthermore, the effects of eugenol at low

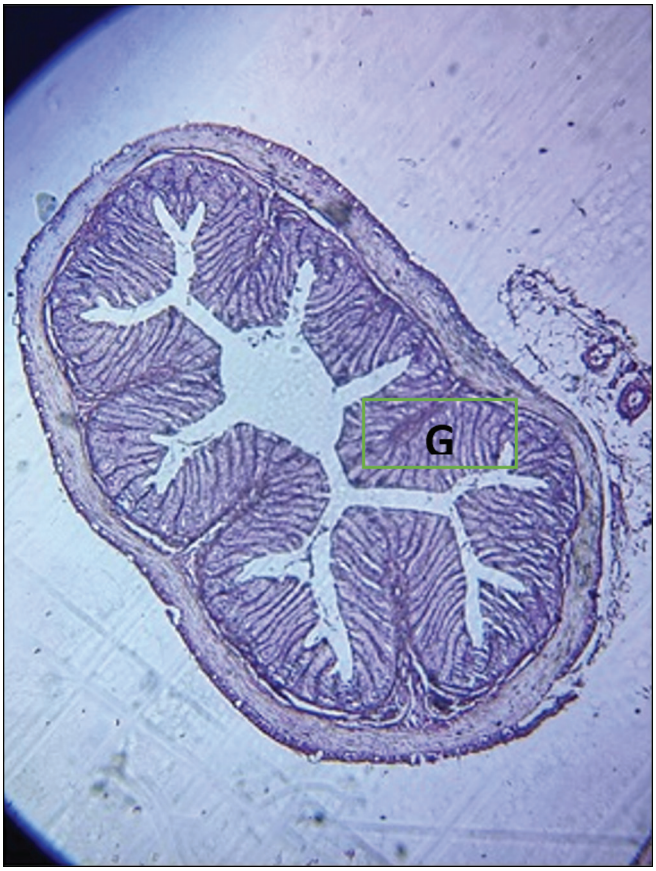

Figure 5: Cross-section of the colorectal region in the treated group, induced with UC and treated with 400 mg/kg for 60 days, exhibits histological abnormalities. These include hyperactivity of goblet cells (G), hyper mucus production, thickening of mucosal layer and myenteric plexus. The histological section was stained with H&E and observed at 40× magnification

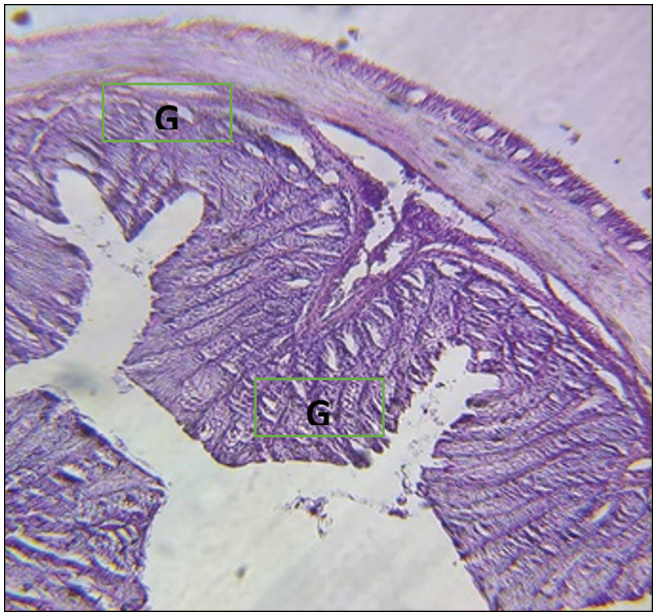

Figure 6: Cross-section of the colorectal region in the treated group, induced with UC and treated with 400 mg/kg for 60 days exhibits histological abnormalities. These include normal structure with normal border with hyperactivity of goblet cells (G). The histological section was stained with H&E and observed at 40× magnification

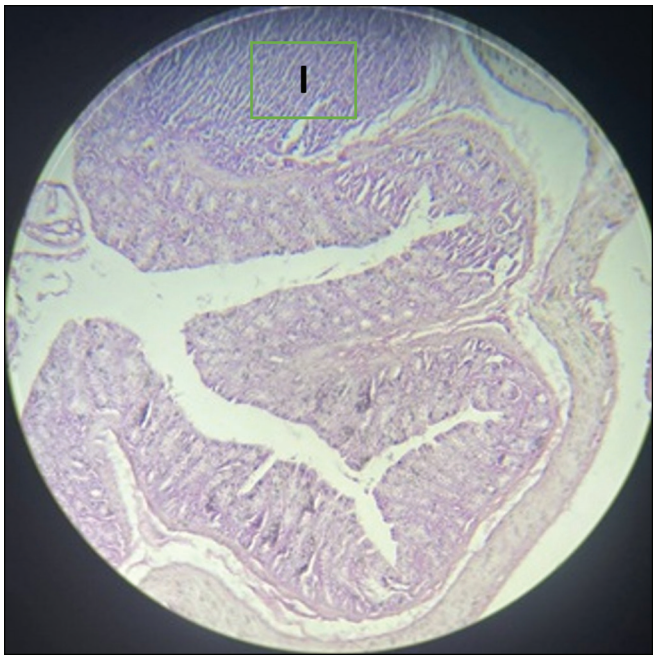

Figure 7: Cross-section of the colorectal region in the treated group, induced with UC and treated with 600 mg/kg of plant extract for 60 days, exhibits a normal structure with normal border. However, there is evidence of a chronic inflammatory response (I) present. The histological section was stained with H&E and observed at 40× magnification

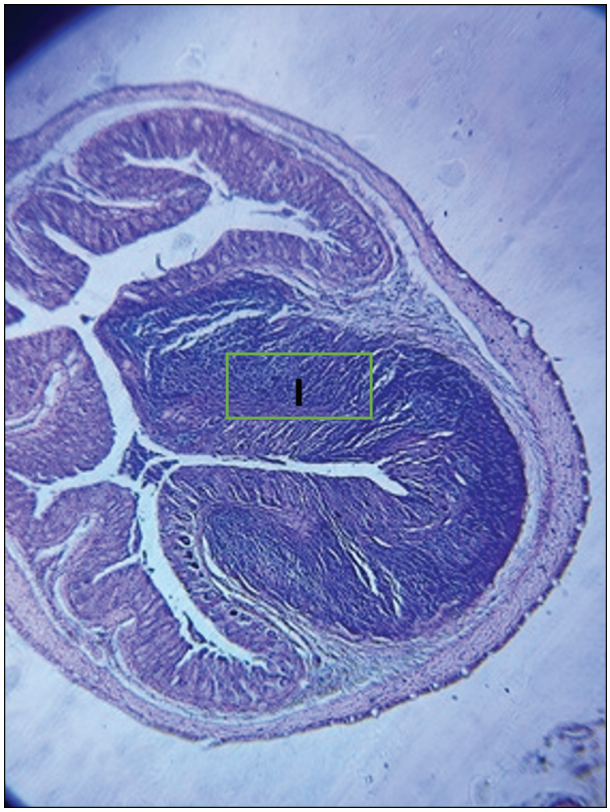

Figure 8: Cross-section of the colorectal region in the treated group, induced with UC and treated with 800 mg/kg of plant extract for 60 days, exhibits a normal structure with normal border. Notably, there is evidence of a present immune response. The histological section was stained with H&E and observed at 40× magnification

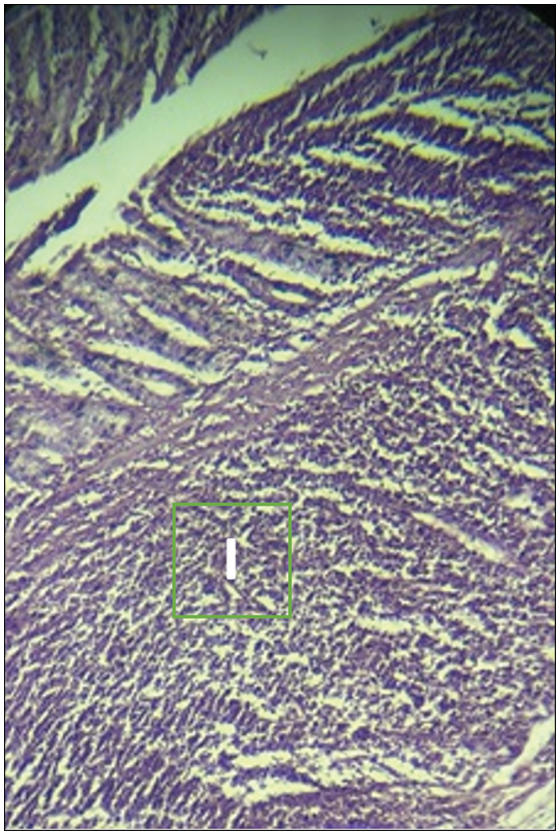

Figure 9: Cross-section of the colorectal region in the treated group, induced with UC and treated with 800 mg/kg of plant mixture for 60 days, exhibits a normal structure with normal border. Notably, there is evidence of a present immune response (I). The histological section was stained with H&E and observed at 40× magnification

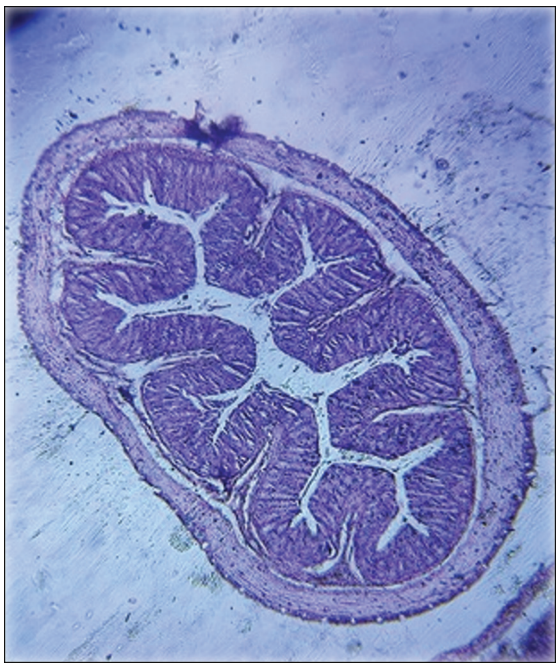

Figure 10: Cross-section of the colorectal region in the treated group, induced with UC and treated with 800 mg/kg of plant mixture for 60 days, exhibits normal structure with normal border. The histological section was stained with H&E and observed at 40× magnification

concentrations also depend critically on the prevention of neutrophil migration. Conversely, oral eugenol treatment at doses of 100 mg/kg exacerbates the ulceration process. This suggests that eugenol may be feasible for treating UC.[35]

On the other hand, Longo *et al*.,[35] examined the potential therapeutic benefits of eugenol, the primary bioactive ingredient in clove (*S. aromaticun*) essential oil, for stomach repair. Using an acetic acid-induced ulcer model, five groups of female Wistar rats were treated twice daily for 7 or 14 days with either vehicle (1 mL/kg, p.o.), eugenol (1, 10, or 100 mg/kg, p.o.), or omeprazole (20 mg/kg, p.o.). The application of eugenol sped up the healing process of the ulcerated area, as determined by macroscopic, microscopic, and biochemical tests. Eugenol's therapeutic properties included rescuing the histological architecture and returning superoxide dismutase and catalase activity to normal levels. Moreover, eugenol (1 mg/kg, p.o) reduced gastric mucosal myeloperoxidase activity, which is compatible with our results showing reduction of ROS and MDA, Longo *et al*.[35] added that mucin secretion was increased. However, after 7 days of treatment, eugenol at a dose of 100 mg/kg improved the ulcerated area by 49%, while a dose of 10 mg/kg for 7 or 14 days had no effect. As a result, even though using large quantities of eugenol can have unfavorable effects by worsening gastric lesions, the compound's antiulcer potential is clear and achievable at reasonable concentrations.[35]

The second probability reason for ulcer recovery is the presence of quercetin in *S. aromaticum* L. Sotnikova *et al*.[36] revealed that quercetin slowed down the synthesis of the inflammatory enzymes such as lipoxygenase and cyclooxygenase *in vitro*. They also found that it suppressed the expression of nitric oxide synthase, nitric oxide synthesis, and TNF-α. Quercetin's anti-inflammatory properties were further supported by several *in vivo* animal studies.[37] According to Alarcón de la Lastra *et al*. (1994), oral pretreatment with a high dose of quercetin (200 mg/kg) shielded the rat stomach mucosa from ethanol-induced necrosis. Studies examining quercetin glycoside, or quercitrin, for its acute and long-term anti-inflammatory properties in experimental colitis models produced varying conclusions.[36]

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Conflicts of interest
There are no conflicts of interest.

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