Original Article

The Possible Protective Role of Arabic gum in Suppression of Acetic Acid-Induced Ulcerative Colitis (A Histological and Immunohistochemical Study)

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ABSTRACT

Introduction: Ulcerative colitis is a serious health disease that is becoming more common around the world. It is characterized by an acute, chronic, and relapsing inflammatory illness manifested by abdominal discomfort and diarrhea. Arabic gum is a natural gummy exudate with a variety of therapeutic benefits.

Aim of the Work: This work aimed to evaluate the role of arabic gum (A.G.) on suppression of acetic acid (A.A.) induced ulcerative colitis in adult male albino rat using different histological and immunohistochemical techniques.

Materials and Methods: The current study involved forty adult male albino rats that were randomly divided into four groups: group I represented the control group, group II was given A.G. (7.5gm/kg/day) orally for consecutive 10 days, group III received 4% A.A. (2 ml/rat) for induction of colitis, and group IV received arabic gum for 7 days, before induction of colitis and continued for 3 days after it in the same dose and manner as groups II & III. The colon specimens were processed for different histological and immunohistochemical techniques. Morphometrical and statistical studies were also performed.

Results: The group of A.A. showed destruction of crypts with formation of cyst like structure and loss of surface epithelium. Dilated blood vessels, hemorrhage, and heavy inflammatory cellular infiltration were also detected. Ultrastructurally, columnar cells showed widening of the intercellular spaces, rarified cytoplasm, and swollen mitochondria. Moreover, their nuclei appeared irregular with condensed chromatin. As regards goblet cells, there was coalescence of their secretory granules. Furthermore, significant decrease in the mean number of goblet cells, while the mean optical density of COX-2 immunostaining and the microscopic score showed a highly significant increase, when compared with control group. Additionally, the group of A.G.+ A.A. showed suppression of ulcerative colitis changes.

Conclusion: Ulcerative colitis changes could be ameliorated by arabic gum.

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Key Words: Arabic gum, acetic acid, colon, COX-2, ultrastructure.

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INTRODUCTION

Inflammatory bowel diseases (IBD), include Crohn's disease (CD) and ulcerative colitis (UC), are gastrointestinal chronic inflammatory disorders. Genetic, environmental, and gut microbial factors all have a role in the etiology and pathogenesis of IBD. Pain, vomiting, weight loss, diarrhea, and bloody stool are all symptoms of UC[1].

The clinical and histological findings of experimental acute colitis in rats induced by intrarectally injecting them with acetic acid are similar to those of human ulcerative colitis. Moreover, A.A-colitis model is used to study innate immune responses and its significance in the development of mucosal inflammation and barrier re-establishment[2].

Arabic gum is a natural gummy exudate obtained from the trees of Acacia species (Acacia senegal and Acacia seyal). A.G. is a dietary fiber that contains high amount of carbohydrates and little protein content. The sugars arabinose and ribose were found and separated from arabic gum. The A.G. was traditionally used to treat chronic renal failure, abdominal discomfort and inflamed intestinal mucosa. Weight loss, antihypertensive, antihyperlipidemic, anticoagulant, antibacterial, antidiabetic, anti-inflammatory, antioxidant, nephroprotective, and other medical effects of A.G. have recently been discovered[3,4].

For all previous criteria of arabic gum, the current study was designed to evaluate its possible protective effect against ulcerative colitis histopathological changes.

MATERIALS AND METHODS

Reagents

Acetic acid was purchased from El Nasr Pharmaceutical Chemicals Company (No. A0198111, Oubour, Qalyubia, Egypt) in concentration of 100%. For reaching the required concentration (4%), we added 60 ml distilled water to 40 ml A.A.

Arabic gum was purchased from Elnasr for food industries (No. 929, Khartoum, Sudan) in a powder form that was dissolved in distilled water. The required dose per rat was achieved by adding 1.5 mL of warm distilled water to 1.5 gm of A.G.

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Induction of colitis

After an overnight fast, colitis was induced in rats by intrarectal injection of 4% acetic acid (2 mL) through a lubricated catheter , which was inserted into the rectum to a depth of 4.5 cm under low-dose ether anesthesia. The rats were kept in Trendelenburg position during rectal instillation and for 1 min after instillation to prevent leakage of the solution. Some rats of the control group were treated also, but instead of 4% AA, they received an equal volume of 0.9% saline by intrarectal injection[5,6].

Animals

The present study involved 40 adult male albino rats with an average weight (200– 250 grams). They were adapted to animal house conditions for 2 weeks. Our experiment was approved by the local ethical committee of the Faculty of Medicine, Tanta University, Egypt (Approval number: 35285).

Study design

The rats were randomly divided into four main groups (10 rats each) as following:

Group I: represented the control group that were divided into 3 subgroups:

- Subgroup (i): 4 rats kept without any treatment until the end of the experiment.
- Subgroup (ii): 3 rats, each one received 1.5ml of distilled water (the diluting vehicle for arabic gum) for 10 successive days.
- Subgroup (iii): 3 rats, each one received 2ml of 0.9% saline once by intrarectal injection.

Group II (A.G.-group): each rat received 1.5 ml of arabic gum prepared solution (7.5gm/kg/day) orally for successive 10 days[7].

Group III (A.A.-group): on the 8th day from the start of the experiment, colitis was induced by single intrarectal injection with 4% acetic acid (2 ml/rat)[5].

Group IV (A.A.+A.G.- group): rats received arabic gum for 7 days before the induction of colitis. On the 8th day, colitis was induced. Next, arabic gum treatment was continued till the 10th day in the same dose and manner as groups II & III respectively.

Finally, on the 11th day, all animals were anaesthetized with a 50 mg/kg intraperitoneal injection of sodium pentobarbital[8]. The colon specimens were excised, cleaned and processed for different histological and immunohistochemical techniques.

Light microscopic preparation

For histological examination, colon of all groups was fixed in formalin solution and processed for paraffin blocks. 5-µm histological colonic sections were cut and exposed to the following techniques:

1. Routine hematoxylin and eosin for the general examination of the colon tissue[9].
2. Periodic acid Schiff (PAS) reagent: to assess mucopolysaccharides and brush border of colonic cells[9].
3. Immunohistochemical staining cyclooxygenase 2 (COX-2):

Cyclooxygenase 2 (COX 2) production is induced by interleukin 1b (IL-1b) and tumor necrosis factor alpha (TNF-α). COX-2 can convert arachidonic acid to prostaglandins which is linked with the incidence of inflammation. Sections were deparaffinised, rehydrated and were covered by 0.3% H_2O_2 for 15 minutes to block endogenous peroxidase activity. After incubation of the sections in the microwave, antigen retrieval was done with citrate buffer. The tissue sections were treated with the following primary antibody, cyclooxygenase 2 (COX-2); dilution 1: 400-800 (clone SP21, Master Diagnostica, Spain, cat no. PG27B) overnight at room temperature. The streptavidin biotin complex detection method is used in the second step of staining. Finally, the sections were stained for 5-10 minutes with diaminobenzidine (DAB) solution (as chromogen), rinsed three times in PBS for two minutes each, and counterstained with Mayer hematoxylin. Negative control was done by the similar previous steps without addition the primary antibody. Positive control was urinary bladder carcinoma. The positive immunoreactivity for COX 2 appeared as brown cytoplasmic staining in the immunoreactive cells[10,11].

All slides were examined and photographed using a light microscope (Olympus, Japan) with a built-in camera in the Histology and Cell Biology department, Faculty of Medicine, Tanta University.

Microscoping scoring of colonic changes was performed according to modified Cagin[12] using the following criteria: Mucosal epithelium (ulceration); lamina propria (inflammatory cell infiltrate); crypts loss, submucosa (hemorrhage, edema and infiltration of inflammatory cells). Colonic mucosal damage was evaluated on a 0 4 scale. Grading criteria were as the followings: Grade 0, normal appearance; grade 1, slight injury (0-25% involvement); grade 2, moderate injury (25-50%involvement); grade 3, severe injury (50-75% involvement); and grade 4, extensive full thickness injury (>75%). The microscopic grade of each tissue was calculated as the sum of the scores given to each criterion.

Morphometric study

Ten separate non-overlapping randomly selected fields from each slide of each group were quantified according to the following measurements using image J software (National Institute of Health, Bethesda, Maryland, USA):

1. The mean number of goblet cells in Periodic Acid Schiff (X 400)[13].

2. The mean optical density of COX2 immunoreactivity (X 400)[14].
3. Microscoping scoring of the colonic mucosal damage[12].

Statistical analysis

The estimated numbers were compared, and statistical analyses were performed using one-way analysis of variance (ANOVA) and Turkey's test for group comparison. The mean and standard deviation were used to express all of the data. If the probability *P-value* was less than 0.05, the differences were considered significant, and if the *P-value* was less than 0.001, the differences were considered highly significant[15].

Electron microscopic preparation

Transmission electron microscopy (TEM) was used to investigate the ultrastructure. Colonic specimens were preserved in solution containing 1 % glutaraldehyde/ 1 % paraformaldehyde. The tissues were then post-fixed with osmium tetroxide.

After that, the specimens were dehydrated in a series of ascending alcohol, coated with propylene oxide, and implanted in epoxy resin. Semithin sections (0.5 µm) and ultrathin sections (80-90nm) were cut using ultramicrotome. Semithin sections were stained with 1% toluidine blue for light microscopic examination. Ultrathin sections were double-stained with uranyl acetate and lead citrate[16].

RESULTS

In the present work, no mortality was recorded throughout the experimental period. All subgroups of the control group showed no difference in the histological results. So, it was referred to subgroups (i, ii& iii) as the control group. As regards group II (A.G.-group) it also showed no difference in the macroscopic examination, histological results or statistical analysis when compared with control group.

Macroscopic examination of the colon

Macroscopic examination of group III (A.A.-group) showed extensive macroscopic colonic damage, in the form of hemorrhagic areas of the colonic wall with hugely dilated lumen when compared to control group that showed no changes (Figures 1 A,B). Meanwhile group IV (A.A.+A.G.- group) showed few changes in the form of redness, and edematous wall (Figure 1 C).

Light Microscopic Examination

Hematoxylin and Eosin stained sections

Examination of colon sections of control group was identical to the known histological structure of the normal colon. The wall of the colon was formed of mucosa, submucosa, musculosa and serosa. The mucosa was divided into 3 layers; surface epithelium, lamina propria, and muscularis mucosa. The surface epithelium was formed of simple columnar absorptive cells (with acidophilic cytoplasm and basal oval nuclei) and goblet cells (with foamy cytoplasm and basal flattened nuclei). Regarding the lamina propria, it contains regularly arranged, closely packed crypts (intestinal glands), and numerous lymphocytes (Figures 2,3,4).

Meanwhile, examination of group III (A.A.-group) showed variation in the changes which were focal in some sections and diffuse in the other sections. Destruction of crypts with formation of cyst like structure was noticed. Moreover, loss of surface epithelium with cellular debris in the lumen was observed. Additionally, most of the columnar cells appeared with dark nuclei, while goblet cells showed apparent decrease in their number (Figures 5,6,7,8). Dilated blood vessels, hemorrhage, and heavy inflammatory cellular infiltration extending from the lamina propria to the submucosa were also detected (Figures 5,6,7).

Examination of group IV (A.A.+A.G.- group) showed almost normal histological structure of the colon. The surface epithelium was intact in some areas while the intestinal crypts appeared long, straight and parallel to each other. Moreover, columnar and goblet cells appeared nearly normal. Some dilated blood vessels were observed in the submucosa (Figures 9,10).

Periodic Acid Schiff (PAS) stained sections

Examination of PAS stained sections obtained from control group revealed intact PAS positive thin brush border of columnar cells and magenta red goblet cells (Figure 11). While examination of group III (A.A.-group) exhibited interrupted brush border and apparent decrease in the number of goblet cells (Figure 12). Moreover, examination of group IV (A.A.+A.G.- group) showed intact PAS positive in some areas of the brush border and goblet cells appeared magenta red (Figure 13).

Cyclooxygenase-2 (COX- 2) immune-stained sections

Examination of sections from control group showed apparent weak COX-2 positive cytoplasmic immunoreaction in both surface and glandular epithelial cells (Figure 14). On the other hand, group III (A.A.-group) revealed apparent strong COX-2 positive cytoplasmic immunoreaction in surface and glandular epithelium (Figure 15). Regarding group IV (A.A.+A.G.- group), there was apparent weak COX-2 positive cytoplasmic immunoreaction with focal strong positive reaction in some cells (Figure 16).

Morphometric results & statistical analysis

The mean number of goblet cells of group III (A.A.-group) showed a highly significant decrease ($p < 0.001$) when compared to control group. On the other hand, group IV (A.A.+A.G.- group) showed a significant increase ($p < 0.05$) when compared to group III. Moreover, there was non-significant decrease in the mean number of

goblet cells in group IV when compared to control group ($p > 0.05$). (Table 1, Histogram A).

The mean optical density of COX-2 immunostaining in group III (A.A.-group) showed a highly significant increase ($p < 0.001$) when compared to control group while group IV (A.A.+A.G.- group) revealed a highly significant decrease ($p < 0.001$) when compared to group III. Meanwhile, there was non-significant increase ($p > 0.05$) in the optical density of group IV when compared to control group (Table 1, Histogram B).

The microscopic score of group III (A.A.-group) showed a highly significant increase when compared to control group ($p < 0.001$), while group IV (A.A.+A.G.- group) showed a highly significant decrease in the microscopic scoring when compared to group III ($p < 0.001$). Group IV also showed non-significant increase ($p > 0.05$) in the scoring when compared to control group (Table 1, Histogram C).

Electron microscopic results

Examination of ultrathin sections obtained from colon of control group showed absorptive columnar cells with closely packed regularly arranged microvilli at their apical surfaces, well developed Golgi, mitochondria and basal oval euchromatic nuclei (Figures 17). These columnar cells were attached to each other by junctional complexes (Figure 18). Goblet cells were also detected having multiple mucin granules at the distended apical portion, RER, and nuclei at their basal compressed part (Figures 19). The lamina propria showed the intestinal crypts containing goblet cells with their characteristic mucin granules (Figure 20). Meanwhile, examination of ultrathin sections of group III (A.A.-group) revealed presence of exfoliated debris in the lumen of the colon (Figure 21). Regarding the columnar cells, their apical microvilli were short and irregular while their cytoplasm showed rarefaction, vacuoles and swollen mitochondria. Moreover, their nuclei appeared with irregular outlines and some of them had condensed chromatin. There was also widening of the intercellular spaces (Figures 22,23 (A,B),24). As regards goblet cells, there was coalescence of their secretory granules (Figure 24). Lamina propria exhibited dilated blood vessels, fibroblast like cells with apparently deposition of collagen and inflammatory cellular infiltration mainly with eosinophils (Figures 25,26). Meanwhile, group IV (A.A.+A.G.- group) showed more or less normal ultrastructure of the colon. The columnar cells showed nearly normal microvilli, mitochondria, and oval euchromatic nuclei with some irregularity in their outlines. Some columnar cells showed vacuoles and rarefaction in their cytoplasm. Goblet cells appeared with nearly normal mucin granules (Figures 27,28).

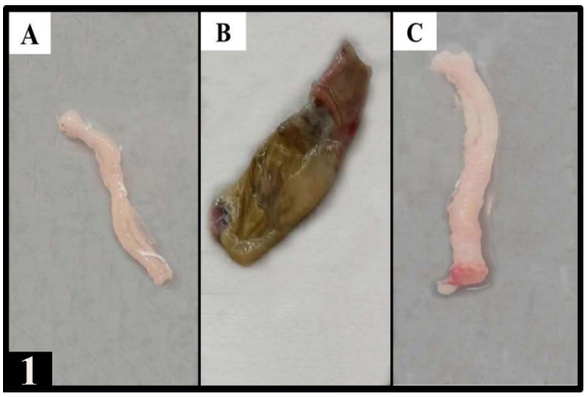


Fig. 1: A photograph showing the macroscopic examination of the colon, (A): control group showing colonic wall with no changes. (B): group III (A.A.-group) showing hemorrhagic areas of the colonic wall with hugely dilated lumen. (C): group IV (A.A.+A.G.- group) showing areas of redness, and mild edematous wall.

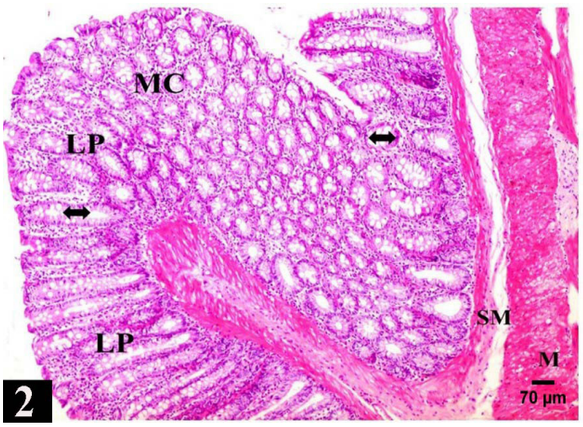


Fig. 2: A photomicrograph of a section in the colon of the control group showing mucosa (MC), submucosa (SM) and musculosa (M). Notice lamina propria (LP) containing closely packed simple tubular intestinal glands ↔ (H &E x 100).

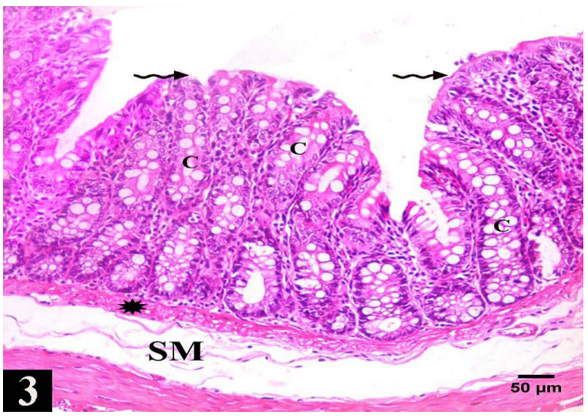


Fig. 3: A Photomicrograph showing the mucosa of control group formed of surface epithelium (zigzag arrows), lamina propria containing regularly arranged, tightly packed crypts (intestinal glands) (C) and muscularis mucosa (asterix). Submucosa (SM) is also observed (H &E x 200).

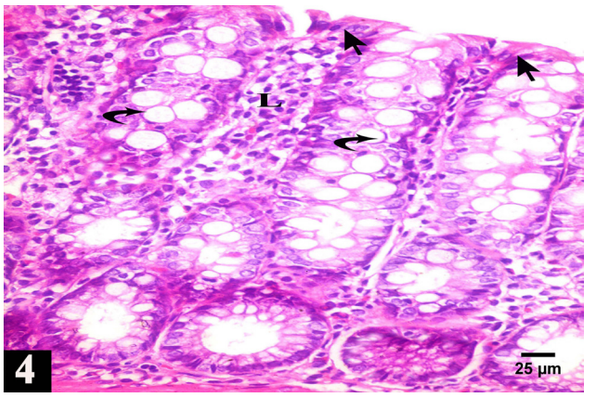


Fig. 4: A Photomicrograph of a section in the colon of control group showing the simple columnar absorptive cells (arrows) with acidophilic cytoplasm and basal oval nuclei. Goblet cells (curved arrows) with foamy cytoplasm and basal flattened nuclei are also noticed. Numerous cells most probably lymphocytes (L) are observed in the lamina propria (H &E x 400).

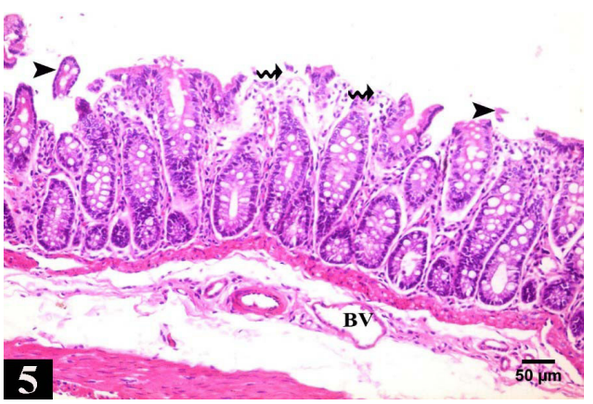


Fig. 5: A photomicrograph of a section in the colon of group III (A.A.- group) showing loss of surface epithelium in some areas (zigzag arrows) with cellular debris in the lumen (arrow heads). Dilated blood vessels (BV) in the submucosa are also observed (H &E x 200).

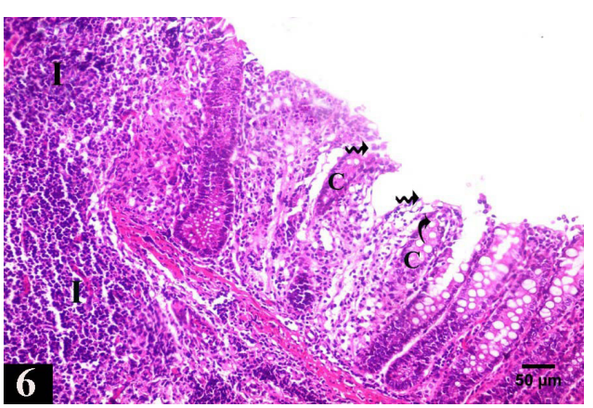


Fig. 6: A photomicrograph of a section in the colon of group III (A.A.- group) showing focal loss of surface epithelium (zigzag arrows) and destruction of the crypts (C). Notice decrease in the number of goblet cells (curved arrow). Heavy inflammatory cellular infiltration (I) is also observed (H &E x 200).

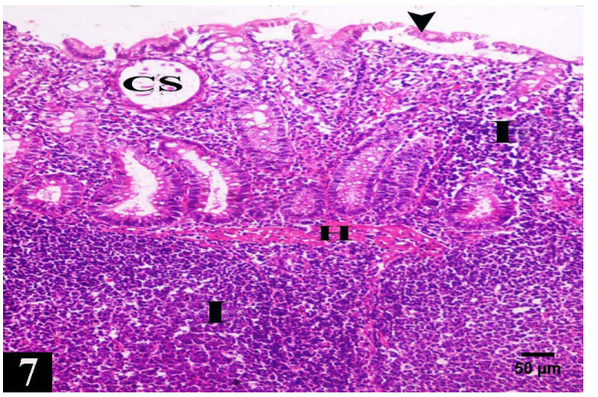


Fig. 7: A photomicrograph of a section in the colon of group III (A.A.- group) showing focal desquamation of the surface epithelium (arrow head), with cyst formation (CS) in the crypt. Hemorrhage (H) and heavy inflammatory cellular infiltration (I) are also noticed (H &E x 200).

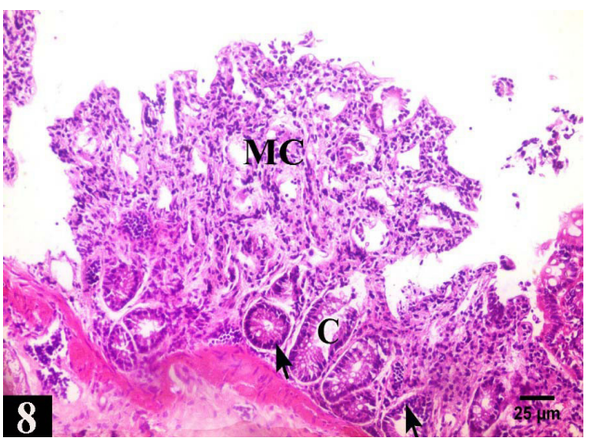


Fig. 8: A photomicrograph of a section in the colon of group III (A.A.- group) showing focal destructed disorganized mucosa (MC) with apparent decrease in goblet cells. Severe destruction of the crypts (C) is observed. Notice dark stained nuclei of the columnar cells (arrows) (H &E x 400).

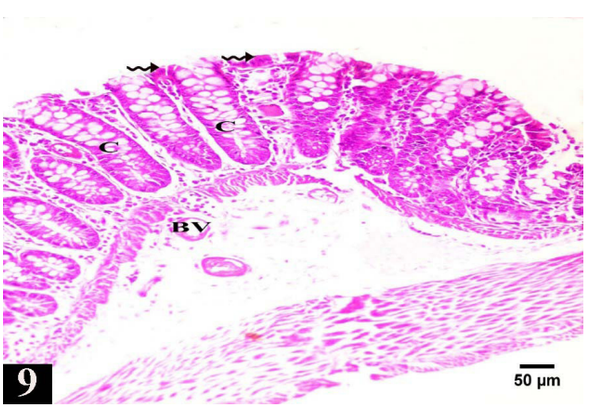


Fig. 9: A Photomicrograph of a section in the colon of group IV (A.A.+A.G.- group) showing area of intact surface epithelium (zigzag arrows) and tightly packed crypts (C). Dilated blood capillaries (BV) are observed in the submucosa (H &E x 200).

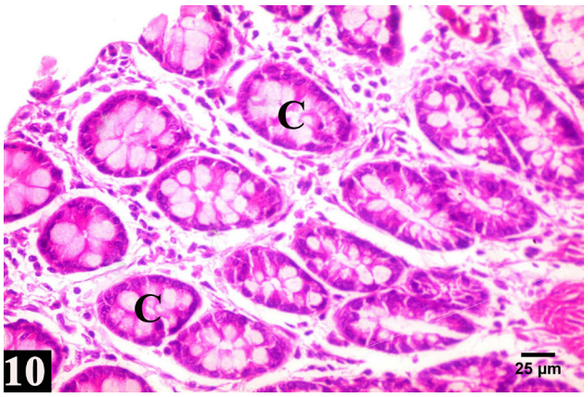


Fig. 10: A Photomicrograph of a section in the colon of group IV (A.A.+A.G.- group) showing intact crypts (C) with intact columnar and goblet cells (H &E x 400).

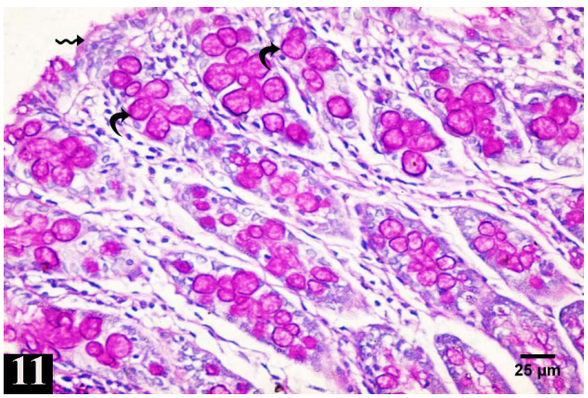


Fig. 11: A Photomicrograph of a section in the colon of the control group showing PAS positive thin brush border (zigzag arrow) and magenta red goblet cells (curved arrows) (PAS Mic. Mag. X 400).

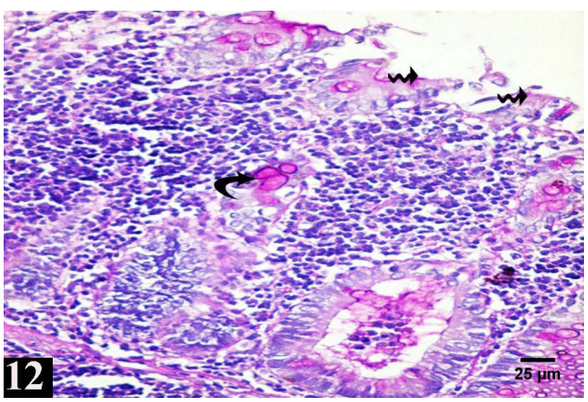


Fig. 12: A Photomicrograph of a section in the colon of group III (A.A.- group) showing thin interrupted brush border (zigzag arrows) and apparent decrease in the number of goblet cells (curved arrow) (PAS Mic. Mag. X 400).

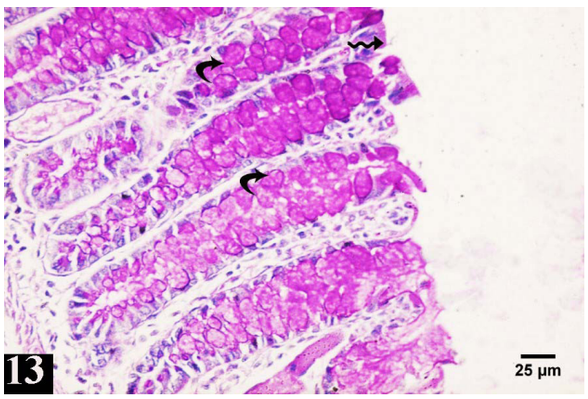


Fig. 13: A Photomicrograph of a section in the colon of group IV (A.A.+A.G.- group) showing PAS positive reaction in some areas of the brush border (zigzag arrow) and magenta red goblet cells (curved arrow) (PAS Mic. Mag. X 400).

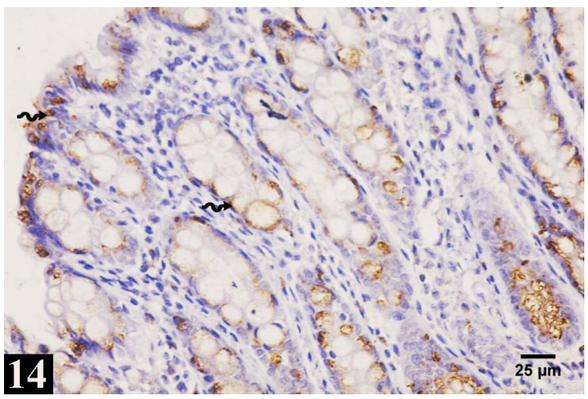


Fig. 14: A photomicrograph of a section in the colon of control group showing apparent weak COX-2 positive cytoplasmic immunoreaction (zigzag arrows) in both surface and crypt epithelial cells (COX-2 immunostaining x 400).

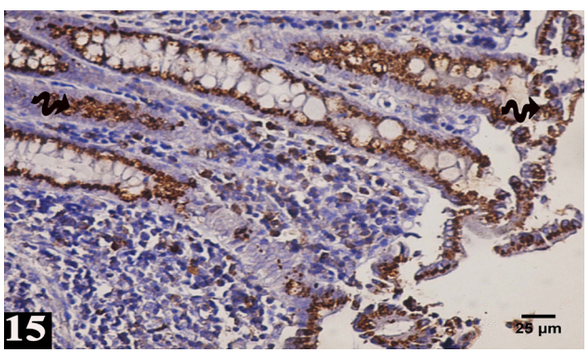


Fig. 15: A photomicrograph of a section in the colon of group III (A.A.-group) showing apparent strong COX-2 positive cytoplasmic immunoreaction (zigzag arrows) in surface and glandular epithelium (COX-2 immunostaining x 400).

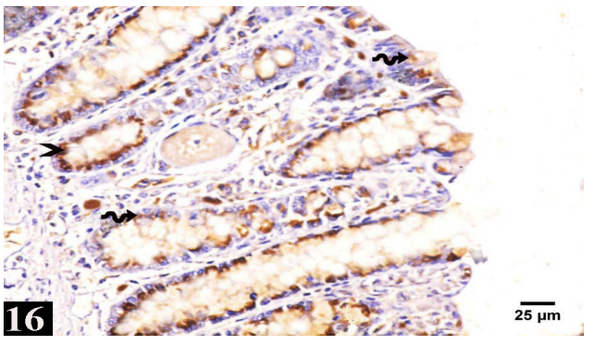


Fig. 16: A photomicrograph of a section in the colon of group IV (A.A.+A.G.- group) showing apparent weak COX-2 positive cytoplasmic immunoreaction in most columnar and goblet cells (zigzag arrows) alternating with focal strong positive reaction in some cells (arrowhead). (COX-2 immunostaining x 400).

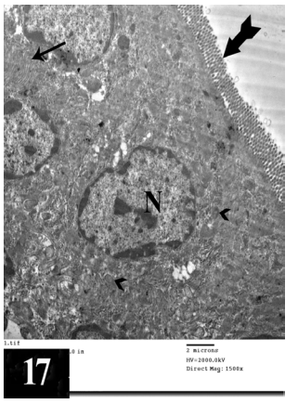


Fig. 17: Electron micrograph of the colon of control group showing the columnar absorptive cells with apical closely packed regularly arranged microvilli (bifid arrow). The cytoplasm contains well developed Golgi apparatus (arrow) and mitochondria (arrow heads) while the nuclei (N) are oval and euchromatic (Mic. Mag x 1500).

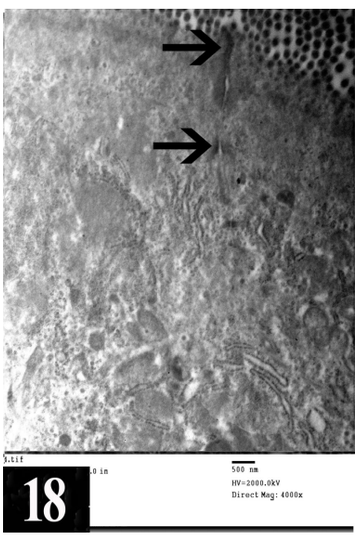


Fig. 18: Electron micrograph of the colon of control group showing the columnar cells and junctional complexes (thin arrows) between them (Mic. Mag x 4000).

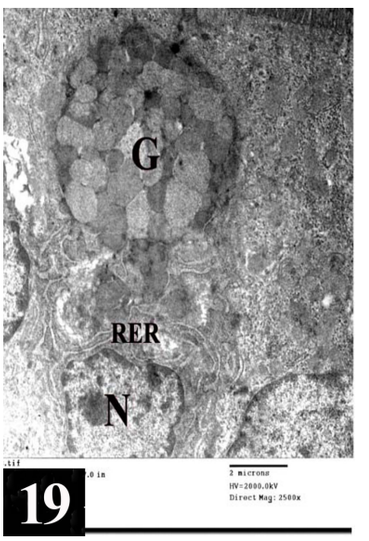


Fig. 19: Electron micrograph of the colon of control group showing goblet cell containing multiple mucin granules (G), RER and nucleus (N) in its basal compressed part (Mic. Mag x 2500).

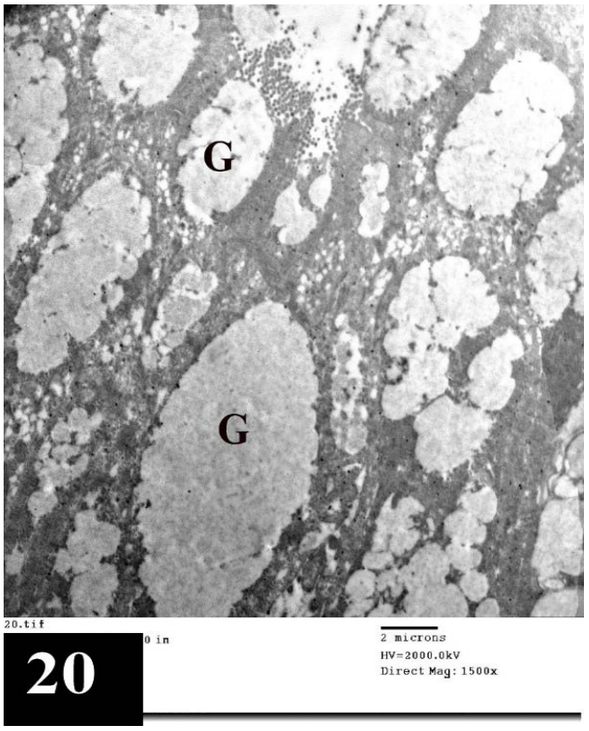


Fig. 20: Electron micrograph of the colon of control group showing the goblet cells of the intestinal crypts with their electron lucent mucin granules (G) (Mic. Mag x 1500).

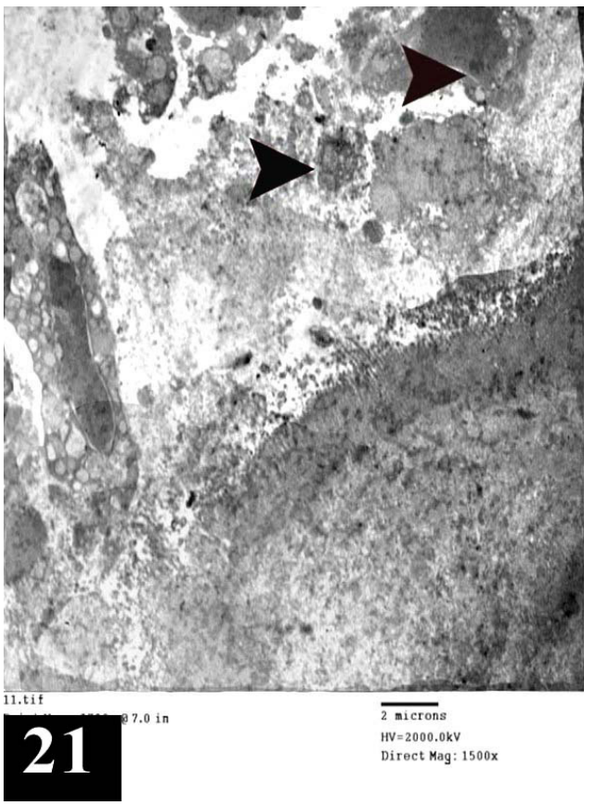


Fig. 21: Electron micrograph of the colon of group III (A.A.-group) showing exfoliated debris (arrow heads) in the lumen (Mic. Mag x 1500).

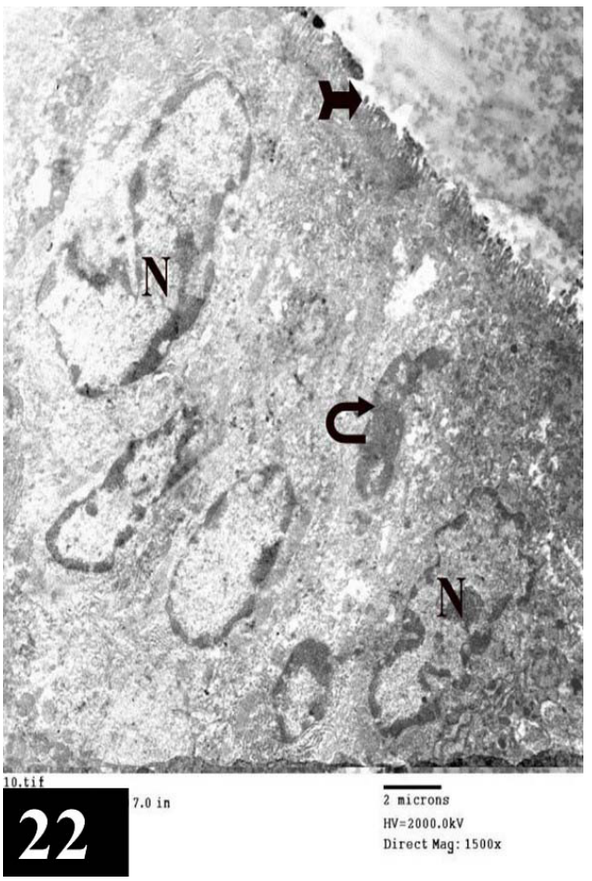


Fig. 22: Electron micrograph of the colon of group III (A.A.-group) showing irregular nuclei of columnar cells (N) and some of them appear with condensed chromatin (curved arrow). Notice short irregular microvilli (bifid arrow) (Mic. Mag x 1500).

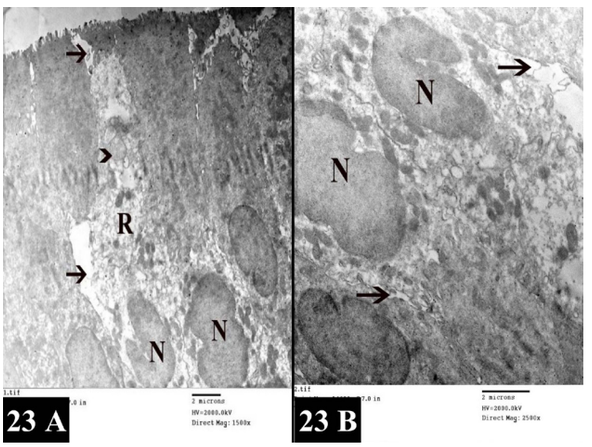


Fig. 23 (A& B): Electron micrographs of the colon of group III (A.A.-group) showing widening of the intercellular spaces (arrows), rarefaction (R) of the cytoplasm and swollen mitochondria (arrow head). Irregular nuclei of columnar cells (N) are also noticed (Mic. Mag x 1500 (A) & x 2500 (B)).

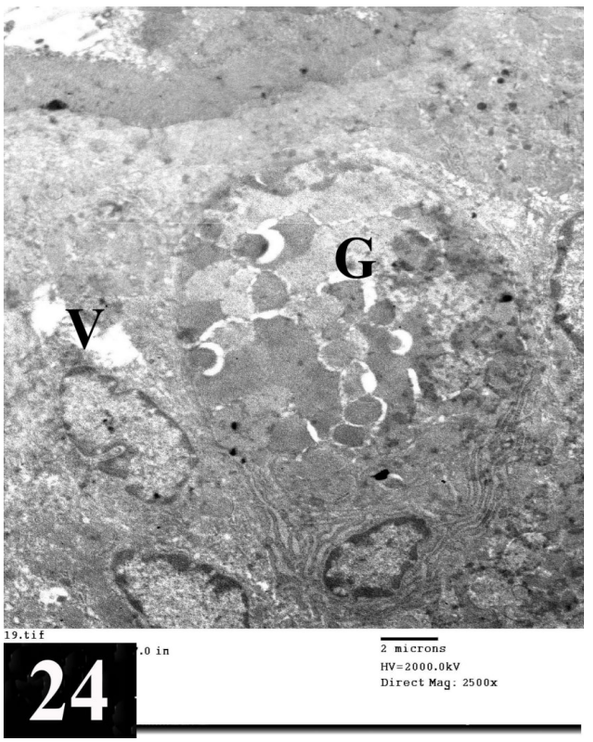


Fig. 24: Electron micrograph of the colon of group III (A.A.-group) showing vacuolated columnar cells (V). Notice goblet cells with coalescence of their mucin granules(G) (Mic. Mag x 2500).

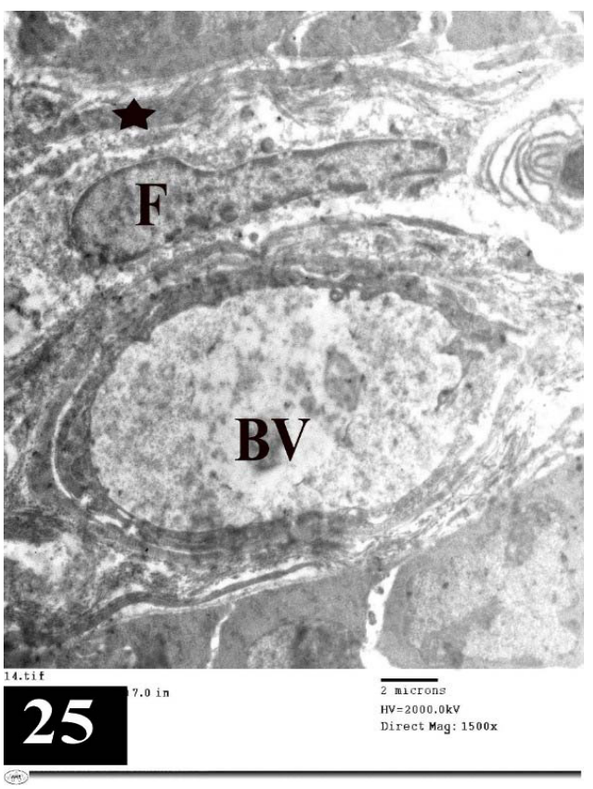


Fig. 25: Electron micrograph of the colon of group III (A.A.-group) showing the lamina propria with dilated blood vessels (BV), fibroblast like cell (F), and collagen fibers in between (star) (Mic. Mag x 1500).

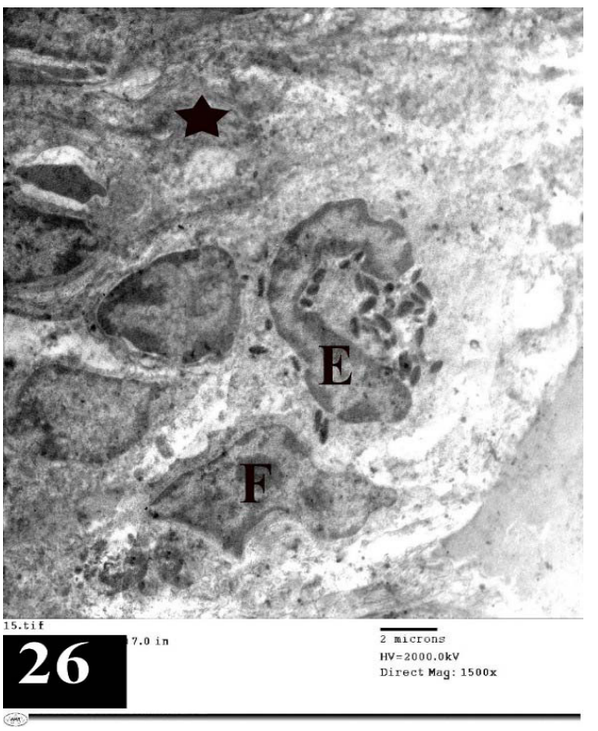


Fig. 26: Electron micrograph of the colon of group III (A.A.-group) showing fibroblast like cell (F), eosinophil (E) with its characteristic cytoplasmic granules and collagen fibers (star) (Mic. Mag x 1500).

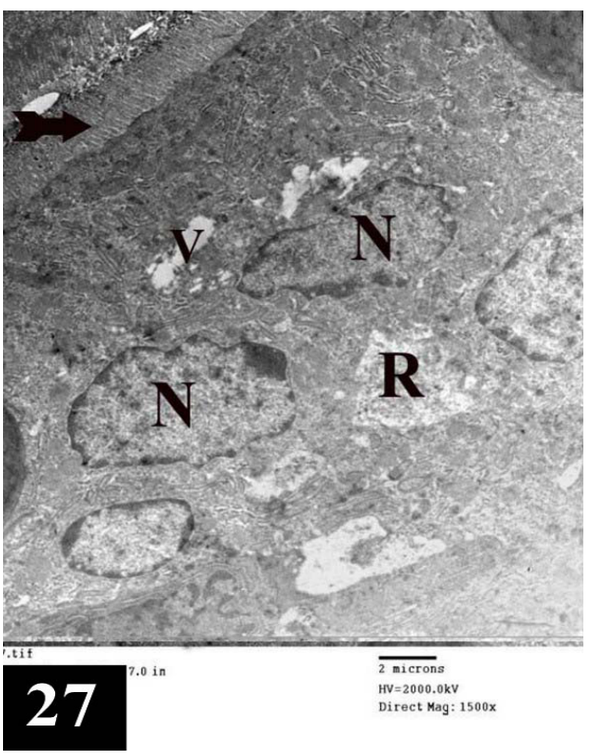


Fig. 27: Electron micrograph of colon of group IV (A.A.+A.G.- group) showing the columnar cells with their regular microvilli (bifid arrow) and nearly normal oval euchromatic nuclei (N). Some vacuoles (V) and rarefaction (R) in the cytoplasm are noticed (Mic. Mag x 1500).

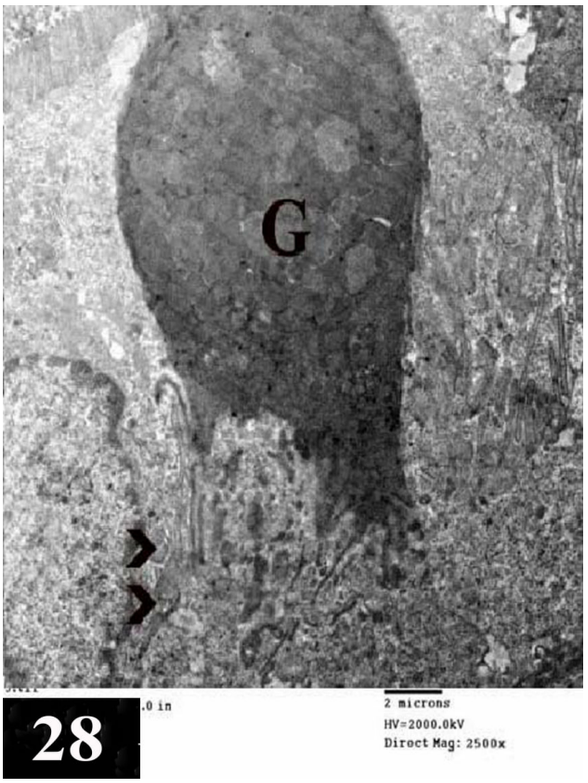
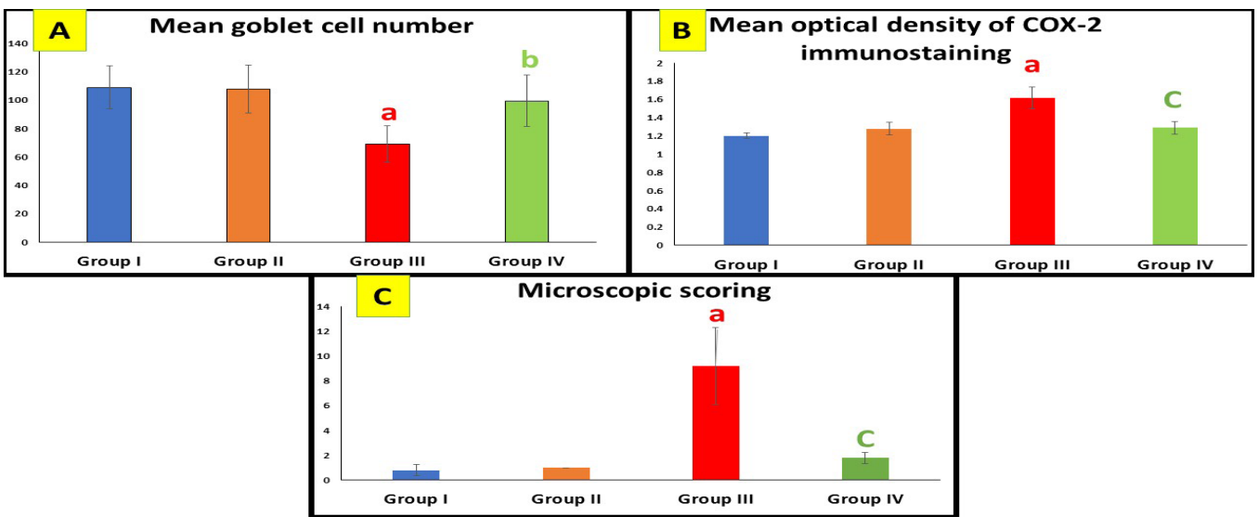


Fig. 28: Electron micrograph of the colon of group IV (A.A.+A.G.- group) showing columnar cell having mitochondria (arrow heads). Goblet cells with their apical part having mucin granules (G) (Mic. Mag x 2500).

Table 1: Morphometrical and statistical analysis of different studied groups

Parameters \ Groups	Group I	Group II	Group III	Group IV
	Mean ± SD			
Mean goblet cell number	109±15	107.71±17	69.14 ±13[a]	99.57 ±18[b]
Mean optical density of COX-2 immunostaining	1.2 ± 0.03	1.28±0.07	1.62±0.12[a]	1.29±0.07[c]
Mean microscoping scoring	0.8±0.45	1±0	9.2±3.11[a]	1.8±0.45[c]

Data are shown as Mean ± standard deviation (SD), [a] indicates a highly significant versus control, [b] indicates a significant versus group III and [c] indicates a highly significant versus group III respectively.



Histogram: (A) showing mean number of goblet cells, **(B)** showing mean optical intensity of COX-2 immunostaining and **(C)** showing mean microscopic scoring between different studied groups.

DISCUSSION

Inflammatory bowel diseases (IBD) are groups of chronic inflammatory disorders of the gastrointestinal tract that include Crohn's disease and ulcerative colitis. The pathogenesis of IBD may be attributed to dysregulated immune response against intestinal commensals[17]. Several medications have shown therapeutic efficacy in IBD patients, but they are expensive, having side effects, and become resistant after long use. Natural products are being considered as supplemental therapies that may play an important role in IBD treatment. Because of their high availability and few adverse effects, these alternative medicines have generated a lot of interest[18].

A rat model of ulcerative colitis induced by acetic acid closely resembles the pathology of human ulcerative colitis. Mucosal ulceration, weight loss, hemorrhage, and inflammation are examples of colonic changes. In these disorders, inflammatory cells enter the affected colon. Inflammatory mediators such as cytokines, iNOS (inducible nitric oxide synthase), COX-2, 5-lipoxygenase enzymes, and arachidonic acid metabolites are released. Furthermore, reactive oxygen species (ROS) are also produced, resulting in oxidative damage[2,6]. Moreover, some authors[19] added that ROS attack the cellular macromolecules, cell membrane lipids, cell proteins and DNA triggering their peroxidation in a continuous cycle, thus rendering it more susceptible to oxidative tissue damage, delaying recovery of the mucosa.

In the present study, macroscopic examination of A.A.-group showed hugely dilated lumen. This finding goes in line with some scientists[20] who stated that the dilation of colon is considered a complication of UC. They attributed the incidence of dilatation to extension of colonic inflammation outside the mucosa to the underlying tissues. They added that loss of contractility as a result of inflammatory response leads to the accumulation of gas and fluid within the lumen and subsequent colonic dilatation.

Light microscopic and ultrastructural analysis of the A.A.-group revealed histological changes in the colonic mucosa, including desquamation, exfoliation, loss of surface epithelium (erosions) and ulceration which may be due to the direct harmful effect of acetic acid on intestinal epithelial cells. Some authors[21] stated that application of A.A. disturbed the colonic mucus, which plays an important defensive role against chemically induced ulceration. They attributed the increased production of oxygen and accumulation of free radical to vascular dilatation and white blood cells gathering, as well as an increase in blood flow. Moreover, some scientists[22] also observed ulceration and sloughing of the surface epithelium and explained these changes to immunological processes and ROS which contribute significantly to the occurrence of tissue injury.

The present study showed that the architecture of crypts was destructed and distorted in the form of cyst formation. The previous finding goes in line with some other studies[19,23]. Moreover, cyst formation may be followed by neutrophilic permeation and formation of crypt abscess.

The irregular dark nuclei found in A.A.-treated groups may denote precancerous lesion. This explanation is in agreement with some authors[24] who recorded that the molecular and pharmacological processes relating colonic inflammation with cancer are unknown, with the possibility of involvement of tumor-initiating DNA damage and interference with tumor-suppressive mechanisms. On the other hand, some scientists[12] attributed the nuclear changes to apoptotic process induced by oxidative stress, and they added that apoptotic cell death also changes epithelial cell barrier and permits infiltration by microorganisms.

Widening of spaces between epithelial cells that was observed ultrastructuraly in A.A.-group may be attributed to disruption of tight junctions as documented by some authors[25]. They stated that defects in the construction and function of apical junctional complexes are concerned in both animal and human models of IBD.

Dilated congested blood vessels in the lamina propria and submucosal vascular congestion or even hemorrhage were observed in the current study and other previous studies[12]. These findings may be attributed to vasodilator substances released by the blood vessels into the blood, triggering vasodilatation and stagnation of blood[26,27].

Previous studies[6,28] observed heavy inflammatory cellular infiltration in both lamina propria and submucosa of the colon in acetic acid induced ulcerative colitis and this goes in line with the current study. Moreover some authors[6] stated that neutrophil infiltration of inflamed mucosa is one of the primary histological features of IBD.

Light microscopic examination of A.A. group using H&E and PAS stain revealed decrease in the number of goblet cells that was confirmed by morphometric results. Additionally, coalescence of mucin granules was observed ultrastructuraly. Similar findings were recorded by other studies[6,29]. Goblet cell depletion can be explained as a result of tissue damage during the inflammatory process. In the same way, some scientists[30] stated that the decreased number of mucus containing goblet cells was one of the primary hallmarks of acute inflammation during UC.

The current study light and morphometric results in A.A.-treated group showed strong COX2 immunoexpression which is one of the inflammatory proteins expressed excessively in the inflamed areas as noted previously by some authors[31]. This goes in line with another study[32] who reported that COX-2 level in the colon was significantly increased in acetic acid induced colitis compared to the control group. Moreover, some scientists[33] stated that COX2 expression is increased significantly in experimental colitis models and in patients with IBD.

Vacuolation, swollen mitochondria, and rarefaction of the cytoplasm of columnar cells observed in this study, may be attributed to acute cell swelling as recorded by

some authors[34]. They stated that acute cell swelling is an initial phase in the progression of cell death that is caused by Ca2+ precipitate in the cytosol and in organelles, especially mitochondria.

Light, ultrastructural examination and microscopic scoring of the colon of rats treated with A.G. showed restoration of normal histological architecture. The decrease of inflammation caused by A.G. in the present study is in agreement with a previous study by some scientists[35] who stated that A.G. has anti-inflammatory, and immunomodulatory effects. A.G. might also scavenge oxidants like ROS and free radicals and prevent the release of proinflammatory cytokines by activated macrophages and leukocytes, both of which contribute to tissue damage in UC. This goes in line with some studies[36,37] who studied the antioxidant properties of A.G.

Some authors[38] stated that A.G. binds with cations as calcium and magnesium which enhance the healing of the gastrointestinal ulcers. They added that A.G. transforms into a gelatinous state and absorbs a large amount of water, therefore, acting as a mechanical laxative. Moreover, A.G. does not prevent the consumption of vitamin A, one of the important factors in ulcer healing. Furthermore, previous study[39] showed that A.G. improve peptic ulcer in experimental rats.

CONCLUSION AND RECOMMENDATIONS

Based on the current study and from all previously mentioned histological and immunohistochemical results, we recommend administration of A.G. for people who are suffering from UC for restoration of histoarchitecture of the colon. Meanwhile, more investigations and more researches on humans are required to confirm the results of the animal studies.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

الدور الوقائي المحتمل للصمغ العربي في تثبيط التهاب القولون التقرحي المستحث بحمض الأسيتيك (دراسة نسيجية وهستوكيميائية مناعية)

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المقدمة: ان التهاب القولون التقرحي هو مرض خطير أصبح أكثر شيوعًا في جميع أنحاء العالم. والذي يتميز بالالتهاب الحاد اوالمزمن اوالمتكرر ومن اعراضه عدم راحة البطن والإسهال. اما الصمغ العربي فهو عبارة عن إفراز صمغي طبيعي وله مجموعة متنوعة من الفوائد العلاجية.

الهدف من البحث: لقد كان الهدف من البحث هو تقييم دور الصمغ العربي في تثبيط التهاب القولون التقرحي المستحث بحمض الأسيتيك باستخدام طرق الفحص النسيجية والهستوكيميائية المناعية.

المواد وطرق البحث: تتضمنت هذه الدراسة اربعين ذكراً من الجرذان البيضاء البالغة. تم تقسيمهم عشوائيا الى اربعة مجموعات :المجموعة الأولى تمثل المجموعة الضابطة. المجموعة الثانية أعطيت الصمغ العربي بجرعة (٧,٥جم / كجم / يوم) عن طريق الفم لمدة ١٠ أيام متتالية. اما المجموعة الثالثة فقد تم احداث التهاب القولون عن طريق تلقي الفئران ٤ ٪ من حمض الأسيتيك (٢ مل / جرذ). كما تناولت المجموعة الرابعة الصمغ العربي لمدة ٧ أيام، قبل احداث التهاب القولون واستمر تناول الصمغ العربي لمدة ٣ أيام بعد احداث الالتهاب بنفس جرعة وطريقة المجموعتين الثانية والثالثة. وتمت معالجة عينات القولون لتقنيات نسيجية وكيميائية مناعية مختلفة. كما تم إجراء دراسات قياسية وإحصائية.

النتائج: أظهرت المجموعة الثالثة تدمير التجاويف مع تكوين شكل يشبه الكيس وفقدان سطح الخلايا الظهارية. ووجد ايضا تمدد بالأوعية الدموية ونزيف وتسلل للخلايا الالتهابية. واظهر الفحص باستخدام المجهر الالكتروني، اتساعًا للمساحات بين الخلايا العمودية، وتآكل السيتوبلازم بها ، كما لوحظ انتفاخ الميتوكوندريا بداخلها. علاوة على ذلك ، ظهرت انوية هذه الخلايا غير منتظمة مع تكثف الكروماتين. وفيما يتعلق بالخلايا الكأسية ، لوحظ اندماج الحبيبات الإفرازية بها. علاوة على ذلك ، ظهر انخفاض ملحوظ في متوسط عدد الخلايا الكأسية ، بينما أظهر متوسط كثافة لون رد الفعل المناعي للسيكلواوكسيجناس ٢ والمقياس المجهري زيادة عالية وملحوظة ، عند مقارنتها بمجموعة التحكم. بالإضافة إلى ذلك ، فإن المجموعة الرابعة أظهرت اثباط تغيرات الالتهاب القولون التقرحي.

الخلاصة: يمكن تقليل تغيرات التهاب القولون التقرحي باستخدام الصمغ العربي.