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Protective Effect of Agomelatine against Acetic Acid-Induced Ulcerative Colitis in Male Albino Rats

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Abstract

Background: Ulcerative colitis (UC) is a form of idiopathic inflammatory bowel disease (IBD) with repeated and widespread mucosal inflammation of the rectum and colon. It is characterized by cycles of acute inflammation, ulceration and colonic mucosal bleeding. It is listed as one of the modern refractory diseases by the World Health Organization (WHO).Agomelatine which is atypical antidepressant with a unique receptor profile and works as a melatonin receptor (MT_1 and MT_2) agonist and a 5-HT_{2C} receptor antagonist.
Aim of study: This study aimed to evaluate the possible protective effects of agomelatineversus mesalazine on experimentally induced UC and to explore the underlying mechanisms of its anti-inflammatory and antioxidant effects in UC.
Materials and Methods: Forty-eight male albino rats weighting 180-200 gm/rat were randomly allocated to six groups(8 rats for each group) as follows: **Group I** {Control group}, **Group II**{Vehicle-Pretreated group}, **Group III** {Acetic Acid (AA)-group}, **Group IV**{Small dose Agomelatine-Pretreated UC group (Ago-10)}, **Group V**{Large dose Agomelatine-Pretreated UC group (Ago-40)}, and **Group VI** {Mesalazine (Mes)-Pretreated UC group}.The drugs were administrated orally by oral gavage for 14 days before induction of UC and continued for 2 days after induction. Ulcerative colitis was induced on the 15th day by instillation of 4% acetic acid rectally in groups (III, IV, V and VI). After 48 hours, animals were sacrificed and colon samples were collected. To estimate the severity of AA-induced UC and the effect of agomelatine and mesalazine, the following parameters had been measured: disease activity index (DAI), colon weight/body weight **(**CW/BW) ratio,ulcer index (UI),macroscopic scoring, microscopic scoring, Sirtuin1 (SIRT1), p38 mitogen-activated protein kinase (p38 MAPK), pro-inflammatory markers (tumor necrosis factor {TNF}-α&interleukin-1 beta {IL-1 }) and oxidative stress parameters (nuclear factor-erythroid-related factor 2{Nrf-2}, heme oxygenase-1 {HO-1} and superoxide dismutase {SOD}).
Results: In the AA-group, theDAI, CW/BW ratio,UI, macroscopic scoring and microscopic scoring were significantlyincreased, SIRT1 level was significantly decreased and p38 MAPK was significantly increased when compared to the control groups.The pro-inflammatory cytokines (TNF-α& IL-1) were significantly increased and the oxidative stress parameters (Nrf2, HO-1and SOD) were significantly decreased as compared to the control groups. Pretreatment with agomelatine significantly reduced

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the DAI, CW/BW ratio, UI, macroscopic scoring and microscopic scoring.Also, SIRT1 level was significantly increased and p38 MAPK was significantly decreased in a dose-dependent manner when compared to the AA-group.The pro-inflammatory cytokines (TNF-α& IL-1) were significantly decreased while the oxidative stress parameters (Nrf2, HO-1and SOD) were significantly increased as compared to the AA-group in a dose-dependent manner. However, better results were observed with a dose of 40 mg/kg/day which was insignificantly different from that of mesalazine 100 mg/kg pretreated group as a standard therapy.

Conclusion: It can be concluded that agomelatine has a dose dependent protective effects against AA- induced colitis in male albino rats which was insignificantly different from that obtained by mesalazine the standard therapy. Agomelatine may exert these protective effects through MT receptors-dependent mechanisms which could explain the anti-inflammatory and antioxidant effects of agomelatine in experimentally induced UC.

Keywords: Ulcerative Colitis, Agomelatine,Sirtuin1,P38 Mitogen Activated Protein Kinase.

1. Introduction:

Ulcerative colitis (UC), a life-long recurrent relapsing-remitting disorder, is a type of debilitating chronic inflammatory bowel diseases(IBD) of the colon that causes a superficial mucosal inflammation in a continuous fashion extending from the rectum to the more proximal colon, in varying extents**(1).**The precise etiology of UC is not well-understood, it is generally hypothesized to be a multifactorial condition induced by a combination of genetic, environment, and gut microbiota which triggers the luminal mucosa leading to an exaggerated and inappropriate immune response **(2).**

The current treatment for UC is based on the using of anti-inflammatory drugs, such as amino salicylates, glucocorticoids, immunosuppressants and anti-tumor necrosis factor-α monoclonal antibodies. However, the conventional treatments are limited by incomplete effectiveness and/or adverse reactions **(3).** So, colectomy is needed in up to 15% of patients with UC due to failed medical therapy or corticosteroid dependence and hence, there is considerable interest in finding an alternative treatment for this debilitating disease **(4).**

Although the pathophysiology of UC is not completely understood, inflammation and oxidative stress play a vital role in the pathogenesis of UC which is associated with an elevation in various inflammatory markers including interleukin-1 beta (IL-1), tumor necrosis factor (TNF)-α, nuclear factor kappa B (NF-κB) and cyclooxygenase-2 (COX-2) and elevated production of free oxygen radicals that leads to an increased oxidative damage. Moreover, inflammation along with oxidative stress may lead to DNA damage, which may further contribute to the process of carcinogenesis **(5).**

Sirtuins (SIRTs) are highly conserved nicotinamide adenine dinucleotide ($NAD^{+)}$-dependent protein deacetylases and/or adenosine diphosphate (ADP) ribosyl transferases that are important for regulation of metabolism, development, tumorigenesis, as well as aging and longevity **(6).** The mammalian genome encodes seven sirtuins, silent information regulator-1 (SIRT1) to SIRT7, among which, SIRT1 is the most evolutionally conserved NAD^{+}-dependent protein deacetylase.Recently, activators of SIRT1 have demonstrated protective effects against chemically induced colitis **(7).**

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Studies have suggested a critical role of SIRT1 in regulation of intestinal inflammation and tissue homeostasis, it has been reported to suppress inflammation by inhibiting NF-κB and p38mitogen-activated protein kinase (p38 MAPK) pathways which are responsible for the elevated levels of pro-inflammatory cytokines through deacetylation and so inactivation of these pathways **(8).**

Agomelatine, which is an antidepressant with a novel mechanism ofaction being a melatonergic agonist for melatonin 1 (MT1) and MT2receptors and a serotonin (5-HT2C) receptor antagonist **(9).** Studies have suggested that agomelatine is a drug that can be used to treat symptoms of depression and anxiety and to improve neuropathic pain caused by diabetes mellitus **(10).**

In several ischemia reperfusion models, in brain and ovaries, agomelatine decreased injury by increasing the antioxidant properties and anti-inflammatory properties and also, it was found to reduce dose-dependent injury in heart **(11)**.

Agomelatine has greater affinity for melatonin receptors (MT_1 and MT_2) and longer half-life than melatonin. Melatonin receptors were identified and characterized in the gastrointestinal tract (GIT) of various species.In the GIT, melatonin receptors are found most commonly in jejunal and colonic mucosa. Melatonin has been shown to be a potentantioxidant that affects many physiological functions including secretion, motility, digestion and absorption of the GIT. In addition, melatonin has anti-inflammatory effect that may contribute to the protection of the gastrointestinal mucosa **(12).**

We aimed in this study to evaluate the possible protective effects of agomelatine versus mesalazine on experimentally induced UC and to explore the underlying mechanisms of its anti-inflammatory and antioxidant effects in UC.

2. Material and Methods:

2.1. Drugs and Chemicals:

Agomelatine powderwas purchased fromSigma-Aldrich, St Louis, MO, USA,acetic acid 4% solution&diethyl etherwere kindly supplied fromCID Pharmaceutical Co. (Giza, Egypt),dimethyl sulfoxide (DMSO)&mesalazine powder and thiopentalwere purchased fromSigma-Aldrich, St Louis, MO, USA, normal Saline 0.9% solution were purchased from Nile Co. (Giza, Egypt).

2.2. Animals and ExperimentalDesign*:*

Forty-eight male albino rats weighing 180-200 grams/rat. They were housed under standard environmental conditions (temperature: 22±2c; humidity: 50%-55%; 12 hours light/dark cycle) and feeding a standard rodent chow diet and water ad libitum. The animals were deprived of food but allowed free access to tap water for 12 hours prior to induction of UC. The study protocol was approved by the local Animal Ethical Committee of Zagazig University, Egypt. All experimental procedures were carried out in accordance with the guidelines set forth by the National Institutes of Health,USA.

The rats were randomly divided into six groups,with each group comprising8 rats, as follow:***Group (I):***Control group;***Group (II):***Vehicle-pretreated group **)13)**;***Group (III):***Acetic acid (AA)-group**(14);*Group (IV):*** Small dose agomelatine-pretreated UC group (Ago-10) in which rats received 10 mg/kg/day agomelatine dissolved in 1% DMSO per os (p.o.)**(15);*Group (V):*** Large dose agomelatine-pretreated UC group (Ago-40) in which rats received 40 mg/kg/day agomelatine dissolved in 1% DMSOp.o. **(16)**

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and ***Group (VI):*** Mesalazine (Mes)-pretreated UC group in which ratsreceived mesalazine at a dose of 100 mg/kg/day dissolved in distilled water p.o. which is used as a reference standard **(17).**UC was induced on the 15th day ***in groups III to VI*** by intrarectal administration of 2 ml of 4% acetic acid (AA), while ***group (I and II)*** received 2 ml of 0.9% normal saline solution intrarectally instead.

Agomelatine and mesalazine were freshly prepared and administrated orally for 14 days before and 2 days after induction of colitis. Control group received 2 mL/kg/day of distilled water p.o. for 14 days before and 2 days after intrarectal injection of 0.9% normal saline solution.Vehicle-pretreated group and AA group were received 1ml/kg/day of 1% DMSO p.o. for 14 daysbefore and 2 days after intrarectal injection of 0.9% normal saline solution and acetic acid, respectively.

2.3. Induction of Ulcerative Colitis:

Animals were pretreated with agomelatine and mesalazine for 14 days along with the normal diet. On the 14thday animals' weights were measured, then animals were kept fasting for 12 hours (overnight) with a free access to water, then in the next morning of the 15thday UC was induced in groups (III, IV, V and VI) under light anesthesia using low-dose ether anesthesia**.** Firstly, colons of rats were washed via enema with 2 ml saline followed by palpation of the lower abdomen to dispose any residual feces then AA [2 ml/kg of 4% v/v in saline (pH:2.4)] was instilled slowly into the colon for 30 s using a polyurethane cannula (2 mm in diameter) inserted through the anus for 6 cm. During this process rats were kept in Trendelenburg position and were maintained in that position for 30 seconds to prevent the leakage of instilled AA, and the rest of the solution was aspirated **(18).**

Rats of Control non-ulcerative colitis groups（group Ⅰ and Ⅱ）underwent the same procedure using equal volume of normal saline instead of AA solution. After 48 hours, animals were sacrificed, and colon samples were collected **(19).**

2.4. Preparation of colonic tissue sample

Firstly, we measured animals' weights just before scarification. Then all animals were sacrificed under deep anesthesia using thiopental sodium 40 mg/kg intraperitoneally (I.P) **(20).** The Abdomen was opened by a longitudinal incision and colon was exposed. Colon weight of each rat was measured after animal scarification then the distal 6cm-portion of the colon was removed for macroscopic evaluation. After that, the proximal 3 cm was maintained in formalin 10% for microscopic studies and the distal 3 cm was snap-frozen in liquid nitrogen and stored at −80°C for laboratory investigation.

2.5. Evaluation of the disease activity:

Disease activity was quantified by the disease activity index (DAI), which utilizes a scoring system for evaluating weight loss, stool consistency, and rectal bleeding. Body weightwas recorded at the beginning of the experiment, before induction of UC and before scarification of animals to determine the percentage of body weight loss while stool consistency and rectal bleeding were recorded after induction of UC.

Body weight loss(0, none; 1, 1−5%; 2, 6−10%; 3, 11−20%; and 4, >20%), **diarrhea** (0, normal; 1, soft stool but still formed; 2, very soft stool; 3, mild diarrhea and 4, severe diarrhea), and **rectal bleeding** (0, normal; 1, positive hemoccult; 2, blood traces in stool visible; 3, mild bleeding; 4, severe bleeding). We used the average values of those parameters to calculate DAI values **(21)**according to thefollowing equation:

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$$\text{DAI} = \frac{\text{body weight loss score + diarrhea score + rectal bleeding score}}{3}$$

The presence of occult blood in stool was determined using stool guaiac test for fecal occult blood (g FOBT) which is a test used for detection of hidden (occult) blood in the stool **(22).** The stool guaiac test involves smearing some feces onto absorbent paper that has been treated with chemical material. Hydrogen peroxide is then dropped onto the paper; if trace amounts of blood are present, the paper will change color in one or two seconds. This method works as the heme component in hemoglobin has a peroxidase-like effect, rapidly breaking down hydrogen peroxide **(23).**

2.6. Colon weight/body weight ratio (CW/BW):

Body weights of the rats were measured before scarification using digital weight scale. Colon weight of each rat was measured after animal scarificationto estimate CW/BW ratio as an indicator of inflammation and edema of colonic tissue **(24).**

2.7. Determination of the ulcer area and ulcer index (UI):

Area of ulcer lesion was calculated by keeping the tissue on the graph paper. Each box of graph paper was considered as $1mm^2$ in area and the number of cells was counted and the ulcer area was determined while UI was determined byusing the following equation:

$$\text{UI} = \frac{\text{Total area of ulcer } (mm^2)}{\text{Total area of the colon specimen } (mm^2)}$$

The UI for each group was calculated as the mean lesion score of all rats in that group; and the inhibition ratio was calculated using the following equation **(25).**

$$\text{\% Inhibition} = \frac{\text{UI of AA-group} - \text{UI of treated groups}}{\text{UI of AA-group}} \text{ X } 100$$

2.8. Macroscopic assessment of mucosal injury:

Macroscopic assessment was evaluated according to The Wallace scoring system **(Table 1),**which is a semiquantitative scoring system that takes into account the area of inflammation and the presence or absence of ulcers **(26).** Examination and scoring were done using magnifying lens by two different observers blinded to the groups to eliminate our bias.

Table 1: Morphological injury scoring system:

Morphologic injuries	***Score***
No ulcer or inflammation	**0**
No ulcer with local hyperemia	**1**
Ulceration without hyperemia	**2**
Ulceration and inflammation at one site only	**3**

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Two or more sites of ulceration and inflammation	**4**
Ulceration extending more than 2 cm	**5**

2.9. Microscopic scoring of colonic damage:

Tissue specimens prepared for histopathological examination were fixed in 10% formalin and embedded in paraffin wax. For histological examination, 5-µm sections was deparaffinized, rehydrated using a graded ethanol series (100%, 90%, and70%) and stained with hematoxylin and eosin (H&E). An assessment of histopathological colonic damage was performed with consideration for the following parameters:Morpho-architectural distortion of crypts, cryptitis, ulceration, glandular atrophy, and submucosal edema. Each one of those parameters was scored according to the following **Table (2).**The final scores for each sample were calculated as the sum of those scores **(27).**

Table 2: Microscopic scoring system

Parameter	***Score***
Morpho-architectural distortion of crypts	0, absent;1 ,mild (present in less than 10% of examined tissue); 2, moderate (present in 10%–50% of examined tissue); and 3, intense (present in over 50% of examined tissue).
Cryptitis	0, absent;1 ,mild (present in less than 10% of examined tissue); 2, moderate (present in 10%–50% of examined tissue); and 3, intense (present in over 50% of examined tissue).
Ulceration	0, absent;1 ,mild (present in less than 10% of examined tissue); 2, moderate (present in 10%–50% of examined tissue); and 3, intense (present in over 50% of examined tissue).
Glandular atrophy	0, absent;1 ,mild (present in less than 10% of examined tissue); 2, moderate (present in 10%–50% of examined tissue); and 3, intense (present in over 50% of examined tissue).
Submucosal edema	0, absent;1 ,mild (present in less than 10% of examined tissue); 2, moderate (present in 10%–50% of examined tissue); and 3, intense (present in over 50% of examined tissue).

2.10. Biochemical assays:

2.10.1. Determination ofColonicSirtuin1 (SIRT1):

ColonicSIRT1level was measured by Rat SIRT1 ELISA Kit supplied from mybiosource, USAaccording to the method described by**(25).**

2.10.2. Determination ofColonicp38mitogen-activated protein kinase(p38 MAPK):

Colonicp38 MAPKwas measured byRat MAPK ELISA Kit supplied from LifeSpan Bio sciences, Inc, USAaccording to the method described by**(28).**

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2.10.3. Determination of ColonicInflammatory Markers:

ColonicTNF-αwas measured by RatTNF-αELISA Kit supplied from LifeSpan Bio sciences, Inc, USAaccording to the method described by**(29)**and colonicIL-1 was measured by Rat IL-1 ELISA Kit supplied from mybiosource, USAaccording to the method described by**(30).**

2.10.4. Determination of ColonicOxidative Stress Markers:

Colonic Nuclear factor-erythroid-related factor 2(Nrf-2)was measured by Rat Nrf-2 ELISA Kit supplied from mybiosource, USAaccording to the method described by**(31),**colonic Heme oxygenase-1 (HO-1)was measured by Rat HO-1 ELISA Kit supplied from mybiosource, USAaccording to the method described by**(32)**and colonic superoxide dismutase (SOD) was measured by Rat SOD ELISA Kit supplied from mybiosource, USA according to**(33).**

2.11. StatisticalAnalysis:

The obtained results were tabulated as means ± standard error of mean (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 **(34).**The differences were considered to be significant when $p<0.05$.Statistical Package of Social Sciences (SPSS) computer software (version 26) was used to carry out the statistical analysis.

3. Results:

3.1.Effect of agomelatine on disease activity index (DAI):

Rats which received AA exhibited features of UC with marked body weight loss, diarrhea and rectal bleeding leading to significant ($p<0.05$) increase in DAI compared to the control groups. Pretreatment with Ago (10 & 40 mg/kg/day) and Messignificantly($p<0.05$) decreased the DAI scores when compared to the AA group; however, these scores were significantly ($p<0.05$) higher than the control groups. The effect of Ago on DAI was dose-dependent and was not significantly different from that of Mes (the standard therapy) **(Table 3).**

3.2.Effect of agomelatine on colonic weight/body weight (CW/BW)ratio:

Rectal administration of AA caused significant increase in the colonic weight/ Body weight ratio when compared to control groups. Pretreatment with Ago (10 and 40 mg/kg/day) and Mes significantly ($p<0.05$) decreased the CW/BW ratio as compared to the AA group. However, CW/BW ratio showed no significant difference between rats pretreated with Ago (40 mg/kg/day) and those pretreated with Mes 100 mg/kg/day**(Table 3).**

3.3.Effect of agomelatine on ulcer area and ulcer index (UI):

Rats pretreated with Ago showed significant ($p<0.05$) and dose-dependent (10 and 40 mg/kg/day) reduction in ulcer area and index as compared to the AA group and thus showed significant ($p<0.05$)increase in % of inhibition against ulcer development. There were insignificant differences between rats pretreated with Ago 40 mg/kg/day and rats pretreated with Mes 100 mg/kg/day and control animals as regard to ulcer area and UI which powerfully support cytoprotective and anti-ulcer effects of agomelatine **(Table 3).**

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Table(3): The effect of administration of single daily oral doses of Ago 10, 40 mg/kg and Mes 100 mg/kg for 14 days before and 2 days after induction of UC in male albino rats on DAI, CW/BW ratio, UI and % of inhibition of ulcer devolvement.

Parameter / Groups (n=8)	DAI	CW/BW ratio	UI	%ofinhibition
control group	0.00±0.00 ^{A}	0.307±0. ^{A}	0.00±0.00 ^{A}	--
Vehicle-Pretreated group	0.00±0.00 ^{A}	0.310±0. ^{A}	0.00±0.00 ^{A}	--
AA-group	2.3±0.1 ^{B}	0.862±0. ^{B}	0.318±0.03 ^{B}	--
Ago-10 group	1.3±0.07 ^{C}	0.622±0.	0.11±0.01 ^{C}	65.41%
Ago-40 group	0.486±0. 02 ^{D}	0.439±0.	0.037±0.003 ^{A}	88.36%
Mes-group	0. 441±0.03 ^{D}	0.425±0. ^{D}	0.029±0.002 ^{A}	90.88%

Results are presented as Mean±Standard Error (SE). Values within the same column with different superscript capital letters are significantly ($p<0.05$) different, n: number of rats in each group.

3.4.Effect of agomelatine on macroscopic examination:

Macroscopic assessment of colonic mucosa of rats of controlgroup &vehicle-pretreated groupshowed normal smooth mucosa with no ulcers, hemorrhage or pseudo polyps **(figure1A&1B).** Animals that received AA only showed a significant ($p<0.05$) increase in macroscopic scores as compared to control groups. As the macroscopic examination showed continuous hemorrhagic large ulcerated area and elevated edematous mucosal pseudo polyps **(figure 1C).** Pretreatment with Ago (10 and 40 mg/kg/day) resulted in significant ($p<0.05$) and dose-dependent decrease in the macroscopic scores as compared to the AA group with higher significant ($p<0.05$) improvement in the Ago (40 mg/kg/day)-pretreated group (**figure 1D&1E**). There were insignificant differences between rats pretreated with Ago 40 mg/kg/day and rats pretreated with Mes 100 mg/kg/day**(figure 1F),** which powerfully support the protective effect of Ago against UC in rats. In both groups the macroscopic examination showed mild edema and minimal mucosal redness without erosions or ulcers (**Table 4).**

3.5.Effect of agomelatine on microscopic examination:

Microscopic assessment of colonic mucosa ofcontrol group &vehicle-pretreated group showed normal architecture with mild inflammation **(figure 2A&2B).** On the other hand, colon sections of rats treated only with AA demonstrated severe mucosal ulceration, mucosal dysplasia, distortion of colon crypt, edema and marked inflammatory cell infiltration **(figure 2C).** Pretreatment with Ago (10 and 40 mg/kg/day) resulted in significant($p<0.05$) and dose-dependent decrease in the microscopic scores compared with AA group with greater significant ($p<0.05$) improvement in the Ago (40 mg/kg/day)-pretreated group (**figure 2D&2E**).

There were insignificant differences between rats pretreated with Ago 40 mg/kg/day and rats pretreated with Mes 100 mg/kg/day**(figure 2F).** In both groups, the microscopic examination of the colon sections showed nearly normal mucosal architecture, mild

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inflammation in submucosa with normal muscle thickness **(Table 4).**

Table(4): The effect of administration of single daily oral doses of Ago 10, 40 mg/kg and Mes 100 mg/kg for 14 days before and 2 days after induction of UC in male albino rats on macroscopic and microscopic scoring of colon tissue.

Parameter / Groups (n=8)	Macroscopic scoring	Microscopic scoring
Control group	0.00 ± 0.00^{A}	0.33 ± 0.01^{A}
Vehicle-Pretreated group	0.00 ± 0.00^{A}	0.51 ± 0.03^{A}
AA-group	6.7 ± 0.4^{B}	5.5 ± 0.4^{B}
Ago-10 group	4.8 ± 0.3^{C}	3.5 ± 0.1^{C}
Ago-40 group	2.2 ± 0.1^{D}	2.55 ± 0.1^{D}
Mes-group	2 ± 0.1^{D}	2.25 ± 0.1^{D}

Results are presented as Mean±Standard Error (SE). Values within the same column with different superscript capital letters are significantly ($p<0.05$) different, n: number of rats in each group

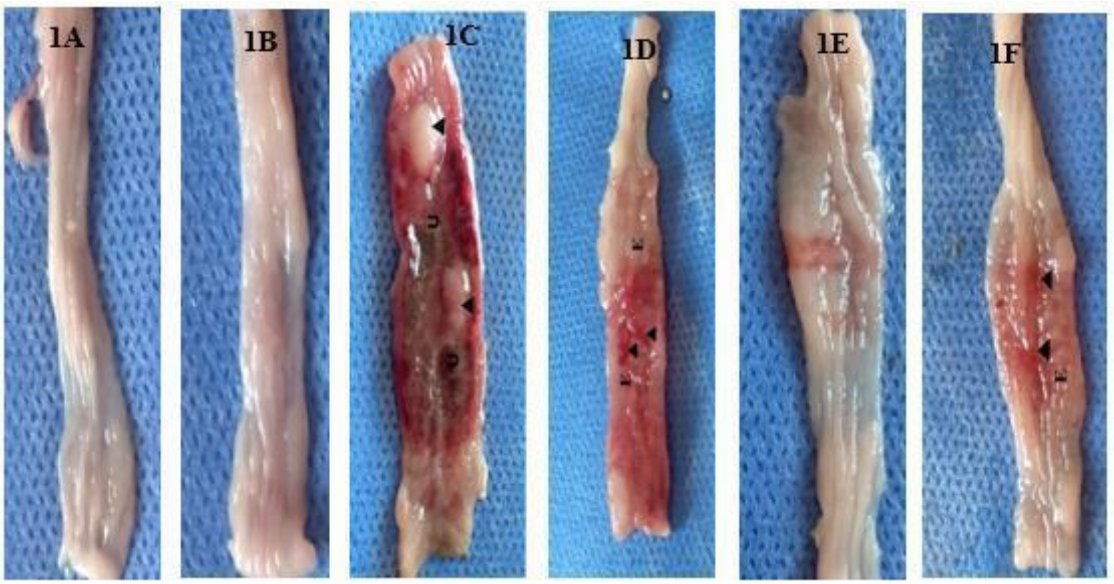

FIG. 1. Representative photo macrographs of rat's colon tissues.A: Control groups showing normal smooth mucosa with no macroscopic changes (no ulcers or hemorrhage or pseudo polyps) (score 0).**B:** Vehicle pretreated group showing normal smooth mucosa with no macroscopic changes (no ulcers, hemorrhage, pseudo polyps) (score 0).**C:**AA-group showing continuous hemorrhagic large ulcerated area (U) and elevated edematous mucosal pseudo polyp (arrowhead) (score 6).**D:**Ago-10 group showing mucosal edema (E) with mild bleeding (arrowhead) (score 4).**E:**Ago-40 group showing mucosal redness (arrow) without erosions or ulcers (score 1).**F:**Mes-group showing mild edema (E) and minimal mucosal redness (arrowhead) without erosions or ulcers (score 1)

3.6. Effect of agomelatine on SIRT1 and p38 MAPK:

As shown in table 5, rectal administration of AA induced significant ($p<0.05$) decrease

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in SIRT1 level as compared to control rats without colitis. Pretreatment with Ago (10and 40 mg/kg/day) significantly ($p<0.05$) inhibited AA-induced reduction in SIRT1 level. There were insignificant differences between rats pretreated with Ago 40 mg/kg/day and rats pretreated with Mes 100 mg/kg/day**.**

P38 MAPK pathway is also important in the pathogenesis of UCand has been previously shown to be target of SIRT1; therefore, we investigated the role of this pathway in the observed anti-inflammatory effect of agomelatine in AA-induced colitis. Pretreatment with Ago (10 and 40 mg/kg/day) significantly ($p<0.05$) reduced AA-induced upregulation of p38 MAPK levels. Better results were obtained with Ago 40 mg/kg/day which was insignificantlydifferent from that of Mes 100 mg/kg/day**(Table 5).**

Table (5): The effect of administration of single daily oral doses of Ago 10, 40 mg/kg and Mes 100 mg/kg for 14 days before and 2 days after induction of UC in male albino rats on values of SIRT1 and p38 MAPK in colon tissue.

Parameter / **Groups (n=8)**	SIRT1 (ng/gm)	P38 MAPK (pg/gm)
Control Group	18.25±0.3 ^{A}	9.02±0.4 ^{A}
Vehicle-Pretreated Group	17.78±0.3 ^{A}	9.26±0.6 ^{A}
AA-Group	2.7±0.09 ^{B}	35.7±0.9 ^{B}
Ago-10 Group	7.2±0.4^{C}	26.9±0.6^{C}
Ago-40 Group	12.3 ±0.4 ^{D}	13.9±0.6^{D}
Mes-Group	12.7±0.6 ^{D}	13.6 ±0.5^{D}

Results are presented as Mean±Standard Error (SE), Values within the same column with different superscript capital letters are significantly ($p<0.05$) different, n: number of rats in each group.

3.7.Effect of agomelatine on pro-inflammatory cytokines:

As shown in figure 3 (A&B), AA administration showed a significant ($p<0.05$) increase in the level of pro-inflammatory cytokines as colonic TNF-α and IL-1βlevels when compared with control groups. Pretreatment with Ago (10 and 40 mg/kg/day) caused significant ($p<0.05$) and dose-dependent decrease in those pro-inflammatory cytokines as compared to AA group. Better results were obtained in the Ago(40 mg/kg/day)-pretreated group which was insignificantly different from that of Mes100 mg/kg/day.

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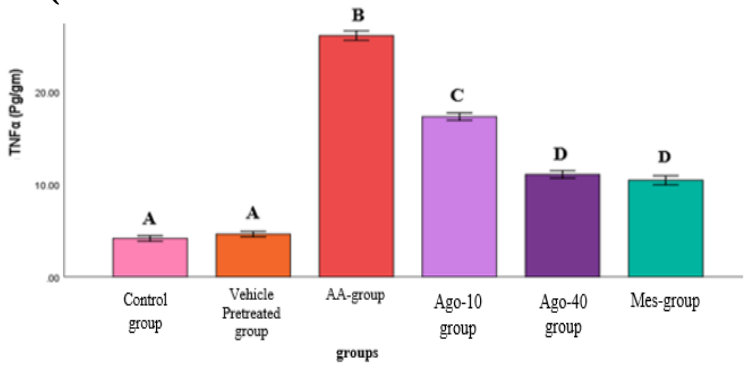

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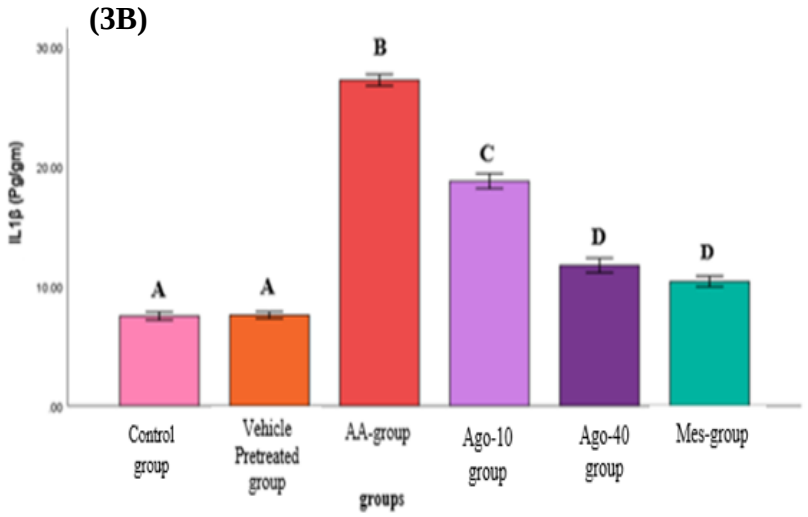
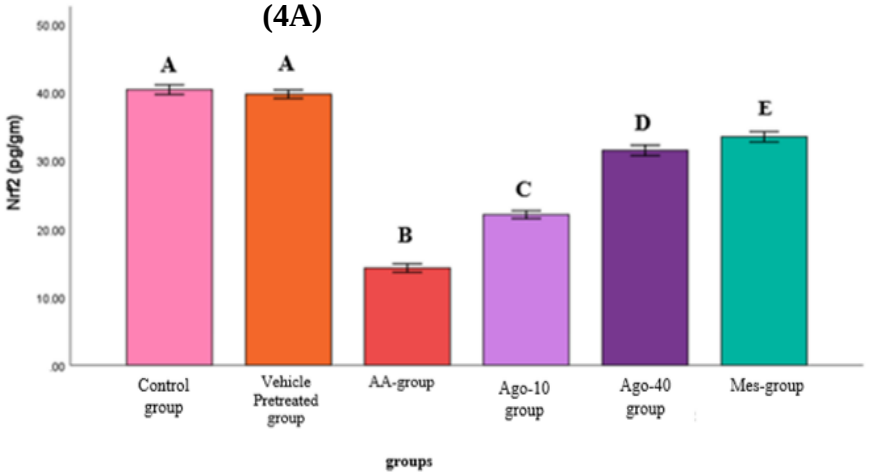


Figure 3: The effect of administration of single daily oral doses of Ago 10, 40 mg/kg and Mes 100 mg/kg for 14 days before and 2 days after induction of UC in male albino rats on **A:**TNF-α**, B:**IL-1β levels in colon tissue.Data are presented as Mean± Standard Error (SE), Values within the same column with different superscript capital letters are significantly ($p<0.05$) different, n: number of rats in each group.

3.8. Effect of agomelatine on oxidant/antioxidant status:

In the AA group, Nrf-2, HO-1 and SOD levels were significantly ($p<0.05$) lower than that of the control groups. Rats pretreated with Ago (10 and 40 mg/kg/day) showed significant ($p<0.05$) and dose-dependent increase in the levels of Nrf-2, HO-1 and SOD as compared to the AA group. Better results were obtained in the Ago (40 mg/kg/day)-pretreated group. Regarding SOD levels, there were no significant difference between Ago(40 mg/kg/day)-pretreated group and Mes (100 mg/kg/day)-pretreated group **(Figure 4A, B&C).**



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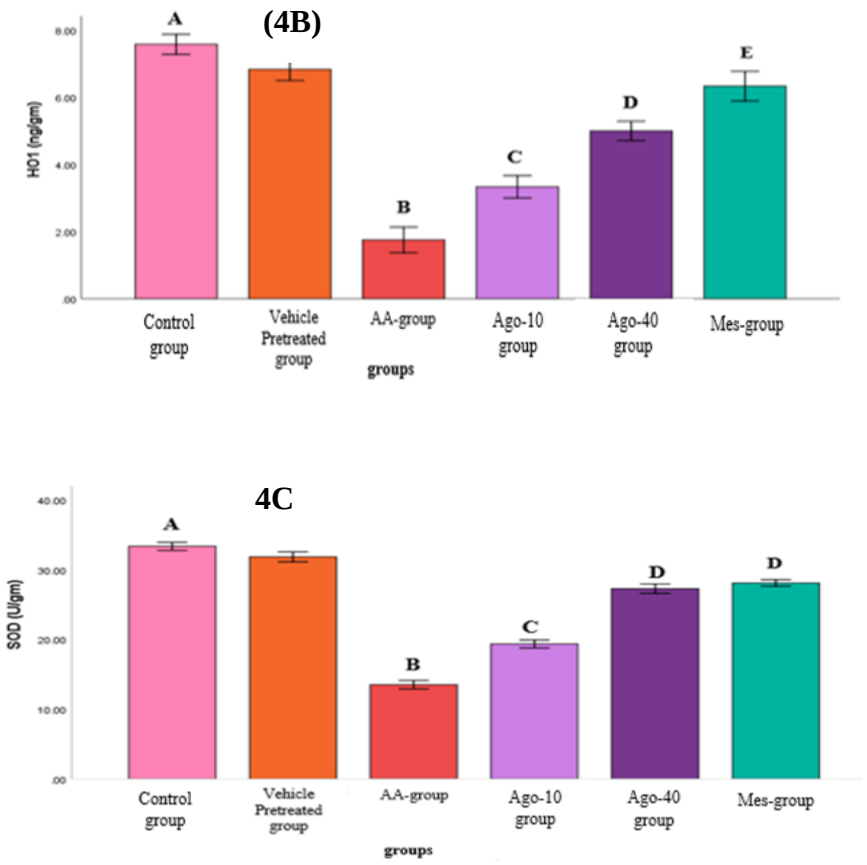
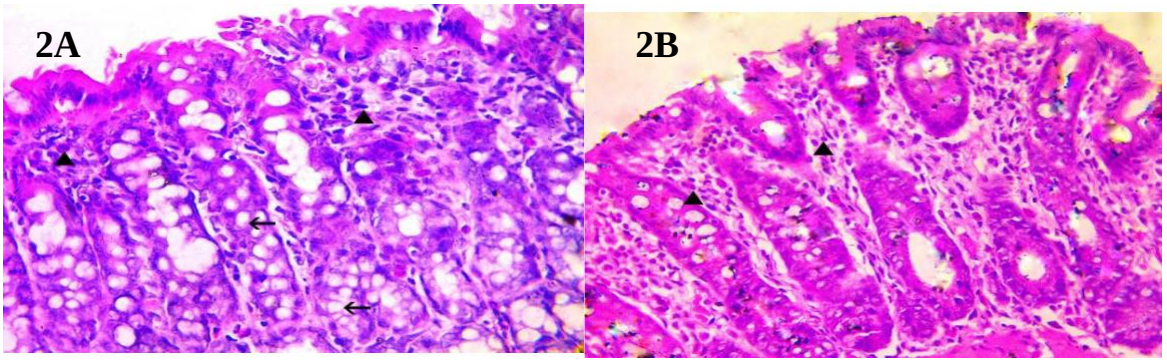


Figure 4: The effect of administration of single daily oral doses of Ago 10, 40 mg/kg and Mes 100 mg/kg for 14 days before and 2 days after induction of UC in male albino rats on **A:**Nrf-2,**B:**HO-1, **C:**SOD levelsin colon tissue.Data are presented as Mean±Standard Error (SE), Values within the same column with different superscript capital letters are significantly ($p<0.05$) different, n: number of rats in each group.



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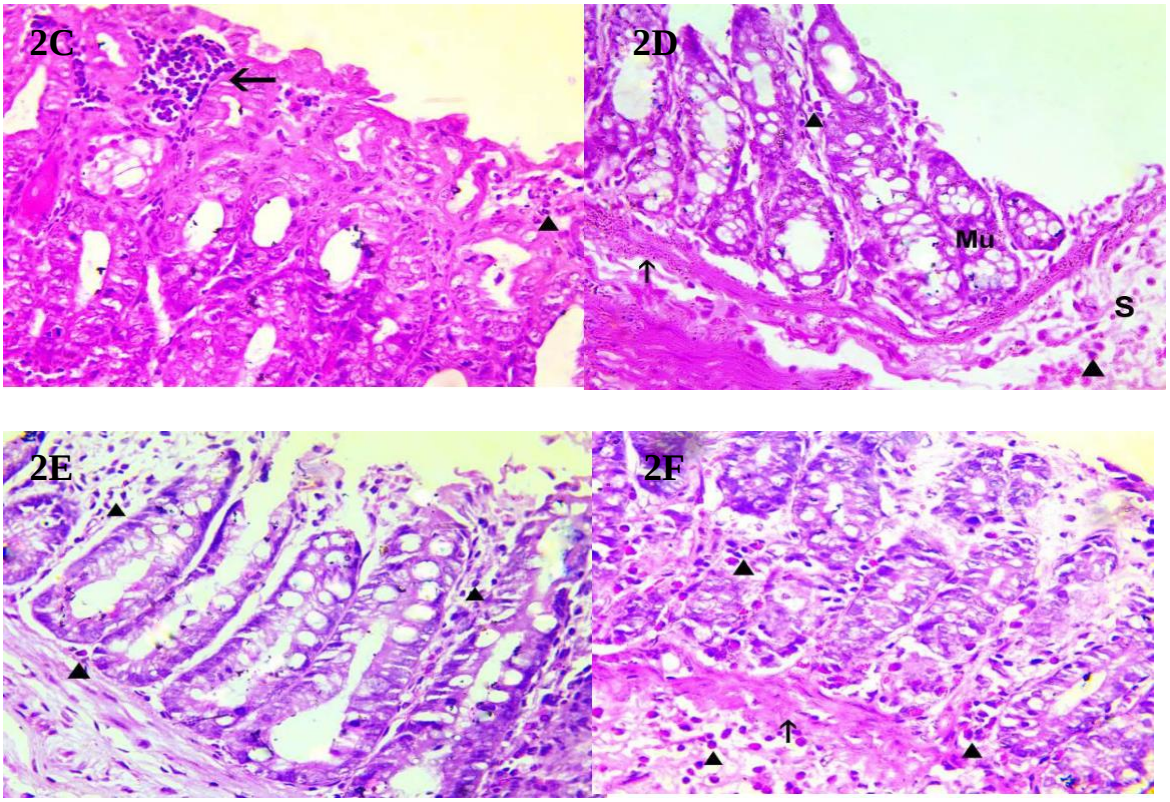

FIG.2. Representative photomicrographs of rat colon sections stained with hematoxylin and eosinX400. A: Control group showing normal mucosal architecture and preserved goblet cells (arrows) with mild inflammatory cells (arrowheads) infiltrating in-between the mucosal glands (score 1). **B:** Vehicle-Pretreated group showing normal mucosal architecture with mild inflammatory cells (arrowheads) infiltrating in-between the mucosal glands (score 1).**C:**AA-group showing crypt abscess (arrow) formation and inflammatory cells(arrowhead) in-between the mucosal glands (score 7). **D:** Ago-10 group showing disturbed mucosal architecture (loss of mucosal glands at the middle), mild inflammation (arrowheads) infiltrating mucosa and submucosa and prominent muscularis mucosa (arrow), (score 4), Mu: Mucosa, S: Submucosa. **E:** Ago-40 group showing nearly normal mucosal architecture, mild inflammation (arrowheads) infiltrating mucosa, and submucosa (score 2). **F:** Mes-group showing mild disturbance of mucosal architecture (some areas show loss of some glands), prominent muscularis mucosa (arrow), and mild inflammation (arrowheads) infiltrating the mucosa (score 2).

4. Discussion:

To the best of our knowledge, the present study is the first work that demonstratesthe potential protective effects of agomelatine against experimentally induced UC; however, melatonin, a major hormone produced mainly in the pineal gland is present in high concentration in the GIT and is known to be synthesized in the enterochromaffin cells of the intestine, where it plays a vital role in maintaining the GIT physiology **(35).**Melatonin has shown protective effects against Trinitrobenzene sulfonic acid (TNBS)-induced colitis in rats**(36)** and significantly improved experimentally Dextran sodium sulfate (DSS) -induced colitis in mice**(35)**.Similar results for the use of melatonin in IBD existed in clinical conditions**(37).**These protective effects were due to its ability to reduce the elevated levels of pro-inflammatory cytokines (TNF-α, IL-1 , and IL-6), myeloperoxidase (MPO) and malondialdehyde and by increasing the levels of reduced glutathione (GSH) and SOD.

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In the current study, we chose the AA-model for induction of UC which resulted in a significant increase in the DAI and CW/BW ratio reflecting the severity and extension of the disease**.** These results are in agreement with **(38); (39)**and could be resulted from decreased body weight due to decreased food and water intake, per rectal bleeding, malabsorption, catabolic cytokines and anorexiawhile colon weight is increased by tissue edema, cellular infiltration and hyperemia of mesenteric arteries and thickening of their walls**(40).** The detected diarrhea may be resulted from the released TNF-α, IL-1β, IL-6 and IL-8 cytokines from the activated white blood cells causing loss of function of ionic channels leading to impaired sodium/chloride absorption, water retention in the lumen and diarrhea **(41)**. In the present study, pretreatment with agomelatine significantly reduced the DAI and CW/BW ratio in a dose-dependent manner. In line with the current study, **(42)**whofound thatagomelatine has gastroprotective effects against indomethacin-induced gastric ulcer damage in rats in a dose-dependent manner by decreasing the pro-inflammatory cytokine levels and reduction of reactive oxygen species (ROS)production through suppression of NF-κB expression.

The intrarectal administration of AA resulted in a significant increase in the ulcerative index and inducedpathological changes of colonic tissue very similar to thepathological changes seen in human UC. These pathological changes are due to conversion of AA to protonated form which diffuses into the epithelium and later dissociates to form protons. This leads to the acidification of intracellular architecture, infiltration of inflammatory cells, mobilization of granulocytes and macrophages to the inflamed epithelial layer and overproduction of pro-inflammatory mediators, such asMPO, IL-1β, and TNF-α **(27).** In the present work, pretreatment with agomelatine significantly improved theulcerative indexand pathological changes of colonic tissue in a dose-dependent manner. our results in agreement with **(43)**whoreported thatagomelatine has anti-ulcerative effect against ethanol induced gastric ulcer damage in rats in a dose-dependent manner and significantly improved the ulcer area and ulcerative index which is consistent with the present study.

In atrial to explore the underlyingmechanisms of agomelatine's protective effect against AA-induced UC,we investigated the effect of agomelatine on SIRT1. Several studies had revealed the importance of SIRT1 in the pathophysiology of many diseases including cardiac, renal, metabolic and neurodegenerative diseases **(44),** the deacetylase activity of sirtuins leads to the removal of the lysine-linked acetyl group of the target protein **(45).**The intrarectal administration of AA resulted in a significant decrease in SIRT1 level.

The lack of SIRT1 activityresulted in activation of NF-κB which causes an increased transcription of pro-inflammatory cytokines (IL-1 , IL-6, IL-8, IL-16, and TNF-α) which are crucial in the IBD sustained cycle of intestinal barrier disruption **(46).**

In the present work, pretreatment with agomelatine significantly increased SIRT1 level in a dose-dependent manner which in agreement with **(47).** The upregulation of SIRT1expression by agomelatine may be attributed to being a potent agonist at MT_1 and MT_2which are G protein-coupled receptors mainly G_i**(48).** In addition to the Gi/cAMP pathway, MT receptors has been suggested to couple to the G_q. Through G_q-coupling or the dissociation of βγ subunits of G_i, MRs phosphorylate PI3K/AKTsignaling pathway, which activates peroxisome proliferator-activated receptor gamma co-activator-1α (PGC-1α), PGC-1α translocate into the nucleus and activates the SIRT1 mRNA transcription **(49).(50)** also reported that activation of melatonin receptors could also

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activates SIRTs through activation of adenosine monophosphate-activated protein kinase (AMPK) enzyme. Recently, it has been demonstrated that MT1 receptor is located in the outer mitochondrial membrane where it inhibits cyclic adenosine mono phosphate(cAMP) production in isolated mitochondria, inhibit cytochrome c release and activate SIRTs in particularly SIRT3 which is located in the mitochondria matrix **(51).**

The upregulation of SIRT1 might underlie the anti-inflammatory effect of agomelatine. SIRT1by interacting with the RelA/p65 subunit of NF-κB, inhibits NF-κB transcription by deacetylating RelA/p65 protein at lysine 310. Furthermore, SIRT1 activation delays TNF-α induced recruitment of NF-κB complexes containing RelA/p65 and p50 proteins to NF-κB-regulated gene promoters. This NF-κB/SIRT1 negative loop has been established in several experimental models **(52).**

p38 MAPK is an important member of the MAPK family, significantly increased after intracolonic administration of AA in rats and plays an important role in amplifying the inflammatory reaction by continuous production of TNF-α and IL-1β. Inhibitors of p38 MAPK have been shown to be effective in inhibiting the production of pro-inflammatory cytokines, such as ILs and NF-κB (**53).** In the present study, the down regulation of p38 MAPK by agomelatine could be attributed to activation of MT receptors and upregulation of SIRT1.**(54)** reported that MT1receptor by coupling to G_i proteins upon melatonin binding, blocks the accumulation of cAMP and potentially inhibits the activity of protein kinase A (PKA) in breast cancer cell then the cAMP/PKA pathway cross-talks with diverse signaling pathways, including the PKC/ERK1/2 and p38 MAPK leading to cross inhibition of ERK and p38 MAPK pathways**(54)**. SIRT1 activation is known also to suppress inflammation via inhibition of p38MAPK activation **(8).** It was found that SIRT1 deacetylated Apoptosis signal regulating kinase1(ASK-1**(**,c-Jun N-terminal kinase (JNK) and p38MAPK activation in ischemic-reperfused cardiomyocytes**.** Vice versa, SIRT1 inhibitor sirtinol significantly induced p38 activation to induce mitochondrial mediated apoptosis **(55).**

In the current study, the upregulation of SIRT-1 together with down-regulation of p38 MABK could be the reason for agomelatine's significant and dose-dependent reduction in the levels of TNF-α and IL-1β.In line with our results, **(56)**and**(57)** who reported that agomelatine has a hepatoprotective effect against paracetamol-induced liver damage and renal protective effects against contrast-induced nephrotoxicity in rats by decreasing the levels of cytokines including TNF-α, IL-6, IL-1β and NF-κB.

Oxidative stress results from imbalance between ROS production and elimination. It is considered a potentialtriggering factor for the development of colon inflammation in humans and animal models of colitis **(58).**Nrf-2 plays an important role in the defense against inflammation and oxidative stress possibly by activation of cellular antioxidant machinery and by suppression of NF-κB mediated proinflammatory signaling pathways**(59).**An interplay between Nrf-2 and NF-κBis suggested by**(60)** who documented that Nrf-2 activators were found to be able to prevent IκK/IκB phosphorylation and NF-κB p65 nuclear translocation, consequently inactivating NF-κB signaling.In the current study, pretreatment with agomelatine suppressed the AA-induced oxidative stress as shown by lowering theNrf-2, HO-1 and SOD levels. In line with our results, **(61)** whofound that melatonin and agomelatine protected against brain damage after permanent ischemic stroke by activating the Nrf-2/HO-1pathway increasing their levels and the level of SOD.

This anti-oxidant effect of agomelatine could be attributed toupregulation of SIRT1 which was reported to deacetylate Nrf-2 allowing its liberation from its repressor Keap1

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then Nrf-2 translocases into the nucleus where it forms a heterodimer with one of the sMaf proteins and binds to the antioxidant response elements (ARE)in the promoter region of antioxidative stress response genes resulting in mitochondrial biogenesis and stimulation of synthesis of antioxidant enzymes such as HO-1 and SOD **(62).** Furthermore, melatonin receptors activation could regulate the expression of various genes that control the production of a number of proteins, among them are the main endogenous antioxidant enzymes, such as glutathione reductase (GR),catalase(CAT),Glutathione peroxidase (GPx) and SOD and could reduce the expression of pro-oxidant, such as inducible nitric oxide synthase(iNOS), and pro-inflammatory enzymes, such as COX-2 **(63).**

Our study showed a protective effect of agomelatine against AA-induced colitis in rats which was insignificantly different from that obtained by mesalazine the standard therapy. This protective effect could be explained by the anti-inflammatory effect of agomelatine which could be related to SIRT1 activation which in turn down regulate NF-κB pathway beside down regulation of p38 MAPK pathway, resulting in reduction of pro inflammatory cytokines like TNF-α and IL-1 . In addition, the anti-oxidant properties of agomelatine resulted from activation of Nrf-2/HO-1 signaling pathway which could be activated also by activation of SIRT1 pathway and its direct radical scavenger activity. Agomelatine may exert these protective effects through MT receptors-dependent mechanisms.

5. Conclusion

It can be concluded that agomelatine has a dose-dependent protective effects against AA-induced colitis in male albino rats which was insignificantly different from that obtained by mesalazine the standard therapy. Agomelatine may exert these protective effects through MT receptors-dependent mechanisms which could explain the anti-inflammatory and antioxidant effects of agomelatine in experimentally induced UC.

6. Conflict of Interest:

Noconflictofinterest.

7. References

1. **Gajendran, M., Loganathan, P., Jimenez, G., Catinella, A. P., Ng, N., Umapathy, C., Hashash, J. G. (2019):** A comprehensive review and update on ulcerative colitis. Disease-a-month, 65(12), 100851.

2. **Shouval, D. S., Rufo, P. A. (2017):** The role of environmental factors in the pathogenesis of inflammatory bowel diseases: a review. JAMA pediatrics, 171(10), 999-1005.

3. **Steinhart, A. H., Fernandes, A. (2015):** Clinical practice guidelines for the medical management of nonhospitalized ulcerative colitis: the patient perspective. Canadian Journal of Gastroenterology and Hepatology, 29(6), 294-296.

4. **Dignass, A., Lindsay, J. O., Sturm, A., Windsor, A., Colombel, J. F., Allez, M., Van Assche, G. (2012):** Second European evidence-based consensus on the diagnosis and management of ulcerative colitis part 2: current management. Journal of Crohn's and Colitis, 6(10), 991-1030.

5. **Sánchez-Fidalgo, S., Cárdeno, A., Sánchez-Hidalgo, M., Aparicio-Soto, M., Villegas, I., Rosillo, M. A., de la Lastra, C. A. (2013):** Dietary unsaponifiable fraction from extra virgin

olive oil supplementation attenuates acute ulcerative colitis in mice. European Journal of Pharmaceutical Sciences, 48(3), 572-581.\

6. **Guarente, L. (2013):** Calorie restriction and sirtuins revisited. Genes & development, 27(19), 2072-2085.

7. **Pu, Z., Han, C., Zhang, W., Xu, M., Wu, Z., Liu, Y., Wu, M., Sun, H., Xie, H. (2019):** Systematic understanding of the mechanism and effects of Arctigenin attenuates inflammation in dextran sulfate sodium-induced acute colitis through suppression of NLRP3 inflammasome by SIRT1. Am J Transl Res 11, 3992−4009.

8. **Luo, W., Jin, Y., Wu, G., Zhu, W., Qian, Y., Zhang, Y., Li, J., Zhu, A., Liang, G. (2019):**Blockage of ROS and MAPKs-mediated inflammation via restoring SIRT1 by a new compound LF10 prevents type 1 diabetic cardiomyopathy. Toxicol. Appl. Pharmacol. 370, 24−35.

9. **Pierre Olié, J., & Kasper, S. (2007):** Efficacy of agomelatine, a MT1/MT2 receptor agonist with 5-HT2C antagonistic properties, in major depressive disorder. International Journal of Neuropsychopharmacology, 10(5), 661-673.

10. **Karaiskos, D., Tzavellas, E., Ilias, I., Liappas, I., Paparrigopoulos, T. (2013):** Agomelatine and sertraline for the treatment of depression in type 2 diabetes mellitus. International journal of clinical practice, 67(3), 257-260.

11. **Yapca, O. E., Borekci, B., Turan, M. I., Gulapoglu, M. (2014):** The effect of agomelatine on oxidative stress induced with ischemia/reperfusion in rat ovaries. Advances in clinical and experimental medicine: official organ Wroclaw Medical University, 23(5), 715-721.

12. **Brzozowska, I., Strzalka, M., Drozdowicz, D., J Konturek, S., Brzozowski, T. (2014):**Mechanisms of esophageal protection, gastroprotection and ulcer healing by melatonin. Implications for the therapeutic use of melatonin in gastroesophageal reflux disease (GERD) and peptic ulcer disease. Current pharmaceutical design, 20(30), 4807-4815.

13. **Gil, F. J. N., Huete-Toral, F., Crooke, A., Godinez, C. O. D., Carracedo, G., Pintor, J. (2019):** Effect of melatonin and its analogs on tear secretion. Journal of Pharmacology and Experimental Therapeutics, 371(1), 186-190.

14. **Al-Rejaie, S. S., Abuohashish, H. M., Al-Enazi, M. M., Al-Assaf, A. H., Parmar, M. Y., Ahmed, M. M. (2013):**Protective effect of naringenin on acetic acid-induced ulcerative colitis in rats. World journal of gastroenterology: WJG, 19(34), 5633.

15. **Ozcan, M., Canpolat, S., Bulmus, O., Ulker, N., Tektemur, A., Tekin, S., Kelestimur, H. (2019):** Agomelatine pretreatment prevents development of hyperglycemia and hypoinsulinemia in streptozotocin-induced diabetes in mice. Fundamental & clinical pharmacology, 33(2), 170-180.

16. **Papp, M., Litwa, E., Gruca, P., Mocaër, E. (2006):** Anxiolytic-like activity of agomelatine and melatonin in three animal models of anxiety. Behavioural pharmacology, 17(1), 9-18.

17. **Mahdavi, N. S., Talebi, A., Minaiyan, M. (2019):**Ameliorative effect of galantamine on

acetic acid-induced colitis in rats. Research in pharmaceutical sciences, 14(5), 391.

18. **Zeytunlu, M., Korkut, M., Akgün, E., Firat, O., Aynaci, M., Içöz, G., ÖZÜTEMİZ, Ö. (2004):** The comparative effects of calcium channel blockers in an experimental colitis model in rats. Turk J Gastroenterology, 15(4), 243-249.

19. **Manna, M. J., Abu-Raghif, A., Al-Saraf, K. M. (2017):**Therapeutic effect of sildenafil in experimental colitis through anti-oxidative stress and inhibition of adhesion molecules. J Pharm Sci Res, 9(9), 1615-1623.

20. **Khodir, A. E., Said, E., Atif, H., ElKashef, H. A., Salem, H. A. (2019):** Targeting Nrf2/HO-1 signaling by crocin: Role in attenuation of AA-induced ulcerative colitis in rats. Biomedicine & Pharmacotherapy, 110, 389-399.

21. **Niu, X., Zhang, H., Li, W., Wang, Y., Mu, Q., Wang, X., Yao, H. (2015):** Protective effect of cavidine on acetic acid-induced murine colitis via regulating antioxidant, cytokine profile and NF-κB signal transduction pathways. Chemico-biological interactions, 239, 34-45.

22. **Beg, M., Singh, M., Saraswat, M. K., Rewari, B. B. (2002):** Occult gastrointestinal bleeding: detection, interpretation, and evaluation. Journal Indian Academy of Clinical Medicine, 3(2), 153-158.

23. **Rockey, D. C. (1999):** Occult gastrointestinal bleeding. New England Journal of Medicine, 341(1), 38-46.

24. **Ko J., Lam F., Cheung A. (2005):** Amelioration of experimental colitis by Astragalus membranaceus through anti-oxidation and inhibition of adhesion molecule synthesis. World J. Gastroenterol. 11: 5787–5794.

25. **Kumar, V. S., Rajmane, A. R., Adil, M., Kandhare, A. D., Ghosh, P., Bodhankar, S. L. (2014):** Naringin ameliorates acetic acid induced colitis through modulation of endogenous oxido-nitrosative balance and DNA damage in rats. Journal of biomedical research, 28(2), 132.

26. **Wallace, J. L., Keenan, C. M., Gale, D., Shoupe, T. S. (1992):** Exacerbation of experimental colitis by nonsteroidal anti-inflammatory drugs is not related to elevated leukotriene B4 synthesis. Gastroenterology, 102(1), 18-27.

27. **Bastaki, S. M., Al Ahmed, M. M., Al Zaabi, A., Amir, N., Adeghate, E. (2016):** Effect of turmeric on colon histology, body weight, ulcer, IL-23, MPO and glutathione in acetic-acid-induced inflammatory bowel disease in rats. BMC complementary and alternative medicine, 16(1), 1-14.

28. **Versteeg, H. H., Nijhuis, E., van den Brink, G. R., Evertzen, M., Pynaert, G. N., van Deventer, S. J., Peppelenbosch, M. P. (2000):** A new phosphospecific cell-based ELISA for p42/p44 mitogen-activated protein kinase (MAPK), p38 MAPK, protein kinase B and cAMP-response-element-binding protein. Biochemical Journal, 350(3), 717-722.

29. **Old, L. J. (1985):**Tumor necrosis factor. Science, 230, 630-633.

30. **March, C. J., Mosley, B., Larsen, A., Cerretti, D. P., Braedt, G., Price, V., Cosman, D. (1985):** Cloning, sequence and expression of two distinct human interleukin-1 complementary DNAs. Nature, 315(6021), 641-647.

31. **Kanninen, K., Malm, T. M., Jyrkkänen, H. K., Goldsteins, G., Keksa-Goldsteine, V., Tanila, H., Koistinaho, J. (2008):** Nuclear factor erythroid 2-related factor 2 protects against beta amyloid. Molecular and Cellular Neuroscience, 39(3), 302-313.

32. **Maines, M. D. (1988):** Heme oxygenase: function, multiplicity, regulatory mechanisms, and clinical applications. The FASEB Journal, 2(10), 2557-2568.

33. **Adachi, T., Ohta, H., Yamada, H., Futenma, A., Kato, K., Hirano, K. (1992):** Quantitative analysis of extracellular-superoxide dismutase in serum and urine by ELISA with monoclonal antibody. Clinicachimica acta, 212(3), 89-102.

34. **Armitage, P.,Berry, G. (1994):** Bayes' theorem. Statistical Methods in Medical Research., Armitage P, Berry G., 3rd Ed., Blackwell, Oxford, pp71-77.

35. **Jena, G., Trivedi, P. P. (2014):** A review of the use of melatonin in ulcerative colitis: experimental evidence and new approaches. Inflammatory bowel diseases, 20(3), 553-563.

36. **Tahan, G., Gramignoli, R., Marongiu, F., Aktolga, S., Cetinkaya, A., Tahan, V., Dorko, K. (2011):** Melatonin expresses powerful anti-inflammatory and antioxidant activities resulting in complete improvement of acetic-acid-induced colitis in rats. Digestive diseases and sciences, 56(3), 715-720.

37. **Rakhimova, O. Y., Rakhimova, O. Y. (2010):** Use of melatonin in combined treatment for inflammatory bowel diseases. Therapeutic archive, 82(12), 64-68.

38. **Saber, S., Khalil, R. M., Abdo, W. S., Nassif, D., El-Ahwany, E. (2019):** Olmesartan ameliorates chemically induced ulcerative colitis in rats via modulating NFκB and Nrf-2/HO-1 signaling crosstalk. Toxicology and applied pharmacology, 364, 120-132.

39. **Elshazly, S. M., Elhassanny, A. E., Mahmoud, N. M. (2020):** Cilostazol protects against acetic acid-induced colitis in rats: possible role for cAMP/SIRT1 pathway. European Journal of Pharmacology, 881, 173234.

40. **Paunovic, B., Deng, X., Khomenko, T., Tolstanova, G., Szabo, S., Sandor, Z. (2011):** Molecular Mechanisms of Basic Fibroblast Growth Factor Effect on Healing of Experimental Ulcerative Colitis in Rats. Gastroenterology, 140(5), S-143.

41. **Wenzl, H. H. (2012):** Diarrhea in chronic inflammatory bowel diseases. Gastroenterology Clinics, 41(3), 651-675.

42. **Eraslan, E., Tanyeli, A., Güler, M. C., Kurt, N., Yetim, Z. (2020):** Agomelatine prevents indomethacin-induced gastric ulcer in rats. Pharmacological Reports, 1-8.

43. **Shukla, P., Porwal, A., Roy, S., Chaturvedi, S., Tripathi, S., Arya, N. (2017):** Preliminary study on antiulcer effect of agomelatine and its potentiation with pyridoxine. International Journal of Basic & Clinical Pharmacology, 6(11), 2566.,

44. **Ma, K., Lu, N., Zou, F., Meng, F. Z. (2019):** Sirtuins as novel targets in the pathogenesis of airway inflammation in bronchial asthma. European journal of pharmacology, 865, 172670.

45. **Sauve, A. A., Wolberger, C., Schramm, V. L., Boeke, J. D. (2006):** The biochemistry of sirtuins. Annu. Rev. Biochem., 75, 435-465.

46. **Targan, S. R., Karp, L. C. (2005):** Defects in mucosal immunity leading to ulcerative colitis. Immunological reviews, 206(1), 296-305.

47. **Savran, M., Aslankoc, R., Ozmen, O., Erzurumlu, Y., Savas, H. B., Temel, E. N., Boztepe, S. (2020).** Agomelatine could prevent brain and cerebellum injury against LPS-induced neuroinflammation in rats. Cytokine, 127, 154957.

48. **Mayo, J. C., Sainz, R. M., Gonzalez Menendez, P., Cepas, V., Tan, D. X., Reiter, R. J. (2017):** Melatonin and sirtuins: a "not-so unexpected" relationship. Journal of pineal research, 62(2), e12391.

49. **Song, C., Zhao, J., Fu, B., Li, D., Mao, T., Peng, W., Zhang, Y. (2017):**Melatonin-mediated upregulation of Sirt3 attenuates sodium fluoride-induced hepatotoxicity by activating the MT1-PI3K/AKT-PGC-1α signaling pathway. Free Radical Biology and Medicine, 112, 616-630.

50. **Yu, L., Gong, B., Duan, W., Fan, C., Zhang, J., Li, Z., Wang, H. (2017):** Melatonin ameliorates myocardial ischemia/reperfusion injury in type 1 diabetic rats by preserving mitochondrial function: role of AMPK-PGC-1α-SIRT3 signaling. Scientific Reports, 7(1), 1-13.

51. Gbahou, F., Cecon, E., Viault, G., Gerbier, R., Jean-Alphonse, F., Karamitri, A., **Jockers, R. (2017):** Design and validation of the first cell-impermeant melatonin receptor agonist. British journal of pharmacology, 174(14), 2409-2421.

52. **Yeung, F., Hoberg, J. E., Ramsey, C. S., Keller, M. D., Jones, D. R., Frye, R. A., Mayo, M. W. (2004):** Modulation of NF-κB-dependent transcription and cell survival by the SIRT1 deacetylase. The EMBO journal, 23(12), 2369-2380.

53. **Topcu-Tarladacalisir, Y., Akpolat, M., Uz, Y. H., Kizilay, G., Sapmaz-Metin, M., Cerkezkayabekir, A., Omurlu, I. K. (2013):** Effects of curcumin on apoptosis and oxidoinflammatory regulation in a rat model of acetic acid−induced colitis: The roles of c-Jun N-terminal kinase and p38 mitogen-activated protein kinase. Journal of medicinal food, 16(4), 296-305.

54. **Mao, L., Yuan, L., Slakey, L. M., Jones, F. E., Burow, M. E., Hill, S. M. (2010):** Inhibition of breast cancer cell invasion by melatonin is mediated through regulation of the p38 mitogen-activated protein kinase signaling pathway. Breast Cancer Research, 12(6), 1-14.

55. **Yang, H., Gu, Z. T., Li, L., Maegele, M., Zhou, B. Y., Li, F., Zhao, K. S. (2017):** SIRT1 plays a neuroprotective role in traumatic brain injury in rats via inhibiting the p38 MAPK pathway. Acta PharmacologicaSinica, 38(2), 168-181.

56. **Karakus, E., Halici, Z., Albayrak, A., Polat, B., Bayir, Y., Kiki, İ., Aksak, S. (2013):** Agomelatine an antidepressant with new potent hepatoprotective effects on paracetamol-induced liver damage in rats. Human & experimental toxicology, 32(8), 846-857.

57. **Karaman, A., Diyarbakir, B., Durur-Subasi, I., Kose, D., Özbek-Bilgin, A., Topcu, A., Alper, F. (2016):** A novel approach to contrast-induced nephrotoxicity: the melatonergic agent agomelatine. The British journal of radiology, 89(1061), 20150716.

58. **Biasi, F., Leonarduzzi, G., Oteiza, P. I., Poli, G. (2013):** Inflammatory bowel disease: mechanisms, redox considerations, and therapeutic targets. Antioxidants & Redox Signaling, 19(14), 1711-1747.

59. **Crespo, I., Miguel, B. S., Laliena, A., Álvarez, M., Culebras, J. M., González-Gallego, J., Tuñón, M. J. (2010):** Melatonin prevents the decreased activity of antioxidant enzymes and activates nuclear erythroid 2-related factor 2 signaling in an animal model of fulminant hepatic failure of viral origin. Journal of pineal research, 49(2), 193-200.

60. **Li, W., Khor, T. O., Xu, C., Shen, G., Jeong, W. S., Yu, S., Kong, A. N. (2008):** Activation of Nrf2-antioxidant signaling attenuates NFκB-inflammatory response and elicits apoptosis. Biochemical pharmacology, 76(11), 1485-1489.

61. **Chumboatong, W., Khamchai, S., Tocharus, C., Govitrapong, P., Tocharus, J. (2020):** Agomelatine protects against permanent cerebral ischaemia via the Nrf2-HO-1 pathway. European journal of pharmacology, 874, 173028.

62. **Xue, F., Huang, J. W., Ding, P. Y., Zang, H. G., Kou, Z. J., Li, T., Yan, W. J. (2016):** Nrf2/antioxidant defense pathway is involved in the neuroprotective effects of Sirt1 against focal cerebral ischemia in rats after hyperbaric oxygen preconditioning. Behavioral brain research, 309, 1-8.

63. **E Camacho, M., D Carrion, M., C Lopez-Cara, L., Entrena, A., A Gallo, M., Espinosa, A., Acuña-Castroviejo, D. (2012):** Melatonin synthetic analogs as nitric oxide synthase inhibitors. Mini reviews in medicinal chemistry, 12(7), 600-617