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EVALUATION THE PROTECTIVE ROLE OF MELATONIN IN ULCERATIVE COLITIS IN MICE

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ABSTRACT

Background: Recent studies have identified the involvement of the inflammatory pathway and the oxidative stress pathway in the pathogenesis of ulcerative colitis. **Objectives of the study:** to investigate the weight loss, colon weight to length ratio, and histological improvement in a mouse model of acetic acid -induced colitis received a preventive melatonin. **Materials and methods:** 48 mice were divided into 4 groups (n =12): group A: normal group; group B: control group; group C: melatonin pretreatment group at a 100 mg/kg; group D: mice were treated with mesalamine as a reference drug for ulcerative colitis. Ulcerative colitis was induced intrarectal administration of 2 ml 6% (v/v) AA in 0.9% NaCl using a soft cannula. Colitis was induced in the seventh day of experiment and finally all mice were killed in the eightieth day under deep ether anaesthesia. **Results:** The highest value of weight loss improvement was recoded in group C (4.29%) with no significant differences between treatment and protective groups ($p > 0.05$). Additionally, the colon weight to length ratio recorded for treatment and protective groups were not significantly differ between each other ($p>0.05$). Histological examination showed that melatonin with 100 mg/kg dose reduced the depth of ulcers (1.000±1.14), without a statistically significant difference with mesalamine group (0.375±0.740). **Conclusion:** The protective effect of melatonin against ulcerative colitis induced in mice was demonstrated. Melatonin alleviated the symptoms of ulcerative colitis, the weight loss in ulcerative colitis was limited. Moreover, colon weight/length ratio and histological parameters improved.

KEYWORDS: Ulcerative colitis – Intestinal – Melatonin.

INTRODUCTION

Ulcerative colitis (UC), which has had increasing incidence rates at the global level,[1] is a chronic idiopathic form of Inflammatory Bowel Disease (IBD) characterized by persistent inflammation with no space for normal tissue between the affected tissues. The inflammation is superficial to the intestinal mucosa, extending variably from the rectum to the cecum. It is characterized by remissions interspersed with acute flares of the disease.[2] Genetic and environmental factors play a crucial role in the onset of UC. Over 200 loci associated with the risk of UC have been identified through genetic analyses of UC.[3,4] Additionally, several factors like diet, smoking, sleep habits, cleanliness, and antibiotic use play a role in the development of UC, and the intestinal microbiota also impacts the risk of UC.[5]

Studies have suggested that the microbiota comprises a virtual superorgan, because of its numerous critical roles in human physiology and metabolism.[6] The microbiota's roles consist of extraction indigestible nutrients from food, synthesizing vitamins, regulating secretions and motility in the intestines, and teaching the immune system to generate mucosal tolerance. Mucosal tolerance is crucial, as UC is primarily characterized by inadequate control of the mucosal immune response resulting from the absence of mucosal tolerance and impaired protective innate immunity against dysbiotic microbiota. Indeed, all chronic illnesses are connected to the intestinal flora,

with the most compelling evidence of intestinal pathogenesis seen in cases of UC.[7]

On other hand, UC results in higher intestinal permeability, potentially creating favorable conditions for gut bacteria to move to different organs. Elevated levels of lipopolysaccharide (LPS) are linked to increased bacterial translocation, leading to inflammation and tissue injury in experimental and pathological situations.[8,9]

Disease activity is defined by the presence of neutrophil-mediated epithelial involvement, which may infiltrate the crypt epithelium results in cryptitis, or neutrophils may accumulate in the lumen of the crypts results in called crypt abscesses.[10] The chronic condition is defined by the distortion of the crypts, which become short, characterized by a space between the bottom of the crypts and the upper border of the muscularis layer, and the branching of the crypts. Histological evaluation in severe cases of UC includes erosion and ulceration of the intestinal mucosa, while in moderate cases, cryptitis and crypt abscesses are noted.[11]

The majority of medications used to treat UC target a single aspect, are not very effective, and are too expensive for many individuals. So, agents with the ability to impact several molecular pathways, with decreased side effects, and lower costs, show great promise in treating UC.[12] Additionally, UC has been linked to local and systemic damage, with inflammation and oxidative stress playing a role.[13] Therefore, a suitable anti-inflammatory and antioxidant agent might be necessary to help decrease the severity of UC and the resulting systemic damage.[12]

Melatonin (N-acetyl-5-methoxytryptamine) was discovered and characterized as an endogenous hormone produced and released by the pineal gland in the 1960s.[14] Numerous studies have shown that oral administration of melatonin eases symptoms of UC. The primary actions of melatonin include reducing the levels of matrix metalloproteinases,[15] controlling immunological damage through macrophage activity,[16] reducing oxidative stress identified by high lipid peroxide levels,[17] lowering inflammation-promoting cytokine levels,[18] inhibiting nitric oxide production, and nuclear factor-kappa beta (NF-kb) activity.[18]

In summary, substantial evidence indicates melatonin has powerful antioxidant properties.[19] It could be beneficial as an antioxidant for various diseases. Certain physical and chemical attributes make melatonin more advantageous than other antioxidant molecules in certain aspects.[20]

While there are many studies showings the positive effects of melatonin on UC, there is few researches on how melatonin protect from body weight loss, intestinal histological changes induced by UC. The purpose of this study was to elucidate the protective role of melatonin against local and systemic damage in mice with UC.

MATERIALS AND METHODS

Animals

All animal experimental procedures were approved by the Research Ethics Committee of the Faculty of Pharmacy Tishreen University, Syrian Arab Republic. Moreover, experiments on animals were performed in accordance with the Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA) guidelines.

Tests were performed on 36 male White Balb-c mice (23-30 g). All the animals were housed in a controlled environment at ambient temperature (20-24 C), humidity between 40% to 60%, and a regulated pattern of 12 hours of light followed by 12 hours of darkness (Figure 1). Standard laboratory animal feed and water were provided ad libitum. The animals were allowed to adjust to the experimental conditions for a week before the experiment began.

Figure 1: White Balb-c mice.

Colitis Induction

This study was based on an animal model of acetic acid-induced UC. Mice were fasted for 24 h with free access to water. After anesthesia with ether, the disease was induced by intrarectal administration of 2 ml 6% (v/v) AA in 0.9% NaCl using a soft cannula. Mice were kept in a head-down position for 30 s to prevent acid leakage. 2 ml of air was applied before the catheter was withdrawn to allow the acetic acid to diffuse completely into the colon (Figure 2).

Figure 2: UC induction using acetic acid.

Dosing Studies

The sample was divided into four groups (n = 12) as follow:

1. Group A (normal group): mice in healthy status
2. Group B (control group): mice received intraperitoneal saline injection for eight days.
3. Group C (protective group): pre-treatment with 100 mg/kg/day of melatonin (MELATONIN, UNIPHARMA, DAMASCUS, SYRIA) by intraperitoneal injection.
4. Group D: received oral mesalamine (MESALAMINE, AVENZOR FOR PHARMACEUTICAL INDUSTRIES, DAMASCUS, SYRIA) for eight days.

Colitis was induced in the seventh day of experiment and finally all mice were killed in the eightieth day under deep ether anaesthesia. The weight of mice was measured from the first day until the end of the experiment, and then the percentage of change in the animal's body weight was calculated.

Colon Weight to Length Ratio

Abdomens of mice were opened immediately after killing, and the colon was excised from the cecum to the anus (Figure 3). It was freed from adhering adipose tissue, opened longitudinally, and washed with 0.9% saline to remove fecal matter. The colon was gently dried between two filter papers and then carefully stretched and the distance from the colo-cecal junction to the anus was measured in cm and then placed on an electronic balance to measure weight in grams. The ratio of colon weight to colon length was calculated as an indirect marker of inflammation (Figure 4).

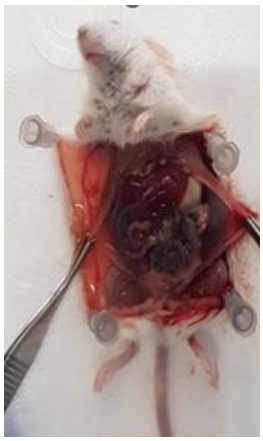
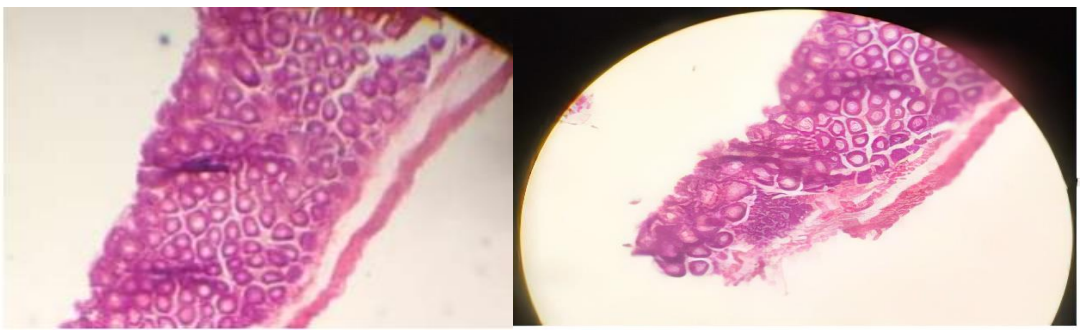


Figure 3: opening abdomen of killed mouse.

Figure 4: Colon weight to length ratio measurement.

Histological Study

The colon tissue samples taken for histological examination were fixed overnight in 4% neutral buffered formalin, processed and sectioned (4 mm thick), and stained with haematoxylin and eosin. Each colon was examined and evaluated by two independent observers. The assessment criteria of the histological score were modified according to Dieleman et al.[21] using a scoring system (Figure 5).



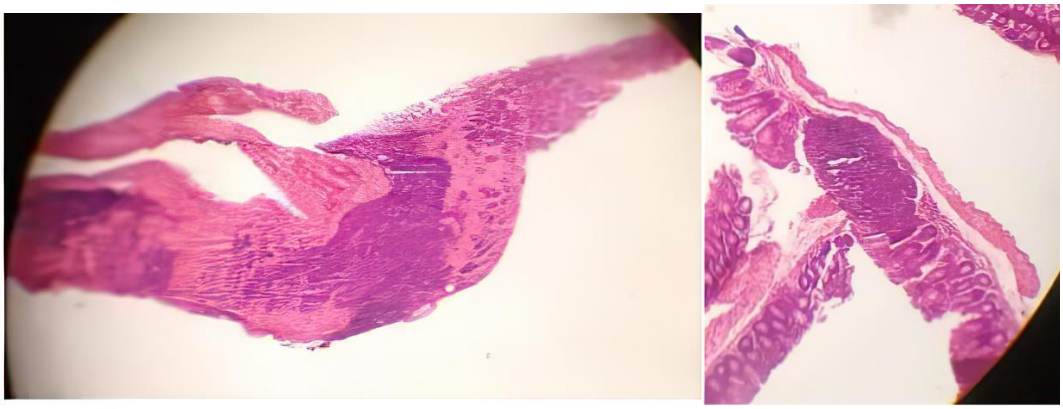


Figure 5: Histological examination of colon tissue samples.

Statistical Analysis

Data that were obtained were analyzed using the statistical software IBM SPSS version 20 (SPSS, Inc., Chicago, IL, USA). Data were expressed as means (SD), the difference between the means of groups was evaluated with one way analysis of variance Statistically significant interactions was followed up with post hoc analyses and a p value of less than 0.05 was considered significant.

RESULTS

The means and standard deviations of the percentage of weight change of mice are presented in Table 1. One-way ANOVA revealed significant differences in percentage of weight change values; the highest value was recoded in group B. Post Hoc multiple comparisons revealed statistically significant decrease in percentage of weight change when compare group B and group C with group D ($p < 0.05$). Moreover, there were significant differences between control group and protective group ($p < 0.05$).

Table 1: Means and standard deviations of the percentage of weight change of mice.

Group	Mice Number	percentage of weight change (%)	
		Mean	standard deviation
A	12	6.922	3.90
B	12	-18.03	6.97
C	12	-0.92	5.63
D	12	4.29	1.17

According to the results of Tukey's test, no treatment group (group B) produced significantly higher colon weight to length ratio than those of other groups ($p<0.05$). The colon weight to length ratio recorded for treatment and protective groups were not significantly differ between each other ($p>0.05$) as shown in Table 2.

Table 2: Means and standard deviations of Colon Weight to Length Ratio.

Group	Mice Number	Colon Weight to Length Ratio (g/cm)	
		Mean	standard deviation
A	12	0.038	0.008
B	12	0.098	0.043
C	12	0.059	0.015
D	12	0.041	0.009

Histological study results are presented in Table 3 and Figure 6. Statistical analysis revealed that the histological changes for normal, protective and treatment groups were not significantly differ between each other ($p>0.05$). however, they were significantly higher than those of no treatment group ($p<0.05$).

Table 3: Means and standard deviations of Histological analysis.

Group	Mice Number	Histological analysis	
		Mean	standard deviation
A	12	0.125	0.353
B	12	3.750	0.462
C	12	1.000	1.410
D	12	0.375	0.740

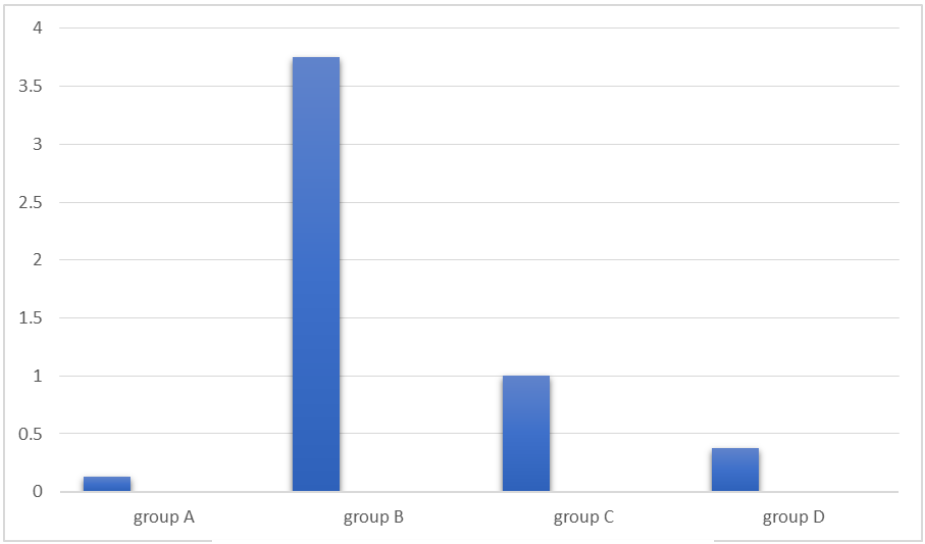


Figure 6: Histological score of study groups.

DISCUSSION

Ulcerative colitis is a chronic inflammatory disorder that affects the mucous membrane of the colon, which may affect parts or all of it, leading to a series of symptoms such as abdominal pain, bloody diarrhea, in addition to a decrease in the quality of life of patients and sleep disturbances.[22] Oxidative stress is a strong factor in the development and exacerbation of ulcerative colitis.[23] Melatonin plays a variety of roles, such as acting as an antioxidant, memory formation, and reducing blood pressure.[24] Furthermore, numerous past researches have demonstrated that both oral and rectal melatonin administration can inhibit UC.[25] Melatonin is found in high levels in the digestive system, and removing the pineal gland has no effect on the levels of melatonin in the gut. While it is commonly believed that IBD disrupts the balance between the gut microbiota and the host immune system,[26] the impact of melatonin on protective of UC is still unclear. In this study, we demonstrated that intraperitoneal administration of melatonin to mice protects against acetic acid-induced UC. Furthermore, mice pretreated with melatonin showed similar improvements to those treated with mesalamine, with no statistically significant differences.

The acetic acid-induced UC mouse model is a standard chemical model for acute colitis, is easily induced, and is widely used as an experimental model for ulcerative colitis, exhibiting diverse pathophysiological manifestations, and mimicking the hallmarks of human UC.[27] The clinical features of weight loss, decreased mucus production, and colon weight gain due to edema and inflammation are common manifestations of this model.[28] In current study, the use of acetic acid resulted in a significant inflammatory response, as evidenced by the weight loss, and increased colon weight/length of the mice.

We observed that administration melatonin at a dose of 100 mg/kg limit weight loss compared to the control group, which may be due to the anti-inflammatory properties of melatonin, through which ulcers are reduced and thus pain is reduced, the intestinal absorptive function is improved, and the mouse's appetite for food is improved without discomfort. These observations were agree with previous studies; Pan et al. recorded a decrease in the rate of weight loss when applying melatonin at a dose of 20 mg/kg to mice in which ulcerative colitis was induced with dextran sodium sulfate.[28] Also, Chamanara et al. demonstrated the effectiveness of melatonin in reducing the rate of weight loss in a model of colitis induced by trinitrobenzene sulfonic acid in rats.[29] This study also showed that induction of UC with acetic acid in mice caused an increase in colon weight and a decrease in its length, and thus an increase in the ratio of colon weight/length, which is an indirect indicator of UC due to the edema and inflammation occurring in the colon.

In view of histology, Colonic edema, ulcers, and necrosis were observed, as well as neutrophil infiltration into the lamina propria, cryptitis and abscesses, vascular congestion in the submucosa, and loss of colonic epithelial cells, which is consistent with previous studies.[30] These signs significantly decreased in melatonin-pretreated group, with a marked improvement in the colonic epithelium, as well as a decrease in neutrophil and inflammatory cell infiltration into the colonic tissue. This evidence supports our finding that melatonin has a highly beneficial protective effect in mice with colitis.

Although further study such as conventional knock-out system is also required to confirm the exact mechanisms of melatonin and to exclude many confounding factors, this study suggests that melatonin may be useful in therapeutics for UC.

CONCLUSIONS

Under the limitations of the present study, few conclusions can be drawn.

The therapeutic potential of melatonin in protective of UC in mice induced by acetic acid, as melatonin alleviated the symptoms of UC, and the weight loss that occur in UC were limited. It also reduced edema in the colon and showed an improvement in histological parameters.

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CONSENT FOR PUBLICATION
Not applicable.

COMPETING INTERESTS
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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