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Comparative Study between the Effect of Ondansetron, Mesalazine, and their Combination on Oxazolone-Induced Colitis In Albino Rats

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Abstract

Background: Ulcerative Colitis (UC) is an inflammatory bowel disorder that represents a common health problem. 5 Hydroxy Tryptamine 3 (5-HT_3) receptors are widely distributed in the gut and 5-HT_3 receptor antagonists have been reported to have anti-inflammatory effects.

Objectives: Evaluates the possible effects of ondansetron, mesalazine and their combination in oxazolone induced-colitis in albino rats.

Methods: This experiment was performed on 50 male Wister albino rats divided into 5 equal groups; (Group I) control group received intrarectal vehicle of ethanol then oral 0.5% carboxymethyl cellulose daily for 21 days, (Group II) untreated induced-colitis group received intrarectal oxazolone then oral 0.5% carboxymethyl cellulose daily, (Group III) oxazolone induced-colitis rats were treated by mesalazine (100mg/kg/day by oral gavage) for 21 days, (Group IV) oxazolone induced-colitis rats were treated by ondansetron (2mg/kg/day) by intraperitoneal injection for 21 days, (Group V) oxazolone induced-colitis rats were treated by mesalazine and ondansetron in the same dosage regimen. Animal body weights, occurrence of diarrhea, and rectal bleeding were recorded daily over the experiment to calculate Disease Activity Index (DAI), at the end of the study all rats were sacrificed, portions of distal colons were dissected and processed for assessment of myeloperoxidase activity, tumor necrosis factor-α levels, caspase-3 activity, histopathological examination and immunohistochemistry of Toll Like Receptor 2 (TLR2).

Results: The present study showed that treatment with ondansetron produce significant decrease in the whole studied parameters, also its combination with mesalazine produce significant decrease in all studied parameters when compared to untreated induced-colitis group as well as produce better response than each drug alone.

Conclusion: These findings suggest that ondansetron produce promising effects in oxazolone induced-colitis which mimics UC in human. Its combination with mesalazine exhibited synergistic effects superior to each monotherapy. It could be recommended to verify these results in further clinical studies.

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Key Words: *Oxazolone – 5-Hydroxy Tryptamine 3 (5-HT_3) – Ondansetron.*

Introduction

ULCERATIVE Colitis (UC) represents one of the major forms of Inflammatory Bowel Disease (IBD). It is chronic, relapsing, non-transmural inflammatory condition that affects the large bowel for a variable length with a continuous caudo-cranial extension without patchiness or skip lesions. Typical features are mucosal ulcerations, bloody diarrhea, rectal tenesmus and the increased susceptibility to the development of Colorectal Cancer (CRC) [1].

The pathogenesis is complex; it is characterized by aberrant immune responses to environmental and gut microbial triggers in genetically susceptible hosts [2]. UC is still incurable disease in spite of the continuous medical advances. There is a great need for additional therapeutics that focused on the development of new formulations with minimal side effects, improved patient compliance, and therefore better clinical outcomes [3,4].

Chemical-induced models are the most widely used models; one of them is oxazolone induced-colitis model. Oxazolone is haptenating agent used to study the pathological process that involved in UC. It mediates Th2 immune response that is the pathologic feature of UC [5].

Mesalazine (also known as mesalamine or 5-amino salicylic acid, 5-ASA) has a well-established role in the management of UC. It is the first line treatment in active and inactive mild to moderate

UC [6]. It has a selective positive effect on UC in inducing remission, preventing relapse and possibly reducing the risk of cancer [7].

5-Hydroxy tryptamine 3 (5-HT_3) receptors are ligand gated cation channels; that are not only distributed in brain and spinal cord but also widely distributed in the Gastrointestinal Tract (GIT) [8]. These receptors are involved in several functions in the GIT as secretory, peristalsis, emetic and pain responses. Several findings suggest that endogenous 5-HT has pro-inflammatory effects that are mediated via 5-HT_3 receptors in the GIT [9].

Ondansetron is one of the commonly used selective 5-HT_3 receptor antagonists; it is used mainly for the management of nausea and vomiting caused by chemotherapy, radiation therapy, gastroenteritis, and surgery [10]. Nevertheless, its possible role in immune modulation and for treatment of UC is still not fully evaluated.

The aim of this experiment was to investigate the possible anti-inflammatory, immune modulatory and anti apoptotic effect of ondansetron on experimental model of UC.

Material and Methods

Chemicals and drugs:

4-Ethoxymethylene-2-phenyl-2-oxazolin-5-one (oxazolone) was obtained from Sigma-Aldrich Chemical Co. Ondansetron (Emerst, ampoule 4mg/2ml) was obtained from Global Pharmaceutical Industries, Egypt. Mesalazine (Pentasa, tablets 500mg) was obtained from FERRING international center S.A St. Prex, Switzerland, prepared using 0.5% Carboxymethyl Cellulose (CMC) to a final concentration 500mg/15ml and other chemicals are products of Sigma Aldrich Chemical Co., unless indicated otherwise.

Animals groups and treatment protocols:

The current study was carried out in Medical Pharmacology Department, Faculty of Medicine, Tanta University, Egypt, in accordance with the guidance of the Ethical Committee of Medical Research, Faculty of Medicine, Tanta University, Egypt (Approval code: (30368/06/15) in april 2016. It was conducted on 50 adult male Wister albino rats weighing 150-200g obtained from experimental animal colony of Tanta University. Rats were housed in wire mesh cages at room temperature, at constant 12/12 hours dark/light cycle and allowed for 15 days for acclmatization and had free access to standard chew and water through the whole duration of experiment, and they were divided into 5 equal groups (10 rats for each).

Induction of colitis [11]*:*

Experimental colitis was induced in rats by Oxazolone. It was given in a dose of 300 µL of 5% (w/v) oxazolone in absolute alcohol for presensitization on skin as follows:

2cm X 2cm area on the back of each animal was shaved then Oxazolone was applied topically on the exposed skin to induce an allergic reaction followed by intra rectal administration of 450 µL of 5% oxazolone in 50% ethanol solution into the colon on 5th and 7th days as follows:

Rats were fasted 24 hours with free access to water before induction of colitis then they were anaesthetized by ether then using 1mm diameter fine rubber catheter inserted in the colon through the rectum to about 4cm proximal to the anal verge. The animals were kept in a vertical head down position for 45 seconds by holding them up from their tails after intra rectal injection to ensure even distribution of oxazolone solution through the colon [11].

Animal grouping:

Group I (control group): Rats were received vehicle of intra rectal 50% ethanol in the 5 th & 7th days followed by administration of 0.5% CMC orally daily for 21 days.

Group II (untreated induced-colitis group): Rats were received oxazolone intrarectal followed after 2 hours by administration of 0.5% CMC orally daily for 21 days.

Group III (mesalazine treated): Oxazolone induced-colitis rats were treated by mesalazine (100mg/kg/day by oral gavage) [12-14] daily started 2 hours post induction of colitis for 21 days.

Group IV (ondanstron treated): Oxazolone induced-colitis rats were treated by ondansetron (2mg/kg by i.p injection) [4] daily started 2 hours post induction for 21 days.

Group V (combination treated): Oxazolone induced-colitis rats were treated by mesalazine and ondansetron in the same dosage regimen mentioned above for 21 days.

Measurements:

Animal body weights, occurrence of diarrhea, and rectal bleeding recorded daily over the experiment. The Disease Activity Index (DAI) calculated according to the following formula, DAI=[% body weight loss + diarrhea score + rectal bleeding score]/3 [15] where the occurrence of diarrhea was defined as fecal matter adherent to anal verge. Rectal bleeding was defined as any visible blood

seen on the anal verge. The absence/presence of either diarrhea or rectal bleeding was given a score of 0/1; respectively **[16]**.

Tissue sampling and processing:

At the end of the study (at the 21 day post-induction) all rats were sacrificed by means of ether inhalation, abdomen and thorax were opened and the distal 8cm of the colon were dissected, opened longitudinally along its mesenteric border and rinsed 3 times with ice cold saline to remove extraneous material, and divided into two portions: One portion was weighted & homogenized with tissue homogenizer for preparations of tissue homogenate in the following ratio [1 colonic tissue: 10 Phosphate Buffered Saline (PBS) 10mM (pH 7.4)] to be used for determination of different parameters. Tissue homogenate was centrifuged at 12,000 rpm for 20 minutes at 4ºC and the resultant supernatant was assayed for the different estimations. The other portion was processed for Histopathological Examination (H & E) and immunohistochemical staining of Toll Like Receptor 2 (TLR2).

- Spectrophotometric assay of tissue protein levels: A product from Biodiagnostic Company, Egypt, was assayed according to Biuret method **[17]**.
- Caspase-3 activity by using ELISA kit for rat supplied by Glory Science Company, Catalog No. (CK-E90597), Egypt according to the manufacturer instructions. The corresponding levels were expressed as ng/mg colonic tissue protein **[18]**.
- Spectrophotometric assay of Myeloperoxidase (MPO) enzyme activity: It was measured using O-dianisidine and H_2O_2. H_2O_2 acts as oxidizing agent and the reaction catalyzed by MPO present in the sample. The change in absorbance was measured spectrophotometrically at 460nm. Results were represented as μm/min/mg tissue weight for colonic tissue **[19]**.
- Tissue tumor necrosis factor-alpha (TNF- a) levels were assayed using ELISA kits purchased from Biokit Company following manufacturer instructions then the results were represented as pg/mg colonic tissue protein.

Histopathological examination:

The second portions of distal colons were immediately fixed in 10% formalin. Paraffin sections were done (5 micron) and stained with Hematoxylin and Eosin (H & E) and examined by light microscope for histopathological changes.

Immunohistochemical staining of Toll Like Receptor 2 (TLR2):

Four micrometer sections were obtained from the paraffin embedded specimens from distal colons and stained with TLR2 then immunohistochemical scoring was done by qualitative estimation of stain intensity as follows: Negative or zero; mild positive or (+1); moderate positive or (++2); strong positive or (+++3) **[20]**.

Statistical analysis:

Statistical analysis of the obtained results was conducted by Scientific Program of Social Science (SPSS) for windows Version 23 (SPSS, Chicago, Illinois, USA). The values were expressed as the mean, Standard Error of Mean (SEM), one-way analysis of variance (ANOVA) was used for multiple comparisons of parametric values to evaluate the statistical significance between experimental groups followed by post-Hoc test (Tukey's test). Kruskal-Wallis test which is a non-parametric test equivalent to one-way ANOVA followed by Mann-Whitney U-test to test the difference between groups of non-parametric data. The correlation study was calculated using Pearson's correlation for parametric data and spearman's correlation for non-parametric data. p-value <0.05 was considered significant.

Results

The induction of colitis by oxazolone showed a statistically significant increase in the assessed parameters in colonic tissue as MPO and caspase -3 activitiy, as well as TNF-ct levels when compared to control group. In addition this group is manifested by high score of DAI.

Treatment of oxazolone induced-colitis by either mesalazine, ondansetron or both of them exhibited significant decrease in DAI in comparison to untreated oxazolone induced-colitis. Treatment of oxazolone induced-colitis by both mesalazine and ondansetron exhibited non-significant difference in DAI in comparison to monotherapy either by mesalazine or ondansetron (Table 1). Also, treatment of oxazolone induced-colitis by either mesalazine, ondansetron or both of them showed significant decrease of the biochemical parameters when compared to untreated oxazolone induced -colitis group (Table 2). When compared to each other, treatment of induced-colitis by either mesalazine or ondansetron showed non-significant difference in the studied parameters.

When the combination group is compared with mesalazine treated group, it showed significant

decrease in MPO activity, caspase-3 activity and TNF-α levels in colonic tissue, while non-significant decrease in DAI.

When the combination group is compared with ondansetron treated group, it showed significant decrease in TNF-α levels, caspase-3 activity in colonic tissue, while non significant decrease in DAI and colonic tissue MPO activity.

Histopathological examination results:

Histopathological Examination (H & E) of colonic tissue of control group (Group I) showed normal mucosa covered by normal epithelium, the crypts are straight and unbranched with normal population of goblet cells, normal lamina propria and normal muscularis mucosa as shown in Fig. (1) while histopathological examination of colon sections from Group II (untreated oxazolone induced-colitis) showed erosions of the mucous membrane and ulcer formation with lamina propria infiltration with inflammatory cells formed of polymorph nuclear leukocytes, lymphocytes, oxyphilic cells and cystic dilatation of intestinal glands Fig. (2), with crypt abscess formation Fig. (3).

In Group III (mesalazine treated), histopathological examination of colon sections showed restoration of part of normal pattern of mucosa, minimal infiltration of lamina propria with inflammatory cells, minimal congestion and healed crypt abscess Fig. (4), also histopathological examination of colon sections of Group IV (ondansetron treated) showed restoration of normal pattern of mucosa, healed ulcers, minimal infiltration of inflammatory cells and edema Fig. (5) as well as histopathological examination of colon sections of oxazolne induced colitis treated by both mesalazine and ondansetron (Group V) showed better response manifested as normal mucosa, inflammatory infiltrate was scanty and crypts were totally restored Fig. (6).

Immunohistochemical staining of TLR2:

Immunohistochemical examination of the colon of the control group was negative for TLR2 immunoreactivity in intestinal epithelial cells Fig. (7) while Immunohistochemical examination of the colon of the untreated induced-colitis group showed strong positive TLR2 immunoreactivity which appeared as dark brownish cytoplasmic discoloration of intestinal epithelial cells Fig. (8).

In mesalazine treated group, there was mild positive TLR2 immunoreactivity which appeared as brownish cytoplasmic discoloration of intestinal epithelial cells Fig. (9) and in ondansetron treated group, there was mild to moderate positivity for TLR2 immunoreactivity Fig. (10). Also combination group showed apparently negative TLR2 immunoreactivity Fig. (11).

Immunohistochemical scoring of TLR2:

In group II; there was a significant increase in TLR2 immunostaining. In addition, treatment of oxazolone induced-colitis by either mesalazine, ondansetron or both of them exhibited significant decrease in TLR2 immunostaining in comparison to Group II. Treatment of oxazolone induced-colitis by both mesalazine and ondansetron exhibited non significant decrease in TLR2 immunostaining in comparison to Group III and significant difference in comparison to Group IV (Table 3).

There was positive significant correlation between DAI & colonic tissue MPO activity, colonic tissue TNF-α level, colonic tissue caspase-3 activity respectively while there was positive non-significant correlation between DAI and TLR2 immunostaining in untreated induced-colitis group (Table 4).

Table (1): Comparative statistics of Disease Activity Index (DAI) among studied groups.

Groups / Parameter	Group II (Untreated) (n=10)	Group III (Mesalazine) (n=10)	Group IV (Ondansetron) (n=10)	Group V (Mesalazine + Ondansetron) (n=10)	One-way ANOVA F value (p-value)
DAI	6.600±0.936	2.969±0.772 p_1 0.004	1.816±0.405 p_1 0.000 p_2 NS	2.680±0.583 p_1 0.002 p_3 NS p_4 NS	9.040 (p 0.000)

*n : Number.
DAI : Disease Activity Index.
NS : Non Significant.
* : Significant at p-value <0.05; values expressed as mean ± SEM, post Hoc Tukey,s test:
p1 versus Group II (untreated oxazolone induced colitis).
p2 versus Group III (Mesalazine treated).
p3 versus Group III (Mesalazine treated).
p4 versus Group IV (ondansetron treated).

Table (2): Comparison of values of biochemical parameters among different studied groups.

Groups Parameter	Group I (Normal) (n=10)	Group II (Untreated) (n=10)	Group III (Mesalazine) (n=10)	Group IV (Ondansetron) (n=10)	Group V (Mesalazine + Ondansetron) (n=10)	*One-way ANOVA F value (p-value)
• Colonic tissue TNF-α levels (pg/mg tissue protein)	2.23 ±0.161	10.2 ±0.753 p_1 0.000	6.74 ±0.698 p_2 0.001	5.95 ±0.519 p_2 0.000 p_3 NS	3.66±0.496 p_2 0.000 p_4 0.003 p_5 0.047	29.386 (p 0.000)*
• Colonic tissue caspase-3 activity (ng/mg tissue protein)	0.048 ±0.007	0.270 ±0.027 p_1 0.000	0.123 ±0.0124 p_2 0.000	0.127 ±0.0134 p_2 0.000 p_3 NS	0.060±0.008 p_2 0.000 p_4 0.046 p_5 0.029	32.55 (p 0.000)*
• Colonic tissue MPO activity (μM/min/mg tissue protein)	1428.405 ±194.84	5117.262 ±503.909 p_1 0.000	3535.273 ±483.89 p_2 0.045	2578.03 ±350.11 p_2 0.000 p_3 NS	1976.88±317.98 p_2 0.000 p_4 0.049 p_5 NS	14.067 (p 0.000)*

*TNF : Tumour Necrosis Factor.
MPO : Myeloperoxidase.
NS : Non Significant.
* : Significant at p-value <0.05; values expressed as mean ± SEM, post Hoc Tukey,s test:
p1 versus Group I (control).
p2 versus Group II (untreated oxazolone induced-colitis).
p3 versus Group III (mesalazine treated).
p4 versus Group III (mesalazine treated).
p5 versus Group IV (ondansetron treated).

Table (3): Comparative statistics of TLR2 immunostaining among studied groups.

Groups Parameter	Group I (Normal) (n=10)	Group II (Untreated) (n=10)	Group III (Mesalazine) (n=10)	Group IV (Ondansetron) (n=10)	Group V (Mesalazine + Ondansetron) (n=10)	Kruskal-Wallis Test χ^2 value (p-value)
TLR2 immunostaining	0 (0.25)	3 (1) p_1 0.000	1 (1) p_2 0.001	2 (1) p_2 0.005 p_3 NS	1 (1) p_2 0.000 p_4 NS p_5 0.005	33.838 p 0.000

*TLR2 : Toll Like Receptor 2.
NS : Non Significant.
* : Significant at p-value <0.05; values expressed as median (IQR), Mann-Whitney U Test:
p1 versus Group I (control).
p2 versus Group II (untreated oxazolone induced-colitis).
p3 versus Group III (mesalazine treated).
p4 versus Group III (mesalazine treated).
p5 versus Group IV (ondansetron treated).

Table (4): Correlation between DAI & other markers in untreated induced-colitis group.

Correlation coefficient		
	DAI	
	r	p-value
Colonic tissue MPO activity [1]	0.939	p 0.000
Colonic tissue Caspase-3 activity [1]	0.920	p 0.000
Colonic tissue TNF-α level [1]	0.900	p 0.000
TLR2 immunostaining [2]	0.124	p 0.732

1: Pearson's correlation.
2: Spearman's correlation.

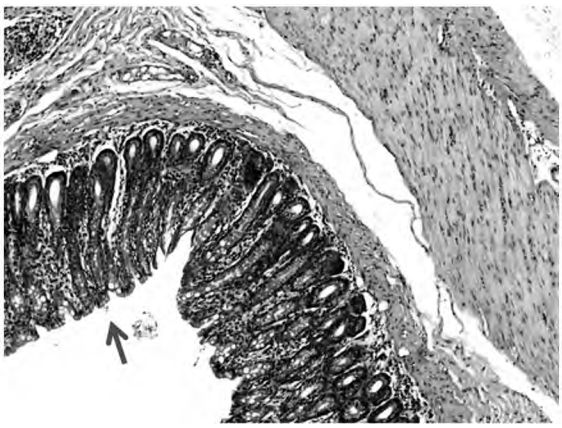

Fig. (1): Colon section in the control group (Group I) showing normal colonic mucosa with normal crypts and normal lamina propria (H & E X200).

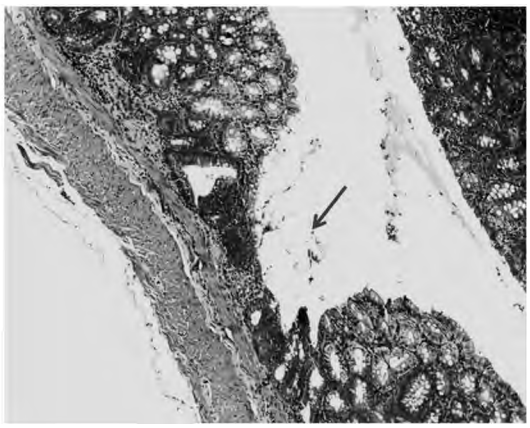

Fig. (2): Colon section in the untreated induced-colitis group (Group II) showing ulcer formation (H & E X200).

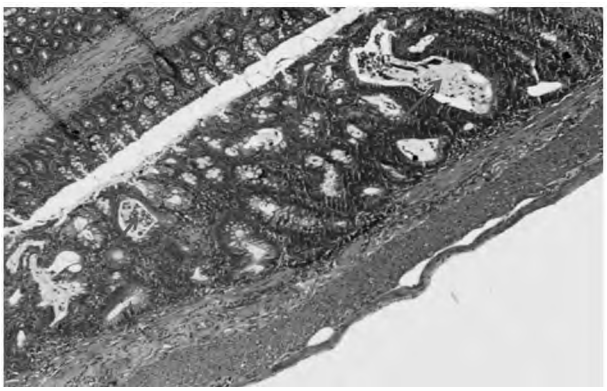

Fig. (3): Colon section in the untreated induced-colitis group (Group II) showing mucosal infiltration of epithelium with multiple crypt abscess formation (H & E X200).

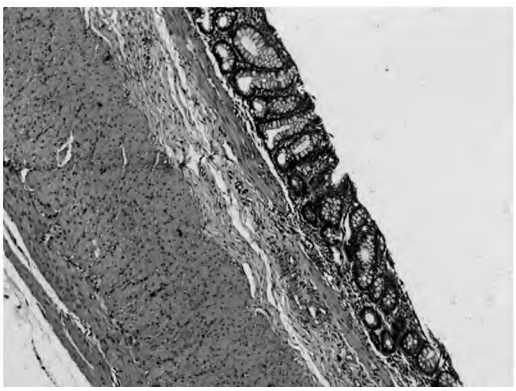

Fig. (4): Colon section in mesalazine treated group (Group III) showing restoration of part of normal pattern of mucosa (H & E X200).

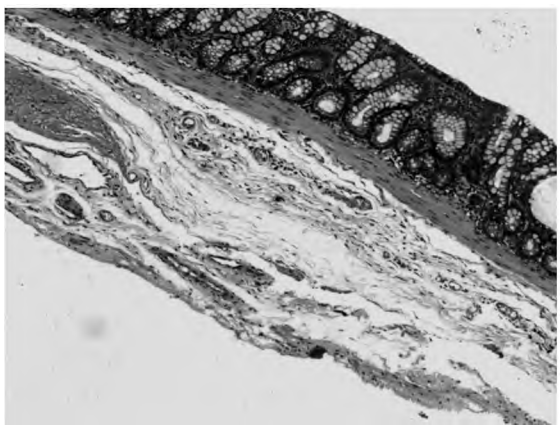

Fig. (5): Colon section in ondansetron treated group (Group IV) showing dilated colonic glands with abundant goblet cells, minimal inflammatory infiltration and minimal dilated blood vessels in lamina propria and submucosa (H & E X200).

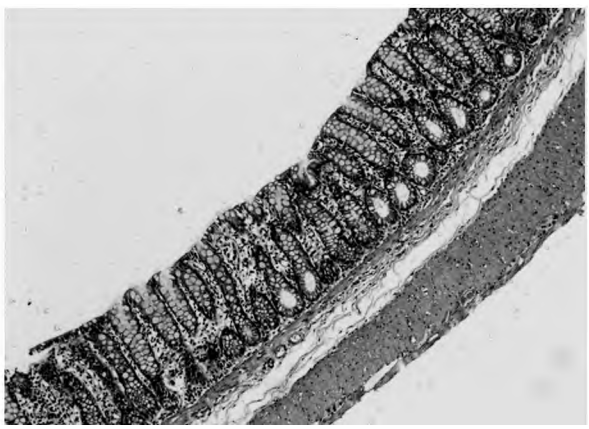

Fig. (6): Colon section in combination treated group (Group V) showing apparently normal epithelium with minimal inflammatory infiltrate, absent crypt abscess and absent inflammatory edema (H & E X200).

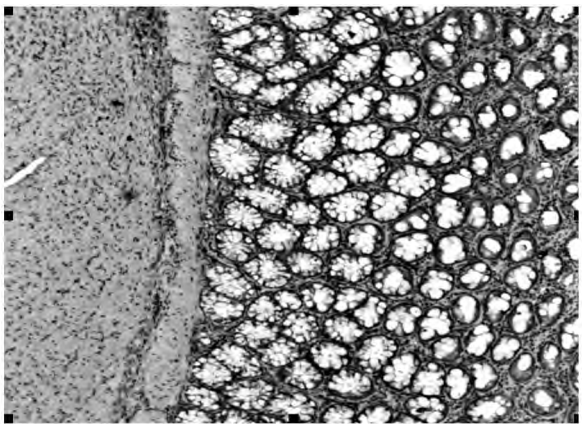

Fig. (7): Section in the colon of control group (Group I) showing normal negativity staining (TLR2 immunostaining X200).

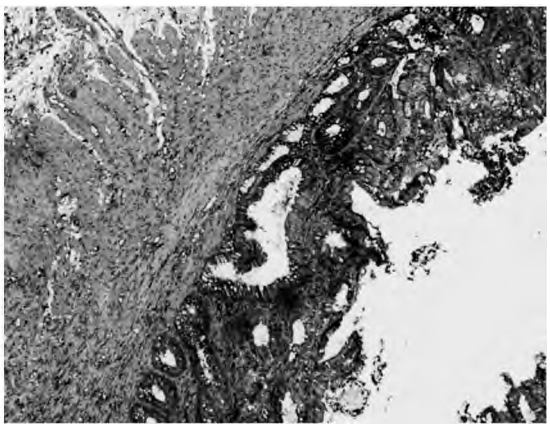

Fig. (8): Section in the colon of untreated induced-colitis group (Group II) showing strong positively staining in the lining epithelium (TLR2 immunostaining X200).

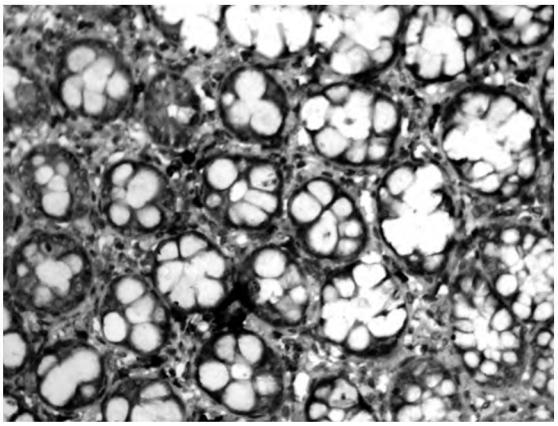

Fig. (9): Section in the colon of mesalazine treated group (Group III) showing mildly positively staining in the lining epithelium (TLR2 immunostaining X400).

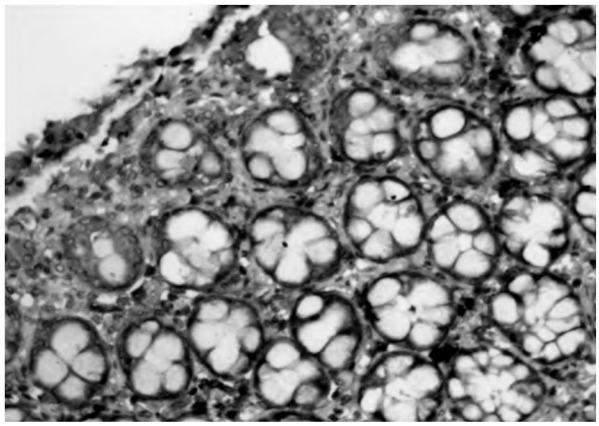

Fig. (10): Section in the colon of ondansetron treated group (Group IV) showing mild to moderate (++) positively staining in the lining epithelium (TLR2 immunostaining X400).

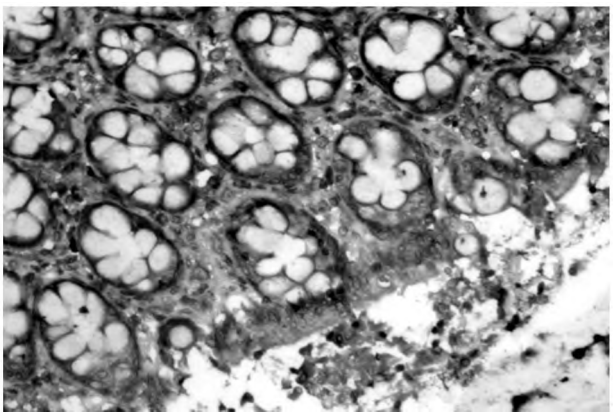

Fig. (11): Section in the colon of combination group (Group V) showing apparently negative staining in the lining epithelium (TLR2 immunostaining X400).

Discussion

Ulcerative Colitis (UC) is a lifelong inflammatory bowel disorder. The precise etiology of UC remains not fully cleared, so complete curative medical therapy is not yet available **[21]**. Oxazolone induced-colitis model shows the main fundamental features of UC, including the same morphological pattern, also it mediates Th2 immune pattern that mimics UC rather than Crohn's Disease (CD) **[5,22-25]**. In the present study the induction of colitis by oxazolone showed significant increase in TNF- a levels as shown in several studies that reported that TNF-a have been elevated in UC and proposed to play an integral role in its pathogenesis **[26,27]** as pathogenic bacteria trigger intestinal inflammation by secreting enterotoxins that increase epithelial cell permeability and impairing epithelial cell metabolism resulting in increased uptake of antigens, bacterial products and endotoxins into lamina propria; followed by activation of immune cells; secretion of proinflammatory cytokines and finally mucosal damage occurs **[28]**. Also, González-Ramírez et al., **[29]** confirmed that cytokine secretion plays an important role in the development of colitis disease & increased level of TNF-a observed

from oxazolone-group denoted the presence of an inflammatory process. In addition to that, the present work showed a significant positive correlation between highly scored DAI and elevated levels of TNF-α, this relationship proved the implication of pro-inflammatory cytokines in the pathogenesis of UC. This finding is in agreement with LU et al., [30] and Olsen et al., [26] who found a positive correlation between severity of disease and increased levels of TNF-α in patients with active UC during their clinical trial. MPO which is an enzyme found predominantly in the azurophilic granules of neutrophils, was measured as a quantitative index of neutrophil activation and inflammation [31]. It is significantly increased in oxazolone induced colitis which is in agreement with a previous study by Abdin [32] who showed that there was a significant increase in the MPO activity that significantly positively correlated with high DAI. In addition, our study showed a significant increase in caspase-3 activity in oxazolone induced colitis group which suggest epithelial apoptosis in the colon, which is in accordance with Arab et al., [33] that showed that there was an increase of caspase-3 mRNA expression which is a reliable indicator for apoptosis in experimental colitis. Also, Karatepe et al., [34] showed that caspase-3 activity was significantly increased in colonic tissues of colitis induced rats and stated that the most important activators of apoptosis that promote DNA-damage are enzymes acting on caspase-3 activity on programmed cell death.

Toll Like Receptors (TLRs) are a pattern recognition receptors which play an important role in innate immune system, their function are to detect the invasion of pathogen and initiate responses, they are involved in immune disease, cancer and their activation occur in the inflammatory cascade [35]. TLR stimulation or inhibition manipulate the immune response in a way of therapeutic value, as TLR agonists are immune system enhancers which often used for treatment of type I allergy, cancer & infectious diseases. On the other hand TLR antagonists play a therapeutic role in suppressing overactive immune responses as in chronic inflammation and autoimmune diseases [36]. Fan and Liu [20] evaluated the expression pattern of TLRs in the colonic mucosa of UC patients, they found overexpression of TLR2, TLR4, TLR9 by polymerase chain reaction and immunohistochemical staining when compared to normal controls, while immunohistochemical staining of TLR1, TLR3 was non significant when compared to controls, they suggest that TLR2, TLR4, TLR9 expressin may be important in the biological pathogensis of UC, TLR alterations in the innate response system may contribute to the pathogenesis of UC. Tan et al., [37] showed a positive correlation between TLR2, TLR4, TLR9 expression with DAI which indicated that the stronger and extensive expression of TLR2, TLR4, TLR9 the more intestinal injury.

In the present study, mesalazine treated group showed significant decrease in studied parameters. These results agree with Yao et al., [14] who demonstrated that mesalazine significantly decrease DAI, also intestinal epithelial cells apoptosis significantly decreased. Also, Zhang et al., [11] and Ming et al., [38] showed that 5-ASA treatment significantly reduce TNF-α levels in oxazolone induced colitis. In recent studies (Chiu et al., [39]; Varga et al., [40]) they reported that mesalazine decrease colonic MPO enzyme activity in experimental model of colitis and significantly decreased the plasma TNF-α level.

Ondansetron is one of the commonly used selective 5-HT_3 receptors antagonists. It is reported that 5-HT_3 receptor is expressed in monocytes, macrophages, and dendritic cells and modulates the production of inflammatory cytokines such as IL-1β and IL-6 [10].

In the present study, ondansetron treated group showed significant decrease in studied parameters. These results in accordance with Motavallian-Naeini et al., [4], who studied the effect of ondansetron on TNBS colitis, showed that ondansetron induces significant decrease in MPO activity and significant decrease in TNF-α also significant decrease in histopathological alteration, this findings explain that ondansetron is likely to reduce pro inflammatory cytokines by blocking serotonin receptors of intestinal macrophages, also explain that diminution of MPO activity denotes the ability of ondansetron to decline neutrophil infiltration to the inflamed tissue. Also, antiapoptotic effect of ondansetron was reported in other diseases as Yasuda et al., [41] showed that ondansetron ameliorate 5-flurouracil induce intestinal mucositis by suppression of apoptosis. In addition Tsukamoto et al., [10] confirmed the anti inflammatory effect of ondansetron on acute pancreatitis as it leads to significant reduction of MPO positive cells in the pancreas, Maehara et al., [42] showed that ondansetron inhibit the infiltration of CD68-positive macrophages and decrease the mRNA expression of MCP-1, TNF-α, IL-1β, IL-6 in a post-operative ileus model. In a previous study by Liu et al., [43], they demonstrated that ondansetron significantly decrease hepatic myeloperoxidase activity & TNF-α levels leading to attenuation of hepatic injury

via p38 MAPK-dependent pathway in a rat haemorrhagic shock model. 5-HT $_3$ receptor also has been demonstrated to express in immune cells including T lymphocytes, also T cell function can be modulated by 5-HT as T cell activation and proliferation is potentiated by activation of 5-HT $_3$ receptors present on these cells **[44]**. So, there was an association between certain autoimmune diseases as Rheumatoid Arthritis and high circulating levels of 5-HT, and this association reinforces its role as immunomodulatory **[45]**. So, there was experimental evidence that 5-HT receptor antagonist treatment may provide beneficial immunomodulatory effects. In this study, ondansetron treatment show significant decrease in TLR2 immunostaining which is a pattern recognition receptor that is expressed in a wide variety of cell types including immune cells as macrophages and T cells **[46,47]**. This evidenced the immunomodulatory effect of ondansetron in an immune based experimental pattern of colitis.

These findings suggest that ondansetron produce promising effects in oxazolone induced-colitis which mimics UC as it decreases inflammation, apoptosis, and TLR2 expression and improve histopathological picture that's all reflected as amelioration in disease activity index. Although combination of mesalazine and ondansetron did not provide significant additional amelioration on the disease activity when compared to each monotherapy either by mesalazine or ondansetron, nevertheless; this combination exhibited synergistic effect superior to each monotherapy in regard to improvement of inflammation, antiapoptotic and immunomodulatory effect. This could be contributed to short period of the current study and it could be recommended to be verified in further long-term experiments and other clinical studies.

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References

1- Di SABATINO A., BIANCHERI P., ROVEDATTI L., MacDONALD T.T. and CORAZZA G.R.: Recent advances in understanding ulcerative colitis. Internal and Emergency Medicine, 7: 103-11, 2012.

2- DALAL S.R. and CHANG E.B.: The microbial basis of inflammatory bowel diseases. The Journal of Clinical Investigation, 124: 4190-6, 2014.

3- FAKHOURY M., NEGRULJ R., MOORANIAN A. and AL-SALAMI H.: Inflammatory bowel disease: Clinical aspects and treatments. J. Inflamm. Res., 7: 113-20, 2014.

4- MOTAVALLIAN-NAEINI A., MINAIYAN M., RABBANI M. and MAHZUNI P.: Anti-inflammatory effect of ondansetron through 5-HT $_3$ receptors on TNBS-induced colitis in rat. EXCLI Journal, 11: 30, 2012.

5- RANDHAWA P.K., SINGH K., SINGH N. and JAGGI A.S.: A review on chemical-induced inflammatory bowel disease models in rodents. The Korean Journal of Physiology & Pharmacology, 18: 279-88, 2014.

6- CRISCUOLI V., MODESTO I., ORLANDO A. and COTTONE M.: Mesalazine for the treatment of inflammatory bowel disease. Expert Opinion on Pharmacotherapy, 14: 1669-78, 2013.

7- HAUSO Ø., MARTINSEN T.C. and WALDUM H.: 5-Aminosalicylic acid, a specific drug for ulcerative colitis. Scandinavian Journal of Gastroenterology, 50: 933-41, 2015.

8- FARBER L., HAUS U., SPATH M. and DRECHSLER S.: Physiology and pathophysiology of the 5-HT $_3$ receptor. Scandinavian Journal of Rheumatology. Supplement, 119: 2-8, 2004.

9- UTSUMI D., MATSUMOTO K., AMAGASE K., HORIE S. and KATO S.: 5-HT $_3$ receptors promote colonic inflammation via activation of substance P/neurokinin-1 receptors in dextran sulphate sodium-induced murine colitis. British Journal of Pharmacology, 173: 1835-49, 2016.

10- TSUKAMOTO A., SUGIMOTO T., ONUKI Y., SHINODA H., MIHARA T., et al.: The 5-HT $_3$ Receptor Antagonist Ondansetron Attenuates Pancreatic Injury in Cerulein-Induced Acute Pancreatitis Model. Inflammation, 1-7, 2017.

11- ZHANG H.Q., DING T.T., ZHAO J.S., YANG X. and ZHANG H.X., et al.: Therapeutic effects of Clostridium butyricum on experimental colitis induced by oxazolone in rats. World J. Gastroenterol., 15: 1821-8, 2009.

12- HORI Y., HOSHINO J., YAMAZAKI C., SEKIGUCHI T., MIYAUCHI S. and HORIE K.: Effects of chondroitin sulfate on colitis induced by dextran sulfate sodium in rats. The Japanese Journal of Pharmacology, 85: 155-60, 2001.

13- HIROTANI Y., MIKAJIRI K., IKEDA K., MYOTOKU M. and KUROKAWA N.: Changes of the peptide YY levels in the intestinal tissue of rats with experimental colitis following oral administration of mesalazine and prednisolone. Yakugaku Zasshi, 128: 1347-53, 2008.

14- YAO J., CAO X., ZHANG R., LI Y.X., XU Z.L., et al.: Protective effect of baicalin against experimental colitis via suppression of oxidant stress and apoptosis. Pharmacognosy Magazine, 12: 225, 2016.

15- COOPER H.S., MURTHY S., SHAH R. and SEDERGRAN D.: Clinicopathologic study of dextran sulfate sodium experimental murine colitis. Laboratory investigation; a Journal of Technical Methods and Pathology, 69: 238-49, 1993.

16- ABDIN A.A.: Targeting sphingosine kinase 1 (SphK1) and apoptosis by colon-specific delivery formula of resveratrol in treatment of experimental ulcerative colitis in rats. European Journal of Pharmacology, 718: 145-53, 2013.

17- FLEURY P. and EBERHARD R.: [Determination of proteins by photometric, biuret method, according to the technique of Gornall]. Annales de Biologie Clinique, 9: 453-66, 1951.

18- LORENTE L., MARTIN M.M., ARGUESO M., RAMOS L., SOLE-VIOLAN J., et al.: Serum caspase-3 levels and mortality are associated in patients with severe traumatic brain injury. B.M.C. Neurology, 15: 228, 2015.

19- XIA Y. and ZWEIER J.L.: Measurement of myeloperoxidase in leukocyte-containing tissues. Analytical biochemistry, 245: 93-6, 1997.

20- FAN Y. and LIU B.: Expression of Toll-like receptors in the mucosa of patients with ulcerative colitis. Experimental and Therapeutic Medicine, 9: 1455-9, 2015.

21- MAGRO F., GIONCHETTI P., ELIAKIM R., ARDIZZONE S., ARMUZZI A., et al.: Third European Evidence-Based Consensus on Diagnosis and Management of Ulcerative Colitis. Part 1: Definitions, diagnosis, extra-intestinal manifestations, pregnancy, cancer surveillance, surgery, and ileo-anal pouch disorders. Journal of Crohn's and Colitis, 2017.

22- BOIRIVANT M., FUSS I.J., CHU A. and STROBER W.: Oxazolone colitis: A murine model of T helper cell type 2 colitis treatable with antibodies to interleukin 4. The Journal of Experimental Medicine, 188: 1929-39, 1998.

23- HELLER F., FUSS I.J., NIEUWENHUIS E.E., BLUMBERG R.S. and STROBER W.: Oxazolone colitis, a Th2 colitis model resembling ulcerative colitis, is mediated by IL-13-producing NK-T cells. Immunity, 17: 629-38, 2002.

24- WANG X., OUYANG Q. and LUO W.J.: Oxazolone-induced murine model of ulcerative colitis. Chinese Journal of Digestive Diseases, 5: 165-8, 2004.

25- KIESLER P., FUSS I.J. and STROBER W.: Experimental models of inflammatory bowel diseases. CMGH Cellular and Molecular Gastroenterology and Hepatology, 1: 154-70, 2015.

26- OLSEN T., GOLL R., CUI G., HUSEBEKK A., VONEN B., et al.: Tissue levels of tumor necrosis factor-alpha correlates with grade of inflammation in untreated ulcerative colitis. Scandinavian Journal of Gastroenterology, 42: 1312-20, 2007.

27- SANDS B.E. and KAPLAN G.G.: The role of TNFalpha in ulcerative colitis. Journal of Clinical Pharmacology, 47: 930-41, 2007.

28- BAI A. and OUYANG Q.: Probiotics and inflammatory bowel diseases. Postgraduate Medical Journal, 82: 376-82, 2006.

29- GONZÁLEZ-RAMÍREZ A.E., GONZÁLEZ-TRUJANO M.E., OROZCO-SUÁREZ S.A., ALVARADO-VÁSQUEZ N. and LÓPEZ-MUÑOZ F.J.: Nerol alleviates pathologic markers in the oxazolone-induced colitis model. European Journal of Pharmacology, 776: 81-9, 2016.

30- LU Y.T., GAO J. and YAO G.Q.: The Experimental Study Of The Related Cytokines For In Patients With Ulcerative Colitis. Modern Preventive Medicine, 7: 013, 2005.

31- LORIA V., DATO I., GRAZIANI F. and BIASUCCI L.M.: Myeloperoxidase: A new biomarker of inflammation in ischemic heart disease and acute coronary syndromes. Mediators of inflammation, 2008.

32- ABDIN A.A.: Targeting sphingosine kinase 1 (SphK1) and apoptosis by colon-specific delivery formula of resveratrol in treatment of experimental ulcerative colitis in rats. Eur. J. Pharmacol., 718: 145-53, 2013.

33- ARAB H.H., AL-SHORBAGY M.Y., ABDALLAH D.M. and NAS SAR N.N.: Telmisartan attenuates colon inflammation, oxidative perturbations and apoptosis in a rat model of experimental inflammatory bowel disease. PloS one, 9: e97193, 2014.

34- KARATEPE O., ALTIOK M., BATTAL M., KAMALI G., KEMIK A., et al.: The effect of progesterone in the prevention of the chemically induced experimental colitis in rats. Acta Cirurgica Brasileira, 27: 23-9, 2012.

35- TARTEY S. and TAKEUCHI O.: Pathogen recognition and Toll-like receptor targeted therapeutics in innate immune cells. International Reviews of Immunology, 1-17, 2017.

36- BASITH S., MANAVALAN B., LEE G., KIM S.G. and CHOI S.: Toll-like receptor modulators: A patent review (2006-2010). Expert opinion on therapeutic patents, 21: 927-44, 2011.

37- TAN Y., ZOU K.F., QIAN W., CHEN S. and HOU X.H.: Expression and implication of toll-like receptors TLR2, TLR4 and TLR9 in colonic mucosa of patients with ulcerative colitis. Journal of Huazhong University of Science and Technology [Medical Sciences], 34: 785-90, 2014.

38- MING Z., JIA Y., YAN Y., PANG G. and CHEN Q.: Amelioration effect of bovine casein glycomacropeptide on ulcerative colitis in mice. Food and Agricultural Immunology, 26: 717-28, 2015.

39- CHIU C.T., KUO S.N., HUNG S.W. and YANG C.Y.: Combined Treatment with Hyaluronic Acid and Mesalamine Protects Rats from Inflammatory Bowel Disease Induced by Intracolonic Administration of Trinitrobenzenesulfonic Acid. Molecules, 22: 904, 2017.

40- VARGA G., UGOCSAI M., HARTMANN P., LAJKÓ N., MOLNÁR R., et al.: Acetylsalicylic acid-tris-hydroxymethyl-aminomethane reduces colon mucosal damage without causing gastric side effects in a rat model of colitis. Inflammopharmacology, 1-11, 2017.

41- YASUDA M., KATO S., YAMANAKA N., IIMORI M., MATSUMOTO K., et al.: 5-HT3 receptor antagonists ameliorate 5-_fluorouracil-induced intestinal mucositis by suppression of apoptosis in murine intestinal crypt cells. British Journal of Pharmacology, 168: 1388-400, 2013.

42- MAEHARA T., MATSUMOTO K., HORIGUCHI K., KONDO M., IINO S., et al.: Therapeutic action of 5-HT3 receptor antagonists targeting peritoneal macrophages in post-operative ileus. British Journal of Pharmacology, 172: 1136-47, 2015.

43- LIU F.C., LIU F.W. and YU H.P.: Ondansetron attenuates hepatic injury via p38 MAPK-dependent pathway in a rat haemorrhagic shock model. Resuscitation, 82: 335-40, 2011.

44- FIEBICH B.L., AKUNDI R.S., SEIDEL M., GEYER V., HAUS U., et al.: Expression of 5-HT3A receptors in cells of the immune system. Scandinavian Journal of Rheumatology. Supplement, 119: 9-11, 2004.

45- ARREOLA R., BECERRIL-VILLANUEVA E., CRUZ-FUENTES C., VELASCO-VEL, #XE1, et al.: Immunomodulatory Effects Mediated by Serotonin. Journal of Immunology Research, 2015: 21, 2015.

46- HAUSMANN M., KIESSLING S., MESTERMANN S., WEBB G., SPOTTL T., et al.: Toll-like receptors 2 and 4 are up-regulated during intestinal inflammation. Gastroenterology, 122: 1987-2000, 2002.

47- HART A.L., AL-HASSI H.O., RIGBY R.J., BELL S.J., EMMANUEL A.V., et al.: Characteristics of intestinal dendritic cells in inflammatory bowel diseases. Gastroenterology, 129: 50-65, 2005.

دراسة مقارنة بين تأثير عقار الآوندانسترون، عقار الميسالازين، وكلاهما فى علاج إلتهاب القولون المستحث بواسطة الآوكسازولون فى الجرذان البيضاء

إن إلتهاب القولون التقرحى هو مرض إلتهاب الآمعاء الذى يعتبر مشكلة صحية شائعة، إن مستقبلات السيرتيونين (٥ إتش تى)-٣ واسعة الآنتشار فى الجهاز الهضمى، كما تم الإبلاغ آن مضادات مستقبلات السيرتيونين (٥ إتش تى)-٣ لها آثار مضادة للإلتهابات.

والهدف من هذه الدراسة هو تقييم الآثار المحتملة من عقار الآوندانستيرون، الميسالازين آو كلاهما فى إلتهاب القولون الناجم عن الآوكسازولون فى الجرذان البيضاء.

\الطرق: آجريت الدراسة على خمسين جرذ آبيض مقسمين إلى خمس مجموعات متساوية كالآتى:

- مجموعة (١): المجموعة الضابطة، وتم إعطاؤها ٥٠٪ من حامل الإيثانول داخل المستقيم فى اليوم الخامس والسابع يليها كربوكسى ميثيل سليلوز عن طريق الفم لمدة ٢١ يوما.
- مجموعة (٢): مجموعة إلتهاب القولون الغير معالجة، وتم إعطاؤها الآوكسازولون داخل المستقيم فى اليوم الخامس والسابع يليها كربوكسى ميثيل سليلوز عن طريق الفم لمدة ٢١ يوما.
- مجموعة (٣): وفيها تم علاج الجرذان المصابة بإلتهاب القولون بعقار الميسالازين (١٠٠مجم/كجم/يوم) عن طريق الفم لمدة ٢١ يوما.
- مجموعة (٤): وفيها تم علاج الجرذان المصابة بإلتهاب القولون بعقار الآوندانسترون (٢مجم/كجم/يوم) داخل الغشاء البريتونى لمدة ٢١ يوما.
- مجموعة (٥): وفيها تم علاج الجرذان المصابة بإلتهاب القولون بعقار الميسالازين والآوندانستيرون فى نفس نظام الجرعة المذكورة لمدة ٢١ يوما.

وقد تم تسجيل آوزان الجسم الحيوانية، حدوث الإسهال ونزيف المستقيم يوميا طوال التجربة لحساب مؤشر نشاط المرض، فى نهاية الدراسة (فى اليوم ٢١ بعد إستحداث المرض)، تم ذبح جميع الجرذان وتم تشريح آجزاء القولون البعيدة وتم معالجتها لتقييم لقياس الآتى: نشاط كاسبيز ٣، نشاط مايلوبيروكسيدز، مستوى عامل تنكرز الورم آلفا، مستوى البروتين الكلى للآنسجة، بالإضافة إلى التحليل الباثولوجى للآنسجة، والظهور الكيميائى الخلوى المناعى لمستقبلات شبيه تول-٢.

\النتائج: الدراسة الحالية آظهرت آن علاج إلتهاب القولون المستحث بعقار الآوندانسترون آحدث إنخفاض ذات دلالة إحصائية فى جميع القياسات بالمقارنة بمجموعة إلتهاب القولون المستحث الغير معالجة، وآظهرت الدراسة آيضا آن المزيج بين عقارى الميسالازين والآوندانسترون آعطى نتائج آفضل من إستخدام كل عقار بمفرده.

\الإستنتاج: وتشير هذه النتائج إلى آن عقار الآوندانستيرون آنتج آثار واعدة فى إلتهاب القولون المستحث بالآوكسازولون الذى يحاكى إلتهاب القولون التقرحى فى الإنسان، كما آن المزج بين عقارى الآوندانستيرون والميسالازين آظهر تآثيرات التآزر متفوقة على كل عقار بمفرده فيما يتعلق بتحسين الإلتهاب، ومكافحة موت الخلايا المبرمج والتآثير المناعى. ويمكن آن يوصى للتحقق من هذه النتائج فى مزيد من الدراسات السريرية.