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Original article

The Possible Ameliorative Effects of Basil Oil on the Colitis Induced by Dextran Sodium Sulfate in Adult Male Albino Rats (Histological , Immunohistochemical and Scanning Electron Microscopic Studies)

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Abstract:

Background: One serious medical condition is ulcerative colitis. Basil is known to have anti-inflammatory effect. Aim: Evaluation of the beneficial effects of basil oil on a rat model of colitis produced by dextran sodium sulfate (DSS). **Materials &Methods:** 40 adult male albino rats were alienated into 4 groups randomly. Each group was 10. control group: 10 rats were alienated equally into 2 subgroups: Subgroup Ia: Five rats weren't given any treatment. Subgroup Ib: Five rats were given corn oil in a dose of 1ml /kg / once daily for 10 days. Basil oil-treated group: was given basil oil 0.2% diluted in corn oil as 1ml of the solution /kg/day once daily for 10 days. DSS-treated group: was given DSS 5% in tap water in a 5 mL solution containing 25 mg of DSS / kg /day once daily for 10 days. DSS and basil oil-treated group: was given DSS 5% and basil oil as the same dose, duration of basil oil group, and DSS-treated group. **Results:** DSS increased disease activity index (DAI). It produced a disrupted histological architecture of the colonic mucosa. A statistically significant decrease in PAS reaction was observed, while collagen deposition and iNOS immuno-expression increased when compared to control group. Administration of basil oil with DSS resulted in reduction in DAI and restoration of colon architecture. the PAS reaction increased, while collagen deposition and iNOS immuno-expression decreased, with no statistical difference with the control group. **Conclusion:** Basil oil decreases histological alterations in induced ulcerative colitis.

Keywords: colitis, basil oil, DSS; anti-inflammatory.

Introduction

Water, nutrients, and electrolytes from partially digested food are absorbed in the colon, which subsequently compacts the feces and sends them on their way to the rectum [1].

Crohn's disease, ulcerative colitis, and other chronic inflammatory gastrointestinal conditions are collectively referred to as inflammatory bowel disease (IBD). IBD results from intestinal immune system dysregulation brought on by a variety of hereditary and environmental causes [2]. Over the past few decades, IBD has become more common worldwide, particularly in developed nations [3]. Anti-inflammatory medications, corticosteroids, and immunosuppressants are typically used to treat IBD in order to reduce symptoms and avoid problems [4].

A sulfated polysaccharide called dextran sodium sulfate (DSS) is used to artificially cause ulcerative colitis in rats. [5] DSS damages epithelial cells by acting as a direct chemical toxin on the colonic epithelium. [6]

Ocimum basilicum, a herbaceous plant belonging to the Lamiaceae family that is grown worldwide and has long been used in cooking, is the source of basil oil. [7] The phenolic components that provide basil oil its antioxidative qualities include flavonoids, phenolic acids, rosmarinic acid, and aromatic chemicals. [8] Due to its antioxidant and anti-inflammatory qualities, basil oil is useful for a variety of conditions. [9] Hepatic fibrosis, diabetes, asthma, anemia, and brain impairment have all been found to be improved by basil oil. [10]

This study aimed to evaluate the effectiveness of basil oil in treating ulcerative colitis in adult male albino rats induced by dextran sodium sulfate.

Material and Methods

Chemical

1.Dextran sodium sulfate (DSS)

DSS 5% powder was bought from Sigma-Aldrich in Egypt. 100 milliliters of water were used to dissolve 500 mg DSS. 5 mg DSS was present in 1 ml of solution. Each rat (200 g) was given 1 ml of the solution orally each day using an insulin syringe, followed by 2 ml of water. This was done because diluted 5 ml of DSS, which contained 25 mg of DSS, was administered daily per kilogram of rat. [11]

2.Basil oil 400µl /kg was bought from El Hawag company for the extraction and processing of natural oils. 19 milliliters of corn oil were mixed with one milliliter of basil oil. Each 200g rat was given 0.2 ml of the solution orally daily using an insulin syringe, with 1 ml of the solution containing 0.2% basil oil administered daily per kilogram of rat. [12]

Animals

Forty adult male albino rats weighing between 180 and 220 grams were employed in this investigation. They were between 10 and 12 weeks old. The rats were housed at room temperature in specially designed cages in the Anatomy Department, Faculty of Medicine, Benha University. After a one-week acclimatization period, the study was carried out from January 5, 2024, to March 12, 2024. They were given commercial food and an ample supply of tap water. There was a 12-hour dark/light cycle in the rats' cage. The experimental protocols were approved by Benha University Ethics Committee in Animal Research **(MS 7-7-2023).**

Experimental Protocols

Forty rats were divided into 4 equal groups (10 rats per group)

Group I (control group):

Ten rats were divided equally into 2 subgroups:

Subgroup Ia: Five rats were fed on a standard diet without any drug for 10 days.

Subgroup Ib: Five rats were given corn oil in a dose of 1ml /kg body weight/day using an insulin syringe orally once daily for 10 days.

Group II (DSS-treated group):

The rats of this group were given DSS 5% in tap water in a dose of 5ml of the solution containing 25 mg of DSS / kg body weight /day using an insulin syringe orally once daily for 10 days.

Group III (Basil oil-treated group):

The rats of this group were given basil oil 0.2% diluted in corn oil in a dose of 1ml of the solution /kg body weight/day using an insulin syringe orally once daily for 10 days.

Group IV (DSS plus Basil oil-treated group):

10 rats were given (DSS 5% and basil oil concomitantly as the same dose and duration as group II and III.

In order to determine the preclinical data, such as body weight change, stool consistency, bloody stool, and rectal bleeding, each rat was monitored twice a day during the experiment. These characteristics were then averaged to estimate the disease activity index (DAI). Stool consistency (0: normal, 2: loose stool, or 4: diarrhea), bloody stool (0: negative, 2: positive, or 4: excessive bleeding), and body weight change (0: <1%, 1:1%–5%, 2:5%–10%, 3:10%–15%, or 4: >15%) were the criteria used to assign scores [(5)].

After a ten-day experiment, the rats were induced to slumber by inhaling ether. The intestine was exposed by making a midline abdominal incision, and the distal 5 cm of the colon was removed and washed with regular saline. After being preserved for 24 hours at room temperature in a 4% formaldehyde solution, the colon tissue was placed in increasing alcohol grades, embedded in paraffin, and then sliced into sections that were 4 µm thick. After the samples were taken, the animals were disposed of by burning in the incinerator of Benha University Hospital.

Light microscopic study

Prior to being prepared into paraffin blocks, the specimens were preserved in 10% neutral formalin. Hematoxylin and eosin were used to stain the blocks after they were cut into 5-mm slices in order to analyze the histological structure. [(13)] , using periodic acid-Schiff (PAS) to assess changes in the stomach mucosa's synthesis of glycoproteins [(14),] using Masson's trichrome to identify the deposition of collagen fibers [(15).]

Before being washed with phosphate-buffered saline (PBS) for the immunohistochemistry examination, paraffin slices were deparaffinized in xylene for 25 minutes at 60 °C in an oven. They were rehydrated with graded alcohol. The slices were then treated with 0.1% hydrogen peroxide for 30 minutes in order to inhibit endogenous peroxidase. Additionally, 10-mM sodium citrate buffer with a pH of 6.0 (1:10 dilution) was used for antigen retrieval, and it was cooked in a microwave for 25 minutes. Primary antibodies and inducible nitric oxide synthase (iNOS) (1:200 dilution; catalog number PA3-030A; Lab Vision, USA; Thermo Fisher Scientific) were applied to the tissue slices, which were then left in a moist chamber at 4 °C for the entire night. Following three PBS rinses, the slides were treated with a diluted 1:200 secondary goat anti-mouse IgG peroxidase-conjugated antibody for 30 minutes at room temperature in a wet chamber. A brown precipitate was produced at the antigen positions as a result of the staining process being completed by diaminobenzidine (DAB) incubation. Following counterstaining with Mayer's hematoxylin, the sample was dehydrated in graded ethanol, cleaned with xylene, and then mounted with DPX [(16).] When seen under a light microscope, brown stains indicated a good immunohistochemical reaction. In the Anatomy Department of the Faculty of Medicine at Benha University in Egypt, slide visualization and picture photography were carried out. In order to do this, a Nikon Eclipse 80i upright microscope (Japan, Nikon Corporation) equipped with a digital camera (ToupTek Europe, Ultramacro Ltd., UK) was utilized.

Scanning electron microscopic study

After being immersed in 2.5% buffered gluteraldehyde for 24 hours, specimens examined by electron microscopy were dehydrated using ethyl alcohol (50, 70, 80, 90, and 100%). Two times, for fifteen minutes each, the tissues were further dehydrated in 100% acetone and then in a 1:1 mixture of acetone and absolute alcohol. A Baltek CPD030 critical point drier (Eindhoven, The Netherlands) was then used to dry the specimens using liquid carbon dioxide. The Baltek SCD005 (The Netherlands, Eindhoven) was used to sputter coat the specimens with gold after they had been placed on aluminum stubs. Each animal's colon was examined using a Philips XL30 scanning electron microscope, which is housed in the Faculty of Medicine's Histology Department at Mansoura University [17]

Morphometric study

Measurements of collagen fiber deposition (in sections stained with masson's trichrome), immuno-expression of iNOS, and mean area % of PAS were taken using immunoassay photomicrographs. Data was imported into an Excel file and subjected to statistical analysis. The overall magnification power used for these measurements was ×200. Using an image analysis tool (Java; NIH, Bethesda, Maryland, USA), the measurements were made in ten non-overlapping microscopic regions of the colon tissue for each rat. The photomicrographs were taken under identical exposure settings for morphometric analysis.

Statistical analysis

The mean±SD was used to display the data. To establish that $P \leq 0.05$ was significant, post hoc multiple comparisons and one-way analysis of variance (ANOVA) were conducted using SPSS software (v.16; Chicago, USA).

Results:

Gross changes of the rat colon :

Group I and Group II: Showed an intact mucosa, no hyperemia or oedema.

Group III: Showed severe hyperemia with obvious congestion, oedema, and atrophy.

Group IV: Showed apparently intact mucosa, no thickened wall.

Results of Disease Activity Index (DAI)

From day four until day zero, the DSS group had significantly higher DAI levels, as evidenced by the prevalence of blood in feces, diarrhea, and weight loss. The DSS-induced colon pathology was significantly reversed in Group IV after receiving basil oil treatment.

Histological results

H&E results:

Groups I*:* The sections of rat colon revealed intact mucosal architecture, submucosa, musculosa, and serosa. Entire mucosal surface epithelium, including simple columnar epithelium, basal basophilic oval nuclei, and acidophilic cytoplasm, was present and undamaged. The mucosal crypts were straight tubular, regularly arranged, closely related, and occupied the whole thickness of the mucosa. Columnar epithelium was simple and bordered the crypts. Goblet cells had a flask-like form, flattened basal nuclei, and vacuolated cytoplasm. There were inflammatory cells visible in the lamina propria, between the crypts. Submucosa exhibited connective tissue and blood vessels. The musculosa consisted of smooth muscle, and the outermost layer, the serosa, appeared intact. **(Figure ١a and b)**

Group II: The sections of rat colon treated by DSS (group II) revealed erosions on the mucosal surface and shedding of surface epithelium into the lumen. Complete destruction of the crypt’s architecture, which appeared lined with small-sized cells having pyknotic nuclei with cytoplasmic vacuolation and nuclear dissolution in the form of karyolysis. Accumulation of inflammatory cells inside the crypt’s lumen and in the lamina propria was observed. Most of the goblet cells were depleted. Some inflammatory cells in

the submucosa and the blood vessels were dilated and congested**.(Figure 1, c and d)**

Group III: The sections of rat colon revealed intact mucosal architecture, submucosa, musculosa, and serosa. Entire mucosal surface epithelium, including simple columnar epithelium, basal basophilic oval nuclei, and acidophilic cytoplasm, was present and undamaged. The mucosal crypts were straight tubular, regularly arranged, closely related, and occupied the whole thickness of the mucosa. Columnar epithelium was simple and bordered the crypts. Goblet cells have a flask-like form, flattened basal nuclei, and vacuolated cytoplasm. There were inflammatory cells visible in the lamina propria, between the crypts. Submucosa exhibited connective tissue and blood vessels. The musculosa consisted of smooth muscle, and the outermost layer, the serosa, appeared intact. **(Figure 2, a and b)**

Group IV: The sections of rat colon treated by DSS plus Basil oil (Group IV) showed restoration of apparently normal histological architecture of the mucosa of the colon. Apparently intact surface epithelium of the mucosa being formed of simple columnar epithelium with acidophilic cytoplasm and basal oval nucleus. crypts were densely packed and oriented consistently, taking up the whole thickness of the mucosa. Simple columnar cells and flask-shaped goblet cells with flattened basal nuclei and vacuolated cytoplasm lined the crypts. **(Figure ٢, c and d)**

Histochemical results:

Masson's trichrome staining:

Group I: Revealed a trace quantity of collagen fibers in the submucosa and between crypts, which looked like blue-colored strips**. (Figure ٣a)**

Group II: Compared to the control group, there was a marked increase in the amount of collagen fibers deposited in the submucosa and between crypts. **(Figure ٣b)**

Group III: Revealed a trace quantity of collagen fibers in the submucosa and between crypts, which looked like blue-colored strips (**Figure ٣c)**

Group IV): Performed slightly worse than the dss-treated group in terms of collagen fiber deposition in the submucosa and in-between crypts. **(Figure ٣d)**

PAS staining:

Group I: showed numerous magenta-red stained PAS-positive goblet cells in the colonic mucosa covering all thickness of the crypts**. (Figure ٣e)**

Group II: showed apparent depletion of PAS-positive stained goblet cells lining the crypts when associated with the control group**. (Figure ٣f)**

Group III: showed numerous magenta-red stained PAS-positive goblet cells in the colonic mucosa covering all thickness of the crypts**. (Figure, ٣g)**

Group IV: showed a moderate number of magenta red-stained PAS-positive goblet cells in colonic mucosa covering the apical part of the crypt when compared with the DSS-treated group. **(figure, ٣h)**

Immunohistochemical results:

Group I: Showed mild expression of brown cytoplasmic positive immunoreactivity for iNOS in the epithelium covering glands**. (Figure 4a)**

Group II: Showed a marked increase in brown cytoplasmic positive immunoreactivity for iNOS in surface epithelial cells, epithelium covering glands. **(Figure 4b)**

Group III: Showed mild expression of brown cytoplasmic positive immunoreactivity for iNOS in the epithelium covering glands**. (Figure 4c)**

Group IV) showed a decrease in brown cytoplasmic positive immunoreactivity in both epithelial cells covering the glands. **(Figure ٤d)**

Scanning electron microscope results:

Group I: Showed normal cerebriform appearance of the mucosa with regularly arranged narrow crypts and intact surface lining epithelium, which had a velvety appearance. Many goblet cells showed a

button-shaped appearance(empty**),** whose mucins appeared extruded, whereas a few of them appeared distended with mucins**. (Figure ٥a)**

Group II: The mucosa was found to be ulcerated, with the surface lining epithelium being lost and its extrusion onto the surface. Shredded cells obstructed the crypts' lumens. Fusion of some crypts together and widening of the crypt's openings were demonstrated. Fissuring of the mucosa, few goblet cells, and a small amount of extruded mucus were observed on the mucosal surface**. (Figure ٥b)**

Group III: Showed normal cerebriform appearance of the mucosa with regularly arranged narrow crypts and intact surface lining epithelium, which had a velvety appearance. Many goblet cells showed a button-shaped appearance(empty**),** whose mucins appeared extruded, whereas a few of them appeared distended with mucins**. (Figure ٥ c)**

Group IV: Showed normal cerebriform appearance of the mucosa with regularly arranged narrow crypts and intact surface lining epithelium, which had a velvety appearance. Many goblet cells showed a button-shaped appearance(empty), whose mucins appeared extruded, whereas a few of them appeared distended with mucins**. (Figure ٥d)**

Morphometric, statistical results:

The percentage of the mean area of collagen fiber deposition by Masson's trichrome staining was represented in Table 1 and Histogram 1):

There was no significant difference (P>0.05) in the percentage of mean area of collagen fiber deposition between the control group **(group I)** and the group that received basil oil treatment **(group III).**

The percentage of mean area of collagen fiber deposition for the Dextran sodium sulfate (DSS)- treated group **(group II)** was 31.6 ± 2.4%, which showed a significant increase (P ≤ 0.05) when associated with the **control group, group III, and group IV.**

The mean area percentage of collagen fiber deposition for DSS plus Basil oil-treated group **(Group IV),** as compared to the DSS-treated (**group II)** (6.1 ± 1.7%), showed a significant decrease (P≤ 0.05). However, there was no significance when comparing with the **control group** and **group III** (basil oil-treated group).

The percentage of the mean area of the PAS staining reaction was represented in (Table 2).

The control group **(group I)** and group cured with basil oil **(group III)** did not differ significantly in the mean area percentage of PAS staining reaction (P>0.05).

The mean area % of PAS staining reaction for the Dextran sodium sulfate (DSS)treated group **(group II)** was 5.6 ± 3.8 % which showed a significant decrease (P≤0.05) when associated with **the control group, group III, and group IV.**

The mean area % of PAS staining reaction for DSS plus Basil oil treated group **(Group IV)** is 16.4 ± 5.9 % which showed a significant increase (P≤ 0.05) when compared with **group II**, while showed no significance when associated with the **control group and group III.**

The percentage of the mean area of iNOS immuno-expression was represented in (Table 3).

The control group **(group I)** and basil oil-treated group **(group III)** did not exhibit any significant differences in the mean area percentage of iNOS immuno-expression (P> 0.05).

In comparison to **the control, group III, and group IV** , the group treated with Dextran sodium sulfate (DSS), **group II,** had a significantly higher mean area % of iNOS immuno-expression at 29.7 ± 2.6% (P≤ 0.05).

The mean area% of iNOS immuno-expression for DSS plus Basil oil treated group **(Group IV)** was 5.7± 1.1% which shows a significant decrease(P≤ 0.05) when compared with **group II**, while

showed insignificance when compared with the **control group and group III.**

Mean values of the Disease Activity Index for ulcerative colitis in the 4 studied groups were represented in (Table 4):

There was no significant difference noticed in the mean values of the Disease Activity Index(P> 0.05) between the control group **(group I)** and the basil oil-treated group **(group III).**

Mean values of the Disease Activity Index for the Dextran sodium sulfate (DSS)treated group **(group II)** were 10 ± 1.6, which showed a significant increase (P≤ 0.05) when compared with the control group, group III, and group IV.

The mean values of the Disease Activity Index for DSS plus Basil oil treated group **(Group IV)** were 1.8± 0.8, which showed a significant decrease (P≤ 0.05) when compared with **group II**, while showed no significance associated with the **control group** and **group III**.

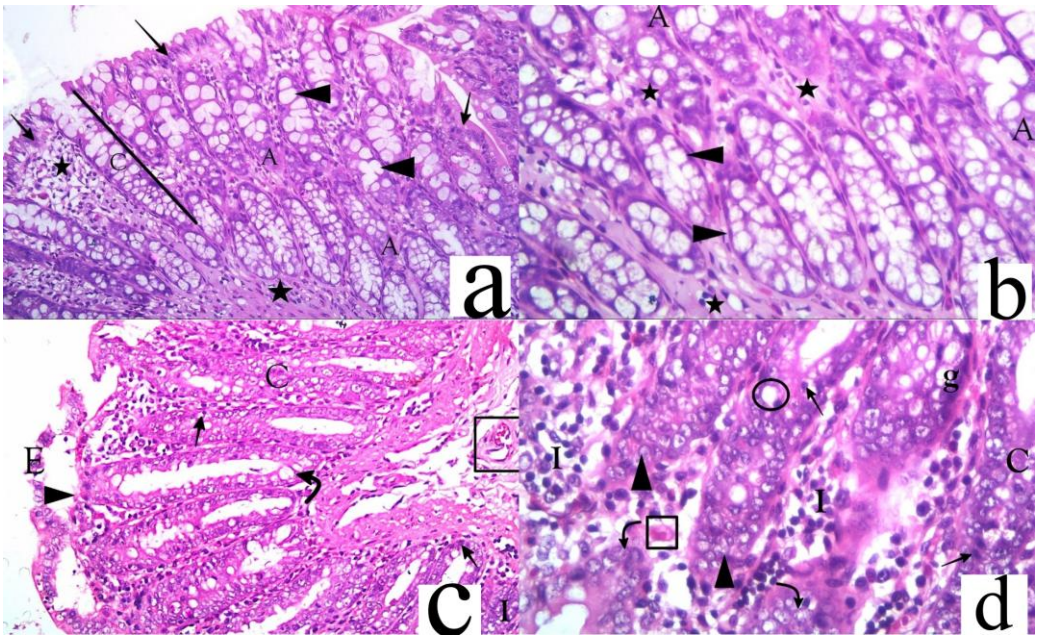


Figure (١): as showing

(A): A photomicrograph of a section of the distal colon of a rodent in the control group (group I) demonstrating the typical histological structure of colon mucosa. An intact surface epithelium of mucosa is composed of a basal oval nucleus and acidophilic cytoplasm found in simple columnar epithelium (arrow). Mucosal crypts (C) are densely packed and regularly arranged, occupying the entire thickness of the mucosa. They are lined with simple columnar cells (A) and flask-shaped goblet cells (arrowhead), which have vacuolated cytoplasm and basal flattened nuclei. The lamina propria (star) contains a significant number of inflammatory cells. **(H &E x 200)**

(b): photomicrograph of a section of the rat distal colon of the control group **(group I),** showing: Normal histological architecture of the mucosa of the colon. Mucosal crypts **(C)** are regularly arranged and tightly packed. The crypts are lined with simple columnar cells**(A)** and flask-shaped goblet cells **(arrowhead)** with vacuolated cytoplasm and basal flattened nuclei. Numerous inflammatory cells are detected in the lamina propria **(star). (H &E: x 400)**

(c): In the distal colon of an adult male albino rat from the DSS-induced colitis group (group II), a photomicrograph was taken. The section exhibited erosions on the mucosal surface (arrowhead) and the discharge of surface epithelium into the lumen (E). The architecture of the crypt (C) has been completely destroyed, and it is surrounded by small cells with profoundly stained nuclei (arrow). Additionally, the lumen of the crypt (I) exhibits the accumulation of inflammatory cells. The number of goblet cells that can be observed is exceedingly low (curved arrows). The spaces between crypts (line) and dilated, congested blood vessels (square) have expanded (H&E: X200).

(d): Photomicrograph of a section in the distal colon of adult male albino rat of DSS induced colitis group (group II) expressing destruction of normal mucosal architecture in the form of Complete destruction of crypt's(C) architecture which is lined with small-sized cells showing cytoplasmic vaculation (circle) and nuclear degeneration in the form of small deeply stained nuclei -pyknosis-(arrow), karyolysis (curved arrow), very few goblet cells can be seen (g)With Massive infiltration with inflammatory cells (I) in the lamina propria . (H&E:X 400)

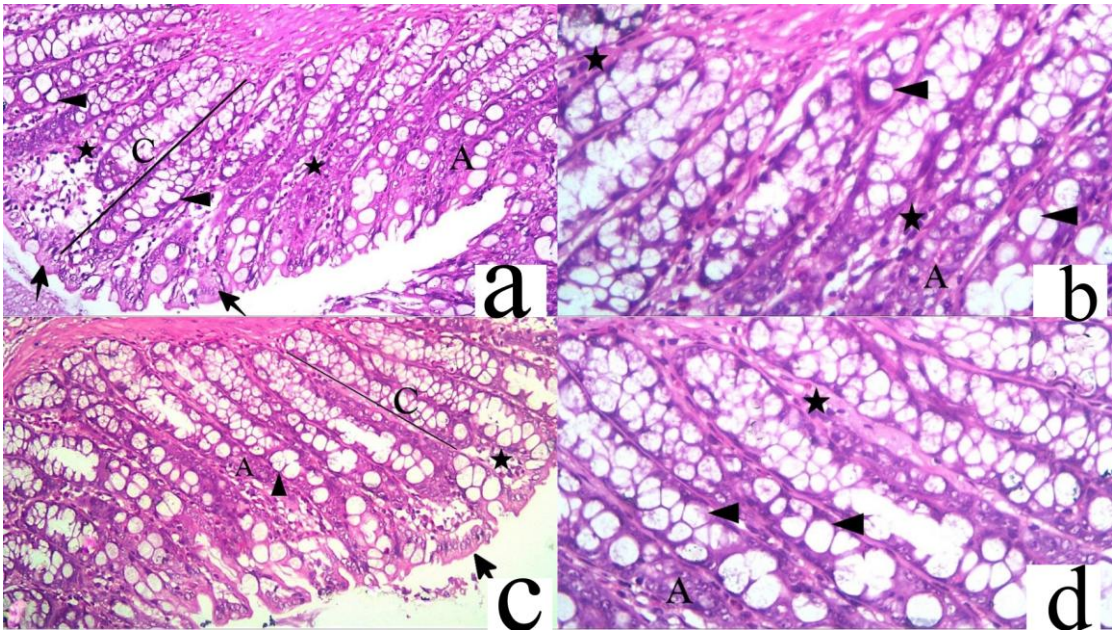


Figure (٢): as showing

(a): a photomicrograph of a section of the distal colon of a rodent that was cured by basil oil: group III Illustrating: The mucosa of the colon's normal histological architecture. Simple columnar epithelium (arrow) with acidophilic cytoplasm and basal oval vesicular nucleus crypts (C) are regularly arranged and densely packed, occupying the entire thickness of the mucosa, forming the intact surface epithelium of the mucosa. A simple columnar cell (A) and flask-shaped goblet cells (arrowhead) with basal flattened nuclei line the crypts and vacuolated cytoplasm. Numerous immune cells are identified in the lamina propria (star). **(H &E: X 200)**

(b): photomicrograph of a section of the rat distal colon of the basil oil-treated group (group III), showing: Normal histological architecture of the mucosa of the colon. Mucosal crypts (C) are regularly arranged and tightly packed. The crypts are lined with simple columnar cells(A)and flask-shaped goblet cells (arrow head) with vacuolated cytoplasm and basal flattened nuclei. Numerous immune cells are detected in the lamina propria (star). (H &E :X 400)

(c): Photomicrograph of a section in the distal colon of an adult male rat of the DSS +basil oil treated group (group IV), Showing Restoration of apparently normal histological architecture of the mucosa of the colon. The mucosa's surface epithelium is composed of simple columnar epithelium (arrow), which has an acidophilic cytoplasm and a basal ovoid nucleus. It appears to be intact. In a regular pattern, crypts (C) are densely packed and occupy the entire thickness of the mucosa. A simple columnar cell (A) and flask-shaped goblet cells (arrowhead) with basal flattened nuclei line the crypts and vacuolated cytoplasm. Numerous immune cells are identified in the lamina propria (star). (H&E.: X200)

(d): A photomicrograph of a section in the distal colon of an adult male rodent from the DSS + basil oil-treated group (group IV). Displaying: The restoration of the mucosa of the colon to an evident normal histological architecture. Apparently, the mucosa's intact surface epithelium is composed of simple columnar epithelium (arrow), which has an acidophilic cytoplasm and a basal oval nucleus. Crypts (C) are densely packed and regularly arranged, occupying the entire thickness of the mucosa. Simple columnar cells (A) and flask-shaped goblet cells (arrowhead) with basal flattened nuclei line the crypts and vacuolated cytoplasm. The lamina propria (star) contains a significant number of immune cells.(H&E: X400)

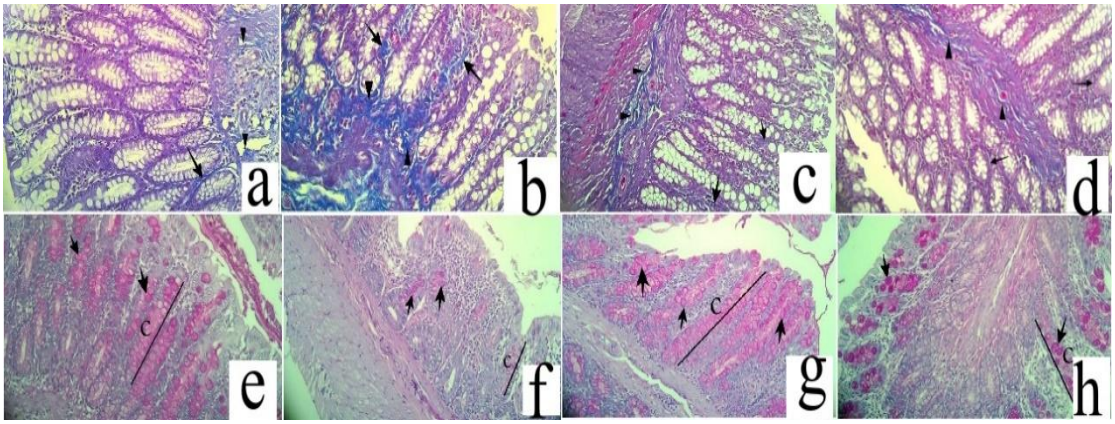


Figure (٣): as showing

(a): A Photomicrograph of Masson's Trichrome-stained section of the distal colon of an adult male albino rat of the control group (Group I), presenting: Minimal amount of collagen fibers (appeared as tinge of blue colored strips in between crypts (arrow) and in the submucosa (arrowhead) (Masson's Trichrome X 200)

(b): Masson's trichrome-stained section of the distal colon of an adult male albino rat from the DSS-persuaded colitis group (Group II) is depicted in a photomicrograph. Deposition of collagen fibers in the submucosa (arrowhead) and between crypts (arrow) is evident (Masson's Trichrome: X 200).

(c): Group III adult male albino rats treated with basil oil had a photomicrograph of their distal colon stained with Masson's trichrome. The image shows: Very few collagen fibers (seen as narrow blue bands in the space amid crypts; arrow) and a concentration in the submucosa; arrowhead (Masson's Trichrome X 200)

(d): A Photomicrograph of Masson's Trichrome-stained section of the distal colon of adult male albino rat of the basil oil + DSS-treated group (Group IV) showing: Mild deposition of collagen fibers in-between crypts (arrow)and in the submucosa (arrow head). (Masson's Trichrome 200)

(e): A photomicrograph of the PAS-stained section of the distal colon of an adult male albino rat of the control group (Group I) showing: Numerous magenta red-stained PAS-positive goblet cells in the colonic mucosa (arrow) covering all thickness of the crypts (c) (PAS: X200)

(f): A photomicrograph of the distal colon of an adult male albino rat in group II (DSS-treated group) that has been PAS-stained and demonstrates the depletion of PAS-positive stained goblet cells (arrow) that line the crypts (c). (PAS: X200)

(g): In the distal colon of adult male albino rats treated with basil oil (Group III), a photomicrograph of the PAS-stained section reveals a plethora of magenta, red-stained PAS-positive goblet cells in the colonic mucosa(arrow). encompassing the entire thickness of the crypts (c) (PAS: X200)

(h): A photomicrograph of the PAS-stained section of the distal colon of an adult male albino rat of the basil oil +DSS treated group (Group IV), presenting: A Moderate number of magenta red-stained PAS-positive goblet cells(arrow) in the colonic mucosa covering the apical part of the crypt (c). PAS (200)

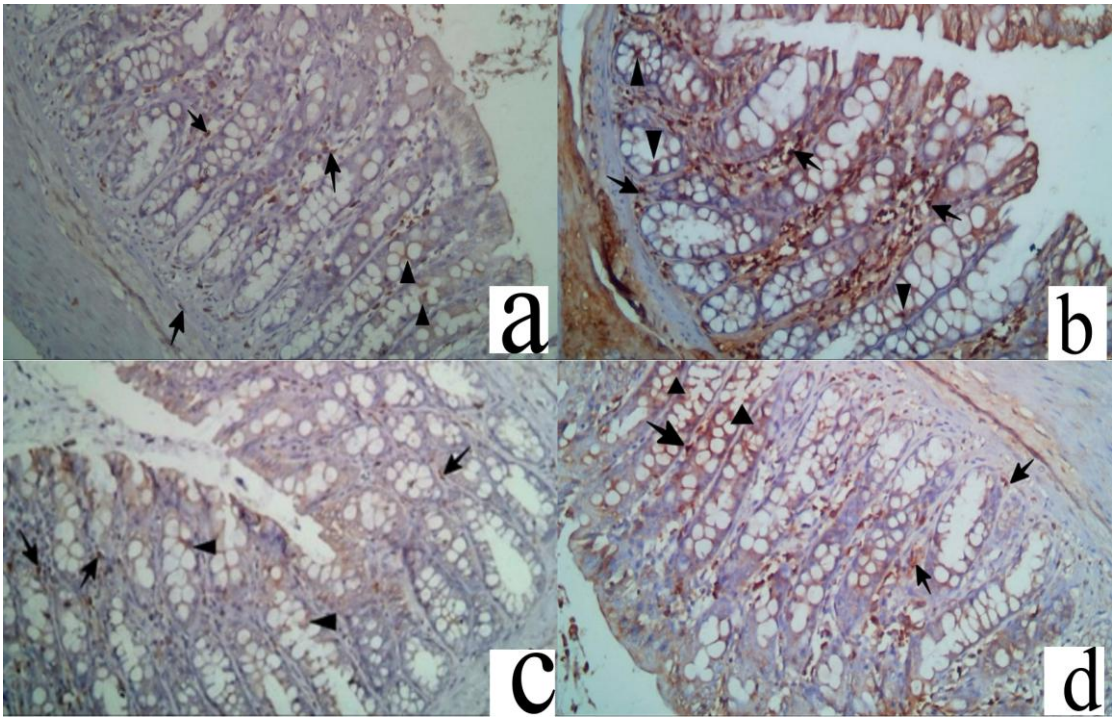


Figure (٤): as showing

(a): Photomicrograph of a section in the distal colon of an adult male albino rat from the Control group (Group I) demonstrates a mild expression of brown cytoplasmic positive immunoreactivity for iNOS in the epithelium covering glands (arrowhead) and a small number of cells in the connective tissue lamina propria of the mucosa (arrow). (iNOS: X 200)

(b): A photomicrograph of a section in the distal colon of an adult male albino rat (Group II) treated with DSS shows a significant increase in brown cytoplasmic positive immunoreactivity for iNOS in surface epithelial cells, epithelium-covering glands (arrowhead), and connective tissue lamina propria cells of the mucosa (arrow). (iNOS: X 200)

(c): In the distal colon of adult male albino rats, basil oil-treated group (Group III) exhibited mild expression of brown cytoplasmic positive immunoreactivity for iNOS in both the surface epithelial cells covering glands (arrow head) and the connective tissue lamina propria cells (arrows) of the mucosa. A photomicrograph of the section is shown. (iNOS: X 200)

(d): Photomicrograph of a section in the distal colon of an adult male albino rat from the basil oil plus DSS-treated group (Group IV). The photomicrograph shows an apparent decrease in brown cytoplasmic positive immunoreactivity in both the epithelial cells covering the glands (arrow head) and the connective tissue lamina propria cells (arrow) of the mucosa. (iNOS: X 200)

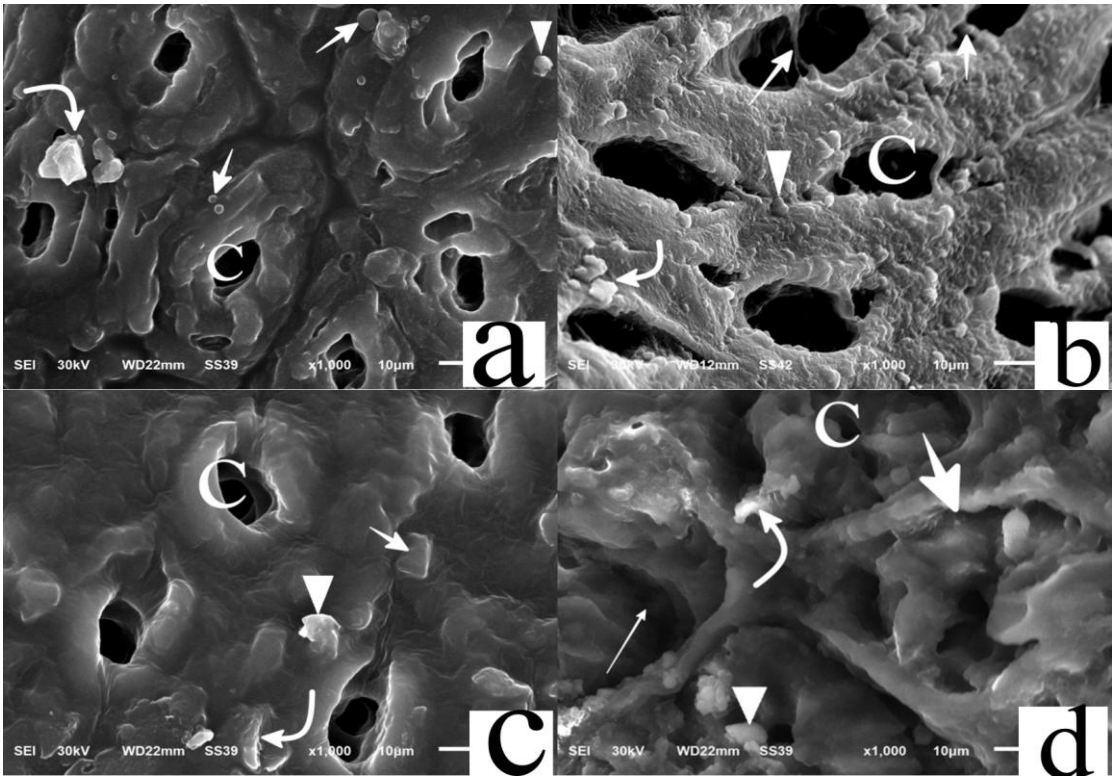


Figure (٥) : as showing

(a): Group I adult male albino rats' scanning electron micrographs of their distal colons reveal: Mucosal crypts (C) with complete surface lining epithelium with velvety appearance; numerous enlarged (arrow head) and empty goblet cells(arrow); and extruded mucous (curved arrows) on the mucosal surface. (SEM: X1000)

(b):A scanning electron micrograph of a section of distal colon of adult male albino rat of DSS induced colitis group (group II), showing: Fissuring of the mucosa (arrows), widening of the crypts openings (C), few goblet cells (arrowhead), and little amount of extruded mucous (curved arrow) on the mucosal surface. (SEM: X1000)

(c): scanning electron micrograph of distal colon section of adult male albino rat of the basil oil-treated group (group III), presenting mucosal crypts (C) that are normal-shaped and have an intact surface lining epithelium, which has a velvety appearance.There are numerous distended and vacant goblet cells (arrowhead) and extruded mucous (curved arrows) on the mucosa's surface (SEM: X1000).

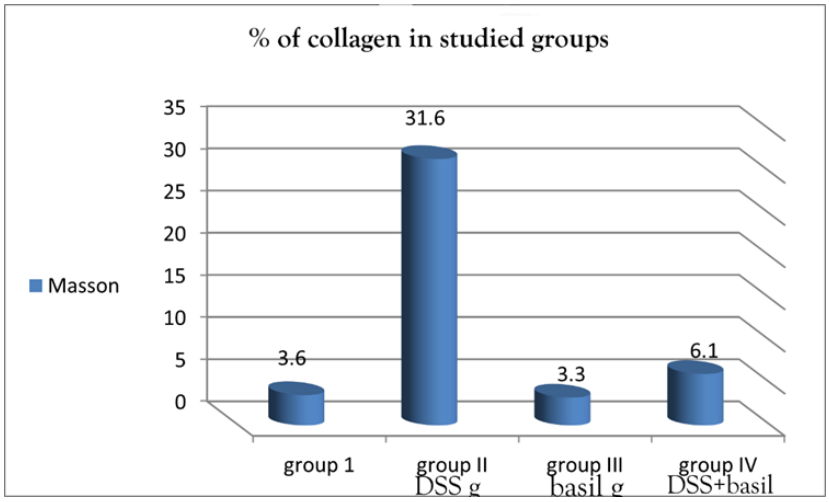
(d): Group IV, which received a combination of basil oil and DSS, was analyzed using scanning electron microscopy. Results exposed surface lining epithelium of mucosal crypts restored to a virtually normal regularity. Take note of the narrow arrows that indicate minor mucosal fissures and the curved arrows that indicate little expansion of the crypt apertures, both of which contain produced mucus. The arrow heads indicate the presence of several enlarged goblet cells. (SEM: X1000)

Table (1) Showing The mean area percentage of collagen fiber deposition in the different studied groups

	Control Group	**Group III (DSS group)**	**Group II (Basil oil group)**	**Group IV (DSS+basil oil group)**
The % of collagen area in the studied groups	3.6 ± 1.2	3.3 ± 0.9 [b]	31.6 ± 2.4 [a, c & d]	6.1 ± 1.7 [c]

Data expressed as mean±SD, *: significance ≤ 0.05 (Bonferroni test)

a: Significance vs Control, b: Significance vs group II, c: Significance vs group III, d: Significance vs group IV



Histogram (1) showing the mean area percentage of collagen fiber deposition in the different studied groups

Table (2) showing the percentage of the mean area of PAS staining reaction in the different studied groups

	Control Group	Group II (DSS group)	Group III (Basil oil group)	Group IV (DSS+basil oil group)
% of PAS in the studied groups	**35 ± 4.8**	**5.6 ± 3.8 [a, c & d]**	**34.3 ± 11.4 [b]**	**16.4 ± 5.9 [c]**

Data expressed as mean±SD, *: significance ≤ 0.05 (Bonferroni test)
a: Significance vs Control, b: Significance vs group II, c: Significance vs group III, d: Significance vs group IV

Table (3) showing the percentage of the mean area of iNOS immuno-expression in the different studied groups

	Control Group	Group II (DSS group)	Group III (Basil oil group)	Group IV (DSS+basil oil group)
% of iNOs area	**2.4 ± 0.9**	**29.7 ± 2.6 [a, c& d]**	**2.9±1 [b]**	**5.7± 1.1 [c]**

Data expressed as mean±SD, *: significance ≤ 0.05 (Bonferroni test)
a: Significance vs Control, b: Significance vs group II, c: Significance vs group III, d: Significance vs group IV

Table (4) showing the disease activity index in the different studied groups

.	Control Group	Group II (DSS group)	Group III (Basil oil group)	Group IV DSS+basil oil group)
Disease activity index (DAI)	**1 ± 0.7**	**0.8± 0.8 [b]**	**10 ± 1.6 [a, c & d]**	**1.8± 0.8 [c]**

Data expressed as mean±SD, *: significance ≤ 0.05 (Bonferroni test)
a: Significance vs Control, b: Significance vs group II, c: Significance vs group III, d: Significance vs group IV

Discussion:

Increased energy expenditure and nutritional deficits, such as malnutrition and malabsorption, are associated with IBD. One of the multifactorial IBDs is ulcerative colitis, a chronic inflammatory disease of the colon (UC). A reduced life expectancy and a higher incidence of colon cancer are associated with UC. Because UC causes colon inflammation, which results in persistent diarrhea and rectal bleeding, it lowers quality of life. **(18)** By reducing the severity of acute episodes and consequences, it is crucial to improve life quality for UC patients. This study is to look at whether basil oil may help the UC rat model.

DSS chosen model for induction of UC is characterized as a reliable, non-invasive, and shares many clinical and pathological features of human UC **(19)**

In this current study, the macroscopic picture of colon sections treated with DSS showed severe hyperemia with obvious congestion, oedema, and atrophy. This finding is in agreement with **(20)**, who observed shortening in colon sections treated with DSS, characterized by hyperemia and mucosal ulceration.

In order to assess disease activity, DAI has been extensively used since it allows for the integration of multiple illness parameters into one value **(21).** By keeping track of rectal bleeding, fecal viscosity, and the pace of body mass reduction twice daily for ten days, we assessed the DAI to see if basil oil could improve the disease activity of UC rats. Compared to the control group and the group that received basil oil treatment, the DSS-treated group's DAI for DSS-induced ulcerative colitis significantly increased.

The same results were recorded by **(22),** who discovered that the rodents that were administered DSS exhibited a significant reduction in weight and a significant rise in DAI scores.

In our current study, H&E analysis of distal colon sections of DSS DSS-treated group demonstrated histological alterations in the colonic mucosa, including erosions and shedding of surface epithelium into the lumen, as well as the destruction of the crypt's architecture, which looked to be lined with tiny cells with deeply stained nuclei (nuclear pyknosis), cytoplasmic vacuolation, and nuclear decline. There was an accumulation of inflammatory cells in the lamina propria and inside the crypt's lumen. Very few goblet cells were seen. These results were in agreement with those of **(23)** and **(24).** They attributed these changes to the direct toxic effect of DSS on the intestinal mucosa.

These findings were similar to those of **(25),** who published that the DSS-treated group showed disruption of crypts' architecture, infiltration of inflammatory cells into the mucosa, and loss of goblet cells.

This comes in agreement with **(26),** who documented a number of pathological alterations in the UC group. The colon tissue's goblet cell architecture was harmed in the mucosa.

The mucosa was found to be ulcerated, with the surface lining epithelium being lost and its extrusion onto the surface. Shredded cells obstructed the lumens of the crypts. To keep the epithelial barrier functioning properly, there must be a constant balancing act between cell proliferation and the death of epithelial cells. Multiple gastrointestinal diseases may manifest when this equilibrium is disrupted by excessive apoptosis **(27).** This was clarified by **(20),** who claimed that tumor necrosis factor (TNF-α) is significantly elevated during DSS-induced colitis and that it communicates through the receptors TNFRII and TNFR1. The TNFRII signaling pathway is related to cell proliferation and enhanced survival, whereas the TNFR1 signaling pathway is related to cell death and cytokine production.

The DSS-treated group in our study showed dilated, clogged blood arteries. According to a prior study, DSS significantly altered the mucosal vascular networks and largely produced vascular leakage. [28]

Consistent with earlier studies, this one found crypt distortion, namely crypt dilatation and cyst formation in the lamina propria. The authors of those earlier studies described the cyst as a huge cystic dilatation with a little "necklace" of cells surrounding the crypt. Meanwhile, DSS has the potential to disrupt the regular communication between intestinal lymphocytes and lead to aberrant regulation of integrin β7 receptor expression, epithelial cells, and extracellular matrix, which might lead to crypt dilatation. [29&30]

Heavy inflammatory cellular infiltration observed in our study was explained by [31] according to the source of inflammatory cellular infiltration in colitis, α4 β 1 & α4 β 7 expression by leukocytes, ICAM-1, and β2 integrins, which are the main mediators of leukocyte recruitment during inflammation, and mucosal address in cell adhesion molecule-1 (MAdCAM-1).

Epithelial cell desquamation observed was explained by [32] that the recruitment and activation of phagocytic leucocytes in the mucosa, as well as the emission of significant quantities of reactive oxygen species (ROS), including superoxide anion, are associated with colonic inflammation. Cellular oxidative damage might arise from this unchecked overproduction of ROS, which could overwhelm defenses. Endogenous antioxidant enzymes, such as superoxide dismutase (SOD), which transforms O2 into H2O2 and is then neutralized to water by catalase or glutathione peroxidase, can neutralize ROS. However, excessive ROS concentrations can attack and inactivate antioxidant enzymes, primarily SOD, which can lead to tissue damage.

Additionally, the percent of mean area of PAS-positive reaction in the DSS-treated group was statistically significantly lower than that in the control group. Similar findings were reported by [33 , 34], who found that the model group's goblet cells atrophied to varied degrees and that the mucus coating on the mucous membrane was diminished.

This comes in accordance with [35]. He claimed that DSS caused goblet cells' mucin contents to seem to decrease along with their PAS response. They linked it to proinflammatory cytokines, especially TNF-α, which play a significant role in illness's pathogenesis. The latter led to increased mucosal inflammation within the colon as mucin was lost from inside goblet cells, the thickness of the colonic mucus barrier was reduced, and the mucosa was exposed to luminal antigens.

Collagen % area was found to be significantly higher in the DSS-treated group compared to the control group in this new investigation. Such a development is in line with [36], who reported that the ulcerative colitis group had significant collagen deposition in the colonic mucosa and submucosa.

This comes in accordance with [37], who documented widespread collagen deposition in the mucosa and submucosa of severely inflammatory colons, which resembles UC fibrosis.

Previous authors [38] stated that the intestinal epithelium's destruction results in inflammation within the intestine. Subsequently, subepithelial myofibroblasts are recruited to the injury site to facilitate tissue regeneration and further extracellular matrix remodeling.

In this investigation, the DSS-treated group exhibited a significantly higher mean percentage of iNOS immuno-expression in the gland-covering epithelium and a few cells in the mucosa's connective tissue lamina propria than the control group. By publishing a comparable discovery, [39], who found that DSS significantly increased iNOS gene expression by 122% compared to the control group.

This was explained by [40], who claimed that excessive levels of reactive oxygen species (ROS) are produced when neutrophils infiltrate. Numerous transcription factors have been shown to be activated by excessive ROS and cytokine production, which heightens the inflammatory response. Among them, proinflammatory genes, such as iNOS, are transcriptionally induced by nuclear factor kappa B (NF-κB).

These results were confirmed with scanning electron microscopic examination of the DSS-treated group, which showed ulceration of the mucosa with loss of surface lining epithelium and its extrusion on the surface. The lumens of the crypts were obstructed by shredded cells. Widening of the crypt's openings was demonstrated. Fissuring of the mucosa, few goblet cells, and a small amount of extruded mucus were observed on the mucosal surface.

This comes in accordance with [41,42]. In the colitis group, the crypts' openings were observed to broaden, and the cells that covered and lined the crypts were lost, resulting in a honeycomb appearance.

Previous studies [43 , 44] have shown that stem or mesenchymal cell damage in the gut can be seen in UC patients. This damage could explain why some patients see the double crypt sign, as well as abnormalities in unit and crypt mouth size.

In our study, the treatment with basil oil was proven to promote the potent efficacy of basil oil in ameliorating the harmful effects of DSS on the colon tissue. Macroscopic picture of the DSS + basil oil-treated group showed intact mucosa, no erosion/ ulceration, or thickened wall.

The same results of [45], who discovered treatment with this plant decreases the wet weight of distal colon segments and major damage score in comparison with the control, which is well correlated with regression in local inflammation scores

In our study, the DSS + basil oil-treated group exhibited a significant reduction in DAI compared to the DSS-treated group, while the control group did not exhibit any significant differences. This comes in accordance with [46] and [47], who stated that basil oil, orally administered, has demonstrated positive benefits in a rat model of acetic acid-induced colitis. A decrease in colon wall (myeloperoxidase) MPO activity, an enzyme implicated in the oxidative damage brought on by colitis, was likewise connected to these effects.

The restoration of the colonic mucosa's normal histological architecture, which was almost identical to that of the control group, supported these findings. The mucosa's intact surface epithelium is composed of simple columnar epithelium with an oval basal nucleus and acidophilic cytoplasm. The crypts were densely packed and oriented consistently, taking up the whole thickness of the mucosa. Simple columnar cells and flask-shaped goblet cells with flattened basal nuclei and vacuolated cytoplasm lined the crypts. The lamina propria has a large number of inflammatory cells.

These findings were in agreement with a recent study of [12]. Researchers found that the histological indicators of inflammation, including tissue damage, edema, and the infiltration of macrophages and lymphocytes into the mucosa, were considerably reduced by basil oil. During experimentally generated colitis, the treatment of basil oil reduced the myeloperoxidase level, demonstrating a strong anti-inflammatory effect.

In this investigation, the mean area percentage of PAS-positive reaction was significantly higher in the group treated with DSS + basil oil compared to the group treated with DSS alone; however, the control group had insignificantly different. That lines up with what we found in [48]. They discovered that the goblet cells of the intestinal crypts responded moderately favorably to the basil oil treatment in the diabetic colon.

In this study, the control group showed no significant difference in mean area percentage of collagen fiber deposition,

while the group treated with DSS showed a significant decrease.

A previous study [49] added that colon sections of basil oil treated diabetic rats showed a mild amount of collagen fibers, as in the control group.

In this study, there was a significant decrease in the percentage of the mean area of iNOS immuno-expression in the DSS and basil oil-treated group in comparison with the DSS-treated group. This finding aligns with [49] and [50]**.** The plant basil extract was found to have anti-inflammatory properties by inhibiting the production of iNOS and NO (nitric oxide).

The crude methanolic extract of basil has an anti-inflammatory effect due to the inhibitory action on key pro-inflammatory cytokine mediators of inflammation (NO and iNOS &TNF-α) [51]

Another author [52] claimed that Tnfrsf9 (a member of the TNF receptor superfamily) may be suppressed by basil's anti-inflammatory properties against inflammation brought on by adipocytes.

In our study, scanning electron microscopic examination of basil oil + DSS-treated group showed apparent restoration of cerebriform appearance of the mucosa with slight dilatation in some of the crypt openings and restoration of normal regularity of crypt arrangement. A moderate amount of goblet cells showed a button-shaped appearance whose mucins appeared extruded, whereas a few of them appeared distended with mucin. These results are in accordance with [48]**,** who discovered that colon sections of diabetic rats treated with basil oil displayed mucosal crypts with large apertures that were almost typical in form, and that the lumen of certain crypts was blocked by mucus released by the rat. However, a scanning electron microscope also revealed an intact mucosal surface with typical crypt apertures and undamaged goblet cells.

The extract from basil is an antioxidant that effectively mitigates potent oxidizing agents, such as hydrogen peroxide, as indicated by research. These effects are ascribed to its composition, which is abundant in substances known to exhibit antioxidant qualities, including rosmarinic acid (RA), flavonoids, and polyphenols. [53]

The primary source of the herbal extracts' antioxidant properties is their ability to absorb free radicals and donate electrons or hydrogen. [53]

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