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Effects of Combined Propranolol or Carvedilol with Mesalazine on Experimentally Induced Ulcerative Colitis in Male Albino Rats

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ABSTRACT

Background: Ulcerative colitis is a chronic disease with a complex etiology. Many studies suggest the coordination of immune and environmental factors in both the initiation and progression of the disease.Oral mesalazine (also known as mesalamine) is a 5-aminosalicylic acid compound used in the treatment of mild to moderate Ulcerative Colitis (UC).Beta blockers are available and widely used drugs with established antioxidant actions, our aim wasto figure out the beneficial effects of propranolol, carvedilol and mesalazineand Their Interaction with Mesalazine on Ulcerative Colitis development, to distinguish which of them offer greater beneficial effect on experimentally induced ulcerative colitis in male albino rats.

Materials and Methods:
The study was done on 50males Wistar albino rats that randomly allocated to five groups, with each group comprising eleven rats except control group composed of six rats. Group I: control group, Group: ulcerative colitis group, Group III:mesalazine pretreated (300 mg/kg/d), Group IV: propranolol (30 mg/kg) + mesalazine (300 mg/kg) group, Group V:carvedilol (30 mg/kg) +mesalazine (300 mg/kg) group. To induce Ulcerative colitis, they were starved from all foods and allowed to drink water for 12 hours. Every rat was housed in separate cage with high mesh to prevent coprophagy (ingestion of hair and feces). They were divided into 5 groups 1st control group (6 rats), 2nd Ulcerative colitis group (11 rats), 3rdmesalazine pretreated group (11 rats). 4thPropranolol plus Mesalazine -pretreated group: (n = 11 rats), 5thCarvedilol plus Mesalazine - pretreated group: (n = 11 rats).

Results:
In UC group, colon W/L ratio, CW/BW ratio were decreased, and it decreased significantly also in Propranolol plus mesalazine group and Carvedilol plus mesalazine group. The mean microscopic scores of mucosal damage were significantly decreased in both Propranolol plus mesalazine and Carvedilol plus mesalazine groups.In carvedilol plus mesalazine group, colonic SOD levelwas significantly higher than that of ulcerative colitis, carvedilol and mesalazine pretreated groups.Carvedilol plus mesalazine group, colonic IL-1βlevel significantly decreased in UC group to lower than that of carvedilol and mesalazine pretreated groups.Propranolol plus mesalazine group, colonic pERK1/2/ non-pERK1/2 ratio significantly) increased. In Carvedilol plus

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Mesalazine pretreated group colonic pERK1/2/ non-pERK1/2 ratio significantlyincreased higher than that of carvedilol and mesalazine pretreated groups.
Conclusion: Propranolol and carvedilol had a beneficial effect against experimentally induced colitis depending on their ability to decreases inflammation, oxidative stress state in rat colon. Carvedilol combination synergized the ameliorative effects of mesalazine compared to mesalazine alone. Thus, carvedilol can be considered the β-blocker of benefit in UC treatment especially during treatment of other coexisting diseases indicated β-blockers. Additional clinical trials are advised to clarify their effectiveness in UC.

Key words: Ulcerative colitis (UC), Mesalazine, Carvedilol, Propranolol.

Introduction

UC is associated with overwhelming levels of pro-oxidants over antioxidants cause cell membrane lipid peroxidation, severe colonic inflammation and migration of neutrophils, basophils with the release of tumor necrosis factor α (TNF α), interferons and interleukins (ILs) as interleukin-1 β (IL-1 β) which play a crucial role in tissue disruption and ulceration **(1)**. The accelerated epithelial cell apoptosis and decreased inflammatory cell apoptosis causes colonic tissue injury and disturbed intestinal functions **(2)**. Genetic susceptibility and environmental triggers can disrupt this balance and lead to inflammation against microbial or "self" antigens **(3).**

The most common comorbidities with UC included cardiovascular illness such as hypertension, Heart failure, pulmonary disease, diabetes mellitus and metabolic syndrome **(4)**. Hypertension or aggravation of already existing hypertension represents a well-known side effect related to the administration of large doses of corticosteroids which often required in the flare-ups of UC to be settled **(5)**.

Beta blockers are widely indicated for the treatment of hypertension, myocardial infarction, congestive heart failure, cardiac arrhythmias and other conditions **(6) .**

Members of β-blockers act as classical receptor antagonists but can also stimulate signaling pathways in a G protein-independent β-arrestin-dependent fashion **(7)**. β-arrestins are identified as scaffolding proteins required for receptor desensitization and found to be important in receptor endocytosis. β-arrestins are also important players in inflammation and consequent pathogenesis of sepsis **(8,9)**, experimental autoimmune encephalomyelitis **(10)** and rheumatoid arthritis. Carvedilol and propranolol stimulate extracellular-signal-regulated kinase (ERK) pathway **(11)** which is one of the major signaling cassettes of the mitogen activated protein kinase (MAPK) signaling pathway. The ERK cascade activation will induce cellular processes that include mainly proliferation and differentiation **(12)**.

Propranolol is a β-adrenergic receptor blocker used for the treatment of hypertension, myocardial infarction, anxiety and tremor. propranolol known to cause reduction in the oxidative stress markers such and recently it shown to display anticancer properties as it showed a decrease risk of head and neck, stomach, colon and prostate cancers in patients receiving propranolol **(13) .**

Carvedilol is a third-generation nonselective β-adrenoceptor blocker used for the treatment of hypertension, heart failure (HF) withreduced ejection fraction (HFrEF) **(14)**, it can inhibit apoptosis, has anti-inflammatory activity, and also has calcium channel blocking activity **(9)**. It is approximately ten folds more potent as an antioxidant than vitamin E **(15) .**

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Carvedilol and Propranolol along with their chronic long-term usage in cardiovascular problems may have potential implication in treatment of inflammatory-based disorders **(16)** which an attractive candidate to be well investigated in UC.We aimed in the current study to figure out the beneficial effects of propranolol, carvedilol and mesalazine and their interaction on UC development and to distinguish which of them offer have greater beneficial effect on experimentally induced ulcerative colitis in male albino rats.

Materials and Methods

Materials

A- Drugs used were:

Propranolol hydrochloride powder (Sigma-Aldrich, St Louis, Missouri, USA), Carvedilol powder (Sigma-Aldrich, St Louis, Missouri, USA), Mesalazine powder (Shire Inc, USA), Acetic acid 4% solution (Sigma-Aldrich, St Louis, MO, USA), Saline solution (0.9% NaCl), Diethyl ether solution

Kits used are:

Kits for estimation of oxidative stress parameters:

Superoxide dismutase (SOD).

Kits for estimation of pro-inflammatory parameters:

Interleukin-1β (IL-1β)

ELISA kit for β arrestin pathway for rat:

For rat Phosphorylated Extracellular signal Regulated Kinase1/2 (pERK1/2) and non-Phosphorylated Extracellular signal Regulated Kinase1/2 (non pERK1/2).

B- Tools:

Soft pediatric venous polyethylene catheter- Surgical instruments- Magnifying lens anddigital weight scale- Ruler- Light microscope in Pharmacology department faculty of medicine Zagazig University.

C- Animals:

Fifty male Wistar albino rats weighing 180-200 grams/rat were purchased from Zagazig Faculty of Veterinary Medicine- animal unit. They were housed under standard environmental conditions (28±2ºC) and 12 hours light/dark cycle. They were allowed free access to food and water ad-labitum. The animals were deprived of food but allowed free access to tap water for 12 hours prior to induction of UC. During fasting, rats were housed each in a separate cage with raised mesh bottom to prevent coprophagy (ingestion of hair and feces). All experimental protocols were approved by the ethical committee at Zagazig University.

Experimental Design

The rats were randomly divided into the following 5 groups:

Group I: Control group: (n = 6 rats)

Rats of this group received 10 mL/kg/day of distilled water orally for 7 days than 1 ml saline was instilled intra-rectally on the 8th day. After that, they were received 10 mL/kg/day of distilled water orally for 3 days.

Group II: Ulcerative colitis (UC) group: (n = 11 rats)

Rats of this group received 10 mL/kg/day of distilled water orally for 7 days then 1 ml of 4% acetic acid was instilled intra-rectally on the 8th day (**22).** After induction of colitis, they were received 10 mL/kg/day of distilled water orally for 3 days.

Group III: Mesalazine -pretreated group: (n =11 rats)

Rats of this group received Mesalazine 300 mg/kg/day **(25)** orally for 7 days before and 3 days after induction of colitis.

Group IV:Propranolol plus Mesalazine -pretreated group: (n = 11 rats)

Rats of this group received 30 mg/kg/day Propranolol plus 300 mg/kg/day mesalazine orally for 7 days before and 3 days after induction of colitis.

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Group V:Carvedilol plus Mesalazine -pretreated group: (n = 11 rats)
Rats of this group received 30 mg/kg/day Carvedilol plus 300 mg/kg/day Mesalazine orally for 7 days before and 3 days after induction of colitis.

Methods
Drugs (Propranolol, Carvedilol and Mesalazine) supplied in powder:
Forms are freshly prepared,dissolved in distilled water and administered by oral gavage (volume for each rat kept constant) using a smooth stainless-steel tube, connecting to an ordinary syringe. The tube introduced into the esophagus to ensure adequate drug delivery and to avoid regurgitation. The animals were pretreated with the drugs for 7days, along with the normal diet then previous drugs administration continued for 3 days after induction of UC. On the 11th day the animals were sacrificed and tissue samples were collected.
Induction of Ulcerative Colitis:
On the 8^{th} day, animals were kept fasting for 12 hours (overnight) and UC was induced next morning in groups II, III, IV, and V. Rats were anaesthetized using low-dose ether then they were kept in Trendlenberg position during the process. An 8 mm fetal Scalp Canula was advanced 8 cm from the anus and 1 ml of 4% acetic acid was given slowly intra-rectally. Then, 2 ml of air was ejected to complete distribution of AA in colon, after that they were kept upside down position for thirty seconds to prevent leakage, as well as the rest of the solution was aspirated with injection of 2 ml air so AA spreads completely into the colon then rinsing the colon with 5 ml saline for 20 seconds **(26)**. Group I (normal control) received 1 ml of normal saline solution intra-rectally instead.
Preparation of colonic tissue samples:
Animals' weights were measured just before sacrification using digital weight scale. Then all animals were sacrificed by cervical dislocation. Longitudinal abdominal incision was done, and the entire colon was dissected totally through proximal end, gently flushed with saline, placed on an ice-cold plate, cleaned of fat and mesentery, and blotted on filter paper to dry. Each colon was weighed and colon weight /body weight ratio. It was used as a parameter to assess the degree of edema and considered as an indicator of severity of colitis **(27).** Also, colon length was measured**.** Then the proximal 5 cm was scored macroscopically and maintained in formalin 10% for microscopic studies. The distal 5 cm was collected in liquid nitrogen and sent for laboratory investigation.
Assessment of oxidative stress parameters in colonic tissue:
Superoxide Dismutase (SOD): The microplate provided in this kit has been pre-coated with an antibody specific to SODand detection Range: 12.5 U/ml – 400 U/ml.
1-Assessment of pro-inflammatory parameters in colonic tissue:
2-Assay of Interleukin-1β (IL-1β) concentrations:IL-1β was assayed by ELISA kit. This assay employs the competitive inhibition enzyme immunoassay technique and detection Range: 62.5 pg/ml-4000 pg/ml.
ELISA kit for β arrestin pathway for rats: Tissue level of Phosphorylated Extracellular signal Regulated Kinase1/2 (pERK1/2) and non-phosphorylated (non p ERK 1/2) and the kit uses a double-antibody sandwich ELISA to assay the level of rat Phosphorylated Extracellular signal Regulated Kinase1/2（p-ERK1/2）and non-phosphorylated (non p ERK 1/2).
Histopathological Examination:
Tissue specimens prepared for histopathological examination were fixed in 10% formalin, put in paraffin and sliced into four-μm sections. Paraffin sections were deparaffinized with xylene, hydrated and stained with H&E for scoring mucosal damage. The grade of inflammation of the colon was evaluated using the scoring system listed in Table 1**(17).**

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Table (1): Histological scoring system:

Histological changes	Score
Loss of mucosal architecture	0-3
Cellular infiltration	0-3
Muscle thickening	0-3
Crypt abscess formation	0-1
Goblet cell depletion	0-1

Biochemical measurements:

For biochemical measurements, the distal 5 cm of the colon quickly removed in liquid nitrogen then homogenized in ice-cold saline.

Tissue Homogenate:

Colon tissue was perfused with (phosphate buffered saline) PBS solution, pH 7.4, Containing 0.16 mg/ ml heparin to remove any red blood cells and clots.

The tissue was homogenized in 5-10 ml cold buffer (i.e., 50 mM potassium phosphate, pH 7.5.) per gram tissue.

The homogenate was centrifuged at 100,000 x g for 15 minutes at 4°C.

The supernatant was removed for assay and stored on ice.

Statistical Analysis

The obtained results were tabulated as means ± SE. Comparison between different groups were made using one-way analysis of variances (one-way ANOVA) followed by Post-Hoc (least significant difference "LSD") tests as described by **Armitage and Berry (18)**.The differences were considered to be significant when $p < 0.05$.Statistical Package of Social Sciences (SPSS) computer software (version 16) was used to carry out the statistical analysis.

Results

Colon W/L ratio in control group was 96.17±3.27 mg/cm. After induction of ulcerative colitis (UC), the animals appeared to have diarrhea, soft stool, and rectal bleeding (in the first 2 days after colitis induction). Colon weight also markedly increased due to tissue edema. Colon W/L ratio was significantly ($p<0.05$) increased from (96.17±3.27) in control group to 174.2±4.09 mg/cm***.***

Colon W/L ratio in mesalazine group significantly ($p<0.05$) decreased from (174.2±4.09 mg/cm) in UC group to 121.2±1.54 mg/cm **(Table 2)***.*

Propranolol plus mesalazine group, colon W/L ratio significantly ($p<0.05$) decreased from (174.2±4.09) in UC group to 120.2±1.14 mg/cm. This value was insignificantly different from mesalazine group**(Table 2)**.

Carvedilol plus mesalazine group colon W/L ratio significantly ($p<0.05$) decreased from (174.2±4.09) in UC group to 109.8±2.36 mg/cm. This value was but insignificantly different from mesalazine group**(Table 2)**.

Colon Weight/ Body Weight in control groupwas 3.837±0.241 mg/gm. After induction of ulcerative colitis (UC) the ratio of the distal colon weight to animal body weight was also markedly increased due to tissue edema. CW/BW ratio value significantly ($p<0.05$) increased from 3.837±0.241 in control group to 8.980±0.420mg/gm **(Table 3)**.

CW/BW ratio in mesalazine group significantly ($p<0.05$) decreased from (8.980±0.420) in UC group to 4.875±0.126 mg/gm. **(Table 3)**.

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Propranolol plus mesalazine group, CW/BW ratio significantly ($p<0.05$) decreased from (8.980±0.420) in UC group to 4.865±0.126 mg/gm. This value was insignificantly different from mesalazine group**(Table 3)**.
Carvedilol plus mesalazine group, CW/BW ratio significantly ($p<0.05$) decreased from (8.980±0.420) in UC group to 4.040±0.125mg/gm. This value was insignificantly different from mesalazine group**(Table 3)**.

Control group, colonic SOD level was 27.17±1.05U/mg. After induction of UC, colonic SOD levelsignificantly ($p<0.05$) decreased from **(**27.17±1.05) in control groupto8.98±0.57U/mg
Mesalazine group colonic SOD level significantly ($p<0.05$) increased from (8.98±0.57)in UC group to 19.67±0.60 U/mg. **(Table 4)**.
In propranolol plus mesalazine group, thevalue ofcolonic SOD level was19.75±0.67U/mg. The value was insignificantly different from mesalazine group **(Table 4)**.
In carvedilol plus mesalazine group, colonic SOD level was 23.18±0.76U/mg. This value was significantly ($p<0.05$) higher than that of ulcerative colitisgroup**(Table 4)**.

In control group, colonic IL-1β level was 31.92±1.12pg/ml. Ulcerative colitis group, colonic IL-1βlevel significantly ($p<0.05$) increased from (31.92±1.12) in control group to 133.40±2.72pg/ml.
Colonic IL-1βvalue in mesalazine group significantly ($p<0.05$) decreased from (133.40±2.72)in UC group to 66.07±2.66 pg/ml. **(Table5)**.
In propranolol plus mesalazine group, thevalue ofcolonic IL-1β level was 60.33±2.92 pg/ml. The value was insignificantly different from mesalazine group **(Table 5)**.
Carvedilol plus mesalazine group, colonic IL-1βlevel significantly ($p<0.05$) decreased from (133.40±2.72)in UC group to 46.12±2.53pg/ml. The value was significantly ($p<0.05$) lower than that of mesalazine pretreated group**(Table 5)**.

In control group, colonic pERK1/2/ non-pERK1/2 ratio was 0.832±0.0361. After induction colitis, colonic pERK1/2/ non-pERK1/2 ratio significantly ($p<0.05$) decreased from (0.832±0.0361) in control group to 0.562±0.0499**(Table 6)**.
In mesalazine group, colonic pERK1/2/ non-pERK1/2 ratio significantly ($p<0.05$) increased from (0.562±0.0499) in UC group to 1.042±0.0543. **(Table 5)**.
Propranolol plus mesalazine group, colonic pERK1/2/ non-pERK1/2 ratio significantly ($p<0.05$) increased from (0.562±0.0499) in UC group to 1.178±0.0523. This valuewas significantly ($p<0.05$) higher than that of ulcerative colitis group. Moreover, this value was insignificantly different from that of mesalazine pretreated group**(Table 6)**.
In Carvedilol plus Mesalazine pretreated group colonic pERK1/2/ non-pERK1/2 ratio significantly ($p<0.05$) increased from (0.562±0.0499) in UC group to 1.523±0.0423. This value was significantly ($p<0.05$) higher than that of mesalazine pretreated group**(Table 6)**.

Light microscopic examination of colon tissues in normal control group, showed normal colonic biopsy, normal mucosal architecture, normal muscle thickness and the mean microscopic score of mucosal damage was 1.500±0.224 **(photo 1,2)**.
Microscopic examination of UC group showed markedly disturbed mucosal architecture, severe inflammation infiltrating mucosa and submucosa with thickening of muscle layer, crypt abscess

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formation and loss of goblet cells in mucosal glands. The mean microscopic score of mucosal damage was significantly (P<0.05) increased from (1.500±0.224) in control group to 8.100±0.277 (**photo 3,4)**.

Mesalazine group microscopic examination showed mild inflammation infiltrating mucosa with moderate muscle thickening **(Photo 5,6)**. The mean microscopic score of mucosal damage significantly (P<0.05) decreased from (8.100±0.277) in UC group to 4.400±0.163.

Propranolol plus mesalazine group microscopic examination showed mild inflammation infiltrating mucosa with mild muscle thickening **(Photo 7,8).** The mean microscopic score of mucosal damage significantly (P<0.05) decreased from (8.100±0.277) in UC group to 3.400±0.306. This value was significantly (p<0.05) lower than that of mesalazine pretreated group.

Carvedilol plus mesalazine group, there were normal mucosal architecture, mild inflammation infiltrating mucosa with normal muscle thickening **(photo 9.10).** The mean microscopic score of mucosal damage significantly (P<0.05) decreased from (8.100±0.277) in UC group to 2.300±0.153. This value was significantly (p<0.05) lower than that of mesalazine pretreated group.

Table (2): The effect (mean ± SE) of mesalazine (300mg\kg) and its combination with propranolol (30mg\kg), carvedilol (30mg\kg) on colon weight/length (W/L) ratio and colon weight/body weight (CW/BW) ratio on experimentally induced ulcerative colitis

Groups	Colon W/L ratio (mg/cm)	CW/BW ratio (mg/gm)
Group I: Control group	96.17 ± 3.27^{A}	3.837 ± 0.241^{A}
Group II: Ulcerative colitis	174.2 ± 4.09^{B}	8.980 ± 0.420^{B}
Group III: Mesalazine-pretreated	121.2 ± 1.54^{C}	4.875 ± 0.126^{A}
Group IV: Propranolol+ plus Mesalazine-pretreated	120.2 ± 1.14^{D}	4.865 ± 0.126^{A}
Group V: Carvedilol plus Mesalazine-pretreated	109.8 ± 2.36^{E}	4.040 ± 0.125^{A}

Table (3): The effect (mean ± SE) of mesalazine (300mg\kg) and its combination with propranolol (30mg\kg) and carvedilol (30mg\kg) on microscopic scores of mucosal damage on experimentally induced ulcerative colitis:

Groups	Microscopic score
Group I: Control group	1.500 ± 0.224^{A}
Group II: ulcerative colitis	8.100 ± 0.277^{B}
Group V: Mesalazine-pretreated	4.400 ± 0.163^{C}
Group IV: Propranolol+ Mesalazine-pretreated	3.400 ± 0.306^{D}
Group VII: Carvedilol+ Mesalazine-pretreated	2.300 ± 0.153^{A}

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Table (4): The effect (mean ± SE) of mesalazine (300mg\kg) and its combination with propranolol (30mg\kg) and carvedilol (30mg\kg) on colonic MDA and SOD levels on experimentally induced colitis:

Groups	SOD (U/mg)
Group I: Control group	27.17 ± 1.05^{A}
Group II: Ulcerative colitis	8.98 ± 0.57^{B}
Group III: Mesalazine-pretreated	19.67 ± 0.60^{C}
Group IV: Propranolol +Mesalazine-pretreated	19.75 ± 0.67^{D}
Group V: Carvedilol +Mesalazine-pretreated	23.18 ± 0.76^{E}

Table (5): The effect (mean ± SE) of mesalazine (300mg\kg) and its combination with propranolol (30mg\kg) and carvedilol (30mg\kg)on colonic IL-1β levels on experimentally induced ulcerative colitis:

Groups	IL-1β (pg/ml)
Group I: Control group	31.92 ± 1.12^{A}
Group II: Ulcerative colitis	133.40 ± 2.72^{B}
Group III: Mesalazine-pretreated	66.07 ± 2.66^{C}
Group IV: Propranolol+ Mesalazine pretreated	60.33 ± 2.92^{D}
Group V: Carvedilol+ Mesalazine pretreated	46.12 ± 2.53^{E}

Table (6): The effect (mean ± SE) of mesalazine (300mg\kg) and the combination withpropranolol (30mg\kg) and carvedilol (30mg\kg)on colonic the pErk1/2 to non pErk1/2 ratio on experimentally induced ulcerative colitis

Groups	pERK1/2 to non pERK1/2 ratio
Group I: Control group	0.832 ± 0.0361^{A}
Group II: Ulcerative colitis	0.562 ± 0.0499^{B}
Group III: Mesalazine-pretreated	1.042 ± 0.0543^{AD}
Group IV: Propranolol+ Mesalazine-pretreated	1.178 ± 0.0523^{CD}
Group V: Carvedilol+ Mesalazine-pretreated	1.523 ± 0.0423^{E}

Photo micrographs representing the pathological effects of propranolol (30mg\kg), carvedilol (30mg\kg), mesalazine (300mg\kg) and their combination on experimentally induced ulcerative colitis:

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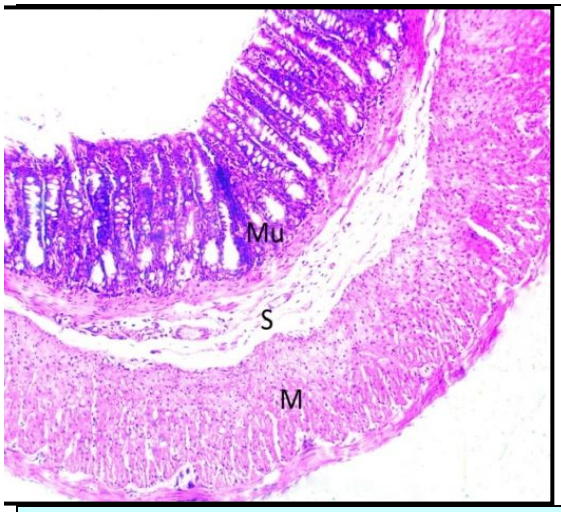
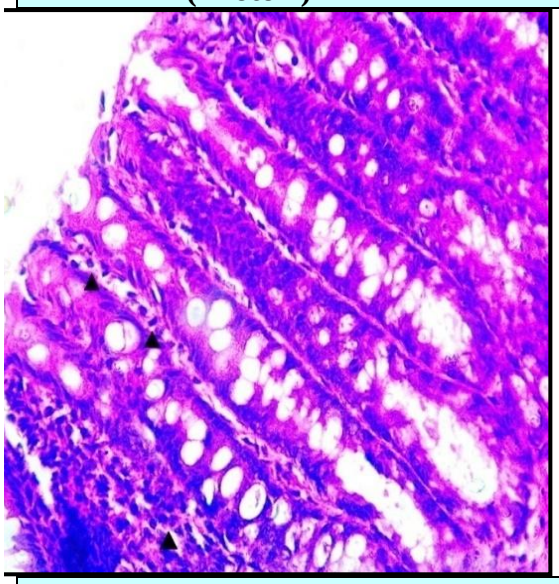
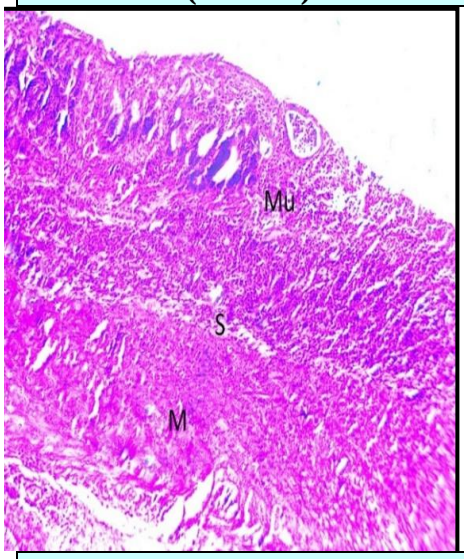
	**Group I:** Control group: showing normal mucosal architecture with normal muscle thickness, Mu: Mucosa, S: Submucosa, M: Musculosa (Hematoxylin and eosin X100).
(Photo 1)	
	**Group I:** Control group: showing normal mucosal architecture with mild inflammatory infiltrate (arrowheads) among the mucosal glands. (Hematoxylin and eosin X400).
(Photo 2)	
	**Group II:** Ulcerative colitis group: showing markedly disturbed mucosal architecture (loss of mucosal glands at right side), severe inflammation infiltrating mucosa and submucosa with moderate thickening of muscle layer, Mu: Mucosa, S: Submucosa, M: Musculosa. (Hematoxylin and eosin X100).
(Photo 3)	

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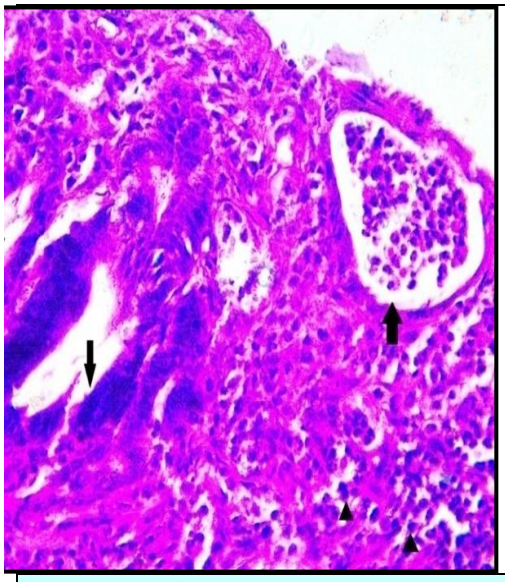
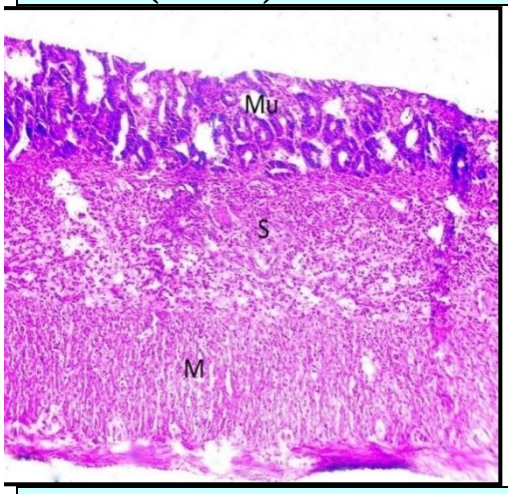
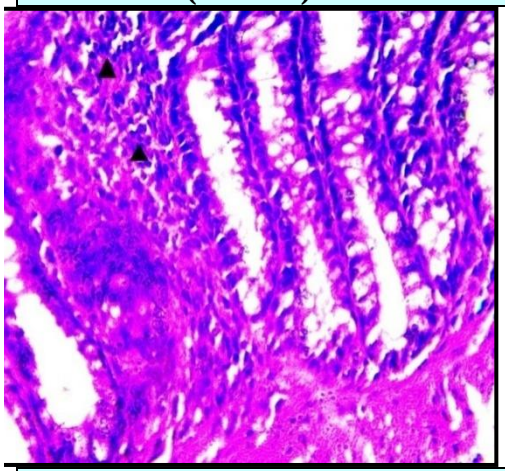
	**Group II:** Ulcerative colitisgroup: showing markedly disturbed mucosal architecture with crypt abscess (upward arrow) formation, severe inflammation (arrowheads) infiltrating mucosa and loss of goblet cells (downward arrows) in mucosal glands. (Hematoxylin and eosin X400).
(Photo 4)	
	**Group III:** Mesalazine-pretreated group: showing nearly normal mucosal architecture, mild inflammation infiltrating mucosa, with moderate muscle thickening, Mu: Mucosa, S: Submucosa, M: Musculosa (Hematoxylin and eosin X100).
(Photo 5)	
	Group III: Mesalazine-pretreated group: showing nearly normal mucosal architecture, mild inflammation (arrowheads) infiltrating mucosa, (Hematoxylin and eosin X400).
(Photo 6)	

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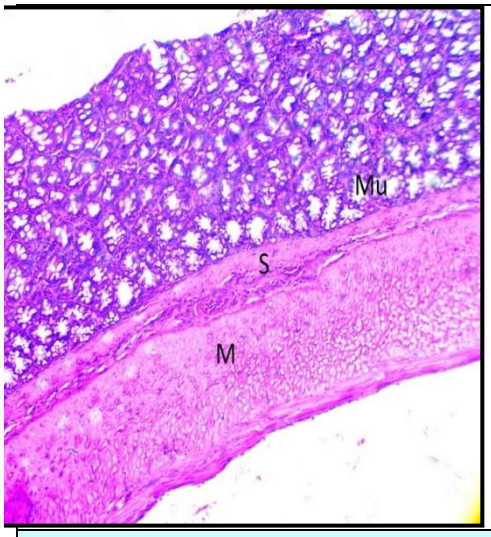
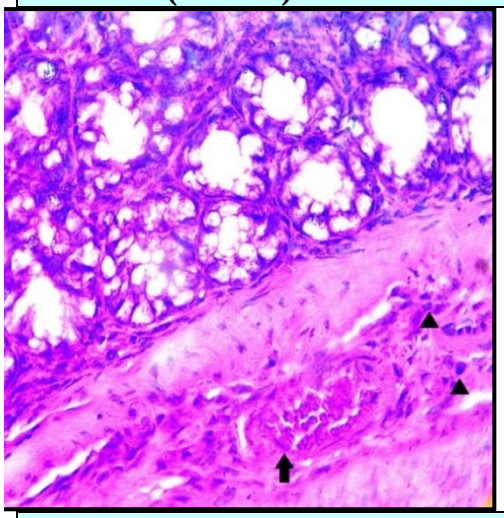
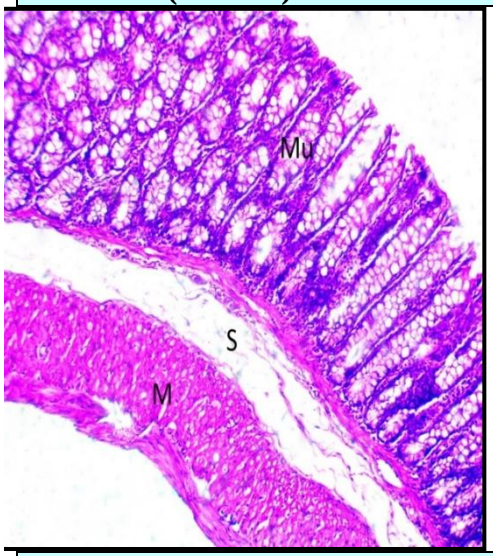
	**Group IV:** Propranolol and Mesalazine-pretreated group: showing normal mucosal architecture, mild inflammation infiltrating mucosa, with mild muscle thickening, Mu: Mucosa, S: Submucosa, M: Musculosa (Hematoxylin and eosin X100).
(Photo 7)	
	**Group IV:** Propranolol and Mesalazine-pretreated group: showing mild inflammation (arrowheads) infiltrating mucosa and submucosa with congested blood vessels in submucosa (upward arrow). (Hematoxylin and eosin X400).
(Photo 8)	
	**Group V:** Carvedilol and Mesalazine-pretreated group: showing normal mucosal architecture, mild inflammation infiltrating mucosa, with normal muscle thickening, Mu: Mucosa, S: Submucosa, M: Musculosa (Hematoxylin and eosin X100).
(Photo 9)	

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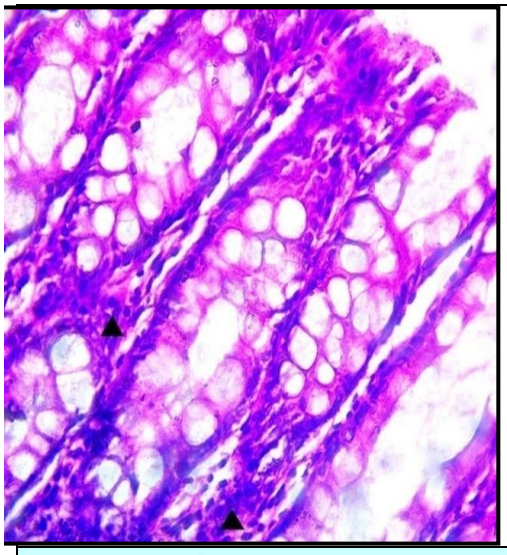	**Group V:** Carvedilol and Mesalazine-pretreated group: showing mild inflammation (arrowheads) infiltrating among mucosal glands. (Hematoxylin and eosin X400).
(Photo 10)	

Discussion

Ulcerative colitis (UC) is a chronic intestinal inflammatory disease characterized by upregulation of pro-inflammatory cytokines and oxidative stress **(19).** The incidence of UC appears to be increasing in the Middle East and Africa and the economic burden of UC is substantial and will undoubtedly continue to rise in African and Middle Eastern countries as the number of diagnosed cases of UC increases **(20)**.

We aimed in our study to investigate the possible protective effect of propranolol and carvedilol and their interaction with mesalazine on acetic acid-induced UC in male albino rats. The following parameters were measured, colon weight/length (W/L) ratio, colon weight/body weight (CW/BW) ratio, & microscopic scoring, oxidative stress parameters in colonic tissue superoxide dismutase (SOD), markers of inflammation in colonic tissue interleukin-1β (IL-1β) and phosphorylated Extracellular signal Regulated Kinase 1 and 2 (pERK1/2) to non-Phosphorylated Extracellular signal Regulated Kinase1/2 (non-pERK1/2) ratio.

Our study results agreed with **Ige et al., (21) who** found that instillation of acetic acid rectally cause increase in colon W/L ratio and CW/BW ratio when demonstrating the role of dietary maize formulations in the healing of experimental ulcerative colitis in male rats.

In the present study, UC rats showed significant deleterious effect on colon structure, microscopically. Microscopically there were shortening and wide separation of mucosal crypts together with sloughing of the surface epithelium forming erosions and ulcers. Extravasated blood between the crypts and crypt abscess were observed and the mucosa showed inflammatory cellular infiltrations. These microscopic insults of colon by acetic acid were supposed to be caused by cascade of reaction mediated by free radicals.

These results are parallel to these obtained by **Sepehrimanesh et al., (22)** who observed that instillation of acetic acid under ether anesthesia intrarectally produce damage to the colon directly by intracellular acidification in experimental-induced UC. **Peyrin–Biroulet et al., (23)** foundsimilar microscopic changes presented clinically in patients**.Sethuraman et al., (24)** also stated that instillation of acetic acid rectally in albino rat's colitis model resulted in an increase in number of inflammatory cells into the colonic tissue in an attempt to protect it from damage

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resulting in marked disturbtion of the mucosal architecture, loss of mucosal glands, severe inflammation infiltrating mucosa and submucosa with thickening of muscle layer.
Regarding oxidative stress and its consequent lipid peroxidation, the results of the present study demonstrated that AA administration resulted in significant increase in MDA level and reduce SOD when compared to control group. **Zhou et al., (25**) reported that AA could aggravate free radicals chain reactions, disrupt the integrity of intestinal mucosa barrier and activate inflammatory mediators.
Excessive production of oxidants in the plasma has been well described by **Yıldırım et al., (26)** who observed that the colonic SOD activity decreased, and MDA level increased by administration of AA compared to control group on experimental AA-induced colitis in rat. These finding agree with **Sethuraman et al., (24) and Shalkami et al., (27)** who reported that 1 ml of 4% acetic acid intrarectally produced a state of oxidative stress through excessive generation of ROS resulting in depletion of these antioxidant enzymes.Also, **Ige et al., (21)** concluded that a single intra-colonic instillation of 7% acetic acid (1 ml/100 g of body weight) intrarectally resulted in antioxidant dyshomeostasis evidenced by elevated MDA level and reduction in SOD on experimental induced ulcerative colitis in male rats.
On the contrary**, Kuralay et al. (28)** reported a significant increase in SOD in acetic acid treated female Swiss rats. The discrepancy between these results and results of the present study may be related to gender differential response to acetic acid.

Concerning the proinflammatory cytokines in the present study, instillation of AA significantly increased colonic IL-1βvalues compared to control group. These results could explain the forementioned histopathological results showing epithelial cell necrosis, edema and necrosis.
Papaconstantinou et al., (29) found that theintense local immune response is associated with increased release of cytokines, in particular, TNF-α and IL-1β that can initiate oxidative burst.

In the present study, the values of phosphorylated Extracellular signal Regulated Kinase 1 and 2 (P-ERK1/2) to non p ERK 1/2 ratio were significantly reduced in UC rats compared to control rats. These results in agreement with **Yu et al., (30)** whofound that p-ERK1/2 protein expression levels were significantly reduced in UC rats compared to control rats**. Zou et al., (31)** reported that Erythropoietin pretreatment attenuated renal I/R injury by promoting activation of ERK1/2 signaling in rat kidneys, which causes the inhibition of apoptosis. The findings agree with those mentioned by **Lv et al., (32)** who concluded that UC oxidative stress and intestinal inflammation resulting relative decrease in protein expression levels of p-ERK1/2 in the colonic mucosa of UC compared to control group inmice ulcerative colitis model through induction of regulatory T cells in colon**.** Also,**Luo et al. (33)** reported that renal cell survival under oxidative injury is strongly suggested to be dependent on ERK1/2 activity and they added that ERK1/2 considered as a mediator of controlling cell survival in response to many stimuli including hypoxia/ischemia.

In the present study, mesalazine pretreatment (300 mg/kg) significantly reduced colon W/L ratio, CW/BW ratio, microscopic scoring as well as it significantly decreased colonic values of inflammatory cytokines IL 1β and significantly increased the colonic SOD level compared to UC group.

This effect was explained by **Almeer et al., (34)** who stated that mesalazine (300 mg/kg/d) for 5 days attenuated the production of inflammatory mediators, such as TNF, and IL-1, as well as scavenging of free oxygen radicals in colonic mucosa of acetic acid treated rats. Similar results were demonstrated by **Rezaei et al., (35)** whoconfirmed the potent antioxidant and anti-inflammatory properties and the efficacy to attenuate colonic tissue injury and oxidative damage on experimental induced ulcerative colitis.

Mesalazine group in the present study significantly increased colonic pERK1/2/ non-pERK1/2 ratio compared to UC group. These findings are in line with **Khare et al., (36)** who stated that mesalazine effect at the cellular level can modulate cell cycle progression through activation of a replication checkpoint, reduce oxidative stress and decrease transcriptional activity of NF-kB.

As regard the co-administration of carvedilol with mesalazine and propranolol with mesalazine in the present work, it was found that carvedilol with mesalazine pretreated group significantly decreased colon W/L ratio, CW/BW ratio, microscopic scoring. Also, it significantly decreased colonic values of MDA, inflammatory cytokines, IL 1β compared to mesalazine pretreated group. On the other hand, propranolol with mesalazine showed insignificant difference when compared with mesalazine group. Regarding the combination interaction, carvedilol combination synergized the ameliorative effects of mesalazine compared to mesalazine alone. As far as we know, no one conducted or explained the same findings in the present study, particularly the combination with mesalazine.

Conclusion

Due to to anti-inflammatory and ant oxidant effects propranolol and carvedilol had a protective effect against rat-induced colitis. Carvedilol combination synergized the ameliorative effects of mesalazine compared to mesalazine alone. Thus, carvedilol can be considered the β-blocker of benefit in UC treatment especially during treatment of other coexisting diseases indicated β-blockers. Additional clinical trials are advised to clarify their effectiveness in UC.

Conflict of Interest: No conflict of interest.

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