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Combination of β-Carotene and Cinnamon Oil with Sulfasalazine for Treatment of Acetic Acid Induced Ulcerative Colitis in Adult Male Albino Rats: Histological and Immunological Study

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Abstract:

Background: Ulcerative colitis (UC) is a common inflammatory bowel disease worldwide. This study evaluated the anti-inflammatory and antioxidant effects of cinnamon oil and β-carotene on acetic acid (AA)-induced UC in rats. **Methods:** 50 adult albino rats were divided into five groups: group I (control), group II (UC induced by 4% AA), group III (UC treated with sulfasalazine 100 mg/kg BW/day), group IV (sulfasalazine + cinnamon oil 2.5 ml/kg BW/day), and group V (sulfasalazine + cinnamon oil + β-carotene 50 mg/kg BW/day). Treatments were given orally for seven days. Colonic tissues were evaluated by biochemical, histological, and immunohistochemical methods. **Results:** Sulfasalazine alone (group III) or with cinnamon oil (group IV) significantly reduced macroscopic damage, ulcer index, and disease activity index compared with UC group. Both groups showed restored antioxidant markers (MDA, CAT, SOD) and improved mucosal regeneration with decreased MPO and caspase-3 expression. Notably, group V, receiving sulfasalazine with cinnamon oil and β-carotene, demonstrated the most pronounced improvement in all parameters, with nearly normal mucosal architecture. **Conclusion:** Combined therapy of sulfasalazine, cinnamon oil, and β-carotene exerted synergistic anti-inflammatory and antioxidant effects, providing greater protection against AA-induced UC than monotherapy or dual therapy.

Keywords: Cinnamon Oil, β-Carotene, Ulcerative Colitis, Sulfasalazine.

Introduction

Ulcerative colitis (UC) is a chronic inflammatory disease affecting submucosal and mucosal layers of large intestine [1]. The World Health Organization (W.H.O) categorizes it as one of the modern refractory diseases [2]. The causes of UC are intricate and multifaceted, involving genetic predispositions, alterations in dietary patterns, dysfunction of intestinal barrier, dysbiosis of microbiota, and abnormal immune responses from the host [3].

Considering these underlying factors, a range of therapeutic strategies has been formulated for UC, which includes the use of 5-aminosalicylic acid medications (such as sulfasalazine), immunosuppressive agents, corticosteroids, surgical procedures, and alternative treatments. Importantly, the application of medicinal plants and their derivatives, such as essential oils, aqueous extracts, and hydroalcoholic extracts, has attracted growing interest among researchers [4].

A possible mechanism that may contribute to UC is oxidative stress, which is initiated by reactive nitrogen species or reactive oxygen species. It can be deduced that certain plants exhibit antioxidant properties and also have antimicrobial effects. These compounds can neutralize both free radicals and excessive bacterial proliferation, thus mitigating the pathological lesions associated with UC [5].

Cinnamon extract derived from Cinnamomum ceylanicum bark, harvested in various Asian nations, has been utilized as a traditional herbal remedy. The primary constituents of cinnamon extract include tannin, cinnamic acid, cinnamic aldehyde, and methyl-hydroxychalcone polymer [6]. Both cinnamon and its derivatives exhibit a range of beneficial properties, counting antimicrobial, antioxidant, anticancer, anti-inflammatory, and antidiabetic effects, and are considered safe for human consumption [7]. Cinnamon is notably rich in polyphenolic compounds, which act as scavengers of free radicals. Researches indicate that total cinnamon extract may provide protection against oxidative damage induced by cadmium, glutamate, bisphenol, and gentamicin [8].

Food and Agriculture Organization of the United Nations and W.H.O recognized β-Carotene as nutritious safe food additive. It is found in numerous orange-hued fruits as well as green leafy and yellow vegetables [9]. The presence of conjugated double bonds and two β-ionone rings at either end of its molecular structure contributes to the high hydrophobicity and non-polarity of β-carotene. Additionally, β-carotene possesses antioxidant, anticancer, and anti-cardiovascular disease properties, along with immunoregulatory functions [10]. Another study demonstrated that administering 40 and 80 mg/kg of β-carotene can mitigate weaning stress in piglets, thereby reducing intestinal inflammation during weaning by modulating gut microbiota [11].

The aim of this study was to detect beneficial effects of β-Carotene and Cinnamon Oil on UC in rodents.

Materials and Methods

This was an experimental study conducted at the Faculty of Medicine, Benha University, Egypt, in April 2024.

Chemicals

Sulfasalazine: obtained from AIT (ACDIMA International Trading) factory, 6th October, Egypt with commercial name colosalazine –EC 500mg tablet. It was prepared as a suspension in the distilled water. Time of study: April 2024

B-Carotene: It is available in capsule form under trade name "Beta carotene forte" and is manufactured by an Arab corporation that specializes in gelatin and pharmaceutical products (MEPACO-MEDIFOOD). Each capsule contains 15 mg of natural B-carotene, which is equal to 250,000 IU of natural vitamin A. It was dissolved in olive oil.

Cinnamon leaf oil (product number W229210, density 1.041 g/mL at 25 °C, Sigma-Aldrich) and **acetic acid** (AA) 4%:

were bought from Sigma-Aldrich, located in St, Missouri, USA, Louis.

CAS Number:
8015-91-6
CAS Number:
8015-91-6

Animals & experimental design

This study employed 50 mature male albino rats, each of which weighed 200-220 grams and had 2months old. These animals were got from laboratory animal branch of Faculty of Veterinary Medicine at Zagazig University in Egypt. In a safe laboratory environment, rats were housed in plastic enclosures and were permitted to consume laboratory chow pellets and water. The temperature was maintained at 20± 2°C.

Randomly, rodents were divided into 5 equal groups after one week of acclimation, with ten rats in each group.

-**Group I** (Control group): Rats were separated into 3 subgroups:

• **Group Ia**: Regular diet was given to four animals.

• **Group Ib**: Distilled water was given to three animals (2 ml/kg) for 7 days through oral gavage.

• **Group Ic:** The last three animals received olive oil (2ml/kg) for 7 days through oral gavage.

-**Group II** (ulcerative colitis (UC) group): 2 ml of 4% AA was administered intrarectally to produce ulcerative colitis [12].

Ulcerative Colitis Induction

Rats were allowed to drink water, but they were starved for a minimum of 24 hours before colitis induction to facilitate the elimination of waste from the colon. A feeding gavage with a diameter of 2 mm was used to provide 2 mL of 4% AA intrarectally under light ether anesthesia. The gavage was inserted 4.5 cm deep into the rectum to promote UC. The rodents were maintained in reversed Trendelenburg position during rectal installation procedure to prevent intracolonic solution leakage [13].

-**Group III** (sulfasalazine group): Sixty minutes after induction of UC, rats were cured by sulfasalazine (colosalazine) dosage 100 mg/kg /day for 1week orally [14].

-**Group IV** (sulfasalazine + cinnamon oil): Rats with UC were received sulfasalazine (100 mg/kg/day) with simultaneous administration of 2.5 ml/kg/day of cinnamon oil through oral gavage for 1 week [15].

-**Group V:** (sulfasalazine+ cinnamon oil + β carotene): After induction of UC, animals were received sulfasalazine (100 mg/ kg BW /day) with concurrent administration of cinnamon oil (2.5 ml/kg /day) and β carotene a (50 mg/ kg BW /day) [9] given orally by gavage for 7 days.

Ethical approval: The Ethical Committee of the Faculty of Medicine, Benha University, reviewed and authorized every aspect of this study (Under number RC 5-8-2023).

Assessment of colitis

A-Macroscopic damage score

On the eighth day, Rats were sacrificed after inhaling an excessive amount of diethyl ether. Following an abdominal dissection, the colon was immediately removed. Colon specimen was meticulously opened longitudinally and rinsed with standard saline. Visual examinations were conducted on the colonic samples. The macroscopic score is determined by a scoring system that ranges from 0 to 6 and is based on the clinical aspects of the colon: 0 denotes absence of inflammation, 1 indicates swelling or redness, 2 indicates redness or edema, 3 indicates one or two ulcers, 4 indicates one large ulcer or more than 2 ulcers, 5 indicates initial necrosis, and 6 indicates severe necrosis [16].

B-Evaluation of Ulcer Index (UI) and the Disease Activity Index (DAI)

-Ulcer area was calculated using a plane glass square. Each cell on glass square was 1 mm² in area, and total number of cells was calculated to determine ulcer area in each colon. UI was estimated using that formula: UI = Macroscopic damage score + Ulcer area [17].

-Animals were checked for three major symptoms: bleeding per rectum, stool consistency, and loss of weight. DAI was intended to assess colitis severity using a scoring system for the mentioned indicators [18] (Table 1).

Table 1: Showing the scoring system for DAI**.**

Score	Body weight loss(%)	Stool consistency	Rectal bleeding
0	No weight loss	Normal	Normal
1	1-5%		
2	6-10%	Loose stool	Mild bleeding
3	11-15%		
4	>15%	Diarrhea	Sever bleeding

DAI= (BW loss score + Rectal bleeding score +Stool consistency score)

Determination of oxidative stress markers

Colon samples were homogenized with PBS on ice after being weighed. Homogenate was then centrifuged for 10 min at 3000 rpm at 4 °C. For assessment of oxidant/antioxidant indicators such as MDA, SOD, and CAT activity, the residue was collected and stored at -70°C [19].

Histological examination

Subsequently, the colon samples were deparaffinized, cleaned, and rehydrated in a graduated ethanol series after being immersed in paraffin and cut into 5 µm sections. The sections were stained with Masson's trichrome and hematoxylin and eosin (H&E) [19].

Immunohistochemistry

Deparaffinization, rehydration, and washing with phosphate buffer (pH 7.4) were performed on the colonic sections prior to incubation with 3% hydrogen peroxide in methanol for immunohistochemical staining. Two cycles of 20 minutes each of microwaving were used to retrieve the antigen. Rabbit polyclonal anti-MPO (ab65871; Massachusetts, Abcam, USA) and rabbit polyclonal anti-caspase-3 (ab49822; Massachusetts, Abcam, USA) were then added to the sections and incubated for a whole night at 4ºC. Sections were cleaned with phosphate buffer, then incubated for 30 min with biotinylated secondary antibody, 30 min with streptavidin peroxidase conjugate, and finally for a short time with the diaminobenzidine (DAB) chromogen. They used Mayer's hematoxylin for counterstaining. The primary antibodies were excluded 2019 to create negative controls [20].

Morphometric study

Image analysis of the photomicrographs was done by using Image J software (Java; NIH, Bethesda, Maryland, USA) to evaluate area percentage of collagen fiber deposition in sectors stained with Masson's trichrome. Also, area percent of MPO and caspase3 immunoreaction were measured in the immuno-stained sections at magnification x200. The morphometric analysis was done on 5 non-overlying fields from 5 distinct sections of 5 different rats in each group. All data were introduced in an excel folder for statistical analysis.

Approval code: Rc 5-8-2024

Statistical analysis

Version 20 of SPSS (SPSS Inc., Illinois, USA, Chicago) was employed to evaluate all data. The results were presented as mean ± standard deviation. Post hoc Tukey's test and one-way ANOVA were implemented. The P value was believed significant when it was ≤ 0.05.

Results

Gross appearance of the colon:

In different groups, degree and colitis intensity prompted by the administration of AA were measured. After AA administration intrarectally to group II, the main signs of ulcerative colitis (UC) were noted, including sever hyperemia, oedema, mucosal necrosis, and ulceration. Group III and group IV exhibited improvement in colonic mucosa with decreased edema, hyperemia and ulceration. However, Group V presented nearly normal colonic mucosa with very minimal hyperemia (Figures 1A-E).

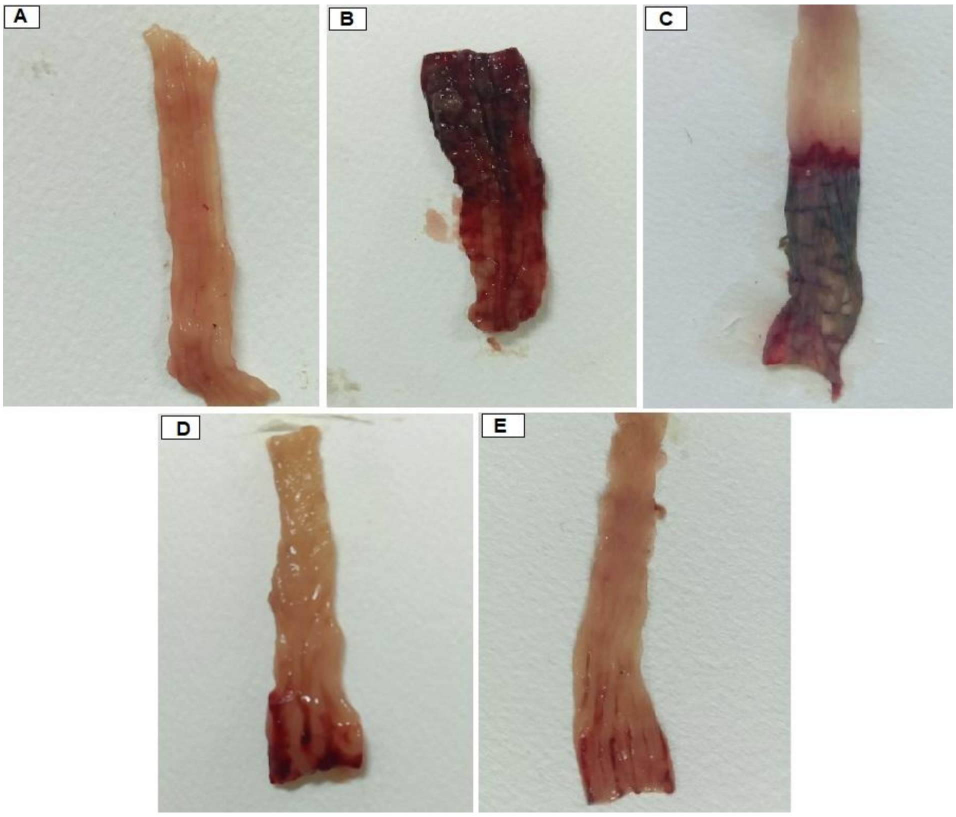

Figure 1: Colon photographs from different experimental groups: **(A)** Group I display the colon's typical mucosa. **(B)** Group II displays severe edema, ulceration, necrosis, and hyperemia of the colon mucosa. **(C)** Group III presents improved colonic mucosa with reduced ulceration, hyperemia, and edema. **(D)** Group IV presents a better recovery in the colonic mucosa with less hyperemia and ulceration.**(E)** Group V's colonic mucosa is almost normal, with very little hyperemia.

Macroscopic parameters of the colon:

Following intra rectal instillation of AA, the animals exhibited severe bloody diarrhea and considerable weight loss demonstrating high DAI, UI in addition to macroscopic damage score were noticeably raised related to control group ($P \leq 0.05$). Sulfasalazine treatment alone (group III) or in combination with cinnamon oil (group IV) markedly ameliorated the effects of AA as there was important decline ($P \leq 0.05$) in macroscopic damage score, UI, and DAI contrasted to group II. Nonetheless, co administration of both cinnamon oil and B carotene with sulfasalazine (in group V) exhibited better enhancement of these parameters that didn't differ significantly from the control rats (table 2, figure 2**).**

Oxidative stress indicators:

Animals in group II founded a significant rise in lipid peroxidation (MDA), indicating significant oxidative stress, while there were major reductions in SOD and CAT levels when compared to control animals ($P \leq 0.05$). Rats cured by sulfasalazine alone (in group III) or in combination with cinnamon oil (in group

IV) showed that the mean MDA concentration significantly reduced (P≤ 0.05) and mean SOD and CAT levels simultaneously rise in relation to group II. Furthermore, co administration of both cinnamon oil and B carotene with sulfasalazine (in group V) restored the MDA level, CAT and SOD activities in the colonic tissues to nearly normal levels (Table 3, figure 3).

Table 2: Macroscopic parameters for ulcerative colitis among different groups.

	Group I (Control group)	Group II	Group III	Group IV	Group V
Macroscopic Lesion Score	0.3 ± 0.5	5.5 ±0.5 a,c,d & e	2.8±0.8 a,b & e	1.8±0.8 a&b	1.0 ±0.6 b&c
Disease Activity Index (DAI)	0.8 ± 0.8	10.3±1.2 a,c,d & e	6.5±1 a,b,d & e	3.8±0.8 a,b,c &e	1.5 ± 1 b, c & d
Ulcer Index (UI)	0.38 ± 0.3	10.6±1.1 a,c,d & e	5.6±1 a,b,d & e	3.4±0.8 a,b,c &e	1.5±0.8 b, c & d

Data stated as mean± SD, analysed by One way ANOVA method followed by Post-hoc Tukey's test. *: Significance ≤ 0.05. a: Significance vs Control, b: Significance vs group II, c: Significance vs group III, d: Significance vs group IV & e: Significance vs group V.

Table 3: Oxidative stress indicators in all studied groups**.**

	Group I (Control group)	Group II	Group III	Group IV	Group V
Malondialdehyde (MDA)(nmol/g tissue)	8.7 ± 0.8	28 ±0.5 a,c,d & e	17.7±0.8 a,b,d & e	13.3±0.8 a,b,c &e	9.5 ± 0.5 b,c & d
Catalase (CAT) (U/g tissue)	0.13 ± 0.01	0.03 ±0.006 a,c,d & e	0.08±0.006 a,b & e	0.09 ±0.006 a,b &e	0.12 ± 0.01 b,c & d
Superoxide dismutase (SOD) (U/g tissue)	3.5 ± 0.3	0.47±0.2 a,c,d&e	1.5±0.1a,b & e	2.1±0.2a,b &e	2.8 ± 0.3 b,c & d

Data stated as means, analysed by One way ANOVA method tailed by Post-hoc Tukey's test. *: Significance ≤ 0.05. a: Significance vs Control, b: Significance vs group II, c: Significance vs group III, d: Significance vs group IV & e: Significance vs group V.

H&E results:

Group I displayed typical histological structure of the colon. The colon mucosa appeared normal with normal crypts as well as numerous goblet cells. Normal submucosa without any inflammatory signs was also observed (Figure 4A). Intra-rectal administration of AA in group II prompted severe mucosal injury and necrosis of the colon. The epithelial mucosa showed ulcerations and desquamated parts. The glandular crypts were atrophied, goblet cells were damaged, and there was marked inflammatory cell infiltration. The submucosa revealed oedema with congested dilated blood vessel (Figure 4B). Group III displayed partial regeneration of the colon mucosa with development of glands in addition to goblet cell repair. However, some inflammatory cell infiltration and congested blood vessel were still observed (Figure 4C). Combination of cinnamon oil with sulfasalazine in group IV produced nearly complete mucosal regeneration with improvement in the mucosal glands that had many goblet cells as well as integrated crypts (Figure 4D). Interestingly, combination of both cinnamon oil and β carotene with sulfasalazine in group V considerably attenuated degree and histological signs severity of cell destruction and refunded normal colonic mucosa's architecture. (Figure 4E).

Masson Trichome staining:

The assessment of distal colon sections stained with Masson's trichrome from group I demonstrated the typical arrangement of very little quantity of collagen fibers between colon glands and in submucosa connective tissue. In contrast, Group II displayed an obvious increase in the collagen fibers distribution in lamina propria among glands as well as in the connective tissue of the submucosa. Group III displayed moderate collagen fiber quantity distributed in the mucosa in addition to the submucosal connective tissues. Group IV presented apparent decrease in collagen fiber amount in between the mucosal glands plus the submucosal connective tissues. Group V presented very minimal quantity of collagen fibers distributed between the mucosal glands that closely similar to the control colon (Figures 5A-E).

Immunohistochemical results:

The examination of MPO immune stained sections from group I exhibited negative MPO immunoreaction in the colonic tissue. However, group II displayed intense brown cytoplasmic coloration of the stromal cells of the colon tissue indicating strong positive MPO immune expression. Group III presented apparent decrease in MPO immune expression in the tissue of the colon. Group IV revealed weak MPO immunoreaction in the colon mucosa while, group V presented very weak expression of MPO immunostaining that closely comparable to the control group (Figures 6A-E).

The evaluation of caspase-3 immune stained sections from group I revealed negative caspase -3 immunoreaction in the glandular and surface epithelial cells. Conversely, Group II displayed powerful positive caspase -3 immune expression in the glandular and surface epithelial cells. In Group III**,** the majority of glandular cells exhibited a moderate immunoreaction, which alternated with focal areas of intense positive cells. Group IV showed moderate immunoreaction for caspase-3 in the colon's glandular cells' cytoplasm. Group V presented the glands of the colon with weak immune expression for caspase-3 (Figures 7A-E).

Table 4: The average value of the collagen fiber area percent in different groups.

	Control Group	**Group II**	**Group III**	**Group IV**	**Group V**
Collagen fiber deposition area%	3.5 ± 1.3	43 ± 4.1 [a,c,d & e]	16.5 ± 2.5 [a,b , d & e]	7.8 ± 1.9 [b & c]	4.4 ± 0.9 [b & c]

Data stated as mean±SD , analysed by One way ANOVA method followed by Post-hoc Tukey's test. *: significance ≤ 0.05. a: Significance vs Control, b: Significance vs group II, c: Significance vs group III, d: Significance vs group IV & e: Significance vs group V .

Table 5: Demonstrating the average values of the area percent of Caspase 3 and MPO immune-expression in every group.

	Control Group	**Group II**	**Group III**	**Group IV**	**Group V**
Caspase area%	5.5 ± 3.7	45.6± 2.6 [a, c ,d & e]	23.4 ± 0.9 [a, b ,d & e]	12.5 ± 2.3 [a,b & c]	6.4 ± 2.9 [b & c]
MPO area %	4.0 ± 2.4	37.3 ± 2.1 [a, c ,d & e]	16 ± 1.8 [a, b ,d & e]	8.2 ± 1.7 [a, b& c]	4.5 ± 2. [b & c]

Data expressed as mean±SD , analysed by One way ANOVA method followed by Post-hoc Tukey's test. *: Significance ≤ 0.05. a: Significance vs Control, b: Significance vs group II, c: Significance vs group III, d: Significance vs group IV & e: Significance vs group V .

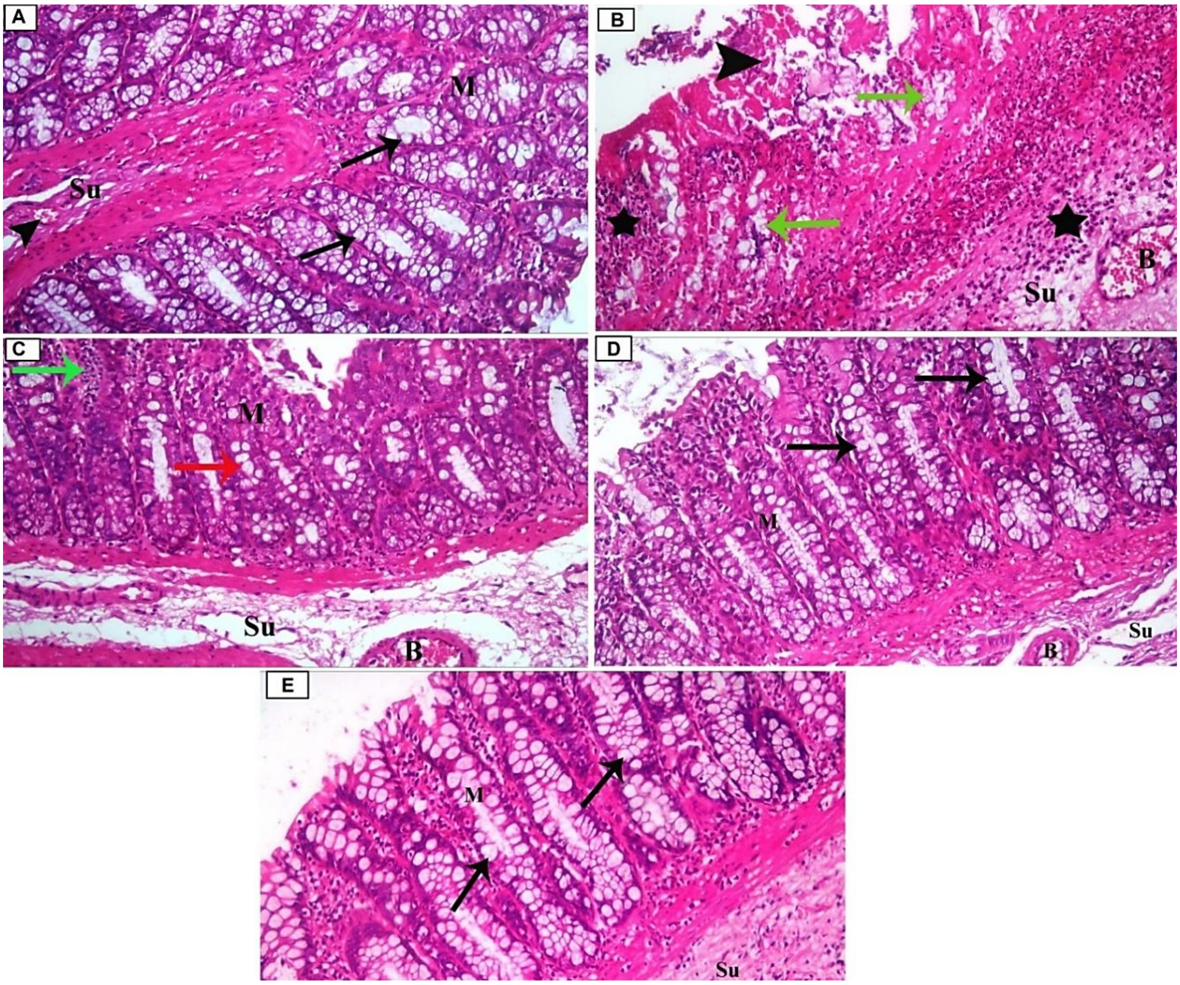

Figure 2: Histological sections from different rat's colon stained with H&E: **(A)** Group I displaying typical histological construction of the colon tissue. The colon mucosa (M) appeared normal with glandular tissue having normal crypts as well as numerous goblet cells (black arrows). Normal submucosa (Su) with no inflammatory signs, normal blood vessel (arrow head)**.(B)** Group II presenting serious necrosis in the mucosa of the colon (arrow head), glandular crypt atrophy, and goblet cell loss (green arrows), severe inflammatory cell infiltration (stars), submucosal (Su) edema with congested dilated blood vessel (B) **.(C)** Group III displaying partial regeneration of the colon mucosa (M) with development of glands in addition to goblet cell repair (red arrow). However, there is some inflammatory cell infiltration (green arrow), partial edema in the submucosa (Su) with congested blood vessel (B). **(D)** Group IV has nearly complete mucosal (M) regeneration with better glands that have goblet cells as well as crypts (black arrows).Submucosa displays partial edema (Su) and normal blood vessel (B). **(E)** Group V demonstrating full restoration of the mucosa (M) of the colon. There is an obvious improvement in glandular tissue with normal crypts and abundant goblet cells (black arrows).The submucosa (Su) appeared normal **(H&E X200).**

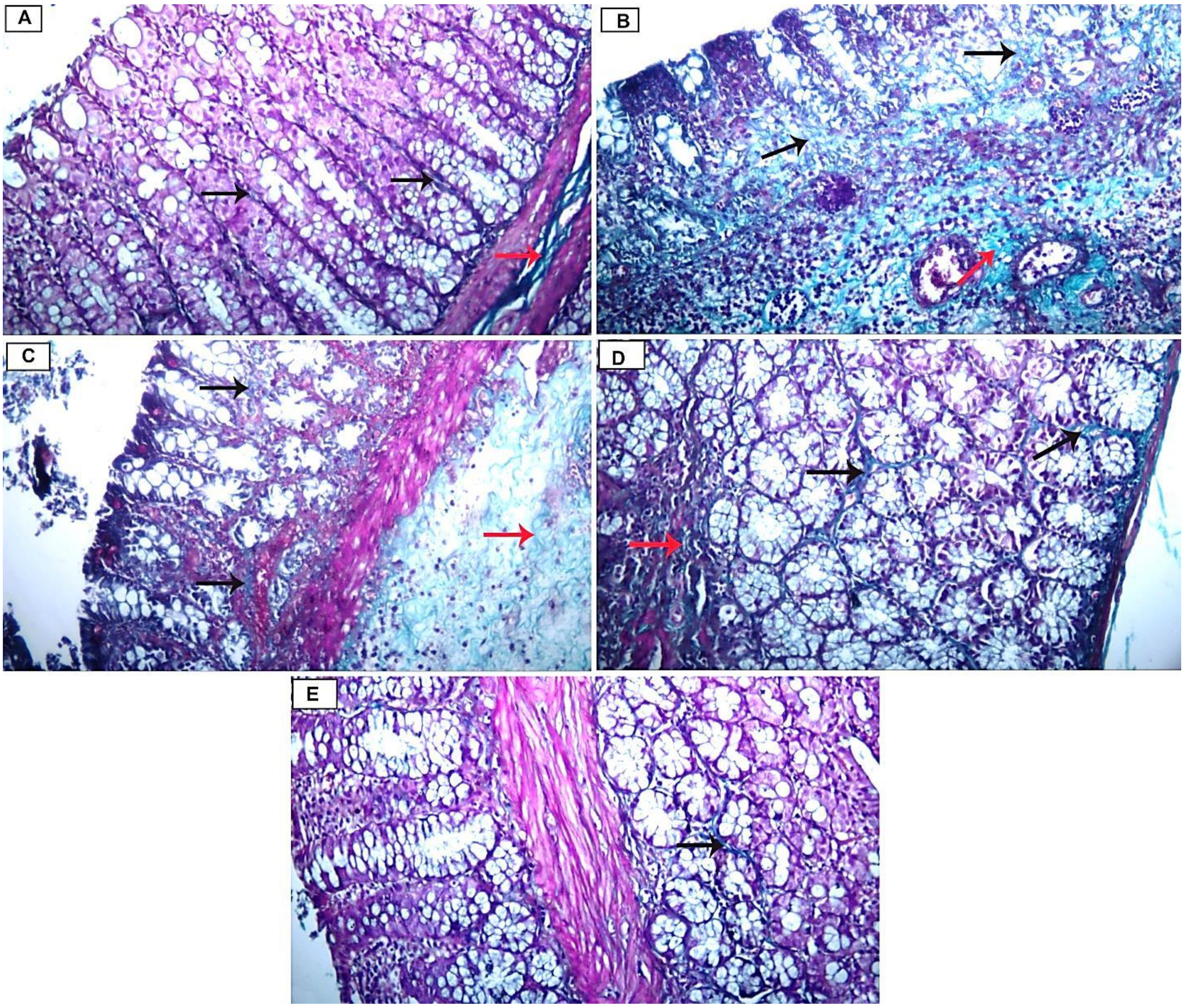

Figure 3: photomicrographs of distal colon's sections from different groups: **(A)** Group I illustrate the typical arrangement of very little quantity of collagen fibers between colon glands (black arrows) and in the connective tissue of the submucosa (red arrow). **(B)** Group II displays an obvious increase in collagen fibers distribution in lamina propria among glands (black arrows) as well as the connective tissue of the submucosa (red arrow). **(c)** Group III displays moderate amount of collagen fibers distributed in mucosa (black arrows) in addition to the submucosal connective tissues (red arrow). **(D)** Group IV presents few collagen fibers in between mucosal glands (black arrows) and in submucosal connective tissues (red arrow**). (E)** Group V presents very minimal quantity of collagen fibers distributed between the mucosal glands of the colon (black arrows**) (Masson Trichome X200).**

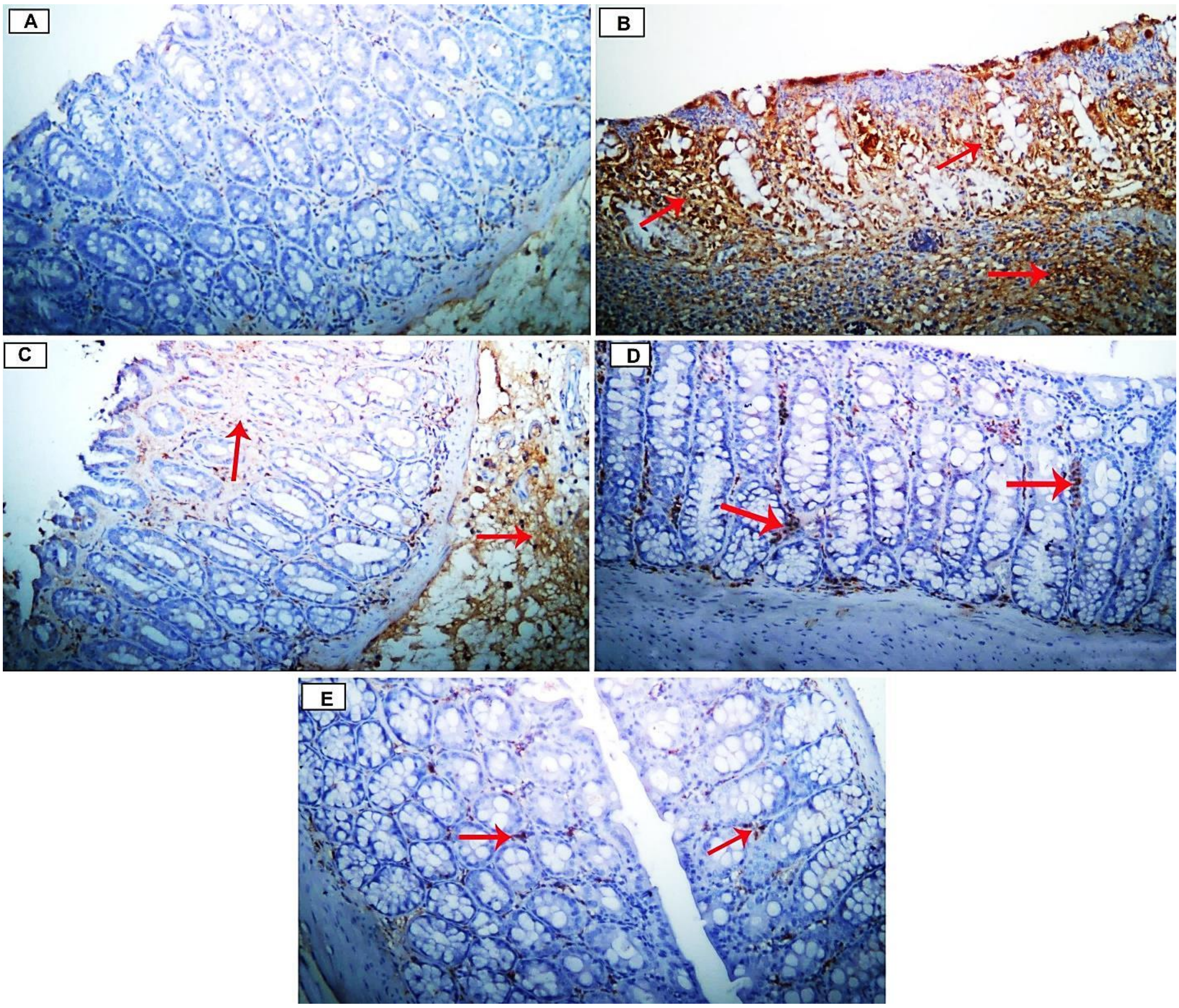

Figure 4: Photomicrographs of MPO immune stained sections from rat's colon: **(A)** Group I exhibiting negative MPO immunoreaction in the colonic tissue. **(B)** Group II displaying intense positive MPO immune expression; brown cytoplasmic color of the stromal cells of the colon tissue (red arrows). **(C)** Group III viewing apparent decrease in MPO immune expression (red arrows) in the tissue of the colon. **(D)** Group IV viewing weak MPO immunoreaction in the colon mucosa (red arrows). **(E)** Group V presenting very weak expression of MPO immunostaining (red arrows) that closely similar to the control group **(MPO immunostaining X 200).**

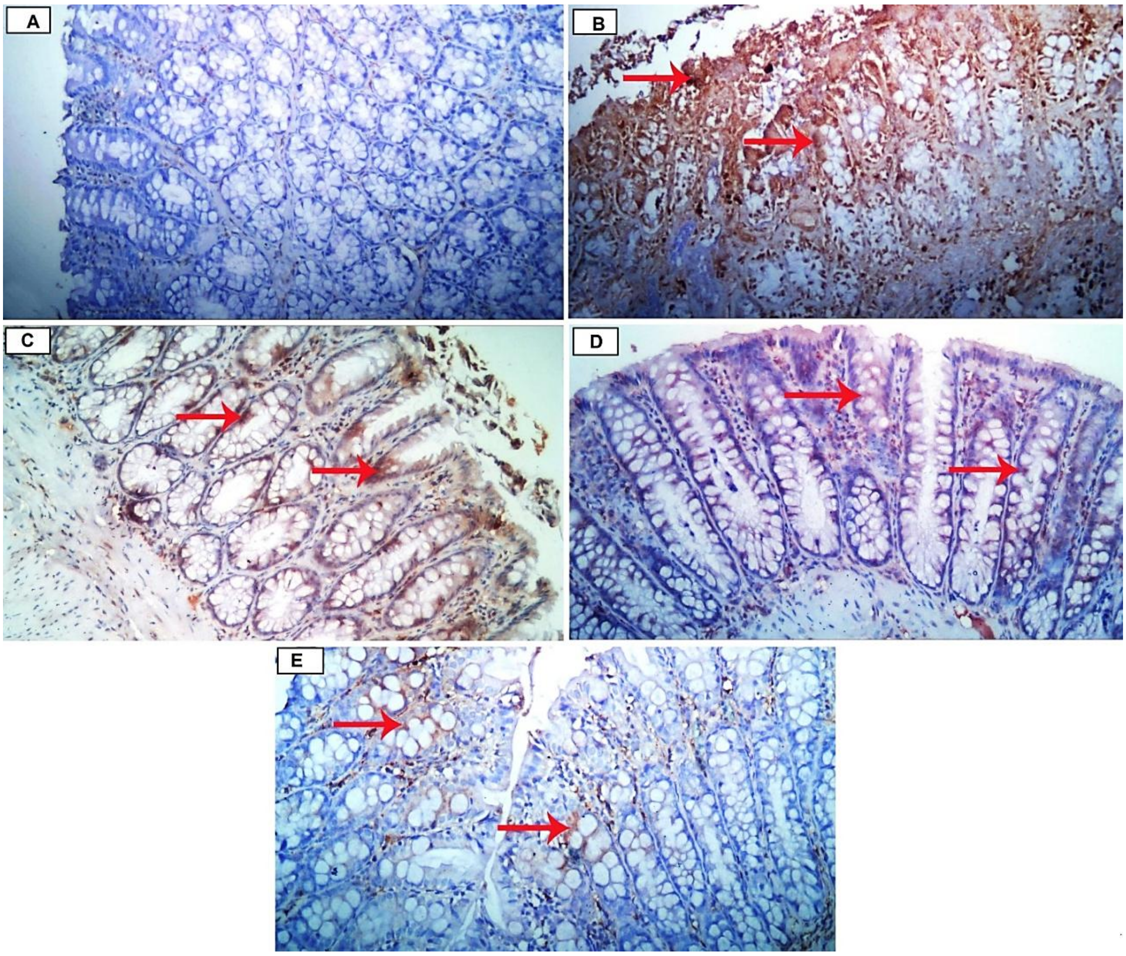

Figure 5: Photomicrographs of caspase-3 immune stained sections from rat's colon:**(A)** Group I presents negative caspase -3 immunoreaction in the glandular and surface epithelial cells .**(B)** Group II displays intense positive caspase -3 immune expression in the glandular and surface epithelial cells (red arrows). In Group III **(C),** the majority of glandular cells exhibit a moderate immunoreaction, which alternates with focal areas of intense positive cells (red arrows).**(D)** Group IV shows moderate immunoreaction in the colon's glandular cells' cytoplasm (red arrows).**(E)** Group V Presents the glands of the colon with weak immune expression for caspase-3 **(caspase-3 immunostaining X 200).**

Morphometric results:

When compared to group I, the average area percentage of collagen deposition in group II presented a notable rise. In contrast to group II, the area percent of collagen deposition significantly decreased in group III ($P < 0.05$). Interestingly, group IV and group V showed a better results as revealed by significant decline in the collagen area percent when compared with group II as well as group III($P \leq 0.05$) (table 4, figure 8).

Mean area percent of MPO and Caspase-3 immune expression presented a significant upsurge in group II, compared to group I. On the other hand, groups III and group IV demonstrated a major decline in the expression of MPO and Caspase-3 related to group II ($P \leq 0.05$) While in group V, the average area percentage of MPO and Caspase-3 immune expression displayed results that didn't differ significantly from the control group (table 5, figure 9).

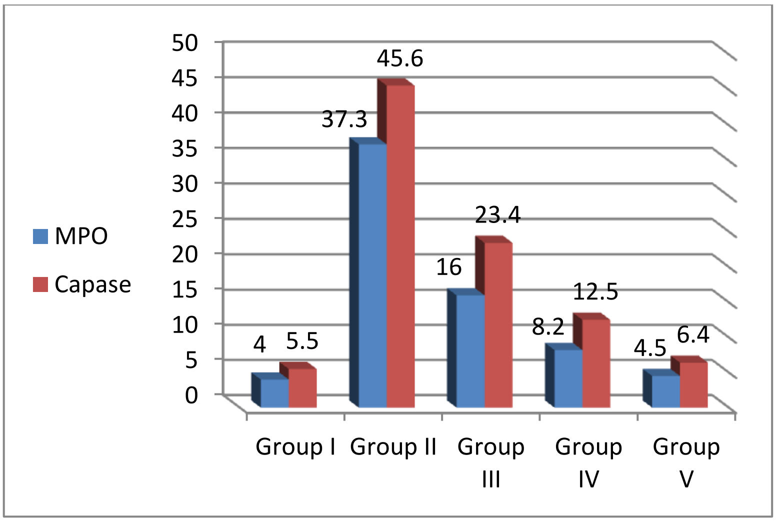

Figure 6: Histogram presenting the average values of the area percent of Caspase 3 and MPO immune-reactivity in every group.

Discussion

Globally, UC is a prevalent form of inflammatory bowel disease (IBD). Ulcerative colitis symptoms included colicky gastrointestinal discomfort, bloody diarrhea, chronic mucosal inflammation, fatigue, nausea, and weight loss **[19]**.

The purpose of this study was to assess anti-apoptotic and anti-inflammatory effects of β-carotene and cinnamon oil on rats with AA-induced ulcerative colitis.

Since acetic acid-induced UC is linked to inflammatory and oxidative responses look like pathophysiology of human IBD, it is the most appropriate model **[21]**.

According to the current study, the UC group's colon showed widespread hyperemia, edema, ulceration, and colonic mucosal necrosis. In groups III and IV, co-administration of sulfasalazine alone or sulfasalazine with cinnamon oil, respectively, resulted in improvements to the colonic mucosa, including a reduction in ulceration and edema. These findings were in line with those of Salamatian et al. who found that whereas colonic instillation of AA resulted in a high DAI score, cinnamon treatment decreased the DAI index [12]. But in group V, the combination of sulfasalazine, cinnamon, and beta-carotene showed almost normal colonic mucosa with very little hyperemia. This was in line with other researcher's theory that β-Carotene can reduce UC brought on by DSS by controlling the gut flora [9].

It is believed that oxidative stress and inflammation are important components of pathophysiological process of UC [22]. Since free oxygen radicals cause the pathophysiology of mucosal damage and the start of apoptosis, they are thought to be a contributing factor of IBD. Thus, compounds with anti-oxidant, antiapoptotic, and anti-inflammatory qualities have been the subject of most research [23].

The tissue's lipid peroxidation process produces MDA as a consequence. MDA levels in plasma rise dramatically in ulcerative colitis, and this is a key indicator of IBD in patients with this condition [24].

SOD, in contrast, is a crucial antioxidant enzyme provides protection. This enzyme's levels in colonic tissues are depleted in UC due to oxidative damage brought on by free radicals. By inhibiting lipid peroxidation and mediating the dismutation of superoxide anion, SOD shields the cells from ulcerative damage [25].

Our findings are agreed with the previously published study showed AA-induced colitis was considered by a marked rise in MDA levels, whereas sulfasalazine treatment of the AA group ameliorated colonic damage, as shown by a marked reduction in colonic MDA levels and a return to normal SOD levels [26].

Rats given sulfasalazine either alone or in conjunction with cinnamon oil in the current study proved a significant reduction in mean MDA levels and a rise in mean SOD levels relative to UC group. Additionally, the MDA and SOD levels in the colonic tissues were returned to almost normal levels when sulfasalazine was administered in combination with cinnamon and beta-carotene (in group V). This goes with Shahid et al. findings, which showed that administering cinnamon restored the antioxidant capacity overall and that the essential oils of cinnamon can reverse lipid peroxidation and nitration brought on by peroxynitrite [27]. The outcomes attained by other authors [12] demonstrated that administering cinnamon to colonic homogenate specimens of colitis rats restored their antioxidant capacity.

Similar to what we found, other study findings suggested that β-carotene might reduce inflammation by altering the gut flora. The intestinal tract can be protected by the gut microbiota, which can also create the body's intestinal mucosal barrier. By lowering inflammatory markers expression, β-Carotene intervention will improve gut microbiota and UC structure [9].

In the present study, UC rats' colon sections stained with H&E revealed atrophied crypts, severe mucosal ulcerations, and a noticeable infiltration of inflammatory cells. This is consistent with a research by Abdelmonaem et al. who found that intrarectal Instillation of AA resulted in considerable histological alterations, including inflammatory infiltrations, colonic thickness, hyperemia, and goblet cell hyperplasia [26]. Other researchers obtained similar results, which validated the existing images [28].

In groups treated with sulfasalazine, the present work successfully demonstrated a considerable improvement in the histological pictures. Accordingly, some studies demonstrated that when sulfasalazine was administered to AA-treated rats, the rats showed surface discontinuity in some places, but epithelial lining began to form, and the crypts developed with a large lumen and were widely spaced [26].

Cinnamon oil and sulfasalazine combined in group IV of the current research resulted in almost total mucosal regeneration and improvement in the mucosal glands with numerous goblet cells and integrated crypts. Correspondingly, the results of Li et al. declared that following cinnamon oil treatment, there was less damage to the epithelial barrier, less infiltration of inflammatory cells, and a lower histological score [29].

Interestingly, we discovered that group V's sulfasalazine, cinnamon oil and β carotene combination significantly reduced histological evidence severity of cell death and reinstated normal architecture of the colonic mucosa. These findings concurred with those of other authors [9] who claimed that β-Carotene administration following DSS-stimulated UC enhanced gland morphology and restored normalcy to the crypts.

Colon sections stained with masson trichrome in UC group showed a clear rise in the dispersion of collagen fibers in our study. This supported findings of Ahmed et al., who founded that slices of distal colon of stained with Masson's trichrome appeared with more abundant Collagen fibers in submucosal and mucosa connective tissue of AA treated group [30].

Similar findings were made by other researchers [31] who discovered that collagen fibers distribution in submucosal and mucosal connective tissues increased statistically significantly in the AA-induced UC group. In contrast, in group V when cinnamon oil and β carotene were combined with sulfasalazine in the current research presented a very little collagen fibers amount scattered between the mucosal glands that were extremely comparable to the control colon. According to Elekhnawy et al., collagen fibers distribution in submucosal and mucosal connective tissues was statistically significantly lower in group IV (the R. aegyptiacum-treated AA-induced UC group) than the AA-induced UC group, but there was insignificantly different when compared to control group [(31)].

Using MPO, an enzyme that is situated in the azurophilic granules of neutrophils, the rate of neutrophil infiltration into a tissue in inflammatory conditions can be indirectly measured. Additionally, MPO is a crucial enzyme that allows reactive oxygen species to spread by penetrating neutrophils [(32)].

Colonic sections from rats exposed to AA showed high positive MPO immune expression, according to the current study. On the other hand, sulfasalazine administration, either alone or in conjunction with cinnamon oil, appeared to reduce MPO immune expression in colon tissue. These outcomes are supported by other authors [(12)] who documented that when AA was instillated into the colon of animals, the amount of MPO activity in the colonic homogenate specimens significantly increased. However, the consumption of cinnamon resulted in a notable reduction in the MPO activity and NO levels in colonic homogenate of colitis animals, when compared to positive control animals. Accordingly, Araruna et al. findings demonstrated that, in contrast to normal group, MPO levels were higher in colitis group [(33)]. It has been claimed that cinnamon inhibits leukocyte attachment and migration via interacting with selectin sialosides, according to the findings of other researchers [(34)].

According to some studies [(21)], the epithelial barrier's integrity is compromised, pathophysiology of UC is advanced by the excessive apoptosis of colon tissue cells. Using caspase-3 immune staining, we assessed each group's colon tissue's apoptosis. According to our findings, the UC group's glandular and surface epithelial cells showed strong caspase-3 immune expression. Similarly, other researchers [(35)] immunohistochemical detection showed that the A.A-treated group had higher levels of caspase-3, an apoptotic marker, than control group [(35)]. This was also consistent with the findings of other researchers [(36)] who found that TUNEL labeling revealed a high number of apoptotic cells in UC group colon, with fluorescence intensity noticeably greater than in the control group.

However, analysis of the sulfasalazine-treated group's caspase-3 immune-stained sections showed that most glandular cells had moderate immunoreaction, which alternated with isolated patches of positive cells. Our findings are supported by a study which found that sulfasalazine-treated AA-induced UC had a moderate Caspase 3 immunoreaction [(35)].

In colon's glandular cells cytoplasm, Rats given both cinnamon oil and sulfasalazine in our study exhibited a mild immunoreaction for caspase-3. This is consistent with some authors [(37)] who declared that hepatic tissues of the control and cinnamon-treated groups showed a negative Caspase 3 reaction upon immunohistochemistry analysis. Interestingly, our data showed that group V's combination of beta-carotene and cinnamon oil had weak immune expression for caspase-3. This was validated by Althurwi et al. findings, which showed that the I/R (ischemic/reperfusion injury) + β carotene group had a much lower percentage of caspase-3 immune-positive cells than the ischemic control rats [(38)].

Additionally, a study by Abdel Kareem and Domouky revealed that the β Carotene group's anti-caspase-3 immunopositivity was lesser than control group, indicating a decrease in proportion of apoptotic active neurons [39].

Conclusion:

In this study, we presented data showing that rats given AA intrarectally had alterations in histopathological and oxidative indices. These changes were diminished by giving these animals sulfasalazine or sulfasalazine combined with cinnamon oil. While co-administration of both cinnamon oil and β carotene with sulfasalazine exhibited better enhancement.

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