EFFECT OF PROBIOTICS VERSUS N- ACETYLCYSTEINE ON ACETIC-ACID-INDUCED ULCERATIVE COLITIS IN ADULT MALE ALBINO RATS: HISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY

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ABSTRACT:

Background: *Several factors compromise the normal operating capacity of the colon. Ulcerative colitis is one of the most frequent forms of gastrointestinal disorders that requires long-term treatment. Probiotic supplementation is increasingly used to treat and prevent gastrointestinal problems. The strong antioxidant N-acetylcysteine (NAC) increases intracellular cysteine and glutathione levels and scavenges reactive oxygen species (ROS).*

Aim of the work: *To compare the therapeutic effects of probiotics versus NAC in adult albino rats with induced UC.*

Material and Methods: *Six groups of sixty animals were separated: control group (I), sub-group, Ia and Ib, Probiotic treated group (II), N-acetylcysteine treated group (III), Induced colitis group (IV); colitis treated with probiotics group (V); and colitis treated with NAC group (VI). After two weeks, blood samples were collected to assess the levels of oxidative stress markers (MDA), (SOD), and (CAT). Colon samples were prepared for histological and immunohistochemical examinations as well as for scanning electron microscopy (SEM).*

Results: *The induced colitis group exhibited a significant reduction in mucosal thickness, with loss of surface epithelium, cells with pyknotic nuclei, and vacuolated cytoplasm. The number of goblet cells was markedly decreased, and cellular infiltration and fibrotic alterations were observed. SEM revealed a widening of the crypt openings. A significant decrease in Ki 67 and an increase in caspase 3 immunoreactivity were also observed. Moreover, the administration of probiotics had more ameliorating effects on these changes than N-acetyl cysteine.*

Conclusion: *Probiotics exhibited superior improvements in biochemical, histological, and morphometric results compared to NAC in inducing colitis through their anti-inflammatory, anti-oxidative, and anti-apoptotic effects*

Keywords: *Colitis, probiotics, NAC, Ki76, caspase 3*

INTRODUCTION:

Ulcerative colitis and Crohn's disease are long-standing diseases called inflammatory bowel diseases (IBDs) with a chronic aggressive progressive course and systemic problems. The incidence of this condition is increasing worldwide. Symptoms include severe abdominal cramps and bloody diarrhea [1]. Several etiological factors, including genetic, immunological, environmental, and microbial factors, are involved in

these diseases. Several pro-inflammatory mediators (cytokines) are produced as a result of these diseases, including Interleukins (IL) (IL1, IL6, IL9, IL13, and IL33), while anti-inflammatory mediators (Transforming growth factor (TGF), interleukins (IL 10, and 37) are downregulated [2]. During mucosal inflammation, disruption of the intestinal barrier leads to abnormal morphology and modification of apical connections, with increased gut permeability [3]. There is no definitive cure for IBDs, and conventional pharmacological drugs have partial efficacy and lack long-term clinical suppression. In addition, prolonged use of pharmacological treatments leads to many side effects. Therefore, new therapeutic alternatives, especially those with antioxidant properties, are needed [4].

Clinical studies have shown greater levels of tumor necrosis factor (TNF) in the intestinal mucosa, feces, and serum of UC patients. TNF is known to induce apoptosis and an inflammatory response in intestinal epithelial cells and may contribute to the initiation and development of disorders of the intestinal barrier [5].

Probiotics are beneficial bacteria that can be ingested or administered into the body. The beneficial effects of probiotic microorganisms on the intestinal epithelium [6] are very important**.** Genera strains, which are the most frequently researched and used probiotics, are Bifidobacterium (Bifidobacterium, Longum adolescentis, Animalis, and Breve) and Lactobacillus (acidophilus, johnsonii, paracasei, casei, fermentum, gasseri, rhamnosus, Plantarum, and salivarius)[7]. These bacteria are components of the gut microflora and are particularly helpful when digesting dairy products such as milk, cheese, and yogurt. The beneficial effects of probiotics on GIT function are ascribed to the repair of microbiota, improved adherence to the intestinal mucosa, and simultaneous inhibition of pathogen adhesion[8]. Probiotics can modulate the gastrointestinal mucosal immune system. Additionally, the competitive exclusion of pathogenic microorganisms, the formation of antibiotic compounds, and an increase in the synthesis of protective intestinal mucus[9]. Enhancement of the immune function barrier and a decrease in inflammatory cytokines are two of the most advantageous consequences[10].

The mucolytic properties of N-acetylcysteine make it useful for treating cystic fibrosis, pneumonia, bronchitis, and paracetamol overdose [11]. As glutathione levels in the body increase when consumed, it exhibits antioxidant and anti-inflammatory properties because it can eliminate reactive oxygen species (ROS)[12]. N-acetylcysteine exerts indirect anti-inflammatory effects through its antioxidant activity, which in turn suppresses the pro-inflammatory transcription factor NFkB[13].

AIM OF THE STUDY:

This study aimed to explain the structural changes in induced colitis and to compare the possible therapeutic effects of probiotics against N-acetylcysteine in adult male albino rat colon with induced colitis using biochemical, histological, and immune-histochemical techniques.

MATERIAL AND METHODS:

Fifty adult male albino rats (12 weeks) were used, each weighing, 180-200 gm. This research was conducted by the guidelines of the Ethical Committee for the Use of LAB Animals NO 6/2023HIST 14, Menoufia University.

Reagents:

1- Probiotic capsule: - contains (Bifidobacterium longum, Lactobacillus acidophilus, and Bifidobacterium bifidum), with one and a half billion cells in each capsule (RITE AID pharmacy,

USA). Each capsule contains 1.5 ×109 CFU per 0.2179 g. Feeding per oral requires 1 ×109 CFU dose, which contains 0.145 gm of probiotic [14].

2- N. acetylcysteine: - (imported by SEDICO Pharmaceutical Company, cited on 6th of October City, Egypt), was prepared by immersing 0.5 grams of NAC powder into 1 ml distilled water. It was administered orally (1 g/kg/day) as a gavage (orogastric gavage) **[15].**

3- Acetic acid preparation: - was prepared at the Faculty of Medicine, Menoufia University, by adding 3 ml of acetic acid to 97 ml of distilled water (3 % concentration) and mixing for use in the experimental groups to induce colitis **[16]**.

Induction of Experimental Colitis:

The animals were fasted overnight and provided plenty of water. A fine rubber catheter was used for intrarectal injection while the rat was in the supine position, and only minimal ether anesthesia was used; 2 ml of acetic acid was infused into the anal verge to a maximum length of 6 cm. During the outward removal of the catheter, 2 ml of air was administered to ensure that the solution was thoroughly distributed throughout the colon **[17].**

Experimental design:

Fifty animals were divided into six groups; this study took two weeks to complete.

1- Group 1, (control groups) including 10 animals was divided into two subgroups. Each group had the same number of animals.

- Subgroup Ia: received a balanced diet and water without medication by oral gavage.

- Subgroup Ib: Each ingested distilled water with a balanced diet by oral gavage.

2- Group II, (probiotic treated): 5 rats received probiotics; 0.145 gm of probiotic dissolved in 2 ml distilled water by oral gavage once daily for 14 days (just before meal) [14].

3- Group III, (NAC treated): 5 rats received NAC, prepared by immersing 0.5 grams of NAC powder into 1 ml of distilled water. It was administered orally (1 g/kg/day) by orogastric gavage [13]**.**

4- Group IV, (colitis group): 10 rats received 2 ml of 3 % Acetic acid was used to induce colitis as previously mentioned.

5- Group V, (colitis received probiotics): include 10 animals, colitis was induced then after 24 h. The rats received probiotics, as previously mentioned.

6- Group VI, (colitis received NAC): 10 rats with induced colitis were treated with NAC, as mentioned previously.

After two weeks, ether inhalation (2 ml) was utilized for anesthesia in a transparent acrylic jar for roughly 2 minutes **[18]**. Blood samples were taken and centrifuged for 10 minutes in heparinized tubes. Plasma samples were kept at -20 °C for biochemical examination. A cut was made in the abdomen to expose the intestine, and six centimeters of the colon, just two centimeters above the anal margin, were obtained and processed for examination using histological, scanning electron microscopy (EM), and immune-histochemical studies.

I- Biochemical studies were conducted in the Central LAB linked to the Biochemistry Department, Menoufia Faculty of Medicine. MDA, SOD, and CAT are indicators of lipid oxidation and oxidative stress **[19]**.

II- Histological study, longitudinal colon sections from each rat were preserved in 10% formaldehyde and treated as paraffin blocks, and slices of the tissue about 5-6 um thickness were acquired for H and E staining to demonstrate the histological structure, and Mallory

trichrome staining to determine the amount of collagen present [20].

III- Scanning EM study [21], sections (1×1 cm) of the colon were cut, preserved in glutaraldehyde solution 2.5% (pH=7.4) for two h at room temperature, washed in PBS, placed in 1% osmium solution, dried, and coated with gold using sputter coated SCD/005. Tissues were mounted in a copper stud and examined using Philips Scanning EM at Tanta within the EM unit of Tanta University's Faculty of Medicine.

IV- Immunohistochemical study [19]**:**

1- **Immunohistochemical results for Ki-67** (proliferative marker) expression using anti-Ki-67 antibodies were nuclear. Hair follicles were used as positive controls for Ki-67 expression.

2- **Immunohistochemical results for activated caspase 3** (apoptotic marker) expression were mainly in the cytoplasm of immune-positive cells. The positive control for caspase-3 was obtained from human tonsillar tissue.

3- **(GFAP), glial fibrillary acidic protein immunohistochemical staining,** and GFAB-positive expression appeared as brown cytoplasmic staining. Brain tissue was used as a positive control for GFAP.

V- Morphometry and Statistics:

In the H and E-stained sections, the whole thickness of the mucosal layer (um) and goblet cell number were quantified, as well as the amount of collagen fibers in various groups using Mallory trichrome staining. Using a 40x (x 400) objective lens of light microscopy, the Quantification of KI 67-positive cells, caspase 3 staining strength, and GFAB intensity were assessed in immunostained sections. Ten distinct images were obtained for each animal. An image analyzer application (Lecia Win, 5 hundred images analyzing system, England) was used at the Menoufia Faculty of Medicine. The mean standard deviation of all data was calculated and then compared using Student's t-test, and analysis of data was performed using the program of statistical package for the Social Science (SPSS) software. Statistical significance was set at $P < 0.05$ [22].

Biochemical results:

Table 1 shows no significant value in MDA between the Ia, Ib, II, and III groups. Group IV exhibited a highly significant elevation ($p < 0.001$) in MDA levels compared with the control group (Histogram 1). A highly significant reduction ($p < 0.001$) in antioxidant activities, SOD, and CAT was observed in group IV. Groups V and VI exhibited a significant reduction in MDA levels and a significant elevation in CAT and SOD activities in the blood (histogram = 1).

Histological results:

In the H and E sections, the colon of the control groups showed normal histological structure. The colon wall was composed of mucosa filled with crypts and covered with surface epithelium, submucosa composed of loose connective tissue, and musculosa contained inner circular and outer longitudinal smooth muscles (Figure 1). The colon mucosa surface was intact. It was bordered by columnar cells with acidophilic cytoplasm and basal oval basophilic nuclei. The crypts appeared straight and tubular with tiny entrances (Figure 2). The crypts were lined by simple columnar epithelium with oval vesicular nuclei at the base. The goblet cells had vacuolated cytoplasm and flattened nuclei at the base, and the connective tissue of lamina propria in between the crypts was also seen (Figure 3). Between the two layers of the musculosa, Auerbach's plexus was found. Enteric glial cells (EGCs), nerve cells, and unmyelinated nerve fibers comprise the plexus (Figure 4). Groups (II and III) had similar structures, more or less like the control group.

Compared to the control group, the colitis group exhibited a highly significant

reduction in mucosal thickness, loss of normal architecture and exfoliated cells within the colon lumen. Columnar cells have vacuolated cytoplasm and pyknotic nuclei. Degenerated goblet cells with small pyknotic nuclei were observed. The connective tissue contained inflammatory cells (Figure 7). Distortion of the submucosa, muscle layer separation with laceration, and cellular infiltration were also observed (Figures 5 and 6). The Auerbach's plexus showed fewer nerve cells and EGCs (Figure 8).

In contrast, analysis of group V (colitis-administered probiotics) revealed an improvement in the histological appearance of the colon. The normal appearance of the mucosa, crypts, submucosa, and musculosa was comparable to that in the control group. However, cellular infiltration was still observed between the crypts. A nearly normal surface columnar epithelium and typical crypt morphology were observed (Figures 9 and 10). Auerbach's plexus in the musculosa showed obvious nerve cells and EGCs compared with the induced colitis group (group IV) (Figure 11).

Examining group VI (colitis treated with NAC) revealed relatively disrupted crypt architecture, tall columnar cells with basal oval nuclei, others with pyknotic nuclei, and vacuolation of the cytoplasm of some cells. Cellular infiltration of the connective tissue of the lamina propria was also observed (Figures 12 and 13). Auerbach's plexus in the musculosa showed nerve cells, unmyelinated nerve fibers, and EGCs (Figure 14).

In addition, Mallory Trichrome-stained colon sections from the control group revealed mild collagen fiber deposition in the lamina propria (Figure 15). Groups (II and III) were more or less like the control group. However, in Group (IV), fiber abundance was observed in the submucosa and corium (Figure 16). Group V was similar to the control group (Figure 17), whereas Group VI exhibited moderate collagen deposition (Figure 18).

Scanning EM results:

Scanning EM of the control group showed a normal intestinal crypt appearance and mucosal surface. There were many goblet cells with mucus on their surface (Fig. 20). A vast carpet of microvilli was observed on the surface of the columnar cells (Figure 19). Groups II and III showed electron microscopy results similar to those of the control group.

Induced colitis animals (group IV) exhibited widened crypt openings with shedding of the epithelium giving a honeycomb appearance, some crypts were closed by desquamation, an area of the ulcer was also seen (Figure 21), and collagen fiber appeared with the presence of extruded mucous and cracks (Figure 22 and 23). Group V (colitis-administered probiotics) had virtually normal mucosal crypts with intact surfaces and mucous extruded on the mucosal surface, similar to the control group (Figure 24). Group VI (colitis treated with NAC) displayed a widening of the crypt openings, an increase in goblet cells, and mucous extrusion on the mucosal surface (Figure 25).

Immunohistochemical analysis

Immunohistochemical results for Ki-67: The control groups had numerous Ki-67 immune-positive nuclei, the induced colitis group showed a relative decrease in Ki-67 immune-positive nuclei, the colitis received probiotics group showed numerous Ki-67 immune-positive nuclei more or less like the control, and the colitis received NAC group showed a moderate number of Ki-67 immune positive nuclei (figure 26).

Immunohistochemical results for activated caspase 3: The control groups showed low cytoplasmic intensity of immune reaction; the induced colitis group, exhibited strong cytoplasmic intensity; and the colitis received probiotics group, with low cytoplasmic intensity as the control group and colitis received NAC group showed moderate cytoplasmic intensity (figure 27).

GFAP immunostaining: Sections of the colon of control groups revealed myenteric plexus between muscle fibers, with strong GFAP-positive EGCs exhibiting strong positive immune intensity in their cytoplasm and processes, and GFAP-negative nerve cells. The induced colitis group showed weak immune intensity for GFAP, and groups IV and VI showed moderately positive GFAP immunoreactivity in EGC (Figure 28).

Morphometric results (Table 2 and Table 3) showed that group IV had a statistically highly significant ($P < 0.001$) reduction in mucosal thickness and the number of goblet cells compared to the control group (Table 1). Groups V and VI showed a statistically significant increase in mucosal thickness and goblet cell number compared to group IV (histograms 2 and 3).

Table 4 shows that Group IV had a highly significant increase in collagen fiber deposition ($P < 0.001$) compared to the control (Table 4). Groups V and VI showed a significant decrease in collagen fiber deposition compared to that in Group IV (histogram 4).

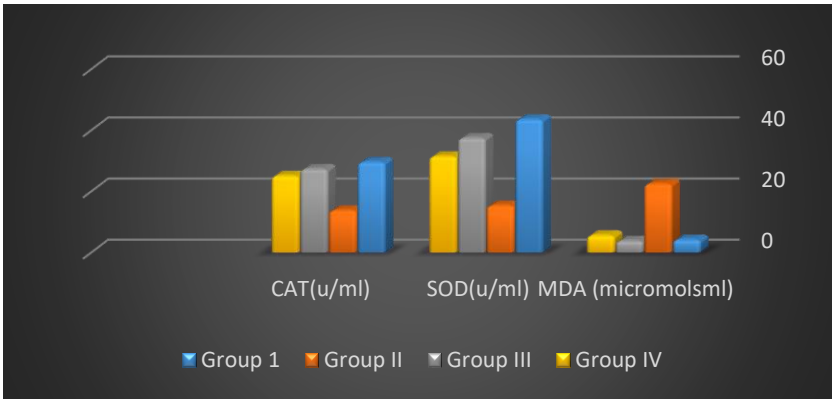
In addition, Table 5 shows that group IV had a highly significant ($P < 0.001$) reduction in the Ki-67 immune positive nuclei number to the control group. However, groups V and VI demonstrated a significant elevation compared to the induced colitis group (histogram 5).

Table 6 also shows that group IV demonstrated a highly significant ($P<0.001$) elevation in the caspase 3 immunoreaction intensity compared to the control group; however, groups V and VI demonstrated a significant ($P<0.001$) reduction in comparison to group II (Histogram 6).

As shown in Table 7, group IV revealed a highly significant ($P < 0.001$) decrease in GFAP immunoreaction intensity compared to the control group. Groups V and VI demonstrated a significant elevation ($P<0.001$) in GFAP immune reaction intensity compared to Group IV (Histogram 7).

Table (1): Effect of colitis on the antioxidant enzyme activities and the level of lipid peroxidation in the plasma of all groups of rats

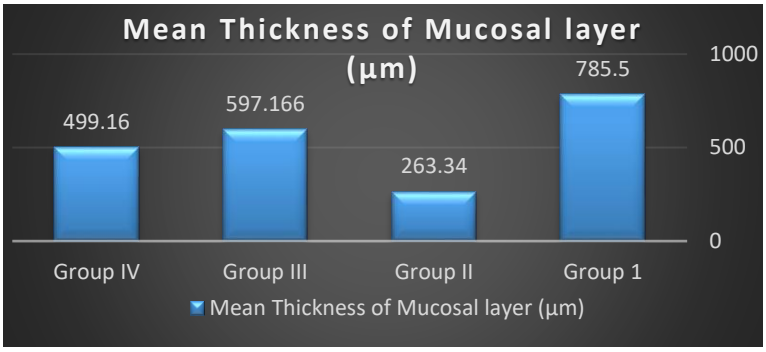
MDA (micromolsml)	4.25 ± 0.47	22.5 ± 4.23**	3.87 ± 0.6*	5.65 ±1.46*
SOD(u/ml)	43.61 ± 1.42	15.39 ± 1.29**	37.51 ± 1.82*	31.49 ± 0.93*
CAT(u/ml)	29.62 ± 1.04	13.82 ± 1.77**	27.38 ± 1.78*	25.14 ± 1.33*



Histogram (1): The Mean of MDA, SOD, and CAT levels in experimental different groups

Table (2): Mean ± SD Thickness of Mucosal layer (µm)in different groups.

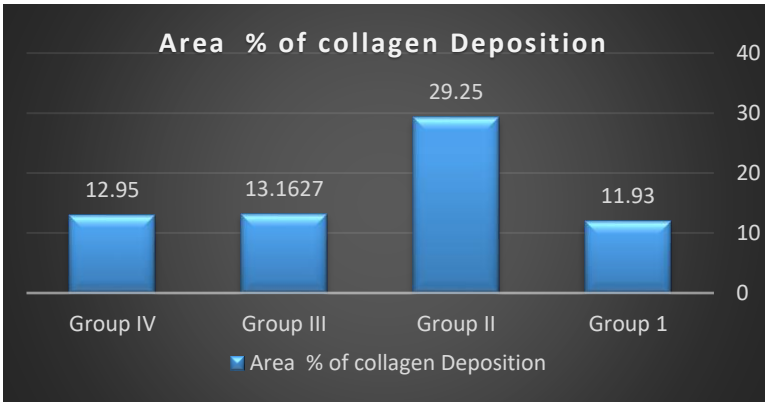
	Group 1	Group II	Group III	Group IV
The thickness of Mucosal layer (µm)	785.5±85 .13	263.34±57.30**	597.166±58 *.188	785.5±85 .13*



Histogram (2): Mean of Thickness of Mucosal layer (µm) in different groups.

Table (3): Number of goblet cells per field at a magnification of 400x $\bar{x}$±SD:

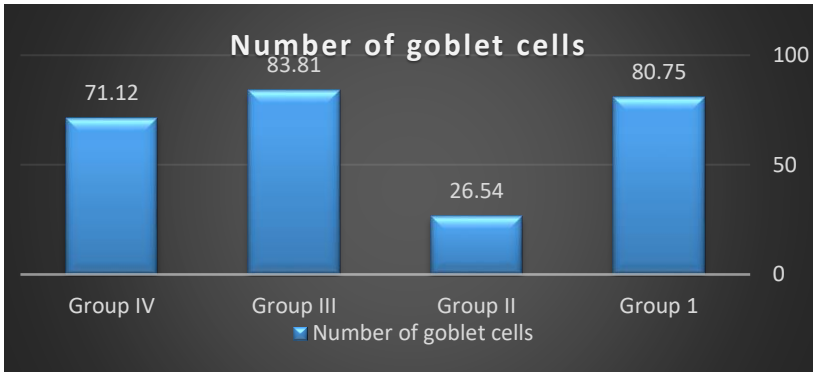
	Group 1	Group II	Group III	Group IV
Number of goblet cells	80.75±3.751	26.54±11.32**	83.81±2.15*	71.12±3.45*



Histogram (3): The mean number of goblet cells in different groups

Table (4): Mean ± SD Area % of collagen Deposition in different groups

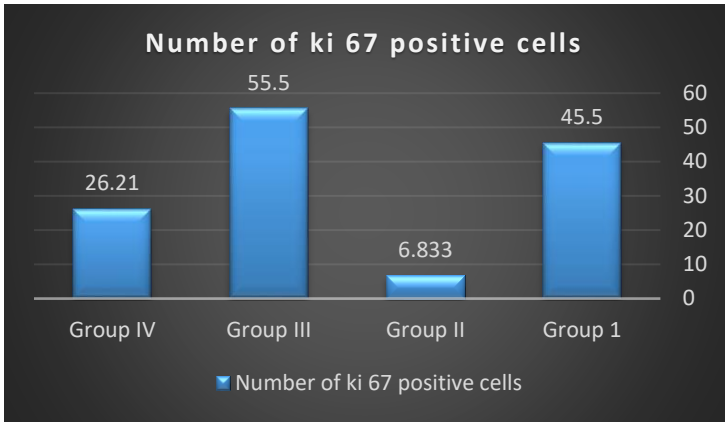
	Group 1	Group II	Group III	Group IV
Area % of collagen Deposition	11.93±1.48	29.25±11.64**	13.1627±1.44*	12.95±1.873*



Histogram (4): the mean area % of collagen Deposition in different groups

Table (5): Mean ± SD Number of ki 67 positive cells in different groups

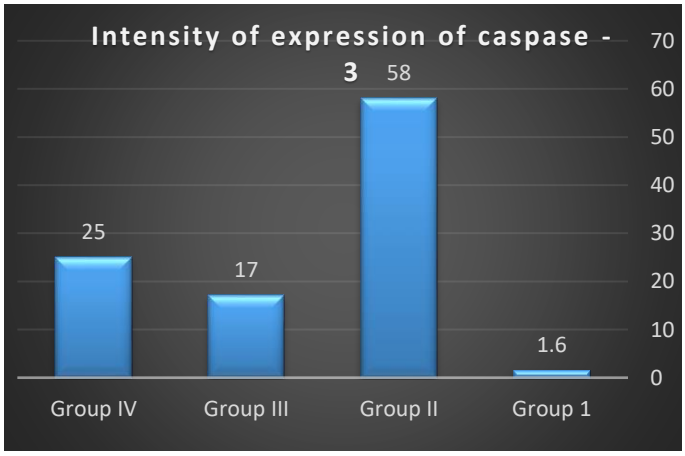
	Group 1	Group II	Group III	Group IV
Number of ki 67 positive cells	45.5±7.12	6.833±3.54**	55.5±7.19*	26.21±11.54*



Histogram (5): Mean Number of ki 67 positive cells in different groups

Table (6): Mean ± SD intensity of expression of caspase- 3 in different groups

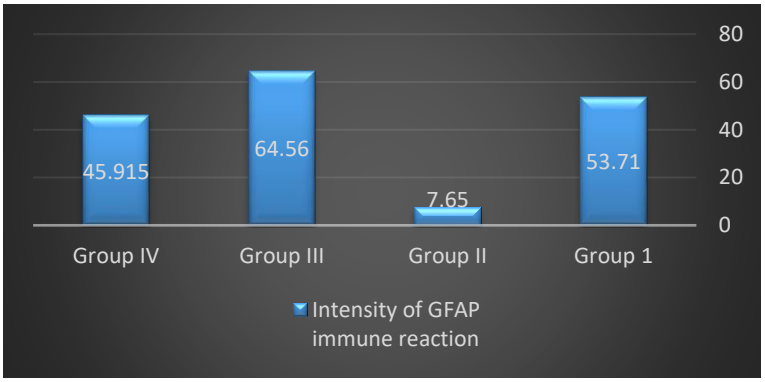
	Group 1	Group II	Group III	Group IV
Intensity of expression of caspase- 3	1.6±1.14	58±1.66**	17±1.58*	25.00±1.14*



Histogram (6): Mean of intensity of expression of caspase- 3 in different groups

Table (7): Mean ± SD of the intensity of GFAP immune reaction of various experimental group

	Group 1	Group II	Group III	Group IV
Intensity of GFAP immune reaction	53.71±5.14	7.65±1.86**	64.56±2.56*	45.915±3.14*



Histogram (7): Mean of intensity of GFAP immune reaction of various experimental group

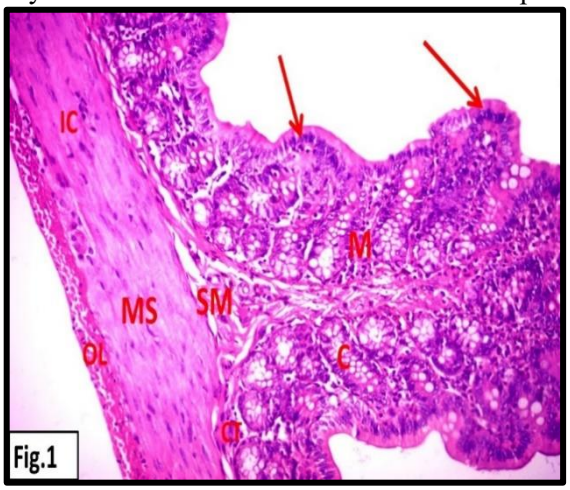


Fig. 1: Photomicrograph of the colon section of a control rat (group I) showing normal mucosa (M) formed of multiple crypts (C) and covered with surface epithelium (arrow), submucosa (SM) composed of loose connective tissue (CT), and musculosa (MS) formed of inner circular (IC) and outer longitudinal (OL) smooth muscle layers. (H & E x 100)

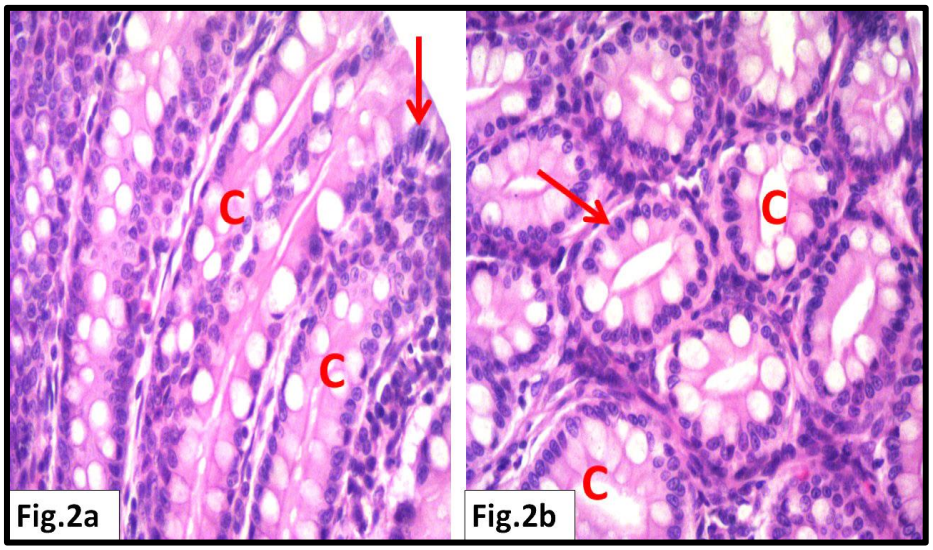


Fig. 2: (2a) Photomicrograph of a longitudinal section of the colon of the control group (group I) showing normal crypt architecture (C) with columnar cells has basal oval nuclei (arrow). (2b) Transverse section of the control colon showing normal crypt architecture (C) with a columnar cell with basal oval nuclei (arrow). (H & E x 200)

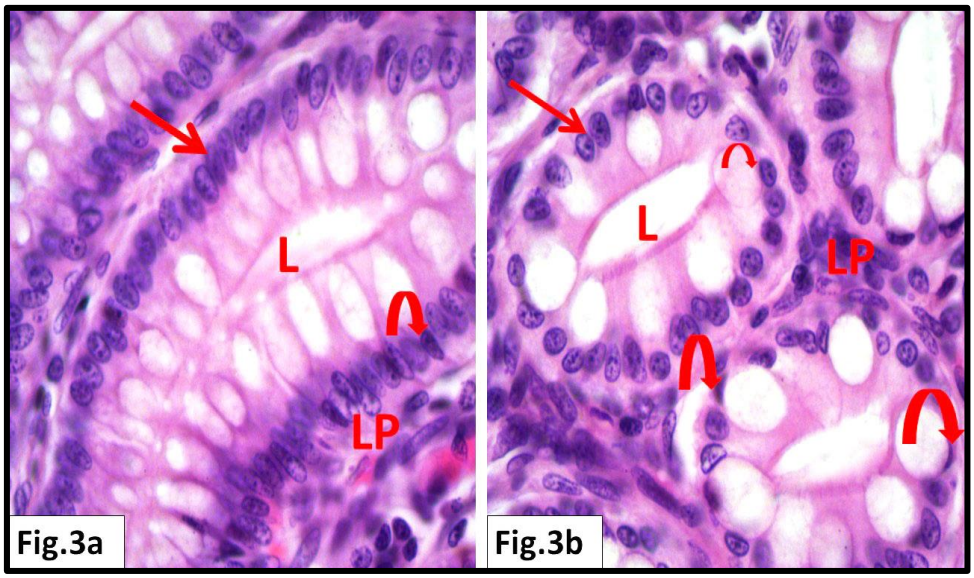


Fig. 3: (3a) Photomicrograph of a longitudinal section of the colon of the control group (group I) showing simple columnar epithelium with basal oval vesicular nuclei and prominent nucleoli (arrows) surrounding the tubular lumen (L). Goblet cells are flask-shaped with vacuolated cytoplasm and basally flattened nuclei (curved arrow). Notice, the connective tissue of lamina propria in between crypts (LP). (3b): A transverse section of the control colon showing the same structure as above (H & E x 400)

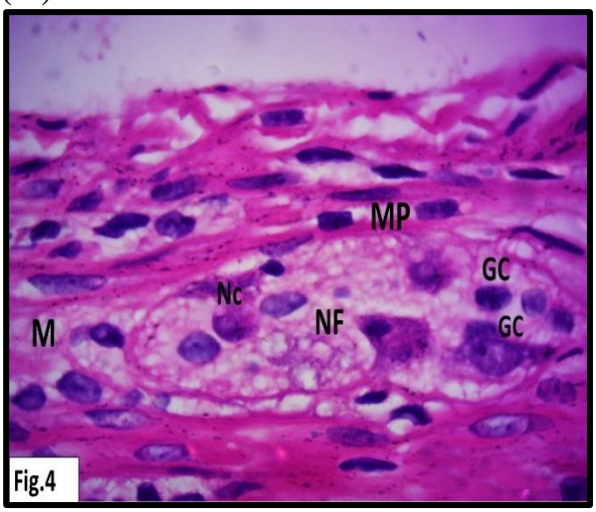


Fig. 4: Photomicrograph of a section of the colon of control group (group I) showing a well-defined myenteric plexus (MP) present between the two layers of the musculosa (M). The plexus consists of numerous nerve cells (NC), enteric glial cells (GC), and a network of unmyelinated nerve fibers (NF). (H & E x 400)

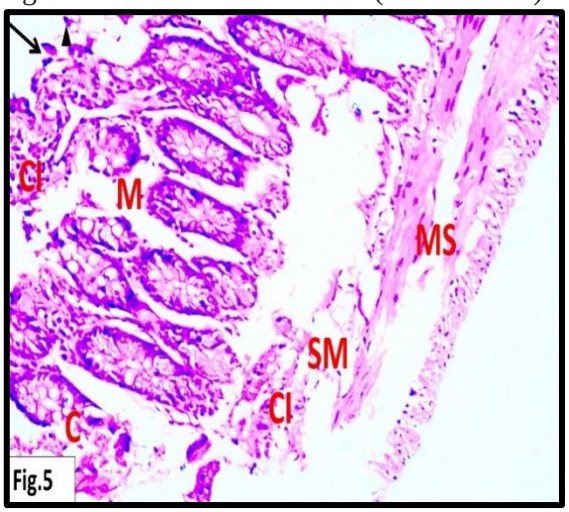


Fig. 5: Photomicrograph of a section of the colon of the colitis-induced group (group IV) showing loss of mucosal architecture (M), loss of surface epithelium (arrow), exfoliated cells in the lumen (arrowhead), and distortion of the submucosa (SM). Note the separation of muscle layers with their laceration (MS) and cellular infiltrations (CI)in the lamina propria and submucosa. (H & E x 100)

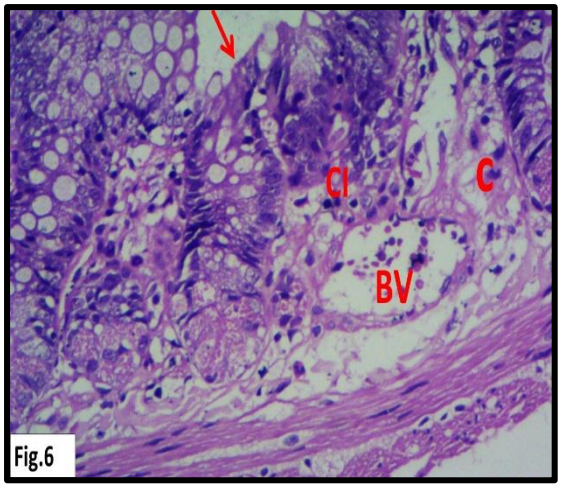


Fig. 6: Photomicrograph of a section of the colon of induced colitis rat (group IV) showing disturbed crypt architecture (C) as loss of surface epithelium (arrow). Severe cellular infiltration (CI), and large blood vessel congestion (BV) in the corium. (H & E x 200)

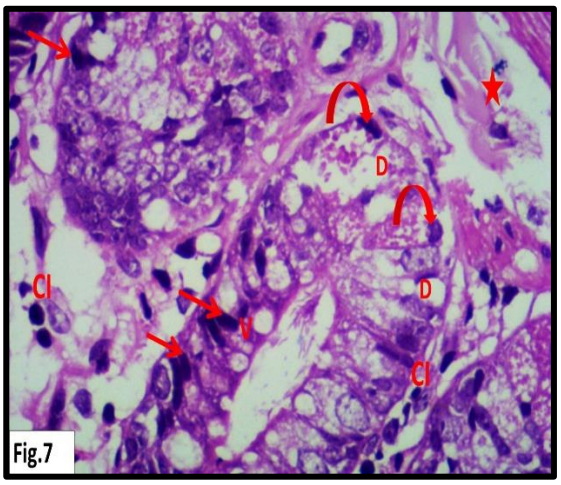


Fig. 7: Photomicrograph of a section of the colon of induced colitis group (group IV) showing disturbed crypts, lined by columnar cells with a darkly stained pyknotic nucleus (arrow) and vacuolation of the cytoplasm (V). The goblet cells are degenerated (D) and others with small darkly stained pyknotic nuclei (curved arrow); note that the connective tissue was filled with cellular infiltration (CI), and degenerated muscle was also observed (star). (H & E x 400)

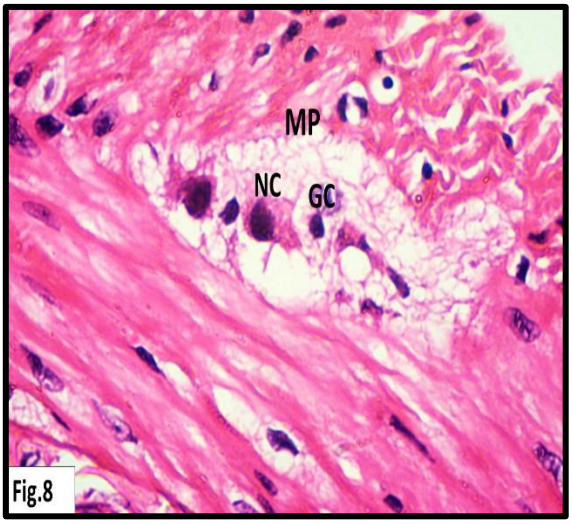


Fig. 8: Photomicrograph of a colon section from an induced colitis rat (group IV) showing a myenteric plexus (MP) with few nerve cells (NC) and few enteric glial cells (GC). (H& E x 400)

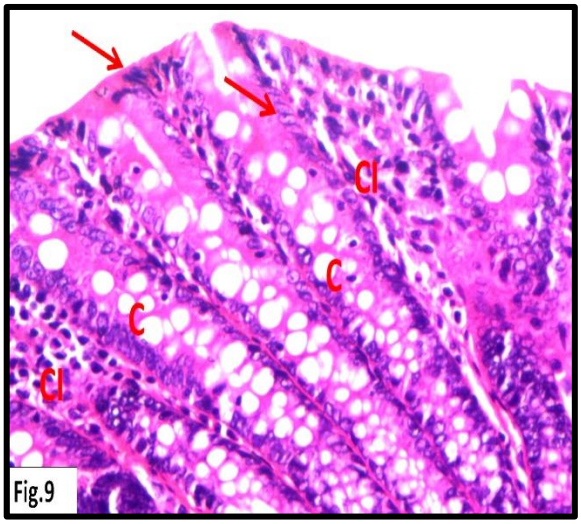


Fig. 9: A photomicrograph of a longitudinal section of the colon of group V (colitis-received probiotics) showing nearly normal crypt architecture (C) formed of surface epithelium with basal oval nuclei (arrow). Cellular infiltration (CI) is observed between connective tissues. (H & E x 200)

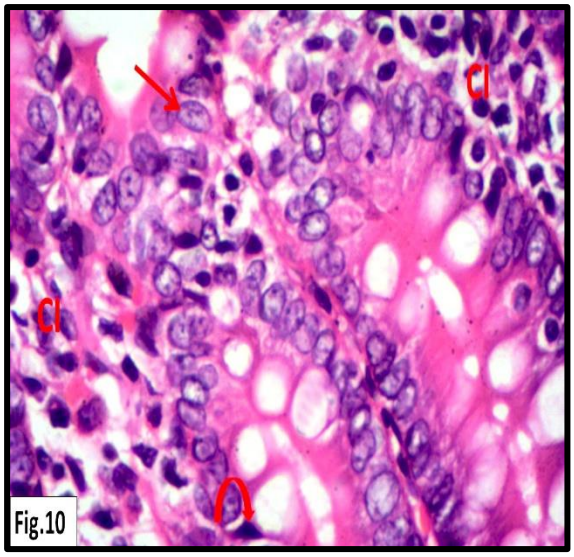


Fig. 10: Photomicrograph of a longitudinal section of the colon of rat group V (colitis-received probiotics) showing a simple columnar epithelium with basal oval vesicular nuclei (arrows). Goblet cells are flask-shaped with vacuolated cytoplasm and basally flattened nuclei (curved arrow). Notice, severe cellular infiltrations (CI) in the lamina propria. (H & E x 400)

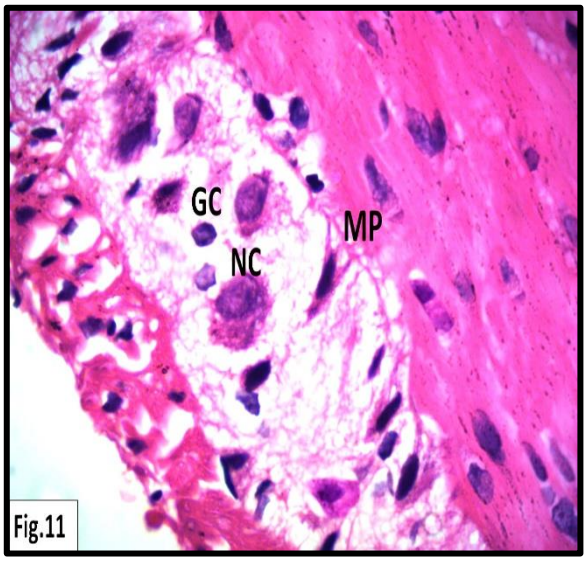


Fig. 11: A photomicrograph of a section of the colon from a group V rat (colitis received probiotics) showing a relatively normal-shaped myenteric plexus (MP) in the musculosa, with an apparent increase in the number of nerve cells (NC) and enteric glial cells (GC). (H & E x 400)

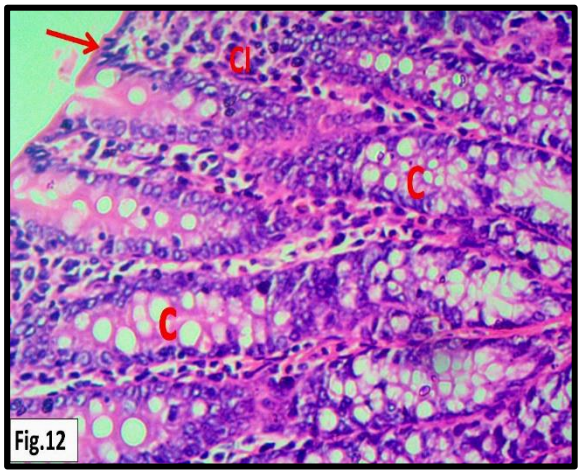


Fig. 12: A photomicrograph of the colon of group VI (colitis received NAC) showing slightly disturbed crypt architecture (C) formed of surface epithelium with basal oval nuclei (arrow). Cellular infiltration (CI) is observed between connective tissues. (H & E x 200)

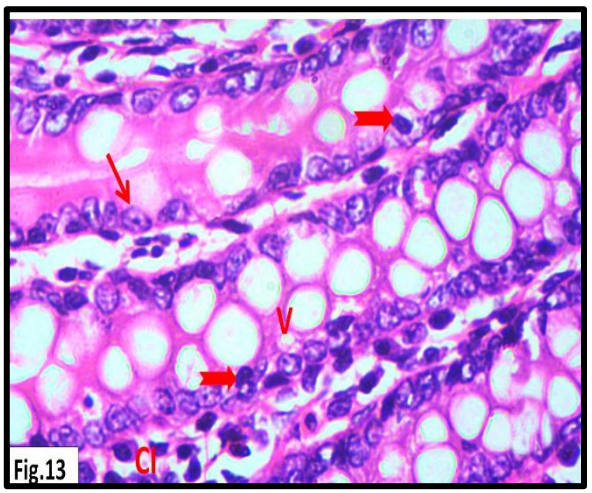


Fig. 13: A photomicrograph of the colon with a longitudinal section of group VI (colitis received NAC) showing simple columnar epithelium with basal oval vesicular nuclei (arrows) but others with pyknotic nuclei (notched arrow). Notice, still cellular infiltrations (CI) in the lamina propria and vacuolation of the cytoplasm of some cells are also seen (V). (H & E x 200)

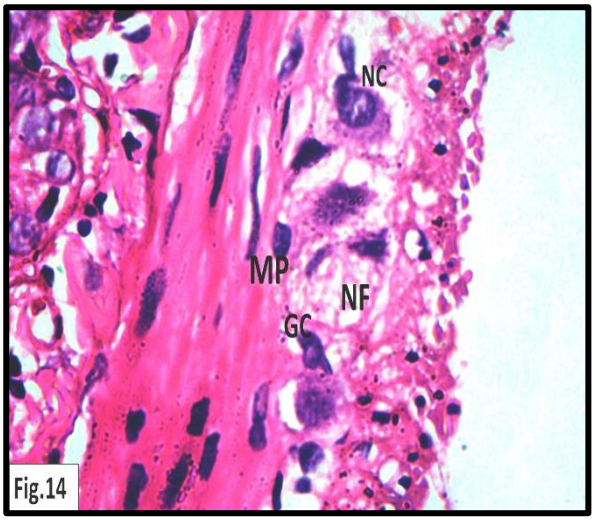


Fig. 14: A photomicrograph of a section of a colon from a rat of group VI (colitis received NAC) showing slightly disturbed myenteric plexus (MP) in the musculosa, with an increase in the number of nerve cells (NC), disturbed enteric glial cells (GC) and unmyelinated nerve fibers (NF). (H & E x 400)

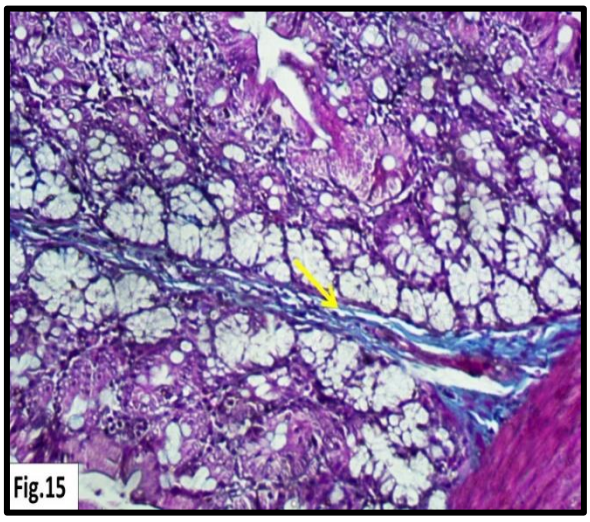


Fig 15: A photomicrograph of Mallory Trichrome-stained sections of the colon of group I exhibiting mild collagen fibers deposition (as tinge blue colored strips), in the lamina propria (arrow). (MT x 200)

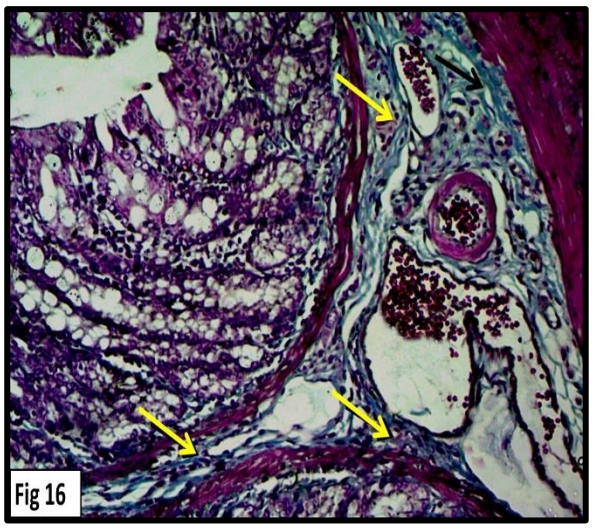


Fig. 16: Photomicrograph of Mallory Trichrome-stained sections of group IV section of the colon showing severe collagen fibers deposition in lamina propria (arrow), and submucosa. (MT x 200)

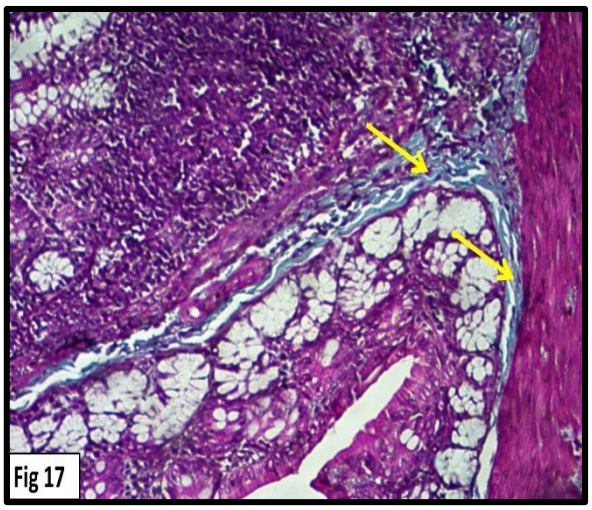


Fig. 17: A photomicrograph of Mallory Trichrome-stained sections of the colon of group V showing mild collagen fibers deposition in the lamina propria and submucosa, (arrow) more or less like the control group. (MT x 200)

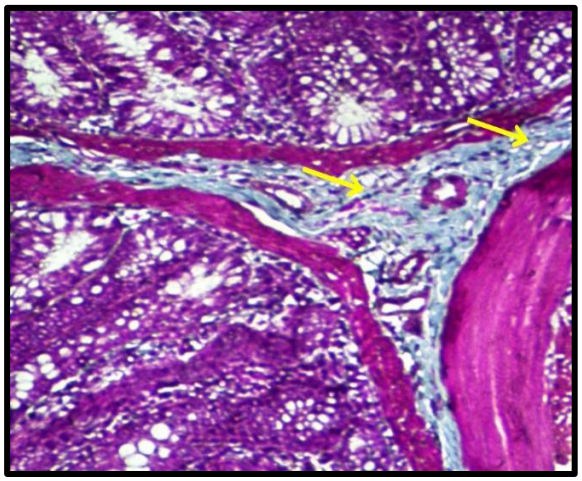


Fig. 18: A photomicrograph of Mallory Trichrome-stained sections of the colon of group VI showing moderate collagen fibers in the submucosa, and lamina propria (arrow). (MT x 200)

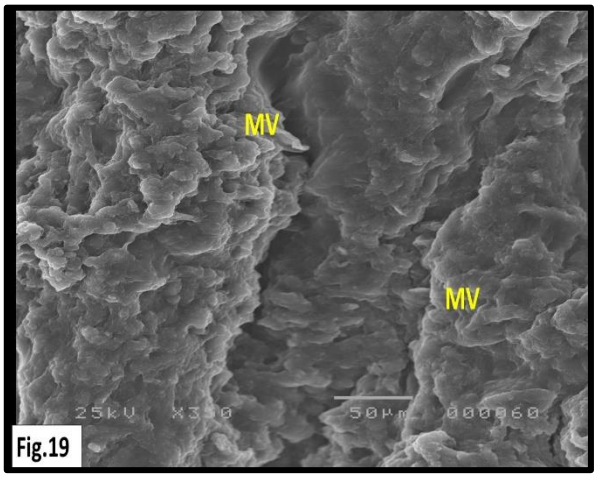


Fig. 19: A scanning electron micrograph, of the control rat (group I) showing the vast carpet of microvilli (MV) covering the surface columnar cells. (SEM x 3500)

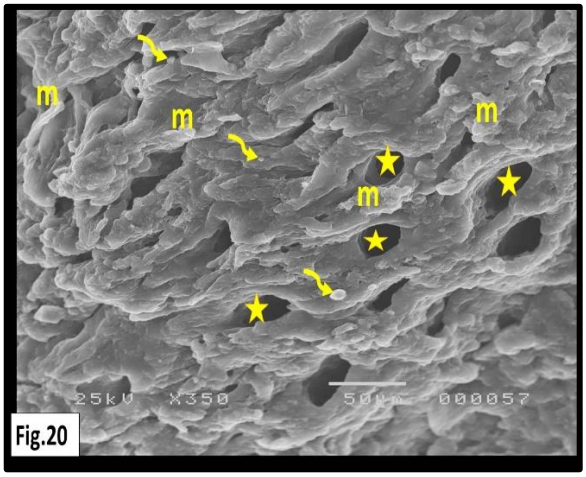


Fig. 20: A scanning electron- micrograph, of the control rat (group I) showing normally shaped mucosal crypts opening (star) with the intact mucosal surface, and numerous goblet cells (curved arrows) Notice: the extruded mucous (M) on the surface of the mucosa. (SEM x 3500)

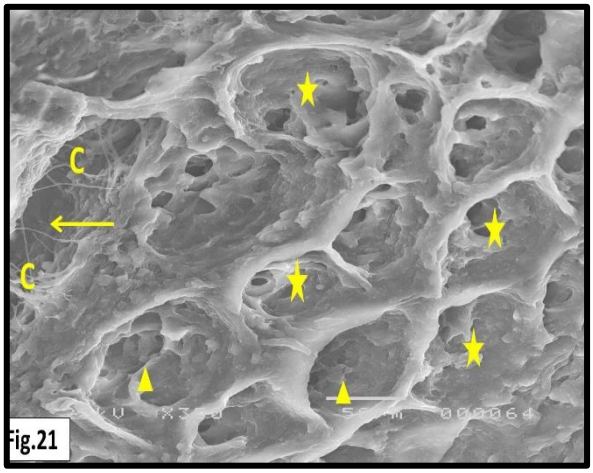


Fig. 21: A scanning electron – micrograph, of the colon of group IV, showing the widening of the openings of the crypts (stars) with the shedding of the epithelium giving a honeycomb appearance, some crypts are closed by desquamation (arrowhead). Note, the presence of an area of ulcer (arrow) and collagen fibers appearance (C). (SEM x 3500)

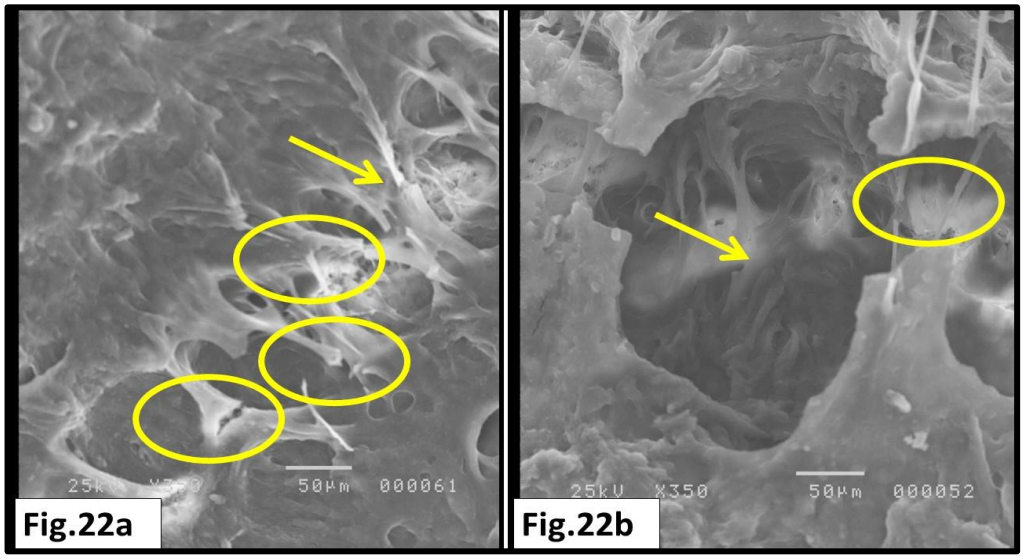


Fig. 22: (22a) A scanning electron - micrograph, of the colon of group IV, showing the area of the ulcer (arrow) and multiple cracks also seen (ellipse). (22b) A scanning electron - micrograph, of the colon of group II, showing the same as 22b. (SEM x 3500)

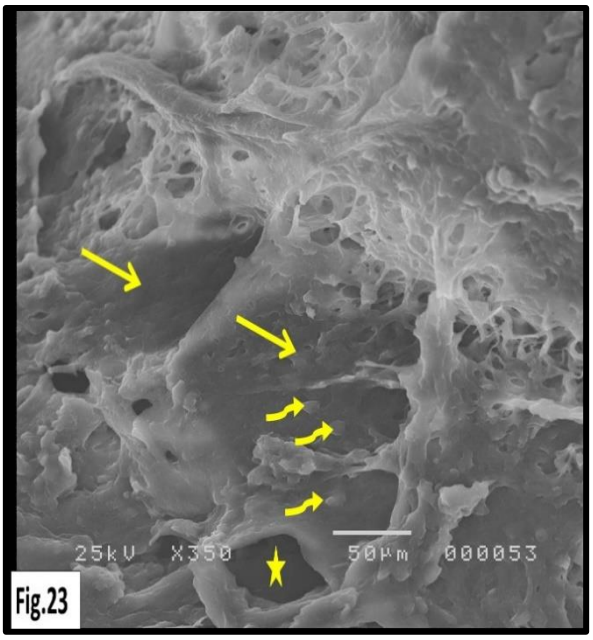


Fig. 23: A scanning electron- micrograph, of the colon of group IV showing multiple areas of ulcers (arrow) widening of the crypts (star), and desquamation of the cells (curved arrow).
(SEM x 3500)

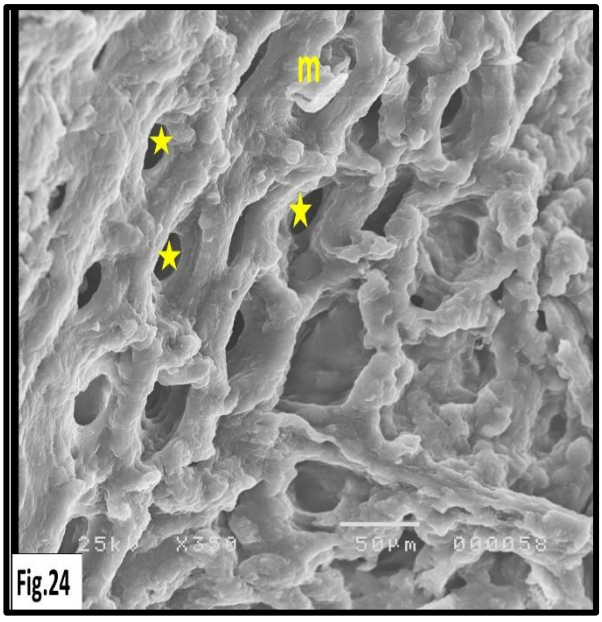


Fig. 24: A scanning electron - micrograph, of the colon of group V (colitis received probiotics) showing nearly normal-shaped mucosal crypts opening (star) with intact mucosal surface and extruded mucous (M) on the surface of the mucosa.
(SEM x 3500)

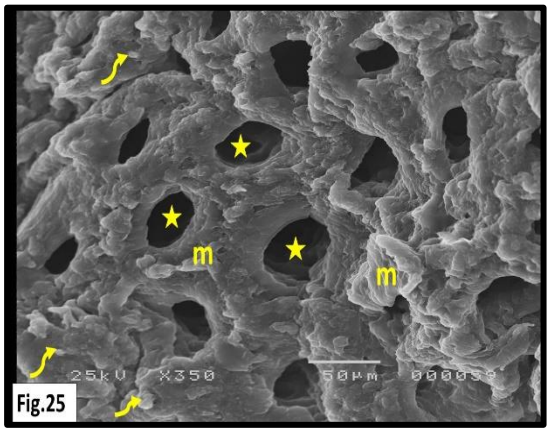


Fig. 25: A scanning electron – micrograph, of the colon of group VI (colitis received NAC), showing the widening of the openings of the crypts (stars), numerous goblet cells (curved arrow), and extruded mucous (M) on the surface of the mucosa. (SEM x 3500)

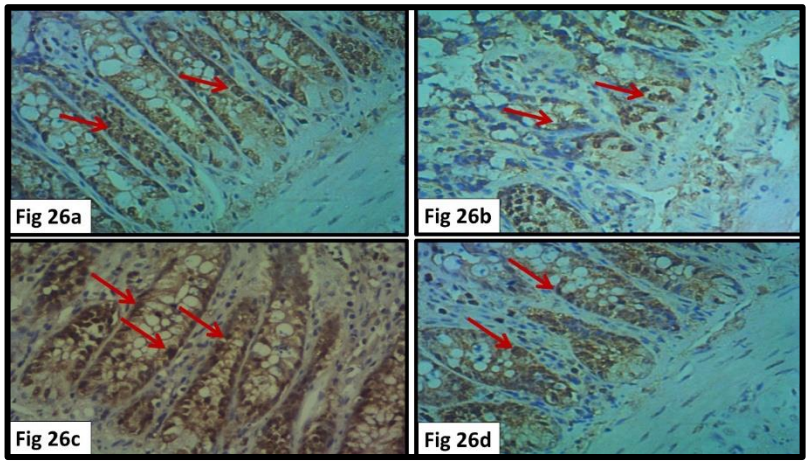


Fig. 26: Ki 67 immunohistochemical staining of the colon showing: (a) Control group (group I) showing numerous Ki-67 immune positive nuclei (arrows). (b) Group IV (induced colitis) shows a relative decrease in Ki-67 immune-positive nuclei (arrows). (C) Group V (colitis-received probiotics) showed numerous Ki-67 immune-positive nuclei (arrows). (d) Group VI (colitis received NAC) showed a moderate number of Ki-67 immune-positive nuclei (arrows). (KI 67 x 200 immunostaining)

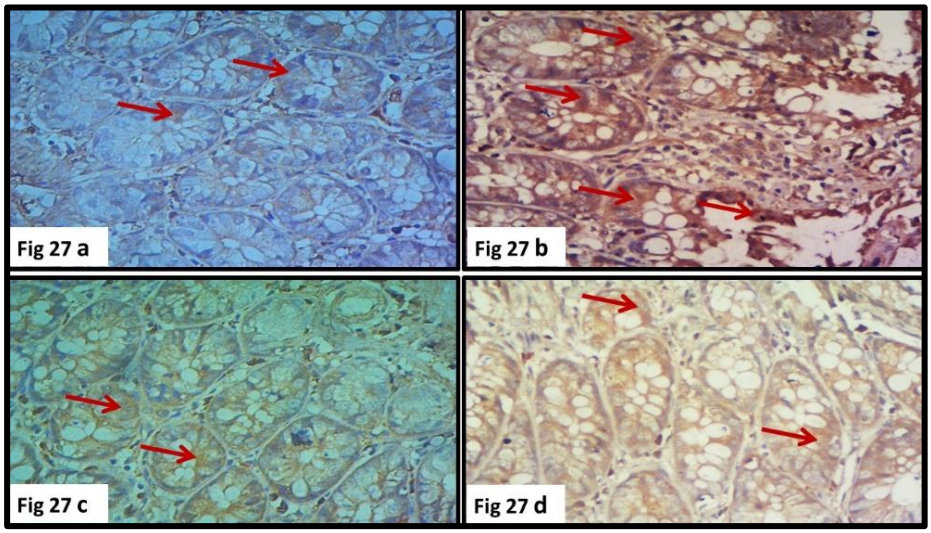


Fig. 27: Caspase 3 immunohistochemical staining, of the colon showing: **(a)** Control group with low caspase – 3 immunoreaction intensity (arrows). (b) Group IV (colitis induced) showed high immunoreaction intensity (arrows) **(c)** Group V (colitis received probiotics) showed low immunoreaction intensity (arrows) **(d)** Group VI (colitis received NAC) showed moderate immunoreaction intensity (arrows). (Caspase 3 x 400 immunostaining)

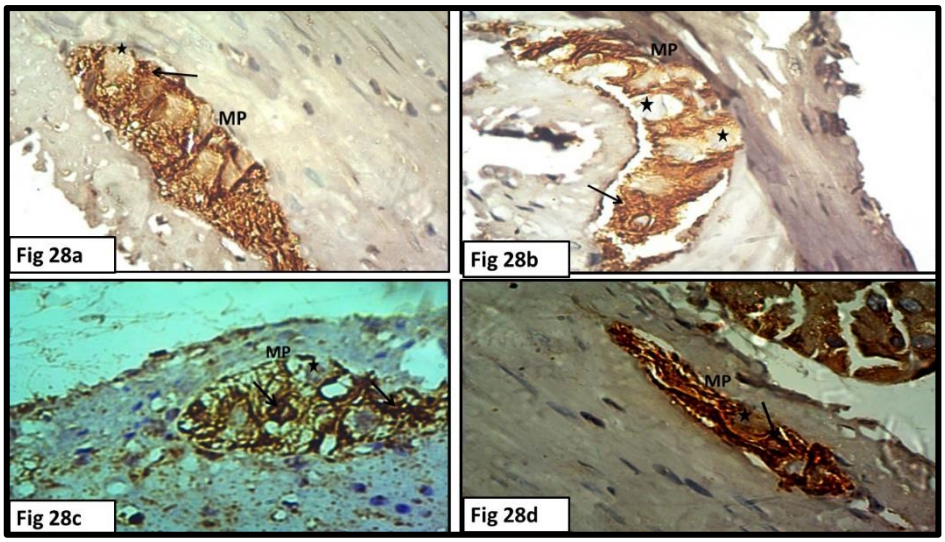


Fig. 28: - a- photomicrograph of a section of the colon of a control rat (group I) showing myenteric plexus (MP) in between muscle fibers, containing numerous GFAP positive enteric glial cells exhibited as strong positive immunoreactions in their cytoplasm and their processes (arrows) and GFAP negative nerve cells (star). b- indued colitis (group IV) showing disturbed myenteric plexus(MP) with few GFAP positive enteric glial cells which have weak positive immunoreactions(arrow) surrounding immune-negative nerve cells (star) C- induced colitis treated with probiotics (group V) showing myenteric plexus(MP) with moderate positive GFAP immune- reaction in enteric glial cells (arrow)surrounding immune-negative nerve cells (star) d- induced colitis treated with acetylcysteine (group VI) showing myenteric plexus(MP) with moderate positive GFAP immune- reaction in enteric glial cells (arrow)surrounding immune-negative nerve cells (star). (GFAP x 400)

DISCUSSION:

UC is a chronic, relapsing IBD. It is caused by interactions between genetic, environmental, nutritional, oxidative stress, immunological, proinflammatory, and gut microbiome variables [23]. Acetic acid injection-induced colitis is a widely studied animal model of UC because it induces pathological and clinical changes similar to those of UC in humans [24].

The induced colitis group exhibited a highly significant elevation in MDA, which indicates an elevation of oxidative stress and lipid peroxidation, as explained by [25]. Colitis significantly decreases the levels of SOD and CAT, which have an essential protective response against oxidative stress [26].

The induced colitis group exhibited a significant decrease in mucosal thickness compared to group I, disturbed mucosal architecture, loss of surface epithelium, exfoliated cells into the lumen and columnar epithelium with small pyknotic nuclei and vacuolated cytoplasm. Degenerated goblet cells with small pyknotic nuclei are also seen. Distortion of the submucosa and cellular infiltrations. These results are consistent with those reported by [1], who used an animal model of UC.

Desquamation and loss of epithelial cells may be caused by the production of ROS in the intestinal mucosa, which impairs blood flow [27]. Columnar cells with pyknotic nuclei and vacuolated cytoplasm may be the result of an imbalance between loss by apoptosis (cell death) and the production of new cells through mitosis (proliferation)[28]. During inflammation, adhesion molecules related to intercellular and mucosal endothelial cells are released and are responsible for leukocyte recruitment in colitis [29]. The statistically proven decline in goblet cell number was caused by an increase in interleukin 18 signaling in the intestinal mucosa, resulting in the gradual depletion of these cells. Interleukin 18 also suppresses goblet cell maturation by modulating the transcriptional

pathway responsible for the formation of goblet cells [30], with decreased mucous secretion impairing the intestinal barrier, which protects the intestinal epithelium against microorganisms, intestinal flora, and any chemicals or noxious agents attacking it. One of the most aggressive pathogenic processes in UC is mucosal ulceration [31].

Compared to the control group, Auerbach's plexus had fewer nerve cells and fewer EGCs. These results are in agreement with a study by[32and33], who postulated that induction of UC leads to alterations in intestinal contraction and reduced neuronal density due to necrosis, apoptosis, and cell death; abnormalities in neurotransmission were also observed. This group's disrupted myenteric plexuses and nerve cells resulted in the constricted colonic wall in certain sections, and enterocolitis, fecal impaction, ulceration, necrosis, and disruption and separation of the colon's wall [34] as seen in this study.

Collagen fibers were abundant in the corium and submucosal connective tissues in the induced colitis group. This is in line with the findings of [1], who demonstrated elevated fiber deposition within the corium and submucosa in the UC model. This is due to elevated collagen protein deposition and pro-collagen mRNA expression observed in experimental colitis [35736] explained that this recurrent inflammation in IBDs resulted in significant tissue fibrosis and colonic stiffness, impeding fluid absorption and peristalsis.

Regarding scanning EM results, induced colitis animals exhibited widened crypt openings with shedding of the epithelium giving a honeycomb appearance, some crypts were closed by desquamation, the presence of an area of ulcer, and collagen fiber appearance, these results were with [35 and 37]. This can be explained by the effects of free radicals and ROS produced during UC [37]. The inflammatory process, upregulation of proinflammatory mediators, and down-regulation of anti-inflammatory mediators cause attenuation of the intestinal epithelium and consequent injury & and damage [38&39].

Immunohistochemical results for Ki-67 showed that group IV (induced colitis) showed a relative decrease in Ki-67 immune positive nuclei this goes with [40] who found that Anti-KI-67 antibody reveals a reduction in epithelial proliferative activity in the UC model.

Immunohistochemical staining for caspase 3 in group IV (induced colitis) showed extensive caspase-3 expression, which was with [41], who found increased expression of caspase 3 in UC induced by acetic acid. This could be explained by the increase in intestinal epithelial cell apoptosis, which occurs in UC and plays an important role in disease development [42].

Immunostaining for GFAP revealed mild GFAP immunoreactions. This may be due to the negative effects of IBDs on the cellular constituents of ENS [43]. Both components of the myenteric plexus, ganglion cells, and EGCs show degeneration in IBD due to inflammation, immune modulation, and oxidative stress [44].

Biochemical analysis revealed that the administration of probiotics for induced colitis resulted in a significant reduction in MDA levels and a significant increase in oxidative markers compared to those in group IV. [9] reported that Lactobacillus plantarum 21 improved oxidative stress in rats with induced UC. Probiotics do not bind to steroids; therefore, their antioxidant properties may be used as an adjunct to conventional treatments [45].

Treatment with probiotics improved the histological picture of the colon by light and EM results compared to the induced colitis group, and a mild amount of collagen fibers. Immunostaining showed numerous Ki-67 immune-positive nuclei, mild caspase 3 expression, and moderate GFAB expression. This can be explained by the ability of

probiotics to enhance mucosal barrier function and decrease the inflammatory response in the gastrointestinal tract by decreasing inflammatory markers (tumor necrosis factor (TNF) alpha, interferon (INF) and interleukin (IL8)) and increasing anti-inflammatory cytokines (IL10 and tumor growth factor (TGF) beta) **[46].**

Probiotics have been scientifically shown to alleviate UC by stimulating primary immunity and changing the inflammation-induced pathogenic process [47] by increasing short-chain fatty acid (SCFA) production, especially butyric acid, and by increasing mucin-3 expression and enhancing the intestinal mucus layer**[48]**, which results in the activation of natural killer cells, lymphocytes, and stimulation of both interferon production and immature leukocytes**[49].** Another study done by**[50]** showed that probiotics have several antioxidant effects as they increased levels of antioxidant enzymes and intestinal disaccharidases, and reduced oxidants.

Numerous studies have shown that probiotics diminish the negative feedback in the intestine by reducing anti-inflammatory cytokines and neutrophil infiltration mechanisms **[51].**

In addition to their anti-inflammatory effects, probiotics can reduce colitis-induced systemic inflammation. Numerous studies have shown that increased gut permeability and vascularity facilitate the transfer of bacterial toxins and luminal microbiota throughout the systemic circulation, resulting in general inflammation **[52]**. Probiotics are useful in the prevention and treatment of UC by modulating gut flora. The favorable effect of probiotics on colitis shown in this study may have been related to the alteration in the structure of the gut microbiota, which was not studied in this investigation. Therefore, more studies are required to evaluate the feedback of natural and sensitized probiotics on the GIT microbiota, flora, and metabolites in animal models and humans. Previous research has shown that the use of probiotics reduces the formation of ROS, such as H_2O_2, thus lowering intestinal damage and inflammation and predicting tumor invasion and proliferation **[53]**. This could be explained by the anti-apoptotic effects of probiotics on both the small and large intestines by increasing SCFA production **[54].**

In this study, group VI (colitis received NAC) showed improvement in the biochemical, light, and EM picture except for a slight disturbance of crypt architecture, some pyknotic columnar epithelium nuclei, and vacuolation of the cytoplasm. Cellular infiltrations in the connective tissue corium. Auerbach's plexus in the musculosa showed a slight arrangement in the nerve cell, the unmyelinated nerve fibers, and EGCs. This advancement is consistent with that of.**[1],** who reported that NAC improves the histopathological features of acetic acid-induced colitis by acting as an anti-inflammatory agent, epithelial regeneration stimulant, and replenishing goblet cells with mucous secretion **[12]** reported that NAC administration led to a significant reduction in intestinal toxicity. NAC activates GSH, leading to the removal of ROS **[52]**. Additionally, NAC can improve the intestinal barrier **[55]**. NAC exerts an anti-inflammatory effect by modulating proinflammatory cytokine production and inhibiting its release **[56]**.

The antioxidant properties of N-acetylcysteine are related to its proliferative nature and modulation of the intracellular redox milieu in damaged cells **[57].** Recently, Wu et al**.** **[58]** revealed that, by scavenging ROS and free radicals, the free thiol group of NAC is responsible for its antioxidant action, since it reacts with RONS **[59].** Moreover, the antioxidative capacity of NAC may be described by its capability to provide cysteine for enhanced glutathione production **[60].** Antioxidants such as glutathione play a pivotal role in cellular health. Glutathione protects the nucleus from ROS by preventing

the breakdown and degeneration of RNA and DNA integrity [61].

Conclusion:

Probiotics showed more potent biochemical, histological, and morphometric effects than NAC, as probiotics improve surface columnar cells, goblet cells, and myenteric plexus by minimizing inflammation and oxidative stress markers and free radicals associated with UC, which is mediated by boosting antioxidants, modulating inflammatory mediators, and increasing antioxidant/oxidant abnormalities. Probiotics could serve as an alternative therapy for UC, as they disable caspase3 and enhance IL-1 signaling pathways, resulting in anti-inflammatory and anti-apoptotic effects, and healthy proliferation. This work is an experimental study, and the results belong to albino rats; therefore, further studies are needed to be clinically applied to humans.

Competing interests:

We have no competing interests to declare.

REFERENCES:

1. **Abd El-Galil, T. I., ElGhamrawy, T. A., & El Sadik, A. O:** The Effect of N. Acetylcysteine and Ginger on Acetic Acid-Induced Colitis in Adult Male Albino Rats: Histological, Immunohistochemical, and Morphometric Study. Journal of Cytology & Histology; 2015, 3(2), 1.
2. **Tatiya-Aphiradee, N.; Chatuphonprasert, W.; Jarukamjorn, K:** Immune response and inflammatory pathway of ulcerative colitis.J. Basic Clin. Physiol. Pharmacol; 2018., 30, 1–10.
3. **Lechuga, S.; Ivanov, A.I:** Disruption of the epithelial barrier during intestinal inflammation: Quest for new molecules and mechanisms. Biochim. Biophys. Acta, 2017, 1864, 1183–1194.
4. **Santiago P, Braga-Neto MB, Loftus EV:** Novel Therapies for Patients with Inflammatory Bowel Disease. Gastroenterol Hepatol (N Y), 2022.
5. **Lee SH**: Intestinal permeability regulation by tight junction: implication on inflammatory bowel diseases. Intestinal research. (2015) 13(1):11-18.
6. **Kangwan, N.; Kongkarnka, S.; Boonkerd, N.; Unban, K.; Shetty,K.; Khanongnuch, C**: Protective Effect of Probiotics Isolated from Traditional Fermented Tea Leaves (Miang) from Northern Thailand and Role of Synbiotics in AmelioratinExperimental Ulcerative Colitis in Mice. Nutrients, 2022, 14, 227.
7. **Varankovich NV, Nickerson MT, and Korber**: Probiotic-based strategies for therapeutic and prophylactic use against multiple gastrointestinal diseases. Frontiers in microbiology. (2015),6:685-692.
8. **Markowiak P and Śliżewska K**: Effects of probiotics, prebiotics, and synbiotics on human health. Nutrients. (2017),9 (9):1021-27. 15.
9. **Ivanovska TP, Mladenovska K, Zhivikj Z, Pavlova MJ, Gjurovski I, Ristoski T and Petrushevska, Tozi L**: Synbiotic loaded chitosan-Ca1alginate microparticles reduces inflammation in the TNBS model of rat colitis. International journal of pharmaceutics. (2017), 527 (1-2):126-134
10. **Li, C., Nie, S.P., Zhu, K.X., Xiong, T., Li, C., Gong, J., and Xie, M.Y:** Effect of Lactobacillus plantarum NCU116 on loperamide-induced constipation in mice. Int J Food Sci Nutr 66, 2015.
11. **Waring WS:** Novel acetylcysteine regimens for treatment of paracetamol overdose. Ther Adv Drug Saf**.** Dec**,** 2012,3(6):305-15.
12. **Zhitkovich A**: N-Acetylcysteine: Antioxidant, Aldehyde Scavenger, and MoreChem Res Toxicol. 2019, (Jul 15).
13. **Khayal EE, Amin DM, and Shabana MA**: The Possible Protective Role of N-Acetyl Cysteine against Titanium Dioxide Nanoparticles Intestinal Toxicity in Adult Male Albino Rats. Ain Shams Journal of Forensic Medicine and Clinical Toxicology, 2019 July, 33: 59-69.

14. **Hussein, Menna Nabil, et al. "**Assessment of the Prophylactic Effect of Probiotics in an Experimental Model of Ulcerative Colitis in Male Albino Rat: Histological and Immunohistochemical Study." Egyptian Journal of Histology 46.1 (2023): 33-48.
15. **Ismael MA, Talbot S, Carbonneau CL, Beauséjour CM, Couture R:** Blockade of sensory abnormalities and kinin B (1) receptor expression by N-acetyl-L- cysteine and ramipril in a rat model of insulin resistance. Eur J Pharmacol , 2010 , 589: 66-72.
16. **Aslan A, Temiz M, Atik E, Polat G, Sahinler N, et al.:** Effectiveness of mesalamine and propolis in experimental colitis. Adv Ther, 2016, **24**: 1085-1097.
17. **Tanideh N, Masoumi S, Hosseinzadeh M, Safarpour AR, Erjaee H, and Koohi-Hosseinabadi O**: Healing effect of pistacia atlantica fruit oil extract in acetic Acid-induced colitis in rats. (2014) Iran J Med Sci. 39(6):522-528.
18. **Nakatsu N, Igarashi Y, Aoshi T, Hamaguchi I, Saito M, Mizukami T, Momose H, Ishii KJ, and Yamada H.** Isoflurane is a suitable alternative to the ether for anesthetizing rats before euthanasia for gene expression analysis. J Toxicol Sci 2017; 42(4):491-497.
19. **Fang J, and Holmgren A:** Thioredoxin reductase is irreversibly modified by curcumin: a novel molecular mechanism for its anticancer activity. J Biol Chem; 2015, 280:25281–25293.
20. **Suvarna KS, Layton C & Bancroft JD:** Bancroft's Theory and Practice of Histological Technique. Elsevier. ; 2018, 8th edition.
21. **Bancroft, J.D, and Layton, C**: Theory and practice of histological technique,7th ed., London: Churchill Livingstone. (2010) Pp. 173-214
22. **Peat J and Barton B:** Medical statistics. A Guide for data analysis and critical appraisal. First Edition. Wiley-Blackwell: 2005, 113-19.
23. **Rafeeq M, Murad HAS, Abdallah HM, El-Halawany AM.** Protective effect of 6-paradol in acetic acid-induced ulcerative colitis in rats. BMC Complementary Medicine and Therapies, 2021; 21:28.
24. **Low D, Nguyen DD, Mizoguchi E.** Animal models of ulcerative colitis and their application in drug research. Drug Des Devel Ther 2013: v.7; PMC3829622.
25. **Soliman, N. A., Keshk, W. A., Rizk, F. H., and Ibrahim, M.A.:** The possible ameliorative effect of simvastatin versus sulfasalazine on acetic acid-induced ulcerative colitis in adult rats. Chemico-biological interactions, 2019;298, 57-65.
26. **Abdelmegid, A.M.; Abdo, F.K.; Ahmed, F.E**: Therapeutic effect of gold nanoparticles on DSS-induced ulcerative colitis in mice concerning interleukin-17 expression. Scientific Reports, 2019; 9(1):10176.
27. **Coskun M**: Intestinal Epithelium in Inflammatory Bowel Disease. Front Med (Lausanne); 2014; 1: 24.
28. **Chassaing B., J.D. Aitken, M. Malleshappa and M. Vijay-Kumar:** "Dextran sulfate sodium (DSS)- induced colitis in mice". Curr. Protoc. Immunol. 2014;104: Unit 15.25.
29. **Abdine NM, Abd El-Hafez AA, Okasha EF and Abo Hassan NF:** Histological Study of the Possible Protective Effect of Royal Jelly on Ulcerative Colitis Induced by Dextran Sodium Sulfate in Adult Male Albino Rat. Med. J. Cairo University, 2018, Vol. 86, No. 5: 2565-2578.
30. **Nowarski R, Jackson R, Gagliani N, De Zoete MR, Palm NW, Bailis W:** "Epithelial IL-18 Equilibrium Controls Barrier Function in Colitis". Cell, 2015: 163 (6): 1444-56.
31. **Bets V. D., Achasova K. M., Borisova M. A., Kozhevnikova E. N., Litvinova E. A.:** Role of mucin 2 glycoprotein and L-fucose in the interaction of immunity and microbiome within the experimental model of inflammatory bowel disease. Biochemistry. 2022; 87 (4), 301–318. 10.1134/S0006297922040010.

32. **da Silva MV, Marosti AR, Mendes CE, et al**: Differential effects of experimental ulcerative colitis on P2X7 receptor expression in enteric neurons. Histochem Cell Biol, 2015; 143: https://doi.org/10. 1007/s00418-014-1270-6.
33. **da Silva MV, Marosti AR, Mendes CE, et al**: Submucosal neurons and enteric glial cells expressing theP2X7 receptor in rat experimental colitis. Acta Histochem; 2017, 119.
34. **Kumar V, Cotran RS, Robbins SL:** Robbin's basic pathology. 7th ed. Philadelphia: W.B. Saunders CO. (2016), 545.
35. **Zhu MY, Lu YM, Ou YX, Zhang HZ & Chen WX**: Dynamic progress of 2,4,6-trinitrobenzene sulfonic acid-induced chronic colitis and fibrosis in a rat model. J Dig Dis; 2012 ,13: 421-429.
36. **Manetti M, Rosa I, Messerini L, Ibba-Manneschi L**: Telocytes are reduced during the fibrotic remodeling of the colonic wall in ulcerative colitis. J Cell Mol Med; 2015, 19: 62-73.
37. **Hamam GG, Raafata MH, and Shoukry Y:** Possible protective effect of dietary extra-virgin olive oil on experimentally induced acute colitis in adult male albino rats: a histological and immunohistochemical study. The Egyptian Journal of Histology, 2014; 37:373-385.
38. **Hashem HR and Sadek AM:** Antioxidant Ameliorating Effect of Setria (Reduced Glutathione) on Experimentally Induced Colitis in Adult Male Albino Rats. The Egyptian Journal of Histology, 2020; Vol. 44, No.1.
39. **MergaY, Campbell B.J, Rhodes J.M.** Mucosal barrier, bacteria, and inflammatory bowel disease: possibilities for therapy. Dig. Dis. 2014, 32(4): 475-483.
40. **Al-Badawi MH, Waly NE, Eid MM, and Soliman NA**: Histopathological Impact of Ginger Loaded Nanoparticle Versus Ginger Extract as A Novel Therapy of Experimentally Induced Acute Ulcerative Colitis, 2022. EJH copyright ©. DOI: 10.21608/ejh.2021.68124.1448.
41. **Ali AA, Abd Al Haleem EN, Khaleel SA, Sallam AS**: Protective effect of cardamon against acetic acid-induced ulcerative colitis. Elsevier, Sp., 2016. o. 1734-1140.
42. **Liu C, Zeng Y, WenY, Huang, and Liu Y**: Natural Products Modulate Cell Apoptosis: A Promising Way for the Treatment of Ulcerative Colitis. Front. Pharmacol 2022, January 31 Volume 13.
43. **Magalhães HIRM and Castelucci P**: Enteric nervous system and inflammatory bowel diseases: Correlated impacts and therapeutic approaches through the P2X7 receptor. World J Gastroenterol; 2021, Dec 14; 27(46): 7909–7924.
44. **Lakhan S,& Kirchgessner[A.]:** Neuroinflammation inInflammatory BowellDiseasee**.** Journal of Neuroinflammation; 2010, volume 7, Article number: 37.
45. **Utku, Ö. G., Karatay, E., Ergül, B., Yilmaz, C., Ekinci, Ö., and Arhan, M.:** TNBS file Olusturulmus Deneysel Rat Kolit Modelinde Tedavide Karisim Probiyotikler Etkin midir? Is the Probiotic Mixture Effective in the Treatment of TNBS-induced Experimental Colitis? Journal of Academic Research in Medicine, 2020; 10(1): 41-48.
46. **Kangwan, N.; Kongkarnka, S.; Boonkerd, N.; Unban, K.; Shetty, K.; Khanongnuch, C:** Protective Effect of Probiotics Isolated from Traditional Fermented Tea Leaves (Miang) from Northern Thailand and Role of Synbiotics in Ameliorating Experimental Ulcerative Colitis in Mice. Nutrients, 2022; 14, 227. https://doi.org/10.3390/nu14010227.
47. **Gerhard D., Sousa F., Andraus RA C, Pardo P.E., Nai G.A., Neto H.B., Messora M.R., et al..** Probiotic therapy reduces inflammation and improves intestinal morphology in rats with induced oral mucositis. Braz Oral Res ;2017, 31, e7.
48. **Soliman MG, Mansour HA, Hassan WA, Shawky E:** Protective effects of amoxicillin and probiotics on colon disorders in an experimental model of acute diverticulitis disease. 2022. Inflammopharmacology.

49. **Dashti N & Zareb Avani M:** Probiotics in the management of Giardia duodenalis: an update on potential mechanisms and outcomes. Naunyn-Schmiedeberg's Archives of Pharmacology, 2021;394:1869–1878.
50. **Depommier, C., Everard, A., Druart, C., Plovier, H., Van Hul, M., Vieira-Silva and Cani, P. D.** Supplementation with Akkermansia muciniphila in overweight and obese human volunteers: A proof-of-concept exploratory study. Nature Medicine, 2019; 25(7): 1096-1103.
51. **Han, Y.; Zhao, T.; Cheng, X.; Zhao, M.; Gong, S.-H.; Zhao, Y.-Q.; Wu, H.-T.; Fan, M.; Zhu, L.-L:** Cortical inflammation is increased in a DSS-induced colitis mouse model. Neurosci. Bull. 2018, 34, 1058–1066.
52. **Górska, A., Przystupski, D., Niemczura, M.J., and Kulbacka, J.:** Probiotic bacteria: a promising tool in cancer prevention and therapy. Current microbiology, **2019**;76, 939-949.
53. **Wu, Y., Wang, B., Zeng, Z., Liu, R., Tang, L., Gong, L., and Li, W.:** Effects of probiotics Lactobacillus plantarum 16 and Paenibacillus polymyxa 10 on intestinal barrier function, antioxidative capacity, apoptosis, immune response, and biochemical parameters in broilers. Poult Sci; 2019, 98, 5028-5039.
54. **Elnagar AMB, Ibrahim A, and Soliman AM:** Histopathological effects of titanium dioxide nanoparticles and the possible protective role of N-acetylcysteine on the testes of male albino rats. Int. J. Fertil. Steril. 2018, 12(3): 249-256.
55. **OZ S, Okay E, Karadenizli A**: N-Acetylcysteine Improves Intestinal Barrier In Partially Hepatectomized Rats. ANZ. J. Surg. 2007, 77: 173–176.
56. **Uraz S, Tahan G, Aytekin H & TahanV**: N-acetylcysteine expresses powerful anti-inflammatory and antioxidant activities resulting in complete improvement of acetic acid-induced colitis in rats. Scandinavian Journal of Clinical and Laboratory Investigation. 2013, Volume 73, - Issue 1.
57. **Chen CY, Cheng KC, Chang AY, Lin YT, Hseu YC, et al.**: 10-Shogaol, an antioxidant from Zingiber officinale for skin cell proliferation and migration enhancer. Int J Mol Sci 2012 **,**13: 1762-1777.
58. **Wu J, Song T, Liu S, Li X, Li G, et al.**: Icariside II inhibits cell proliferation and induces cell cycle arrest through the ROS-p38-p53 signaling pathway in A375 human melanoma cells. Mol Med Rep, 2015; 11: 410-416.
59. **Tenório MCDS, Graciliano NG, Moura FA, de Oliveira ACM, Goulart MOF:** N-acetylcysteine (Nac): Impacts on human health. Antioxidants.; 2021 ,10(6). doi: 10.3390/antiox10060967.
60. **Ezeriņa D, Takano Y, Hanaoka K, Urano Y, Dick TP:** N-Acetyl Cysteine Functions as a Fast-Acting Antioxidant by Triggering Intracellular H 2 S and Sulfane Sulfur Production. Cell Chem Biol, **2018**; 25(4): 447-459.e4. doi: 10.1016/j.chembiol. 2018. 01.011.
61. **Aldini G, Altomare A, Baron G, Vistoli G, Carini M, Borsani L, et al.:** N-Acetylcysteine as an antioxidant and disulfide breaking agent: the reasons why. Free Radic Res.; 2018,52(7):751–62. doi 10.1556/ism. v8i2.11.

تأثير البر وبيوتيك مقابل ان استيل سيستاين على التهاب القولون التقرحي الناجم عن حمض الأسيتيك في ذكور الجرذان البيضاء البالغة. دراسة هستولوجية وهيستوكيميائية مناعية

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هناك عدة عوامل تؤثر على القدرة التشغيلية الطبيعية للقولون. يعد التهاب القولون التقرحي أحد أكثر أشكال اضطرابات الجهاز الهضمي شيوعًا والتي تتطلب علاجًا طويل الأمد. يتم استخدام مكملات البر وبيوتيك بشكل متزايد لعلاج مشاكل الجهاز الهضمي والوقاية منها، ومن مضادات الأكسدة القوية، ان- أسيتيل سيستيين، الذي يرفع مستويات السيستين والغلوتاثيون داخل الخلايا وتنقب على أنواع الأكسجين التفاعلي.

الهدف: مقارنة الآثار العلاجية للبروبيوتيك مقابل ان- أسيتيل سيستيين في الجرذان البيضاء مع التهاب القولون التقرحي المستحث.

المواد والطرق: تم استخدام ست مجموعات منفصلة من 50 حيوانًا: المجموعة الضابطة الأولى، المجموعة الفرعية، Ia، Ib، المجموعة المعالجة بالبروبايوتك (المجموعة الثانية) والمجموعة المعالجة ان- أسيتيل سيستيين (المجموعة الثالثة) التهاب القولون (المجموعة الرابعة) ، التهاب القولون تلقى البر وبيوتيك (المجموعة الخامسة)، والتهاب القولون المعطى ان- أسيتيل سيستيين (المجموعة السادسة).

بعد أسبوعين، تم جمع عينات الدم لتقييم مستويات علامات الإجهاد التأكسدي. تم تحضير عينات القولون للفحوصات النسيجية، والفحوصات المناعية، وكذلك فحص الفحص المجهري الإلكتروني.

النتائج: أظهرت مجموعة التهاب القولون المستحث انخفاضًا كبيرًا في سماكة الغشاء المخاطي مع فقدان الخلايا السطحية، وظهرت الخلايا بانويه داكنة وفراغات بالسيتوبلازم وانخفاض في الخلايا الكأسية مع زيادة التغيرات الليفية المخاطية وتحت المخاطية. أظهر المسح الضوئي الإلكتروني اتساع فتحات الخبايا. لوحظ أيضًا انخفاض كبير في Ki 67 وزيادة في نشاط المناعة لـ caspase 3. علاوة على ذلك، كان لإعطاء البر وبيوتيك تأثيرات مخففة على هذه التغييرات أكثر من ان- أسيتيل سيستيين.

الخلاصة: أظهرت البر وبيوتيك تأثيرات علاجيه أعلى من ان- أسيتيل سيستيين من خلال آثارها المضادة للالتهابات ومضادات الأكسدة ومضادات موت الخلايا المبرمج.