

# Antioxidant Ameliorating Effect of Setria (Reduced Glutathione) on Experimentally Induced Colitis in Adult Male Albino Rats

Original  
Article

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## ABSTRACT

**Introduction:** Ulcerative colitis is a common worldwide health problem. Its etiology remains unknown but it's commonly associated with reduced antioxidant activity and free radicals formation. Glutathione is an important antioxidant present in human body.

**Aim:** To evaluate the possible ameliorating effect of Setria on acetic acid induced ulcerative colitis.

**Materials and Methods:** Thirty six adult male albino rats were divided into four groups. Group I (control): eighteen rats divided equally into three subgroups. Group II: six rats injected acetic acid intrarectally under anesthesia. Group III: six rats received Setria 50 mg/kg/day via an oral gavage. Group IV: six rats received acetic acid then after two days received Setria same dose and route as group II, III. At the end of the experiment, blood samples collected to measure MDA and GSH level. Colon samples were processed for histopathological, immunohistochemical, scanning electron microscopic examination and morphometric analyses.

**Results:** Macroscopic and microscopic grading of the specimens was done. Examination of distal colon sections from group II showed shedding of the surface epithelium with absence of mucosal crypts, hemorrhage and wide area of necrosis. That is accompanied with an apparent increase in submucosa, muscle layer thickness with heavy infiltration by inflammatory cells and vacuolation. PAS sections showed decrease in goblet cells, Mallory trichrome sections increase in collagen deposition in submucosa, Touildine blue section revealed multiple mast cells and iNOS sections showed strong reaction. Scanning electron microscope showed maceration of mucosa, wide cryptal opening with lack of microvilli. Examination of group IV sections showed apparent improvement in almost all layers.

**Conclusion:** Setria had an ameliorating effect on acetic acid induced colitis.

**Received:** 14 February 2020, **Accepted:** 09 April 2020

**Key Words:** Acetic acid induced colitis; iNOS; scanning electron microscope; setria.

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**ISSN:** 1110-0559, Vol. 44, No.1

## INTRODUCTION

Inflammatory bowel disease (IBD) has been a worldwide health-care problem. It includes ulcerative colitis (UC) and Crohn's disease (CD)<sup>[1,2]</sup>.

Ulcerative colitis (UC) is a chronic inflammation in the colonic mucosa with intervals of remissions and exacerbations that can markedly increase the risk of colon cancers by 10-fold. It is affecting the large intestine, involving the rectum, the sigmoid colon, descending colon, and sometimes the entire colon<sup>[3,4]</sup>.

Patients of UC suffer from frequent acute relapses which characterized by recurrent abdominal pain, prolonged diarrhea, and stool with pus, blood, and mucus<sup>[3,5]</sup>.

It is an idiopathic disease and its etiology remains unknown. Several factors are involved in both initiation and progression of colitis like genetic susceptibility, environmental factors and altered immune response<sup>[6,7]</sup>.

UC has been commonly associated with reduced antioxidant capacity as well as increased free radical production such as reactive oxygen and nitrogen species<sup>[1,8]</sup>.

Reactive oxygen species (ROS) have been mentioned to have a significant role in UC pathogenesis. Overproduction of ROS leads to lipid peroxidation (LPO); this can inhibit cellular antioxidant capability finally resulting in prominent colonic inflammation<sup>[1]</sup>. ROS has a main role in oxidative stress and apoptosis development<sup>[8]</sup>.

So, increased oxidant production and decreased antioxidant capacity are well-known characteristics of UC<sup>[9]</sup>.

The acetic acid (AA) induced ulcerative colitis is a widely used in experimental animal to induce inflammation and ulceration in the rectum and the colon in rats; simulating colitis in humans<sup>[6,8]</sup>.

Nowadays, the clinical management of UC is based on using anti-inflammatory agents such as aminosalicylates

(sulfasalazine), corticosteroids and immunosuppressants. The failure of these treatments to be generally efficient with the high incidences of adverse effects makes it necessary to explore new ways of treatment<sup>[3,6]</sup>.

Glutathione is a water-soluble, low molecular weight consists of the tripeptide amino acids<sup>[10]</sup>. In the human body, glutathione exists in both the oxidized disulfide form (GSSG) or reduced (GSH) state. Dynamic balance controls the ratio between GSH and GSSG<sup>[11]</sup>.

GSH is an important antioxidant present in almost all cells in the body<sup>[12]</sup>. Sources of GSH in the intestinal mucosa include biliary supply, intracellular synthesis, and dietary intake. The intestinal lumen receives a large quantity of GSH from diet like fresh fruits, vegetables, and many types of meat<sup>[13,14]</sup>.

It plays a major role in maintaining health and preventing disease through detoxification of endogenous metabolic products<sup>[10,15]</sup>.

GSH is safe for usage as a dietary supplement<sup>[16]</sup>. In many trials, it used for improvement of liver abnormalities, diabetic complications, protection from viral infection, reverse the toxic effects from other amino acids overdose<sup>[11,17]</sup> and even used to treat autism<sup>[18]</sup>. Also, it has anti-melanogenic and antiaging properties<sup>[11,19]</sup>.

The present work aimed to study the possible ameliorating role of oral GSH on acetic acid induced ulcerative colitis in adult male albino rats; this was done using serological and histological methods.

## MATERIALS AND METHODS

### Animals

Thirty six adult male albino rats of Wistar strain (14-16 weeks old); weighing 200- 220 g were used in the present study. Animals were obtained from the animal house of Research Center and Bilharzial Research Unit of Faculty of Medicine, Ain Shams University.

Rats were allowed free access to water and food and were housed in rooms with 12 hours day and night cycle. They kept in good hygienic conditions, well ventilated wire cages and temperature of 21±3°C. Animals were left two weeks for acclimatization before the start of the experiment. All animal procedures were approved by CARE (Committee of Animal Research Ethics) of the Faculty of Medicine at Ain-Shams University.

### Chemicals

- Acetic acid (AA) solution (MW 60.05, A 0078111) was purchased from El-Nasr Pharmaceutical Chemical Co. (ADWIC), Egypt. It was prepared in 0.9% NaCl to get a concentration of AA 4% v/v.
- GSH (Setria®) (Cas NO. 70-18-8) was imported from KYOWA HAKKO BIO CO., LTD (Tokyo, Japan).

### Experimental design and drug administration

Rats were randomly divided into four groups as follows:

**Group I (Control group and sham control):** eighteen rats divided equally into three subgroups:

**Ia:** six rats were not subjected to any procedure and served as a control.

**Ib:** six rats were administered a single dose intracolonic injection of 1 ml saline as a vehicle for AA. Same preparation for this procedure as G II was done.

**Ic:** six rats were administered 1 ml saline by oral gavage for 7th days as a vehicle for Setria.

**Group II (ulcerative colitis group):** Induction of colitis (6rats)

Rats were fasting for 48 hours with free access for water and libitum. Then they were anesthetized by an intraperitoneal injection of 1% sodium pentobarbital in a dose of 50 mg/kg before induction of colitis<sup>[20]</sup>, 2 mm-diameter soft pediatric catheters were lubricated with KY gel and inserted 8-10 cm proximal to the anus. One mL of 4% acetic acid was injected into the catheter<sup>[8]</sup>. The solution was instilled into the colon and rats were maintained in a supine Trendelenburg position for 30 seconds to prevent leakage<sup>[4]</sup>.

**Group III (rats received Setria) (6rats):** Rats received Setria (50 mg/kg/day) dissolved in normal saline by oral gavage through feeding needle as a single daily dose all through the experiment<sup>[10]</sup>.

**Group IV (Setria-AA treated group) (6rats):** Rats received a single dose of AA same route and dose as group II, then two days after start to receive a single daily dose of Setria for 7 consecutive days the same route and dose as group III.

At the end of the experiment, animals were anesthetized by ether inhalation. Blood and tissue samples were collected. Then the colon excised 8-10 cm above the anal margin, opened longitudinally along its mesenteric border and rinsed with cold saline to clean, examined for macroscopically assessment then proceed for histological, immunohistochemical studies and scanning.

### I. Assessments of Colitis

The mucosal injury was assessed macroscopically using the grading scale by Morris and colleagues ranging from 0 to 5 (Table 1).

**Table 1:** Index of macroscopic changes in the colonic mucosa<sup>[9]</sup>

0	No damage
1	mucosal erythema only or focal hyperemia with no ulcers
2	a single site of ulceration with no significant inflammation
3	linear ulcer with inflammation at one site
4	two or more sites of ulceration and inflammation, ulcer <1cm
5	multiple sites of injury and inflammation, ulcer > 1 cm

## II. Histological study

For light microscopic analysis, samples were collected and fixed in 10% formalin for 48 h, dehydrated in graded alcohol and embedded in paraffin. Paraffin blocks were prepared using a microtome, for sectioning at 5  $\mu$ m thickness. Sections were stained with

- Hematoxylin and eosin to assess the general morphology and determine the histological grading (Table 2)<sup>[21]</sup>.
- Periodic acid Schiff for polysaccharides in goblet cell assessment<sup>[21]</sup>.
- Mallory trichrome stain was used to assess the amount of collagen deposition and muscle layer thickness<sup>[21]</sup>.
- Toluidine blue stain was used for the mast cell examination<sup>[21]</sup>.

All stained sections were examined with the light microscope and photographed with Lecia ICC50 W camera.

**Table2:** Histological grading of colitis<sup>[3]</sup>

Feature graded	Grade	Description
Inflammation severity		
	0	None
	1	Mild
	2	Moderate
	3	Sever
Inflammation Extent		
	0	None
	1	Mucosa
	2	Mucosa & Submucosa
	3	Transmural
Crypt damage		
	0	None
	1	Basal 1/3 damaged
	2	Basal 2 /3 damaged
	3	crypts lost, surface epithelium present
	4	crypts lost, surface epithelium lost
Percentage of involvement		
	1	1-25
	2	26-50
	3	51-75
	4	76-100

## III. Immunohistochemical study: Inducible nitric oxide synthetase (iNOS)

The bowel samples were incubated with 10% of rabbit serum at room temperature. Then slides were incubated at 4°C with polyclonal primary antibodies against iNOS (iNOS: Product 15323; dilution 1:200, Abcam, USA). After this period, the slides were washed in buffer and incubated at room temperature for 30 min with the biotinylated goat anti-polyvalent secondary antibody

(Catalog # TP-060-BN, Thermo Scientific, USA). Then were washed, dehydrated and embedded in balsam. Localization was counter-stained in Meyer's hematoxylin. Negative control sections were performed with the same procedure but the primary antibody was non-immune rabbit serum<sup>[22]</sup>.

A photomicrograph of positive control from colon immunostained with iNOS was obtained from Abcam catalogue images accompanied with the primary antibody used in this study<sup>[23]</sup>.

All stained sections were examined with the light microscope and photographed with Lecia ICC50 W camera.

## IV. Scanning electron microscope

Small pieces of the colon were fixed in glutaraldehyde 2.5% and dehydrated by graded ethanol using tissue processor (Leica EM TP, Leica Microsystems; Austria). Then drying by CO<sub>2</sub> critical point drier (Model: Audosamdri-815, Tousimis; Rockville, Maryland, USA).

The samples were examined under scanning electron microscopy (Model: JSM-5500 LV; JEOL Ltd-Japan) by using a high vacuum mode at the Regional center for Mycology and Biotechnology, Cairo. Egypt.

## V. Serological investigation

At the end of the experiment, blood samples were collected from puncture of the retro-orbital plexus using a capillary tube for the measurement serum levels of oxidative enzymes (malondialdehyde (MDA) and glutathione (GSH)). All blood samples were processed at Tumor Markers Oncology Research center, Al-Azhar University.

### • Malondialdehyde measurement

MDA (Colorimetric/Fluorometric Assay Kit Catalog # K739-100) is a byproduct of the arachidonate cycle, its level in serum was determined spectrophotometrically by using trichloroacetic acid (TCA) and 1% thiobarbituric acid (TBA)<sup>[24]</sup>.

### • GSH measurements

Glutathione (GSH) Fluorometric Assay Kit (Catalog #: K264-100) in serum was performed. Samples and standards were added to the coated microtiter plate for 30 min at room temperature<sup>[7]</sup>.

## VI. Quantitative morphometric study

Images were analyzed using computer-based software (Leica Qwin 500; Imaging Systems, Cambridge, UK).

The measurements were done in ten non overlapping fields randomly selected in slides of each animal in each group. The following parameters were measured:

- a. Mean number of PAS-positive goblet cells x200 magnification.

- b. The area of percent of collagen deposition using Mallory trichrome stain at x100 magnification.
- c. Mean thickness of the muscle layer using Mallory trichrome stain at x100 magnification.
- d. The number of mast cells stained with toluidine blue at x400 magnification.
- e. Mean number of iNOS positive cells at x40 magnification.

### VII. Statistical study

Serum levels of GSH, MDA enzymes and the morphometric results were recorded and statistically analyzed. Data were expressed as mean and standard deviation (SD) for the quantitative variable. Data were statistically analyzed using statistical package SPSS version 19 (SPSS Inc., Chicago, USA). Comparisons between groups were done using ANOVA (analysis of variance) followed by post hoc test for multiple comparisons between each 2 groups. The results were considered significant when  $p < 0.05$ .

## RESULTS

During the study period, rats from group II (AA induced colitis) showed no signs of mortality but there were morbidity signs of colitis like calmness, diarrhea and redness around anus. In group IV (Setria-AA treated group) showed no mortality and morbidity recorded with general good condition.

Examination of the rat distal colon sections from group I (a, b, c) and group III showed similar findings with no observable differences. Thus, all these groups will be represented as the control group (I) in figures. Concerning statistical results also group III (Setria group) showed non- statistically significant difference in comparison to the group I ( $P > 0.05$ )

### 1- Macroscopic grading of colitis

Macroscopic examination of the distal colons of rats of group II (AA induced colitis) showed an inflammatory response in the form of edema, hyperemia, hemorrhagic spots, and ulceration in comparison to the control group (group I) (Figure 1). The macroscopic score for group II showed a highly statistically significant increase in comparison to group I ( $P < 0.001$ ).

However, the treatment of rats of induced colitis with Setria (group IV) for 7 days revealed improvement in the macroscopic finding. The macroscopic score for group IV showed a highly statistically decrease in comparison to group II ( $P < 0.001$ ) (Table 3 and Histogram 1).

### 2- Histological Results

#### A. Histological grading of colitis

Hx & E stained colonic sections of rats in group II (colitis group) revealed multiple areas of distorted crypts, vacuolation, necrosis, hemorrhage, submucosal edema and inflammatory infiltration in the submucosa.

Histological grading for group II showed a highly statistically significant increase in comparison to group I ( $P < 0.001$ ).

However, the treatment of rats of induced colitis with Setria (group IV) for 7 days revealed improvement in the histological finding. Histological grading for group IV showed a highly statistically decrease in comparison to group II ( $P < 0.001$ ) (Table 3 and Histogram 2).

#### B. Histological observation

##### Group I and III (control group and Setria group):

Light microscopic examination of hematoxylin and eosin (Hx&E) stained sections of the rat distal colon from the group I showed large intestine layers which are arranged from in to out; mucosa, submucosa, muscle layer and serosa. The mucosa is intact, lined by simple columnar epithelium folded in tubular crypts and contains multiple goblet cells (Figure 2).

Examination of periodic acid Schiff stained (PAS) section of the rat distal colon from the group I showed numerous PAS-positive goblet cells in the colonic mucosa (Figure 3). In Mallory Trichrome stained section examination showed muscle layer thickness and little amount of collagen fibers in the submucosa and in-between the crypts (Figure 4). Moreover, examination of Toluidine Blue stained sections showed apparently absence of mast cells (Figure 5).

Immunohistochemically, iNOS stained section of the rat distal colon from the group I showed a weak immune reaction in the lamina propria of the mucosa (Figure 6).

Scanning electron microscopic examination of sections of the rat distal colon from the group I showed microvilli of the cells with narrow crypt opening full of mucus secretion (Figures 7, 8).

**Group II (AA induced colitis group):** Examination of hematoxylin and eosin (Hx&E) stained sections of the rat distal colon from group II showed mucosal architectural distortion in the form of shedding of the surface epithelium with absence or shortening of mucosal crypts and hemorrhage (Figures 9, 10, 11). Mucosal ulcer and wide area of necrosis were also observed (Figure 10).

There was an apparent increase in submucosa thickness with heavy infiltration by inflammatory cells, hemorrhage, vacuolation and submucosal edema and necrosis (Figures 9, 10, 11). Moreover, plenty of eosinophils and mast cells were noticed (Figure. 12).

The muscle layer showed an apparent increase in thickness with myocyte vacuolation (Figure 9).

Periodic acid Schiff stained section of the rat distal colon from group II showed few PAS-positive goblet cells (Figure 13). Mallory Trichrome-stained section from group II showed an apparent increase in muscle layer thickness with marked deposition of collagen fibers in-between crypts and submucosa (Figure 14). In Toluidine

Blue stained sections showed multiple mast cells in the submucosa (Figure 15).

Examination of immunohistochemically iNOS stained section of the rat distal colon from group II showed a strong immune reaction throughout the mucosa within the lamina propria with cytoplasmic staining (Figure 16).

Scanning electron microscopic examination of sections of the rat distal colon from group II showed widening of the crypts opening with maceration of lining mucosa and increase the gap between the cells and connective tissue (Figures 17, 18). Colonic mucosa cells showed a lack of microvilli with the appearance of areas of fibrous tissue (Figure 18) and areas of necrotic tissue (Figure 19).

**Group IV (Setria-AA treated group):** In group IV, the examination of hematoxylin and eosin (Hx&E) stained sections of the rat distal colon showed apparently normal mucosal epithelium, folds, and regular crypts. The submucosa is slightly wide with few inflammatory cells and the absence of congestion in blood vessels. Muscle layer thickness apparently returned to normal in comparison to control (Figure 20).

Examination of periodic acid Schiff stained section of group IV showed numerous PAS-positive goblet cells (Figure 21). Mallory Trichrome stained sections of group IV showed muscle layer thickness and little amount of collagen fibers deposition (Figure 22). In Toluidine Blue stained sections showed few mast cells in the submucosa (Figure 23). The Immunohistochemical iNOS section of group IV showed weak iNOS immune reaction in the lamina propria of the mucosa (Figure 24).

Scanning electron microscopic examination of sections of the rat distal colon from group IV showed slight dilatation of the crypt opening with smooth lining mucosa and filled with mucus secretion. The disappearance of the necrotic tissue and fibrous tissue between crypts opening were observed (Figures 25, 26).

### 3- Statistical results

#### The number of goblet cells

Group II (AA-induced colitis) showed a highly statistically significant decrease in PAS-positive goblet cells in comparison to the group I ( $P < 0.001$ ). On the other hand, group IV (Setria-AA treated group) showed a statistically significant decrease in comparison to the group I ( $P < 0.05$ ) and a highly statistically significant increase in comparison to group II ( $P < 0.001$ ) (Table 4 and Histogram 3).

#### Area of percent of collagen deposition

In group II (AA-induced colitis) there was a statistically significant increase in area of collagen deposition in comparison to the group I ( $P < 0.05$ ). In group IV (Setria-AA treated group) showed a statistically significant increase in comparison to the group I ( $P < 0.05$ ) and a

statistically significant decrease in comparison to group II ( $P < 0.05$ ) (Table 4 and Histogram 4).

#### Mean thickness of the muscle layer

Muscle layer thickness, in group II (AA-induced colitis) showed a highly statistically significant increase in comparison to the group I ( $P < 0.001$ ). However in group IV (Setria-AA treated group) there was a statistically significant increase in comparison to the group I ( $P < 0.05$ ) and a highly statistically significant decrease in comparison to group II ( $P < 0.001$ ) (Table 4 and Histogram 5).

#### Number of mast cells

Mast cell number in group II (AA-induced colitis) showed a highly statistically significant increase in comparison to the group I ( $P < 0.001$ ). In group IV (Setria-AA treated group) showed a statistically significant increase in comparison to the group I ( $P < 0.05$ ) and a highly statistically significant decrease in comparison to group II ( $P < 0.001$ ) (Table 4 and Histogram 6).

#### Mean number of iNOS positive cells

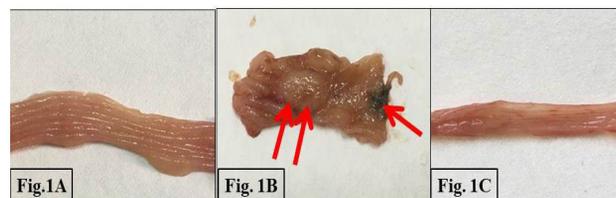
Mean number of iNOS positive cells in group II (AA-induced colitis) showed a highly statistically significant increase in comparison to the group I ( $P < 0.001$ ). In group IV (Setria-AA treated group) showed a statistically significant increase in comparison to the group I ( $P < 0.05$ ) and a highly statistically significant decrease in comparison to group II ( $P < 0.001$ ) (Table 4 and Histogram 7).

#### Serum MDA level

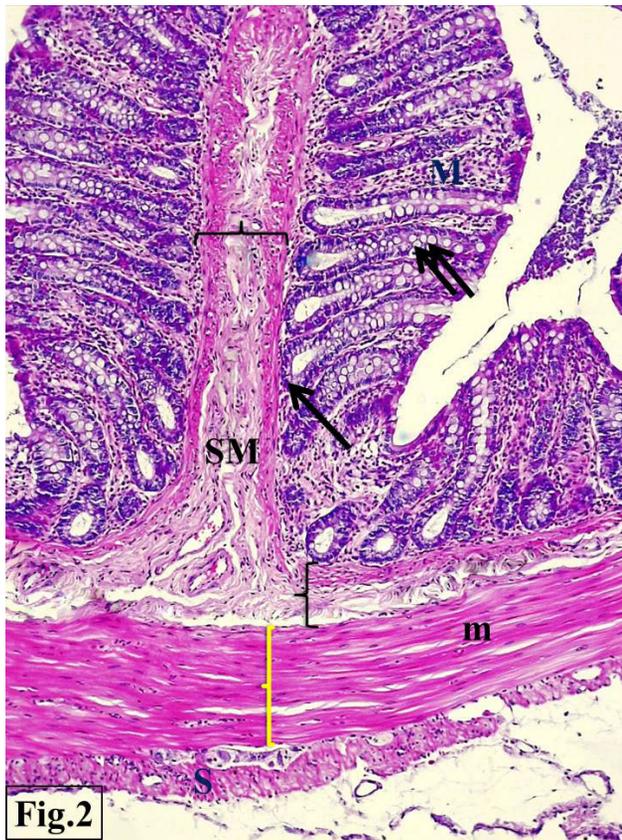
Serum MDA, in group II (AA induced colitis) showed a statistically significant increase in comparison to the group I ( $P < 0.05$ ). In group IV (Setria-AA treated group), it showed a statistically significant decrease in comparison to group II ( $P < 0.05$ ) (Table 5 and Histogram 8).

#### Serum GSH level

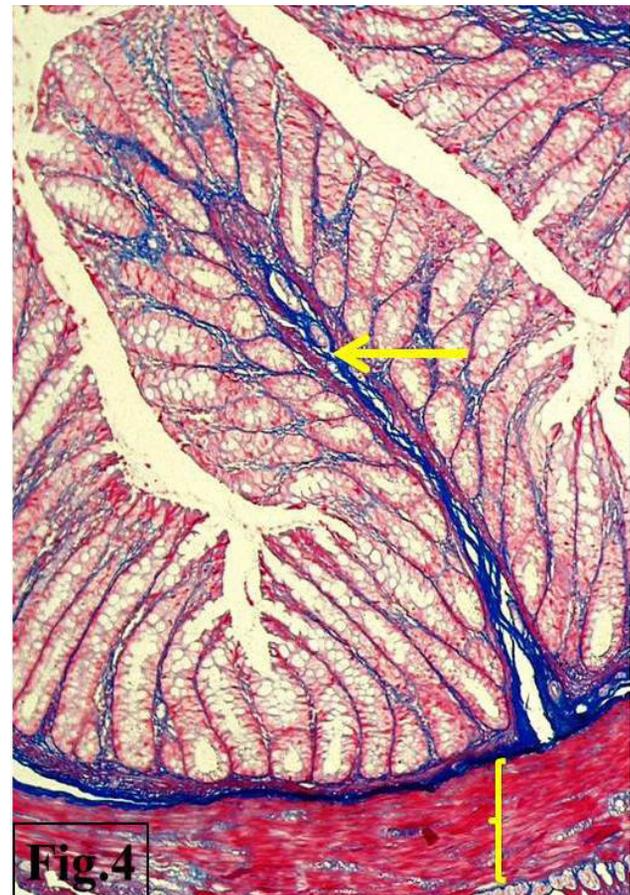
Serum GSH level, in group II (AA induced colitis) were showed a highly statistically significant decrease in comparison to the group I ( $P < 0.001$ ). In group IV (Setria-AA treated group), it showed a highly statistically significant increase in comparison to group II ( $P < 0.001$ ) (Table 5 and Histogram 9).



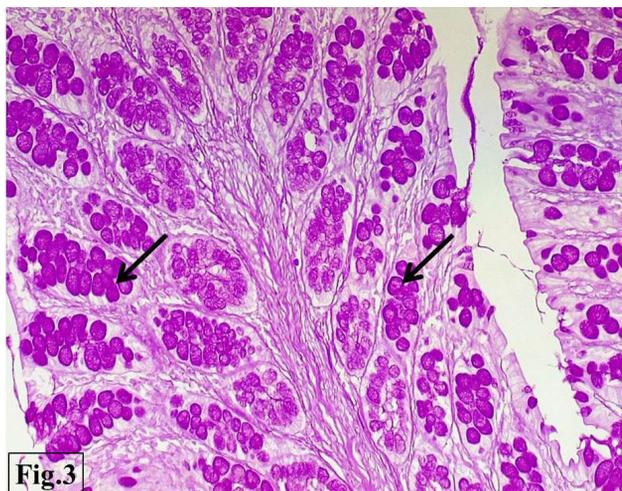
**Fig. 1:** Photomicrographs of the macroscopic appearance of the rat distal colon: A: From the control group, B: From AA induced colitis group showing ulcer (double arrow) and area of necrotic tissue (arrow), C: From Setria treated AA group.



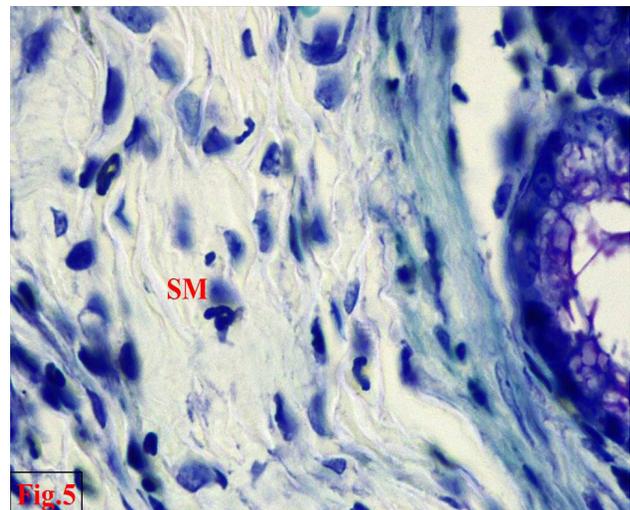
**Fig.2** A photomicrograph of a section of the rat distal colon from the control group (group I) showing layers of the large intestine which is arranged from in to out mucosa (M), submucosa (SM), muscle layer (m) and serosa (s). The mucosa is lined by a simple columnar epithelium folded in tubular crypts (arrow) and contains multiple goblet cells (double arrow). Note submucosal thickness (black line) and muscle layer thickness (yellow line). (Hx&E x100)



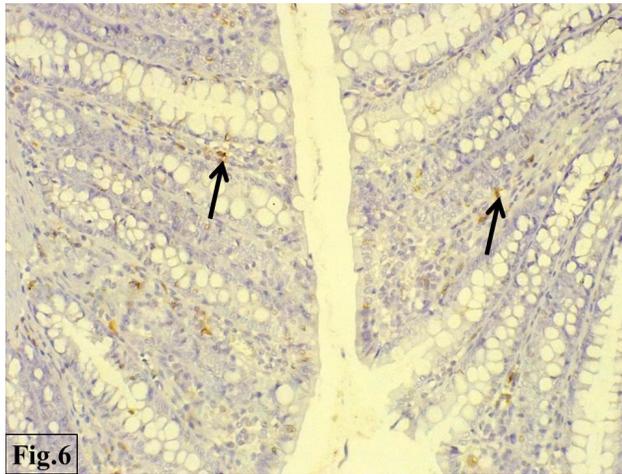
**Fig. 4** A Photomicrograph of Mallory Trichrome-stained section of the rat distal colon from the control group showing the little amount of collagen fibers in the submucosa and in-between crypts (yellow arrow). Notice muscle layer thickness (yellow line). (Mallory Trichrome x100)



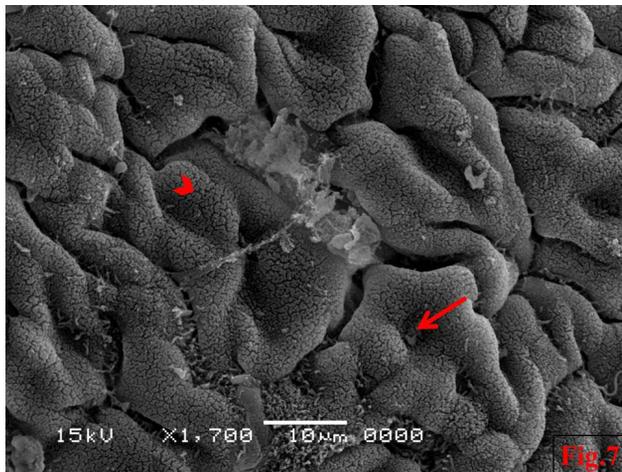
**Fig.3** A photomicrograph of the PAS-stained section of the rat distal colon from the control group showing numerous PAS-positive goblet cells in the colonic mucosa (arrow). (PAS x200)



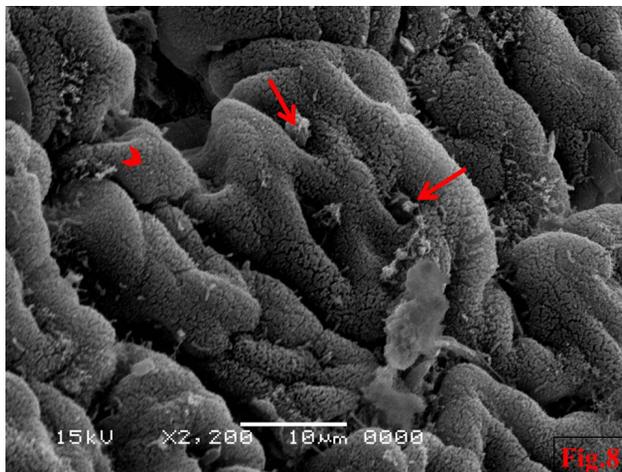
**Fig. 5** A Photomicrograph of the Toluidine blue stained section of the rat distal colon from control group showing apparently no mast cells in the submucosa (SM). (Toluidine blue x1000)



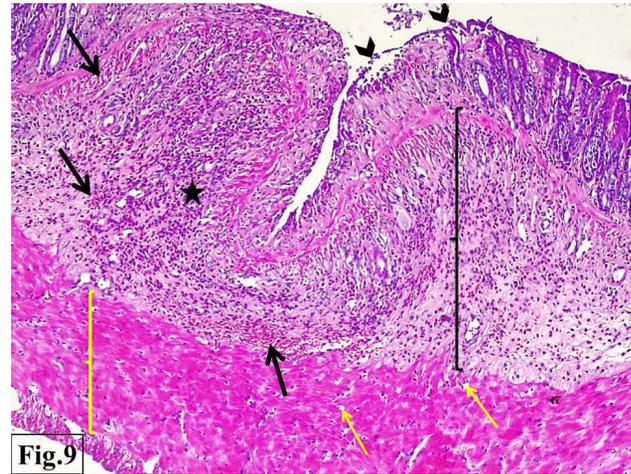
**Fig. 6:** A Photomicrograph of Inducible nitric oxide synthase immune stained section from the control group showing weak iNOS immune reaction in the lamina propria of the mucosa (arrow). (iNOS x200)



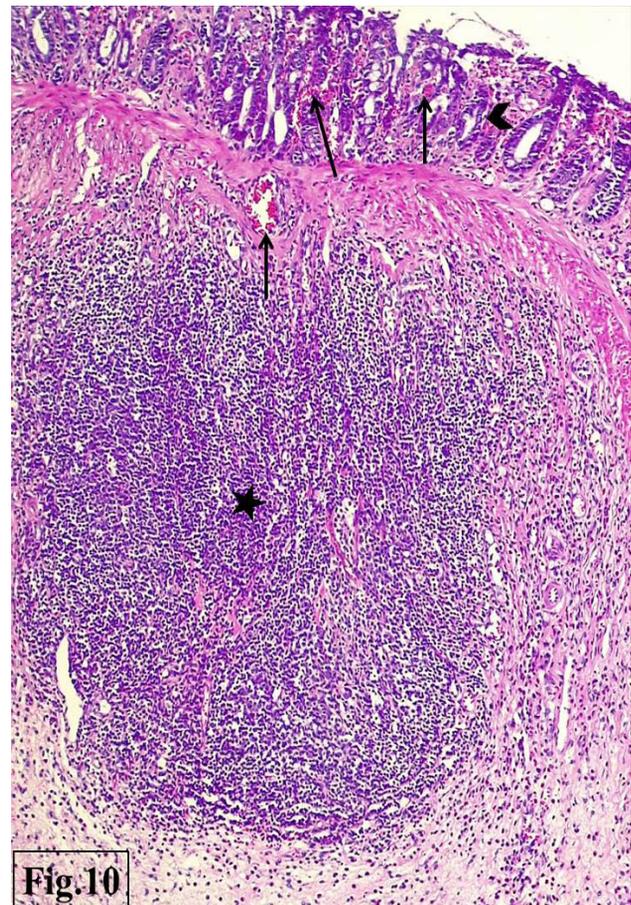
**Fig. 7:** Scanning electron micrograph of a section of the rat distal colon from the control group showing colonic mucosa (arrow head) with its characteristic narrow crypt opening (arrow). (SEM x 1700-Scale bar 10µm)



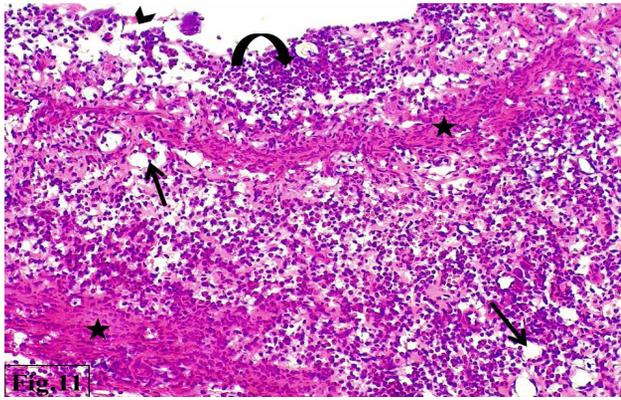
**Fig. 8:** Scanning electron micrograph of a section of the rat distal colon from the control group showing the opening of the crypts containing mucus secretion (arrow) and microvilli of the cells (arrow head). (SEM x 2200-Scale bar 10µm)



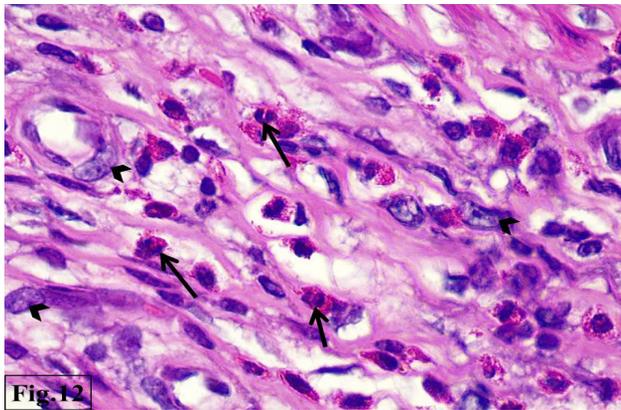
**Fig. 9:** A photomicrograph of a section of the rat distal colon from AA induced colitis group (group II) showing disfigurement of mucosa in the form of shedding of surface epithelium (arrow head) with the absence of mucosal crypts and hemorrhage (double head arrow). There is an apparent increase in submucosa thickness (black line), heavy infiltration by inflammatory cells (asterisk) and hemorrhage (arrow). Notice muscle layer thickness apparently increased (yellow line) with myocyte vacuolation (yellow arrow). (Hx&E x100)



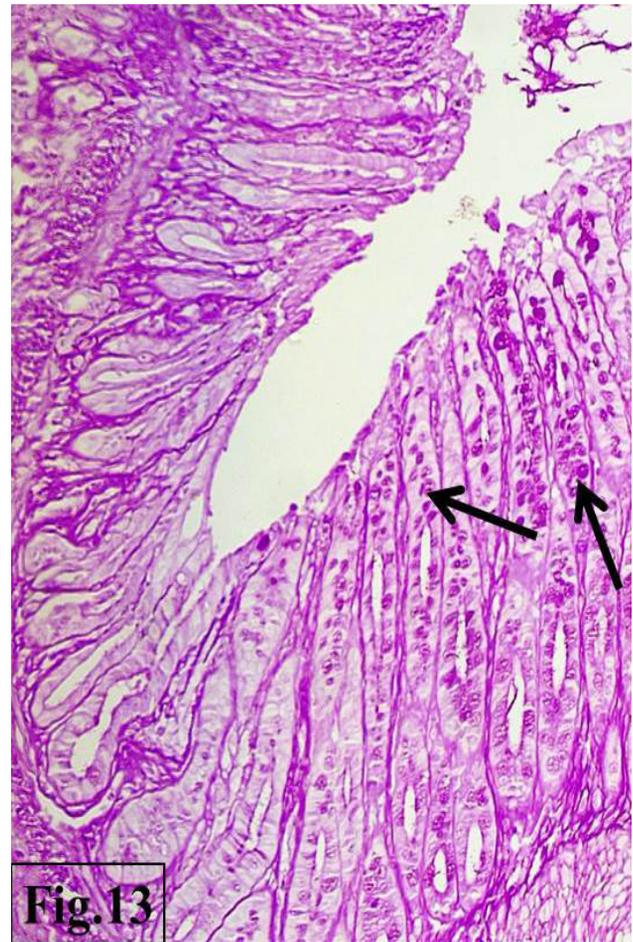
**Fig. 10:** A photomicrograph of a section of the rat distal colon from AA induced colitis group (group II) showing a wide area of mononuclear inflammatory infiltration (asterisk). There is mucosal architectural distortion, including shortening of crypts (arrowhead) and hemorrhage (arrows). (Hx&E x100)



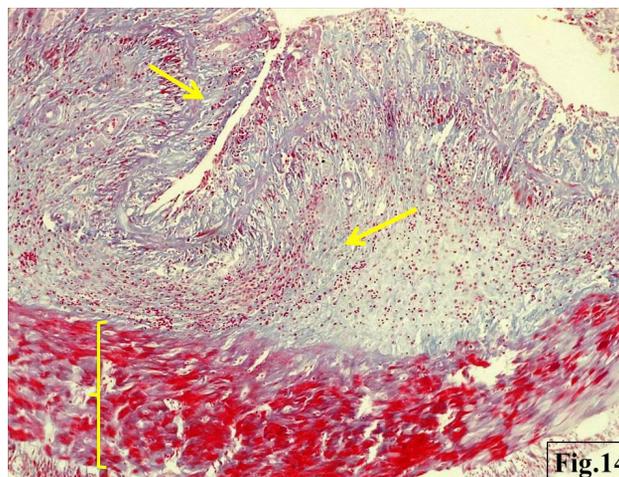
**Fig 11:** A photomicrograph of a section of the rat distal colon from AA induced colitis group (group II) showing wide areas of vacuolation (arrow), submucosal edema and necrosis (asterisk). There was extensive inflammatory infiltration in the mucosa (curved arrow). Shedding of the surface epithelium (arrow head) with the absence of crypts is observed. (Hx&E x200)



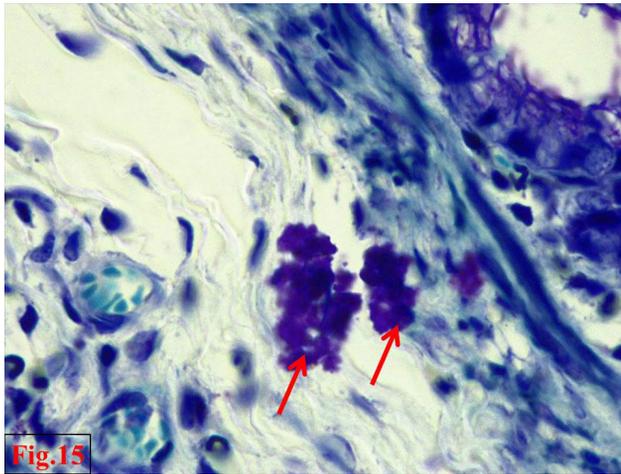
**Fig. 12:** A photomicrograph of a section of the rat distal colon from AA induced colitis (group II) plenty of eosinophils (arrow) and mast cell (arrow head). (Hx&E x1000)



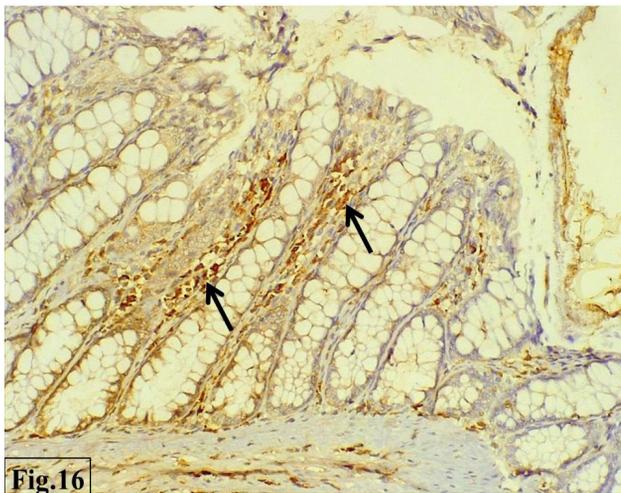
**Fig. 13:** A photomicrograph of PAS-stained section of the rat distal colon from AA induced colitis group showing few PAS-positive goblet cells (arrow). (PASx200)



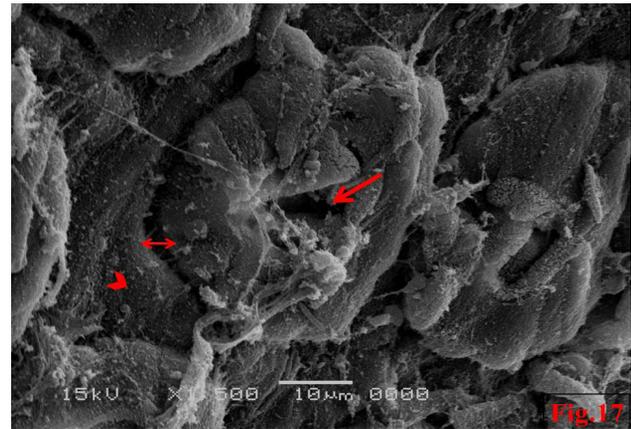
**Fig. 14:** A photomicrograph of Mallory Trichrome-stained section of the rat distal colon from AA induced colitis group showing marked deposition of collagen fibers in-between crypts and submucosa (yellow arrow). Notice muscle layer thickness (yellow line). (Mallory Trichrome x100)



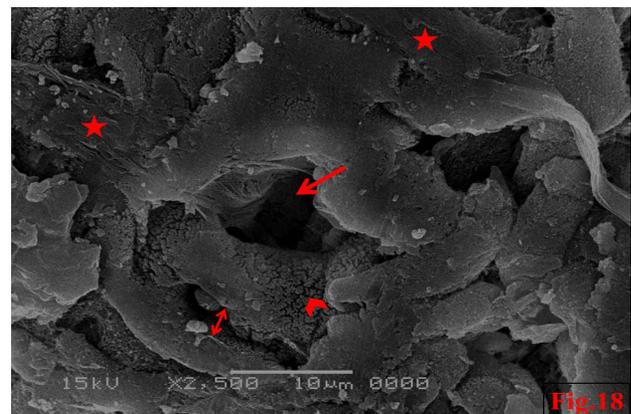
**Fig. 15:** A Photomicrograph of the Toluidine blue stained section of the rat distal colon from AA induced colitis group showing multiple mast cells in the submucosa (arrow). (Toluidine blue x1000)



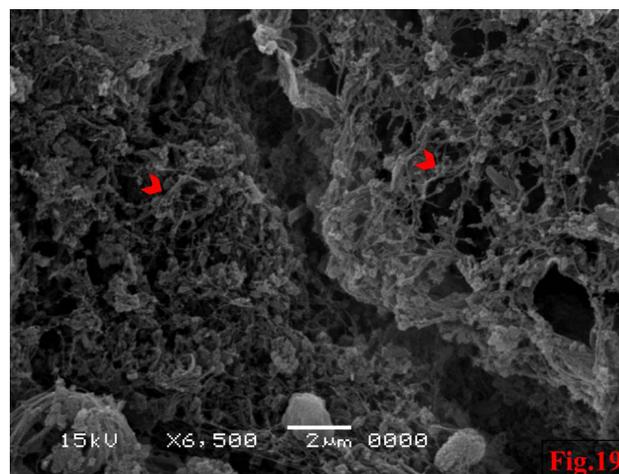
**Fig. 16:** A photomicrograph of Inducible nitric oxide synthase immune stained section from AA induced colitis group showing strong iNOS immune reaction in the lamina propria of the mucosa (arrow). (iNOS x200)



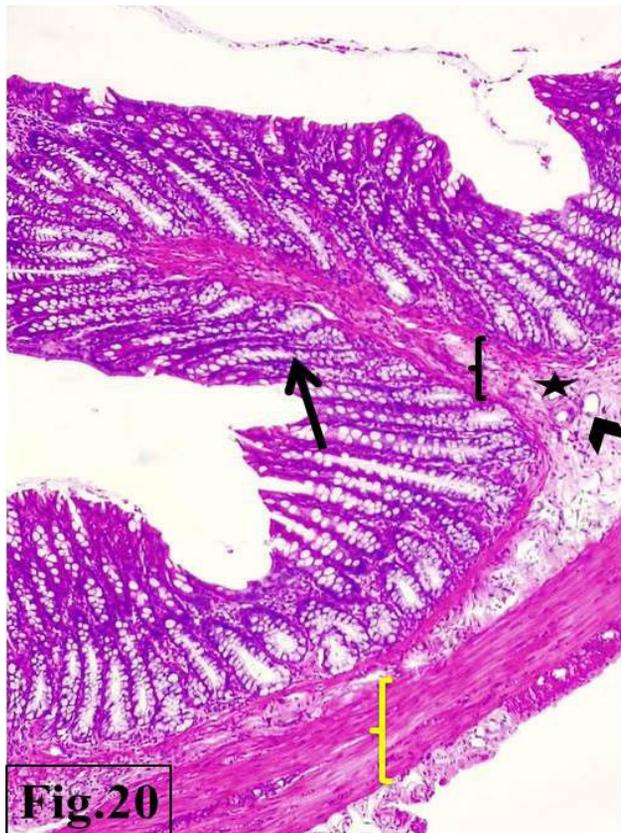
**Fig. 17:** Scanning electron micrograph of a section of the rat distal colon from AA induced colitis group showing widening of the crypt opening (arrow) with lack of microvilli of the cells (arrow head). Notice the gap between cells lining the crypts and connective tissue (double head arrow). (SEM x 1500-Scale bar 10µm)



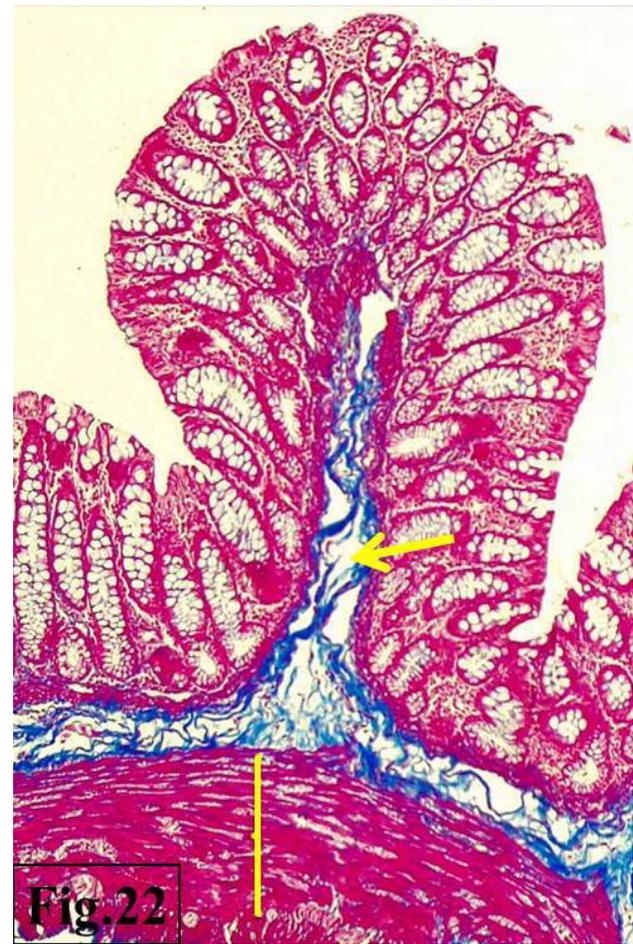
**Fig. 18:** Scanning electron micrograph of a section of the rat distal colon from AA induced colitis group showing widening of the crypt opening (arrow) with maceration (cracks) in the mucosa lining the crypts (arrow head) and a gap between cells lining the crypts and connective tissue (double arrow). Fibrous tissue is observed in the colonic mucosa (asterisk). (SEM x 2500-Scale bar 10µm)



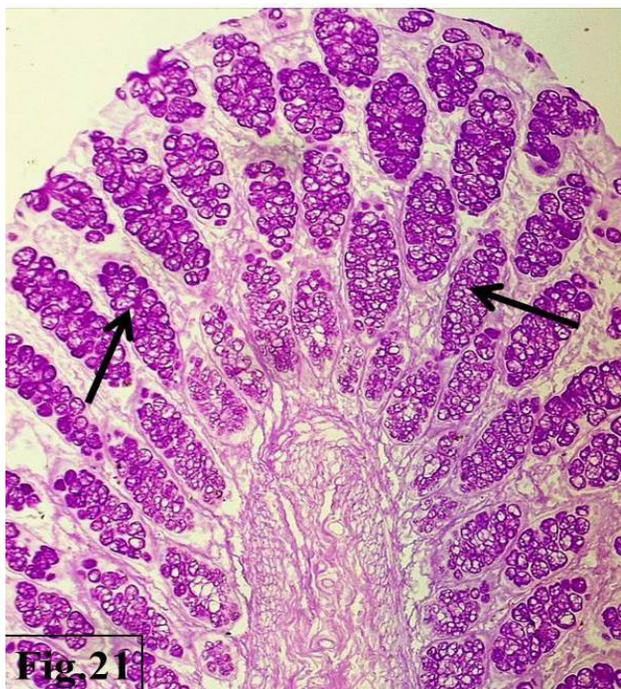
**Fig. 19:** Scanning electron micrograph of a section of the rat distal colon from AA induced colitis group showing necrotic tissue in the colonic mucosa (arrow head) (SEM x 6500-Scale bar 2µm)



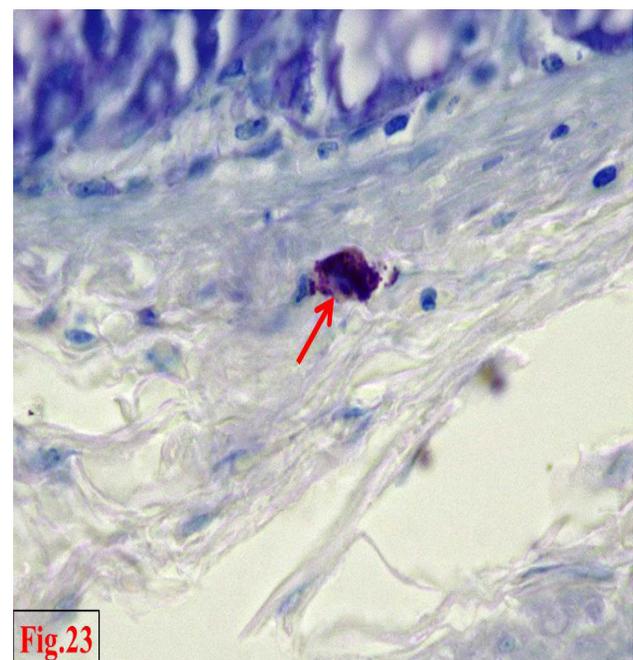
**Fig.20**  
 Fig 20: A photomicrograph of a section of the rat distal colon from the Setria-AA treated group (group IV) showing apparently normal mucosal epithelium, folds, and crypts (arrow). The submucosa is slightly wide (black line) with few inflammatory cells (asterisk) and the absence of congestion in blood vessels (arrow head). Notice muscle layer thickness (yellow line) apparently returned to normal. (H&Ex 100)



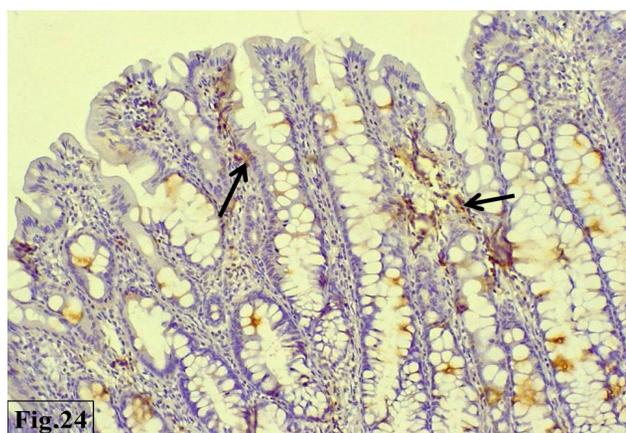
**Fig.22**  
 Fig 22: A photomicrograph of Mallory Trichrome-stained section of the rat distal colon from the Setria-AA treated group showing the little amount of collagen fibers deposition (yellow arrow). Notice muscle layer thickness (yellow line) (Mallory Trichrome x100)



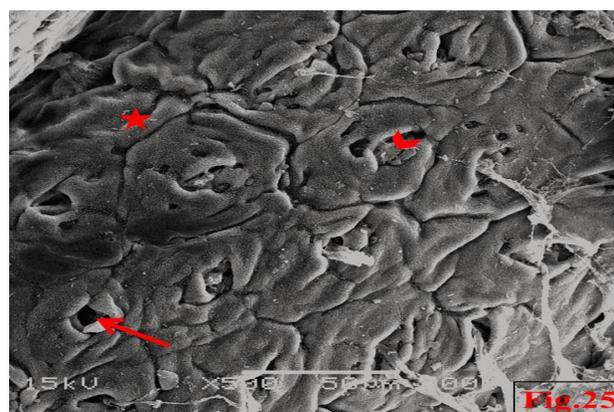
**Fig.21**  
 Fig 21: A photomicrograph of the PAS-stained section of the rat distal colon from the Setria-AA treated group showing numerous PAS-positive goblet cells (arrow). (PASx200)



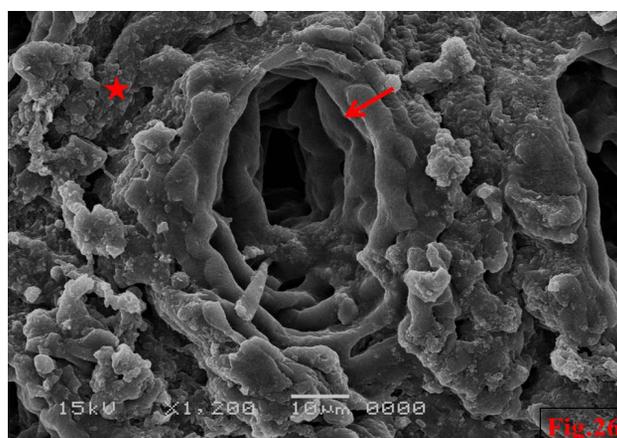
**Fig.23**  
 Fig 23: A Photomicrograph of Toluidine blue stained section of the rat distal colon from the Setria-AA treated group showing mast cell in the submucosa (arrow). (Toluidine blue x1000)



**Fig. 24:** A Photomicrograph of Inducible nitric oxide synthase immune stained sections from Setria-AA treated group showing weak iNOS immune reaction in the the lamina propria of mucosa (arrow). (iNOS x200)



**Fig 25:** Scanning electron micrograph of a section of the rat distal colon from Setria-AA treated group showing slight dilatation in some of the crypt opening (arrow) with mucus secretion inside (arrow head). Notice the colonic mucosa between the openings of the crypt (asterisk). (SEM x 500-Scale bar 50µm)



**Fig. 26:** Scanning electron micrograph of a section of the rat distal colon from the Setria-AA treated group showing the crypt opening (arrow) with smooth lining mucosa. Notice the colonic mucosa between the openings of the crypt (asterisk). (SEM x 1200-Scale bar 10µm)

**Table 3:** Effect of Setria macroscopic and microscopic features of the colon (Mean ± SD)

	G I	G II	G III	G IV
Macroscopic grading	0.6667±0.52	4±0.8944**	0.5±0.5477	0.5±0.5472 <sup>b</sup>
Microscopic grading	0.333±0.516	10.67±2.81**	0.667±0.516	0.833±0.752 <sup>b</sup>

**Table 4:** Effect of Setria on morphometric parameters in the colon after induction of colitis (Mean ± SD)

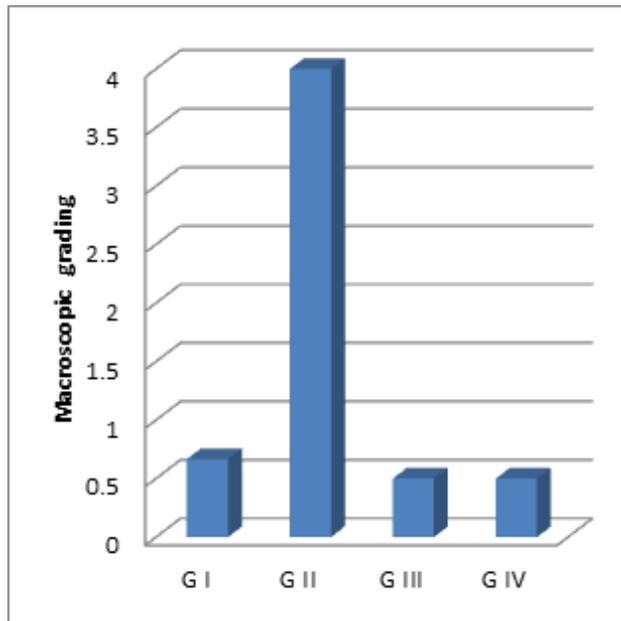
	G I	G II	G III	G IV
Number of goblet cells	76.65±3.781	27.06±12.34**	73.8167±2.15	69.12±3.61 <sup>a,b</sup>
Area of collagen Deposition	10.93±1.484	26.21±11.54*	10.1667±1.45	12.7±1.883 <sup>a</sup>
Mean Thickness of the muscle layer (µm)	83.5±10.949	468.33±96.8**	87.166±3.188	163±21.832 <sup>a,b</sup>
Number of mast cells	0±0	7.33±1.751**	0±0	1.5±1.048 <sup>a,b</sup>
Number of iNOS positive cells	6.833±3.54	45.5±7.12**	7.166±2.483	12.5±3.987 <sup>a,b</sup>

**Table 5:** Effect of Setria on serum MDA, GSH levels after induction of colitis (Mean ± SD)

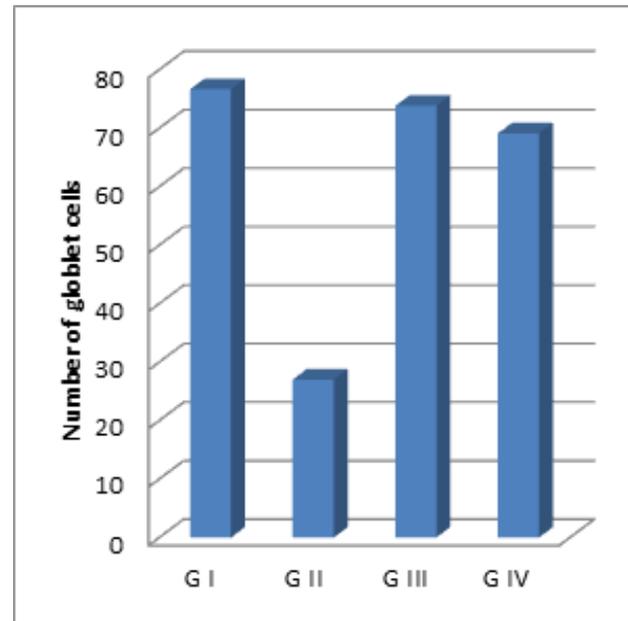
	G I	G II	G III	G IV
MDA (nmol/ml)	2.0833±0.426	8.567±4.045*	1.95±0.3507	1.916±0.348 <sup>a</sup>
GSH (mg/dl)	57.75±1.995	29.6±4.674**	60.05±3.766	56.06±4.096 <sup>b</sup>

Data in Tables (3, 4 & 5) are presented as mean ± SD, mean and analyzed by one-way analysis of variance followed by Bonferroni's multiple comparison test (post-test);  $P < 0.05$  was considered statistically significant in all analyses and  $P < 0.001$  was considered highly statistically significant.

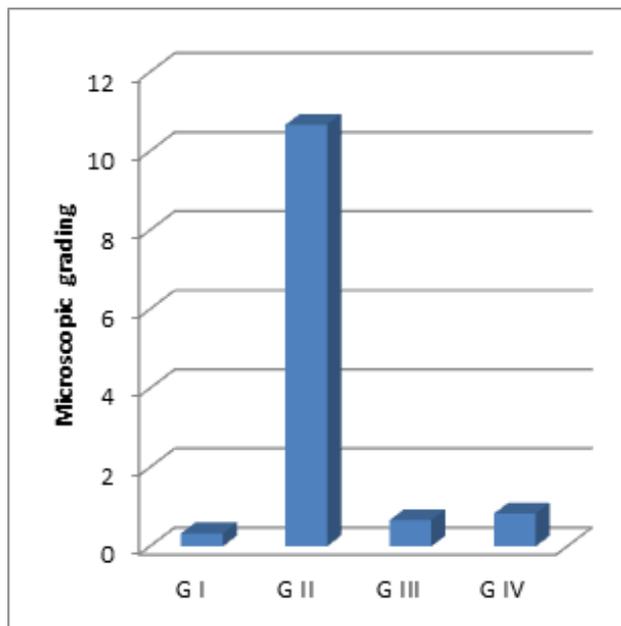
- \* = statistically significant difference from the group I - \*\* = highly statistically significant difference from the group I. - a = statistically significant difference from the group II - b = highly statistically significant difference from group II.



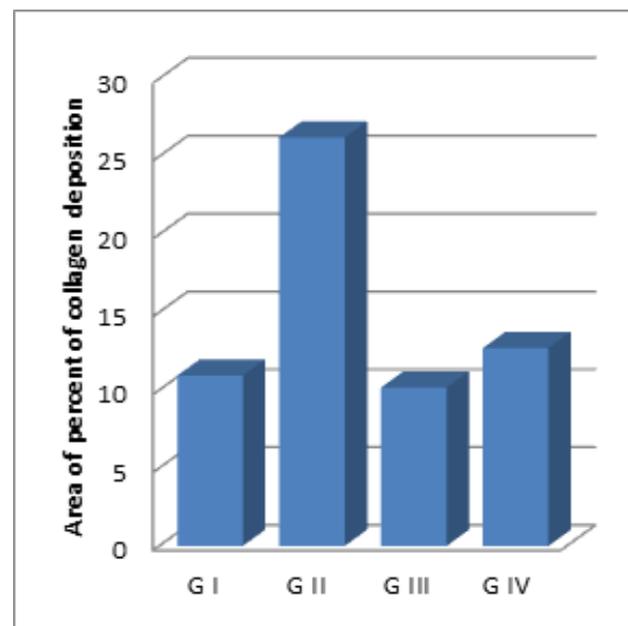
**Histogram 1:** Effect of Setria on macroscopic grading of colitis in different groups.



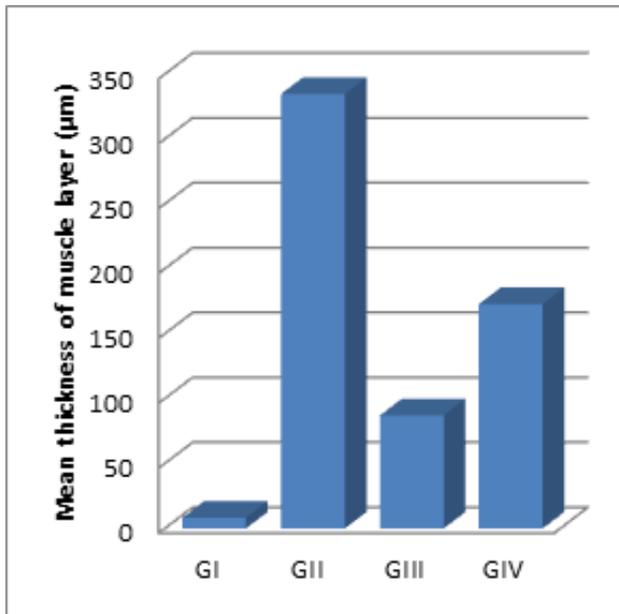
**Histogram 3:** Effect of Setria on the number of goblet cells in different groups.



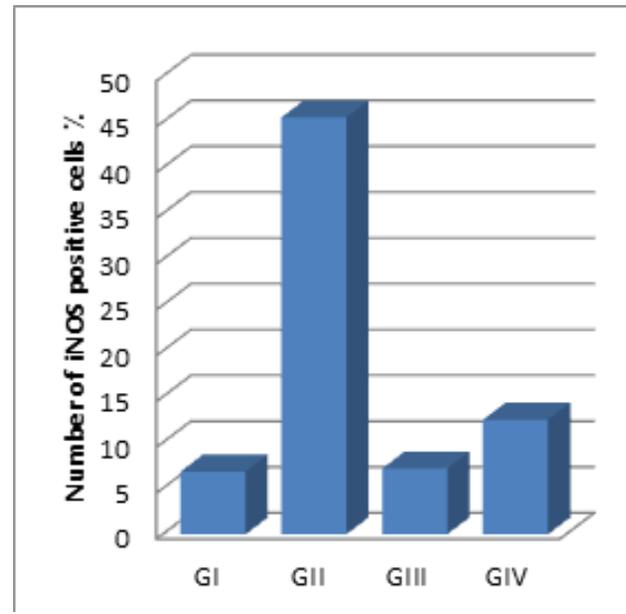
**Histogram 2:** Effect of Setria on histological grading of colitis in different groups.



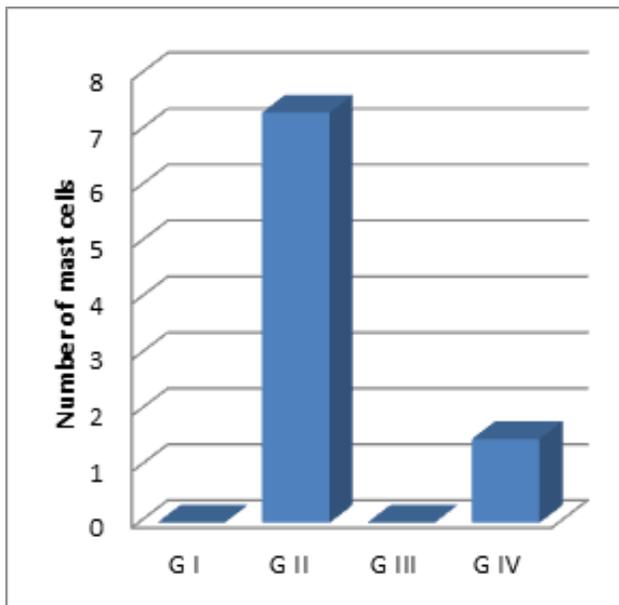
**Histogram 4:** Effect of Setria on the area of percent of collagen deposition in different groups.



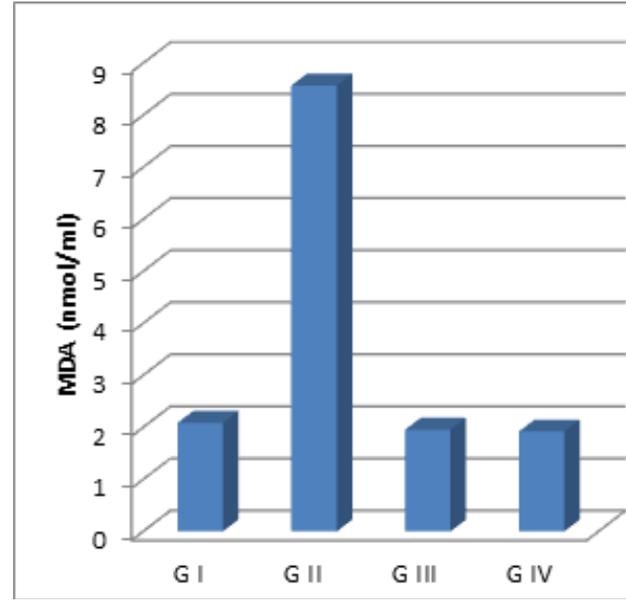
**Histogram 5:** Effect of Setria on the mean thickness of the muscle layer in different groups.



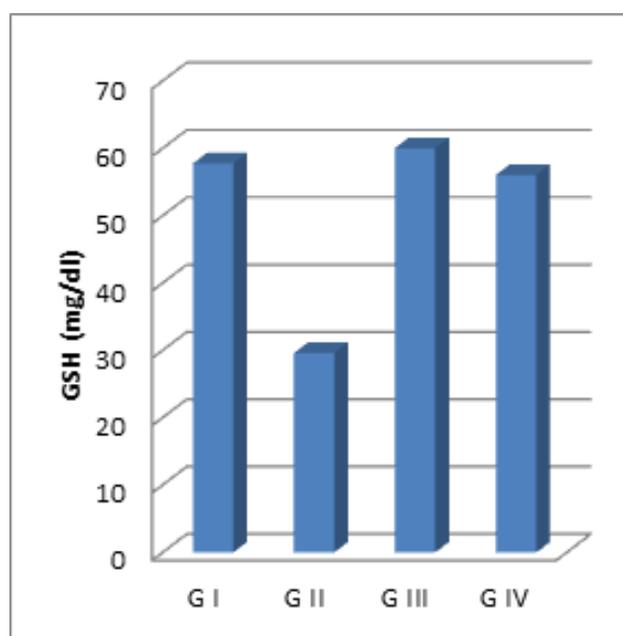
**Histogram 7:** Effect of Setria on the number of iNOS positive cells in different groups.



**Histogram 6:** Effect of Setria on the number of mast cells in different groups.



**Histogram 8:** Effect of Setria on serum MDA level in different groups.



**Histogram 9:** Effect of Setria on serum GSH level in different groups.

## DISCUSSION

Recently, the incidence of inflammatory bowel disease has gradually increased. Ulcerative colitis patients experience cyclic bouts of clinical symptoms<sup>[3,25]</sup>.

AA induced colitis is one of the most frequently used models to induce ulcerative colitis in rodents that mimic the human colitis in many features; clinical, metabolic and histological<sup>[5,26]</sup>.

Oxidative stress had an essential role in the pathogenesis of colitis especially when it is accompanied by decreased antioxidant defense system<sup>[27,28]</sup>.

In the present study, measuring serum levels of MDA and GSH reflected the balance between the anti-oxidative and oxidative systems.

MDA is an indicator of oxidative stress, lipid peroxidation and reflects the degree of tissue damage. In the current study, the MDA serum level showed a statistically significant increase in the colitis group in comparison to the control group. This finding was in agreement with previous studies<sup>[29,30]</sup>. They reported that inflammatory bowel disease is associated with an increase in MDA level and it is an indicator for lipid peroxidation and tissue damage.

During the active lesion in ulcerative colitis and disease progression, inflammatory cell activity increased resulting in free radicals generation. The free radicals diminish the antioxidants and attack the plasma membrane leads to lipid peroxidation and cell damage<sup>[31,32]</sup>.

On the other hand, GSH is a crucial intracellular non-enzymatic antioxidant that has an important role in tissue protection and repair mechanism against tissue injury by free radicals through scavenger properties. In the present study, GSH serum level showed a highly statistically

significant decrease in the colitis group. This finding was in accordance with Franco *et al.* (2009) & Ashry *et al.* (2016)<sup>[6,33]</sup>. They mentioned that during active lesion in AA tissue injury GSH level decreased resulting in disruption of colonic mucosa.

Inflammatory cells lead to the initiation and progression of epithelial injury and the production of reactive oxygen species (ROS) and nitrogen species<sup>[3]</sup>.

In the current study, the examination of hematoxylin and eosin colonic section of group II showed distortion of the normal histological criteria of colonic mucosa. Shedding of the surface epithelium was accompanied by shortened widely spaced mucosal crypts that lined with flat epithelium and marked reduction in goblet cells. These findings were previously reported by Ahmed *et al.* (2012) who mentioned that AA induced colitis is associated with massive epithelial loss, destruction of crypts<sup>[34]</sup>.

Goblet cells are responsible for synthesis of mucin and maintain it as a barrier protecting the epithelium from the intestinal flora and from any attack by chemicals or microorganisms<sup>[35]</sup>.

In the present study, an examination of PAS stained sections revealed a highly statistically significant decrease in the number of goblet cells in group II sections in comparison to the control group. Researchers mentioned before that there was a statistically significant decrease in the number of goblet cells in colitis group sections<sup>[36]</sup>. Others explained cell depletion due to alteration in maturation pattern of mucin in goblet cell leading to accumulation of oxygen species and initiation of the inflammatory process and colon injury<sup>[37,38]</sup>.

There were marked widening of submucosa with intense submucosal inflammatory mononuclear cellular infiltrations with abundant eosinophils and mast cells, submucosal exudate and congested dilated blood vessels. These findings were consistent with earlier studies<sup>[39,40]</sup>.

Some authors explained the widening in submucosa may be due to edema and inflammatory infiltration<sup>[3]</sup>. Other authors clarified that dilated congested blood vessels and submucosal exudate are associated with an increase in inflammatory mediators like nitric oxide<sup>[41]</sup>.

Moreover, inflammatory cell infiltration explained by researchers as a result of an imbalance between oxidant and antioxidant substances that release protease and generate hydroxyl radicals and lipid mediators that accelerate colonic tissue injury<sup>[42]</sup>.

In the current study, the examination of toluidine blue stained sections of group II revealed a highly statistically significant increase in mast cell numbers in comparison to the control group. This finding was in agreement with other investigators who mentioned a significant increase in mast cell number in cases of ulcerative colitis<sup>[4]</sup>.

Researchers found an elevation in serum levels of pro inflammatory mediators like interleukins, cytokines

and prove it has an essential role in the pathogenesis of inflammatory bowel disease. Mast cells could release proinflammatory mediators that are increased in ulcerative colitis pathogenesis<sup>[43]</sup>.

Examination of Mallory trichrome stained sections showed a statistically significant increase in area percentage of collagen fibers when compared to control. The same results detected by other authors<sup>[44]</sup>. Li *et al.* (2014) mentioned that chronic exposure to inflammatory cells converts fibroblast to activated myofibroblasts that leads to initiating the fibrotic process<sup>[45]</sup>.

Moreover, a highly statistically significant increase in muscle layer thickness and myocyte vacuolation detected in the present study. Similar finding described by Ahmed *et al.* (2012) & Hamam *et al.* (2014) they mentioned that inflammatory bowel disease affects bowel smooth muscles like bowel thickness, folding with loss of haustrations<sup>[26,34]</sup>.

There was a highly statistically significant increase in inducible nitric oxide synthase (iNOS) positive cells in the colitis group when compared with control. That was in agreement with Abd El Galil *et al.* (2015) & Olamilosoye *et al.* (2018) they explained that in normal conditions, nitric oxide (NO) expressed in colonic epithelium and produces an anti-inflammatory effect. In the case of ulcerative colitis, NO concentration increased due to overproduction by iNOS and superoxide anion forms free radicals through reacting with the elevated NO<sup>[22,46]</sup>.

Scanning electron microscopic examination revealed a widening of the crypts opening that associated with the replacement of the characteristic mucosal microvilli by fibrous and necrotic tissue. Similar results were detected by Hamam *et al.* (2014)<sup>[26]</sup>.

Setria could reverse the histological, serological and statistical changes associated with colitis. MDA level showed a statistically significant decrease accompanied by a highly statistically significant increase in GSH level in comparison to the colitis group. Examination of Setria Hx&E stained sections showed amelioration of the AA effect as the mucosa appeared lined by columnar epithelium with regular crypts and decrease submucosal and muscle layer thickness. Few inflammatory cells were detected in the submucosa.

Richie *et al.* (2015) mentioned that GSH daily supplement in humans was effective to elevate GSH body stores<sup>[47]</sup>. Researchers proved that jejunal mucosal injury improved by daily oral GSH supplementation through control apoptosis; promote oxygen species metabolism and prevention of oxidative stress<sup>[10]</sup>.

The number of goblet cells was highly statistically significant increased and percent of collagen fibers and immunopositive iNOS cells were statistically significant decreased that when compared to colitis group. Uchida *et al.* (2017) & Olamilosoye *et al.* (2018) they mentioned that treatment with oral GSH has an influence on NO activity and associated with decreased iNOS in the case

of fasting induced jejunal ulcer due to inhibition of ROS by GSH<sup>[13,46]</sup>.

## CONCLUSION

Setria (oral GSH) may be an effective treatment for patients with inflammatory bowel diseases through its antioxidant effect and controlling inflammatory mediators, oxygen and nitrogen species.

## CONFLICT OF INTERESTS

There are no conflicts of interest.

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## الملخص العربي

# تأثير مضادات الأكسدة بالستيريا (الجلوتاثيون المختزل) لتحسين التهاب القولون المستحدث في ذكور الجرذان البيضاء البالغة

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**مقدمة:** التهاب القولون التقرحي هو مشكلة صحية شائعة في جميع أنحاء العالم. لا تزال مسببات المرض غير معروفة ، لكنها مرتبطة بشكل عام بتقليل نشاط مضادات الأكسدة. الجلوتاثيون هو أحد مضادات الأكسدة الهامة الموجودة في جسم الإنسان.

**الهدف:** تهدف هذه الدراسة الي تقييم التأثير المُحسن المحتمل لستيريا على التهاب القولون التقرحي الناجم عن حامض الاستيك.

**المواد والطرق:** ستة وثلاثون من الجرذان الذكور البيضاء استخدمت في هذه الدراسة وتم تقسيمهم الي أربع مجموعات. المجموعة الأولى: كمجموعة ضابطة، تحتوي علي ثمانية عشر جرذا مقسمة بالتساوي إلى ثلاث مجموعات فرعية. المجموعة الثانية: تحتوي على ست فئران تم حقنهم بحمض الاستيك عن طريق القسطره بعد التخدير. المجموعة الثالثة: ست من الجرذان تلقت سيتريا 50 مغ / كغ / يوم عن طريق الفم. المجموعة الرابعة: ست من الجرذان تم حقنها بحمض الأستيك ثم بعد يومين تلقت مستخلص الستيريا بنفس الجرعة وطريقة المجموعات السابقة. في نهاية التجربة، تم سحب عينات الدم وأخذ عينات القولون وتحضيرها للفحص.

**النتائج:** تم تقييم العينات تقييم عيني ومجهري. أظهر فحص عينات القولون للمجموعة الثانية سقوط للخلايا المبطنة للسطح مع وجود نزيف ومساحة واسعة من النخر. يترافق ذلك مع زيادة في سمك الطبقة تحت المخاطية وطبقة العضلات وزيادة كثيفة بالخلايا الالتهابية والتسلل الخلوي. وبفحص عينات حمض شيف الأيودي وجد نقص في خلايا الكأس. وعينات مالوري ثلاثي الألوان زيادة بترسب الكولاجين. وبعينات التولودين الأزرق وجد زيادة بالخلايا البدينة وزيادة بالصبغة الهستوكيميائية المناعية. أظهر الفحص بالمجهر الإلكتروني الماسح تقرحات بالغشاء المخاطي يصاحبه اتساع بفتحات الخلايا. وبفحص أقسام المجموعة الرابعة وجد تحسناً واضحاً في جميع الطبقات تقريباً.

**الاستنتاج:** للستيريا تأثير محسن علي التهاب القولون التقرحي الناتج عن حمض الاستيك.